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Title: Thermal Tolerance and Adaptation in Cnidarians: An Investigation of Host Transcriptomic Responses and Heritable Variation Across Natural Populations.

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Coral reefs have become vulnerable to climate change, with mass bleaching events, the loss of symbiotic algae (Symbiodiniaceae), increasing in both frequency and severity. As climate change continues to threaten the persistence and existence of coral reefs around the world, the biggest question posed for coral reefs is "can they adapt to ongoing climate change threats?" A growing number of studies have recently shown the importance of host transcriptomic responses, evidence of genetic diversity in bleaching susceptibility, and potential adaptive responses in these traits, but there are still gaps in our understanding of these mechanisms and their distribution across corals. Therefore, the research presented in this dissertation addresses 1) the genes and genomic regions associated with genetic variation in bleaching responses, 2) heritability of thermal tolerance traits in natural populations, and 3) the roles of gene expression and symbiont communities in thermal acclimation.

In Chapter 2, I used quantitative genetic and genomic approaches to investigate heritable variation in thermal tolerance in the coral species *Orbicella faveolata*, as well as the genomic basis for this variation. I estimated narrow-sense heritability (h^2) and used a genome-wide association study to identify loci

significantly associated with thermal tolerance, indicating capacity for adaptation in this natural population of corals. In addition, profiling gene expression in corals with contrasting bleaching phenotypes uncovered substantial differences in transcriptional stress responses between heat-tolerant and heat-susceptible corals. In Chapter 3, I quantified variation in thermal tolerance and investigated its genomic basis using *Anthopleura elegantissima,* a model system for corals. Using SNP genotypes to compare anemone aggregations, I estimated clonal repeatability (a proxy for broad sense heritability, H^2) and narrow-sense heritability, revealing substantial heritable variation. Additionally, I conducted a genome-wide association study and found significant genetic markers and genes associated with thermal tolerance. Heterozygote advantage was evident across these markers, indicating a potential role in Cnidarian thermal tolerance. In Chapter 4, I conducted a comparative study across eight coral taxa to explore variation in thermal acclimation capacity at high and low temperatures. I profiled gene expression following acclimation to investigate the functional basis for variation in thermal acclimation and pinpointed genes playing more of a mechanistic role. Additionally, I surveyed changes in algal symbiont communities to investigate changes in symbiont communities during acclimation that may contribute to subsequent changes in thermal tolerance of the holobiont. This study revealed considerable variation across coral taxa and documents potential mechanisms that might explain this variation, information important for modeling biological responses to ocean warming. Together, the work presented here provides insights into the potential for adaptation and acclimation in corals threatened by climate change, and identifies potential genomic regions and genes that may become targets of selection as ocean temperatures continue to rise.

Thermal Tolerance and Adaptation in Cnidarians: An Investigation of Host Transcriptomic Responses and Heritable Variation Across Natural Populations

by
Katherine E. Dziedzic

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Katherine E. Dziedzic, Author

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CONTRIBUTION OF AUTHORS

Dr. Hannah Tavalire developed the heritability pipeline outlined in Chapter 2 and 3.

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DEDICATION

To my parents. Thank you for indulging my curiosity for marine science at such a young age. Without your support and encouragement, I wouldn't be where I am today.
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To Geoffrey. Here's to the last six years and long distance coming to an end.
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CHAPTER 1 – Introduction

Coral reefs are one of the most diverse and complex ecosystems in the world, providing a variety of environmental and economic services, such as sheltering thousands of reef fishes, protecting coastal environments, and serving as a source of income for local communities. However, these reefs are globally threatened as increasing sea surface temperatures due to global warming are causing mass bleaching events worldwide (Heron *et al.*, 2016; Hughes *et al.*, 2017). To predict the ability of corals to adapt to a warming climate, we need to understand how their thermal capacity may change over time through acclimatization, a phenotypic change due to multiple environmental stressors, and adaptation, a change that causes an organism to become better suited to its environment. Acclimatization appears to be a likely contributor to individual and population responses in coral species, but genetic variation in this trait among and within coral species has received little attention. Additionally, while we see considerable variation in bleaching susceptibility across species, it is uncertain whether this variation can contribute to selection and lead to adaptation of these populations over time. The research presented here builds upon existing studies to explore the possible genetic and genomic mechanisms underlying thermal tolerance and thermal acclimation, a phenotypic change due to a single environmental stressor through experimental manipulation. I test for genetic contributions to thermal tolerance in cnidarians, estimate heritability of thermal tolerance in natural populations of cnidarians, and compare capacities of thermal acclimation across species spanning the coral phylogenetic tree. This work provides insights for future studies of coral bleaching response and thermal adaptation in the face of rising sea surface temperatures, as well as useful information to aid in coral reef management and restoration.

Coral Reefs and their Dinoflagellate Partners

Reef-building corals (Order Scleractinia) live in high light, nutrient-poor tropical waters near the equatorial region from about 30°N to 30°S and exhibit substantial diversity in morphology (i.e. plating, branching, massive, etc.) and life history strategies

(i.e. brooding vs. broadcast spawning, horizontal vs. vertical symbiont transmission) (Baird *et al.*, 2009a; Harrison, 2011; Veron, 2011; Darling *et al.*, 2012; Veron, 2013). Differences in morphologies allow reefs to form complex structures, providing habitats for an incredible diversity of marine life such as reef fishes and invertebrates (Glynn & Enochs, 2011). In addition, these reefs offer a multitude of economic services for countries and cities living in close proximity to reefs, providing income through tourism and fishing industries (Barbier *et al.*, 2011; Spalding *et al.*, 2017). They also serve as natural wave barriers to protect coastlines from storm surge and large destructive waves (Ferrario *et al.*, 2014) and provide medicinal compounds that can be used to treat cancers and other diseases (Bruckner, 2002; Cooper *et al.*, 2014). While it is often hard to account for all the services coral reef ecosystems provide and therefore their value, some estimates say coral reefs are valued at more than $11 trillion annually (Hoegh-Guldberg *et al.*, 2018; Mehvar *et al.*, 2018).

The symbiosis between corals and single-celled dinoflagellate algae in the family Symbiodiniaceae allows these ecosystems to thrive in high light, nutrient-poor waters. The success of corals at obtaining nutrients in tropical waters is in part due to their ability to feed heterotrophically, but also from reduced organic carbon translocated from the algal symbiont (Muller-Parker *et al.*, 2015). Symbionts translocate the majority of photosynthetically fixed carbon compounds (e.g. glucose, glycerol, and/or amino acids) to the host, and in return the coral provides inorganic nitrogen, phosphorus, and carbon for photosynthesis, in addition to a high-light environment and refuge from herbivory (Muscatine *et al.*, 1984; Davy *et al.*, 2012). The majority of the food corals receive is from the symbiont, and often it is enough to meet energetic demands such as reproduction and building of the calcium carbonate skeleton that forms the reef structure (Muller-Parker *et al.*, 2015). In fact, the presence of algal symbionts can enhance calcification by altering the inorganic chemistry within the gastrovascular cavity and/or the extracellular matrix within the coral tissue, and/or by producing organic molecules directly used in calcification (Al-horani *et al.*, 2003; Holcomb *et al.*, 2011; Davy *et al.*, 2012).

Coral-Symbiodiniaceae relationships are diverse, with some corals species harboring one or a few dominant symbiont types, while others host several background populations of different species (Baker, 2003; Mieog *et al.*, 2007; Silverstein *et al.*, 2012; Quigley *et al.*, 2014; Cunning *et al.*, 2015b). Some Symbiodiniaceae species (e.g. *Breviolum minutum* and *Cladicopium goreaui*) act as generalists and are found in association with multiple coral hosts, whereas other species within the same genus have very specific host preferences (e.g. *Breviolum endomadracis* within corals of the genus *Madracis*) (Baker, 2003; LaJeunesse *et al.*, 2004; Stat *et al.*, 2009; Thornhill *et al.*, 2009; LaJeunesse *et al.*, 2010; Franklin *et al.*, 2012; Smith *et al.*, 2017). Many studies that have examined specific relationship patterns between certain host and symbiont species have seen increased host thermal tolerance during stress (Rowan *et al.*, 1997; Baker *et al.*, 2004; Rowan, 2004; Berkelmans & van Oppen, 2006; Jones & Berkelmans, 2010; Cunning *et al.*, 2015b; Silverstein *et al.*, 2015, 2017), or increased fixed carbon translocation to the host (Fitt, 2000; Cantin *et al.*, 2009; Jones & Berkelmans, 2010; Cunning *et al.*, 2015a), allowing the host to grow at faster rates. These associations can remain stable over time with relatively constant symbiont densities within the host and no partner switches, or the communities can change due to seasonal influences of temperature, salinity, and light exposure (Jones *et al.*, 2008; Bellantuono *et al.*, 2012a; Cunning *et al.*, 2015b; Silverstein *et al.*, 2015). Most importantly, these relationships can change when exposed to certain extreme environmental stressors. Environmental perturbations to this relationship can have serious consequences for both partners, particularly when they undergo stress such as increased temperature.

Coral Bleaching: Breakdown of the Coral-Dinoflagellate Symbiosis

Rising ocean temperatures and ocean acidification due to anthropogenic CO_2 emissions are posing the greatest threats to coral reefs (Pandolfi, 2003; Hughes *et al.*, 2017, 2018). Corals live near their thermal limits and therefore are extremely sensitive to temperature increases, with warming of just 1-2°C often causing severe stress. Specifically, increasing temperatures can cause bleaching events – the breakdown of the symbiotic relationship between corals and their symbionts – across individuals and

populations on both a local and global scale.

The dynamic relationship between the algal symbiont and coral host relies on the ability of photosynthesis to occur in the algae so that organic carbon can be produced and effectively translocated to the host coral. In general, as the coral and its algal symbiont undergo thermal stress, reactive oxygen species (ROS) are produced through a back-up of excitation energy at photosystem II (PSII) in the chloroplasts within the algal symbionts (Weis, 2008). Production of these ROS is thought to occur through one of three ways: (1) damage to PSII, particularly at the reaction center D1 protein, which can cause a backup of excitation energy and dysfunction within PSII; (2) inhibition of the dark reaction (Calvin-Benson cycle) can cause a decline in carbon fixation and therefore reduced consumption of ATP and NADPH within the light reaction, causing a backup of excitation energy; or (3) through direct damage to thylakoid membranes in the symbiont's chloroplasts causing energetic uncoupling in both PSI and PSII (Jones *et al.*, 1998; Warner *et al.*, 1999; Douglas, 2003; Tchernov *et al.*, 2004; Weis, 2008; Lesser, 2011; Oakley & Davy, 2018). As electrons build up from any of these mechanisms, ROS begins to increase in the symbiont and/or the host. Antioxidant defense mechanisms within the symbiont (e.g. superoxide dismutase (SOD), ascorbate peroxidase, glutathione peroxidase) and host reduce and detoxify these ROS agents, repair oxidative stress damage, and prevent further oxidative stress within the animal. However, as ROS begin to accumulate, these defense mechanisms can become overwhelmed and the relationship between host and symbiont begins to break down, also referred as 'dysbiosis' or the symbiosis dysregulation.

There are also signaling events within the host that can cause bleaching to take place. In addition to ROS leaking directly from symbiont cells into host cells, ROS can be produced from mitochondria within the coral host (Dunn *et al.*, 2012). In the host's mitochondrial electron transport chain (ETC), ROS production can begin to increase with the onset of increased temperatures (Turrens, 2003; Dunn *et al.*, 2012). In addition to producing ROS via the ETC, mitochondria also store calcium, and under stress calcium may increase (Görlach *et al.*, 2015; Bertero & Maack, 2018). Studies have shown that calcium-binding proteins play an important role in thermal stress responses alongside

heat shock and antioxidant proteins within the host cell (Ganot *et al.*, 2011; Bellantuono *et al.*, 2012b; Weston *et al.*, 2015; Oakley *et al.*, 2017). In addition, the endoplasmic reticulum is important in regulating protein synthesis and protein folding, mechanisms that are particularly sensitive to temperature. As proteins become unfolded or misfolded, they become toxic and their accumulation induces the unfolded protein response (Walter & Ron, 2011). Thermal stress studies in the sea anemone *Exaiptasia pallida* (commonly called Aiptasia), the coral *Acropora hyacinthus*, and other coral species have shown consistent upregulation of protein folding and degradation proteins (Maor-Landaw *et al.*, 2014; Ruiz-Jones & Palumbi, 2017; Traylor-Knowles *et al.*, 2017a). Lastly, nitric oxide (NO) is a reactive nitrogen species that may play a role in the bleaching cascade, acting as a toxin in animal cells (Weis 2008). In anemones and corals, NO has been shown to increase dramatically with the onset of increased temperatures, and addition of NO to cnidarians can cause bleaching (Perez & Weis, 2006; Bouchard & Yamasaki, 2008; Hawkins *et al.*, 2013).

Ultimately, oxidative stress that causes an increase in ROS and dysfunctional defense mechanisms in the host, algal symbiont, or both causes the coral to lose its symbionts. This can happen via multiple mechanisms, including *in situ* degradation, exocytosis, host cell detachment, apoptosis, autophagy and/or necrosis (Weis 2008; Oakley & Davy 2018). Without re-colonization of symbionts after stress, reef-building corals (those with obligate symbioses compared to facultative corals) will eventually die. However, coral species and their symbionts have varying thermal and oxidative stress susceptibilities; therefore, understanding their individual roles is vital to understanding how this symbiosis is maintained and how dysbiosis is prevented. Importantly, bleaching thresholds can change and have been shown to differ across host species and symbiont associations, raising questions about the roles of the host and symbionts and the mechanisms they may use to become tolerant and adapt to changing conditions.

Mechanisms of Thermal Tolerance and Adaptation in Cnidarians

Bleaching events have increased in frequency and severity over the last three decades, with the last decade being the most devastating to populations all around the

world. In some areas, more than 50% of coral reefs were lost in as little as one year. If bleaching trends continue, models predict >90% of reef species may face long-term degradation (van Hooidonk, 2013). However, the thresholds that induce coral degradation can change over time, Models that consider both environmental conditions and changing thresholds suggest that the fate of corals during the next century may be strongly affected by long-term adaptive changes (D'Angelo *et al.*, 2015). Changes in bleaching thresholds may occur in populations through adaptation (Meyer *et al.*, 2009a; Coles & Riegl, 2013; Palumbi *et al.*, 2014) or in individual corals through acclimatization (Jones & Berkelmans, 2010; Oliver & Palumbi, 2011).

Adaptation

Adaptation through genetic change can play a large role in allowing populations to persist in a changing environment. Genetic variation is the "currency for natural selection" (Császár *et al.*, 2010), allowing individuals to adapt to changing environmental conditions and increasing the survival and reproduction of more fit genotypes in the population (Barrett & Schluter, 2008). In long-lived, clonal species, such as corals that build reefs, the adaptive potential of the organism is best estimated by the clonal or broad-sense heritability, i.e., the proportion of phenotypic variation that is due to genetic factors (Falconer & Mackay, 1996).Thus, the genetic basis of a particular trait, in this case thermal tolerance, determines the adaptive potential of that trait in a given population.

For rapid adaptation to occur in a population, there has to be sufficient genetic variation in fitness-related traits needed for survival. A great deal of genetic variation already exists in coral thermal tolerance, as evidenced in the substantial variation in bleaching susceptibility across and within populations of corals (Bay & Palumbi, 2014; Kenkel & Matz, 2016; Bay *et al.*, 2017; Kirk *et al.*, 2018; Thomas *et al.*, 2018; Dziedzic *et al.*, 2019). However, the environmental and genetic contributions to variation in thermal tolerance across coral species is still unknown (Császár *et al.*, 2010). Some studies have provided evidence for adaptive potential via genetic variation in adult corals, demonstrating thermal tolerance differences between local populations (Jokiel & Brown,

2004; Smith-Keune & Van Oppen, 2006; Oliver & Palumbi, 2011; Riegl *et al.*, 2011; Barshis *et al.*, 2013; Coles & Riegl, 2013; Bay & Palumbi, 2014; Palumbi *et al.*, 2014; Howells *et al.*, 2016; Kenkel & Matz, 2016; Bay *et al.*, 2017; Matz *et al.*, 2018). Other studies have shown considerable heritable variation in coral larvae (Dixon *et al.*, 2015; Kenkel *et al.*, 2015), highlighting the role of genotype in determining thermal tolerance limits. Others have characterized variation in algal symbionts (e.g., Császár *et al.*, 2010). These examples shed light on mechanisms of coral adaptation, but questions still remain regarding rates of adaptation in the coral host, particularly in adult populations that are already experiencing the effects of climate change. Considerations around life history strategies of the coral host are important when estimating rates of adaptation in natural populations of corals. Because corals are slow-growing and take years to reach reproductive maturity (~10-15 years for some coral species), adaptive changes may take decades, rates slower than what is needed to keep pace with the current rates of warming.

To estimate selection responses in corals and consider rates of adaptation, heritability in thermal tolerance needs to be quantified. Adaptive responses to selection can be assessed in populations using the selection differential (i.e. the difference between a population's mean trait before vs. after selection) and narrow-sense heritability (h^2) (Falconer & Mackay 1996). This quantitative approach uses the Breeder's equation to estimate the expected evolutionary change in a trait per generation. Currently, very few studies provide heritability estimates for coral species and their algal symbionts. Despite this gap, estimates on the fate of corals can be calculated by considering generation times and response to selection in a handful of species. One study used empirical measurements of bleaching thresholds, biologically realistic assumptions for the rates of adaptive responses, and generation times of corals to estimate that it may take 67 years for some corals to adapt to a 1.5°C increase (Baird *et al.* 2013). Another study used an evolutionary quantitative genetics model with heritability estimates and a variety of host traits to reveal that adaptation may be possible in some populations, as long as there is enough heritable variation ($h^2 > 0.5$) and selection pressures are strong (Colton *et al.* unpublished data). However, Hoegh-Guldberg and colleageus (2007) are less optimistic and concluded that corals may lack the adaptive capacity to the current rates of warming.

These concerns over rates of adaptation have led many to focus on acclimatization to high temperatures as the primary mechanism for thermal tolerance.

Acclimatization

Variation in the capacity for corals to withstand thermal stress may be especially important in the short term. Acclimatization, the ability of an individual to adjust its phenotype during the duration of its lifetime, signifies a response to a variety of natural environmental stressors. On the other hand, acclimation refers to a phenotypic shift related to a single environmental variable (e.g., temperature) (Gates & Edmunds, 1999). More specifically, thermal acclimation is associated with experimental manipulation as researchers can focus on one environmental variable by controlling for other factors. For corals, acclimation may be due to gene expression changes in the coral host (Voolstra et al. 2009; Seneca et al. 2010; Bellantuono et al. 2012a; Kenkel et al. 2013; Louis et al. 2017), varying associations with certain algal symbionts (Baker *et al.*, 2004; LaJeunesse *et al.*, 2004; Van Oppen *et al.*, 2005; Jones *et al.*, 2008; Cunning *et al.*, 2015a, 2015b), or influences from local environmental conditions (Brown *et al.*, 2002; Howells *et al.*, 2011; D'Angelo *et al.*, 2015). In order to pinpoint more at-risk species, it is important to consider these three responses together and determine their overall contributions to coral thermal tolerance (Barshis *et al.*, 2010).

Acclimation can occur more rapidly than adaptation, and has been widely viewed as important components of biological responses to a changing climate (Bay *et al.*, 2013; Palumbi *et al.*, 2014; Putnam & Gates, 2015). Currently, there is evidence for diverse responses in acclimation potential in the coral host. Studies identify a variety of acclimation effects across multiple coral taxa and environmental conditions. For instance, Rodolfo-Metalpa et al. (2014) found little to no thermal acclimation in populations of the Mediterranean coral *Oculina patagonica*, despite a gradual two-week exposure to increasing temperature. Furthermore, Howells et al. (2013) found evidence in the field for population-specific responses of *Acropora millepora* and their symbionts, suggesting that local adaptation and thermal history may limit acclimatization potential in some populations of corals. On the contrary, in a laboratory experiment by Bellantuono et al.

(2012b), *Acropora millepora* colonies were relatively more tolerant to bleaching when exposed to a 10-day acclimation period as compared to their non-acclimated counterparts. Another study that acclimated *Acropora nana* to stable and variable temperature regimes found individuals to be more thermally tolerant after only 7-11 days in acclimation treatments (Bay & Palumbi, 2015). They also found striking transcriptional differences in acclimated and non-acclimated corals, indicating the importance of an acclimation period in responding to thermal stress events. Across natural populations, Kenkel et al., (2013) demonstrated strong genetic partitioning, as well as strong differentiation between gene expression profiles, providing evidence for acclimatization in different habitats.

Overall, while there is evidence for acclimatization in some populations of corals, the differences across these studies in acclimatization and acclimation potential could be due to differences in coral species, symbiont types, and/or locations studied. While there is some evidence for acclimatization in the field and acclimation in the lab, it is still uncertain whether short-term exposure provides any benefit for coral thermal tolerance and whether this mechanism of bleaching resistance occurs in every coral species.

Symbiont Switching and/or Shuffling

Apart from mechanisms within the coral host, functional and physiological differences among symbiont types may help corals become tolerant during periods of thermal stress. Symbiont communities can change temporarily over time. These changes may include “symbiont shuffling”, which involves adjusting the abundance of major symbiont species, or “symbiont switching”, in which symbiont species are changed to readily available or favorable types (Baker, 2003; Baker *et al.*, 2004; Jones *et al.*, 2008; Cunning *et al.*, 2015b; Silverstein *et al.*, 2015). The dynamics of partner associations before, during, and after bleaching events is still largely unknown, but evidence suggests that there are mechanisms of thermal tolerance in certain host-symbiont associations. However, these mechanisms may not exist in every reef-building coral species, and it is imperative to understand how these relationships may evolve and adapt to future conditions.

Genomic and Transcriptomic Insights into Thermal Tolerance

High-throughout DNA sequencing technologies have enabled researchers to apply a wide range of genomic and transcriptomic methods to study the mechanisms underlying thermal tolerance in coral populations. Genomic and transcriptomic studies generate a large amount of sequence data (i.e., tens of thousands of genes), allowing researchers to explore the correlation of traits (e.g. bleaching tolerance or susceptibility, bleaching recovery, growth, etc.) and environmental conditions (Barshis, 2015). The diversity of genes and molecular responses uncovered from these studies has pointed to many potential mechanisms that could facilitate adaptation. As we continue to explore these targets, we are beginning to unravel more specific, sequence-level details about host- and symbiont-specific responses to stress and responses of specific host-symbiont combinations.

Insights into Host Mechanisms

Over the past two decades, the number and quality of genomic and transcriptomic resources for cnidarian host species has increased dramatically. More than 15 coral genomes and 20 transcriptomes have become publicly available (Meyer *et al.*, 2009b; Shinzato *et al.*, 2014; Traylor-Knowles *et al.*, 2011; Medina *et al.*, 2011; Polato *et al.*, 2011; Shinzato *et al.*, 2011; Kitchen *et al.*, 2015; Anderson *et al.*, 2016; Mansour *et al.*, 2016; Voolstra *et al.*, 2017; Kenkel & Bay, 2017; ReFuGe 2020 Consortium, 2017; Cunning *et al.*, 2018; Ying *et al.*, 2018). These resources and their associated studies are finding diverse gene expression patterns, gene sequence differences, and genetic variation across species and populations, which provide evidence for thermal tolerance in corals. Transcriptomic studies in cnidarians have shown that thermal stress-induced bleaching strongly affects gene expression profiles. This can happen through up-regulation of heat shock proteins, antioxidant enzymes, apoptosis and autophagy proteins, and protein folding genes, and down-regulation of calcium homoeostasis and ribosomal proteins during early onset of heat stress (DeSalvo *et al.* 2008; Voolstra *et al.* 2009; Barshis *et al.* 2013; Palumbi *et al.* 2014; Kenkel and Matz 2016; Ruiz-Jones and Palumbi 2017). Comparative genomics emphasize the importance of immunity and apoptotic genes for

responses to stress (Shinzato *et al.*, 2011; Bhattacharya *et al.*, 2016; Cunning *et al.*, 2018) and coral acid-rich proteins (CARPs), collagens, and adhesion proteins for calcification (Bhattacharya *et al.*, 2016). Not only are these resources providing insights into the molecular basis of responses to environmental stress, but they are also helping to improve our understanding of the onset and maintenance of symbiosis.

Insights into Symbiont Mechanisms

There is an extraordinary amount of genetic diversity across symbiont types. We are beginning to appreciate the complexity of these relationships and the importance of physiological differences between symbiont species in response to environmental stressors such as ocean acidification, nutrient levels, and temperature (Parkinson *et al.*, 2015, 2016; LaJeunesse *et al.*, 2018). Transcriptomic studies on various Symbiodiniacea species illustrate strong evolutionary divergence between species, with functional differences in genes involved in protein folding responses and maintaining the thylakoid membrane of the chloroplast during stress (Ladner *et al.*, 2012; Palumbi *et al.*, 2014; Parkinson *et al.*, 2016). Differences in these antioxidant and biochemical responses across symbiont species may explain the variation of thermal sensitivity when associated with different hosts (Abrego *et al.*, 2008; Baums *et al.*, 2013; Parkinson *et al.*, 2015). In fact, a recent comparative genomic study of multiple Symbiodiniacea species has provided insights into genome organization, structure, and gene content documenting differences in protein domains that may account for physiological differences across species (Aranda *et al.*, 2016). While these resources provide an impressive first glimpse at the functional basis of species-specific responses to environmental stress, we are just beginning to understand the complexity of responses in host-symbiont associations.

Anemones as Model Systems

Despite the need to understand coral-specific mechanisms to thermal tolerance, we can use model systems to investigate shared mechanisms of tolerance using other cnidarians such as the sea anemones Aiptasia and *Anthopleura elegantissima*. These anemones associate with Symbiodiniaceae, can be manipulated to induce bleaching, and

genomes and transcriptomes are available for both (Muller-Parker & Davy, 2001; Weis *et al.*, 2008; Voolstra, 2013; Baumgarten *et al.*, 2015; Kitchen *et al.*, 2015; Macrander *et al.*, 2018). Using these genomic resources, we can improve our understanding of this important cnidarian-dinoflagellate symbiosis, specifically highlighting similar stress response mechanisms and conserved pathways across Class Anthozoa (Schwarz & Weis, 2003; Dunn *et al.*, 2004; Muller-Parker *et al.*, 2007; Davy *et al.*, 2012; Bellis *et al.*, 2016; Matthews *et al.*, 2017; Macrander *et al.*, 2018). *Anthopleura elegantissima* is a temperate anemone living on the west coast of North America as far north as Alaska and as south as Baja, California. These anemones live in thermally variable intertidal environments with air and water temperatures changing up to 20°C in a day (Helmuth et al. 2002; Bingham et al. 2011). Because they are exposed to such extreme variations in environmental parameters and have thrived in these conditions, we can use this anemone to ask general questions about mechanisms of cnidarian thermal tolerance. Past studies using *A. elegantissima* have studied symbiosis onset and breakdown and have related their findings to the cellular and molecular players driving coral reef responses to stress (Reynolds *et al.*, 2000; Schwarz & Weis, 2003; Richier *et al.*, 2008; Macrander *et al.*, 2018). While there are limitations to comparing corals and these temperate anemones, such as habitat differences (i.e. nutrient-poor versus nutrient-rich environments), carbonate skeletons, etc., we are finding important similarities that can further characterize responses in the cnidarian host.

Dissertation Outline

The coral reef crisis has demanded the attention of coral researchers worldwide, who are searching for answers about the fate of coral reef ecosystems in the next 100 years. With the advancements in genomic and transcriptomic resources, studies have begun to reveal potential physiological and molecular mechanisms driving thermal tolerance differences across coral reef species and populations. Although thermal tolerance has been studied extensively, we still lack answers to many fundamental questions regarding the capacity for corals to increase their thermal tolerance. These processes of acclimatization and adaptation in corals and anemones have often been

considered as separate alternatives, with individual studies emphasizing one over the other as the important driver in future coral responses (e.g., Hoegh-guldberg, 2014; Palumbi *et al.*, 2014). In fact, acclimatization and adaptation are not mutually exclusive, and acclimatization may play an important but under-appreciated role in evolutionary responses to climate change. In this, dissertation I present a collection of studies focused on thermal tolerance and acclimation and the potential for adaptation in corals and anemones.

Specifically, I explore whether genetic variation drives differences in the capacity for thermal tolerance and acclimation and what molecular and physiological mechanisms contribute to this variation. By integrating adaptive and acclimatory responses, I provide a unique perspective on the potential for corals to persist during ongoing climate change. In Chapter 2, I explore heritable variation in bleaching responses and its functional genomic basis in a dominant Caribbean reef-building coral, *Orbicella faveolata.* Using SNP genotyping, I conduct a genome-wide association study to determine if certain loci are indicative of thermal tolerance, estimate heritability of thermal tolerance in a natural population of corals, and profile gene expression in contrasting bleaching phenotypes. Additionally, I link the genomic and transcriptomic datasets to discuss the functional basis of thermal tolerance, a unique opportunity to interpret potential mechanisms of thermal tolerance and adaptation in a natural population of corals. In Chapter 3, I explore genetic variation in thermal tolerance of the temperate sea anemone, *Anthopleura elegantissima*, to aid in our understanding of evolutionary responses to thermal stress in cnidarians. In Chapter 4, I use a comparative transcriptomic approach to study genetic variation in the capacity for corals to acclimate to increasing temperatures, comparing responses across eight reef-building corals in the Indo-Pacific region. I sequenced and annotated six de novo transcriptomes and used these to explore differences in corals from different phylogenetic clades and compare gene expression patterns across different acclimation temperatures. Finally, in Chapter 5, I synthesize findings across these three data chapters and discuss future studies to further our understanding of thermal tolerance and adaptation across species and populations of corals.

References

Abrego D, Ulstrup KE, Willis BL, van Oppen MJH (2008) Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 2273–2282.

Al-horani FA, Al-moghrabi SM, Beer D De (2003) Microsensor study of photosynthesis and calcification in the scleractinian coral, Galaxea fascicularis: active internal carbon cycle. *Journal of Experimental Marine Biology and Ecology*, **288**, 1–15.

Anderson DA, Walz ME, Weil E, Smith MC (2016) RNA-Seq of the Caribbean reef-building coral Orbicella faveolata (Scleractinia- Merulinidae) under bleaching and disease stress expands models of coral innate immunity. *PeerJ*, **4:e1616**, DOI 10.7717/peerj.1616.

Aranda M, Li Y, Liew YJ et al. (2016) Genomes of coral dinoflagellate symbionts highlight evolutionary adaptations conducive to a symbiotic lifestyle. *Scientific Reports*, **6**, 1–15.

Baird AH, Guest JR, Willis BL (2009) Systematic and Biogeographical Patterns in the Reproductive Biology of Scleractinian Corals. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 551–571.

Baker AC (2003) Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of Symbiodinium. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 661–689.

Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Corals' adaptive response to climate change. *Nature*, **430**, 741–741.

Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, Silliman BR (2011) The value of estuarine and coastal ecosystem services. *Ecological Monographs*, **81**, 169–193.

Barrett RDH, Schluter D (2008) Adaptation from standing genetic variation. *Trends in Ecology and Evolution*, **23**, 38–44.

Barshis DJ (2015) Genomic Potential for Coral Survival of Climate Change. In: *Coral Reefs in the Anthropocene*, pp. 133–146.

Barshis DJ, Stillman JH, Gates RD, Toonen RJ, Smith LW, Birkeland C (2010) Protein expression and genetic structure of the coral Porites lobatain an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity? *Molecular Ecology*, **19**, 1705–1720.

Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR (2013)

Genomic basis for coral resilience to climate change. *PNAS*, **110**, 1387–1392.

Baumgarten S, Simakov O, Esherick LY et al. (2015) The genome of Aiptasia , a sea anemone model for coral symbiosis. **112**, 11893–11898.

Baums IB, Polato NR, Xu D et al. (2013) Genotypic variation influences reproductive success and thermal stress tolerance in the reef building coral , Acropora palmata. *Coral Reefs*, **32**, 703–717.

Bay RA, Palumbi SR (2014) Multilocus adaptation associated with heat resistance in reef-building corals. *Current Biology*, **24**, 2952–2956.

Bay RA, Palumbi SR (2015) Rapid acclimation ability mediated by transcriptome changes in reef-building corals. *Genome Biology and Evolution*, **7**, 1602–1612.

Bay LK, Guérécheau A, Andreakis N, Ulstrup KE, Matz M V (2013) Gene Expression Signatures of Energetic Acclimatisation in the Reef Building Coral Acropora millepora. *PLoS ONE*, **8**, 1–10.

Bay RA, Rose NH, Logan CA, Palumbi SR (2017) Genomic models predict successful coral adaptation if future ocean warming rates are reduced. *Science Advances*, **3**, e1701413.

Bellantuono AJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2012a) Resistance to thermal stress in corals without changes in symbiont composition. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 1100–1107.

Bellantuono AJ, Granados-Cifuentes C, Miller DJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2012b) Coral thermal tolerance: tuning gene expression to resist thermal stress. **7**, e50685.

Bellis ES, Howe DK, Denver DR (2016) Genome-wide polymorphism and signatures of selection in the symbiotic sea anemone Aiptasia. *BMC Genomics*, **17**, 1–14.

Berkelmans R, van Oppen MJH (2006) The Role of Zooxanthellae in the Thermal Tolerance of Corals: A “Nugget of Hope” for Coral Reefs in an Era of Climate Change. *Proceedings: Biological Sciences*, **273**, 2305–2312.

Bertero E, Maack C (2018) Calcium signaling and reactive oxygen species in Mitochondria. *Circulation Research*, **122**, 1460–1478.

Bhattacharya D, Agrawal S, Aranda M et al. (2016) Comparative genomics explains the evolutionary success of reef-forming corals. *eLife*, **5**, 1–26.

Bingham BL, Freytes I, Emery M, Dimond J, Muller-Parker G (2011) Aerial exposure and body temperature of the intertidal sea anemone Anthopleura elegantissima. *Invertebrate Biology*, **130**, 291–301.

Bouchard JN, Yamasaki H (2008) Heat stress stimulates nitric oxide production in Symbiodinium microadriaticum: A possible linkage between nitric oxide and the coral bleaching phenomenon. *Plant and Cell Physiology*, **49**, 641–652.

Brown BE, Downs CA, Dunne RP, Gibb SW (2002) Exploring the basis of thermotolerance in the reef coral Goniastrea aspera. *Mar Ecol Prog Ser*, **242**, 119–129.

Bruckner A (2002) Life-Saving Products from Coral Reefs. *Issues in Science and Technology*, **18**, 39–44.

Cantin NE, Oppen MJH, Willis BL, Mieog JC, Negri AP (2009) Juvenile corals can acquire more carbon from high-performance algal symbionts. *Coral reefs*, **28**, 405–414.

Coles SL, Riegl BM (2013) Thermal tolerances of reef corals in the Gulf: A review of the potential for increasing coral survival and adaptation to climate change through assisted translocation. *Marine pollution bulletin*, **72**, 323–332.

Colton MA, Bellis ES, Logan CA et al. Evolutionary Pathways to Coral Persistence if We Act Soon. *In prep*.

Cooper EL, Hirabayashi K, Strychar KB, Sammarco PW (2014) Corals and Their Potential Applications to Integrative Medicine. *Evidence-Based Complementary and Alternative Medicine*, 1–9.

Császár NBM, Ralph PJ, Frankham R, Berkelmans R, van Oppen MJH (2010) Estimating the potential for adaptation of corals to climate warming. *PLoS ONE*, **5**, e9751–e9751.

Cunning R, Silverstein RN, Baker AC (2015a) Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20141725.

Cunning R, Gillette P, Capo T, Galvez K, Baker AC (2015b) Growth tradeoffs associated with thermotolerant symbionts in the coral Pocillopora damicornis are lost in warmer oceans. *Coral Reefs*, **34**, 155–160.

Cunning R, Bay RA, Gillette P, Baker AC, Traylor-Knowles N (2018) Comparative analysis of the Pocillopora damicornis genome highlights role of immune system in coral evolution. *Scientific Reports*, **8**, 1–10.

D'Angelo C, Hume BCC, Burt J, Smith EG, Achterberg EP, Wiedenmann J (2015) Local adaptation constrains the distribution potential of heat-tolerant Symbiodinium from the Persian/Arabian Gulf. *The ISME Journal*, **9**, 2551–2560.

Darling ES, Alvarez-Filip L, Oliver TA, McClanahan TR, Côté IM, Bellwood D (2012) Evaluating life-history strategies of reef corals from species traits. *Ecology Letters*, **15**, 1378–1386.

Davy SK, Allemand D, Weis VM (2012) Cell Biology of Cnidarian-Dinoflagellate Symbiosis. *Microbiology and Molecular Biology Reviews*, **76**, 229–261.

DeSalvo MK, Voolstra CR, Sunagawa S et al. (2008) Differential gene expression during thermal stress and bleaching in the Caribbean coral Montastraea faveolata. *Molecular Ecology*, **17**, 3952–3971.

Dixon GB, Davies SW, Aglyamova GV, Meyer E, Bay LK, Matz MV (2015) Genomic determinants of coral heat tolerance across latitudes. **348**, 2014–2016.

Douglas AE (2003) Coral bleaching - How and why? *Marine Pollution Bulletin*, **46**, 385–392.

Dunn SR, Thomason JC, Le Tissier MDA, Bythell JC (2004) Heat stress induces different forms of cell death in sea anemones and their endosymbiotic algae depending on temperature and duration. *Cell Death and Differentiation*, **11**, 1213–1222.

Dunn SR, Pernice M, Green K, Hoegh-Guldberg O, Dove SG (2012) Thermal stress promotes host mitochondrial degradation in symbiotic cnidarians: Are the batteries of the reef going to run out? *PLoS ONE*, **7**.

Dziedzic K, Elder H, Tavalire H, Meyer E (2019) Heritable variation in bleaching responses and its functional genomic basis in reef-building corals (Orbicella faveolata. *Molecular Ecology*, 1–16.

Falconer DS, Mackay TFC (1996) *Introduction to Quantitative Genetics*, 4th edn. Pearson Education.

Ferrario F, Beck MW, Storlazzi CD, Micheli F, Shepard CC, Airoldi L (2014) The effectiveness of coral reefs for coastal hazard risk reduction and adaptation. *Nature Communications*, **5**, 1–9.

Fitt WK (2000) Cellular growth of host and symbiont in a cnidarian-zooxanthellar symbiosis. *The Biological Bulletin*, **198**, 110–120.

Franklin EC, Stat M, Pochon X, Putnam HM, Gates RD (2012) GeoSymbio : a hybrid , cloud-based web application of global geospatial bioinformatics and ecoinformatics for Symbiodinium – host symbioses. 369–373.

Ganot P, Moya A, Magnone V, Allemand D, Furla P, Sabourault C (2011) Adaptations to endosymbiosis in a Cnidarian-Dinoflagellate association: Differential gene expression and specific gene duplications. *PLoS Genetics*, **7**.

Gates RD, Edmunds PJ (1999) The Physiological Mechanisms of Acclimatization in Tropical Reef Corals. *American Zoologist*, **39**, 30–43.

Glynn PW, Enochs IC (2011) Invertebrates and Their Roles in Coral Reef Ecosystems. In: *Coral Reefs: An Ecosystem in Transition*, pp. 273–325.

Görlach A, Bertram K, Hudecova S, Krizanova O (2015) Calcium and ROS: A mutual interplay. *Redox Biology*, **6**, 260–271.

Harrison PL (2011) Sexual Reproduction of Sclerctinian Corals. In: *Coral Reefs: An Ecosystem in Transition*, pp. 59–85.

Hawkins TD, Bradley BJ, Davy SK (2013) Nitric oxide mediates coral bleaching through an apoptotic-like cell death pathway: evidence from a model sea anemone-dinoflagellate symbiosis. *Federation of American Socities for Experimental Biology*, **28**, 2737.

Helmuth B, Harley CDG, Halpin PM, Donnell MO, Hofmann GE, Blanchette CA (2002) Climate Change and Latitudinal Patterns of Intertidal Thermal Stress. **298**, 1015–1018.

Heron SF, Maynard JA, van Hooidonk R, Eakin CM (2016) Warming Trends and Bleaching Stress of the World's Coral Reefs 1985–2012. *Scientific Reports*, **6**, 38402.

Hoegh-guldberg O (2014) Coral reef sustainability through adaptation: glimmer of hope or persistent mirage? *Current Opinion in Environmental Sustainability*, **7**, 127–133.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ et al. (2007) Coral Reefs Under Rapid Climate Change and Ocean Acidification. *Science*, **318**, 1737–1742.

Hoegh-Guldberg O, Eakin CM, Hodgson G, Sale PF, Veron JEN (2018) Climate Change Threatens the Survival of Coral Reefs Only 12 years to Avoid the Worst Damage. **2015**, 1–4.

Holcomb M, Allemand D, Tambutte S, Venn A, Tambutte E (2011) Live Tissue Imaging Shows Reef Corals Elevate pH under Their Calcifying Tissue Relative to Seawater. *PLoS ONE*, **6**, 1–9.

van Hooidonk R (2013) Temporary refugia for coral reefs in a warming world. *Nature Climate Change*, **3**, 508–511.

Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL, van Oppen MJH (2011) Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change*, **2**, 116–120.

Howells EJ, Berkelmans R, van Oppen MJH, Willis BL, Bay LK (2013) Historical

thermal regimes define limits to coral acclimatization. *Ecology*, **94**, 1078–1088.

Howells EJ, Abrego D, Meyer E, Kirk NL, Burt JA (2016) Host adaptation and unexpected symbiont partners enable reef-building corals to tolerate extreme temperatures. *Global Change Biology*, **22**, 2702–2714.

Hughes TP, Kerry JT, Álvarez-Noriega M et al. (2017) Global warming and recurrent mass bleaching of corals. *Nature*, **543**, 373–377.

Hughes TP, Kerry JT, Baird AH et al. (2018) Global warming transforms coral reef assemblages. *Nature*, **556**, 492–496.

Jokiel PL, Brown EK (2004) Global warming, regional trends and inshore environmental conditions influence coral bleaching in Hawaii. *Global Change Biology*, **10**, 1627–1641.

Jones A, Berkelmans R (2010) Potential costs of acclimatization to a warmer climate: growth of a reef coral with heat tolerant vs. sensitive symbiont types. *PLoS ONE*, **5**, e10437.

Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U (1998) Temperature-induced bleaching of corals begins with impairment of the CO2 fixation mechanism in zooxanthellae. *Plant, Cell and Environment*, **21**, 1219–1230.

Jones AM, Berkelmans R, van Oppen MJ., Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 1359–1365.

Kenkel CD, Bay LK (2017) Novel transcriptome resources for three scleractinian coral species from the Indo-Pacific. *GigaScience*, **6**, 1–4.

Kenkel CD, Matz M V (2016) Gene expression plasticity as a mechanism of coral adaptation to a variable environment. *Nature Publishing Group*, **1**, 1–6.

Kenkel CD, Meyer E, Matz M V. (2013) Gene expression under chronic heat stress in populations of the mustard hill coral (Porites astreoides) from different thermal environments. *Molecular Ecology*, **22**, 4322–4334.

Kenkel CD, Setta SP, Matz M V (2015) Heritable differences in fitness-related traits among populations of the mustard hill coral, Porites astreoides. *Heredity*, **115**, 509–516.

Kirk NL, Howells EJ, Abrego D, Burt JA, Meyer E (2018) Genomic and transcriptomic signals of thermal tolerance in heat-tolerant corals (Platygyra daedalea) of the Arabian/Persian Gulf. *Molecular Ecology*, **27**, 5180–5194.

Kitchen SA, Crowder CM, Poole AZ, Weis VM, Meyer E (2015) De Novo Assembly and Characterization of Four Anthozoan (Phylum Cnidaria) Transcriptomes. *G3: Genes, Genomes, Genetics*, **5**, 2441–2452.

Ladner JT, Barshis DJ, Palumbi SR (2012) Protein evolution in two co-occurring types of Symbiodinium: an exploration into the genetic basis of thermal tolerance in Symbiodinium clade D. *BMC Evolutionary Biology*, **12**, 217.

LaJeunesse TC, Thornhill DJ, Cox EF, Stanton FG (2004) High diversity and host specificity observed among symbiotic dinoflagellates in reef coral communities from Hawaii. *Coral reefs*, **23**, 596–603.

LaJeunesse TC, Pettay DT, Sampayo EM et al. (2010) Long-standing environmental conditions, geographic isolation and host-symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus Symbiodinium. *Journal of Biogeography*, **37**, 785–800.

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. *Current Biology*, **28**, 2570-2580.e6.

Lesser MP (2011) Coral Bleaching: Causes and Mechanisms. In: *Coral Reefs: An Ecosystem in Transition*, pp. 405–419.

Louis YD, Bhagooli R, Kenkel CD, Baker AC, Dyall SD (2017) Gene expression biomarkers of heat stress in scleractinian corals: Promises and limitations. *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology*, **191**, 63–77.

Macrander JC, Dimond JL, Bingham BL, Reitzel AM (2018) Marine Genomics Transcriptome sequencing and characterization of Symbiodinium muscatinei and Elliptochloris marina , symbionts found within the aggregating sea anemone Anthopleura elegantissima. *Marine Genomics*, **37**, 82–91.

Mansour TA, Rosenthal JJC, Brown CT, Roberson LM (2016) Transcriptome of the Caribbean stony coral Porites astreoides from three developmental stages. *GigaScience*, **5**, 1–6.

Maor-Landaw K, Karako-Lampert S, Ben-Asher HW, Goffredo S, Falini G, Dubinsky Z, Levy O (2014) Gene expression profiles during short-term heat stress in the red sea coral Stylophora pistillata. *Global Change Biology*, **20**, 3026–3035.

Matthews JL, Crowder CM, Oakley CA et al. (2017) Optimal nutrient exchange and immune responses operate in partner specificity in the cnidarian-dinoflagellate symbiosis. *Proceedings of the National Academy of Sciences*, **114**, 201710733.

Matz M V, Treml EA, Aglyamova G V, Bay LK (2018) Potential for rapid genetic

adaptation to warming in a Great Barrier Reef coral. *PLoS Genetics*, 1–19.

Medina M, Hannah B, Morrison C et al. (2011) Orbicella faveolata Genome Project. *http://montastraea.psu.edu/genome/*.

Mehvar S, Filatova T, Dastgheib A, Steveninck EDR Van, Ranasinghe R (2018) Quantifying Economic Value of Coastal Ecosystem Services : A Review. *Journal of Marine Science and Engineering*, **6**, 1–18.

Meyer E, Davies S, Wang S, Willis BL, Abrego D (2009a) Genetic variation in responses to a settlement cue and elevated temperature in the reef-building coral Acropora millepora. *Mar Ecol Prog Ser*, **392**, 81–92.

Meyer E, Aglyamova G V, Wang S et al. (2009b) Sequencing and de novo analysis of a coral larval transcriptome using 454 GSFlx. *BMC genomics*, **10**, 219.

Mieog JC, Oppen MJH, Cantin NE, Stam WT, Olsen JL (2007) Real-time PCR reveals a high incidence of Symbiodinium clade D at low levels in four scleractinian corals across the Great Barrier Reef: implications for symbiont shuffling. *Coral reefs*, **26**, 449–457.

Muller-Parker G, Davy SK (2001) Temperate and tropical algal-sea anemone symbioses. *Invertebrate Biology*, **120**, 104–123.

Muller-Parker G, Pierce-Cravens J, Bingham BL (2007) Broad thermal Tolerance of the Symbiotic Dinoflagellate Symbiodinium muscatinei (Dinophyta) in the Sea Anemone Anthopleura elegantissima (Cnidaria) from Northern Latitudes. *Journal of Phycology*, **43**, 25–31.

Muller-Parker G, D'Elia CF, Cook CB (2015) Interactions Between Corals and Their Symbiotic Algae. In: *Coral Reefs in the Anthropocene*, In: Birkel edn, pp. 99–116. Springer, Dordrecht.

Muscatine L, Falkowski PG, Porter JW, Dubinsky Z (1984) Fate of Photosynthetic Fixed Carbon in Light- and Shade-Adapted Colonies of the Symbiotic Coral Stylophora pistillata. *Proceedings. Biological sciences / The Royal Society*, **222**, 181–202.

Oakley CA, Davy SK (2018) Cell Biology of Coral Bleaching. In: *Coral Bleaching*, pp. 189–211.

Oakley CA, Durand E, Wilkinson SP, Peng L, Weis VM, Grossman AR, Davy SK (2017) Thermal Shock Induces Host Proteostasis Disruption and Endoplasmic Reticulum Stress in the Model Symbiotic Cnidarian Aiptasia. *Journal of Proteome Research*, **16**, 2121–2134.

Oliver TA, Palumbi SR (2011) Do fluctuating temperature environments elevate coral thermal tolerance? *Coral reefs*, **30**, 429–440.

Van Oppen MJH, Mahiny AJ, Done TJ (2005) Geographic distribution of zooxanthella types in three coral species on the Great Barrier Reef sampled after the 2002 bleaching event. *Coral Reefs*, **24**, 482–487.

Palumbi SR, Barshis DJ, Traylor-Knowles N, Bay RA (2014) Mechanisms of reef coral resistance to future climate change. *Science*, **344**, 895–898.

Pandolfi JM (2003) Global Trajectories of the Long-Term Decline of Coral Reef. *Science*, **301**, 955–958.

Parkinson JE, Banaszak AT, Altman NS, LaJeunesse TC, Baums IB (2015) Intraspecific diversity among partners drives functional variation in coral symbioses. *Scientific reports*, **5**, 12.

Parkinson JE, Baumgarten S, Michell CT, Baums IB, LaJeunesse TC, Voolstra CR (2016) Gene Expression Variation Resolves Species and Individual Strains among Coral-Associated Dinoflagellates within the Genus Symbiodinium. *Genome biology and evolution*, **8**, 665–680.

Perez S, Weis VM (2006) Nitric oxide and cnidarian bleaching: an eviction notice mediates breakdown of a symbiosis. *Journal of Experimental Biology*, **209**, 2804–2810.

Polato NR, Vera JC, Baums IB (2011) Gene discovery in the threatened elkhorn coral: 454 sequencing of the Acropora palmata transcriptome. *PLoS ONE*, **6**, e28634–e28634.

Putnam HM, Gates RD (2015) Preconditioning in the reef-building coral Pocillopora damicornis and the potential for trans-generational acclimatization in coral larvae under future climate change conditions. *Journal of Experimental Biology*, **218**, 2365–2372.

Quigley KM, Davies SW, Kenkel CD, Willis BL, Matz M V., Bay LK (2014) Deep-sequencing method for quantifying background abundances of Symbiodinium types: Exploring the rare Symbiodinium biosphere in reef-building corals. *PLoS ONE*, **9**.

ReFuGe 2020 Consortium (2017) The ReFuGe 2020 Consortium—using "omics" approaches to explore the adaptability and resilience of coral holobionts to environmental change. *Frontiers in Marine Science*, **2**.

Reynolds WS, Schwarz JA, Weis VM (2000) Symbiosis-enhanced gene expression in cnidarian-algal associations: cloning and characterization of a cDNA, sym32, encoding a possible cell adhesion protein. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **126**, 33–44.

Richier S, Rodriguez-Lanetty M, Schnitzler CE, Weis VM (2008) Response of the symbiotic cnidarian Anthopleura elegantissima transcriptome to temperature and

UV increase. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, **3**, 283–289.

Riegl BM, Purkis SJ, Al-Cibahy AS, Abdel-Moati MA, Hoegh-Guldberg O (2011) Present limits to heat-adaptability in corals and population-level responses to climate extremes. *PLoS ONE*, **6**, e24802–e24802.

Rodolfo-Metalpa R, Hoogenboom MO, Rottier CC, Ramos-Espla A, Baker AC, Fine M, Ferrier-Pagès CC (2014) Thermally tolerant corals have limited capacity to acclimatize to future warming. *Global Change Biology*, **20**, 3036–3049.

Rowan R (2004) Coral bleaching: Thermal adaptation in reef coral symbionts. *Nature*, **430**, 742.

Rowan R, Knowlton N, Baker A, Jara J (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature*, **388**, 265–269.

Ruiz-Jones LJ, Palumbi SR (2017) Tidal heat pulses on a reef trigger a fine-tuned transcriptional response in corals to maintain homeostasis. *Science Advances*, **3**, 1–10.

Schwarz JA, Weis VM (2003) Localization of a Symbiosis-Related Protein, Sym32, in the Anthopleura elegantissima-Symbiodinium muscatinei Association. *Biological Bulletin*, **205**, 339–350.

Seneca FO, Forêt S, Ball EE, Smith-Keune C, Miller DJ, Oppen MJH (2010) Patterns of Gene Expression in a Scleractinian Coral Undergoing Natural Bleaching. *Marine Biotechnology*, **12**, 594–604.

Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, Fujie M (2011) Using the Acropora digitifera genome to understand coral responses to environmental change. *Nature*, **476**, 320–323.

Shinzato C, Inoue M, Kusakabe M (2014) A snapshot of a coral "holobiont": A transcriptome assembly of the scleractinian coral, Porites, captures a wide variety of genes from both the host and symbiotic zooxanthellae. *PLoS ONE*, **9**.

Silverstein RN, Correa AMS, Baker AC (2012) Specificity is rarely absolute in coral-algal symbiosis: implications for coral response to climate change. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 2609–2618.

Silverstein RN, Cunning R, Baker AC (2015) Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Global Change Biology*, **21**, 236–249.

Silverstein RN, Cunning R, Baker AC (2017) Tenacious D: Symbiodinium in clade D remain in reef corals at both high and low temperature extremes despite impairment.

The Journal of Experimental Biology, **220**, 1192–1196.

Smith-Keune C, Van Oppen M (2006) Genetic structure of a reef-building coral from thermally distinct environments on the Great Barrier Reef. *Coral reefs*, **25**, 493–502.

Smith EG, Ketchum RN, Burt JA (2017) Host specificity of Symbiodinium variants revealed by an ITS2 metahaplotype approach. *The ISME Journal*, **11**, 1500–1503.

Spalding M, Burke L, Wood SA, Ashpole J, Hutchison J (2017) Mapping the global value and distribution of coral reef tourism. *Marine Policy*, **82**, 104–113.

Stat M, Pochon X, Cowie ROM, Gates RD (2009) Specificity in communities of Symbiodinium in corals from Johnston Atoll. *Marine Ecology Progress Series*, **386**, 83–96.

Tchernov D, Gorbunov MY, de Vargas C, Narayan Yadav S, Milligan AJ, Haggblom M, Falkowski PG (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings of the National Academy of Sciences*, **101**, 13531–13535.

Thomas L, Rose NH, Bay RA, López EH, Morikawa MK, Ruiz-Jones L, Palumbi SR (2018) Mechanisms of Thermal Tolerance in Reef-Building Corals across a Fine-Grained Environmental Mosaic: Lessons from Ofu, American Samoa. *Frontiers in Marine Science*, **4**, 1–14.

Thornhill DJ, Xiang Y, Fitt WK, Santos SR (2009) Reef Endemism , Host Specificity and Temporal Stability in Populations of Symbiotic Dinoflagellates from Two Ecologically Dominant Caribbean Corals. *PLoS ONE*, **4**, 1–12.

Traylor-Knowles N, Granger BR, Lubinski TJ et al. (2011) Production of a reference transcriptome and transcriptomic database (PocilloporaBase) for the cauliflower coral, Pocillopora damicornis. *BMC Genomics*, **12**, 585.

Traylor-Knowles N, Rose NH, Sheets EA, Palumbi SR (2017) Early transcriptional responses during heat stress in the coral Acropora hyacinthus. *Biological Bulletin*, **232**, 91–100.

Turrens JF (2003) Mitochondrial formation of reactive oxygen species. *Journal of Physiology*, **552**, 335–344.

Veron JEN (2011) Coral Taxonomy and Evolution. In: *Coral Reefs: An Ecosystem in Transition*, pp. 37–45.

Veron J (2013) Overview of the taxonomy of zooxanthellate Scleractinia. *Zoological Journal of the Linnean Society*, 1–24.

Voolstra CR (2013) A journey into the wild of the cnidarian model system Aiptasia and

its symbionts. *Molecular Ecology*, **22**, 4366–4368.

Voolstra CR, Schnetzer J, Peshkin L, Randall CJ, Szmant AM, Medina M (2009) Effects of temperature on gene expression in embryos of the coral Montastraea faveolata. *BMC genomics*, **10**, 627.

Voolstra CR, Li Y, Liew YJ et al. (2017) Comparative analysis of the genomes of Stylophora pistillata and Acropora digitifera provides evidence for extensive differences between species of corals. 1–14.

Walter P, Ron D (2011) The Unfolded Protein Response: From Stress Pathway to Homeostatic Regulation. *Science*, **334**, 1081–1086.

Warner ME, Fitt WK, Schmidt GW (1999) Damage to photosystem II in symbiotic dinoflagellates: A determinant of coral bleaching. *PNAS*, **96**, 8007–8012.

Weis VM (2008) Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *The Journal of experimental biology*, **211**, 3059–3066.

Weis VM, Davy SK, Hoegh-Guldberg O, Rodriguez-Lanetty M, Pringle JR (2008) Cell biology in model systems as the key to understanding corals. *Trends in Ecology and Evolution*, **23**, 369–376.

Weston AJ, Dunlap WC, Beltran VH, Starcevic A, Hranueli D, Ward M, Long PF (2015) Proteomics Links the Redox State to Calcium Signaling During Bleaching of the Scleractinian Coral Acropora microphthalma on Exposure to High Solar Irradiance and Thermal Stress . *Molecular & Cellular Proteomics*, **14**, 585–595.

Ying H, Cooke I, Sprungala S et al. (2018) Comparative genomics reveals the distinct evolutionary trajectories of the robust and complex coral lineages. *Genome biology*, **19**, 175.

CHAPTER 2 – Heritable variation in bleaching responses and its functional genomic basis in reef-building corals (*Orbicella faveolata*)

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Abstract

Reef-building corals are highly sensitive to rising ocean temperatures, and substantial adaptation will be required for corals and the ecosystems they support to persist in changing ocean conditions. Genetic variation that might support adaptive responses has been measured in larval stages of some corals, but these estimates remain unavailable for adult corals and the functional basis of this variation remains unclear. In this study, we focused on the potential for adaptation in *Orbicella faveolata*, a dominant reef-builder in the Caribbean. We conducted thermal stress experiments using corals collected from natural populations in Bocas del Toro, Panama, and used multilocus SNP genotypes to estimate genetic relatedness among samples. This allowed us to estimate narrow-sense heritability of variation in bleaching responses, revealing that variation in these responses was highly heritable (h^2=0.58). This suggests substantial potential for adaptive responses to warming by natural populations of *O. faveolata* in this region. We further investigated the functional basis for this variation using genomic and transcriptomic approaches. We used a publicly available genetic linkage map and genome assembly to map markers associated with bleaching responses, identifying twelve markers associated with variation in bleaching responses. We also profiled gene expression in corals with contrasting bleaching phenotypes, uncovering substantial differences in transcriptional stress responses between heat-tolerant and heat-susceptible corals. Together, our findings contribute to the growing body of evidence that natural populations of corals possess genetic variation in thermal stress responses that may potentially support adaptive responses to rising ocean temperatures.

Introduction

Coral reefs are one of the most diverse and complex ecosystems in the world. They provide habitat for hundreds of thousands of invertebrates and fish, protect coastal environments, and support a variety of resources for local communities. Unfortunately, the invaluable ecosystem services they provide are at risk of being lost as coral reefs worldwide continue to decline. Coral reefs are particularly sensitive to increases in sea surface temperature and have undergone worldwide degradation as the oceans have

warmed (Brown, 1997; Hoegh-Guldberg & Jones, 1999; Baker *et al.*, 2004; Eakin *et al.*, 2009). Bleaching events, which reflect the breakdown of symbiotic relationships between corals and dinoflagellates (Symbiodiniaceae*,* formerly *Symbiodinium spp.* (LaJeunesse *et al.*, 2018)) resulting from environmental stress, have increased in frequency and severity over the past few decades (Hughes, 2003; Donner *et al.*, 2005; Hoegh-Guldberg *et al.*, 2007). In the past three years alone, 30-50% of coral reefs have declined in some areas along the Great Barrier Reef (Hughes *et al.*, 2017). This dramatic decline in such a short period of time demands an increased understanding of the potential for these ecosystems to persist into the future.

In order to persist, corals will need to increase their thermal tolerance to cope with ocean warming. It is already known that coral species have differing thermal capacities due to extrinsic factors such as variation in their environment, as well as intrinsic mechanisms to deal with acute and long-term stress events, such as varying associations in symbiont type (Baker *et al.*, 2004; Van Oppen *et al.*, 2005; Jones *et al.*, 2008) or changes in gene expression in the coral host (Bellantuono *et al.*, 2012a; Kenkel *et al.*, 2013). Importantly, bleaching thresholds for some species have been shown to change over time (Fitt *et al.*, 2001; Grottoli *et al.*, 2014). Models that consider both environmental conditions and these changing thresholds suggest that the fate of corals during the next century may be strongly affected by long-term adaptive changes in bleaching thresholds (Donner *et al.*, 2005; D'Angelo *et al.*, 2015). Changes in bleaching thresholds may occur in populations through adaptation (Meyer *et al.*, 2009; Coles & Riegl, 2013; Palumbi *et al.*, 2014), or in individual corals through acclimatization (Jones & Berkelmans, 2010; Oliver & Palumbi, 2011).

Adaptation through genetic change can play a large role in allowing populations to persist in a changing environment. Genetic variation within populations in fitness-related traits, including resistance to environmental stress, supports adaptive responses to selection (Falconer & Mackay, 1996; Barrett & Schluter, 2008). Predicting the adaptive potential of a trait requires an understanding of the proportion of phenotypic variation resulting from genetic factors (Falconer and Mackay, 1996). However, the relative contributions of environmental and genetic factors to variation in thermal tolerance of

corals remain poorly understood (Császár *et al.*, 2010). Some studies have provided evidence for corals' adaptive capacity, demonstrating thermal tolerance differences between local populations (Palumbi *et al.*, 2014; Howells *et al.*, 2016) and considerable heritable variation in thermal tolerance in coral larvae (Dixon *et al.*, 2015) and algal symbionts (Császár *et al.*, 2010). These examples have provided an important first demonstration that genetic potential for adaptation exists in natural populations, but many questions still remain.

Global sea surface temperatures are predicted to rise 1-2°C by the end of the century, and thermally sensitive organisms like reef-building corals will require substantial adaptive responses. Adaptive responses to selection depend on the change in a population's phenotypic mean and the narrow-sense heritability (h^2), the proportion of total phenotypic variance that is due to additive genetic factors (Falconer and Mackay, 1996). Quantitative estimates of this parameter allow us to estimate the expected evolutionary change in a trait per generation (Visscher *et al.*, 2008; Morrissey *et al.*, 2012). In order to estimate selection responses in corals and consider rates of adaptation, we need to quantify heritability in thermal tolerance. Currently, very few studies provide heritability estimates for coral species and their algal symbionts, particularly in natural populations (Meyer *et al.*, 2009b; Dixon *et al.*, 2015; Kenkel *et al.*, 2015). Previous studies have focused on larval stages for important advantages in experimental design, leaving it unclear whether the high heritabilities estimated in larval responses to elevated temperatures (Meyer *et al.*, 2009, Dixon *et al.*, 2015) can be generalized to understand responses to selection on the adult stage. Further, since the heritability of a trait is specific to a particular population and environment in which it is measured, it remains unclear whether previous estimates of h^2 from Indo-Pacific Acroporids can be generalized to evaluate adaptive potential in other regions and species.

The Caribbean has seen dramatic reductions in coral cover over the last thirty years (Hughes & Tanner, 2000; Gardner, 2003) and the potential for existing populations to recover or adapt to changing ocean conditions remains unknown. To understand the potential for adaptation by corals in this region, we investigated the mechanisms that may enable long-term adaptation by investigating heritable variation in thermal tolerance and

its genomic basis in *Orbicella faveolata*, a dominant reef-builder in the Caribbean. Our studies aim to quantify the contribution of genetic factors to variation in thermal tolerance of corals, and identify genetic markers and genes associated with this variation. Together our findings providing new insights into the potential for adaptive changes in corals' thermal tolerance during ongoing climate change.

Materials and Methods

Sampling and thermal stress experiment

To study natural variation in thermal tolerance of corals, we measured responses to thermal stress in corals sampled from a natural population. For these experiments, we sampled 43 colonies of *Orbicella faveolata* from seven reef sites around the Bocas del Toro, Panama archipelago in 2015 (Figure 2.1a). Large intact colonies were extracted off the reef and tissue samples were collected and stored in RNAlater for genotyping (Scientific Permit No. SC/A-28-14). Each colony was cut into nine smaller uniform fragments (387 fragments total) with approximately 15-20 polyps per fragment. Fragments were maintained at ambient temperature in aquaria at the Smithsonian Tropical Research Institute (STRI) on Isla Colon, Bocas del Toro for one week prior to experimentation. Initial photographs of each individual fragment were taken before experiments began.

To estimate thermal tolerance, we exposed replicate fragments from each colony to a thermal stress treatment and measured their bleaching responses. Three randomly chosen fragments from each colony were maintained at control conditions (ambient seawater temperature of 29°C) while the remaining six fragments were ramped approximately 0.1°C every two hours to an elevated temperature treatment of 31°C for two weeks and 32°C for an additional two weeks. Corals were maintained for 4 weeks in normal and elevated temperatures, monitoring pH and salinity daily. Corals were monitored by daily visual inspection to evaluate bleaching response using the Coral Watch color scorecard, and the effects of temperature stress were scored as the number of degree heating weeks (DHW) required to induce bleaching. The experiment was terminated when approximately half of the fragments were bleached. Photographs were

taken at the end of 4 weeks (approximately 5 DHW) and tissues were sampled and stored in RNAlater.

Multilocus SNP genotyping of coral colonies

To estimate genetic relatedness and test for genetic associations with thermal tolerance, we conducted multilocus SNP genotyping on all coral colonies. To that end, we extracted genomic DNA from each colony using the Omega bio-tek E.Z.N.A. Tissue DNA Kit (Omega Bio-tek, Norcross, GA). We used the 2bRAD (Restriction Site-Associated DNA) protocol for SNP genotyping, a streamlined and cost-effective method for genome-wide SNP genotyping (Wang *et al.*, 2012). For these libraries we used the reduced tag representation method previously described (Wang *et al.*, 2012), using selective adaptor with overhangs ending in "NR" to target ¼ of the AlfI sites in the genome. This approach made it possible to analyze the number of samples included here on a limited budget, a tradeoff between marker number and sample numbers. We combined these libraries in equimolar amounts for sequencing in a single lane of 50 bp SE reads on Illumina HiSeq 3000 at OSU's Center for Genome Research and Biocomputing (CGRB).

We analyzed the resulting data using a 2bRAD reference our research group has recently produced and used for a linkage map (Snelling *et al.*, 2017). Since the reference was produced from larval stages that naturally lack algal symbionts, no special filtering was required to eliminate algal reads in these samples from adult tissue. We conducted this analysis as previously described for de novo analysis of corals (Wang *et al.*, 2012; Howells *et al.*, 2016). Briefly, we filtered reads prior to analysis to exclude any low quality or uninformative reads (Joint Genome Institute, 1997), then aligned reads to the reference using SHRiMP (Rumble *et al.*, 2009) and called genotypes based on nucleotide frequencies at each position (calling loci homozygous if a second allele was present at less than 1%, heterozygous if present at > 25%, and leaving the genotype undetermined at intermediate frequencies where genotypes cannot be confidently determined from allele frequencies) (Wang *et al.*, 2012). Genotypes for each colony were called with a permissive threshold of ≥ 5x coverage to call as many loci as possible for this genome

wide survey of associations with bleaching responses. The scripts used for this analysis are available at (https://github.com/Eli-Meyer/2brad_utilities).

Profiling algal symbionts with amplicon sequencing (ITS2)

To control for variation in the algal symbiont communities of each coral, which can contribute to variation in thermal tolerance of the holobiont (host plus associated algal and microbial symbionts) (Abrego *et al.*, 2008; Howells *et al.*, 2011), we sequenced the symbiont community in each colony using Sanger and Illumina amplicon sequencing. First, we amplified ITS2 using PCR primers previously described for studies of Symbiodiniaceae diversity (LaJeunesse, 2002), and sequenced the resulting amplicons using Sanger Sequencing. The resulting sequences were compared with multiple known ITS2 sequences from all formerly described *Symbiodinium* clades A-H (Hunter *et al.*, 2007; Cunning *et al.*, 2015b). Using our symbiont sequences and these reference ITS2 sequences, we created an alignment in the program MEGA (Kumar *et al.*, 2017). A maximum likelihood phylogenetic tree was created with all known and unknown sequences to determine which clades our coral samples fell into. The dominant symbiont type was assigned for each sample by comparing the phylogenetic tree of unknown and known samples.

To confirm these results and evaluate whether our samples included mixed symbiont populations, we prepared additional ITS2 amplicon libraries for high-throughput sequencing on Illumina. We prepared these libraries using forward (5'-TACACGACGCTCTTCCGATCTGAATTGCAGAACTCCGTG-3') and reverse (5'-ACGTGTGCTCTTCCGATCGGATCCATATGCTTAAGTTCAGCGGGT-3') primers, and sequenced libraries using 300 bp PE read chemistry on Illumina MiSeq at OSU's Center for Genome Research and Biocomputing (CGRB). We filtered reads to exclude any low quality reads (<20), removed reads lacking the expected amplicon primer sequence, and removed orphan reads. After filtering, paired reads from each sample were merged and were imported into dada2 (Callahan *et al.*, 2016). Using dada2, we further filtered samples for missing data and removed chimeric sequences. In this way, we identified valid amplicon sequence variants (ASVs) and described the abundance of each

ASV in each sample. Finally, we created a BLAST database containing a diversity of annotated ITS2 sequences and the ITS2 sequence from the *Orbicella faveolata* host (Cunning *et al.*, 2015b) and identified the clade of each ASV by comparison with this database.

We identified the dominant symbiont type in each colony based on the consensus of Sanger and Illumina sequencing results. While Sanger data lack resolution to describe mixtures of algal symbiont clade types, we interpret these sequences as the dominant symbiont types in each sample based on the presence of a single dominant haplotype in sequencing chromatograms. For Illumina, we quantified the proportion of each sequence variant in each sample and assigned a dominant clade if sequence variants were present >80% and a mix of symbionts if <80%. We included the dominant or mixed clade type(s) for each colony in quantitative models of bleaching responses to evaluate the contribution of variation in the dominant symbiont type to variation in thermal tolerance.

Quantifying bleaching responses

To quantify bleaching in each fragment, we used qPCR to estimate the abundance of algal symbionts relative to host cells (Cunning *et al.*, 2015b). We quantified collected samples after stress experiments in qPCR reactions. DNA from all fragments (control and heat-stressed from each colony) was extracted using an organic phase extraction. All qPCR reactions were run on an Eppendorf Realplex 4 machine using the SYBR and ROX filters. Each reaction consisted of 7.5 µL SensiFAST SYBR Hi-ROX master mix (Bioline, Taunton, MA), 4.3 µl NFW, 0.6 µl each of forward and reverse 10-µM primers, and 2 µl of genomic DNA (10ng total) in a final volume of 15 µl. The thermal profile for each reaction consisted of an initial denaturing step of 95°C for 2 min, followed by 40 cycles of: 95°C for 5 s, annealing temperature of 60°C for 30 s, and then 72°C for 30 sec. All control and heat-stressed samples were run using the same reaction parameters and were analyzed together. In addition, one sample was included on every plate as an inter-plate calibrator. We quantified host cells using host actin loci using the forward (5'-CGCTGACAGAATGCAGAAAGAA-3') and reverse (5'-CACATCTGTTGGAAGGTGGACA-3') primers, as previously described (Cunning *et*

al., 2015b). To quantify Symbiodiniaceae in each sample we used a pair of universal primers developed based on multiple sequence alignments of the cp23S-rDNA locus from multiple Symbiodiniaceae species (https://www.auburn.edu/~santosr/sequencedatasets.htm). We identified regions that were sufficiently conserved to design primers suitable for qPCR (53-76 and 169-189 in that alignment). We conducted qPCR with primers (5'-CTACCTGCATGAAACATAGAACG -3' and 5'- CCCTATAAAGCTTCATAGGG -3') to determine the total amount of symbiont cells present after experimentation in control and experimental conditions. Host cell quantifications (C_T values) were subtracted from symbiont cell quantifications to calculate the ΔC_T value in each colony, a measure of the ratio of symbiont cells to host cells, for both control and experimental conditions. The ΔC_T stress value was subtracted from the ΔC_T control value to generate $\Delta\Delta C_T$ values, representing the symbiont density. Then, we used these $\Delta\Delta C_T$ values for each colony to calculate the fold change of symbiont abundance ($2^{-\Delta\Delta Ct}$), which were then log-transformed to compare across colonies, which will be referred to as "log fold change". Additionally, we calculated the variation within stress and control samples separately. The ΔC_T values from stress and control samples were calculated as described above, and these ΔC_T values were then compared to a reference control sample to generate $\Delta\Delta C_T$ values, allowing us to normalize the values for comparison. These values represent variation between colonies, and will be referred to as "colony variation in stress samples" and "colony variation in control samples". We analyzed these qPCR data on relative symbiont density of each fragment to evaluate the effects of genotype, origin, and symbiont type on bleaching responses.

Estimating heritability of variation in bleaching responses

Estimating the heritability of this variation in bleaching responses requires information on genetic relationships among subjects, which is initially unknown in samples collected from a natural population. For our study, we inferred genetic relatedness among samples based on multilocus SNP genotypes, and then used the genetic relatedness matrix derived from these SNPs to estimate genetic variance

components. For this analysis we used the 'related' package in R and used the method described by Queller & Goodnight to calculate genetic distance between samples (Queller & Goodnight, 1989; Muir & Frasier, 2015; Tavalire *et al.*, 2018). After developing this matrix of genetic relatedness among samples, we analyzed variation in bleaching responses in the context of these relationships to estimate heritability. Using the R package 'regress', we created a linear mixed model with symbiont clade type and population source as fixed effects (site where samples were collected) (Tavalire *et al.*, 2018). This analysis accounted for variation in thermal bleaching responses attributable to these specific factors. We estimated narrow-sense heritability and the associated standard error based on the phenotypic variation remaining after accounting for these known sources of variance, using the *h2G* function in the R package 'gap' (Zhao, 2007).

Testing for genetic associations with bleaching responses

To identify genetic markers associated with variation in bleaching responses, we tested for associations at each SNP locus using linear mixed models including SNP genotypes as a random effect and population source as a fixed effect. To account for errors arising from multiple tests, we converted controlled false discovery rate at 0.05 using the pFDR procedure (Storey, 2003). The multilocus SNP genotypes obtained from 2bRAD made it possible to test for associations between bleaching phenotypes and genotypes at each locus. Combining SNP data and the linkage map for this species (Snelling *et al.*, 2017), we searched for genomic regions underlying variation within more thermally tolerant phenotypes. We used the R package 'rrBLUP' to test for associations between bleaching responses and genotypes at each locus, accounting for genetic structure in the population using an additive relationship matrix produced from SNP genotypes. We used the *A.mat* function to calculate the additive relationship matrix, considering all loci with no more than 5% missing data, then used the GWAS function to conduct association tests, requiring allele frequencies > 0.08 (a second allele was detected at least 3 times), and included source population as a fixed effect. Once significant SNPs were found, we searched genomic scaffolds to examine neighboring genes. Based on an integrated genomic resource our group has recently developed by combining the linkage

map with transcriptome and genome assemblies (Snelling *et al.*, 2017), we calculated linkage disequilibrium (LD) blocks in cM for each SNP based on <10% recombination frequency. We searched within each LD block to identify genes linked to each SNP.

Profiling gene expression in heat-tolerant and susceptible colonies

To evaluate whether genomic regions associated with heat tolerance include genes differentially expressed between heat-tolerant and susceptible genotypes, we profiled transcriptional responses in a subset of corals demonstrating contrasting phenotypes (3 heat-tolerant, collected at Isla Bastimentos; 3 heat-susceptible collected at Isla Solarte) (Figure 2.1a and 2). RNA was extracted from replicate fragments from each colony using the Omega Bio-tek E.Z.N.A. Tissue RNA Kit (Omega Bio-tek, Norcross, GA). RNA was then used to prepare 3' tag-based cDNA libraries for expression profiling (Meyer *et al.*, 2011). Samples were individually barcoded and combined in equal ratios for multiplex sequencing. We sequenced these libraries repeatedly on multiple runs because incompatibilities between the versions of the library preparation primers and the recently updated sequencing platforms resulted in very low sequencing yields. The first run was on the HiSeq 3000 platform at OSU's CGRB, the second run on HiSeq 4000 at the University of Oregon's Genomic and Cell Characterization Core Facility, and the third run on MiSeq at OSU's CGRB. After sequencing, we processed the raw reads to remove non-template regions introduced during library preparation, and excluded reads with long homopolymer regions (>20bp) and low-quality reads with a Phred score of <30. All filtering steps were conducted using publicly available Perl scripts from https://github.com/Eli-Meyer/rnaseq_utilities. We mapped the high quality reads against the transcriptome for this species (Anderson *et al.*, 2016) using a short-read aligner software SHRiMP (Rumble *et al.*, 2009), and counted unique reads aligning to each gene to produce count data for statistical analysis of gene expression in each sample.

We tested for differential gene expression using a negative binomial model in the R package 'DESeq2' (Love *et al.*, 2014). We tested for changes in gene expression by evaluating changes in stress-induced expression across samples in control and stress treatments. Our models tested for effects of treatment (control versus heat stress

treatment) and bleaching response (susceptible versus tolerant) as main effects, and their interaction (treatment x response). We identified differentially expressed genes (DEGs) controlling the false discovery rate at 0.05. To identify patterns of differential expression among the interaction effect DEGs, we conducted hierarchical clustering of expression patterns, subdividing the tree into clusters of correlated genes using the *cutree* function in R (Oksanen, 2010).

Results

Sequencing yield and SNP genotyping

To analyze genetic relationships among corals and associations with bleaching responses, we conducted multilocus SNP genotyping using a sequencing-based approach (2bRAD). Altogether, we sequenced 150 million high-quality reads, averaging 3.87 million reads per colony. We mapped these reads to a reference previously developed from aposymbiotic larvae, ensuring the loci being genotyped are derived from the coral host rather than the algal symbionts. We genotyped >700 kb at ≥ 5x coverage in each sample (Table 2.1), identifying a large number of putative polymorphisms (35,067 loci). We further filtered genotypes to minimize missing data and genotyping errors, identifying a set of 5,468 high-quality SNPs that we used for all subsequent analyses.

Symbiodiniaceae communities in host colonies and bleaching responses

To identify the dominant symbiont type or mixed symbiont communities in each coral colony, we sequenced ITS2 amplicons using Sanger and Illumina sequencing. In an effort to identify the dominant clade present in each colony, we classified the origin of each Sanger sequence by constructing a maximum likelihood tree including diverse representatives from Symbiodiniaceae, formerly described as *Symbiodinium* clades A-H (Hunter *et al.*, 2007; Cunning *et al.*, 2015b). This analysis identified all sequences as members of clades A-D (Figure 2.1b; Figure 2.7), and revealed differences in symbiont types across samples from different sites.

To confirm and expand on these results, we analyzed high-throughput ITS2 sequence data from the same samples. For this analysis we used a BLAST database with

known ITS2 sequences (Cunning *et al.*, 2015b) to classify the proportion of sequence variants in each sample originating from each symbiont species (or clade) (Figure 2.1b; Figure 2.8). Most colonies contained a dominant sequence variant (>80%), while only two colonies showed a mixed community with two clade types. We considered both Sanger and Illumina data to assign the dominant or mixed symbiont community for each colony. Comparing these data revealed that the symbiont type identified from a single Sanger sequencing reaction in each sample corresponded to the dominant type identified in deep sequencing for nearly all samples in both datasets (26/29). For a small number of sample (3/29), the symbiont type identified from Sanger sequencing corresponded to a minor component of the community identified by Illumina sequencing rather than the dominant type. Illumina libraries for the remaining 14 samples were unsuccessful due to host contamination, so their identities were assigned based on Sanger sequencing. Overall, there was strong agreement between the assignments of symbiont type between Sanger and Illumina sequence data (Figures 2.7 and 2.8).

After 4 weeks in thermal stress at 31°C and 32°C, we saw considerable variation in bleaching among stressed fragments, while symbiont density changed very little across control samples (Figure 2.3). While there was variation between colonies, there was little to no variation in bleaching among fragments from the same colony (Figure 2.2). We quantified symbiont densities in each fragment using qPCR, and estimated the bleaching response of each colony as the log fold change between stressed and control fragments (Figure 2.3 and Figure 2.9). Colonies showed substantial variation in both their initial symbiont densities and their bleaching responses, based on both visual examination of the fragments and qPCR analysis of relative symbiont abundance (Figure 2.2 and 2.3). Most colonies bleached in response to thermal stress, but the extent of these bleaching responses varied considerably (Figure 2.3).

Heritable variation in thermal tolerance in a natural population

To investigate heritable variation in thermal tolerance, we combined SNP data with bleaching responses measured by qPCR. We conducted a mixed model analysis to determine which factors to include in our heritability and association models. While

population source had significant effects on thermal tolerance (p=0.0014), symbiont type had no effect (p=0.06). However, to be conservative, we included all factors in our REML mixed model to partition variation in thermal tolerance into genetic and non-genetic variance components. We estimated genetic relatedness among samples based on multilocus SNP genotypes, and then partitioned variance into genetic and non-genetic variance components in an 'animal model' (Wilson *et al.*, 2010) based on this genetic relatedness matrix. On average, pairwise genetic distances between colonies (calculated as the proportion of divergent alleles) between samples was 0.098 (range: 0.001 - 0.176). This analysis revealed that after accounting for effects of source and symbiont type, phenotypic variation in bleaching responses (log-fold change values) was highly heritable, with a narrow-sense heritability (h^2) of 0.58 (SE=0.22). Taken alone, this estimate suggests substantial potential for adaptive responses to ocean warming in this population (but see Discussion for additional considerations).

Genomic basis for variation in thermal tolerance

To understand the genomic basis for this variation in thermal tolerance, we used our SNP genotypes to test for associations between bleaching responses and genotypes. For this analysis, we conducted a series of linear mixed models testing for the effect of genotype at each locus while accounting for population structure. To visualize regions of the genome showing strong association with thermal tolerance, we mapped the results from statistical tests onto the integrated map, plotting $-\log_{10}$(p-value) for each marker by linkage group and position (Figure 2.4). After multiple test corrections, we found twelve markers significantly associated with bleaching; three markers when examining the log fold change between control and stressed samples, three markers when examining colony variation in control samples, and six markers when examining colony variation in stress samples (FDR $\leq$ 0.05). We emphasize that these three analyses of the symbiont densities are not intended to represent independent traits, but different aspects of biological variation relevant for thermal stress (bleaching response and colony variation).

To identify the genomic positions of these SNPs were located and the genes linked to each marker, we used the integrated map (Snelling *et al.*, 2017) to search for

genes closely linked (within an LD block) with each marker. Our SNPs fell onto linkage groups 2 (two SNPs), 3 (two SNPs), 4, 5, 6, 7 (two SNPs), 8, 9 and 16 (Figure 2.4). Within the LD blocks around our SNPs, we used the integrated map to identify genes linked to each marker (Table 2.3). All genes identified in this analysis can be found in the published manuscript Table 2.4 (https://doi.org/10.1111/mec.15081).

This analysis identified several groups of genes previously implicated in stress responses of corals or other Cnidarians, including genes with roles in oxidative stress responses, regulation of protein folding or degradation, and regulation of apoptosis. Genes linked to markers on LG2 included peroxiredoxin, a redox regulation protein for oxidative stress and genes associated with apoptosis (protein NLRC3). Genes linked to the markers on LG 3 were mucin proteins, ubiquitin protein ligases, caspase, a potassium voltage-gated channel protein, cellular tumor antigen p53, a gene associated with apoptosis; cytochrome 450, a protein involved in defense against chemical stressors; the chaperone DnaJ homolog involved in preventing inappropriate unfolding of proteins; heat shock protein 70, and glutathione s-transferase, a key enzyme in enhancing the oxidative stress response. Genes on LG 6 included ubiquitin protein ligases and potassium voltage-gated channel proteins. Catalase, apoptosis-inducing factor proteins, sodium-potassium transporting proteins involved in ion transport, tyrosine kinase receptor proteins involved in responding to oxidants and tumor necrosis receptor-associated proteins, important regulators of the apoptosis pathway were all linked to the markers on LG 7. Genes linked to the marker on LG 8 included mucin proteins and protein disulfide-isomerase, part of the unfolded protein response pathway. Genes linked to the markers on LG 9 and 16 included tyrosine kinase receptor proteins and tumor necrosis receptor-associated proteins, and ubiquitin protein ligases.

We also found several novel groups of genes that were not expected based on prior studies but were repeatedly observed across multiple markers and linkage groups in our study, suggesting a possible functional role for these genes in bleaching responses. These included 5-hydroxytryptamine (serotonin) receptors (5 genes altogether, linked to markers on LG 2, 3, 9, and 16). Similarly, we repeatedly found that galanin receptors were linked to bleaching associated markers (10 galanin receptor genes linked to

bleaching-associated markers on LG 3, and 6). Galanins are neuropeptides classically associated with activities in the brain and peripheral nervous system, that have recently been shown to play diverse roles in innate immunity, inflammation, and energy metabolism (Lang *et al.*, 2014). We also found multiple collagen proteins (8 collagen genes linked to bleaching-associated markers on LG 2, 3, 6, 7, and 8). The possible functions of these genes in coral stress responses is not clear, but the repeated observation that these genes are linked to bleaching-associated markers on multiple scaffolds and linkage groups suggests that variation in these genes may contribute to variation in bleaching responses.

Differences in transcriptional responses of tolerant and susceptible phenotypes

To further investigate the mechanisms of thermal tolerance, we profiled gene expression in contrasting phenotypes. For this dataset, we chose three heat-tolerant colonies and three susceptible colonies (Figure 2.2). The three heat-tolerant colonies were collected at Isla Bastimentos and contained clade C and D symbiont types, while the three heat-susceptible colonies were collected at Isla Solarte and all contained clade B symbionts (Figures 2.1 and 2.2). These sites were approximately 15 km from one another and Isla Bastimentos exhibited more protection from wave action than Isla Solarte. Comparing bleaching responses, colonies from Isla Solarte had an average log-fold value of -0.8 (susceptible to bleaching) whereas colonies from Isla Bastimentos had an average value of 0.1 (tolerant to bleaching) (Figure 2.3).

Using a tag-based RNASeq approach (Meyer *et al.*, 2011), we prepared sequencing libraries for all 36 fragments (six colonies with six fragments, three control and three heat-stress fragments). We sequenced our libraries three times, once on Illumina HiSeq 3000, Illumina HiSeq 4000, and Illumina MiSeq, and all sequenced reads from all three runs were combined. In total, 63.9 million raw reads were produced, with approximately 1.73 million reads per sample. The majority of these passed quality and adaptor filtering (93%) leaving 59.4 million HQ reads for expression analysis (Table 2.2).

Using a negative binomial model, we tested for changes in gene expression, evaluating differences in stress-induced expression. Our model tested for the effect of

bleaching response, whether the colonies were bleached or unbleached, the effect of treatment, whether the fragments were in control or heat-stress, and the interaction effect between type and treatment. We found 737, 104, and 187 differentially expressed genes (DEGs) when testing for main effects of type and treatment, and their interaction, respectively. The interaction between type and treatment on gene expression can be visualized in a heatmap of expression for these DEGs (Figure 2.5), where heat tolerant colonies (red bars in figure 2.5) generally express these genes at higher levels than heat susceptible colonies (light blue bars in figure 2.5) regardless of treatment. Heatmaps for the effects of treatment and type are shown in Figure 2.10. A complete list of differentially expressed genes in each category is provided in the published manuscript Table 2.5 (https://doi.org/10.1111/mec.15081).

A substantial number of genes showed significant type × treatment effects, where the effects of treatment on expression differed between tolerant and susceptible corals. To characterize these interactions, we averaged expression for each gene in both susceptible and tolerant phenotypes for each treatment. Gene expression profiles were categorized into two dominant patterns. In the first pattern, genes were expressed at higher levels in heat-tolerant corals and were downregulated during thermal stress, and expressed at lower levels in heat-susceptible corals but upregulated during thermal stress. We found 159 genes in this category (Figure 2.6a). The second pattern was the opposite: genes that were expressed at higher levels and upregulated during thermal stress in heat-tolerant corals were down-regulated in susceptible corals (Figure 2.6b). The remaining 33 genes formed a third cluster with similar patterns as 6b but with more variation across genes (not shown).

Finally, we compared differentially expressed genes and those genes within the gene neighborhoods of our significant linkage groups. Genes differentially expressed as a function of type, treatment or their interaction all contained ubiquitin protein ligases. In addition, when examining differentially expressed genes in the type effect, we found multiple collagen genes, mucins, as well as DnaJ proteins, glutathione peroxidase, and peptidyl-prolyl cis-trans isomerases, genes known to have a potential role in response to heat stress.

Discussion

Our study provides some of the first quantitative estimates for heritability of variation in bleaching responses of corals. This builds upon larval studies (Meyer *et al.*, 2009a, 2011; Dixon *et al.*, 2015) that have demonstrated substantial heritability in responses to elevated temperatures, but left uncertainty in whether these findings extended to adult corals with intracellular algal symbionts and the energetic demands of calcification. Our findings confirm that some coral populations harbor similar genetic variation in thermal tolerance traits of adult coral colonies. These parameters have been studied in Indo-Pacific Acroporids, but to our knowledge no quantitative estimates for heritability of thermal tolerance were previously available for corals in the Robust clade (Fukami *et al.*, 2008; Meyer *et al.*, 2009a, 2011; Kitahara *et al.*, 2010; Baums *et al.*, 2013; Dixon *et al.*, 2015) or any other Caribbean corals. This is an important consideration because heritability of a trait is specific to the population and environment under study, suggesting caution in generalizing results from Indo-Pacific larval studies of Acroporids to evaluate potential adaptive responses in the deeply diverged groups of corals that dominate Caribbean reefs (Meyer *et al.*, 2009a, 2011; Baums *et al.*, 2013; Dixon *et al.*, 2015; Kenkel *et al.*, 2015; Lohr & Patterson, 2017).

To investigate the functional basis for this variation in bleaching responses, we conducted genomic and transcriptomic studies comparing allele frequencies and transcriptional stress responses in these corals. We found genetic markers significantly associated with thermal tolerance, and used the integrated genomic resource developed from a genetic linkage map and a genome sequence assembly to identify some of the genes linked to these markers. We found that transcriptional responses of heat-tolerant corals to thermal stress are markedly different from those of heat-susceptible colonies. We identified just under 200 genes differentially expressed as a function of type × treatment interactions, which were generally expressed at higher levels in tolerant corals and regulated in opposite directions by tolerant and susceptible corals in response to thermal stress.

This study builds on growing evidence that coral populations harbor genetic variation that may support adaptation to ocean warming. These questions are especially

pressing for Caribbean corals, where reefs have declined severely over the last few decades (Hughes & Tanner, 2000). Since genetic variation supporting heritable variation in traits under selection is species- and population-specific, measuring these parameters in Caribbean populations is vital for understanding the future of these ecosystems. Our study documents considerable genetic variation in thermal tolerance for a population of the mountainous star coral, *Orbicella faveolata*, an important reef-builder throughout the Caribbean.

Our data suggests the genetic potential for substantial adaptive responses to selection for thermal tolerance in this population. Responses to selection can be modeled with the univariate breeder's equation to estimate the expected rate of adaptation within a single generation (Falconer and Mackay, 1996). These predictions require empirical estimates for the narrow-sense heritability of the trait under selection, the proportion of phenotypic variation attributable to additive genetic variation (Falconer and Mackay, 1996). While it has been clear for some time that corals possess substantial variation in thermal tolerance, in part resulting from acclimatization or association with different algal symbionts (Fitt *et al.*, 2001; Howells *et al.*, 2011; Oliver & Palumbi, 2011; Silverstein *et al.*, 2012), the variation attributable to genetic factors in the coral host has remained understudied. This genetic variation will determine the adaptive responses of corals in the immediate future, since rapid adaptation relies on standing genetic variation in natural populations (Barrett & Schluter, 2008). Our study contributes novel information on this potential for adaptation to ocean warming, confirming that heritability of bleaching responses in adult corals can be comparable to the high heritability of thermal tolerance observed in some previous larval studies (Dixon *et al.*, 2015).

Importantly, these estimates of h^2 express genetic potential for adaptation, and other factors may constrain the adaptive responses that are actually realized in nature. The breeder's equation expresses the rate of adaptive change within a single generation, requiring that we account for generation times to convert these estimates into units of adaptive change per decade or century. Massive corals like *Orbicella* are slow-growing and while direct estimates of generation time are unavailable for *O. faveolata* itself, comparisons with similar slow-growing massive corals suggests that these corals

probably begin reproduction at ~ 5 years old and reach peak reproductive output around 10-15 years (Babcock, 1991). These life-history considerations impose inherent constraints on the rates of adaptation in this species, since even "rapid" adaptive changes occurring in a single generation would take 5-15 years to affect populations of adult corals. Additionally, correlations among traits can alter responses to selection relative to univariate predictions (Lande & Arnold, 1983; Houle, 1991; Falconer & Mackay, 1996; Lynch & Walsh, 1998). In these cases, selection for one trait affects the distribution of not only that trait, but also indirectly affects the distributions of correlated traits (Falconer & Mackay, 1996; Lynch & Walsh, 1998). Negative correlations among fitness related traits may constrain adaptive responses to selection (Etterson & Shaw, 2001), while positive correlations may facilitate adaptive responses (Agrawal & Stinchcombe, 2009). These correlations can change in different environments (Messina & Fry, 2003; Sgrò & Hoffmann, 2004), so describing these effects is also required for understanding responses to selection. Future studies should investigate the possibility that trait correlations may constrain adaptive responses in corals, preventing these populations from achieving the rapid adaptive responses that h^2 estimates suggest are possible.

The development of sequencing-based approaches for multilocus SNP genotyping has made genomewide association studies (GWAS) a widely used tool for identifying markers associated with traits of interest (Schlötterer *et al.*, 2015). These approaches map statistical associations between genetic markers and traits onto a genomic reference to identify regions of the genome underlying variation in the trait. Such an analysis obviously requires a genomic resource for mapping, and this requirement has limited the application of these approaches in many non-model systems. Despite limitations in the genomic resources available for *O. faveolata*, we used an integrated resource our group has recently established (Snelling *et al.*, 2017) to map statistical associations with bleaching responses onto the *O. faveolata* genome. It is important to acknowledge that our study was underpowered with only 43 genotypes (logistical constraints prevented us from further sampling, in this case). Despite the low power of our sampling design, we succeeded in identifying genetic markers associated with variation in bleaching

responses. These likely represent the loci with the largest effects on thermal tolerance in these samples, with additional loci of smaller effects remaining undetected.

The markers we identified are linked to biologically interesting genes that could contribute to host thermal tolerance. For example, we found gene functions involved in oxidative stress, neural response, ubiquitination, protein folding regulation, and apoptosis. Glutathione s- transferase functions as an antioxidant enzyme in response to reactive oxygen species and has been shown to increase during thermal stress (Downs *et al.*, 2002; DeSalvo *et al.*, 2008; Polato *et al.*, 2010). Linkage with this gene could indicate an important role for oxidative stress response to reactive oxygen species (ROS) production during stress induced by increasing sea surface temperatures or pathogens (Downs *et al.*, 2002; DeSalvo *et al.*, 2008; Polato *et al.*, 2010). Voltage-gated proteins have been characterized in *Nematostella vectensis* demonstrating the importance of these proteins in maintaining cellular homeostasis, regulation of movement, and feeding (Moran *et al.*, 2015). The process of ubiquitination labels proteins for degradation and expression of ubiquitin protein ligases may play an important role in increased tolerance to heat-stress (Finley *et al.*, 1987; Pickart, 2001; Welchman *et al.*, 2005; Shahsavarani *et al.*, 2012). For corals, these genes are highly correlated with increased thermal tolerance and are typically up-regulated in heat-stress corals with more damaged proteins (DeSalvo *et al.*, 2008; Barshis *et al.*, 2010; Lundgren *et al.*, 2013; Bay & Palumbi, 2015).

Some of the most interesting genes found are those involved in protein folding. The DnaJ chaperone plays an important role in the unfolded protein response (UPR) and is typically seen up regulated in response to elevated temperatures (≥ 32°C) in species such as *Acropora hyacinthus* (Ruiz-Jones & Palumbi, 2017) and *Stylophora pistillata, Porites sp.* and *Acropora eurystoma* (Maor-Landaw & Levy, 2016). This protein is also a co-chaperone of Hsp70, making it an important marker for thermal stress in corals (Cyr *et al.*, 1994; Walter & Ron, 2011). The close proximity (within a LD block of <10% recombination frequency) to our SNPs suggests a possible role for these genes in determining variation in thermal tolerance among colonies of *O. faveolata*.

We also found genes repeatedly observed across multiple markers and linkage groups, but their function and relationship to thermal tolerance in corals is unknown.

These genes included galanin receptors, 5-hydroxytryptamine (serotonin) receptors, and collagen proteins. Galanin receptors are known to modulate neural responses and have been shown to play an important role in responses to stress, such as pain, emotional stimuli, and disease (Mitsukawa *et al.*, 2009; Lang *et al.*, 2014; Sciolino *et al.*, 2015). In cnidarians, 5-hydroxytryptamine (serotonin) receptors may serve as a neural signaling molecule and radiolabeling studies have localized the distribution of these proteins around nerve tissues within host cells (Hajj-Ali & Anctil, 1997; Dergham & Anctil, 1998; Westfall *et al.*, 2000). Lastly, collagen is a component of the extracellular matrix and may be related to wound healing and regeneration in cnidarians (Reitzel *et al.*, 2010; Stewart *et al.*, 2017). Despite these genes having an unknown role in thermal tolerance, their continued expression and linkage to significant SNPs indicate they may contribute to tolerance in the coral host.

In addition to genetic analysis, high-throughput sequencing has also enabled widespread application of RNA-Seq approaches to profile gene expression (Wang *et al.*, 2009a). These methods have been widely adopted to study transcriptional responses to thermal stress in corals (DeSalvo et al. 2008; Voolstra et al. 2009; Leggat et al. 2011; Meyer et al. 2011; Oliver and Palumbi 2011; Bellantuono et al. 2012a, b; Barshis et al. 2013; Kenkel et al. 2013; Palumbi et al. 2014). One finding that has emerged consistently from these studies is the observation that corals vary widely in their transcriptional and phenotypic responses to thermal stress (Hunter, 1993; Ayre *et al.*, 1997; Marshall & Baird, 2000; Baums *et al.*, 2013). Many studies have demonstrated variation in gene expression among coral phenotypes, both in natural populations and in controlled studies (López-Maury *et al.*, 2008; DeSalvo *et al.*, 2010; Meyer *et al.*, 2011; Granados-Cifuentes *et al.*, 2013).

Here, we built upon these studies by quantifying variation in transcriptional responses to thermal stress in the context of known genetic relationships and thermal tolerance phenotypes. We found that heat-tolerant and -susceptible corals differed substantially in their responses to thermal stress. While our study demonstrates differences in gene expression between these contrasting phenotypes, we do not consider the contribution of the symbiont type and microclimate, which may be important factors

influencing these patterns. Focusing on the genes differentially expressed as a function of the type × treatment interaction, we identified a cluster of genes that were expressed at higher levels in heat-tolerant corals than their susceptible counterparts, and were down-regulated during thermal stress whereas susceptible corals up-regulated the same genes (Figure 2.6a). These included genes associated with protein metabolism (ribosomal protein genes, E3 ubiquitin protein ligase, and ubiquitin-conjugating enzyme E2), regulation of apoptosis (cathepsin-L and AP-1), and genes associated with calcium-binding (calcium-binding protein CML19, calretinin, neurocalcin, and a voltage-dependent L-type calcium channel). We also identified a cluster of genes showing the opposite pattern (up-regulated by heat-tolerant corals during thermal stress), which included a fluorescent protein. These proteins are commonly reported in studies of Cnidarian stress responses (Smith-Keune & Dove, 2008; Rodriguez-Lanetty *et al.*, 2009; Roth & Deheyn, 2013), and our findings provide additional evidence these genes may play a role in variation among corals' thermal tolerances.

Differentially expressed genes that overlapped with our association study included collagen genes, mucins, DnaJ proteins, and glutathione s-transferase. Inferring functional consequences from gene expression profiles is always uncertain, but these patterns suggest that thermal tolerance phenotypes in corals may be achieved in part by down-regulating energetically expensive processes such as protein synthesis, and in part by altering expression of the regulatory machinery controlling apoptosis.

Overall, our study provides a novel perspective on the potential for corals to adapt to ocean warming by estimating heritability of variation in thermal tolerance for a Caribbean reef-builder. We found that corals sampled from a natural population in Panama varied widely in their bleaching responses during an experimental thermal stress treatment. We used multilocus SNP genotyping to infer genetic relatedness among corals and estimate narrow-sense heritability (h^2) for variation in bleaching responses, revealing that variation in this trait is primarily attributable to additive genetic variation. This suggests substantial genetic potential for adaptation to ocean warming in this population, although the complexities of multivariate selection suggest caution in predicting responses to selection from a single trait. We used the same SNP genotypes to test for

associations between bleaching responses and genotypes at each marker, identifying genetic markers for bleaching responses that can be directly applied in restoration and conservation efforts to identify heat-tolerant corals. We used expression profiling to demonstrate that heat-tolerant corals respond to thermal stress differently than susceptible corals, and functional analysis of the differentially expressed genes suggests differential regulation of protein metabolism and apoptosis in heat-tolerant corals. Our findings provide crucial data for models aiming to predict corals' adaptation to ocean warming, and identify genetic markers for thermal tolerance that may be useful for restoration efforts as conservation biologists work to reverse the global degradation of coral populations resulting from changing ocean conditions.

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Data Accessibility

Reference numbers for data in public repositories: sequence data archived at NCBI's Sequence Read Archive (SRA) under the BioProject PRJNA413258.
Scripts used for analysis can be found at https://github.com/Eli-Meyer.

Figures and Tables

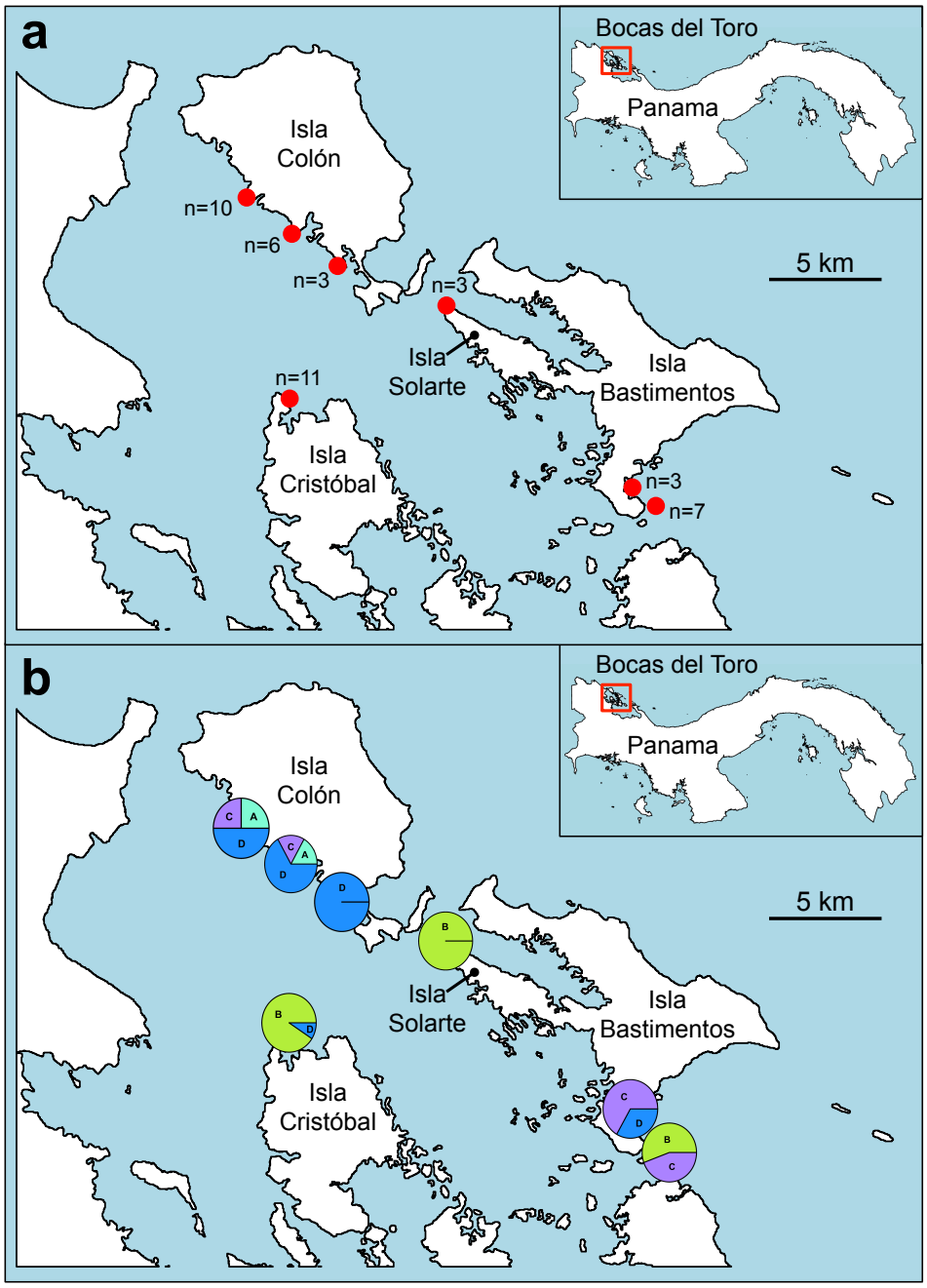

Figure 2.1. Map of Collection Sites around Bocas del Toro Archipelago, Panama. (a) Map of the seven locations where coral genotypes were collected around the archipelago. (b) Proportion of dominant symbiont types found at each site across colonies collected.

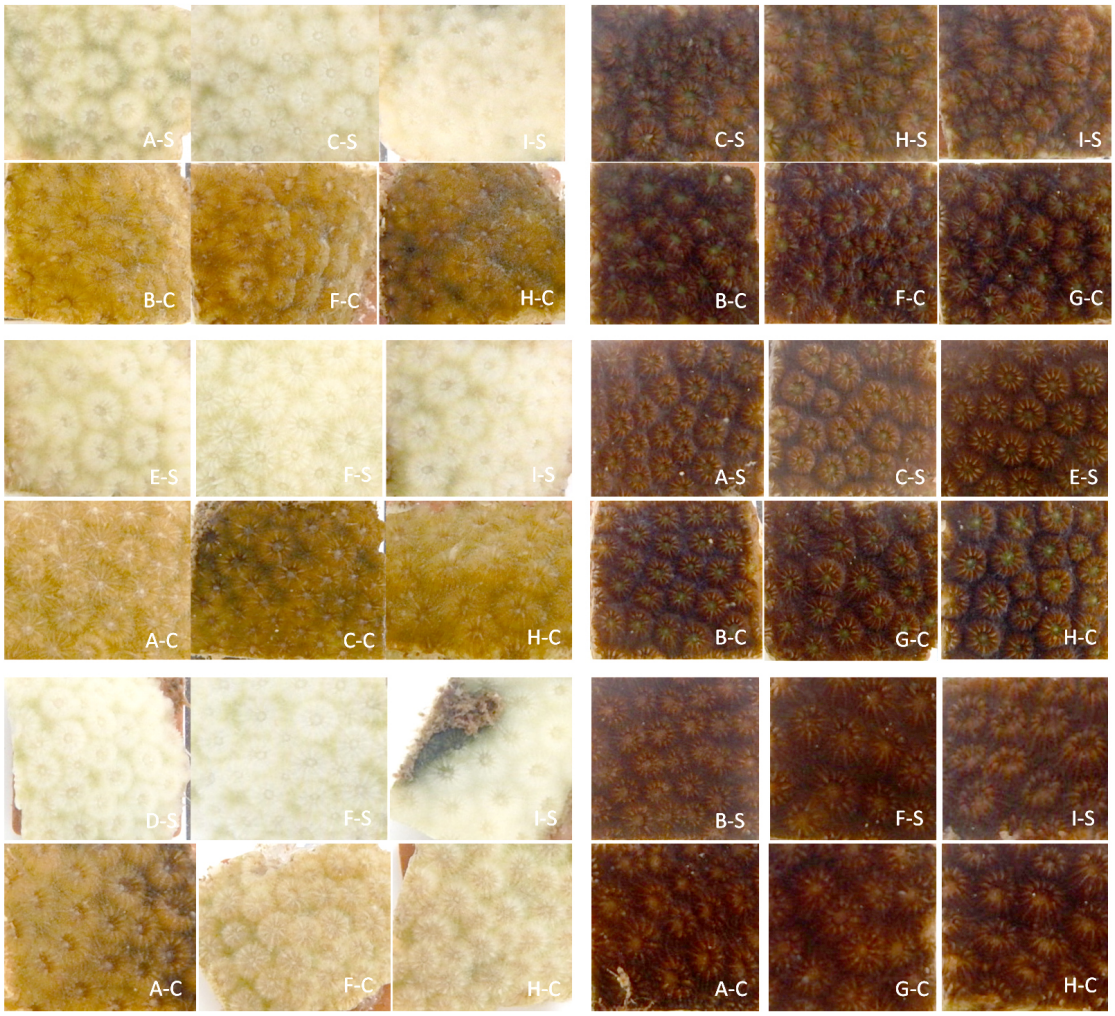

Figure 2.2. An example of the striking contrast between bleaching phenotypes of heat-susceptible and -tolerant corals sampled for this study. Each panel of six images represents fragments from a single colony, with control fragments indicated with "-C" (bottom of each panel) and heat-stressed fragments indicated with "-S" (bottom of each panel). Bleaching responses varied widely among colonies, but very little among fragments prepared from each colony.

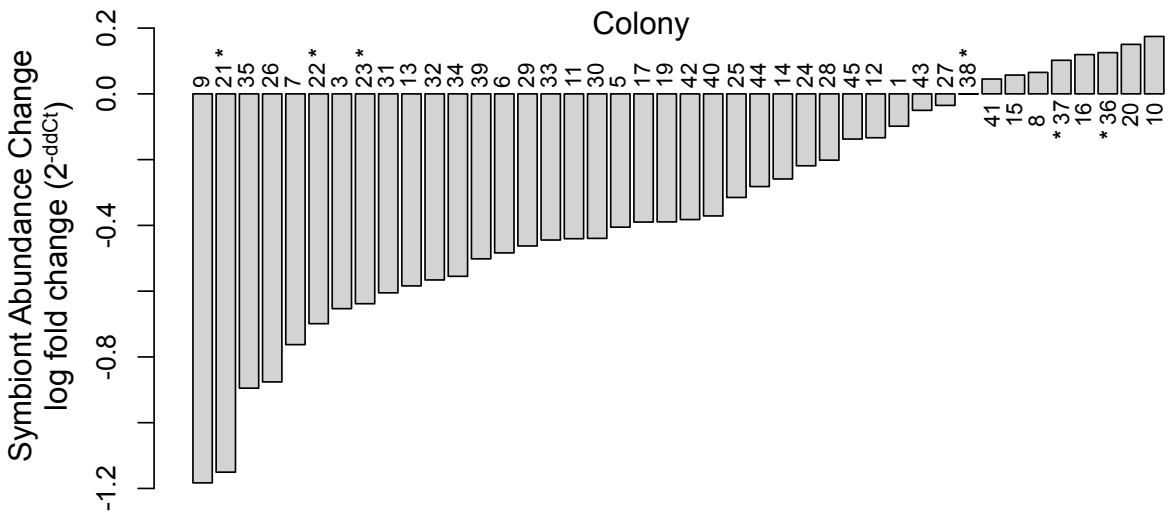

Figure 2.3. Quantification of algal symbiont densities using qPCR reveals variation in bleaching phenotypes. Bars represent the log fold change (2-ΔΔCt) of symbiont abundance between control and stress samples across colonies after four weeks in control and experimental conditions. Starred colonies indicate those selected for RNASeq, showing contrasting abundances.

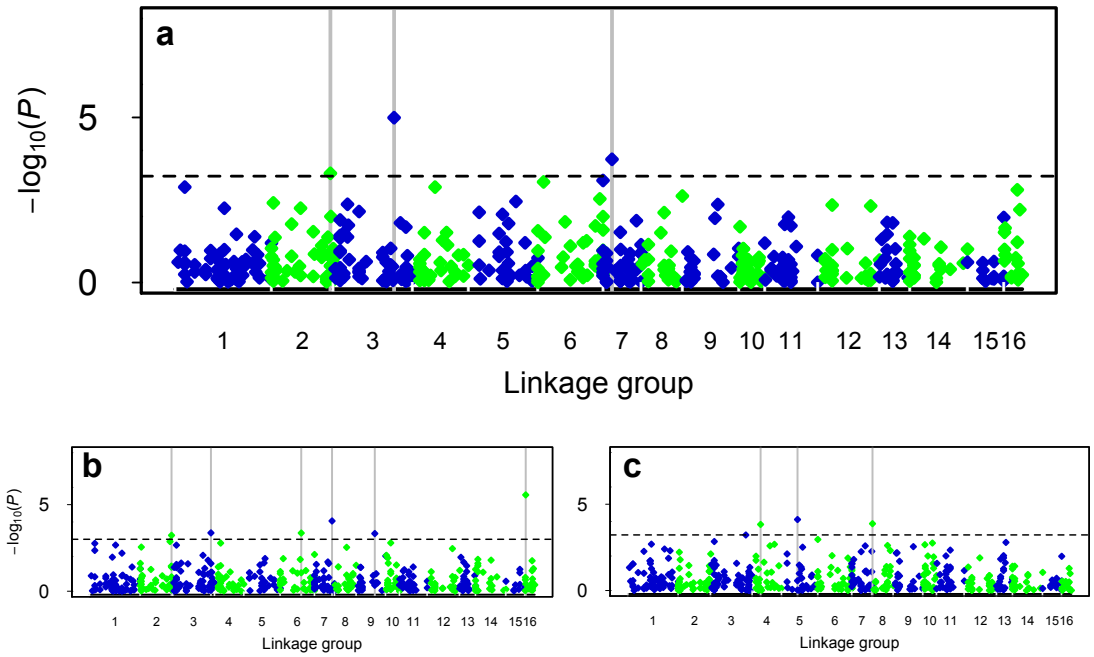

Figure 2.4. Mapping statistical associations between SNP genotypes and bleaching responses onto the linkage map identifies genomic regions associated with thermal tolerance in *O. faveolata*. a) using the log fold change between control and stress bleaching responses, b) colony variation in stress samples, and c) colony variation in control samples. Genetic markers are mapped against the linkage groups, indicated by alternating colors. Three markers on linkage groups 3, 5 and 7 were significantly associated (gray lines) with variation in bleaching responses during thermal stress, six markers on linkage groups 2, 3, 6, 7, 9 and 16 were significantly associated with variation in symbiont change in stress samples, and three markers on linkage groups 4, 5 and 8 were significantly associated with variation in symbiont change in control samples (FDR<0.05).

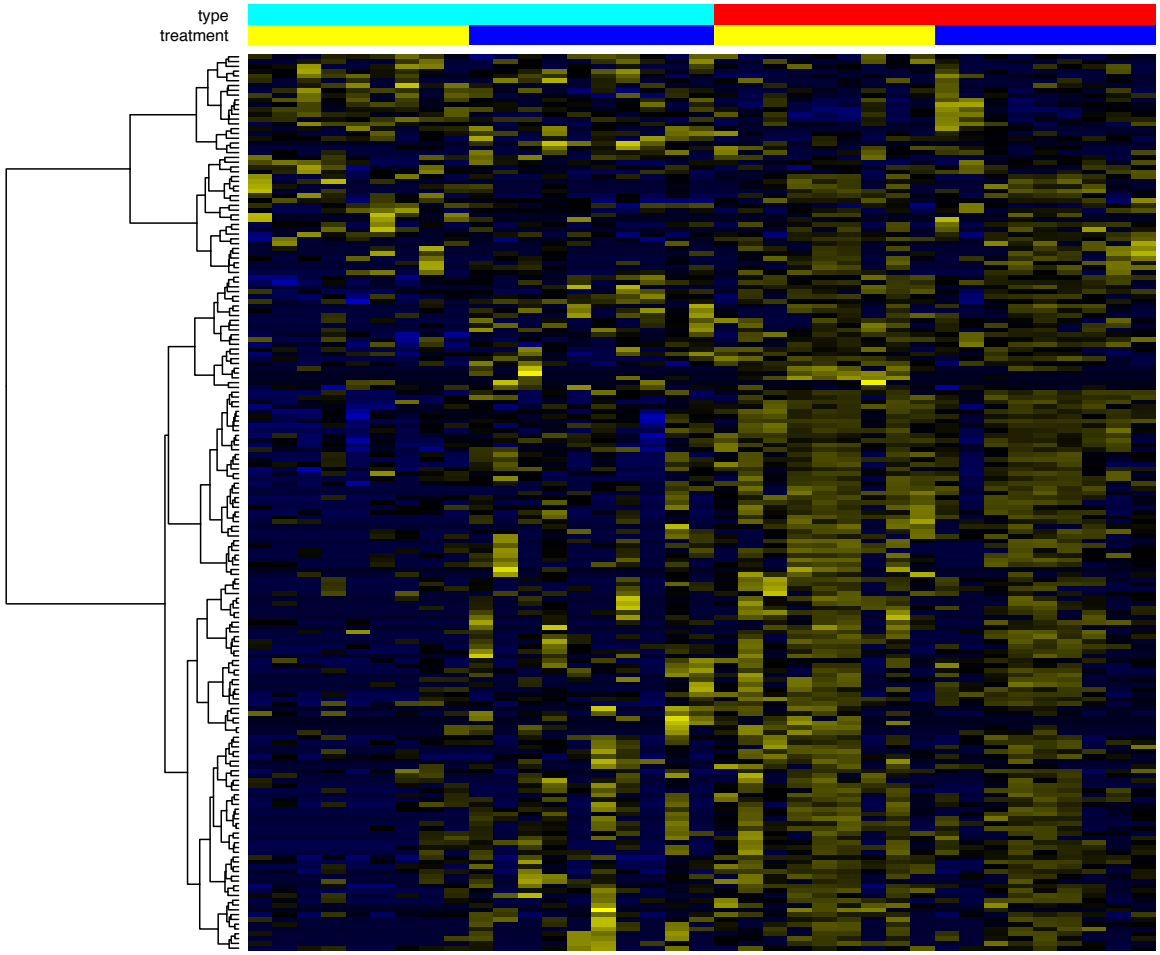

Figure 2.5. Heatmap showing relative expression of genes that were differentially expressed between heat-tolerant and heat-susceptible corals. The 187 DEG in this category are shown here, with samples and genes grouped with hierarchical clustering based on similarity in gene expression patterns. In the heatmap, blue indicates low expression, black moderate expression, and yellow indicates high expression. Colored bars indicate the type and treatment of each sample included in this analysis; red type refers to tolerant phenotypes, light blue refers to susceptible phenotypes, yellow bars are control samples, and dark blue bars are samples in heat stress treatment.

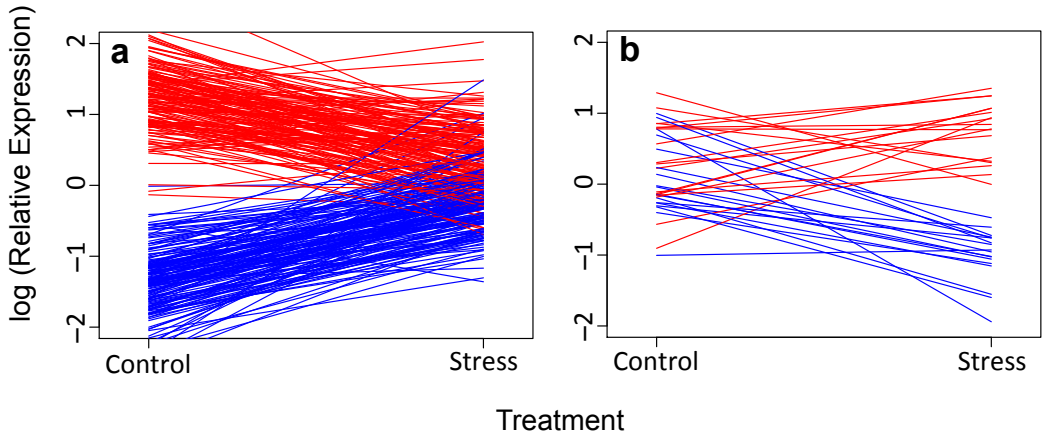

Figure 2.6. Type × treatment effects on gene expression fall into two general categories. a) 159 genes were downregulated in heat-tolerant corals (red) and upregulated in heat-susceptible corals (blue). b) 18 genes showed contrasting expression changes, but in the opposite directions: up-regulated in heat-tolerant corals and down-regulated in heat-susceptible corals. The remaining 33 genes showed similar patterns to (b) but were less consistent across genes, forming a third cluster (not shown).

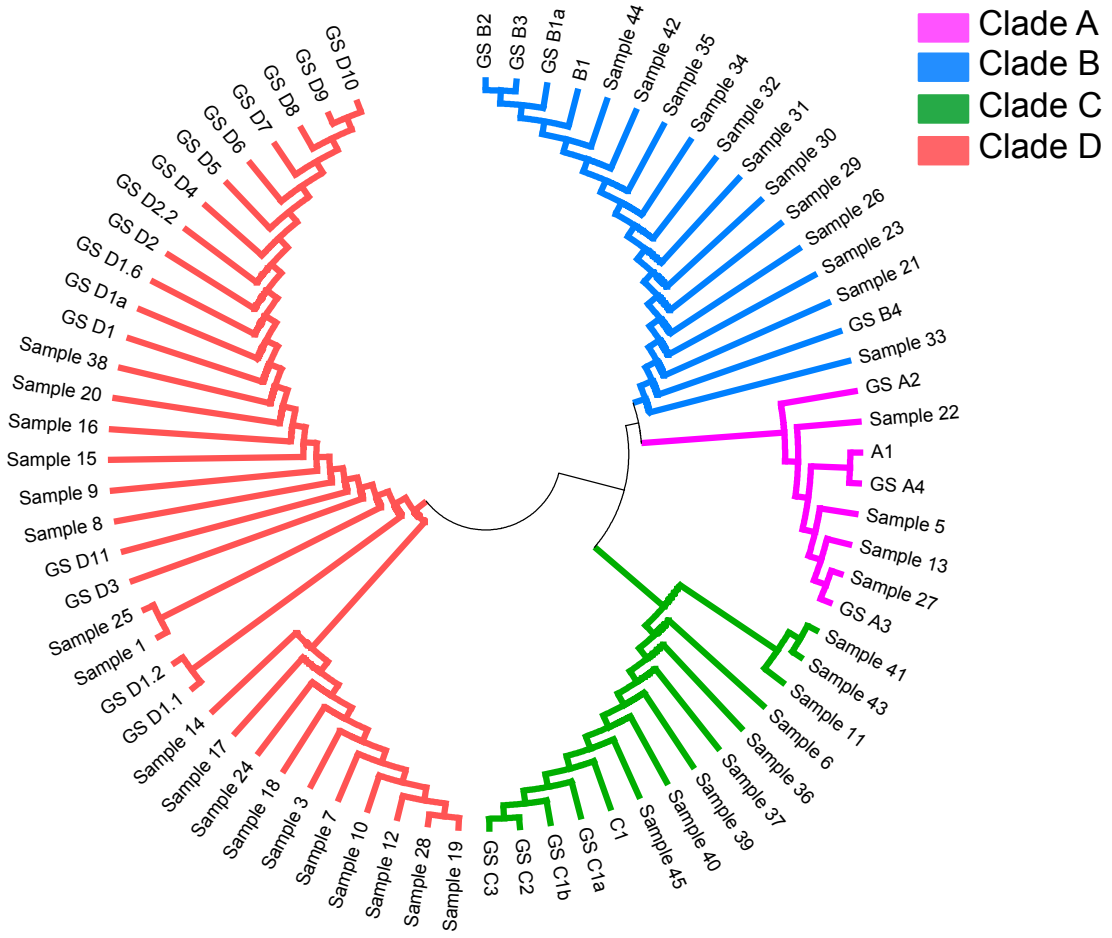

Figure 2.7. Sanger sequence data results for all samples (colonies). Sanger sequence data resolved a dominant clade, A-D for all samples.

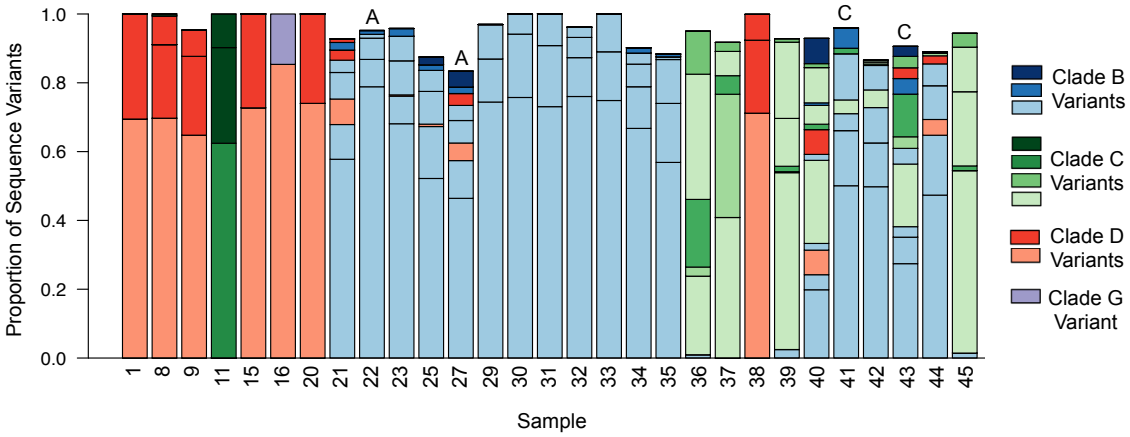

Figure 2.8. Illumina MiSeq sequence data results for all samples (colonies). Symbiont variants (Clades B, C, D and G) are shown as a proportion within each sample. Letter above a column indicates samples that demonstrate disagreement between Sanger result and ITS amplicon result when assigning dominant symbiont clade.

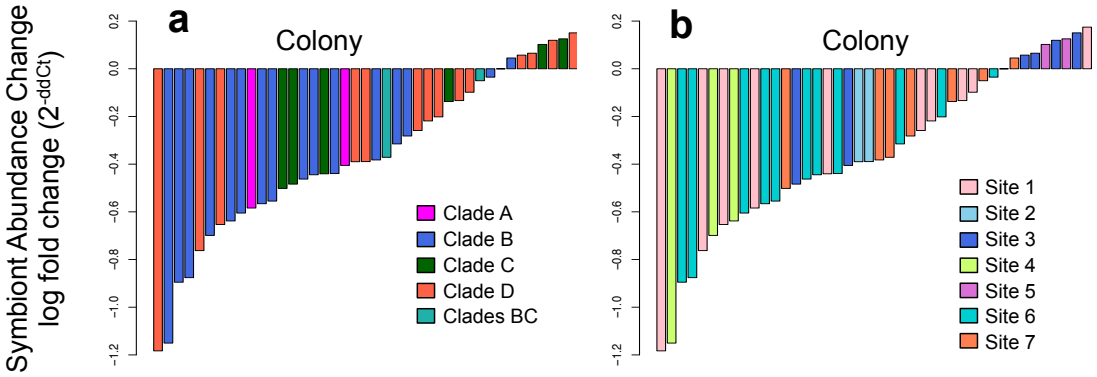

Figure 2.9. Quantification of algal symbiont densities using qPCR reveals variation in bleaching phenotypes as a function of a) symbiont types and b) site. Bars represent the log fold change (2-ΔΔCt) of symbiont abundance between control and stress samples across colonies after four weeks in control and experimental conditions.

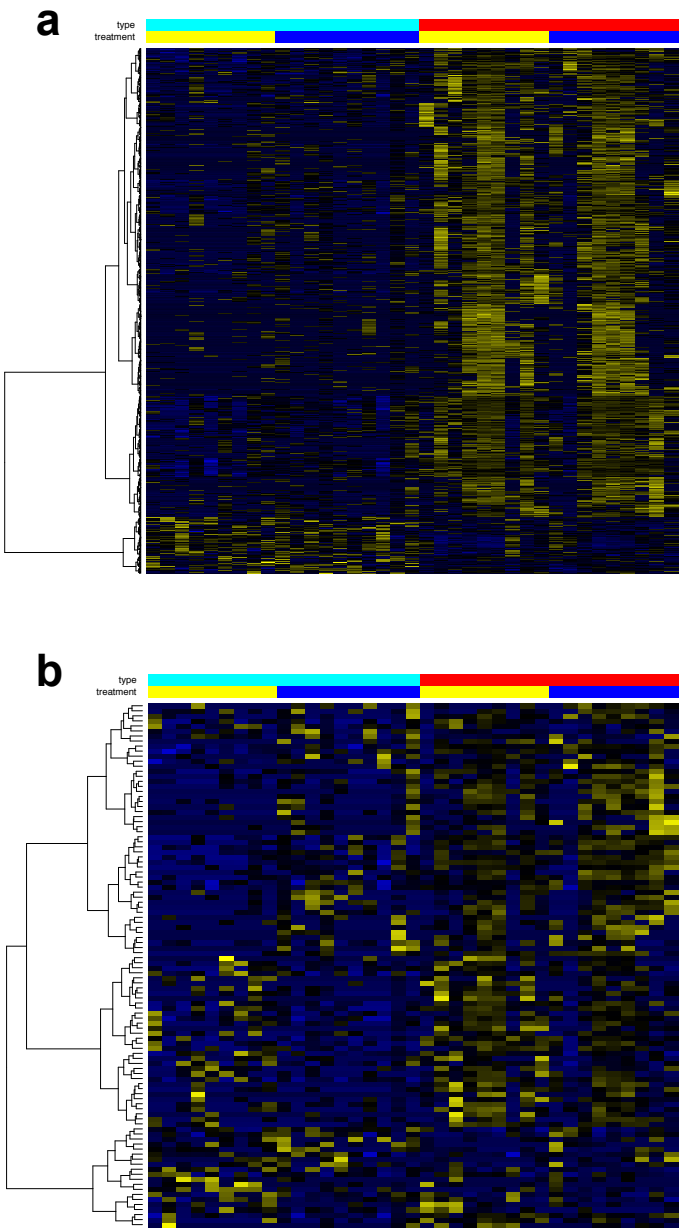

Figure 2.10. Heatmap showing relative expression of genes that were differentially expressed a) between heat-tolerant and heat-susceptible corals (type effect; 737 DEGs) and b) between heat stressed and control samples (treatment effect; 104 DEGs). In both heatmaps, blue indicates low expression, black moderate expression, and yellow indicates high expression. Colored bars indicate the type and treatment of each sample included in this analysis; red type refers to tolerant phenotypes, light blue refers to susceptible phenotypes, yellow bars are control samples, and dark blue bars are samples in heat stress treatment.

Table 2.1. Summary of sequencing yields, processing, and mapping efficiencies for 2bRAD sequencing libraries.

No. samples	43
Raw sequencing depth (millions)	203.3
HQ sequencing depth (millions)	150
HQ reads per sample (millions)	3.87
Mb genotyped (>5x coverage)	>700 kb
Putative polymorphisms	35,067
SNPs (>5x coverage)	5,468

Table 2.2. Summary of sequencing yields, processing, and mapping efficiencies for RNASeq sequencing libraries.

No. samples	51
No. biological replicates	2
Raw sequencing depth (millions)	203.3
HQ sequencing depth (millions)	160.6
HQ reads per sample	1.80
Mapping efficiency	84.97%
Genes quantified	7,733

Table 2.3. Information about Linkage Disequiblium blocks, maximum recombination frequency, and number of genes of each significant SNP.

	Linkage Group (LG)	Max Recombination Frequency	cM length of LD block	No. of Genes
Fold Change between Control and Stress				
denovoLocus4622	2	0.096	3.937356593	282
denovoLocus5063	3	0.075	19.013261	309
denovoLocus10421	7	0.07	4.790380542	96
Control				
denovoLocus8891	4	0.08	4.12	0
denovoLocus15681	5	0.001	0	0
denovoLocus12166	8	0.069	8.142038203	172
Stress				
denovoLocus3061	2	0.096	3.937356593	282
denovoLocus3945	3	0.078	4.924473345	211
denovoLocus3845	6	0.048	5.474121518	76
denovoLocus13690	7	0.096	23.38772539	328
denovoLocus44551	9	0.072	11.72419073	197
denovoLocus10427	16	0.079	9.044720409	266

Table 2.4. Complete list of genes linked to SNP markers significantly associated with variation in bleaching responses (within 10 cM of the markers) can be found here: https://doi.org/10.1111/mec.15081

Table 2.5. Complete list of genes differentially expressed as a function of temperature, thermal tolerance type, or their interactions can be found here: https://doi.org/10.1111/mec.15081

References

Abrego D, Ulstrup KE, Willis BL, van Oppen MJH (2008) Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 2273–2282.

Agrawal AF, Stinchcombe JR (2009) How much do genetic covariances alter the rate of adaptation? *Proceedings of the Royal Society B: Biological Sciences*, **276**, 1183–1191.

Anderson DA, Walz ME, Weil E, Smith MC (2016) RNA-Seq of the Caribbean reef-building coral Orbicella faveolata (Scleractinia- Merulinidae) under bleaching and disease stress expands models of coral innate immunity. *PeerJ*, **4:e1616**, DOI 10.7717/peerj.1616.

Ayre DJ, Hughes TP, Standish RJ (1997) Genetic differentiation, reproductive mode, and gene flow in the brooding coral Pocillopora damicornis along the Great Barrier Reef, Australia. *Mar Ecol Prog Ser*, **159**, 175–187.

Babcock RC (1991) Comparative demograpgy of three species of scleractinian corals using age- and size-dependant classifications. *Ecological Monographs*, **61**, 225–244.

Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Corals' adaptive response to climate change. *Nature*, **430**, 741–741.

Barrett RDH, Schluter D (2008) Adaptation from standing genetic variation. *Trends in Ecology and Evolution*, **23**, 38–44.

Barshis DJ, Stillman JH, Gates RD, Toonen RJ, Smith LW, Birkeland C (2010) Protein expression and genetic structure of the coral Porites lobatain an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity? *Molecular Ecology*, **19**, 1705–1720.

Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR (2013) Genomic basis for coral resilience to climate change. *PNAS*, **110**, 1387–1392.

Baums IB, Polato NR, Xu D et al. (2013) Genotypic variation influences reproductive success and thermal stress tolerance in the reef building coral , Acropora palmata. *Coral Reefs*, **32**, 703–717.

Bay RA, Palumbi SR (2015) Rapid acclimation ability mediated by transcriptome changes in reef-building corals. *Genome Biology and Evolution*, **7**, 1602–1612.

Bellantuono AJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2012a) Resistance to thermal stress in corals without changes in symbiont composition. *Proceedings of the Royal*

Society B: Biological Sciences, **279**, 1100–1107.

Bellantuono AJ, Granados-Cifuentes C, Miller DJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2012b) Coral thermal tolerance: tuning gene expression to resist thermal stress. **7**, e50685.

Brown BE (1997) Coral bleaching: causes and consequences. *Coral Reefs*, **16**, S129–S138.

Callahan BJ, Mcmurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2 : High-resolution sample inference from Illumina amplicon data. **13**.

Coles SL, Riegl BM (2013) Thermal tolerances of reef corals in the Gulf: A review of the potential for increasing coral survival and adaptation to climate change through assisted translocation. *Marine pollution bulletin*, **72**, 323–332.

Császár NBM, Ralph PJ, Frankham R, Berkelmans R, van Oppen MJH (2010) Estimating the potential for adaptation of corals to climate warming. *PLoS ONE*, **5**, e9751–e9751.

Cunning R, Silverstein RN, Baker AC (2015) Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20141725.

Cyr DM, Langer T, Douglas MG (1994) DnaJ-like proteins: molecular chaperones and specific regulators of Hsp70. *Trends in Biochemical Science*, **19**, 176–181.

D'Angelo C, Hume BCC, Burt J, Smith EG, Achterberg EP, Wiedenmann J (2015) Local adaptation constrains the distribution potential of heat-tolerant Symbiodinium from the Persian/Arabian Gulf. *The ISME Journal*, **9**, 2551–2560.

Dergham P, Anctil M (1998) Distribution of serotonin uptake and binding sites in the cnidarian Renilla koellikeri: An autoradiographic study. *Tissue and Cell*, **30**, 205–215.

DeSalvo MK, Voolstra CR, Sunagawa S et al. (2008) Differential gene expression during thermal stress and bleaching in the Caribbean coral Montastraea faveolata. *Molecular Ecology*, **17**, 3952–3971.

DeSalvo MK, Sunagawa S, Voolstra CR (2010) Transcriptomic responses to heat stress and bleaching in the elkhorn coral Acropora palmata. *Marine Ecology Progress Series*, **402**, 97–113.

Dixon GB, Davies SW, Aglyamova GV, Meyer E, Bay LK, Matz MV (2015) Genomic determinants of coral heat tolerance across latitudes. **348**, 2014–2016.

Donner SD, Skirving WJ, Little CM, Oppenheimer M, Hoegh-Gulberg O (2005) Global assessment of coral bleaching and required rates of adaptation under climate change. *Global Change Biology*, **11**, 2251–2265.

Downs CA, Fauth JE, Halas JC, Dustan P, Bemiss J, Woodley CM (2002) Oxidative stress and seasonal coral bleaching. *Free Radical Biology and Medicine*, **33**, 533–554.

Eakin CM, Lough JM, Heron SF, Stednick JD (2009) Climate variability and change: Monitoring data and evidence for increased coral bleaching stress. *Coral Bleaching*, **205**, 41–67.

Etterson JR, Shaw RG (2001) Constraint to Adaptive Evolution in Response to Global Warming. *Science*, **151**, 151–154.

Falconer DS, Mackay TFC (1996) *Introduction to Quantitative Genetics*, 4th edn. Pearson Education.

Finley D, Ozkaynak E, Varshavsky A (1987) The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation, and other stresses. *Cell*, **48**, 1035–1046.

Fitt W, Brown B, Warner M, Dunne R (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral reefs*, **20**, 51–65.

Fukami H, Chen CA, Budd AF et al. (2008) Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (Order Scleractinia, Class anthozoa, phylum cnidaria). *PLoS ONE*, **3**, e3222.

Gardner TA (2003) Long-Term Region-Wide Declines in Caribbean Corals. *Science*, **301**, 958–960.

Granados-Cifuentes C, Bellantuono AJ, Ridgway T, Hoegh-Guldberg O, Rodriguez-Lanetty M (2013) High natural gene expression variation in the reef-building coral Acropora millepora: potential for acclimative and adaptive plasticity. *BMC Genomics*, **14**, 228.

Grottoli AG, Warner ME, Levas SJ et al. (2014) The cumulative impact of annual coral bleaching can turn some coral species winners into losers. *Global Change Biology*, **20**, 3823–3833.

Hajj-Ali I, Anctil M (1997) Characterization of a serotonin receptor in the cnidarian Renilla koellikeri: A radiobinding analysis. *Neurochemistry International*, **31**, 83–93.

Hoegh-Guldberg O, Jones RJ (1999) Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. *Mar Ecol Prog Ser*, **183**, 73–86.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ et al. (2007) Coral Reefs Under Rapid Climate Change and Ocean Acidification. *Science*, **318**, 1737–1742.

Houle D (1991) Genetic Covariance of Fitness Correlates : What Genetic Correlations are Made of and Why it Matters. *Evolution*, **45**, 630–648.

Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL, van Oppen MJH (2011) Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change*, **2**, 116–120.

Howells EJ, Abrego D, Meyer E, Kirk NL, Burt JA (2016) Host adaptation and unexpected symbiont partners enable reef-building corals to tolerate extreme temperatures. *Global Change Biology*, **22**, 2702–2714.

Hughes TP (2003) Climate Change, Human Impacts, and the Resilience of Coral Reefs. *Science*, **301**, 929–933.

Hughes TP, Tanner JE (2000) Recruitment Failure, Life Histories, and Long-Term Decline of Caribbean Corals. *Ecology*, **81**, 2250–2263.

Hughes TP, Kerry JT, Álvarez-Noriega M et al. (2017) Global warming and recurrent mass bleaching of corals. *Nature*, **543**, 373–377.

Hunter CL (1993) Genotypic Variation and Clonal Structure in Coral Populations with Different Disturbance Histories. *Evolution*, **47**, 1213–1228.

Hunter RL, LaJeunesse TC, Santos SR (2007) Structure and evolution of the rDNA internal transcribed spacer (ITS) region 2 in the symbiotic dinoflagellates (Symbiodinium, Dinophyta). *Journal of Phycology*, **43**, 120–128.

Joint Genome Institute (1997) BBTools. http://jgi.doe.gov/data-and-tools/bbtools/.

Jones A, Berkelmans R (2010) Potential costs of acclimatization to a warmer climate: growth of a reef coral with heat tolerant vs. sensitive symbiont types. *PLoS ONE*, **5**, e10437.

Jones AM, Berkelmans R, van Oppen MJ., Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 1359–1365.

Kenkel CD, Meyer E, Matz M V. (2013) Gene expression under chronic heat stress in populations of the mustard hill coral (Porites astreoides) from different thermal environments. *Molecular Ecology*, **22**, 4322–4334.

Kenkel CD, Setta SP, Matz M V (2015) Heritable differences in fitness-related traits among populations of the mustard hill coral, Porites astreoides. *Heredity*, **115**, 509–

516.

Kitahara M V, Cairns SD, Stolarski J, Blair D, Miller DJ (2010) A Comprehensive Phylogenetic Analysis of the Scleractinia (Cnidaria, Anthozoa) Based on Mitochondrial CO1 Sequence Data (ed DeSalle R). *PLoS ONE*, **5**, e11490.

Kumar S, Stecher G, Tamura K (2017) MEGA7 : Molecular Evolutionary Genetics Analysis Version 7 . 0 for Bigger Datasets. **33**, 1870–1874.

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. *Current Biology*, **28**, 2570-2580.e6.

Lande R, Arnold SJ (1983) The Measurement of Selection on Correlated Characters. *Evolution*, **37**, 1210–1226.

Lang R, Gundlach AL, Holmes FE, Hobson SA, Wynick D, Hokfelt T, Kofler B (2014) Physiology, Signaling, and Pharmacology of Galanin Peptides and Receptors: Three Decades of Emerging Diversity. *Pharmacological Reviews*, **67**, 118–175.

Leggat WW, Seneca FF, Wasmund KK, Ukani LL, Yellowlees DD, Ainsworth TDTD (2011) Differential responses of the coral host and their algal symbiont to thermal stress. *PLoS ONE*, **6**, e26687.

Lohr KE, Patterson JT (2017) Intraspecific variation in phenotype among nursery-reared staghorn coral Acropora cervicornis (Lamarck, 1816). *Journal of Experimental Marine Biology and Ecology*, **486**, 87–92.

López-Maury L, Marguerat S, Bähler J (2008) Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. *Nature Reviews: Genetics*, **9**, 583–593.

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology*, **15**, 1–21.

Lundgren P, Vera JC, Peplow L, Manel S, van Oppen MJH (2013) Genotype environment correlations in corals from the Great Barrier Reef. *BMC Genetics*, **14**, 1.

Lynch M, Walsh B (1998) *Genetics and Analysis of Quantitative Traits*. Sinauer Sunderland, MA, 980 pp.

Maor-Landaw K, Levy O (2016) Gene expression profiles during short-term heat stress; branching *vs.* massive Scleractinian corals of the Red Sea. *PeerJ*, **4**, e1814.

Marshall PA, Baird AH (2000) Bleaching of corals on the Great Barrier Reef:differential susceptibilities among taxa. *Coral reefs*, **19**, 155–163.

Messina FJ, Fry JD (2003) Environment-dependent reversal of a life history trade-off in the seed beetle Callosobruchus maculatus. *Journal of Evolutionary Biology*, **16**, 501–509.

Meyer E, Davies S, Wang S, Willis BL, Abrego D (2009a) Genetic variation in responses to a settlement cue and elevated temperature in the reef-building coral Acropora millepora. *Mar Ecol Prog Ser*, **392**, 81–92.

Meyer E, Aglyamova G V, Wang S et al. (2009b) Sequencing and de novo analysis of a coral larval transcriptome using 454 GSFlx. *BMC genomics*, **10**, 219.

Meyer E, Aglyamova G V, Matz M V (2011) Profiling gene expression responses of coral larvae (Acropora millepora) to elevated temperature and settlement inducers using a novel RNA□Seq procedure. *Molecular Ecology*, **20**, 3599–3616.

Mitsukawa K, Lu X, Bartfai T (2009) Bidirectional regulation of stress responses by galanin in mice: Involvement of galanin receptor subtype 1. *Neuroscience*, **160**, 837–846.

Moran Y, Barzilai MG, Liebeskind BJ, Zakon HH (2015) Evolution of voltage-gated ion channels at the emergence of Metazoa. *Journal of Experimental Biology*, **218**, 515–525.

Morrissey MB, Parker DJ, Korsten P, Pemberton JM, Kruuk LEB, Wilson AJ (2012) The prediction of adaptive evolution: Empirical application of the secondary theorem of selection and comparison to the breeder's equation. *Evolution*, **66**, 2399–2410.

Muir P, Frasier T (2015) Related : an R package for analysing pairwise relatedness from codominant molecular markers related : an R package for analysing pairwise relatedness from codominant molecular markers. *Molecular Ecology Resources*, **15**, 557–561.

Oksanen J (2010) Cluster analysis: tutorial with R. *University of Oulu, Oulu*, 1–8.

Oliver TA, Palumbi SR (2011) Do fluctuating temperature environments elevate coral thermal tolerance? *Coral reefs*, **30**, 429–440.

Van Oppen MJH, Mahiny AJ, Done TJ (2005) Geographic distribution of zooxanthella types in three coral species on the Great Barrier Reef sampled after the 2002 bleaching event. *Coral Reefs*, **24**, 482–487.

Palumbi SR, Barshis DJ, Traylor-Knowles N, Bay RA (2014) Mechanisms of reef coral resistance to future climate change. *Science*, **344**, 895–898.

Pickart CM (2001) Mechanisms underlying ubiquitination. *Annual Review of Biochemistry*, **70**, 503–33.

Polato NR, Voolstra CR, Schnetzer J et al. (2010) Location-specific responses to thermal stress in larvae of the reef-building coral Montastraea faveolata. *PLoS ONE*, **5**, e11221–e11221.

Queller DC, Goodnight KF (1989) Estimating Relatedness Using Genetic Markers. *Evolution*, **43**, 258–275.

Reitzel AM, Sullivan JC, Traylor-knowles N, Finnerty JR (2010) Genomic Survey of Candidate Stress-Response Genes in the Estuarine Anemone Nematostella vectensis. *Genomics*, **214**, 233–254.

Rodriguez-Lanetty M, Harii S, Hoegh-Guldberg O (2009) Early molecular responses of coral larvae to hyperthermal stress. *Molecular Ecology*, **18**, 5101–5114.

Roth MS, Deheyn DD (2013) Effects of cold stress and heat stress on coral fluorescence in reef-building corals. *Scientific reports*, **3**, 1421.

Ruiz-Jones LJ, Palumbi SR (2017) Tidal heat pulses on a reef trigger a fine-tuned transcriptional response in corals to maintain homeostasis. *Science Advances*, **3**, 1–10.

Rumble SM, Lacroute P, Dalca A V., Fiume M, Sidow A, Brudno M (2009) SHRiMP: Accurate mapping of short color-space reads. *PLoS Computational Biology*, **5**, 1–11.

Schlötterer C, Kofler R, Versace E, Tobler R, Franssen SU (2015) Combining experimental evolution with next-generation sequencing: a powerful tool to study adaptation from standing genetic variation. *Heredity*, **114**, 431–440.

Sciolino NR, Smith JM, Stranahan AM, Freeman KG, Edwards GL, Weinshenker D, Holmes P V. (2015) Galanin mediates features of neural and behavioral stress resilience afforded by exercise. *Neuropharmacology*, **89**, 255–264.

Sgrò CM, Hoffmann AA (2004) Genetic correlations, tradeoffs and environmental variation. *Heredity*, **93**, 241–248.

Shahsavarani H, Sugiyama M, Kaneko Y, Chuenchit B, Harashima S (2012) Superior thermotolerance of Saccharomyces cerevisiae for efficient bioethanol fermentation can be achieved by overexpression of RSP5 ubiquitin ligase. *Biotechnology Advances*, **30**, 1289–1300.

Silverstein RN, Correa AMS, Baker AC (2012) Specificity is rarely absolute in coral-algal symbiosis: implications for coral response to climate change. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 2609–2618.

Smith-Keune CC, Dove SS (2008) Gene expression of a green fluorescent protein homolog as a host-specific biomarker of heat stress within a reef-building coral. *Marine Biotechnology*, **10**, 166–180.

Snelling J, Dziedzic K, Guermond S, Meyer E (2017) Integrating genomic resources for a threatened Caribbean coral (Orbicella faveolata) using a genetic linkage map developed from individual larval genotypes. *bioRxiv*, **2925759**, 1–40.

Stewart ZK, Pavasovic A, Hock DH, Prentis PJ (2017) Transcriptomic investigation of wound healing and regeneration in the cnidarian Calliactis polypus. *Scientific Reports*, **7**, 41458.

Storey JD (2003) The positive false discovery rate: A Bayesian interpretation and the q-value. *Annals of Statistics*, **31**, 2013–2035.

T L (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine biology*, **141**, 387–400.

Tavalire HF, Beechler BR, Buss PE et al. (2018) Context-dependent costs and benefits of tuberculosis resistance traits in a wild mammalian host. *Ecology and Evolution*, **8**, 12712–12726.

Visscher PM, Hill WG, Wray NR (2008) Heritability in the genomics era — concepts and misconceptions. *Nature Reviews Genetics*, **9**, 255–266.

Voolstra CR, Schnetzer J, Peshkin L, Randall CJ, Szmant AM, Medina M (2009) Effects of temperature on gene expression in embryos of the coral Montastraea faveolata. *BMC genomics*, **10**, 627.

Walter P, Ron D (2011) The Unfolded Protein Response: From Stress Pathway to Homeostatic Regulation. *Science*, **334**, 1081–1086.

Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews: Genetics*, **10**, 57–63.

Wang S, Meyer E, McKay JK, Matz M V (2012) 2b-RAD: a simple and flexible method for genome-wide genotyping. *Nature Methods*, **9**, 808–810.

Welchman RL, Gordon C, Mayer RJ (2005) Ubiquitin and ubiquitin-like proteins as multifunctional signals. *Nature Reviews Molecular Cell Biology*, **6**, 599–609.

Westfall J a, Elliott SR, MohanKumar PS, Carlin RW (2000) Immunocytochemical evidence for biogenic amines and immunogold labeling of serotonergic synapses in tentacles of Aiptasia pallida (Cnidaria, Anthozoa). *Invertebrate Biology*, **119**, 370–378.

Wilson AJ, Réale D, Clements MN et al. (2010) An ecologist's guide to the animal model. *Journal of Animal Ecology*, **79**, 13–26.

Zhao JH (2007) gap: Genetic Analysis Package. *Journal of Statistical Software*, **23**, 1–18.

CHAPTER 3 – Genetic variation in thermal tolerance in the temperate anemone, *Anthopleura elegantissima*

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Abstract

The intertidal sea anemone *Anthopleura elegantissima* experiences large temperature fluctuations on a daily basis, with internal body temperatures varying by more than 20°C. Understanding how these environmentally tolerant cnidarians survive and maintain symbiotic relationships in the face of such extreme thermal variation may provide important insights into the mechanisms used by their relatives, the reef-building corals to cope with rising ocean temperatures. To study genomic mechanisms underlying variation in thermal tolerance of anemones, we subjected over 500 anemones from 63 colonies to control (12°C) or heat-stress conditions (23°C) for two weeks. We quantified bleaching susceptibility using qPCR, identifying heat-tolerant or heat-susceptible colonies for further study. We profiled transcriptional responses in these tolerant and susceptible anemones, revealing strong transcriptional responses to thermal stress (>2,400 differentially expressed genes, DEG), which differed significantly among colonies (colony x treatment interaction; 128 DEG). Next, we analyzed variation in bleaching responses of individual anemones in the context of colony identities and multi-locus SNP genotypes to estimate clonal repeatability (proxy for broad-sense heritability, H^2=0.59) and narrow-sense heritability (h^2=0.45), revealing substantial heritable variation in this population of anemones. We used the same SNP genotypes to test for genome-wide patterns of association between genotype and thermal tolerance, using the linkage map and draft genome assembly developed for this species by our research group. This analysis revealed four markers associated with thermal tolerance, in two genomic regions. Interestingly, heterozygote advantage in thermal tolerance is clearly evident at three of these markers, suggesting that genome-wide heterozygosity might play a role in variation in Cnidarians' thermal tolerance. Together, these findings demonstrate substantial genetic variation in thermal tolerance across anemones and identify genes and genetic markers associated with this variation, highlighting the value of this system as a model for the study of environmental stress in symbiotic Cnidarians.

Introduction

Symbiosis between cnidarian hosts and dinoflagellate endosymbionts play important roles in the marine environment, particularly in coral reef ecosystems and other marine animals such as anemones. This mutualism is responsible for generating highly productive ecosystems and allows both partners to thrive and persist in an otherwise nutrient-poor environment. However, this symbiosis can breakdown rapidly with the onset of various stressors, such as increased temperature (Weis, 2008; Baird *et al.*, 2009b; Leggat *et al.*, 2011). Over the last 30 years, bleaching events, the breakdown of the association between the cnidarian host and dinoflagellate endosymbiont, have increased in severity and frequency along the equatorial region (Hughes, 2003; Donner *et al.*, 2005; Hoegh-Guldberg *et al.*, 2007).

As scleractinian corals become increasingly threatened, it is imperative that we understand how this symbiotic relationship is maintained and breaks down, the genetic basis of thermal tolerance, and their capacity for adaptation (Weis, 2008; Meyer *et al.*, 2009a; Császár *et al.*, 2010; Kenkel *et al.*, 2013; Palumbi *et al.*, 2014). Since coral reef ecosystems are fragile and not experimentally tractable, attention has turned to other cnidarians as models for coral reefs, such as the anemones *Exaiptasia pallida* and *Anthopleura elegantissima*, which both harbor dinoflagellate symbionts similar to those found in corals (Muller-Parker & Davy, 2001; Weis *et al.*, 2008). Using these model organisms, we can study stress response mechanisms to infer conserved stress response pathways within this phylum and understand if and how these processes may be working across coral reef species. With an expanding repertoire of genomic and transcriptomic resources for these model species, we are beginning to understand how these organisms respond to stress and how these processes relate to coral reef species (Donner *et al.*, 2005; Nicholas H. Putnam *et al.*, 2007; Shinzato *et al.*, 2011; Coles & Riegl, 2013; Baumgarten *et al.*, 2015; Kitchen *et al.*, 2015; Kenkel & Bay, 2017; Matthews *et al.*, 2017; Oakley *et al.*, 2017).

Anthopleura elegantissima is an aggregating anemone that forms genetically identical clones. These anemones can be found in the intertidal along the West coast of North America and they experience dramatic temperature fluctuations both daily and

seasonally. In the summer, an anemone's internal body temperature can change by 20°C or more and photosynthetic rates can significantly decline as the tide changes (Muller-Parker *et al.*, 2007; Bingham *et al.*, 2011). Anemones in Oregon, specifically at the sites Boiler Bay or Strawberry Hill, experience daily maximums around 24 – 25°C, and are exposed to extreme intertidal conditions for 3-5 hours during the middle of the day (11 am – 1 pm) (Helmuth *et al.*, 2002). This contrast in environmental susceptibility, particularly temperature fluctuations, makes this an ideal model system for asking questions about thermal tolerance in a natural population. Using these anemones, we can ask whether there are genetic factors, genomic regions, or specific genes associated with variation in thermal tolerance.

Previous studies conducted on *A. elegantissima*, have revealed diversity in thermal tolerance among clonal aggregations living in dynamic environmental conditions. In particular, a study by Coleman *et al.* showed substantial differences in emersion stress tolerances among *A. elegantissima* aggregations (genotypes). Low intertidal anemones significantly upregulated heat shock proteins after the first tidal cycle, but their overall survivorship did not differ from high intertidal anemones (Coleman *et al., in prep*). Instead, clonal aggregations played a significant role in survivorship, demonstrating genetic variation in emersion stress tolerance. In addition, transcriptional responses to increased temperature and UV light include genes such as protein biosynthesis, regulation of biological processes, and catalytic activity (Richier *et al.*, 2008). Exploring the gene expression of the symbiont, *Breviolum muscatinei* (formerly *Symbiodinium muscatinei*) found in *A. elegantissima,* links several genes to stress response pathways (e.g. heat shock proteins, ion transports), shedding light on how this vital symbiosis is maintained through regulation of existing genes (Rodriguez-Lanetty *et al.*, 2006; Macrander *et al.*, 2018). However, despite many studies presenting variation in responses in both the host and symbiont, no studies have addressed genetic variation that may contribute to thermal tolerance capacity in this system and across cnidarian species.

Climate change impacts are dramatically affecting the survival of coral reefs around the world, and the need to understand how and if these species will adapt to the changing climate is of the utmost priority. Here, we use *Anthopleura elegantissima* as a

model system to explore the potential for genetic change in a natural population. This study takes advantage of the experimentally tractable anemone system to gain insights into the role of thermal tolerance in cnidarians and evaluate the mechanisms these organisms may use to respond to stress. We investigate heritable variation in thermal tolerance and investigate to what extent it is genetically determined by exploring patterns of gene expression between heat-tolerant and heat-susceptible anemones and identify genetic markers and genes associated with thermally tolerant individuals. Our findings highlight potential mechanisms these anemones and their relatives, the reef-building corals, might use to adapt to climate change conditions.

Materials and Methods

Sample collection, thermal stress trials and experiments

We collected anemones at Strawberry Hill, OR during low tide on August 2-3, 2016. Eight random individual anemones were collected from each of 100 separate aggregations found in the lowest part of the intertidal zone (Figure 3.1a). We brought live anemones to Hatfield Marine Science Center, Newport OR where they were placed in outdoor flow-through water tables and maintained at ambient conditions for 3 months prior to experimentation. This period served to acclimate them to common conditions and minimize any variation resulting from their previous environmental conditions. Anemones from each aggregation were kept together in flow-through containers and were spot fed crushed mussels each week. At the time of collection, we also sampled tentacle clips from each colony for SNP genotyping, and preserved these in a nucleic acid stabilization buffer (RNA*later*; Qiagen, CA).

To identify thermal stress treatments appropriate for studying variation in stress responses of these anemones, we first conducted stress trials testing temperatures ranging from realistic ambient temperatures (12-14°C) to a maximum temperatures of 30°C. This temperature was chosen based on previous estimates for the maximum anemones experience at this intertidal site and has been used in previous experimental studies (Helmuth *et al.*, 2002; Muller-Parker *et al.*, 2007). Based on anemone survival percentage and overall phenotypic appearance (visible bleaching and death), we identified a critical

temperature of 23°C to test thermal tolerance across aggregations. High temperatures (26-30°C) showed >75% death within one week, while 23°C showed visible bleaching and variation (<10% death) across individual anemones from multiple aggregations.

Anemones from each clonal aggregation were arbitrarily assigned (3-4 anemones per treatment) to control and stress treatments (Figure 3.1b, c, and d). Tentacle clips of all anemones were taken prior to experimentation and preserved in RNAlater. Anemones in the control condition were maintained at an ambient seawater temperature of 12°C while anemones in the heat stress treatments were ramped (~0.1°C per hour) to 23°C for two weeks. Anemones were monitored daily for signs of visible bleaching and their survival scored based on tentacle retraction when animals were removed from the water and re-expansion when returned to the water; the loss of these responses was scored as mortality. Dead anemones were rapidly removed from the experimental treatment. After two weeks, tentacle clips of anemones in both treatments were again sampled and stored in RNAlater for further analysis.

Quantifying thermal stress responses

To evaluate effects of thermal stress on the symbiotic association, we quantified changes in the abundance of algal symbionts using quantitative PCR (qPCR). We used the host ATPase gene as a reference gene to normalize signals from symbiont cells to the amount of host tissue in each sample. We developed forward (5'-CACCAACACGAGCTCTGACT-3') and reverse (5'-GAAGAGTTGCTAGGCCGTGT-3') primers for this target using Primer3, confirmed the efficiency of these primers using a dilution series prepared from anemone DNA (5 ng uL^{-1}). To evaluate whether non-specific amplification of symbiont DNA contributed to the qPCR signal interpreted as host, we made mixes of known amounts of host and symbiont cells and quantified each mixture with the host primer to ensure that symbiont cells present in the samples did not mask the host signal, demonstrating strong (>96%) specificity to anemone DNA. To quantify Symbiodiniaceae in each sample we used a pair of universal primers developed based on multiple sequence alignments of the cp23S-rDNA locus (Dziedzic *et al.*, 2019, Dziedzic *et al.*, *in prep*). We conducted qPCR using these primers (5'-

CTACCTGCATGAAACATAGAACG -3' and 5'- CCCTATAAAGCTTCATAGGG -3') to determine the total amount of symbiont cells present after experimentation in control and heat stress conditions. All reactions were run on an Eppendorf Realplex 4 machine and consisted of 7.5 µL SensiFAST SYBR Hi-ROX master mix (Bioline, Taunton, MA), 4.3 µl NFW, 0.6 µl each of forward and reverse 10-µM primers, and 2 µl of genomic DNA (10ng total) in a final volume of 15 µl. The thermal profile for each reaction consisted of an initial denaturing step of 95°C for 2 min, followed by 40 cycles of: 95°C for 5 s, annealing temperature of 60°C for 30 s, and then 72°C for 30 sec. All samples were run using the same reaction parameters and were analyzed together. In addition, we included one sample on every plate to serve as an inter-plate calibrator to ensure consistency in amplification across plates.

To compare changes in symbiont density across treatments, we first calculated the ratio of symbiont cells to host cells (ΔC_T) in each sample by subtracting host cell quantifications (C_T values) from symbiont cells (C_T). The ΔC_T value from the initial time point was subtracted from the ΔC_T value from the post-stress time point to generate $\Delta\Delta C_T$ values, representing the change in symbiont density over time. We calculated fold-change of symbiont densities in each anemone from these data as $2^{-\Delta\Delta CT}$. To get a metric of thermal tolerance for each aggregation, we divided the average fold change ($2^{-\Delta\Delta CT}$) in anemones from the control treatment from the average fold change ($2^{-\Delta\Delta CT}$) in anemones from the stress treatment.

Profiling gene expression in individuals with contrasting thermal capacity

To identify genes that may play a functional role in thermal tolerance, we compared gene expression profiles between anemones in heat stress and control treatments. After two weeks of experimental conditions, anemone tentacle clips were sampled for gene expression analysis. Based on variation in bleaching responses in image analysis, we selected 12 contrasting phenotypes: six anemone aggregations that showed visible signs of stress (i.e. bleaching) compared to initial images (heat-susceptible), and six aggregations that showed no signs of stress (heat-tolerant). We sampled both control and heat-stress anemones from all selected colonies (48 total samples). Based on images

and qPCR data for all anemones from each colony, we labeled each anemone in this analysis as “heat-susceptible” or “heat-tolerant”. RNA was extracted using the Omega Bio-tek E.Z.N.A. Tissue RNA Kit (Omega Bio-tek, Norcross, GA). Purified RNA was used to prepare 3’ tag-based cDNA libraries for expression profiling using unique barcodes for each sample (Meyer *et al.* 2011). Libraries were combined in equal ratios for sequencing on 50bp SE HiSeq 2500 at the Oregon Health and Science University’s Massively Parallel Sequencing Shared Resource (MPSSR) Facility. After sequencing, we processed raw reads to remove non-template sequences, exclude reads with long homopolymer regions (>20bp) and exclude low-quality reads with no more than 10% having Phred scores <30. Scripts used for filtering steps can be found at https://github.com/Eli-Meyer/rnaseq_utilities. We mapped the high quality reads against the transcriptome for this species (Kitchen *et al.*, 2015) using SHRiMP, a short-read aligner software (Rumble *et al.*, 2009). Finally, we counted unique reads that aligned to each gene (subcomponents in the Trinity assembly; Haas *et al.*, 2013) to produce count data in each sample for statistical analysis.

We tested for differential gene expression using a negative binomial model in the R package ‘DESeq2’ (Love *et al.* 2014). To explore this variation in gene expression, we considered three models: 1) the effects of “treatment” (control vs. stress), “phenotype” (heat-susceptible vs. heat-tolerant colonies), and their interaction; 2) the effects of “treatment”, and “aggregation”, and their interaction; and 3) the effect of anemone aggregation on expression in the heat stress treatment alone. We conducted multiple test corrections across all genes to control false discovery rate (FDR) at ≤ 0.05 (Benjamini & Hochberg, 1995).

To further explore variation in gene expression between heat-susceptible and heat-tolerant anemones, we compared expression between phenotypes in control and stress treatments separately, quantifying the average change in expression for each phenotype in each treatment. We then conducted a linear regression between these expression responses to identify genes responding differently in the contrasting phenotypes. This analysis made it possible to assign each differentially expressed gene (DEG) to one of three categories: consistent responses in both phenotypes (i.e. up-

regulated or down-regulated in both), opposing responses in these phenotypes (one up-regulated and one down-regulated); or genes affected by treatment in one phenotype but not in the other.

To identify clusters of co-regulated genes associated with thermal tolerance, we clustered gene expression patterns using signed 'WGCNA' (Weighted Gene Co-Expression Network Analysis) (Langfelder & Horvath, 2008). Network-based approaches like this are used to describe correlations between large sets of genes and help pinpoint specific pathways that may be co-regulated as part of a coordinated transcriptional stress response. We used samples in the stress treatment to construct our modules and explore correlations with thermal tolerance following online tutorials and publically available scripts (Langfelder & Horvath, 2008, 2014; Kenkel & Matz, 2016). First, we normalized gene expression data using the variance stabilization procedure in DESeq2, and then conducted Pearson correlations for all gene pairs across all samples to produce a similarity matrix of gene expression (including the sign of the gene expression; hence the term signed WGCNA). These expression correlations were transformed into connection strengths through a power adjacency function, using a soft threshold power of 11. We then performed hierarchical clustering of genes based on topological overlap to identify groups of genes whose expression co-varied across samples. The expression of each module was summarized as an "eigengene", calculated as the first principal component of all the genes within a module. These modules were related to thermal tolerance phenotypes across samples to determine module-trait correlations. The direction of the module eigengene indicates the strength of the correlation. Finally, once significant associations between modules and thermal tolerance were found, we performed enrichment analysis on the genes found within each significant module using ErmineJ version 3.02 (Lee *et al.*, 2005). Gene set enrichment analyses were performed with the gene score resampling (GSR) method on p-values associated with each gene, using the median score for each gene set with 10,000 iterations. We identified groups of enriched genes (>2 genes) based on their functional annotation and the top 10 unique Gene Ontology (GO) categories were examined.

Multilocus SNP genotyping

To test for genetic associations and estimate genetic relatedness, we conducted genome-wide SNP genotyping of all aggregations using the 2b-RAD (Restriction Site-Associated DNA) approach for SNP genotyping (Wang *et al.* 2012). This method has been in diverse invertebrate systems, including our previous analysis of quantitative genetic variation in a reef-building coral (Dziedzic *et al.*, 2019). We extracted DNA from all aggregation samples (samples taken during initial collection) using the Omega bio-tek E.Z.N.A. Tissue DNA Kit (Omega Bio-tek, Norcross, GA). We quantified libraries using qPCR and libraries in equimolar amounts for sequencing in a single lane of 50 bp SE reads on Illumina HiSeq 3000 at OSU's Center for Genome Research and Biocomputing (CGRB).

Prior to analysis, we filtered reads to exclude any low quality or uninformative reads, then aligned reads to the reference and called genotypes based on nucleotide frequencies at each position. We analyzed the resulting data using a 2bRAD reference our research group has recently produced and used for a linkage map (https://datadryad.org/review?doi=doi:10.5061/dryad.3jt1tp7). Since the reference was produced from sperm samples that lack algal symbionts, no special filtering was required to eliminate algal reads in our anemone samples. We determined genotypes using the same pipeline described in a previous study (Dziedzic *et al.*, 2019) Briefly, we called loci homozygous if a second allele was present at less than 1%, heterozygous if present at > 25%, and left the genotype undetermined at intermediate frequencies where genotypes cannot be confidently determined from allele frequencies. Genotypes were called with a threshold of ≥ 10x to call as many loci as possible for this genome wide survey of associations with bleaching responses. The scripts used for this analysis are available at (https://github.com/Eli-Meyer/2brad_utilities).

Estimating heritability

To estimate the proportion of variation in thermal tolerance attributable to variation among aggregations, we calculated clonal repeatability by partitioning variance into within-aggregation and between-aggregation components. Clonal repeatability is a

measure of phenotypic variance across individuals and is equivalent to broad-sense heritability (Falconer & Mackay, 1996). We calculated clonal repeatability using linear mixed-effects models implemented in the 'rptR' package (Nakagawa & Schielzeth, 2010). For this analysis we used the thermal tolerance measurement calculated from qPCR to estimate repeatability, modelling aggregation (genotype) as a random effect.

To estimate narrow sense heritability, we followed a similar protocol as outlined in Dziedzic et al. 2019. We used multilocus SNP genotypes to infer relatedness between aggregations and create a genetic relatedness matrix using the 'related' package in R, using the method described by Queller & Goodnight to calculate genetic distance between samples (Queller & Goodnight, 1989; Muir & Frasier, 2015). We created a linear mixed model with aggregation as a random effect using the R 'regress' package (Tavalire *et al.*, 2018). Using the thermal tolerance measurement, we estimated narrow-sense heritability and the associated standard error based on the phenotypic variation, using the *h2G* function in the R package 'gap' (Zhao, 2007).

Testing for genotype-phenotype associations

To identify genomic regions underlying variation in thermal tolerance, we conducted an association study using the same SNP genotypes and thermal tolerance data described above. At each locus, we tested for effects of genotype on thermal tolerance using linear mixed models analysis of variance, similar to the approach outlined in Dziedzic et al. 2019. We used individual thermal tolerance measurements from qPCR to examine and correlate responses between stress and control treatments. To control for errors arising from multiple tests, we used the pFDR at 0.05 (Storey, 2003). To evaluate genomic patterns in these relationships, we analyzed these SNP data in the context of a genetic linkage map our research group has recently developed for this species (https://datadryad.org/review?doi=doi:10.5061/dryad.3jt1tp7). We used the R package 'rrBLUP' to test for associations between thermal tolerance and genotype at each locus. We used the *A.mat* function to calculate an additive relationship matrix, considering no more than 5% missing data across all loci. We then used the *GWAS* function to conduct association tests with allele frequencies > 0.08. Once significant SNPs were found, we

explored genotypes in the "tolerant" and "susceptible" phenotype groups to determine if particular genotypes were associated more with either phenotype, as well as characterize genomewide heterozygosity. Additionally, we mapped differentially expressed genes from the "colony" effect, the interaction effect between colony and treatment, and the genes showing different patterns in contrasting phenotypes found in RNAseq analysis (described above) onto the genome and linkage map to determine where genes were located in relation to significant SNPs.

Results

Stress responses in anemone aggregations

After 2 weeks in thermal stress at 23°C, we saw considerable variation in stress responses in anemones from different aggregations. We quantified symbiont densities in each anemone individual using qPCR, and estimated the bleaching response of each aggregation as the log fold change between stressed and control treatments (Figure 3.2). Aggregations showed substantial variation in both their initial symbiont densities and their bleaching responses, based on qPCR analysis of relative symbiont abundance (Figure 3.2). About half of the aggregations bleached in response to thermal stress, but the extent of these bleaching responses varied considerably.

Transcriptional responses to heat stress in tolerant and susceptible phenotypes

To understand the variation in responses after heat stress, we profiled gene expression in tolerant and susceptible phenotypes across 12 anemone aggregations (genotypes), six susceptible and six tolerant aggregations (48 anemone individuals total). We sequenced the libraries on Illumina HiSeq 2500, which produced a total of 218 million raw reads and approximately 4.4 million reads per sample. After quality and adaptor filtering, approximately 210 million high-quality reads remained (96.5%) for expression analysis (Table 3.1).

To test for changes in gene expression after stress conditions, we ran a negative binomial model using DESeq2. First, we evaluated the interaction between treatment and phenotype. While treatment had a strong effect (>3,000 DEGs), variation in

transcriptional responses within each phenotypic group obscured differences between phenotypic groups. To explore these differences, we evaluated the interaction between treatment and anemone aggregation. This revealed significant interactions between aggregation and treatment (128 DEGs). Identifying expressed genes responding differently to stress in these different colonies. To further explore these effects, we analyzed expression by colony in the stress treatment alone. This analysis identified a set of 402 DEGs showing significant differences in expression among colonies. Genes differentially expressed in the treatment × aggregation interaction included carbonic anhydrase, ubiquitin ligases, thioredoxin, and calcium binding proteins. Genes differentially expressed as a function of colony in the heat stress treatment included collagen proteins, ubiquitin-ligases, apoptosis proteins, glutathione peroxidase, and a tumor necrosis factor receptor. All annotated differentially expressed genes can be found in Table 3.3 as well in Appendix Table B1.

We further explored the difference in treatments by analyzing the phenotypes separately within each treatment (heat-susceptible versus heat-tolerant in just control, and stress). We correlated the log-fold change in expression between heat-susceptible and heat-tolerant anemones to evaluate whether these groups differed in transcriptional responses to stress. We found 58 genes that had either opposite patterns of expression between the two phenotypes or genes that remained unchanged in one phenotype but not the other (pvalue<0.05) (Figure 3.3). Although few of these genes could be identified based on sequence similarity with known genes from other systems, we were able to identify a putative tumor necrosis factor receptor gene showing opposing responses to stress (up-regulated in heat-susceptible anemones and down-regulated in heat-tolerant anemones). In addition, we found collagen proteins, potassium channel proteins, and a gene involved in regulating the apoptotic process (NACHT domain protein), all of which were upregulated in heat-tolerant anemones and unchanged in heat-susceptible anemones. All annotated differentially expressed genes can be found in Table 3.3 as well in Appendix Table B1.

We explored the correlation among gene expression levels to identify groups of co-regulated genes associated with thermal tolerance using signed WGCNA. Forty-nine

modules were identified in this analysis, one of which was significantly associated with tolerance across aggregations (Figure 3.4a). The genes in M13 module (162 genes total) were positively correlated with thermal tolerance, with a Pearson's correlation coefficient equal to 0.45 (p=0.007) (Figure 3.4a,b). Functional enrichment analysis of this module revealed that several functional categories were more strongly associated with the module than expected by chance (Figures 3.4c). Inspection of the gene annotations within this module also revealed several groups of genes highly represented (>10 genes in each group): collagen genes, ubiquitin-hydrolases, mannose receptors, glutamine amido-transferases, calcium binding proteins, and aldehyde/alcohol dehydrogenases. Genes found within this module are provided in Table 3.4 and Appendix Table B2.

SNP genotyping

To explore genetic relationships and genetic associations with stress responses, we conducted multilocus SNP genotyping on all clonal aggregations of anemones using 2bRAD. We sequenced a total of 314 million high quality reads, with an average of 4.9 million reads per clonal aggregation. We mapped these reads to a denovo reference generated from aposymbiotic larvae, providing us with loci derived only from the anemone host and not algal symbiont contaminants. We genotyped >700kb at ≥ 10x coverage across each anemone sample and identified 41,148 polymorphic loci (Table 3.1). We filtered genotypes to reduce the number of missing data (low-coverage samples and loci) and minimize genotyping errors, providing us with a total of 8,966 high quality SNPs that we used to estimate heritability and search for genetic associations with thermal tolerance (see below).

Heritable variation in thermal tolerance

To estimate heritability in this population of anemones, we investigated both the clonal repeatability (proxy for broad-sense heritability, H^2) and narrow sense heritability (h^2). For both estimates, we included clonal aggregation as a random effect and used phenotypic thermal tolerance measurements for every individual in all aggregations. Using the linear mixed effects model in rptR, we estimated clonal repeatability to be

equal to 0.59 (SE=0.086). To estimate narrow sense heritability, we calculated genetic relatedness among samples based on multilocus SNP genotypes (Queller & Goodnight, 1989). Using these genetic relatedness values, phenotypic variation in stress responses was found to be highly heritable, with a narrow-sense heritability (h^2) of 0.45 (SE=0.11). In fact, these estimates are consistent with the expected relationship between clonal repeatability and narrow sense heritability. H^2 is an upper bound estimate on narrow-sense heritability (h^2) as it also includes effects due to dominance and epistasis, so clonal repeatability should be higher than h^2 estimates of narrow sense (Falconer & Mackay, 1996; Lynch & Walsh, 1998)

Genomic basis for variation in thermal tolerance

To explore the genomic basis for this variation in thermal tolerance, we combined our SNP genotypes with phenotypic measurements of stress to test for genetic associations. We mapped each marker by linkage group and position with its corresponding $-\log_{10}$(p-value) from association tests to show regions of the genome strongly associated with thermal tolerance (Figure 3.5a). This analysis identified two regions significantly associated with thermal tolerance: three markers on linkage group (LG) 1 and one marker on LG 9 (FDR ≤ 0.05).

We also explored the distribution of genotypes at each significant locus in tolerant and susceptible anemones and documented an interesting pattern. Using a linear mixed model, we found significant heterozygosity associated with the tolerance phenotype at three of the four SNPs, SNP21192 (pvalue<0.0028) and SNP29722 (pvalue<0.0048) on LG1 and SNP8170 (pvalue< 0.00035) on LG9, whereas the opposite was true for SNP8423 (pvalue<0.0068) on LG1 (Figure 3.6). This result points to the intriguing possibility of heterozygote advantage for stress tolerance in this system, but see Discussion for addition considerations.

Furthermore, we explored genes linked to each marker by mapping differentially expressed genes from our RNAseq analysis onto the genome and linkage map (Figure 3.5b). We plotted the $-\log_{10}$(p-value) values for all genes onto the linkage map and searched for genes within 5 cM around each significant SNP, locating 6 genes linked to

SNPs on LG1 and 1 gene linked to the SNP on LG9. The set of genes found in close proximity to our SNPs on LG1 included a methyltransferase, tubulin-gamma complex protein, syntaxin, a heat shock protein, and three unannotated genes. We identified a single gene linked to the marker on LG9, a phosphofructokinase protein.

Discussion

With climate change continuing to threaten marine ecosystems, it is essential we understand how these organisms are currently responding to thermal stress and the mechanisms they may use to adapt to increasing sea surface temperatures. Our study elucidates possible mechanisms of thermal tolerance and provides estimates for heritability of variation in bleaching responses in a temperate anemone population. The results from this study build on the growing body of thermal tolerance studies on symbiotic anemones (Muller-Parker *et al.*, 2007; Bingham *et al.*, 2011; Dimond *et al.*, 2011). We found that temperate anemones harbor substantial genetic variation in thermal tolerance. Our study identified genetic markers associated with this variation, and documented differences in transcriptional responses to thermal stress among heat-tolerant and heat-susceptible anemones.

Gene expression analysis is a powerful tool for studying responses to environmental stress. This method allows for simultaneous evaluation of expression patterns of thousands of genes, providing global insights into which genes may play a mechanistic role in thermal tolerance. In our study we found a strong transcriptional response to thermal stress, which differed significantly between anemones from different aggregations. Genes differentially expressed in the interaction effect between treatment and aggregation included carbonic anhydrase, ubiquitin-related, and redox-related genes, all of which have been repeatedly identified in studies exploring cnidarian responses to heat stress (Downs *et al.*, 2002; Maor-Landaw & Levy, 2016; Ruiz-Jones & Palumbi, 2017). Genes differentially expressed in the effect of colony in the heat stress treatment included apoptosis inducing factors, collagen proteins, ubiquitin ligases and glutathione peroxidase. Ubiquitin-ligases and glutathione peroxidases are known for their role in labeling certain proteins for degradation and providing an antioxidant response in relation

to increases in reactive oxygen species, respectively (Downs *et al.*, 2002; Welchman *et al.*, 2005; Barshis *et al.*, 2010; Polato *et al.*, 2010; Bay & Palumbi, 2015). Carbonic anhydrases have been studied in anemones and corals, demonstrating their importance in regulating the inorganic carbon transport system when associated with symbionts (Weis, 1991; Weis & Reynolds, 1999; Bertucci & Tambutté, 2011; Bertucci *et al.*, 2013). We find that variation across individual anemone aggregations may be related to variation in bleaching responses across treatments. This finding is consistent with the conclusion from quantitative genetic and genomic analysis: that different aggregations of anemones vary in thermal tolerance in part because of genetically determined differences. These findings builds on the growing body of evidence that genetic factors in the animal host contribute to variation in thermal tolerance of the holobiont, and emphasize the value of the aggregating anemone system for studies of thermal tolerance in symbiotic cnidarians.

We identified a set of genes showing opposing patterns of regulation between thermal tolerance phenotypes (Figure 3.3). Specifically, we found a tumor necrosis receptor factor (TNRF) that was upregulated in heat-susceptible anemones (log fold change >6) and down-regulated in heat-tolerant anemones (log fold change <4), posing as a positive mechanism for thermal tolerance in this population of anemones. TNRF proteins are central to responses such as apoptosis and programmed cell death (Bradley & Pober, 2001; Shen & Pervaiz, 2006) and have been widely conserved in metazoans, emphasizing their general adaptive importance (Quistad *et al.*, 2014). For corals and anemones, this gene has shown a strong correlation with thermal tolerance (DeSalvo *et al.*, 2010; Mansfield *et al.*, 2017; Traylor-Knowles *et al.*, 2017b; Zhou *et al.*, 2017; Thomas *et al.*, 2018). We also found a gene part of the NACHT protein domain, one of the domains of NOD-like receptors (NLRs) in the innate immune system (Koonin & Aravind, 2000; Ghosh *et al.*, 2011). These receptors recognize intracellular pattern molecules and regulate inflammatory and apoptotic pathways within an organism (Koonin & Aravind, 2000; Damiano *et al.*, 2004; Rast & Messier-Solek, 2008; Ghosh *et al.*, 2011). Across the genome of *Acropora digitifera,* there are high number of NACHT domain containing proteins and therefore may play a large role in innate immune responses (Shinzato *et al.*, 2011).

Module expression in WGCNA and correlations with thermal tolerance traits highlighted one group of genes positively correlated with thermal tolerance (Figure 3.4). These genes included >10 collagen genes, ubiquitin-hydrolases, mannose receptor, calcium binding proteins, and aldehyde/alcohol dehydrogenases. Collagen proteins are important for immune responses such as wound healing and tissue regeneration in invertebrates (Reitzel *et al.*, 2010; Chang *et al.*, 2012; Stewart *et al.*, 2017). Previous studies in corals have shown increased expression of collagen genes in thermally tolerant corals compared to susceptible corals (Barshis *et al.*, 2013; Kenkel *et al.*, 2013), and also found this gene genetically linked to thermal tolerance traits (Dziedzic *et al.*, 2019). Ubiquitin proteins (such as ubiquitin hydrolase and ligase) are known for their role in labeling certain proteins for degradation and signaling in cells (Welchman *et al.*, 2005; Barshis *et al.*, 2010; Bay & Palumbi, 2015; Wright *et al.*, 2017). We also found genes involved in calcium binding. Loss of calcium homeostasis in cnidarians may be linked to stress in the endoplasmic reticulum, which could lead to an increase in the unfolded protein response (Weston *et al.*, 2015; Ruiz-Jones & Palumbi, 2017). Enrichment of the genes within this module showed two interesting categories, genes involved in lipid transport and cell surface receptor signaling. Previous studies examining UV and temperature stress on anemones find upregulation of lipid metabolism genes, which may be due to tissue damage from increased reactive oxygen species (ROS) within the host (Richier *et al.*, 2008; Moya *et al.*, 2012). Increased ROS can trigger intracellular signaling responses that in turn could lead to symbiosis breakdown between the cnidarian host and its algal partner (Weis, 2008). Overall, these genes may indicate a possible mechanism of dealing with increased ROS due to thermal stress across anemone aggregations between heat-tolerant and heat-susceptible phenotypes.

Recent studies using genetic maps have provided new insights into the way organisms organize their genomes, genome function, and how evolution has or could potentially occur (Wang *et al.*, 2009b). For cnidarians, we have gathered information on how their genomes are organized and function, but more specifically, which regions of the genome may be under selection. Here, we explored associations between thermal tolerance and SNPs using a linkage map and draft genome developed for *Anthopleura*

elegantissima. We found genetic markers associated with thermal tolerance, and using the genetic linkage map, we were able to put them into a genomic context. We found four significant SNPs that were strongly associated with thermal tolerance across anemone aggregations, with three markers found on linkage group (LG) 1 and one marker on LG 9 Figure 3.5). These loci may represent the loci having the largest effect on variation in thermal tolerance within this population, and therefore should be compared to responses in other cnidarian populations to determine if these loci are acting similarly.

To examine the functional basis for variation in bleaching responses, we compared our transcriptional responses with genomic analyses to further understand the genes that are contributing to this variation as well as the markers that may explain the difference in tolerance. We searched for genes possibly linked to each of these significant SNPs by combining the positions of our differentially expressed genes with the genome for *A. elegantissima*. On LG1, we found a methyltransferase, tubulin-gamma complex protein, syntaxin, and heat shock protein 70 (HSP70) all within 0-2.8 cM to our SNPs. Previous studies on HSP70 have shown that up-regulating this gene allows various invertebrates to survive in extreme intertidal conditions (Hofmann & Somero, 1995; Hamdoun *et al.*, 2003; Tomanek & Sanford, 2003; Jeno & Brokordt, 2014; Kim *et al.*, 2014). Specifically for anemones, when exposed to emersion stress that raised their body temperature to 30°C, upregulation of HSP70 allowed anemones to survive across all intertidal zones (Snyder & Rossi, 2004). Finding this gene possibly linked to a significant SNP and region of the genome, as well as seeing this gene upregulated in our transcriptional profiling dataset, shows the importance of this regulatory mechanism in response to stress. In addition, methyltransferases have been shown to be highly expressed in coral species in response to stress (Schwarz *et al.*, 2008; Dixon *et al.*, 2014, 2016; Putnam *et al.*, 2016; Li *et al.*, 2018). Our finding of association with thermal tolerance here in anemones may indicate a conserved response over evolutionary time. In addition, a phosphofructokinase protein was found on LG9. Phosphofructokinase (PFK) is an important enzyme part of the glycolysis pathway to generate energy (ATP) (Fernie *et al.*, 2004). As cnidarians undergo thermal stress, they have been shown to regulate metabolic pathways such as oxidative phosphorylation, the TCA cycle, and glycolysis in

both the host and symbiont (Hillyer *et al.*, 2016). For the host, down-regulating PFK may indicate that carbohydrate products are decreasing due to reduced levels being translocated from the symbiont (Hillyer *et al.*, 2016). Linkage with this gene may indicate a functional role in helping the anemone regulate its metabolism during periods of stress or when living aposymbiotically and forced to find nutrients externally, but its overall role needs to be explored further.

Additionally, we explored the genotypes of tolerant and susceptible anemones at each of our SNPs. We found significantly more heterozygous heat-tolerant individuals at two SNPs on LG1 and one SNP on LG9 (Figure 3.6), suggesting the possibility of heterozygote advantage for thermal tolerance in this system. Heterozygote advantage occurs when the heterozygous genotype shows greater fitness than either homozygous genotype, allowing genetic variation to be maintained in natural populations (Hansson & Westerberg, 2002; Conner & Hartl, 2004). Finding sites that are associated with heterozygosity may indicate heterozygote advantage in this fitness related trait, allowing the frequency of these heterozygote loci to increase in the population. Additionally, observing heterozygote advantage may explain the stability of these populations over time in such a dynamic and harsh environment (Mitton, 1993; Sellis *et al.*, 2011; Westram *et al.*, 2018). While we find evidence this mechanism may be playing a role in stabilizing anemone responses, this also may suggest the opportunity for heterozygote advantage to play a role in maintaining genetic variation in natural populations of corals and allow corals with heterozygote advantage at certain fitness-related loci to adapt to changing conditions (Mitton, 1997; Bellis *et al.*, 2016; Sellis *et al.*, 2016). For clonal species like anemones and corals, heterozygote advantage can be effectively fixed and propagated in clones, which may be an important mechanism for an evolutionary response to climate change (Chapman *et al.*, 2011; Coulson & Clegg, 2015). Future studies that pinpoint particular trait loci associated with these and other adaptation-relevant physiological traits can determine if heterozygosity is stabilizing population responses, and if selection is taking place.

Heritability is a critical measurement when predicting the potential of a trait for selection (Visscher *et al.*, 2008). For cnidarians, we are primarily concerned with the trait

of thermal tolerance, and need to understand if populations can respond to selection over time. In natural populations, it is often difficult to measure heritability with traditional pedigree approaches, because relationships between individuals are unknown (Mousseau *et al.*, 1998; Visscher *et al.*, 2008; Stanton-Geddes *et al.*, 2013). Using just a few thousand SNPs can provide reliable estimates of heritability and provide a tool for us to continue asking these questions in different environmental conditions across different populations of the same or similar species (Stanton-Geddes *et al.*, 2013; Dziedzic *et al.*, 2019). This makes 2bRAD is well-suited for inferring genetic relatedness among individuals, since the reduced-representation options allow cost-effective estimation of genetic relatedness regardless of genome size or SNP frequencies (Wang *et al.*, 2012; Stanton-Geddes *et al.*, 2013).

Our data suggest the genetic potential for adaptive responses to selection for thermal tolerance within this population. This represents one of the largest genomic studies to date in cnidarians, with 63 genotypes and 500+ individuals. With this sample size, we were able to provide estimates for clonal repeatability and narrow-sense heritability using SNP genotype data and phenotypic measurements of thermal tolerance. We estimated narrow-sense heritability (h^2) to be 0.45 (SE=0.11), which is more than half of the repeatability estimate (R=0.59, SE=0.086), indicating that majority of the phenotypic variation is explained by additive genetic variance. Moreover, favorable epistatic combinations within this population can become fixed and then propagated clonally across anemone aggregations, allowing local adaption to arise. Overall, heritability within this population of anemones is high and considering both genetic factors and life history strategies of this anemone, thermal tolerance can potentially be selected for in this population.

More broadly, we are seeing a devastating impact to coral reef ecosystems as sea surface temperatures continue to increase, but corals remain experimentally intractable and their conservation status complicates large-scale sampling. To understand cnidarian-specific mechanisms underlying thermal tolerance, model cnidarian systems provide vital tools for investigating shared pathways of thermal stress response (Muller-Parker & Davy, 2001; Weis *et al.*, 2008). Using genomic and cellular resources and tools, we can

begin to improve our understanding of this important cnidarian-dinoflagellate symbiosis. We can pinpoint genes that play a role in the onset, maintenance, and breakdown of the symbiosis and associate these as cnidarian-host specific or symbiont specific pathways (Davy *et al.*, 2012). In our study, we saw genes that have already been described in other thermal tolerance studies, and therefore provide more evidence that these genes play an important role in cnidarian response to stress (see above). Because anemones are more distantly related, we can hypothesize that some of these genes or groups of genes, or even regions of the genome may be conserved over evolutionary time. Therefore, these genomic and transcriptomic analyses in anemones can begin to pinpoint specific areas or highlight priorities to study in coral species.

While anemones are closely related to corals and we can broadly characterize mechanisms of thermal tolerance, it is important to recognize the caveats to using this system. First and foremost, anemones lack carbonate skeletons and therefore we cannot generalize our conclusions to the energetics of the host (Muller-Parker & Davy, 2001; Weis *et al.*, 2008; Davy *et al.*, 2012). And most obviously, this is a temperate anemone and therefore does not experience the same environmental conditions as corals. These anemones see a much more dramatic temperature regime, and are exposed to other stressors than just water temperature (e.g. emersion stress, irradiance) (Muller-Parker & Davy, 2001; Weis *et al.*, 2008). Additionally, these anemones live in nutrient rich environments and have facultative associations with their symbionts, in contrast to the nutrient-poor coral reef environment and obligate nature of the coral-algal symbiosis (Muller-Parker & Davy, 2001; Hiebert & Bingham, 2012; Bingham *et al.*, 2014). Despite these ecophysiological differences, thermal tolerance studies on *Anthopleura* species can provide insights into algal population dynamics and bleaching mechanisms and begin to elucidate the mechanisms each symbiotic partner may use to combat and cope with stress. Past studies have used *Anthopleura elegantissima* to study symbiosis onset and breakdown and relate their findings to the cellular and molecular players driving coral reef responses to stress (Reynolds *et al.*, 2000; Schwarz & Weis, 2003; Richier *et al.*, 2008; Macrander *et al.*, 2018). In our experimental study, we also find shared functional pathways that drive stability in this symbiosis and show genes that are known to be

involved in the response to oxidative stress in this partnership. We saw considerable variation in anemone's response to thermal stress. In fact, we saw bleaching responses similar to those measured in corals, a response not often seen and documented in the lab or intertidal setting. Using this variation, we were able to further explore the genetic underpinnings of this variation and further relate stress responses to other populations of this anemone as well as cnidarians in general.

Overall, our study provides a novel perspective on genetic variation and its contribution to thermal tolerance and adaptation in this population of *Anthopleura elegantissima*. We used expression profiling to demonstrate that anemones respond to thermal stress differently than control anemones, with opposite patterns of expression for some genes in heat-susceptible and heat-tolerant anemones. Through functional analysis of these differentially expressed genes and enrichment across groups of genes, we find genes primarily involved in processes such as ubiquitination, calcium binging, response to unfolded proteins, and apoptosis and programmed cell death across heat tolerant anemones. We used multilocus SNP genotyping to infer genetic relatedness among anemone aggregations and estimate clonal repeatability (R) and narrow-sense heritability (h^2) for variation in bleaching responses. We found that a substantial fraction of variation in this trait is additive genetic variation, suggesting substantial genetic potential for adaptation to ocean warming in this population. We also identified genetic markers linked to thermal tolerance, markers that are one or within close proximity to a heat shock protein and methyltransferase. These findings highlight some potential mechanisms for adaptation to increasing sea surface temperatures within a Cnidarian species.

Acknowledgments

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Data Accessibility

Reference numbers for data in public repositories: sequence data archived at NCBI's Sequence Read Archive (SRA) under BioProject PRJNA542929.
Scripts used for analysis can be found at https://github.com/Eli-Meyer.

Figures and Tables

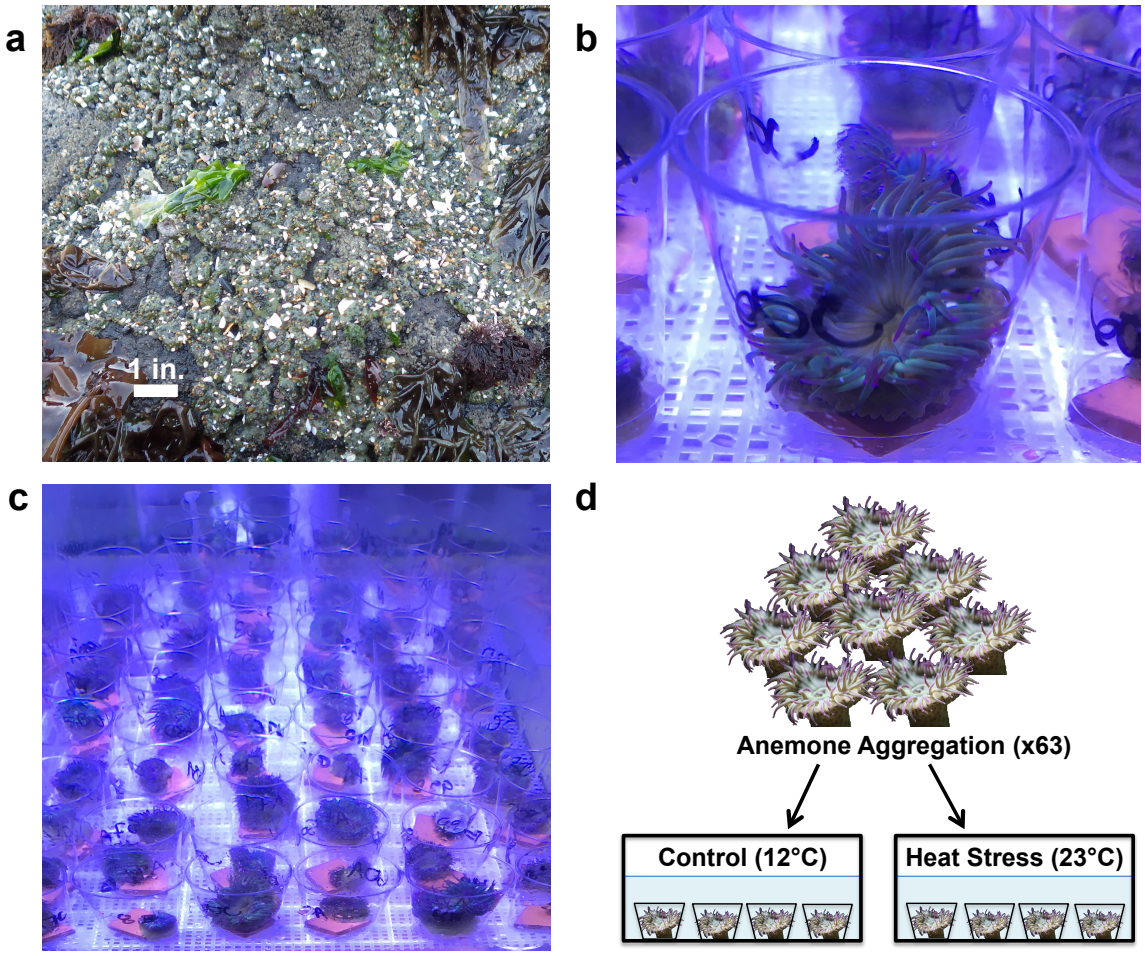

Figure 3.1. Anemone aggregations collected at Strawberry Hill, site on the coast of Oregon. a) Example of one anemone aggregation collected at Strawberry Hill. b) Example of an individual anemone isolated in plastic cups. c) Example of isolated anemones from multiple aggregations randomized in each treatment. d) Experimental setup for stress and control treatments for each aggregation of anemones.

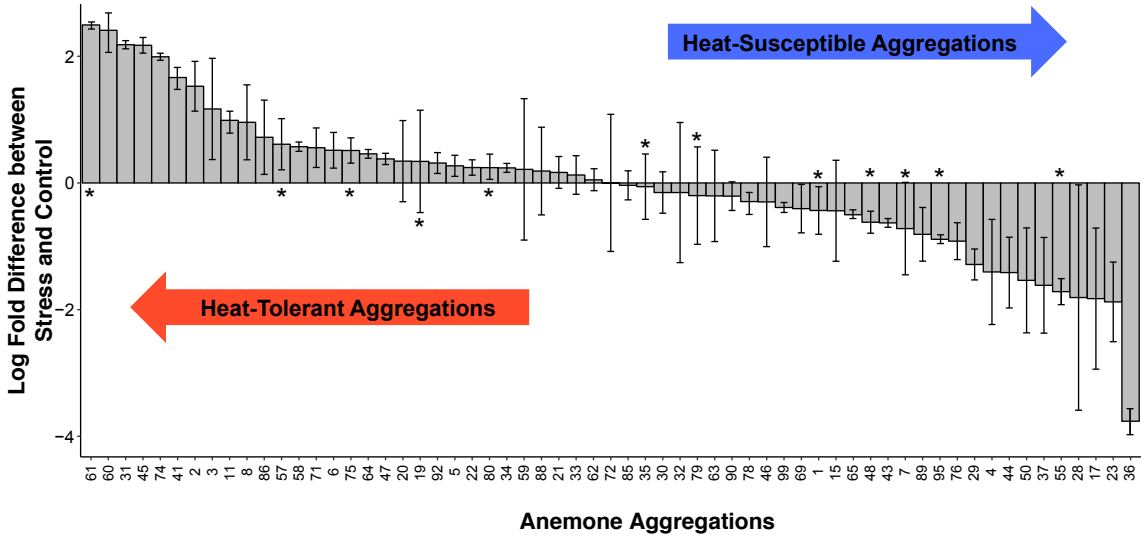

Figure 3.2. Quantification of algal symbiont densities using qPCR shows variation across anemone aggregations. Each bar represents the average difference within each anemone aggregation (n=8, 4 in control and 4 in stress), calculated as the difference in log-fold change ($2^{-\Delta\Delta Ct}$) in symbiont abundance in stress samples subtracted from the log-fold change in control samples. Error bars represent variation in responses across all anemones within each aggregation. (*) indicates samples that were included in RNAseq analysis.

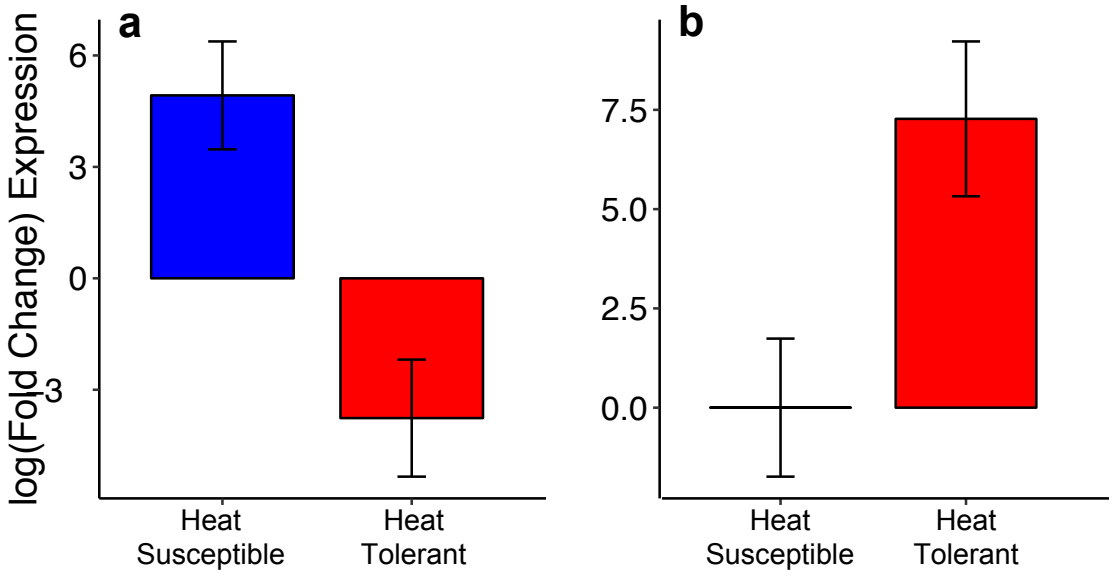

Figure 3.3. Examples of the two dominant patterns when exploring gene expression across heat-susceptible and heat-tolerant anemones as a function of phenotype in differential expression analysis. a) First dominant pattern showing differentially expressed genes were upregulated in heat-susceptible corals (blue) and downregulated in heat-tolerant corals (red). b) Second pattern that shows differentially expressed genes were unchanged in heat-susceptible anemones but upregulated in heat-tolerant corals. Error bars indicate variation in gene expression across anemone samples in either phenotype for these examples.

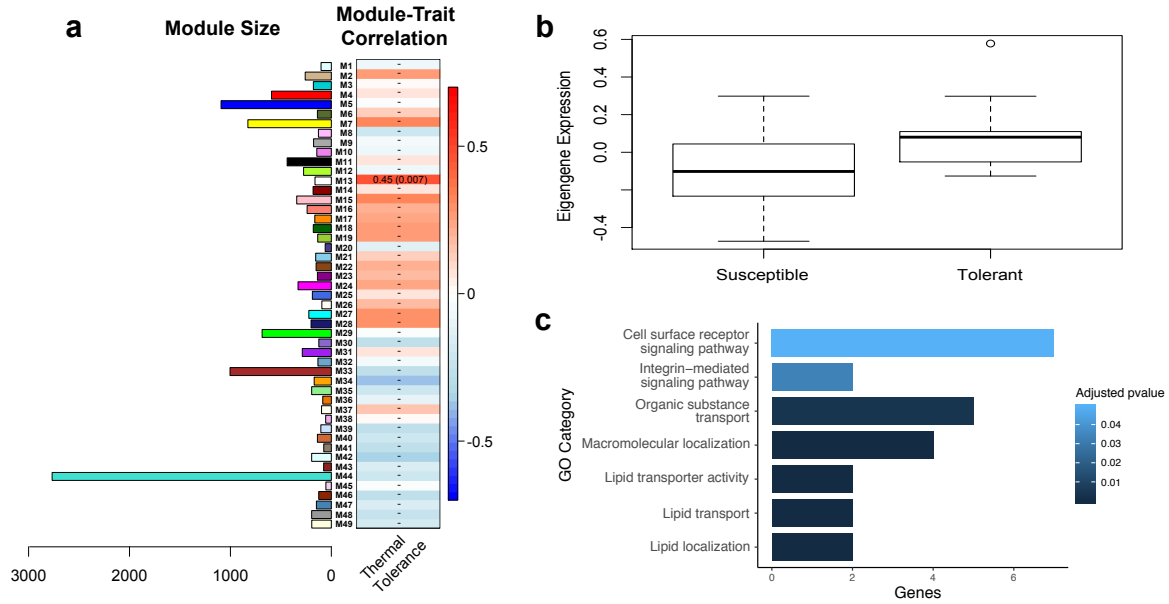

Figure 3.4. Module assignment and correlation to thermal tolerance across anemone aggregations. a) Forty-nine modules were clustered together using a matrix of VSD transformed counts in the R package WGCNA (Weighted Gene Co-Expression Network Analysis). The number of genes found within each module is indicated as the module size and are presented as color bars to the left of each individual module. The module-trait correlation is presented in the graph to the right of each module, with the strength of the correlation indicated by color (red is indicative of a strong positive correlation and blue a strong negative correlation). The module that was significantly associated with thermal tolerance is presented with p-value indicated in parentheses. b) Comparing module eigengene expression for module 13 (pvalue < 0.007) across heat-susceptible and heat-tolerant anemones in the stress treatment. c) Enrichment (adj. pvalue <0.05) for GO categories in module 13 across molecular, biological, and cellular functional categories.

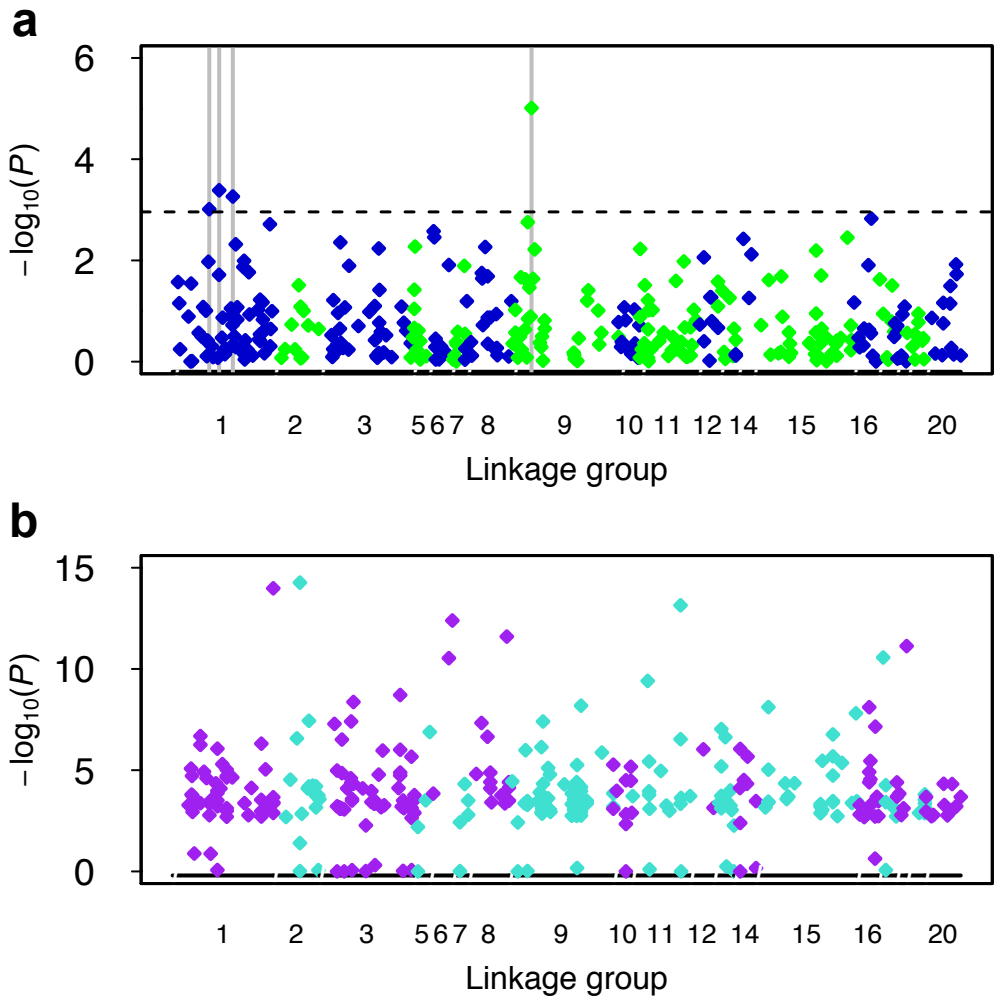

Figure 3.5. Mapping of statistical associations between SNP genotypes and bleaching responses on the genetic linkage map identifies genomic regions associated with thermal tolerance in *A. elegantissima* and differentially expressed genes. a) Genome wide association study reveals 4 significant SNPs. Symbols represent individual genetic markers, and markers on adjacent linkage groups are represented by alternating colors. The dashed line indicates the significance threshold (FDR<0.05). b) Differentially expressed genes mapped onto the linkage map by linkage group, which are represented by alternating colors.

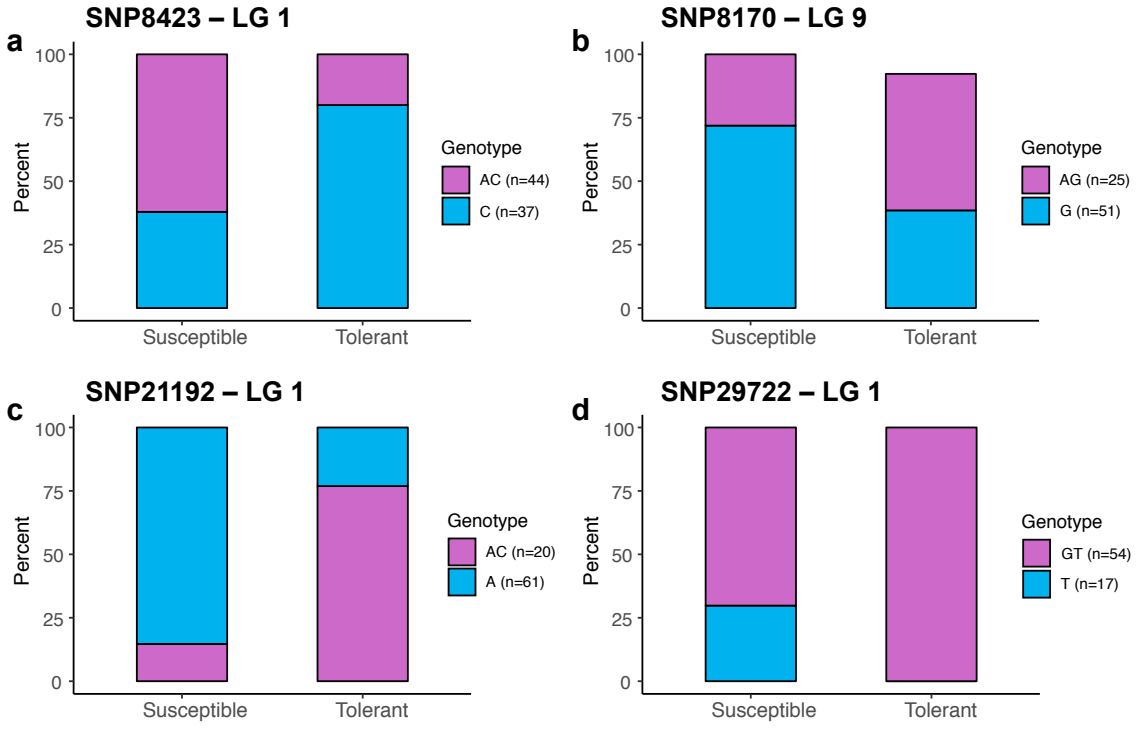

Figure 3.6. Comparing the genotypes of our four significant SNPs to examine heterozygosity across heat-susceptible and heat-tolerant anemones. Two of the SNPs on LG 1 (c and d) showed greater heterozygotes in the tolerant phenotype, as well as the SNP on LG 9 (b).

Table 3.1. Summary of sequencing yields, processing, and mapping efficiencies for RNASeq sequencing libraries.

No. samples	48
No. biological replicates	1
Raw sequencing depth (millions)	218
HQ sequencing depth (millions)	210
HQ reads per sample	4.4
Mapping efficiency	82.1%

Table 3.2. Summary of sequencing yields, processing, and mapping efficiencies for 2bRAD sequencing libraries.

No. samples	63
Raw sequencing depth (millions)	320
HQ sequencing depth (millions)	314
HQ reads per sample (millions)	4.9
Mb genotyped (>5x coverage)	>700 kb
Putative polymorphisms	41,148
SNPs (>10x coverage)	8,966

Table 3.3. Genes differentially expressed when testing for the interaction effect (anemone aggregation × treatment), aggregation effect only in heat stress samples, and genes showing varying patterns of expression in heat-susceptible (HS) vs. heat-tolerant (HT) anemones. Genes presented here are annotated, which is a subset of all 588 DEGs. Unannotated genes can be found in Appendix Table B1.

Effect	Transcript Name	Gene Description	pvalue
Interaction	comp11602_c0	3-dehydroquinate_synthase/O-methyltransferase_fusion_	8.13E-05
	comp74387_c0	5'_nucleotidase_	6.28E-05
	comp62699_c0	Androglobin_(Fragment)_	5.22E-05
	comp10156_c0	Ankyrin_repeat_protein	3.18E-04
	comp54483_c0	Ankyrin-3_	6.20E-05
	comp90860_c0	Axonemal_dynein_light_chain_domain-containing_protein_1_	4.30E-04
	comp8532_c0	cAMP-responsive_element-binding_protein-like_2_	1.02E-04
	comp7398_c0	Carbonic_anhydrase_	1.55E-08
	comp10843_c1	Cast_multi-domain_protein_	2.60E-17
	comp37523_c0	cDNA_FLJ61470	9.82E-05
	comp7131_c0	Conserved_protein_	1.73E-06
	comp6533_c0	Cytadherence_high_molecular_weight_protein_2_	3.99E-02
	comp19172_c0	DBH-like_monooxygenase_protein_1_homolog_(Fragment)_	9.28E-04
	comp8149_c0	E3_ubiquitin-protein_ligase_DZIP3_	1.72E-03
	comp11395_c0	EGF-like_domain-containing_protein_	3.69E-05
	comp602_c0	Endoglucanase_	3.76E-17
	comp38015_c0	Epididymal_secretory_protein_E1_	3.63E-08
	comp4167_c2	Fibroblast_growth_factor_receptor_c_(Fragment)_	8.84E-04
	comp206062_c0	Forkhead_box_protein_j3_	4.58E-05
	comp1090_c1	G_protein_coupled_receptor_98-like_protein_	5.33E-06
	comp751_c0	GCC2_and_GCC3_domain-containing_protein_	4.55E-03
	comp2613_c0	Green_fluorescent_protein_as(S)FP499_	5.62E-06
	comp59041_c0	Guanylate-binding_protein	1.13E-06
	comp38590_c0	Hemicentin-1_	5.98E-04
	comp517_c0	Histone_H1-delta_	3.60E-04
	comp7135_c0	Homeobox_protein_meis	4.60E-05
	comp75426_c0	Hydrocephalus-inducing_protein_	7.92E-05
	comp18910_c0	Janus_kinase_and_microtubule-interacting_protein_3_(Fragment)_	4.16E-04
	comp4259_c0	KIF13B_protein_(Fragment)_	2.92E-11

	comp50600_c0	Kinesin_family_member_3A_(Predicted)_	2.72E-04
	comp10832_c0	Kinesin_light_chain-like_protein_	2.83E-05
	comp26451_c0	Kinesin-C_	4.88E-09
	comp19889_c0	Kinesin-related_protein_1_	2.16E-06
	comp125282_c0	Klebsiella_pneumoniae_subsp._rhinoscleromatis_strain_SB3432	1.59E-20
	comp29429_c0	Lebercilin_	8.17E-05
	comp46175_c0	LIM-type_zinc_finger-containing_protein_	8.54E-06
	comp12710_c0	Lipase_family_protein_	1.22E-04
	comp39678_c0	Long-chain-fatty-acid--CoA_ligase_1_	4.15E-04
	comp21218_c0	Low-density_lipoprotein_receptor	4.95E-04
	comp1676_c0	Metalloproteinase_inhibitor_4_	1.09E-05
	comp1168_c0	MGC132398_protein_	7.30E-05
	comp9587_c0	Myol_protein_	7.49E-05
	comp53021_c0	Myosin-IIIb_(Fragment)_	9.33E-08
	comp4555_c0	Nerve_growth_factor_receptor-like_protein_(Fragment)_	1.10E-04
	comp7653_c0	Neurexin_IV	2.76E-04
	comp103751_c0	Non-ribosomal_peptide_synthase_	1.84E-05
	comp24888_c0	Ojoplano_variant_B_	1.04E-14
	comp173205_c0	Patched_1_(Fragment)_	2.32E-05
	comp93052_c0	Poly_[ADP-ribose]_polymerase_14_	1.40E-05
	comp13680_c0	Polyadenylate-binding_protein_2_(Fragment)_	5.08E-04
	comp346_c0	Probable_serine_incorporator_	2.72E-11
	comp7611_c0	Protein_CBG24309_	8.94E-06
	comp41385_c0	Protein_couch_potato_	4.69E-05
	comp42240_c0	Protein_FAM184A_isoform_1_(Fragment)_	7.90E-06
	comp2424_c0	Protein_FAM46A_	7.19E-04
	comp41638_c0	Putative_n-acetylglucosaminyltransferase_i_	2.91E-04
	comp13359_c2	Putative_rootletin_(Ciliary_rootlet_coiled-coil_protein)_(Fragment)_	4.69E-05
	comp333821_c0	Putative_tick_transposon_(Fragment)_	4.58E-04
	comp35081_c0	S1L_	3.24E-06
	comp126974_c0	Spectrin_beta_chain	3.13E-05
	comp26085_c0	Thioredoxin_domain-containing_protein_3-like_protein_	4.44E-05
	comp5658_c1	THO_complex_subunit_2_	1.91E-04
	comp223_c0	Thrombospondin_type_1_repeat-containing_protein_2_(Precursor)_	5.88E-05
	comp44123_c0	Tolloid-like_protein_1_	6.02E-04
	comp81840_c0	TPR_repeat-containing_protein_	1.46E-06
	comp8007_c0	Trichohyalin	4.73E-05
	comp11455_c0	UDP-N-acteylglucosamine_pyrophosphorylase_1_	7.50E-04

	comp806_c0	Villin_	7.99E-05
	comp64773_c0	Viral_A-type_inclusion_protein	1.07E-06
	comp131869_c0	WD_repeat-containing_protein_52_(Fragment)_	6.87E-04
	comp40399_c0	WD_repeat-containing_protein_60_	2.92E-04
	comp70219_c0	Zgc:153272_	1.42E-05
	comp2241_c0	Zgc:175248_protein_	4.28E-09
	comp74954_c0	Zinc_transporter_6_	5.36E-05
Colony only	comp38846_c0	28S_ribosomal_protein_S24	8.82E-04
	comp152_c0	40S_ribosomal_protein_S9	4.45E-05
	comp234_c0	60S_acidic_ribosomal_protein_P1_	3.62E-04
	comp188_c0	60S_ribosomal_protein_L27_	8.52E-04
	comp38764_c0	Ankyrin_repeat-containing_protein_	8.95E-06
	comp86876_c0	Ankyrin-1_	3.99E-05
	comp119034_c0	Apoptosis-stimulating_of_p53_protein_2_	2.46E-05
	comp7248_c0	Arf-GAP_with_coiled-coil	1.30E-03
	comp74747_c0	ATP-binding_cassette	2.65E-04
	comp26761_c0	Avd_protein_	5.17E-08
	comp60489_c0	Brevican_core_protein_(Fragment)_	1.89E-04
	comp59724_c0	Chromosome_transmission_fidelity_protein_8_homolog_	1.65E-05
	comp225_c0	Cold_shock_domain_protein_	3.36E-05
	comp124266_c0	Collagen_alpha-3(VI)_chain_	1.53E-03
	comp42309_c0	Collagen_alpha-6(VI)_chain_	1.38E-05
	comp178668_c0	Collagen_triple_helix_repeat-containing_protein_	7.66E-09
	comp40995_c0	Conserved_oligomeric_Golgi_complex_subunit_5_isoform_1_(Fragment)_	4.37E-04
	comp7131_c0	Conserved_protein_	1.73E-06
	comp131_c0	Cytochrome_C_	3.51E-06
	comp1806_c0	Cytochrome_c_oxidase_polyprotein_Vb_	1.65E-03
	comp833_c21	Cytochrome_P450_family_17_polypeptide_2_	1.03E-04
	comp1697_c0	Cytochrome_P450_likeTBP_	5.34E-11
	comp1203_c0	Dolichyl-diphosphooligosaccharide-protein_glycosyltransferase_(Fragment)_	1.88E-03
	comp71146_c0	Dysferlin_	2.02E-03
	comp23518_c0	E3_ubiquitin-protein_ligase_CHFR_	4.38E-04
	comp8149_c0	E3_ubiquitin-protein_ligase_DZIP3_	1.72E-03
	comp259_c0	Eef1d_protein_	1.18E-04
	comp890_c0	EF_hand_domain_protein_	1.46E-08
	comp4379_c0	EGF-like_peptide_SHTX-5_	4.40E-05
	comp602_c0	Endoglucanase_	3.76E-17
	comp48204_c0	Endonuclease-reverse_transcriptase_	4.36E-05
	comp21379_c0	EW135_	2.82E-05

	comp46728_c0	Ferredoxin-fold_anticodon-binding_domain-containing_protein_1_homolog_	1.19E-05
	comp2644_c0	Fibroblast_growth_factor_receptor_	4.01E-05
	comp2644_c0	Fibroblast_growth_factor_receptor_	4.01E-05
	comp169862_c0	Gem-associated_protein_6-like_protein_	3.42E-04
	comp101843_c0	GF23793_	1.81E-05
	comp571_c2	Glutathione_synthetase_(Fragment)_	2.80E-08
	comp40858_c0	Glycine_cleavage_system_protein_H_	1.90E-04
	comp4325_c0	Gram-negative_bacteria-binding_protein_	3.89E-10
	comp2613_c0	Green_fluorescent_protein_as(S)FP499_	5.62E-06
	comp292_c0	Green_fluorescent_protein_as(S)FP499_	7.81E-04
	comp50221_c0	HEAT_repeat-containing_protein_2_	2.06E-04
	comp74094_c0	Hemagglutinin/amebocyte_aggregation_factor_	3.87E-04
	comp253_c3	Heme_binding_protein_	2.06E-03
	comp175_c0	Heme-binding_protein_1_	1.98E-04
	comp967_c0	Hexokinase_type_2_	1.88E-03
	comp118252_c0	Histone_acetyltransferase_MYST2_	5.30E-06
	comp18910_c0	Janus_kinase_and_microtubule-interacting_protein_3_(Fragment)_	4.16E-04
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	comp125486_c0	LOC398523_protein_(Fragment)_	5.35E-06
	comp13313_c0	LOC733325_protein_(Fragment)_	1.97E-03
	comp98501_c0	M-phase_phosphoprotein_6_	8.15E-04
	comp31180_c0	Map3k7_protein_	2.93E-04
	comp145810_c0	Matrix_metalloproteinase-9_(Fragment)_	2.37E-09
	comp49808_c0	Mature_parasite-infected_erythrocyte_surface_antigen_	1.54E-03
	comp36265_c0	MGC78867_protein_	7.27E-05
	comp44555_c0	MGC83526_protein_	3.93E-08
	comp791_c0	Minicollagen_4_	1.95E-05
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	comp1136_c0	Mytimacin-1_	5.68E-04
	comp43755_c0	NADPHdependent_FMN_and_FAD_containing_oxidoreductase-like_protein_	1.58E-03
	comp156933_c0	NFX1-type_zinc_finger-	7.23E-04

		containing_protein_1_(Fragment)_	
	comp235618_c0	NFX1-type_zinc_finger-containing_protein_1_(Fragment)_	6.75E-04
	comp41182_c0	Nonfibrillar_collagen_	4.49E-04
	comp10480_c0	Nucleoside_diphosphate_kinase_	3.55E-05
	comp646_c0	Nucleoside_diphosphate_kinase_	1.99E-03
	comp70729_c0	ORF2-encoded_protein_(Fragment)_	1.00E-03
	comp70764_c0	PDZ_domain-containing_RING_finger_protein_3_	3.20E-04
	comp32083_c0	PHD_finger_protein_3_(Fragment)_	6.47E-06
	comp15635_c0	Phospholipase_A2_isozymes_PA3A/PA3B/PA5_	2.00E-04
	comp140501_c0	Pkd2	2.45E-04
	comp10946_c0	PNPLA3_(Fragment)_	3.51E-04
	comp93052_c0	Poly_[ADP-ribose]_polymerase_14_	1.40E-05
	comp39795_c0	Potassium_channel_protein_(Fragment)_	1.23E-03
	comp592_c0	Potassium_channel_toxin_AETX_K_	5.38E-09
	comp32094_c0	Probable_ATP-dependent_RNA_helicase_DDX31_(Fragment)_	1.93E-03
	comp39243_c0	Probable_extracellular_nuclease_	4.30E-04
	comp20435_c0	Proteasome_subunit_beta_type_	1.22E-03
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	comp19739_c0	Protein_CBR-NAS-13_	2.02E-04
	comp5651_c0	Protein_ETHE1_	7.65E-04
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	comp11462_c0	Putative_tick_transposon_(Fragment)_	1.17E-03
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	comp393_c0	Putative_ubiquitin_C_variant_10_	8.04E-04
	comp78667_c0	Ras-related_protein_Rab-27A_	9.15E-05
	comp1722_c1	RBL2_protein_	1.95E-05
	comp110919_c0	Reverse_transcriptase	1.71E-07
	comp17306_c0	Rhamnospondin-2_(Fragment)_	6.94E-05
	comp1405_c1	Ribosome_biogenesis_protein_NSA2-like_protein_	1.91E-04
	comp3152_c0	Robo3_(Fragment)_	4.63E-04
	comp696_c0	RRNA_intron-encoded_homing_endonuclease_	5.12E-21

	comp215659_c0	Serine_acetyltransferase_	3.44E-09
	comp4949_c3	Serine_acetyltransferase_	2.03E-20
	comp4949_c3	Serine_acetyltransferase_	2.03E-20
	comp28643_c0	Serine_protease_27_	4.06E-13
	comp91864_c0	Sialin	7.97E-05
	comp2841_c0	SIN3-like_protein_A	1.73E-03
	comp30_c0	Small_cysteine-rich_protein_2_	2.11E-04
	comp1259_c0	Small_integral_membrane_protein_14_	1.57E-04
	comp102148_c0	Solute_carrier_family_9	3.86E-04
	comp1033_c0	Spectrin_beta_chain	4.24E-04
	comp9649_c0	Steroid_17-alpha-hydroxylase_	4.23E-05
	comp82294_c0	Strain_CBS138_chromosome_F_complete_sequence_	3.17E-04
	comp48762_c0	Svil_protein_	1.07E-03
	comp20432_c0	Syntaxin-17_	1.67E-03
	comp0_c1	Tar1p_	6.56E-05
	comp16870_c0	Taurine_catabolism_dioxygenase_TauD	6.13E-04
	comp1106_c0	Testican-3_	9.36E-06
	comp123329_c0	Tetratricopeptide_TPR_2_	3.73E-04
	comp24_c0	Toxin_2c2_(Precursor)_	3.15E-04
	comp24_c0	Toxin_2c2_(Precursor)_	3.15E-04
	comp2153_c0	Transcript_antisense_to_ribosomal_rna_protein_	9.11E-10
	comp23609_c0	Transcript_antisense_to_ribosomal_RNA_protein_(Fragment)_	7.95E-05
	comp225381_c0	Transcription_factor_E2F7_	3.13E-04
	comp2151_c12	Tumor_necrosis_factor_receptor-associated_factor_2_	1.66E-03
	comp3064_c1	Two-domain_arginine_kinase_	4.76E-04
	comp2358_c0	U-AITX-Bgr3d_protein_	2.48E-07
	comp5032_c0	U-AITX-Bgr3d_protein_	6.36E-04
	comp8472_c0	U-AITX-Bgr3d_protein_	4.04E-07
	comp2504_c1	U2_small_nuclear_ribonucleoprotein_	2.28E-05
	comp1142_c0	Vigilin-like_protein_(Fragment)_	5.25E-04
	comp116867_c0	Viral_A-type_inclusion_protein_repeat	3.90E-04
	comp37745_c0	Viral_A-type_inclusion_protein	1.32E-05
	comp29161_c0	Viral_A-type_inclusion_protein	5.25E-04
	comp17419_c0	Viral_A-type_inclusion_protein	1.95E-03
	comp21334_c0	WGS_project_CABT00000000_data	1.29E-03
	comp4976_c0	Xin_actin-binding_repeat-containing_protein_1_	1.47E-06
	comp2241_c0	Zgc:175248_protein	4.28E-09
HS vs. HT	comp214081_c0	ATP-binding_cassette_sub-family_C_member_3_(Fragment)_	3.15E-04

	comp1603_c2	BF-DED-NACHT_(Fragment)_	3.41E-02
	comp3944_c0	Casein_kinase_I_isoform_alpha_	7.44E-04
	comp6533_c0	Cytadherence_high_molecular_weight_protein_2_	3.99E-02
	comp13369_c0	Cytosolic_phospholipase_A2_	9.59E-04
	comp166584_c0	Excision_repair_cross-complementing_rodent_repair_deficiency	3.74E-03
	comp751_c0	GCC2_and_GCC3_domain-containing_protein_	4.55E-03
	comp67813_c0	GL15118_	2.34E-04
	comp7872_c0	NVHD115-ANTP_class_homeobox_protein_(Fragment)_	6.37E-03
	comp592_c0	Potassium_channel_toxin_AETX_K_	5.38E-09
	comp5864_c0	Potassium_channel_toxin_BcsTx3_	6.02E-03
	comp3374_c0	Protein_ATF-8_	8.01E-04
	comp5651_c0	Protein_ETHE1_	7.65E-04
	comp133889_c0	Putative_rna-binding_polyribonucleotide_nucleotidyltransferase_(Fragment)_	5.35E-03
	comp83437_c0	Sodium-dependent_phosphate_transporter_2_	5.41E-03
	comp8967_c0	TNF_receptor-associated_factor_6_	1.43E-03
	comp44123_c0	Tolloid-like_protein_1_	6.02E-04
	comp305429_c0	UPF0553_protein_v1g230591_	3.36E-04
	comp21611_c0	VMMP-Lio1_(Fragment)_	3.15E-04

Table 3.4. Genes found highly correlated with thermal tolerance using WGCNA analysis. Genes presented here are annotated, which is a subset of all 162 genes. Unannotated genes can be found in Appendix Table B2.

Transcript Name	Gene Description	Pvalue
comp11530_c0	AAEL007038-PA_	2.98E-03
comp2828_c0	AAEL009795-PA_	1.79E-03
comp8668_c0	Adenosylhomocysteinase_	1.33E-03
comp7297_c0	Alcohol_dehydrogenase_class-3_	3.76E-02
comp1043_c0	Alpha-2-macroglobulin_	8.08E-02
comp72190_c0	Alpha-catulin_(Fragment)_	1.76E-03
comp3644_c0	Antileukoproteinase-like_protein_(Fragment)_	5.75E-03
comp102650_c0	ATP-binding_cassette_sub-family_C_member_3_(Fragment)_	4.71E-02
comp3906_c1	Bic-C_protein_	1.29E-05
comp13519_c0	Calcium_binding_EGF_domain_containing_protein_	7.98E-03
comp81872_c0	Caldesmon	3.92E-02
comp112161_c0	Calumenin_	1.06E-03
comp41071_c0	Cation-dependent_mannose-6-phosphate_receptor_	1.93E-02
comp1963_c0	CD109-like_molecule_	6.60E-06
comp138048_c0	CD63_antigen_	1.62E-04
comp17978_c0	cDNA_FLJ60734	3.41E-05
comp79823_c0	Cell_polarity_protein_alp11_	4.82E-06
comp168037_c0	Cephalosporin_hydroxylase_	3.09E-02
comp42544_c0	CG2264	3.67E-04
comp10853_c0	Cntnap5b_protein_(Fragment)_	6.35E-02
comp92798_c0	Coiled-coil_domain-containing_protein_132_(Fragment)_	3.44E-03
comp3491_c0	COL5A1_collagen_type_V_alpha_1_(Fragment)_	3.48E-05
comp9094_c0	Collagen_alpha-1	1.88E-05
comp1107_c0	Collagen_alpha-1(II)_chain_	8.34E-04
comp2697_c0	Collagen_alpha-1(V)_chain_preproprotein_(Fragment)_	1.30E-04
comp133410_c0	Collagen_alpha-1(XX)_chain_	1.56E-02
comp1477_c1	Collagen_alpha-3(VI)_chain_	3.11E-06
comp14985_c0	Collagen_alpha-5(VI)_chain_(Fragment)_	3.47E-02
comp562_c0	Collagen_alpha-6(VI)_chain_	1.95E-03
comp57665_c0	Collagen_triple_helix_repeat_containing_protein_	3.50E-04
comp483_c0	Collagen_type_I_alpha_1_	4.63E-05
comp56784_c0	Collagen-like_protein_	8.38E-04
comp1834_c0	Collagen	6.91E-04
comp44396_c0	Complement_component_C3_	6.14E-03
comp23468_c0	CUB_and_sushi_domain-containing_protein_1_	1.05E-01
comp141167_c0	Dynein-1-beta_heavy_chain	4.69E-04

comp259_c0	Eef1d_protein_	8.84E-04
comp57680_c0	EF-hand_domain-containing_protein_C3orf25-like_protein_	1.56E-02
comp29570_c0	EGF_domain-containing_protein_	6.10E-03
comp43708_c0	Endoplasmic_reticulum_aminopeptidase_1_	1.75E-02
comp13244_c0	Endothelin-converting_enzyme_1-like_	1.02E-03
comp2746_c0	Equistatin_(Precursor)_	1.48E-02
comp118870_c0	EW135_	6.96E-02
comp3225_c0	Follistatin-related_protein_1_(Fragment)_	3.32E-05
comp2391_c0	Follistatin	6.96E-05
comp73667_c0	Gamma-2-syntrophin_	3.94E-03
comp85120_c0	Gamma-glutamyltranspeptidase_	5.87E-06
comp173890_c0	GF20726_	1.20E-01
comp14398_c0	GI11576_	1.78E-02
comp192477_c0	GI13049_	1.57E-03
comp30816_c0	Glutamine_amidotransferase_subunit_pdxT_	8.29E-05
comp63691_c0	Glycine_receptor	5.71E-02
comp2152_c0	GP2_THP-like_protein_(Fragment)_	1.16E-05
comp13366_c0	GP2_THP-like_protein_(Fragment)_	7.84E-06
comp175_c1	Heme-binding_protein_1_	2.74E-03
comp1626_c0	Heme-binding_protein_2_	5.79E-05
comp12813_c0	Hemicentin-1_	4.49E-07
comp22218_c0	Leprecan-like_protein_	1.08E-07
comp154182_c0	Lgtn_protein_	5.26E-02
comp19438_c2	Lissencephaly-1_homolog_	8.85E-03
comp1228_c1	LOC100036716_protein_	3.63E-08
comp4328_c1	LOC100036716_protein_	4.15E-04
comp679_c1	LOC100124952_protein_	1.44E-07
comp8825_c0	LOC100158609_protein_	2.51E-03
comp25445_c0	Low_density_lipoprotein_receptor_adapter_protein_1_(Fragment)_	1.45E-02
comp482145_c0	Lysosome_membrane_protein_II_	8.34E-03
comp89652_c0	Macrophage_receptor_	1.79E-03
comp74028_c0	Myol_protein_	2.10E-03
comp9587_c0	Myol_protein_	4.59E-05
comp61954_c0	Myotubularin_	5.70E-05
comp70771_c0	N-acetyl-beta-glucosaminyl-glycoprotein_4-beta-N-acetylgalactosaminyltransferase_1_(Fragment)_	2.87E-02
comp68693_c0	NEDD8-conjugating_enzyme_UBE2F_	1.13E-01
comp41182_c0	Nonfibrillar_collagen_	8.15E-06
comp124512_c0	Nucleolar_protein_14_	6.11E-03
comp21974_c0	Oncoprotein-induced_transcript_3_protein_(Fragment)_	2.99E-04

comp21974_c1	Oncoprotein-induced_transcript_3_protein_(Fragment)_	5.22E-06
comp47717_c0	PF05960_family_protein_	1.15E-03
comp23095_c0	Phosphodiesterase_4D	2.36E-03
comp7348_c0	Polycomb_complex_protein_BMI-1_(Fragment)_	6.29E-04
comp3374_c0	Protein_ATF-8_	9.20E-02
comp37008_c0	Protein_CBR-HIM-4_	9.75E-07
comp25313_c0	Protein_Wnt_(Fragment)_	1.30E-04
comp82540_c0	Protein_Zfp808_	2.08E-04
comp14982_c0	Receptor_protein_tyrosine_phosphatase_LAR_(Fragment)_	1.42E-02
comp37788_c0	Regulator_of_chromosome_condensation_(RCC1)_repeat_domain_containing_protein_	6.50E-02
comp1187_c0	Retinal_dehydrogenase_1_	6.41E-05
comp5251_c0	Secreted_frizzled-related_protein_4_	7.50E-03
comp29206_c0	Si:ch211-125e6.14_protein_	3.15E-04
comp1906_c0	Sushi_	6.80E-06
comp125674_c0	Sushi_domain-containing_protein_(Fragment)_	9.01E-04
comp15130_c0	TAR_DNA-binding_protein_43_	2.06E-02
comp2479_c0	Testican-2_	2.57E-05
comp1894_c0	Thyrotroph_embryonic_factor_	3.90E-03
comp2791_c0	Titin_(Fragment)_	6.63E-03
comp2039_c0	Titin	1.81E-04
comp541_c0	Tropomyosin_alpha-1_chain_	2.25E-03
comp1141_c0	Trypsin_(Fragment)_	3.27E-02
comp72709_c0	UBX_domain-containing_protein_1_	8.13E-03
comp10197_c0	Vacuolar_protein_sorting-associated_protein_35_	1.08E-02
comp3310_c1	Virulence-associated_trimeric_autotransporter_	4.77E-04
comp1941_c1	Vitellogen-2_like_protein_(Fragment)_	3.67E-08
comp3824_c0	Vitellogenin_	7.64E-08
comp25143_c0	WAP_four-disulfide_core_domain_protein_1_(Fragment)_	1.08E-02
comp132026_c0	Zinc_finger_B-box_domain_containing_protein_1_	3.24E-02

References

Baird AH, Bhagooli R, Ralph PJ, Takahashi S (2009) Coral bleaching: the role of the host. *Trends in ecology & evolution*, **24**, 16–20.

Barshis DJ, Stillman JH, Gates RD, Toonen RJ, Smith LW, Birkeland C (2010) Protein expression and genetic structure of the coral Porites lobatain an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity? *Molecular Ecology*, **19**, 1705–1720.

Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR (2013) Genomic basis for coral resilience to climate change. *PNAS*, **110**, 1387–1392.

Baumgarten S, Simakov O, Esherick LY et al. (2015) The genome of Aiptasia , a sea anemone model for coral symbiosis. **112**, 11893–11898.

Bay RA, Palumbi SR (2015) Rapid acclimation ability mediated by transcriptome changes in reef-building corals. *Genome Biology and Evolution*, **7**, 1602–1612.

Bellis ES, Howe DK, Denver DR (2016) Genome-wide polymorphism and signatures of selection in the symbiotic sea anemone Aiptasia. *BMC Genomics*, **17**, 1–14.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society B*, **57**, 289–300.

Bertucci A, Tambutté S (2011) A New Coral Carbonic Anhydrase in Stylophora pistillata. *Marine Biotechnology*, **13**, 992–1002.

Bertucci A, Moya A, Tambutté S, Allemand D, Supuran CT, Zoccola D (2013) Carbonic anhydrases in anthozoan corals — A review. *Bioorganic & Medicinal Chemistry*, **21**, 1437–1450.

Bingham BL, Freytes I, Emery M, Dimond J, Muller-Parker G (2011) Aerial exposure and body temperature of the intertidal sea anemone Anthopleura elegantissima. *Invertebrate Biology*, **130**, 291–301.

Bingham BL, Dimond JL, Bingham BL (2014) Symbiotic state influences life-history strategy of a clonal cnidarian. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 1–8.

Bradley JR, Pober JS (2001) Tumor necrosis factor receptor-associated factors (TRAFs). *Oncogene*, **29**, 6482–6491.

Chang SW, Flynn BP, Ruberti JW, Buehler MJ (2012) Molecular mechanism of force induced stabilization of collagen against enzymatic breakdown. *Biomaterials*, **33**,

3852–3859.

Chapman RW, Mancia A, Beal M et al. (2011) The transcriptomic responses of the eastern oyster, Crassostrea virginica, to environmental conditions. *Molecular Ecology*, **20**, 1431–1449.

Coles SL, Riegl BM (2013) Thermal tolerances of reef corals in the Gulf: A review of the potential for increasing coral survival and adaptation to climate change through assisted translocation. *Marine pollution bulletin*, **72**, 323–332.

Conner JK, Hartl DL (2004) *A Primer of Ecological Genetics*.

Coulson T, Clegg S (2015) Selection on heritable heterozygosity but no response to selection. Why? *bioRxiv*, 1–8.

Császár NBM, Ralph PJ, Frankham R, Berkelmans R, van Oppen MJH (2010) Estimating the potential for adaptation of corals to climate warming. *PLoS ONE*, **5**, e9751–e9751.

Damiano JS, Oliveira V, Welsh K, Reed JC (2004) Heterotypic interactions among NACHT domains: implications for regulation of innate immune responses. *Biochemical Journal*, **381**, 213–219.

Davy SK, Allemand D, Weis VM (2012) Cell Biology of Cnidarian-Dinoflagellate Symbiosis. *Microbiology and Molecular Biology Reviews*, **76**, 229–261.

DeSalvo MK, Sunagawa S, Voolstra CR (2010) Transcriptomic responses to heat stress and bleaching in the elkhorn coral Acropora palmata. *Marine Ecology Progress Series*, **402**, 97–113.

Dimond JL, Bingham BL, Muller-Parker G (2011) Seasonal stability of a flexible algal-cnidarian symbiosis in a highly variable temperate environment. *Limnology and Oceanography*, **56**, 2233–2242.

Dixon GB, Bay LK, Matz M V (2014) Bimodal signatures of germline methylation are linked with gene expression plasticity in the coral Acropora millepora. *BMC Genomics*, **15**, 1–11.

Dixon GB, Bay LK, Matz M V (2016) Evolutionary Consequences of DNA Methylation in a Basal Metazoan. *Molecular Biology and Evolution*, **33**, 2285–2293.

Donner SD, Skirving WJ, Little CM, Oppenheimer M, Hoegh-Gulberg O (2005) Global assessment of coral bleaching and required rates of adaptation under climate change. *Global Change Biology*, **11**, 2251–2265.

Downs CA, Fauth JE, Halas JC, Dustan P, Bemiss J, Woodley CM (2002) Oxidative stress and seasonal coral bleaching. *Free Radical Biology and Medicine*, **33**, 533–

554.

Dziedzic KE, Kirk NL, Meyer E A universal Symbiodiniaceae primer for quantitative PCR (qPCR) and its application for symbiont detection. *In prep*.

Dziedzic K, Elder H, Tavalire H, Meyer E (2019) Heritable variation in bleaching responses and its functional genomic basis in reef-building corals (Orbicella faveolata. *Molecular Ecology*, 1–16.

Falconer DS, Mackay TFC (1996) *Introduction to Quantitative Genetics*, 4th edn. Pearson Education.

Fernie AR, Carrari F, Sweetlove LJ (2004) Respiratory metabolism: glycolysis, the TCA cycle and mitochondrial electron transport. *Current Opinion in Plant Biology*, **7**, 254–261.

Ghosh J, Lun CM, Majeske AJ, Sacchi S, Schrankel CS, Smith LC (2011) Invertebrate immune diversity. *Developmental and Comparative Immunology*, **35**, 959–974.

Haas BJ, Papanicolaou A, Yassour M et al. (2013) De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature protocols*, **8**, 1494–512.

Hamdoun AM, Cheney DP, Cherr GN (2003) Phenotypic Plasticity of HSP70 and HSP70 Gene Expression in the Pacific Oyster (Crassostrea gigas): Implications for Thermal Limits and Induction of Thermal Tolerance. *The Biological Bulletin*, **205**, 160–169.

Hansson B, Westerberg L (2002) On the correlation between heterozygosity and fitness. *Molecular Ecology*, **11**, 2467–2474.

Helmuth B, Harley CDG, Halpin PM, Donnell MO, Hofmann GE, Blanchette CA (2002) Climate Change and Latitudinal Patterns of Intertidal Thermal Stress. **298**, 1015–1018.

Hiebert TC, Bingham BL (2012) The effects of symbiotic state on heterotrophic feeding in the temperate sea anemone Anthopleura elegantissima. *Marine Biology*, **159**, 939–950.

Hillyer KE, Tumanov S, Villas-bo S, Davy SK (2016) Metabolite profiling of symbiont and host during thermal stress and bleaching in a model cnidarian – dinoflagellate symbiosis. *Journal of Experimental Biology*, **219**, 516–527.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ et al. (2007) Coral Reefs Under Rapid Climate Change and Ocean Acidification. *Science*, **318**, 1737–1742.

Hofmann GE, Somero GN (1995) Evidence for protein damage at environmental temperatures: seasonal changes in levels of ubiquitin conjugates and HSP70 in the

intertidal mussel Mytilus trossulus. *The Journal of Experimental Biology*, **198**, 1509–1518.

Hughes TP (2003) Climate Change, Human Impacts, and the Resilience of Coral Reefs. *Science*, **301**, 929–933.

Jeno K, Brokordt K (2014) Nutritional status affects the capacity of the snail Concholepas concholepas to synthesize Hsp70 when exposed to stressors associated with tidal regimes in the intertidal zone. *Marine Biology*, **161**, 1039–1049.

Kenkel CD, Bay LK (2017) Novel transcriptome resources for three scleractinian coral species from the Indo-Pacific. *GigaScience*, **6**, 1–4.

Kenkel CD, Matz M V (2016) Gene expression plasticity as a mechanism of coral adaptation to a variable environment. *Nature Publishing Group*, **1**, 1–6.

Kenkel CD, Meyer E, Matz M V. (2013) Gene expression under chronic heat stress in populations of the mustard hill coral (Porites astreoides) from different thermal environments. *Molecular Ecology*, **22**, 4322–4334.

Kim B, Rhee J, Jeong C, Soo J, Soo G, Lee Y, Lee J (2014) Heavy metals induce oxidative stress and trigger oxidative stress-mediated heat shock protein (hsp) modulation in the intertidal copepod Tigriopus japonicus. *Comparative Biochemistry and Physiology, Part C*, **166**, 65–74.

Kitchen SA, Crowder CM, Poole AZ, Weis VM, Meyer E (2015) De Novo Assembly and Characterization of Four Anthozoan (Phylum Cnidaria) Transcriptomes. *G3: Genes, Genomes, Genetics*, **5**, 2441–2452.

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**, 223–224.

Langfelder P, Horvath S (2008) WGCNA : an R package for weighted correlation network analysis. *BMC Bioinformatics*, **9**, 1–13.

Langfelder P, Horvath S (2014) Tutorials for the WGCNA Package. *UCLA*.

Lee HK, Braynen W, Keshav K, Pavlidis P (2005) ErmineJ : Tool for functional analysis of gene expression data sets. *BMC Bioinformatics*, **6**, 1–8.

Leggat WW, Seneca FF, Wasmund KK, Ukani LL, Yellowlees DD, Ainsworth TDTD (2011) Differential responses of the coral host and their algal symbiont to thermal stress. *PLoS ONE*, **6**, e26687.

Li Y, Liew YJ, Cui G et al. (2018) DNA methylation regulates transcriptional homeostasis of algal endosymbiosis in the coral model Aiptasia. *Science Advances*,

4, 1–11.

Lynch M, Walsh B (1998) *Genetics and Analysis of Quantitative Traits*. Sinauer Sunderland, MA, 980 pp.

Macrander JC, Dimond JL, Bingham BL, Reitzel AM (2018) Marine Genomics Transcriptome sequencing and characterization of Symbiodinium muscatinei and Elliptochloris marina , symbionts found within the aggregating sea anemone Anthopleura elegantissima. *Marine Genomics*, **37**, 82–91.

Mansfield KM, Carter NM, Nguyen L et al. (2017) Transcription factor NF-κB is modulated by symbiotic status in a sea anemone model of cnidarian bleaching. *Scientific Reports*, **7**, 1–14.

Maor-Landaw K, Levy O (2016) Gene expression profiles during short-term heat stress; branching *vs.* massive Scleractinian corals of the Red Sea. *PeerJ*, **4**, e1814.

Matthews JL, Crowder CM, Oakley CA et al. (2017) Optimal nutrient exchange and immune responses operate in partner specificity in the cnidarian-dinoflagellate symbiosis. *Proceedings of the National Academy of Sciences*, **114**, 201710733.

Meyer E, Davies S, Wang S, Willis BL, Abrego D (2009) Genetic variation in responses to a settlement cue and elevated temperature in the reef-building coral Acropora millepora. *Mar Ecol Prog Ser*, **392**, 81–92.

Mitton JB (1993) Theory and Data Pertinent to the Relationship between Heterozygosity and Fitness. In: *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives*, pp. 17–41.

Mitton JB (1997) *Selection in Natural Populations*.

Mousseau TA, Ritland K, Heath DD (1998) A novel method for estimating heritability using molecular markers. **80**, 218–224.

Moya A, Ganot P, Furla P, Sabourault C (2012) The transcriptomic response to thermal stress is immediate, transient and potentiated by ultraviolet radiation in the sea anemone Anemonia viridis. *Molecular Ecology*, **21**, 1158–1174.

Muir P, Frasier T (2015) Related : an R package for analysing pairwise relatedness from codominant molecular markers related : an R package for analysing pairwise relatedness from codominant molecular markers. *Molecular Ecology Resources*, **15**, 557–561.

Muller-Parker G, Davy SK (2001) Temperate and tropical algal-sea anemone symbioses. *Invertebrate Biology*, **120**, 104–123.

Muller-Parker G, Pierce-Cravens J, Bingham BL (2007) Broad thermal Tolerance of the

Symbiotic Dinoflagellate Symbiodinium muscatinei (Dinophyta) in the Sea Anemone Anthopleura elegantissima (Cnidaria) from Northern Latitudes. *Journal of Phycology*, **43**, 25–31.

Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews*, **85**, 935–956.

Nicholas H. Putnam, Mansi Srivastava, Uffe Hellsten et al. (2007) Sea Anemone Genome Reveals Ancestral Eumetazoan Gene Repertoire and Genomic Organization. *Science*, **317**, 86.

Oakley CA, Durand E, Wilkinson SP, Peng L, Weis VM, Grossman AR, Davy SK (2017) Thermal Shock Induces Host Proteostasis Disruption and Endoplasmic Reticulum Stress in the Model Symbiotic Cnidarian Aiptasia. *Journal of Proteome Research*, **16**, 2121–2134.

Palumbi SR, Barshis DJ, Traylor-Knowles N, Bay RA (2014) Mechanisms of reef coral resistance to future climate change. *Science*, **344**, 895–898.

Polato NR, Voolstra CR, Schnetzer J et al. (2010) Location-specific responses to thermal stress in larvae of the reef-building coral Montastraea faveolata. *PLoS ONE*, **5**, e11221–e11221.

Putnam HM, Davidson JM, Gates RD (2016) Ocean acidification influences host DNA methylation and phenotypic plasticity in environmentally susceptible corals. *Evolutionary Applications*, **9**, 1165–1178.

Queller DC, Goodnight KF (1989) Estimating Relatedness Using Genetic Markers. *Evolution*, **43**, 258–275.

Quistad SD, Stotland A, Barott KL et al. (2014) Evolution of TNF-induced apoptosis reveals 550 My of functional conservation. *Proceedings of the National Academy of Sciences*, **111**, 9567–9572.

Rast JP, Messier-Solek C (2008) Marine Invertebrate Genome Sequences and Our. *Biological Bulletin*, **214**, 274–283.

Reitzel AM, Sullivan JC, Traylor-knowles N, Finnerty JR (2010) Genomic Survey of Candidate Stress-Response Genes in the Estuarine Anemone Nematostella vectensis. *Genomics*, **214**, 233–254.

Reynolds WS, Schwarz JA, Weis VM (2000) Symbiosis-enhanced gene expression in cnidarian-algal associations: cloning and characterization of a cDNA, sym32, encoding a possible cell adhesion protein. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **126**, 33–44.

Richier S, Rodriguez-Lanetty M, Schnitzler CE, Weis VM (2008) Response of the

symbiotic cnidarian Anthopleura elegantissima transcriptome to temperature and UV increase. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, **3**, 283–289.

Rodriguez-Lanetty, Phillips WS, Weis VM (2006) Transcriptome analysis of a cnidarian-dinoflagellate mutualism reveals complex modulation of host gene expression. *BMC Genomics*, **7**, 23.

Ruiz-Jones LJ, Palumbi SR (2017) Tidal heat pulses on a reef trigger a fine-tuned transcriptional response in corals to maintain homeostasis. *Science Advances*, **3**, 1–10.

Rumble SM, Lacroute P, Dalca A V., Fiume M, Sidow A, Brudno M (2009) SHRiMP: Accurate mapping of short color-space reads. *PLoS Computational Biology*, **5**, 1–11.

Schwarz JA, Weis VM (2003) Localization of a Symbiosis-Related Protein, Sym32, in the Anthopleura elegantissima-Symbiodinium muscatinei Association. *Biological Bulletin*, **205**, 339–350.

Schwarz JA, Brokstein PB, Voolstra CR et al. (2008) Coral Life History and Symbiosis: functional genomic resources for two reef building Caribbean corals, Acropora palmata and Montastraea faveolata. *BMC Genomics*, **9**, 97.

Sellis D, Callahan BJ, Petrov DA, Messer PW (2011) Heterozygote advantage as a natural consequence of adaptation in diploids. *Proceedings of the National Academy of Sciences*, **108**, 20666–20671.

Sellis D, Kvitek DJ, Dunn B, Sherlock G, Petrov DA (2016) Heterozygote advantage is a common outcome of adaptation in Saccharomyces cerevisiae. *Genetics*, **203**, 1401–1413.

Shen H-M, Pervaiz S (2006) TNF receptor superfamily-induced cell death: redox-dependent execution. *The FASEB Journal*, **20**, 1589–1598.

Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, Fujie M (2011) Using the Acropora digitifera genome to understand coral responses to environmental change. *Nature*, **476**, 320–323.

Snelling J, Dziedzic K, Guermond S, Meyer E (2017) Integrating genomic resources for a threatened Caribbean coral (Orbicella faveolata) using a genetic linkage map developed from individual larval genotypes. *bioRxiv*, **2925759**, 1–40.

Snyder MJ, Rossi S (2004) Stress protein (HSP70 family) expression in intertidal benthic organisms: the example of Anthopleura elegantissima (Cnidaria: Anthozoa)*. *Scientia Marina*, **68**, 155–162.

Stanton-Geddes J, Yoder JB, Briskine R, Young ND, Tiffin P (2013) Estimating

heritability using genomic data. *Methods in Ecology and Evolution*, **4**, 1151–1158.

Stewart ZK, Pavasovic A, Hock DH, Prentis PJ (2017) Transcriptomic investigation of wound healing and regeneration in the cnidarian Calliactis polypus. *Scientific Reports*, **7**, 41458.

Storey JD (2003) The positive false discovery rate: A Bayesian interpretation and the q-value. *Annals of Statistics*, **31**, 2013–2035.

Tavalire HF, Beechler BR, Buss PE et al. (2018) Context-dependent costs and benefits of tuberculosis resistance traits in a wild mammalian host. *Ecology and Evolution*, **8**, 12712–12726.

Thomas L, Rose NH, Bay RA, López EH, Morikawa MK, Ruiz-Jones L, Palumbi SR (2018) Mechanisms of Thermal Tolerance in Reef-Building Corals across a Fine-Grained Environmental Mosaic: Lessons from Ofu, American Samoa. *Frontiers in Marine Science*, **4**, 1–14.

Tomanek L, Sanford E (2003) Heat-Shock Protein 70 (Hsp70) as a Biochemical Stress Indicator : an Experimental Field Test in Two Congeneric Intertidal Gastropods (Genus: Tegula). *Biological Bulletin*, **205**, 276–284.

Traylor-Knowles N, Rose NH, Palumbi SR (2017) The cell specificity of gene expression in the response to heat stress in corals. *The Journal of Experimental Biology*, **220**, 1837–1845.

Visscher PM, Hill WG, Wray NR (2008) Heritability in the genomics era — concepts and misconceptions. *Nature Reviews Genetics*, **9**, 255–266.

Wang S, Zhang L, Meyer E, Matz M V (2009) Construction of a high-resolution genetic linkage map and comparative genome analysis for the reef-building coral Acropora millepora. *Genome biology*, **10**, R126.

Wang S, Meyer E, McKay JK, Matz M V (2012) 2b-RAD: a simple and flexible method for genome-wide genotyping. *Nature Methods*, **9**, 808–810.

Weis VM (1991) The Induction of Carbonic Anhydrase in the Symbiotic Sea Anemone Aiptasia pulchella. *Biological Bulletin*, **180**, 496–504.

Weis VM (2008) Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *The Journal of experimental biology*, **211**, 3059–3066.

Weis VM, Reynolds WS (1999) Carbonic Anhydrase Expression and Synthesis in the Sea Anemone Anthopleura elegantissima Are Enhanced by the Presence of Dinoflagellate Symbionts. *Physiological and Biochemical Zoology*, **72**, 307–316.

Weis VM, Davy SK, Hoegh-Guldberg O, Rodriguez-Lanetty M, Pringle JR (2008) Cell

biology in model systems as the key to understanding corals. *Trends in Ecology and Evolution*, **23**, 369–376.

Welchman RL, Gordon C, Mayer RJ (2005) Ubiquitin and ubiquitin-like proteins as multifunctional signals. *Nature Reviews Molecular Cell Biology*, **6**, 599–609.

Weston AJ, Dunlap WC, Beltran VH, Starcevic A, Hranueli D, Ward M, Long PF (2015) Proteomics Links the Redox State to Calcium Signaling During Bleaching of the Scleractinian Coral Acropora microphthalma on Exposure to High Solar Irradiance and Thermal Stress . *Molecular & Cellular Proteomics*, **14**, 585–595.

Westram AM, Rafajlović M, Chaube P et al. (2018) Clines on the seashore: The genomic architecture underlying rapid divergence in the face of gene flow. *Evolution Letters*, **2**, 297–309.

Wright RM, Kenkel CD, Dunn CE, Shilling EN, Bay LK, 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**, 1–13.

Zhao JH (2007) gap: Genetic Analysis Package. *Journal of Statistical Software*, **23**, 1–18.

Zhou Z, Zhang G, Chen G et al. (2017) Elevated ammonium reduces the negative effect of heat stress on the stony coral Pocillopora damicornis. *Marine Pollution Bulletin*, **118**, 319–327.

CHAPTER 4 – A comparative study of variation in thermal acclimation and its functional basis in reef-building corals

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Abstract

To survive the predicted and ongoing increases in ocean temperature, reef-building corals will need to develop greater thermal tolerance than observed in most extant populations. These changes could occur over generations through adaptation, or more rapidly through thermal acclimation. Despite the potential importance of acclimation for short-term biological responses to ocean warming, few studies have compared these effects across coral taxa to evaluate the generality of these effects and their functional basis. To address this gap, we conducted a series of laboratory experiments and measured changes in gene expression and algal symbiont profiles. Our study compared thermal acclimation capacity and stress responses across eight coral taxa (*Acanthastrea, Acropora, Favia, Galaxea, Hydnophora, Pocillopora, Porites,* and *Turbinaria*). This design includes a diversity of morphologies nested within each of the major clades of coral diversity (Robust and Complex). To measure variation in thermal tolerance and capacity for thermal acclimation, we subjected fragments from multiple colonies of each taxon to a replicated series of thermal acclimation treatments (24.5, 27, and 30°C), followed by a thermal stress treatment (32°C). We measured the effects of thermal stress as a reduction in algal symbionts following thermal stress treatments, using fluorescence microscopy. To evaluate capacity for acclimation within each taxon, we tested for effects of acclimation temperatures on final symbiont densities after thermal stress. We profiled gene expression following acclimation to investigate the functional basis for variation in the capacity for thermal acclimation. For this analysis, we developed annotated reference transcriptome assemblies for six coral taxa by sequencing normalized cDNA libraries (references were already available for the other two). We also profiled algal symbiont communities in each coral to investigate changes in symbiont communities during acclimation that may contribute to subsequent changes in thermal tolerance of the holobiont (coral host plus its associated symbionts). Together, these measurements reveal substantial variation in the capacity for thermal acclimation across coral taxa, and identify patterns of gene expression and symbiont communities that may contribute to this variation. Our findings highlight that while thermal acclimation may buffer corals against the effects of ocean warming in the short-term, these effects vary

widely across taxa. Further studies of this variation are needed to clarify the contribution of thermal acclimation to the biological responses of corals to ongoing ocean warming.

Introduction

Coral reef ecosystems are now threatened on a global scale. Within the last decade, corals have declined more than 50% in some areas due to annual global mass bleaching events (Hughes *et al.*, 2017, 2018a, 2018b). With sea surface temperatures predicted to rise 1-2°C by the end of the century, coral reefs will need to develop enhanced stress tolerance to ensure their future survival. Currently corals exhibit a great deal of biological variation in bleaching responses, with some individuals or species displaying tolerance to bleaching whereas their neighbors bleach completely (Hughes *et al.*, 2017). While we see diversity in bleaching susceptibility between populations (Guest *et al.*, 2012), species (Marshall & Baird, 2000; Loya *et al.*, 2001; Van Woesik *et al.*, 2011), and regions (Ulstrup *et al.*, 2006; Sully *et al.*, 2019), we still know very little about the mechanisms that are causing this variation.

Adaptive responses to selection take multiple generations, while acclimation occurs within an individual's lifetime. This factor may be especially important (relative to adaptation) in corals because of their long lifespans and generation times. Thermal acclimation, an increase in thermal tolerance resulting from exposure to slightly elevated temperatures, offers a potential route for increased thermal tolerance in corals. Acclimation can occur more rapidly than adaptation, and therefore has been widely viewed as an important component of biological responses to a changing climate (Bay *et al.*, 2013; Palumbi *et al.*, 2014; Putnam & Gates, 2015). For example, colonies of the coral *Acropora millepora* naturally exposed to increased temperatures during daily low tides are more thermally tolerant compared to individuals in more stable regimes, showing the capacity for acclimation (Oliver & Palumbi, 2011; Barshis *et al.*, 2018). Consistent with these field studies, laboratory-based acclimation studies on *Acropora millepora* and *A. nana* demonstrate increased thermal tolerance associated with changes in transcriptional profiles over a 7-11 day acclimation period (Bellantuono *et al.*, 2012; Bay & Palumbi, 2015).

The mechanisms underlying acclimation appear to include multiple factors within both the coral host and algal symbionts, such as changes in gene expression or shifts in symbiont composition. For instance, genes upregulated during a thermal acclimation period prior to heat stress may allow corals to retain symbiont levels at higher rates (Voolstra *et al.*, 2009; Seneca *et al.*, 2010; Bellantuono *et al.*, 2012; Kenkel *et al.*, 2013; Louis *et al.*, 2017). Specifically, up-regulating genes associated with apoptosis, oxidative stress, heat shock, and unfolded protein response, including genes involved in ubiquitination, demonstrates potential mechanisms and genetic basis for acclimation capacity (Barshis *et al.*, 2010; Bellantuono *et al.*, 2012; Bay & Palumbi, 2015; Seneca & Palumbi, 2015). Additionally, associations with certain symbionts may confer tolerance through increased photosynthetic efficiency or decreased perturbations during prolonged stress conditions (Baker *et al.*, 2004; LaJeunesse *et al.*, 2004; Van Oppen *et al.*, 2005; Jones *et al.*, 2008; Cunning *et al.*, 2015a, 2015b).

Bleaching responses have been measured in a variety of ways across the coral holobiont (coral host plus its associated symbionts). To understand contributions of the coral to these bleaching phenotypes, gene expression profiling has become widely used for studies of stress responses and thermal tolerance across taxa. For non-model organisms, transcriptomics is a cost-effective technique to repeatedly sample individuals across multiple time points and multiple experimental conditions to discover and understand functional processes taking place at the molecular level (Wang *et al.*, 2009; Meyer & Manahan, 2010; Conaco *et al.*, 2012; Riesgo *et al.*, 2012). Until recently, few genomic resources have been available for scleractinians corals, limiting the use of genomic tools for the study of how coral reefs will survive ongoing climate change threats. However, with more transcriptomic and genomic resources becoming available in the last decade (Meyer *et al.*, 2009; Shinzato *et al.*, 2011, 2014; Traylor-Knowles *et al.*, 2011; Baumgarten *et al.*, 2015; Kitchen *et al.*, 2015), studies focusing on the molecular processes driving coral reef acclimation and adaptation in the host and symbiont to changing environmental conditions are becoming more accessible (Meyer *et al.*, 2011; Bayer *et al.*, 2012; Barshis *et al.*, 2013; Granados-Cifuentes *et al.*, 2013; Kenkel *et al.*, 2013; Vidal-Dupiol *et al.*, 2013; Pinzón *et al.*, 2015; Parkinson *et al.*, 2016). These

increased resources are facilitating studies that focus on more diverse scleractinian coral species, and are allowing scientists to compare species and morphological types more effectively.

The symbiont community hosted by corals has been shown to influence the tolerance of the resulting holobiont (Rowan *et al.*, 1997; Jones & Berkelmans, 2010; Cunning *et al.*, 2015b; Silverstein *et al.*, 2015). For instance, corals hosting *Durusdinium trenchii* (formerly ITS2-type D1a) or *Cladocopium sp.* (ITS2-type C3) perform better than those with other algal species such as *Cladocopium goreaui* (ITS2-type C1) (Baker *et al.*, 2004; Hume *et al.*, 2013; Smith *et al.*, 2017; Wham *et al.*, 2017). Temporary changes in the relative abundance of certain species of symbionts (symbiont shuffling) or acquiring new symbiont partners from the environment (symbiont switching) can also influence the tolerance of coral species (Baker, 2001; Boulotte *et al.*, 2016).

Thermal acclimation has been documented in corals and considered as a possible route to short-term increases in tolerance for ocean warming, but variation in these responses among species has not been systematically studied. This contrasts with studies on thermal tolerance, which are the subject of extensive ongoing studies (Bellantuono *et al.*, 2012; Barshis *et al.*, 2013; Bay & Palumbi, 2015; Kenkel & Matz, 2016; Ruiz-Jones & Palumbi, 2017; Thomas *et al.*, 2018). More comparative studies need be performed in order to learn which species have the capacity for thermal acclimation and how these responses change so that we can draw conclusions about similarities and differences between species and examine patterns of common stress response genes in various host-symbiont combinations. Additionally, these comparative studies may suggest that there is no capacity for adaptation within certain species, information that is still useful, especially for management and conservation strategies.

Therefore, to investigate the functional basis for variation in the capacity for thermal acclimation, we performed an integrative analysis across coral taxa. First, we explored variation in thermal acclimation capacity through a comparative study across eight coral taxa with varying phylogenetic clades and morphologies. We quantified the effect of acclimation across three acclimation temperatures as well as overall thermal tolerance within each taxon. We profiled gene expression after acclimation to test for

relationships between the extent to which each gene is regulated during acclimation and the effectiveness of the acclimation response in each coral. To do so, we generated reference transcriptomes for six Indo-Pacific reef-building corals. We also measured changes in symbiont communities across coral samples to investigate the role of algal communities in the capacity of acclimation. Our goals in this study were three-fold: 1) to measure variation in thermal acclimation across taxa, 2) to evaluate whether this variation is associated with phylogenetic position or colony morphology, and 3) to identify contributions of the coral host and algal symbionts to thermal acclimation. Comparing these mechanisms at the level of taxon and across morphologies will allow us to broaden our understanding of gene expression profiles and specific groups of genes conserved across the coral phylogeny, as well as how the holobiont is responding.

Materials and Methods

Sample collection and taxa identification

We selected a set of eight Indo-Pacific coral taxa for this comparative study, based on their colony morphologies and phylogenetic relationships. We designed this study to address two fundamental contrasts: a) morphological – branching versus non-branching colony types; and b) genetic – members of the deeply diverged Robust versus Complex clades (Fukami *et al.*, 2008; Kitahara *et al.*, 2010). For this study, we used multiple (n=3-4) coral colonies from *Acanthastrea, Acropora, Favia, Galaxea, Hydnophora, Pocillopora, Porites,* and *Turbinaria* purchased from a coral wholesaler (Quality Marine, CA) (Figure 4.1a). Approximately twenty small fragments were prepared from each colony and glued to small ceramic tiles. Fragments were maintained in a recirculating research aquarium for at least 8 months before experiments began, to allow recovery after fragmentation and to minimize effects of any variation in their thermal histories. During this recovery phase, corals were kept at a constant ambient temperature of 27°C.

To evaluate the species identities provided by the supplier, we conducted Sanger sequencing of the mitochondrial cytochrome c oxidase 1 (CO1) gene using forward (5’-GGTCAACAAATCATAAAGATATTGG-3’) and reverse (5’-

TAAACTTCAGGGTGACCAAAAAATCA-3') primers (Kitahara *et al.*, 2010). This locus is useful for classification because it has been used to construct a phylogeny of corals that to our knowledge includes the largest number of taxa (Kitahara *et al.*, 2010), and has been widely used to infer evolutionary relationships among other cnidarians and invertebrates (Fukami *et al.*, 2008; Kitahara *et al.*, 2010; Geller *et al.*, 2013; Kayal *et al.*, 2013; Kress *et al.*, 2015). We compared sequences with other known CO1 sequences using BLASTn (e-value $\leq 10^{-5}$) and assigned species names based on the top hit. To classify each specimen, we compared our CO1 sequences with other sequences from the same genus (downloaded form the NCBI database; https://www.ncbi.nlm.nih.gov/), including *Nematostella vectensis* as an outgroup. DNA sequences were aligned using MAFFT v7.402 (Katoh *et al.*, 2002) and phylogenetic trees were constructed using maximum likelihood (ML) in RAxML v8.2.12 (Stamatakis, 2014) with the GTRCAT model and 100 bootstrap replicates (Stamatakis, 2016).

Thermal acclimation and heat stress experimental design

To quantify differences in the thermal acclimation capacity across coral taxa, we conducted a long-term, replicated set of experiments in which corals from each taxon were acclimated at a range of different temperatures, then exposed to a thermal stress treatment. For these experiments, we subjected duplicate fragments from each colony to acclimation temperatures of 24.5, 27, or 30°C for two weeks prior to a heat stress temperature of 32°C (Figure 4.1b). Because this series of experiments was conducted over a relatively long period of time, we conducted each acclimation experiment twice to ensure treatments were not confounded with other factors, such as the treatment room or time of year.

Corals were ramped to their acclimation temperatures at a rate of 1°C per day and heat stress temperatures at a rate of 0.1°C per hour. Coral fragments were then maintained at this temperature until they incurred approximately 4 degree heating weeks (DHW). This metric represents a cumulative measurement of thermal stress, where 1 DHW is equivalent to 1°C above average summer maxima temperatures for 1 week (Liu *et al.*, 2013). We chose 29°C as the average summer maximum temperature, a typical

value in many reef habitats at the time of collection (October 2015). Salinity and pH were monitored daily, and partial water changes (~25%) were conducted every two weeks. Coral fragments were photographed before the start of the experiment using fluorescence microscopy (470 nm excitation, 665 longpass emission filter) to measure symbiont abundance. This non-invasive approach made it possible to measure each fragment repeatedly before and after acclimation, and up to 4DHW in stress. In addition, tissue samples of each individual fragment were taken prior to acclimation (prior to any acclimation temperature exposure), after two weeks in acclimation treatments and at the end of heat stress at 4DHW for further analysis.

Measuring bleaching phenotypes

Symbiont density was quantified using fluorescence micrographs to compare the capacity of acclimation and overall thermal tolerance within and across taxa. First, we examined fluorescence images for all images at all time points (before and post-acclimation, and after heat stress). We used ImageJ version 2.0 to measure the red fluorescence intensity (a measurement of the symbiont cells within the host tissue) at each time point (Bellis & Denver, 2017).

Comparing these fluorescence measurements provided a record of changes in symbiont density within each fragment throughout the experiment. We calculated the symbiont density retained for the period of acclimation and heat stress for each coral taxon. We used these data to compare the thermal tolerance profiles of each taxon, based on the decrease in symbiont density as a function of cumulative thermal stress above the summer maximum of 29°C. To compare capacities for acclimation, we estimated effects of acclimation in each taxon as the effect of acclimation temperature on final symbiont density (after the subsequent thermal stress treatment) for the acclimation temperatures at 24.5 and 30°C. We measured symbiont densities at control (27°C) and took the difference between each acclimation temperature and this control measurement to estimate the effect for each coral taxon (ANOVA statistics $p\text{-value}<0.05$).

RNA extraction and preparation of transcriptome libraries

To compare variation in sequences and expression among homologous genes from each taxon, we developed de novo transcriptome assemblies for six of the taxa in our study. For the other two taxa (*Acropora* and *Pocillopora*), reference transcriptomes were already available (Barshis *et al.*, 2013; Vidal-Dupiol *et al.*, 2013). To maximize the diversity of genes included in each library, coral fragments from each taxon were exposed to different conditions prior to sampling; control (27°C, sampled in the daytime), after 24 hours of heat stress (32°C), after 12 hours of darkness, and after 24 hours in hyper salinity stress (40 ppt). Samples were preserved in RNAlater and total RNA was extracted using the Omega Bio-tek E.Z.N.A. Tissue RNA Kit (Omega Bio-tek, Norcross, GA). Extracted RNA from each treatment was purified by precipitating samples with 4M LiCl, quantified using A_{260}, and then pooled by taxa with equal contributions from each treatment. Pooled RNA was then used to prepare normalized cDNA libraries at (>1ug total RNA per library).

We prepared cDNA libraries by normalizing amplified cDNA to enrich libraries for transcripts expressed at low levels, fragmenting the cDNA, and then repairing and ligating adaptors to build sequencing constructs with sample-specific barcodes (Meyer *et al.*, 2011; Kitchen *et al.*, 2015). First-strand cDNA was synthesized using SuperScript II (Invitrogen, CA) and amplified using Q5 DNA polymerase (New England Biolabs, MA) according to the manufacturer's protocol using oligonucleotides shown in Table 4.1. In order to enrich each library for transcripts expressed at low levels, cDNA was normalized using a double-stranded DNA specific nuclease (DSN) (Evrogen, Russia) for 4 hours at 68°C. Normalized cDNA was amplified using Q5 DNA polymerase (New England Biolabs, MA) according to the manufacturer's protocol and modified oligonucleotides (Table 4.1). Amplified normalized cDNA was purified using E.Z.N.A PCR cleanup Kit (Omega Bio-tek, Norcross, GA) and quantified using a spectrophotometer. The normalized and amplified cDNA was then randomly fragmented using sonication in 10-second bursts for a total of 1 minute. Fragmented cDNA was repaired and tailed and then ligated to modified sequencing adaptors (Table 4.1) using T4 DNA Ligase (New England Biolabs, MA) according to the manufacturer's protocol. Finally, ligation constructs were amplified to introduce sequencing primer binding sites and sample-specific barcodes.

Barcoded libraries were size selected by excising the 350-550 bp fraction from a 2% agarose gel. Samples were combined in equal ratios for multiplex sequencing on Illumina HiSeq 3000 to generate one lane of PE 150-bp reads.

Transcriptome assembly, processing, and functional annotation

Prior to assembly and annotation, we first processed raw DNA sequences to exclude low quality or uninformative reads. These filters removed all reads with Phred scores less than 20 at more than 20 bp, all reads with excessive poly-A tails, and reads matching adaptor sequences (Table 4.1). Additionally, we screened all reads for possible contamination from the algal symbiont (Symbiodiniaceae) as previously described (Kitchen *et al.*, 2015) and removed any matches prior to assembly. The protocol and custom scripts used in this study are available online at GitHub (https://github.com/Eli-Meyer). High-quality filtered reads were then assembled using default settings in Trinity v2.0.2 (Grabherr *et al.*, 2013). After assembly, we again screened all reads for biological contaminants and removed them by following the protocol described in Kitchen *et al.* 2015.

To develop these transcriptomes as references for studies of gene expression, we annotated assembled transcripts by assigning putative gene names and functional categories based on comparisons with online databases. We added gene names and gene ontologies (GO) using a BLASTx search (e-value $\leq 10^{-5}$) using the Uniprot Swiss-Prot database (downloaded May 15, 2018). We identified and annotated organelle sequences using BLAST searches against mitochondrial and rRNA databases for *Acropora tenuis* and *Nematostella vectensis*, respectively (van Oppen *et al.*, 2001; Nicholas H. Putnam *et al.*, 2007). Finally, we annotated transcripts using a BLAST search against the *Acropora digitifera* genome (Shinzato *et al.*, 2011), labeling each transcript with its *A. digitifera* homolog to provide a common framework for comparing gene expression profiles among taxa. All scripts used for this analysis are available on https://github.com/Eli-Meyer.

To evaluate the completeness of our transcriptome assemblies, we made sequence comparisons with CEGMA (core eukaryotic genes) (Parra *et al.*, 2007) and with a cnidarian relative, *Nematostella vectensis* (Nicholas H. Putnam *et al.*, 2007). CEGMA

contains universally conserved genes and therefore is a useful reference for comparison with other systems. Comparing our transcriptomes with a close relative (*N. vectensis* in this case) allows us to include additional shared taxon specific genes not found in CEGMA. First, we used BLASTx to compare our transcriptomes with the CEGMA database to determine conserved genes. Then, we compared our transcriptomes with gene models from *N. vectensis* (bit-score ≥50) to identify orthologs. We calculated the Ortholog Hit Ratio (OHR), a metric ranging from 0 to 1 describing the proportion of each *N. vectensis* gene included in our assembled transcripts (O'Neil *et al.*, 2010).

Profiling gene expression

To measure transcriptional responses to acclimation that may contribute to effects on thermal tolerance, we profiled gene expression in each taxa following acclimation at a range of different acclimation temperatures. We selected one fragment from each colony, across all three acclimation temperatures (~120 samples total). RNA was extracted from each fragment using a phenol-chloroform extraction (Chomczynski & Sacchi, 1987, 2006). To remove PCR inhibitors, RNA was precipitated by adding an equal volume of 8M LiCl and then incubating samples at -80°C for 30min. Sample were centrifuged for 30min at 4°C, the supernatant was removed, and nuclease free water was added to the dried RNA pellet. RNA was quantified using a spectrophotometer.

To profile gene expression following acclimation, we used a cost-effective RNA-seq method previously used in corals (Meyer *et al.*, 2011; Lohman *et al.*, 2016). Samples were individually barcoded and combined in equal ratios for multiplex sequencing. Sequencing was done on Illumina HiSeq 3000 at the Center for Genome Research and Biocomputing (CGRB) at Oregon State University. After sequencing, we processed the raw reads to remove non-template regions introduced during library preparation, and excluded reads with long homopolymer regions (>20bp) and low-quality reads with a Phred score of <30. All filtering steps were conducted as outlined in Kitchen et al., 2015 (scripts are available at https://github.com/Eli-Meyer/rnaseq_utilities). We mapped the high quality reads against the transcriptomes for these taxa (Traylor-Knowles *et al.*, 2011; Barshis *et al.*, 2013) using a short-read aligner software SHRiMP (Rumble *et al.*, 2009).

We then counted unique reads aligning to each gene to produce count data for statistical analysis of gene expression in each sample. In order to facilitate comparisons across taxa, we defined homologous groups of genes by comparison with the *Acropora digitifera* genome. In order to determine these homologous genes, we used BLAST searches (e-value $\leq 10^{-5}$) to compare our transcriptomes with the *A. digitifera* genome to identify the gene that each transcript matches best in this reference genome. For differential expression analysis, we used these homologs to make comparisons across coral taxa.

We tested for differential gene expression using a negative binomial model in the R package 'DESeq2' (Love *et al.*, 2014). Within each taxon, we tested for the relationships between gene expression and acclimation temperature to identify genes responding to thermal acclimation treatments. Our model tested for the effect of treatment (acclimation temperatures of 24.5, 27, and 30°C) on overall bleaching response across the duration of the experiment (symbiont density following heat stress relative to symbiont density prior to acclimation treatments). Differentially expressed genes were identified after multiple test corrections (adjusted p-value <0.1, the default threshold in DESeq2). We conducted hierarchical clustering of expression patterns using the *cutree* function in R (Oksanen, 2010). We compared patterns across acclimation temperature to determine if host gene expression after acclimation was predictive of their overall bleaching responses following heat-stress.

Profiling symbiont communities

To investigate the roles of algal symbiont communities in the capacity for acclimation, we profiled samples using amplicon sequencing, targeting the ITS2 locus commonly used for Symbiodiniaceae classification (Green 2014; Quigley *et al.* 2014). The same specimens were profiled repeatedly, prior to and following acclimation treatments and again following thermal stress treatments to evaluate possible changes in type of symbiont in addition to overall density (see above). We prepared additional ITS2 sequencing libraries for high-throughput sequencing on Illumina MiSeq. We prepared these libraries using forward (5'-TACACGACGCTCTTCCGATCTGAATTGCAGAACTCCGTG-3') and reverse (5'-

ACGTGTGCTCTTCCGATCGGATCCATATGCTTAAGTTCAGCGGGT-3') primers, and sequenced libraries using 250 bp PE read chemistry on Illumina MiSeq at OSU's Center for Genome Research and Biocomputing (CGRB). We filtered reads to exclude any low quality reads (<20), removed reads lacking the expected amplicon primer sequence, and removed orphan reads. Due to technical errors during this sequencing run, this version of the manuscript is based on analysis of only the forward read for all samples. However, additional sequences will be generated to obtain both forward and reverse reads.

To determine which symbiont species were present and their overall densities across acclimation temperature and time, we first clustered reads with ≥97% sequence similarity to determine unique sequence variants. First, we screened for non-Symbiodiniaceae sequences by running BLASTn searches against NCBI's 'nt' database (e-value ≤ 10^{-5}). Next, we curated a database of only symbiont sequences representing species from all major taxa and used BLASTn to identify symbiont species. Additionally, we calculated the density of each symbiont species (number of reads within each cluster). We compared percent abundances of species across all time points and acclimation temperatures to characterize changes in the symbiont community of each species following acclimation and subsequent thermal stress.

Results

Coral identification

To explore the phylogenetic relationship across all eight corals as well as confirm their species identification, we sequenced the COI gene for every coral colony. We were able to assign species names based on the top BLASTx hit (Table 4.2). Using a phylogenetic approach, we found that all genus level sequences grouped together in monophyletic clades and grouped together based on current phylogenetic trees (robust versus complex clades) (Figure 4.2) (Fukami *et al.*, 2008; Kitahara *et al.*, 2010). However, not all samples matched with a single species (e.g. *Hydnophora* colonies were spread across the clade) and some matched equally to more than one species (e.g.

Acropora colonies matched to *A. tenuis* and *A. hyacinthus*). Therefore, we refer to corals and make our comparisons at the level of coral taxon as opposed to specific species.

Effects of acclimation on bleaching responses

To investigate the functional basis for variation in the capacity for corals to thermally acclimate, we took a comparative approach using eight coral taxa spanning the coral phylogenetic tree (Figure 4.1). We acclimated corals at a range of different temperatures prior to a thermal stress treatment to quantify the effects of acclimation on thermal tolerance. We quantified the symbiont density retained during acclimation and heat stress and found substantial variation in bleaching responses (Figure 4.3). Symbiont density changed slightly across all acclimation treatments, but patterns are complex. However, the 30°C acclimation temperature may have caused some cumulative stress prior to heat stress treatments, therefore causing strong decreases in densities (e.g. *Acanthastrea* and *Acropora*). *Porites* and *Turbinaria* had the greatest changes in symbiont densities in heat stress following acclimation at 30°C, whereas the other taxa had either slight decreases in symbiont densities (e.g. *Acanthastrea, Galaxea* and *Pocillopora*) or increases in symbiont densities (e.g. *Favia, Hydnophora,* and *Acropora*) (Figure 4.3).

In order to explore the effect of acclimation, we quantified the difference in symbiont density after heat stress following acclimation at 24.5°C and 30°C relative to control conditions (27°C). We found effects of acclimation across taxa for *Pocillopora* (pvalue=0.004), *Hydnophora* (pvalue=0.02), *Turbinaria* (pvalue=0.07), and *Acanthastrea* (pvalue=0.0003). For all four taxa, acclimation at 24.5°C caused strong negative effects when exposed to heat stress (Figure 4.4). *Pocillopora* and *Hydnophora* performed better during heat stress compared to control (27°C), demonstrating a positive effect of acclimation (Figure 4.4). In contrast, *Turbinaria* had strong negative effects of acclimation, while *Acanthastrea* showed almost no effect of acclimation compared to control samples (Figure 4.4).

In addition, we quantified thermal tolerance of each taxon as the symbiont density as a function of cumulative heat stress over time. We found *Favia, Galaxea, Hydnophora*

and *Porites* taxa to be the most thermally tolerant taxa, retaining more than 75% of the symbionts after 17 days at a temperature greater than 29°C (Figure 4.5). In contrast, *Acanthastrea, Acropora, Pocillopora* and *Turbinaria* were not as tolerant, losing 40-60% of their symbionts after cumulative heat stress. These findings are consistent with previous studies that demonstrate the relative thermal tolerance of these taxa, with the exception of *Acanthastrea* and *Turbinaria*, which have been considered to be more hardy to environmental changes (Loya *et al.*, 2001; Van Woesik *et al.*, 2011; Hoey *et al.*, 2016).

Development of reference transcriptomes

To examine the effects of acclimation on gene expression profiles across all eight taxa, we developed reference transcriptomes, along with two previously published transcriptomes. Sequencing yielded 35.3-82.3 million raw PE reads per library. Assembly produced on average ~190,000 transcripts. An average of 55,122 and 23,184 transcripts were assigned UniProt and GO annotations, respectively (Table 4.2), similar to other published de novo transcriptomes for invertebrate species (Kitchen *et al.* 2015; Kenkel & Bay, 2017). Altogether, assembled transcriptomes ranged in size from 10.1Mb to 48.6Mb. Assemblies included many small contigs (on average, 77% were <500bp), and therefore we removed contigs <500bp for comparison across transcriptomes. These sequences were only removed for sequence comparisons because they were unlikely to provide any significant matches in homology searches (Kitchen *et al.* 2015). However, for profiling transcriptomic responses we included all contigs (see below). The average contig length ranged from 715-826 bp and N_{50} ranged from 715-826 bp across all seven transcriptomes (Table 4.2). These are shorter than the typical transcript length in corals and other metazoans, suggesting these assemblies remain somewhat fragmented. GC content across all transcriptomes ranged from 40.05-42.54%. Finally, sequence comparisons (BLASTx, e-value $\leq 10^{-5}$) with the CEGMA database revealed an average of 76.4% (range of 60.2-91.7%) of these conserved genes across all seven transcriptomes (Table 4.2). The median OHR percent ranged from 47-71.1% with an average of 2,469 transcripts above an OHR of 75% (Table 4.2) across all seven transcriptomes, which is comparable to other estimates for cnidarian (Kitchen *et al.*, 2015; Kenkel & Bay, 2017)

and invertebrate (O'Neil *et al.*, 2010; Riesgo *et al.*, 2012) species.

Comparing effects of acclimation on expression by homologous groups

To further investigate the mechanisms of acclimation in the coral host, we profiled gene expression in all taxa across all acclimation temperatures after the acclimation period. We prepared sequencing libraries for all ~120 fragments (all eight taxa, 3-4 colonies per taxa, one fragment per colony, across all three acclimation temperatures). In total, 175.9 million raw reads were produced. The majority of these passed quality and adaptor filtering (91%) leaving 1.3 million HQ reads per sample for expression analysis (Table 4.3). Due to sequencing errors, we were only able to analyze data for four taxa (*Acanthastrea, Hydnophora, Pocillopora,* and *Turbinaria*). We are working to re-sequence samples to gain additional coverage.

To provide a common frame of reference for gene expression profiles in different coral taxa, we compared gene expression profiles between transcripts in each species that were homologous to the same gene in *Acropora digitifera* genome. We found more than 18,000 homologous genes across our four taxa and combined count data for each homolog to run through differential expression analysis. Using these homologous genes, we analyzed gene expression data to evaluate the effects of acclimation temperatures on gene expression profiles. Our model tested for the effect of acclimation treatment (24.5, 27, and 30°C) on expression in each taxon. After multiple test corrections (adjusted pvalue <0.1), we found 22, 11, 58, and 51 differentially expressed genes (DEGs) in *Acanthastrea*, *Hydnophora*, *Pocillopora*, and *Turbinaria*, respectively. The heatmap for the main effect of acclimation treatment is found in Figure 4.6. Interesting DEGs in *Acanthastrea* included a collagen protein, a heat shock protein, and a serine threonine protein kinase. In *Hydnophora* a dehydrogenase protein was differentially expressed. Interesting DEGs in *Pocillopora* included caspase, methyltransferase, thioredoxin, and NACHT domain proteins. In *Turbinaria*, interesting DEGs included BF-NACHT protein, glutathione S-transferase, two heat shock proteins, and a stress protein. A complete list of annotated homologs differentially expressed in all four taxa is provided in Table 4.4.

To characterize these patterns and search for relationships between transcriptional

responses to acclimation and its effects on stress tolerance, we averaged expression for each gene in each acclimation treatment. We conducted hierarchical clustering of expression patterns using the *cutree* function in R, which categorized gene expression profiles into two dominant patterns for all four taxa. One pattern (blue lines in Figure 4.7) showed lower expression in the high acclimation temperature (30°C), while the second pattern (pink) had higher expression at high acclimation. Interestingly, there were no overlapping genes (i.e. the same genes expressed in multiple taxa) across coral taxa, highlighting a unique set of genes regulated by each taxon. Genes in the blue category included cytochrome c oxidase (*in Acanthastrea, Hydnophora,* and *Turbinaria*), serine threonine protein kinase and a heat shock protein (in *Acanthastrea*), dehydrogenase and BF-NACHT domain protein (in *Turbinaria*), and methyltransferase, thioredoxin, caspase and NACHT domain protein (all in *Pocillopora*). Genes in the pink category included collagen (in *Acanthastrea*), and glutathione s-transferase and heat shock proteins (in *Turbinaria*). A list of genes in each pattern can be found in Table 4.4.

Effects of acclimation and thermal stress on symbiont communities

To identify the dominant symbiont type or mixed symbiont communities in each coral colony, we sequenced ITS2 amplicons using Illumina sequencing. We profiled symbiont communities repeatedly, before and after acclimation and again after heat stress (Figure 4.8). Analysis of these sequences revealed that all coral taxa contained algal symbionts belonging to the *Cladocopium* and *Durusdinium* taxa, while some also contained *Symbiodinium* and *Breviolum* (Figure 4.8). Interestingly, most corals contained mixed symbiont communities, and most symbiont communities changed following acclimation. In corals acclimated at low temperatures, symbiont communities were disrupted by thermal stress treatment. Specifically, there was a change in the dominant symbiont type for two of the three taxa (*Pocillopora* bleached and therefore no ITS sequences were generated) (Figure 4.8a). The relative abundance of *Cladocopium sp.* (ITS2 type C3) increased in *Acanthastrea* and *Turbinaria* after acclimation, but *Cladocopium sp.* increased following heat stress. On the other hand, *Durusdinium trenchii* increased after acclimation and heat stress in *Hydnophora*.

In contrast, symbiont communities at higher acclimation temperatures were less affected by thermal stress, there was no change in dominant type for three of the four taxa acclimated at 27°C and for all taxa acclimated at 30°C (Figure 4.8b and c). In other words, for seven of the eight comparisons, the dominant symbiont type following acclimation at high temperatures remained the dominant symbiont type after thermal stress. At 27 and 30°C, the relative abundance of *Cladocopium sp.* increased following heat stress in *Pocillopora* and *Turbinaria* (Figure 4.8b and c). At 30°C, *Acanthastrea* contained a fairly stable *Cladocopium sp.* (ITS2 type C3) throughout the experiment, but had an increase in *Durusdinium trenchii* after heat stress. *Hydnophora* contained mostly *Durusdinium trenchii* throughout the duration of the experiment (Figure 4.8c).

In Indo-Pacific corals, *Durusdinium trenchii* and *Cladocopium sp*. (ITS2 type C3) have been considered more thermally tolerant than other symbiont species within the same genus. Here, we find examples where these symbionts increase either during thermal acclimation or thermal stress periods. In *Acanthastrea* and *Hydnophora*, *Durusdinium trenchii* and *Cladocopium sp*. (ITS2 type C3) increases within the coral host in the high acclimation temperature, but not necessarily in lower acclimation temperature post-heat stress (Figure 4.8). We find similar patterns between *Pocillopora* and *Turbinaria; Pocillopora* has more *Cladocopium sp.* with a small fraction of *Cladocopium sp.* (ITS2 type C3) after heat stress in the high acclimation temperature, whereas *Turbinaria* has more (ITS2 type C3) to start but switches to *Cladocopium sp*. dominant post-heat stress. More so, the dominant symbiont species after stress was *Cladocopium sp.* for eight of the twelve comparisons. Overall, these findings show thermal acclimation caused remodeling of the symbiont communities in order to become more thermally tolerant. Symbiont communities were dynamic in some taxa, responding both to the acclimation and stress treatments, whereas in others the community remained fairly stable over time (e.g. *Hydnophora* and *Pocillopora* at 30°C).

Discussion

Variation in capacity for thermal acclimation in corals

To our knowledge, this study is the first to systematically investigate variation in thermal acclimation across coral taxa in a comparative context. Here, we build on previous studies showing acclimation in individual species (Bellantuono *et al.*, 2012; Edmunds, 2014; Bay & Palumbi, 2015) by comparing eight taxa from diverse morphologies and phylogenetic backgrounds. Our findings (summarized in Table 4.5) demonstrate variation in both the direction and strength of acclimation effects across coral taxa. Our study demonstrates substantial variation in corals' capacities for thermal acclimation, and highlights mechanisms in the coral host and algal symbionts that may contribute to these effects. Identifying the functional basis for this variation in thermal acclimation will improve our ability to predict these effects in different coral taxa. Understanding this variation may be critical for conservation of coral reef ecosystems, since coral assemblages vary widely among regions, and coral populations are declining globally (McClanahan, 2017; Hughes *et al.*, 2018b).

Our study of thermal acclimation contributes to a growing body of work addressing the potential for corals to survive ongoing ocean warming. Comparisons of the bleaching thresholds of modern coral populations with future temperature scenarios have predicted bleaching events will increase in strength and severity (Donner *et al.*, 2005). Variation in these bleaching thresholds across coral populations and species is expected to lead to the "winners" and "losers" in climate change (Fitt *et al.*, 2001; Loya *et al.*, 2001; Van Woesik *et al.*, 2011). Our comparative analysis of thermal tolerance across coral taxa is generally consistent with previous studies (Bellantuono *et al.*, 2012; Edmunds, 2014; Bay & Palumbi, 2015; Ainsworth *et al.*, 2016; Gibbin *et al.*, 2018). For example, we found high thermal tolerance in *Favia, Galaxea, Hydnophora* and *Porites*, and lower tolerance in *Acropora* and *Pocillopora*, consistent with previous studies (Hoey *et al.*, 2016).

Thermal tolerance variation has been studied within coral species to evaluate the potential for adaptive responses to warming (Császár *et al.*, 2010; Davies *et al.*, 2015; Dixon *et al.*, 2015; Kenkel *et al.*, 2015; Kirk *et al.*, 2018; Dziedzic *et al.*, 2019). These studies of coral adaptation highlight potential routes for coral survival, but because corals have long generation times (>10 years in many species), adaptive responses may be

outpaced by the rate of climate change. Therefore, phenotypic plasticity through acclimation of physiological responses may be an essential mechanism for corals to survive in the short term (Pigliucci, 2006). Previous studies exploring rapid acclimation responses within coral species demonstrates changes in thermal tolerance can occur rapidly (within weeks) (Bellantuono *et al.*, 2012; Bay & Palumbi, 2015). In our study, we contribute to these previous studies by adding information across multiple species and highlight substantial variation in acclimation capacities.

The selection of corals chosen for this study provided contrasting colony morphologies (branching versus non-branching) nested within the two major clades of coral diversity (Robust and Complex). Acclimation temperatures significantly affected thermal tolerance for half of the coral taxa studied, and three of four were corals from the Robust clade. Although at face value this may suggest greater capacity for thermal acclimation in this clade, this comparison should be interpreted with caution, since all corals studied showed a trend toward reduced thermal tolerance in cold-acclimated corals, whether significant or not (Figure 4.7). Overall, our data suggest that the acclimation at low temperatures reduces thermal tolerance generally across corals. Among the taxa significantly affected by acclimation at high temperatures, there was substantial variation in the magnitude of these effects, with only two (*Hydnophora* and *Pocillopora*, both in the Robust clade) showing increased thermal tolerance after acclimation at high temperatures. Again, this pattern is consistent with the overall pattern across all species (Figure 4.7). These patterns suggest a slightly greater capacity for thermal acclimation in the Robust clade.

Similarly, our comparisons revealed interesting patterns suggesting relationships between colony morphology and thermal acclimation. Three out of four branching taxa showed significant effects of thermal acclimation on tolerance, but only one out of four non-branching taxa (Table 4.5). Again, consideration of the temperature range suggests caution for this contrast. All cold-acclimated corals showed a trend toward reduced thermal tolerance, regardless of colony morphology. We observed increased tolerance in warm acclimated corals for 2 out of 4 branched taxa (*Hydnophora* and *Pocillopora*),

while the lone Complex coral (*Turbinaria*) significantly affected by acclimation temperature showed no evidence of increased tolerance after warm acclimation.

Taken together, these findings demonstrate substantial variation among coral's capacity for thermal acclimation, and suggest greater capacity in the Robust clade and in branching colony types. Further studies with greater taxon sampling would be required to further evaluate the generality of these patterns.

Roles of gene expression in thermal acclimation

To explore contributions of the coral host, we profiled gene expression in corals acclimated at different temperatures. Exploring the effects of acclimation temperature on gene expression revealed two dominant patterns of expression (Figure 4.7). The first pattern (blue lines) included genes that were down-regulated at high acclimation temperatures (30°C), whereas the second pattern (pink lines) contained genes that were up-regulated at high temperatures. This comparison revealed that the majority of acclimation effects on gene expression involved down-regulation with increasing temperature (Table 4.5). This finding of dampened gene expression at higher acclimation temperatures is consistent with effects reported in previous studies (Bellantuono *et al.*, 2012; Bay & Palumbi, 2015).

Transcriptome sequencing and gene expression profiling have become important tools for the study of corals' thermal stress responses and thermal tolerance (Császár *et al.*, 2010; Barshis *et al.*, 2013, 2018; Dixon *et al.*, 2015; Kenkel *et al.*, 2015; Seneca & Palumbi, 2015; Kirk *et al.*, 2018; Dziedzic *et al.*, 2019). Our study contributes to this field by producing annotated reference transcriptomes for six additional coral species. These new resources expand the range of coral taxa accessible for transcriptomic studies, and our application of these references demonstrates their utility for profiling gene expression.

We found substantial differences between gene expression profiles in corals acclimated at different temperatures. Most of the genes significantly affected by acclimation temperature were down-regulated with increasing acclimation temperatures (blue series in Figure 4.7). Interesting DEGs in this category included a serine threonine

protein kinase (in *Acanthastrea*), a dehydrogenase protein (in *Hydnophora*), caspase, methyltransferase, thioredoxin, fasciclin transmembrane protein (in *Pocillopora*) and NACHT domain proteins (in *Pocillopora* and *Turbinaria*). Another cluster of DEGs affected by acclimation temperature included genes down-regulated with increasing acclimation temperature. Interesting DEGs in this category included a collagen protein (*Acanthastrea*), glutathione S-transferase, two heat shock proteins, and an HSC (heat shock constitutive) stress protein (*Turbinaria*). Conducting this analysis at the level of genes (rather than anonymous genetic markers) facilitates comparisons among taxa and species, making it possible to search for general mechanisms through which corals achieve thermal acclimation.

Interestingly, we find almost no overlap in specific genes expressed by different coral taxa in response to thermal acclimation. At face value, this finding suggests that transcriptional responses to acclimation are highly species-specific and may not be generalizable across coral taxa. However, functional analysis reveals underlying similarities among these transcriptional responses. For instance, we find chaperone proteins such as heat shock proteins up-regulated during acclimation at high temperatures in two of the four taxa, while NACHT protein domains were down-regulated in two taxa. Enzymes associated with oxidative stress responses, such as caspase, thioredoxin (*Pocillopora*), and, serine threonine protein kinase (*Acanthastrea*) were down-regulated, while glutathione S-transferase (*Turbinaria*) was up-regulated at high temperatures.

Many of the genes identified in our study have been in previous studies of coral acclimation and thermal tolerance, such as peroxidasin, heat shock proteins, a constitutive heat shock protein (HSC70), collagen, methyltransferase, and glutathione s-transferase. Peroxidasin is an extracellular matrix protein and is involved in peroxidase activity within coral host cells (DeSalvo *et al.*, 2008). Heat shock protein 70 and 90 play an important role in refolding proteins that have been denatured due to high temperature. Similar studies in corals and other invertebrates, such as snails, found that heat shock proteins were important in regulating an acclimation response over time (Tomanek & Somero, 2002). While we only find a handful of these genes at this time point, other studies show timing of expression of these genes may be upregulated earlier throughout

acclimation and stress (Tomanek & Somero, 2002; Seneca & Palumbi, 2015). Interestingly, we also found up-regulation of HSC70, a constitutively expressed heat shock protein known to act as first line of defense during heat stress before expression of HSP70, the inducible form (Chong *et al.*, 1998). Previous studies have found both proteins to respond together, where up-regulation in HSC70 corresponded with up-regulation in HSP70, to provide increased tolerance during heat shock-induced stress (Chong *et al.*, 1998; Robbart *et al.*, 2004; Carpenter *et al.*, 2010). Collagen and methyltransferase collagen proteins are important for immune responses such as wound healing and tissue regeneration in invertebrates (Reitzel *et al.*, 2010; Chang *et al.*, 2012; Stewart *et al.*, 2017). Previous studies in corals have shown increased expression of collagen genes in corals during before and after heat stress (Barshis *et al.*, 2013; Bay *et al.*, 2013; Kenkel *et al.*, 2013; Dziedzic *et al.*, 2019). These genes have been repeatedly upregulated in corals, and therefore they may play a mechanistic role not only in acclimation capacity, but overall thermal tolerance in the host. Lastly, glutathione s-transferase is an important enzyme part of the oxidative stress response (Downs *et al.*, 2002). This gene was upregulated in *Turbinaria,* a coral that showed no capacity for thermal acclimation, indicating possible oxidative stress throughout the duration of acclimation and therefore subsequent bleaching in heat stress. While corals are known to have striking differences in transcriptional profiles, we are finding genes involved in oxidative stress response, unfolded protein response, and immune function, consistent with other studies in coral and anemone species. These repeated observations of genes expressed across heat stress experiments calls for more pointed observations of how these genes respond across coral species, as well as if these are genes to target for studies like those using assisted evolution techniques or genome editing or knockdown using CRISPR/Cas9 or RNAi (Dunn *et al.*, 2007; van Oppen *et al.*, 2015, 2017; Chen *et al.*, 2018; Cleves *et al.*, 2018).

Roles of the algal symbiont community in thermal acclimation

Here, we profiled the symbiont community repeatedly to determine if communities changed across time and within each taxon, and whether certain symbiont

species were associated with increased acclimation capacity. We found that symbiont communities changed substantially over the course of the experiment. In the low acclimation treatment (24.5°C), post-acclimation symbiont communities were relatively unstable following heat stress, compared to the high (30°C) acclimation treatment where communities stayed more stable following stress These communities may have allowed corals to remain tolerant over the course of the stress. Specifically, when we compare with the acclimation effects for each of the four taxa, symbiont communities do contribute to the effect. While we see correlations with partner switching and thermal acclimation capacity in some corals, the patterns are complex and cannot be easily generalized.

The thermal tolerance of the coral holobiont can also be strongly influenced by the composition of algal symbiont communities. Past studies exploring Indo-Pacific coral reef species and their algal symbiont partners have shown that *Durusdinium trenchii* and *Cladocopium sp*. (ITS2 type C3) may be more thermally tolerant than other symbiont species within the same genus (Howells *et al.*, 2016). Additionally, corals that "switch" to *D. trenchii*-dominated during and after heat stress do not bleach compared to corals that contain their homologous symbiont types. Despite the benefits of added thermal tolerance, *D. trenchii*-colonized hosts offer reduced nutritional exchange and cause the coral to grow at slower rates (Jones & Berkelmans, 2011; Cunning *et al.*, 2015a; Matthews *et al.*, 2017, 2018). These disparities in optimal nutritional exchange and reduced immune function may not allow these host-symbiont combinations to remain intact long term, and therefore reduce the adaptive capacity of partner switching. These partner switches may, however, offer short-term acclimation and tolerance capacity, allowing the coral host to survive and avoid the negative consequences of bleaching (Boulotte *et al.*, 2016).

Conclusions

Integrating these different datasets for the four taxa in which all are available (*Acanthastrea, Hydnophora, Pocillopora* and *Turbinaria*) reveals interesting patterns (Table 4.5). *Hydnophora* was one of the most thermally tolerant taxon in our analysis

(Figure 4.5) and also had the greatest capacity for thermal acclimation at high temperatures (Figures 4.3 and 4.4). Both host gene expression and algal symbiont communities remained stable in this coral, more so than the other three taxa (Figures 4.6 and 4.8). *Pocillopora* also had a positive acclimation effect and a large number of genes down-regulated at high acclimation temperatures (Figure 4.7), while the symbiont communities stayed relatively constant (Figure 4.8). In contrast, *Turbinaria* showed the least benefit of acclimation at high temperatures (Figure 4.4) and the greatest change in gene expression (Figure 4.7) and symbiont communities in both the low and high temperatures (Figure 4.8). Similarly, *Acanthastrea* showed no effect of acclimation at high temperatures (Figure 4.4) and had dramatic increases in gene expression and symbiont communities in the low and high acclimation temperatures. Additionally, *Acanthastrea* showed the lowest thermal tolerance over time (Figure 4.5). Together, we find that Robust corals had a higher capacity for acclimation and greater thermal tolerance than the Complex Clade. These differences may be attributed to gene expression magnitude as well as the specific genes expressed, as well as the stability or shuffling of symbionts throughout acclimation stress. These results show that acclimation capacity varies greatly across coral taxa and the mechanisms are complex across the host and symbiont.

Overall, our study provides novel information about variation in thermal acclimation across coral taxa. Using a combination of experiments and sequencing techniques, we were able to build on previous studies demonstrating acclimation in individual species by comparing responses across multiple coral taxa. We found genes differentially expressed across acclimation treatments, with genes in the lower acclimation temperatures expressed at a higher magnitude compared to the high acclimation temperature. Genes differentially expressed across taxa highlight oxidative stress responses, immune function, and the unfolded protein response, important mechanisms for dealing with acute heat stress. Additionally, we found changes in symbiont communities that may correlate with thermal tolerance patterns across taxa. Coral reef ecosystems are built and supported by the activities of diverse coral taxa, so predicting their responses to warming requires characterizing variation in current thermal

tolerance, capacity for thermal acclimation, and potential for genetic adaptation. Information on this variation is vital for conservation of coral reefs around the world that are dominated by different assemblages of coral species. Our data suggest that thermal acclimation offers a potential route to enhanced thermal tolerance in the short-term, but only for some coral taxa. In addition, our observation of changes in coral gene expression and symbiont communities during thermal acclimation suggest that both partners contribute to variation in thermal acclimation. These effects differed widely among species, emphasizing that coral taxa differ not only in the extent of thermal acclimation, but in the species mechanisms (genes or symbiont types) underlying these effects. Effective application of this information for management and conservation decisions will require further study of this variation and its functional basis.

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Figures and Tables

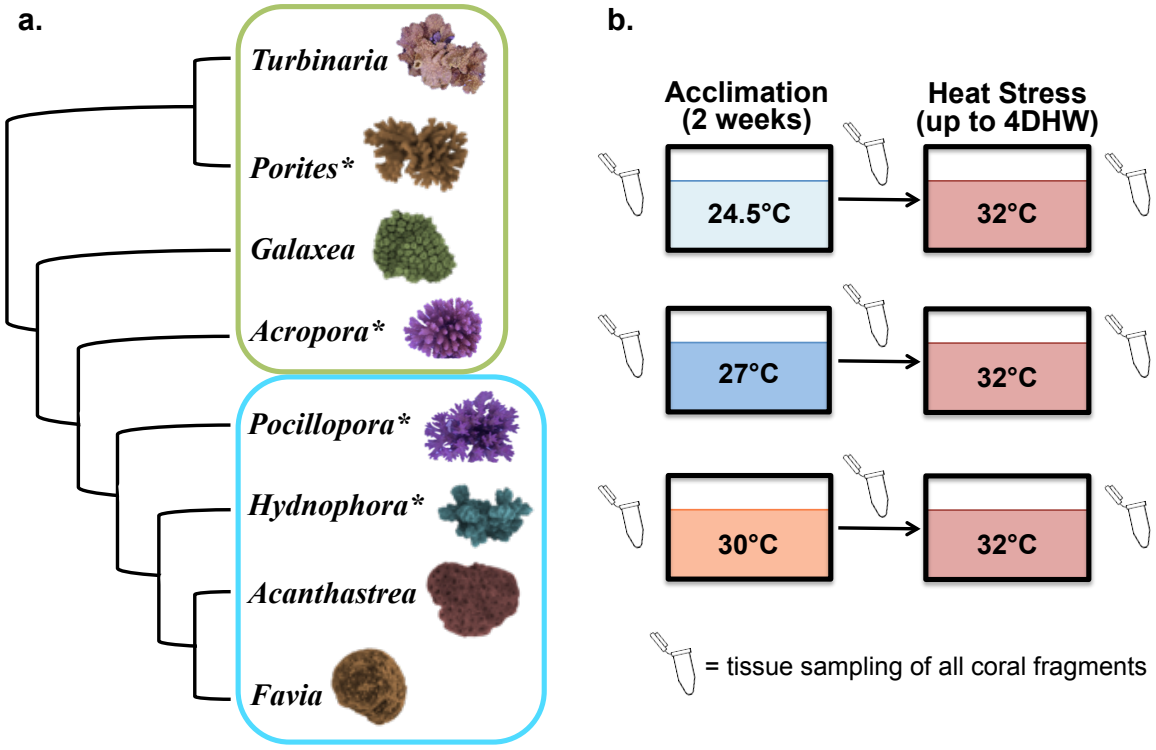

Figure 4.1. Experimental design for studying variation in thermal acclimation of diverse corals. a) Simplified phylogenetic tree showing relationships between the eight coral taxa chosen for this study based on Kitahara *et al.* 2008. The Complex clade is outlined in green and the Robust clade in blue. (*) indicate branching colonies while the absence indicates non-branching colonies. Study includes 3-4 colonies per taxon. b) Design of acclimation experiments: duplicate fragments from each colony were acclimated at different temperatures for 2 weeks, then subjected to thermal stress (32°C, until 4 degree heating weeks of cumulative heat stress was incurred in each treatment). Centrifuge tube icons indicate sampling times.

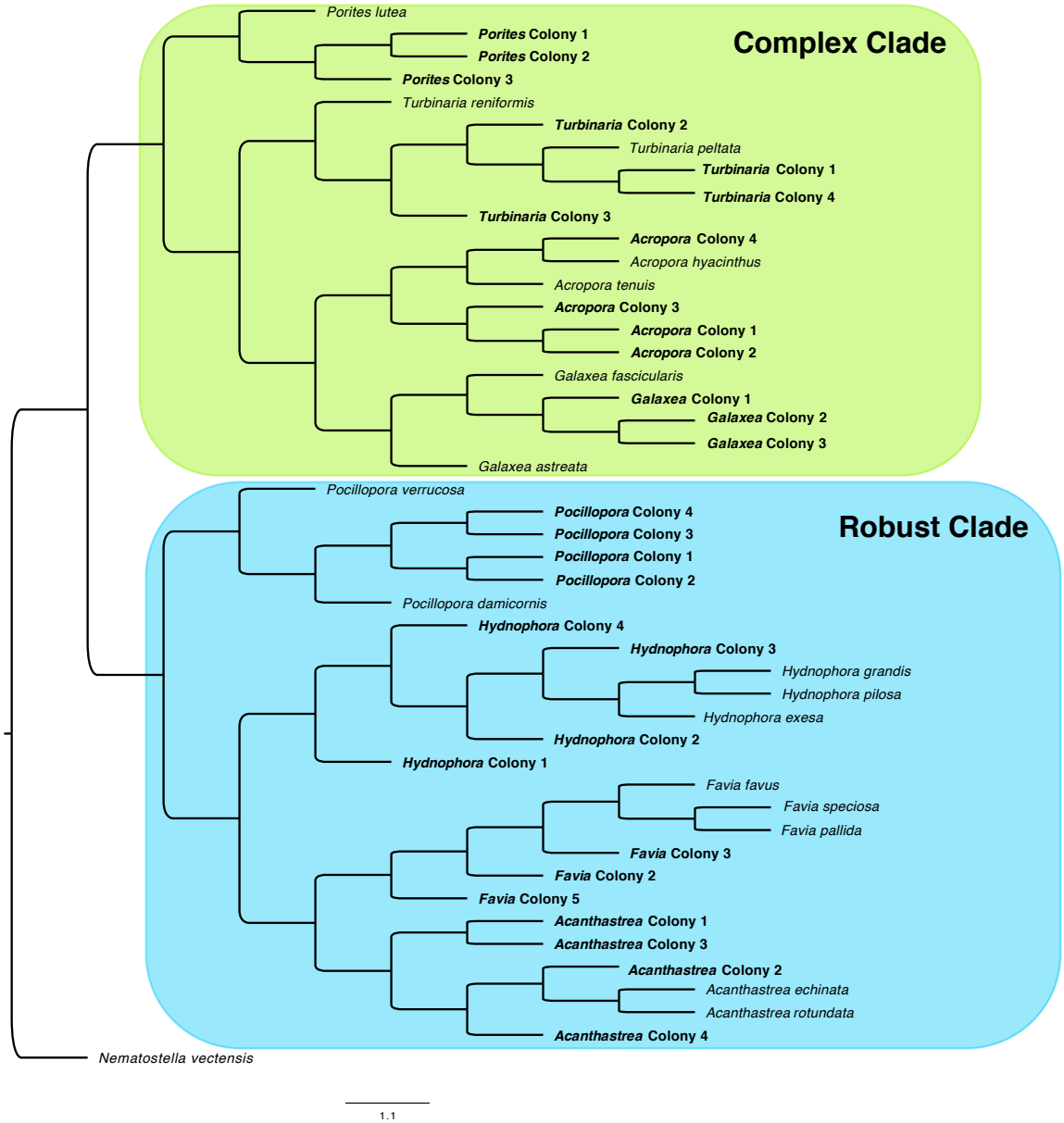

Figure 4.2. Phylogenetic tree of all coral colonies using CO1 sequence data. All coral colonies used in this experiment (bolded) were Sanger sequenced and sequences were compared with known CO1 sequences of other species found within the genus. All taxa and most experimental colonies grouped together. However, there are some colonies that grouped together more closely with other species more so than their original identification.

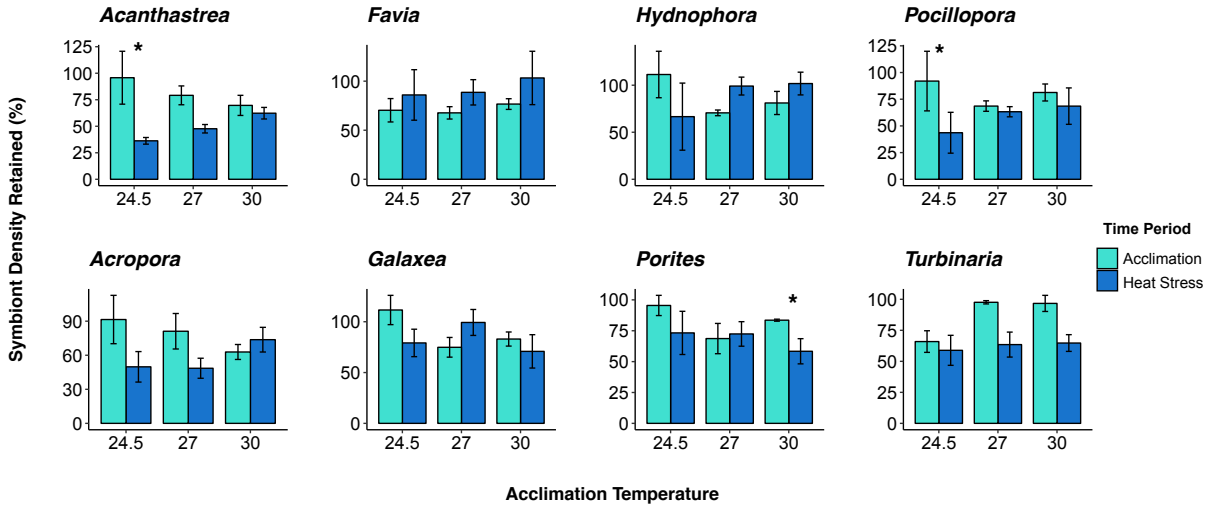

Figure 4.3. Symbiont density retained (percent) during acclimation (24.5°C, 27°C, and 30°C) and heat stress (32°C) time periods. Light blue bars indicate the symbiont density retained across the acclimation period, calculated as the symbiont density post-acclimation divided by the density prior to acclimation. Dark blue bars indicate the symbiont density retained across the heat stress period, calculated as the symbiont density post-heat stress divided by the density post-acclimation. Error bars represent standard error of the mean across all individual coral fragments exposed to each acclimation and heat stress temperature.

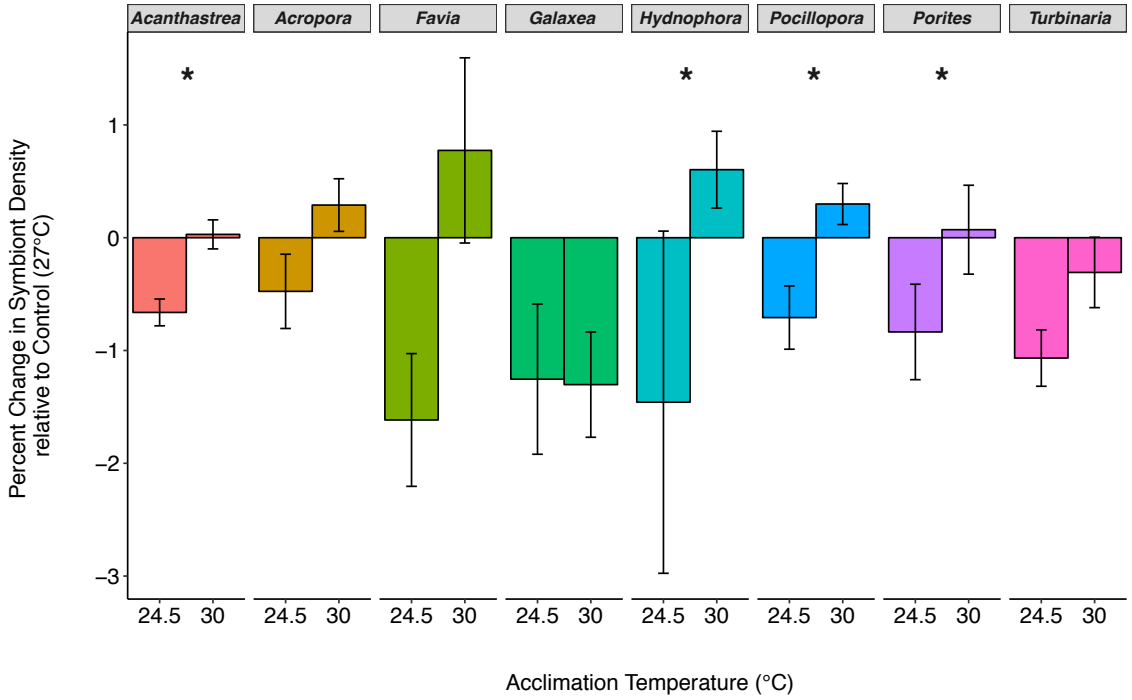

Figure 4.4. The effects of thermal acclimation on bleaching responses during subsequent exposures to thermal stress. Each bar represents the average symbiont density remaining after heat stress treatments in corals acclimated at low or high temperatures (24.5 and 30°C). To enable comparison across taxa in this figure, each pair of bars was normalized by subtracting the post-stress symbiont density in corals acclimated at control temperatures (27°C). Positive values represent acclimated corals that bleached less severely than controls, and negative values represent acclimated corals that bleached more than controls. Error bars represent standard error of the mean across individual coral fragments. (*) indicates significance (p <0.05) testing for the effect of acclimation temperature on final symbiont density.

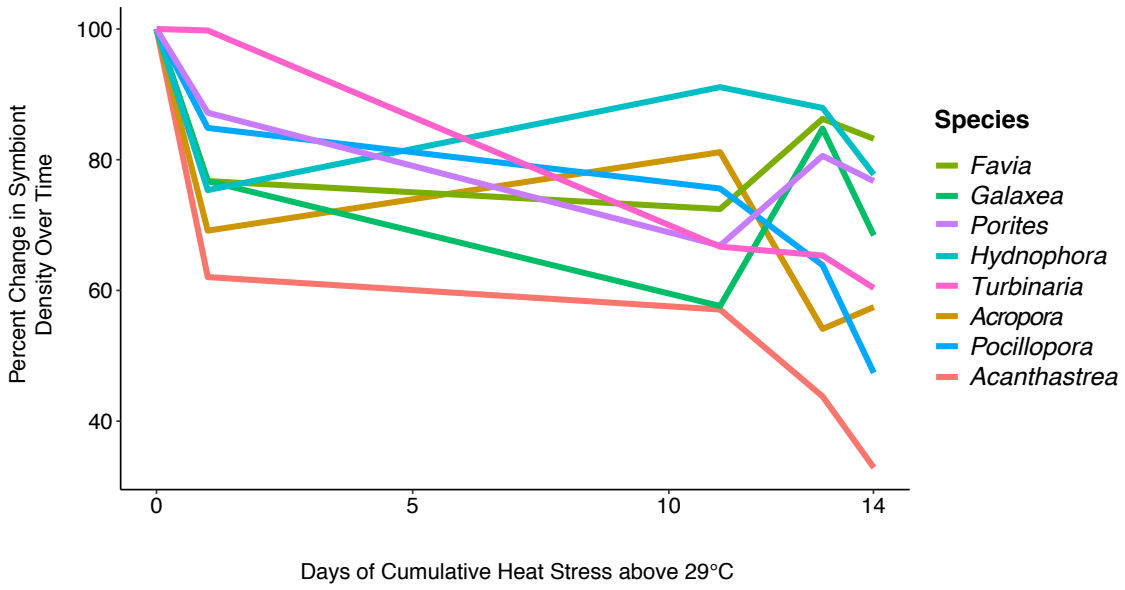

Figure 4.5. Comparison of thermal tolerance profiles across coral taxa. Each line depicts changes in symbiont density relative to the maximum for each taxa. For this analysis, the average symbiont density in post-acclimation and post-stress samples from each taxon were plotted relative to the cumulative thermal stress incurred in each treatment. Cumulative stress was expressed in per day each coral taxa was above the annual summer maximum (days >29°C, a typical maximum summer temperature in reef habitats).

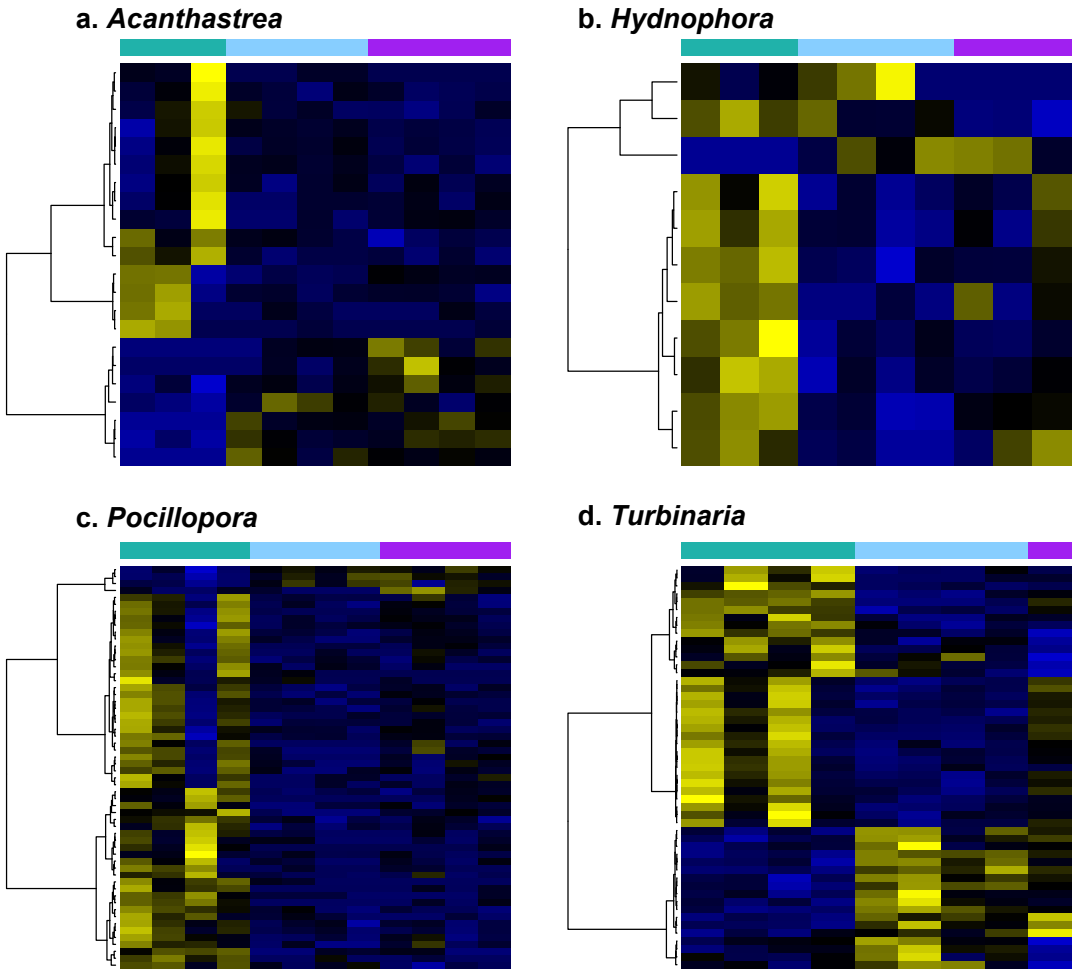

Figure 4.6. Variation in transcriptional responses to acclimation at different temperatures in four coral taxa. Each panel shows differentially expressed genes (DEG) identified in each taxon: a) *Acanthastrea,* b) *Hydnophora*, c) *Pocillopora*, and d) *Turbinaria*. Colored bars above columns indicate acclimation treatment; teal is 24.5°C, light blue is 27°C, and purple is 30°C. Columns represent biological replicates (colonies), and each row represents a single homologous group (one or more transcripts matching a particular *A. digitifera* gene; see Methods for details.) In the heatmap, blue indicates low expression, black moderate expression, and yellow indicates high expression.

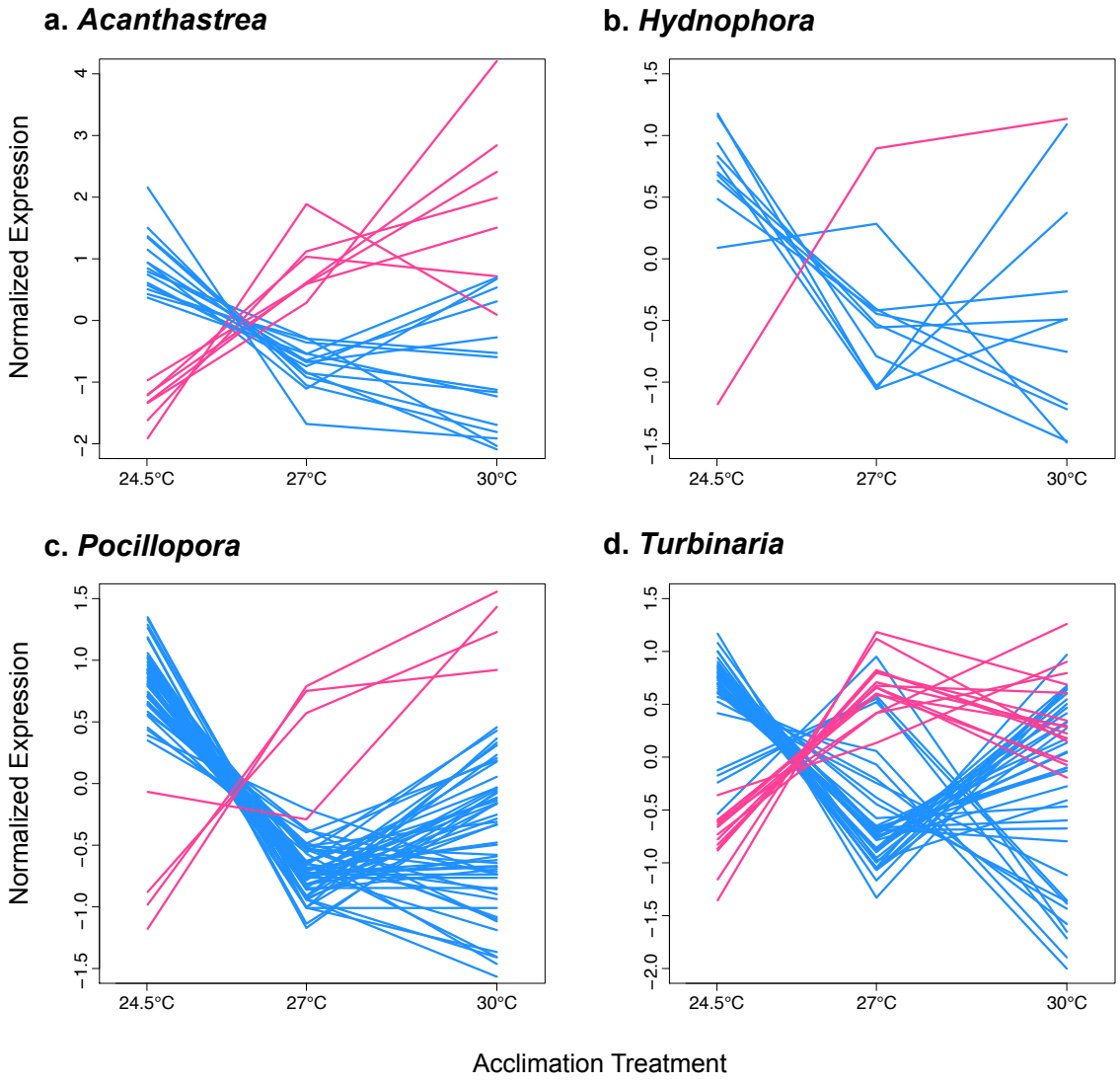

Figure 4.7. Comparing patterns of transcriptional responses to thermal acclimation across coral taxa. Lines depict quantitative changes in expression of differentially expressed genes. Groups of genes showing similar changes in expression are color-coded; blue represents genes down-regulated at high temperatures and pink represents genes up-regulated at high temperatures.

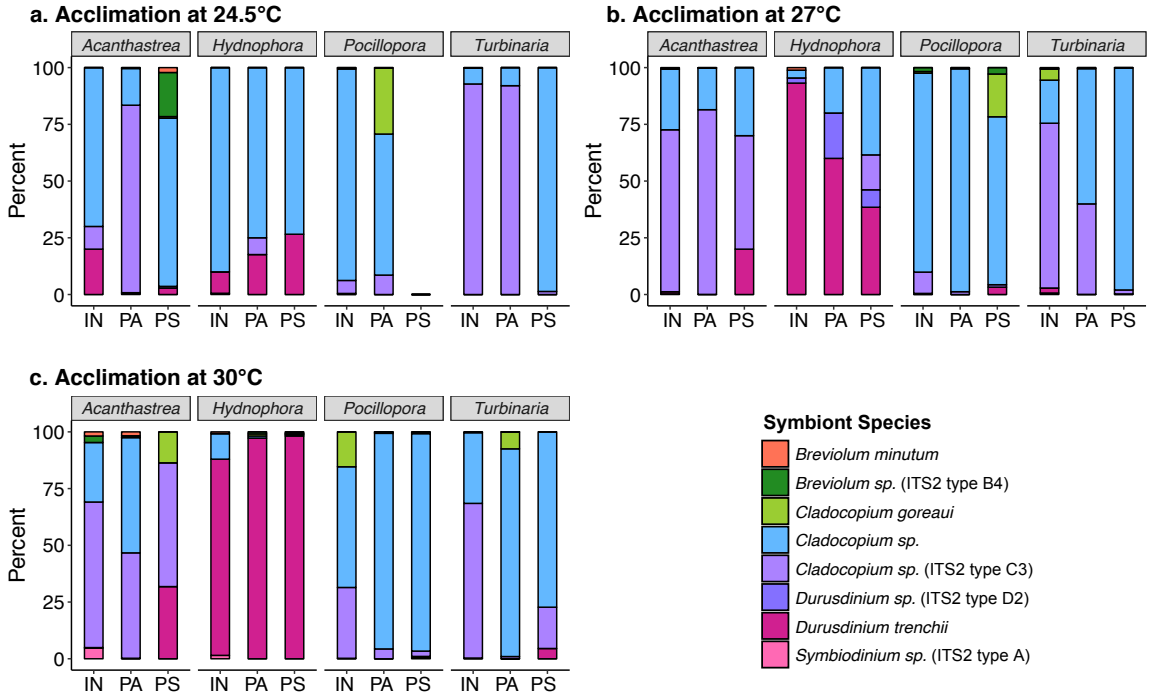

Figure 4.8. Changes in composition of algal symbiont communities during acclimation and thermal stress. Each panel depicts a different acclimation temperature: a) 24.5°C, b) 27°C, and c) 30°C. The taxa for which RNASeq data are available are compared here: *Acanthastrea, Hydnophora Pocillopora*, and *Turbinaria*. Stacked bars depict the symbiont community in each taxon prior to the experiment (IN), post acclimation (PA), and post thermal stress treatment (PS).

Table 4.1. Primer and adaptor sequences for transcriptome library preparations. 'V' indicates any A, G, or C nucleotide at that position. (*) indicates phosphorothioate bond modifications to prevent nuclease degradation. '5Phos' indicates phosphorylation modification on the 5' end.

Primer/Adaptor	**Sequence**
CA1-20TVN	AAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTVN
CAT-TS-YY **(RNA)**	AAGCAGTGGTATCAACGCAGAGTACYYGGG
CA1	AAGCAGTGGTATCAACGCAGAGTAC
PE-Top	ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
HT-Bot	/5Phos/GATCGGAAGAGCACACGTCTGAACTCCAGTCA
BC index	CAAGCAGAAGACGGCATACGAGATAATCGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
HT index	AATGATACGGCGACCACCGAGATCTACACCGAGAACACTCTTTCCCTACACGACGCTCTTCCGATCT

Table 4.2. Assembly statistics for *de novo* transcriptomes for all six coral taxa. Statistics are shown for both the complete assembly (transcripts >200 bp) and the subset of long transcripts (>500 bp). To maximize gene representation for gene expression analysis, we used complete transcriptomes as references.

	Acanthastrea	***Favia***	***Galaxea***	***Hydnophora***	***Porites***	***Turbinaria***
Total number of raw sequencing reads (PE)	65,935,582	82,309,412	69,889,132	50,640,474	73,284,248	35,250,942
Total number of reads after quality filtering (PE)	40,491,718	41,942,123	31,487,376	29,296,957	39,581,850	18,090,526
Total number of contigs	229,519	240,916	80,845	169,696	317,399	190,365
Average contig length (bp)	423	397	371	433	391	419
Maximum contig length	7,285	10,795	3,202	10,771	10,795	10,795
Minimum contig length	201	201	201	201	201	201
N50 (bp)	467	425	385	482	418	456
Mean GC content	40.60	39.15	40.43	42.38	39.53	39.70
Number of transcripts with UniProt annotation	75,245	50,713	24,885	71,636	79,850	55,284
Number of transcripts with GO annotation	32,265	17,369	10,442	42,910	27,531	21,002
Total Mb	97.2	95.6	30	76.6	124.3	78.9
Total number of contigs > 500bp	57,094	51,922	14,018	42,844	65,791	46,105
Average contig length >500bp	787	736	718	826	738	767
n50 > 500bp	783	725	697	837	727	764
Mean GC content	41.50	40.05	42.07	42.54	40.31	40.32
Total Mb	44.9	38.2	10.1	32.2	48.6	35.4
Median OHR %	66.9	47.0	53.3	71.1	55.4	60.2
Number transcripts with OHR 75%	4,062	1,621	1,055	3,765	2,826	2,649
Total % core KOGs	90.2	64.0	60.2	91.7	81.4	81.2

Table 4.3 Summary of sequencing yields, processing, and mapping efficiencies for RNASeq sequencing libraries.

No. samples	120
No. taxa	8
No. colonies	3-4
No. treatments	3
Raw sequencing depth (millions)	175.9
HQ sequencing depth (millions)	160.7
HQ reads per sample (millions)	1.3
Mapping efficiency	62.3%

Table 4.4. Annotated differentially expressed genes (p.adj<0.1) as a main effect of acclimation temperature for *Acanthastrea, Hydnophora*, *Turbinara,* and *Pocillopora*, and the log fold change and cluster assignments in each expression category as shown in Figure 4.7. Log fold change was calculated using DESeq2 for each gene based on the log2 fold change, which is equivalent to the effect size estimate or how much the gene's expression has changed due to acclimation temperature.

Taxon	*A. digitifera* homolog	Gene Description	pvalue	Log FC	Cluster
Acanthastrea	adi_v1.02060	Dextran-binding lectin A	3.21E-11	-10.059	Blue
	adi_v1.18306	Tetratricopeptide TPR 2	5.01E-05	-7.613	Blue
	adi_v1.00911	LOC100158331 protein	2.57E-04	-6.402	Blue
	adi_v1.02251	ATPase, histidine kinase-, DNA gyrase B-, and HSP90-like domain containing protein	1.55E-05	-5.755	Blue
	adi_v1.00933	LReO 3 protein	3.89E-04	-5.021	Blue
	adi_v1.20556	Neuralized PATS1	6.14E-04	-4.615	Blue
	adi_v1.18600	AGAP001193-PA	6.03E-04	-4.266	Blue
	adi_v1.20211	Reverse transcriptase-like protein	1.15E-03	-4.005	Blue
	adi_v1.02255	Cytochrome c oxidase subunit 1	5.35E-04	-3.719	Blue
	adi_v1.16781	Calreticulin	1.09E-03	-3.682	Blue
	adi_v1.16167	Serine threonine protein kinase, putative (Fragment)	1.87E-03	-3.432	Blue
	adi_v1.17188	Zgc;92254	8.78E-04	-3.319	Blue
	adi_v1.16845	AGAP010394-PA (Fragment)	5.35E-05	-2.467	Blue
	adi_v1.10370	Ly6/PLAUR domain-containing protein 2	5.12E-04	4.195	Pink
	adi_v1.10906	Collagen, type VI, alpha 3	9.48E-04	5.832	Pink
	adi_v1.13808	Multicopper oxidase	1.80E-03	5.951	Pink
	adi_v1.22462	Polyprotein	3.74E-05	6.108	Pink
Hydnophora	adi_v1.13321	MGC132201 protein	1.45E-03	-4.537	Blue
	adi_v1.14794	GE13192	1.20E-03	-3.984	Blue
	adi_v1.12931	Egg protein	9.78E-06	-2.314	Blue
	adi_v1.12914	Pre-mRNA processing factor 19	2.10E-05	-2.099	Blue
	adi_v1.04740	LOC100037160 protein (Fragment)	1.98E-03	-1.862	Blue
	adi_v1.02254	Cytochrome c oxidase subunit 2	4.16E-04	-1.747	Blue
	adi_v1.06134	Dehydrogenase/reductase protein, member 7C	1.79E-03	-1.390	Blue
	adi_v1.16383	Rab GTPase	5.39E-05	-1.335	Blue
	adi_v1.09244	Wd-repeat protein	3.65E-04	-1.315	Blue
	adi_v1.07049	Zinc finger protein, putative	1.47E-03	-0.423	Blue
	adi_v1.18772	Iron-sulfur cofactor synthesis protein	2.25E-04	7.127	Pink
Pocillopora	adi_v1.14338	Alanyl-tRNA synthetase domain-	2.36E-05	-5.860	Blue

		containing protein 1			
	adi_v1.13323	OTTXETP00000002638	4.28E-05	-5.774	Blue
	adi_v1.03885	Rpia protein (Fragment)	2.76E-05	-5.620	Blue
	adi_v1.19034	Eukaryotic elongation factor-2 kinase	5.78E-08	-5.242	Blue
	adi_v1.20417	Na+/k+ atpase alpha subunit	1.61E-04	-5.018	Blue
	adi_v1.10904	Hedgling (Fragment)	6.61E-03	-4.858	Blue
	adi_v1.01484	Nalp (Nacht, leucine rich repeat and pyrin domain containing)-related	9.09E-05	-4.702	Blue
	adi_v1.05801	Latrophilin-like receptor	6.40E-03	-4.132	Blue
	adi_v1.03134	Profilin	7.04E-06	-4.115	Blue
	adi_v1.06037	Cyclin dependent kinase 8	2.14E-03	-3.882	Blue
	adi_v1.24192	Putative lipoprotein	1.41E-05	-3.808	Blue
	adi_v1.16690	GJ20076	2.23E-03	-3.359	Blue
	adi_v1.05511	Ift122 protein	2.05E-04	-3.339	Blue
	adi_v1.11267	Transmembrane protein 115	1.27E-03	-3.264	Blue
	adi_v1.10548	TTC6 protein	5.15E-05	-3.248	Blue
	adi_v1.09702	Peroxisomal 3,2-trans-enoyl-CoA isomerase	7.59E-05	-3.018	Blue
	adi_v1.07011	GA10057	2.08E-05	-2.934	Blue
	adi_v1.16098	Thioredoxin-like 4A	1.22E-03	-2.825	Blue
	adi_v1.14151	LOC100145328 protein	1.85E-03	-2.778	Blue
	adi_v1.13871	Zgc:153354	1.05E-02	-2.690	Blue
	adi_v1.04723	Aspartyl aminopeptidase	1.40E-03	-2.639	Blue
	adi_v1.08670	DEAH (Asp-Glu-Ala-His) box polypeptide 15, isoform CRA a	1.08E-02	-2.537	Blue
	adi_v1.10228	GF11387	2.96E-07	-2.493	Blue
	adi_v1.03805	APAF1 interacting protein	9.41E-05	-2.418	Blue
	adi_v1.11899	Copine III	1.64E-03	-2.404	Blue
	adi_v1.16006	TAF2 protein (Fragment)	3.69E-03	-2.321	Blue
	adi_v1.03360	Methyltransferase like 3	4.39E-06	-2.319	Blue
	adi_v1.05385	Pfs, NACHT and Ankyrin domain protein	6.92E-03	-2.299	Blue
	adi_v1.07996	LOC398863 protein	3.05E-04	-2.194	Blue
	adi_v1.13482	LOC100145450 protein	3.33E-04	-2.134	Blue
	adi_v1.00670	Fasciclin II transmembrane protein isoform	5.45E-03	-2.130	Blue
	adi_v1.19611	Cellulase, putative	1.76E-04	-2.102	Blue
	adi_v1.20763	Rfc1 protein	2.80E-03	-2.089	Blue
	adi_v1.20271	Phosphonopyruvate decarboxylase	3.92E-03	-1.892	Blue
	adi_v1.08422	Protein VPRBP, putative	5.36E-04	-1.858	Blue
	adi_v1.16366	Cell cycle control protein 50A	2.00E-05	-1.788	Blue
	adi_v1.09611	Caspase 3/9	2.85E-04	-1.614	Blue

	adi_v1.13394	MAK16-like protein RBM13	2.94E-03	-1.540	Blue
	adi_v1.14911	RAB18, member RAS oncogene family	4.00E-03	-1.538	Blue
	adi_v1.21447	MGC80689 protein	4.67E-04	-1.518	Blue
	adi_v1.04953	Novel protein similar to asparaginases (Fragment)	1.48E-03	-1.500	Blue
	adi_v1.13180	UBX domain-containing protein 2	1.12E-03	-1.474	Blue
	adi_v1.17411	GCN5-like protein	2.55E-03	-1.435	Blue
	adi_v1.23241	Syringomycin synthesis regulator SyrP, putative	5.54E-04	-1.375	Blue
	adi_v1.01184	Macrophage erythroblast attacher, isoform CRA b	8.93E-03	-1.265	Blue
	adi_v1.07881	Regulator of g protein signaling (Fragment)	8.24E-03	-1.080	Blue
	adi_v1.06619	Thiol-disulfide exchange intermediate	5.50E-04	1.696	Pink
	adi_v1.10368	Hedgling (Fragment)	1.37E-03	2.069	Pink
Turbinaria	adi_v1.03235	Metallopeptidase inhibitor 3	2.08E-03	-8.124	Blue
	adi_v1.09043	Transcription factor GETS-1	1.17E-03	-7.659	Blue
	adi_v1.16451	Zgc:123178	2.37E-03	-7.434	Blue
	adi_v1.08645	Complement component C3	7.22E-04	-6.366	Blue
	adi_v1.07806	cDNA FLJ55575, moderately similar to Homo sapiens zinc finger CCCH-type containing 12A (ZC3H12A), mRNA	1.30E-03	-5.838	Blue
	adi_v1.14721	Vascular endothelial growth factor receptor	2.89E-04	-4.357	Blue
	adi_v1.07190	Novel protein similar to vertebrate CUB and Sushi multiple domain containing protein family (Fragment)	1.28E-05	-4.021	Blue
	adi_v1.11786	Transcription elongation factor B polypeptide, putative	4.11E-03	-4.005	Blue
	adi_v1.01661	Zinc finger protein	2.49E-05	-3.994	Blue
	adi_v1.02255	Cytochrome c oxidase subunit 1	2.02E-04	-3.719	Blue
	adi_v1.10753	GI17881	3.06E-03	-3.356	Blue
	adi_v1.10325	Pao retrotransposon peptidase family protein	1.79E-03	-2.698	Blue
	adi_v1.04793	Anion exchanger Ae2.1	1.13E-04	-2.353	Blue
	adi_v1.19175	Sulfatase 1	4.21E-03	-2.049	Blue
	adi_v1.18513	Reverse transcriptase	1.36E-03	-1.289	Blue
	adi_v1.22572	ORF2 protein	1.78E-03	-1.193	Blue
	adi_v1.07648	Polyprotein (Fragment)	4.13E-04	-1.090	Blue
	adi_v1.03932	POL protein	9.98E-04	-1.025	Blue
	adi_v1.20461	DNA-directed RNA polymerase, omega subunit family protein	4.43E-04	-1.024	Blue
	adi_v1.16799	Reverse transcriptase	2.95E-03	-1.008	Blue

	adi_v1.23860	Neurogenic locus notch (Notch)	5.55E-04	-0.996	Blue
	adi_v1.13326	Gag-Pol polyprotein	3.58E-05	-0.839	Blue
	adi_v1.10106	Putative gag protein	1.49E-04	-0.808	Blue
	adi_v1.04310	BF-DED-NACHT (Fragment)	2.80E-06	-0.771	Blue
	adi_v1.24521	Pol-like protein	3.11E-04	-0.749	Blue
	adi_v1.03714	Stromal antigen 1	2.37E-03	-0.637	Blue
	adi_v1.07060	Hedgehog interacting protein-like protein	1.06E-03	-0.492	Blue
	adi_v1.05761	Novel protein (Fragment)	2.52E-03	-0.489	Blue
	adi_v1.18192	LOC100145473 protein	1.65E-04	-0.391	Blue
	adi_v1.04103	Sperm phosphodiesterase 5	8.44E-05	-0.147	Blue
	adi_v1.16781	Calreticulin	9.93E-04	-3.682	Pink
	adi_v1.22354	Stress protein HSC70-2	2.67E-03	0.534	Pink
	adi_v1.07452	Heat shock protein 70	3.12E-03	0.581	Pink
	adi_v1.14106	Peroxiredoxin 4 variant	3.42E-03	1.075	Pink
	adi_v1.09185	cDNA FLJ34642 fis, clone KIDNE2016918, highly similar to UROMODULIN	9.74E-04	1.096	Pink
	adi_v1.20922	Protein disulfide isomerase	4.64E-04	1.216	Pink
	adi_v1.11299	Glutathione S-transferase, putative	3.63E-03	1.253	Pink
	adi_v1.19441	Cathepsin Z	4.04E-06	1.602	Pink
	adi_v1.14149	Heat shock protein gp96	2.09E-08	1.761	Pink
	adi_v1.14792	Ovoperoxidase	1.67E-11	2.172	Pink
	adi_v1.04115	STK38L protein	4.28E-04	2.406	Pink
	adi_v1.12269	Rh type C glycoprotein2a	2.72E-03	2.800	Pink
	adi_v1.17793	Zgc:165490 protein	1.37E-04	2.942	Pink

Table 4.5. Summary of results from acclimation experiments and profiling gene expression and symbiont communities for all eight coral taxa.

	Robust				**Complex**			
	Acanthastrea	***Favia***	***Hydnophora***	***Pocillopora***	***Acropora***	***Galaxea***	***Porites***	***Turbinaria***
Morphology	Massive	Massive	Branching	Branching	Branching	Encrusting	Branching	Plating
Acclimation Effect in 24.5°C	Negative*	Negative	Negative*	Negative*	Negative	Negative	Negative*	Negative
Acclimation Effect in 30°C	None*	Positive	Positive*	Positive*	Positive	Negative	None*	Negative
Thermal Tolerance	Low	High	High	Low	Low	High	High	Low
Dominant Symbiont after acclimation in 24.5°C	*Cladocopium sp.* (ITS2 type C3)		*Cladocopium sp.*	*Cladocopium sp.*				*Cladocopium sp.* (ITS2 type C3)
Dominant Symbiont after heat stress in 24.5°C	*Cladocopium sp.*		*Cladocopium sp.*	NA				*Cladocopium sp.*
Dominant Symbiont after acclimation in 30°C	*Cladocopium sp.* (ITS2 type C3)		*Durusdinium trenchii*	*Cladocopium sp.*				*Cladocopium sp.*
Dominant Symbiont after heat stress in 30°C	*Cladocopium sp.* (ITS2 type C3) and *Durusdinium trenchii*		*Durusdinium trenchii*	*Cladocopium sp.*				*Cladocopium sp.*
No. in Blue Gene Expression Pattern	15		10	54				37
No. in Pink Gene Expression Pattern	7		1	4				14

References

Ainsworth TD, Heron SF, Ortiz JC et al. (2016) Climate change disables coral bleaching protection on the Great Barrier Reef. *Science*, **352**, 338–342.

Baker A (2001) Reef corals bleach to survive change. *Nature*, **411**, 765–766.

Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Corals' adaptive response to climate change. *Nature*, **430**, 741–741.

Barshis DJ, Stillman JH, Gates RD, Toonen RJ, Smith LW, Birkeland C (2010) Protein expression and genetic structure of the coral Porites lobatain an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity? *Molecular Ecology*, **19**, 1705–1720.

Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR (2013) Genomic basis for coral resilience to climate change. *PNAS*, **110**, 1387–1392.

Barshis DJ, Birkeland C, Toonen RJ, Gates RD, Stillman JH (2018) High-frequency temperature variability mirrors fixed differences in thermal limits of the massive coral Porites lobata . *The Journal of Experimental Biology*, **221**, jeb188581.

Baumgarten S, Simakov O, Esherick LY et al. (2015) The genome of Aiptasia , a sea anemone model for coral symbiosis. **112**, 11893–11898.

Bay RA, Palumbi SR (2015) Rapid acclimation ability mediated by transcriptome changes in reef-building corals. *Genome Biology and Evolution*, **7**, 1602–1612.

Bay LK, Guérécheau A, Andreakis N, Ulstrup KE, Matz M V (2013) Gene Expression Signatures of Energetic Acclimatisation in the Reef Building Coral Acropora millepora. *PLoS ONE*, **8**, 1–10.

Bayer TT, Aranda MM, Sunagawa SS et al. (2012) Symbiodinium transcriptomes: genome insights into the dinoflagellate symbionts of reef-building corals. *PLoS ONE*, **7**, e35269–e35269.

Bellantuono AJ, Granados-Cifuentes C, Miller DJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2012) Coral thermal tolerance: tuning gene expression to resist thermal stress. **7**, e50685.

Bellis ES, Denver DEER (2017) Natural Variation in Responses to Acute Heat and Cold Stress in a Sea Anemone Model System for Coral Bleaching. *Biological Bulletin*, **233**, 168–181.

Boulotte NM, Dalton SJ, Carroll AG, Harrison PL, Putnam HM, Peplow LM, van Oppen MJ (2016) Exploring the Symbiodinium rare biosphere provides evidence for

symbiont switching in reef-building corals. *The ISME Journal*, **10**, 1–9.

Carpenter LW, Patterson MR, Bromage ES (2010) Water flow influences the spatiotemporal distribution of heat shock protein 70 within colonies of the scleractinian coral Montastrea annularis (Ellis and Solander, 1786) following heat stress: Implications for coral bleaching. *Journal of Experimental Marine Biology and Ecology*, **387**, 52–59.

Chang SW, Flynn BP, Ruberti JW, Buehler MJ (2012) Molecular mechanism of force induced stabilization of collagen against enzymatic breakdown. *Biomaterials*, **33**, 3852–3859.

Chen B, Feder ME, Kang L (2018) Evolution of heat-shock protein expression underlying adaptive responses to environmental stress. *Molecular Ecology*, **27**, 3040–3054.

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry*, **162**, 156–159.

Chomczynski P, Sacchi N (2006) The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. *Nature Protocols*, **1**, 581–585.

Chong KY, Lai CC, Lille S, Chang C, Su CY (1998) Stable overexpression of the constitutive form of heat shock protein 70 confers oxidative protection. *Journal of Molecular and Cellular Cardiology*, **30**, 599–608.

Cleves PA, Strader ME, Bay LK, Pringle JR, Matz M V. (2018) CRISPR/Cas9-mediated genome editing in a reef-building coral. *Proceedings of the National Academy of Sciences*, **115**, 5235–5240.

Conaco C, Neveu P, Zhou H, Arcila ML, Degnan SM, Degnan BM, Kosik KS (2012) Transcriptome profiling of the demosponge Amphimedon queenslandica reveals genome-wide events that accompany major life cycle transitions. *BMC Genomics*, **13**, 1–19.

Császár NBM, Ralph PJ, Frankham R, Berkelmans R, van Oppen MJH (2010) Estimating the potential for adaptation of corals to climate warming. *PLoS ONE*, **5**, e9751–e9751.

Cunning R, Gillette P, Capo T, Galvez K, Baker AC (2015a) Growth tradeoffs associated with thermotolerant symbionts in the coral Pocillopora damicornis are lost in warmer oceans. *Coral Reefs*, **34**, 155–160.

Cunning R, Silverstein RN, Baker AC (2015b) Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental

change. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20141725.

Davies SW, Scarpino S V, Pongwarin T (2015) The design and analysis of binary variable traits in common garden genetic experiments of highly fecund species to assess heritability.

DeSalvo MK, Voolstra CR, Sunagawa S et al. (2008) Differential gene expression during thermal stress and bleaching in the Caribbean coral Montastraea faveolata. *Molecular Ecology*, **17**, 3952–3971.

Dixon GB, Davies SW, Aglyamova GV, Meyer E, Bay LK, Matz MV (2015) Genomic determinants of coral heat tolerance across latitudes. **348**, 2014–2016.

Donner SD, Skirving WJ, Little CM, Oppenheimer M, Hoegh-Gulberg O (2005) Global assessment of coral bleaching and required rates of adaptation under climate change. *Global Change Biology*, **11**, 2251–2265.

Downs CA, Fauth JE, Halas JC, Dustan P, Bemiss J, Woodley CM (2002) Oxidative stress and seasonal coral bleaching. *Free Radical Biology and Medicine*, **33**, 533–554.

Dunn SR, Phillips WS, Green DR, Weis VM (2007) Knockdown of actin and caspase gene expression by RNA interference in the symbiotic anemone Aiptasia pallida. *Biological Bulletin*, **212**, 250–258.

Dziedzic K, Elder H, Tavalire H, Meyer E (2019) Heritable variation in bleaching responses and its functional genomic basis in reef-building corals (Orbicella faveolata. *Molecular Ecology*, 1–16.

Edmunds PJ (2014) Is acclimation beneficial to scleractinian corals, Porites spp.? *Marine Biology*, **161**, 1531–1542.

Fitt W, Brown B, Warner M, Dunne R (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral reefs*, **20**, 51–65.

Fukami H, Chen CA, Budd AF et al. (2008) Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (Order Scleractinia, Class anthozoa, phylum cnidaria). *PLoS ONE*, **3**, e3222.

Geller J, Meyer C, Parker M, Hawk H (2013) Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources*, **13**, 851–861.

Gibbin EM, Krueger T, Putnam HM, Barott KL, Bodin J, Gates RD, Meibom A (2018) Short-Term Thermal Acclimation Modifies the Metabolic Condition of the Coral Holobiont. *Frontiers in Marine Science*, **5**, 1–11.

Grabherr M, Haas BJ, Yassour M et al. (2013) Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nature Biotechnology*, **29**, 644–652.

Granados-Cifuentes C, Bellantuono AJ, Ridgway T, Hoegh-Guldberg O, Rodriguez-Lanetty M (2013) High natural gene expression variation in the reef-building coral Acropora millepora: potential for acclimative and adaptive plasticity. *BMC Genomics*, **14**, 228.

Guest JR, Baird AH, Maynard JA et al. (2012) Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. *PLoS ONE*, **7**, 1–8.

Hoey AS, Howells E, Johansen JL et al. (2016) Recent advances in understanding the effects of climate change on coral reefs. *Diversity*, **8**.

Howells EJ, Abrego D, Meyer E, Kirk NL, Burt JA (2016) Host adaptation and unexpected symbiont partners enable reef-building corals to tolerate extreme temperatures. *Global Change Biology*, **22**, 2702–2714.

Hughes TP, Kerry JT, Álvarez-Noriega M et al. (2017) Global warming and recurrent mass bleaching of corals. *Nature*, **543**, 373–377.

Hughes TP, Kerry JT, Baird AH et al. (2018a) Global warming transforms coral reef assemblages. *Nature*, **556**, 492–496.

Hughes TP, Anderson KD, Connolly SR et al. (2018b) Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science*, **359**, 80–83.

Hume B, D'Angelo C, Burt J, Baker AC, Riegl B, Wiedenmann J (2013) Corals from the Persian/Arabian Gulf as models for thermotolerant reef-builders: Prevalence of clade C3Symbiodinium, host fluorescence andex situtemperature tolerance. *Marine pollution bulletin*, **72**, 313–322.

Jones A, Berkelmans R (2010) Potential costs of acclimatization to a warmer climate: growth of a reef coral with heat tolerant vs. sensitive symbiont types. *PLoS ONE*, **5**, e10437.

Jones AM, Berkelmans R (2011) Tradeoffs to Thermal Acclimation: Energetics and Reproduction of a Reef Coral with Heat Tolerant Symbiodinium Type-D . *Journal of Marine Biology*, **2011**, 1–12.

Jones AM, Berkelmans R, van Oppen MJ., Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 1359–1365.

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic acids research*, **30**, 3059–66.

Kayal E, Roure B, Philippe H, Collins AG, Lavrov D V. (2013) Cnidarian phylogenetic relationships as revealed by mitogenomics. *BMC Evolutionary Biology*, **13**, 1–18.

Kenkel CD, Bay LK (2017) Novel transcriptome resources for three scleractinian coral species from the Indo-Pacific. *GigaScience*, **6**, 1–4.

Kenkel C, Matz M V (2016) Enhanced gene expression plasticity as a mechanism of adaptation to a variable environment in a reef-building coral. *bioRxiv*, **1**, 059667.

Kenkel CD, Meyer E, Matz M V. (2013) Gene expression under chronic heat stress in populations of the mustard hill coral (Porites astreoides) from different thermal environments. *Molecular Ecology*, **22**, 4322–4334.

Kenkel CD, Setta SP, Matz M V (2015) Heritable differences in fitness-related traits among populations of the mustard hill coral, Porites astreoides. *Heredity*, **115**, 509–516.

Kirk NL, Howells EJ, Abrego D, Burt JA, Meyer E (2018) Genomic and transcriptomic signals of thermal tolerance in heat-tolerant corals (Platygyra daedalea) of the Arabian/Persian Gulf. *Molecular Ecology*, **27**, 5180–5194.

Kitahara M V, Cairns SD, Stolarski J, Blair D, Miller DJ (2010) A Comprehensive Phylogenetic Analysis of the Scleractinia (Cnidaria, Anthozoa) Based on Mitochondrial CO1 Sequence Data (ed DeSalle R). *PLoS ONE*, **5**, e11490.

Kitchen SA, Crowder CM, Poole AZ, Weis VM, Meyer E (2015) De Novo Assembly and Characterization of Four Anthozoan (Phylum Cnidaria) Transcriptomes. *G3: Genes, Genomes, Genetics*, **5**, 2441–2452.

Kress WJ, García-Robledo C, Uriarte M, Erickson DL (2015) DNA barcodes for ecology, evolution, and conservation. *Trends in Ecology and Evolution*, **30**, 25–35.

LaJeunesse TC, Thornhill DJ, Cox EF, Stanton FG (2004) High diversity and host specificity observed among symbiotic dinoflagellates in reef coral communities from Hawaii. *Coral reefs*, **23**, 596–603.

Liu G, Rauenzahn JL, Heron SF et al. (2013) *NOAA Technical Report NESDIS 143 - NOAA Coral Reef Watch 50 km Satellite Sea Surface Temperature-Based Decision Support System for Coral Bleaching Management*.

Lohman BK, Weber JN, Bolnick DI (2016) Evaluation of TagSeq, a reliable low-cost alternative for RNAseq. *Molecular Ecology Resources*, **16**, 1315–1321.

Louis YD, Bhagooli R, Kenkel CD, Baker AC, Dyall SD (2017) Gene expression biomarkers of heat stress in scleractinian corals: Promises and limitations. *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology*, **191**, 63–77.

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology*, **15**, 1–21.

Loya Y, Sakai K, Yamazato K, Nakano Y, Sambali H, van Woesik R (2001) Coral bleaching: the winners and the losers. *Ecology Letters*, **4**, 122–131.

Marshall PA, Baird AH (2000) Bleaching of corals on the Great Barrier Reef:differential susceptibilities among taxa. *Coral reefs*, **19**, 155–163.

Matthews JL, Crowder CM, Oakley CA et al. (2017) Optimal nutrient exchange and immune responses operate in partner specificity in the cnidarian-dinoflagellate symbiosis. *Proceedings of the National Academy of Sciences*, **114**, 201710733.

Matthews JL, Oakley CA, Lutz A et al. (2018) Partner switching and metabolic flux in a model cnidarian–dinoflagellate symbiosis. *Proceedings of the Royal Society B: Biological Sciences*, **285**, 1–10.

McClanahan TR (2017) Changes in coral sensitivity to thermal anomalies. *Marine Ecology Progress Series*, **570**, 71–85.

Meyer EE, Manahan DTDT (2010) Gene expression profiling of genetically determined growth variation in bivalve larvae (Crassostrea gigas). *The Journal of experimental biology*, **213**, 749–758.

Meyer E, Aglyamova G V, Wang S et al. (2009) Sequencing and de novo analysis of a coral larval transcriptome using 454 GSFlx. *BMC genomics*, **10**, 219.

Meyer E, Aglyamova G V, Matz M V (2011) Profiling gene expression responses of coral larvae (Acropora millepora) to elevated temperature and settlement inducers using a novel RNA‐Seq procedure. *Molecular Ecology*, **20**, 3599–3616.

Nicholas H. Putnam, Mansi Srivastava, Uffe Hellsten et al. (2007) Sea Anemone Genome Reveals Ancestral Eumetazoan Gene Repertoire and Genomic Organization. *Science*, **317**, 86.

O'Neil ST, Dzurisin JDK, Carmichael RD, Lobo NF, Emrich SJ, Hellmann JJ (2010) Population-level transcriptome sequencing of nonmodel organisms Erynnis propertius and Papilio zelicaon. *BMC Genomics*, **11**, 1–15.

Oksanen J (2010) Cluster analysis: tutorial with R. *University of Oulu, Oulu*, 1–8.

Oliver TA, Palumbi SR (2011) Do fluctuating temperature environments elevate coral

thermal tolerance? *Coral reefs*, **30**, 429–440.

van Oppen MJH, Catmull J, Mcdonald BJ, Hislop NR, Hagerman PJ, Miller DJ (2001) The Mitochondrial Genome of Acropora tenuis (Cnidaria ; Scleractinia) Contains a Large Group I Intron and a Candidate Control Region. **55**, 1–13.

van Oppen MJH, Oliver JK, Putnam HM, Gates RD (2015) Building coral reef resilience through assisted evolution. *Proceedings of the National Academy of Sciences*, **112**, 1–7.

van Oppen MJH, Gates RD, Blackall LL et al. (2017) Shifting paradigms in restoration of the world's coral reefs. *Global Change Biology*, **23**, 3437–3448.

Van Oppen MJH, Mahiny AJ, Done TJ (2005) Geographic distribution of zooxanthella types in three coral species on the Great Barrier Reef sampled after the 2002 bleaching event. *Coral Reefs*, **24**, 482–487.

Palumbi SR, Barshis DJ, Traylor-Knowles N, Bay RA (2014) Mechanisms of reef coral resistance to future climate change. *Science*, **344**, 895–898.

Parkinson JE, Baumgarten S, Michell CT, Baums IB, LaJeunesse TC, Voolstra CR (2016) Gene Expression Variation Resolves Species and Individual Strains among Coral-Associated Dinoflagellates within the Genus Symbiodinium. *Genome biology and evolution*, **8**, 665–680.

Parra G, Bradnam K, Korf I (2007) Genome analysis CEGMA : a pipeline to accurately annotate core genes in eukaryotic genomes. **23**, 1061–1067.

Pigliucci M (2006) Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology*, **209**, 2362–2367.

Pinzón JH, Kamel B, Burge CA, Harvell CD, Medina M, Weil E, Mydlarz LD (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**, 140214.

Putnam HM, Gates RD (2015) Preconditioning in the reef-building coral Pocillopora damicornis and the potential for trans-generational acclimatization in coral larvae under future climate change conditions. *Journal of Experimental Biology*, **218**, 2365–2372.

Reitzel AM, Sullivan JC, Traylor-knowles N, Finnerty JR (2010) Genomic Survey of Candidate Stress-Response Genes in the Estuarine Anemone Nematostella vectensis. *Genomics*, **214**, 233–254.

Riesgo A, Andrade SCS, Sharma PP et al. (2012) Comparative description of ten transcriptomes of newly sequenced invertebrates and efficiency estimation of

genomic sampling in non-model taxa. *Frontiers in Zoology*, **9**, 1–24.

Robbart ML, Peckol P, Scordilis SP, Curran HA, Brown-Saracino J (2004) Population recovery and differential heat shock protein expression for the corals Agaricia agaricites and A. tenuifolia in Belize. *Marine Ecology Progress Series*, **283**, 151–160.

Rowan R, Knowlton N, Baker A, Jara J (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature*, **388**, 265–269.

Ruiz-Jones LJ, Palumbi SR (2017) Tidal heat pulses on a reef trigger a fine-tuned transcriptional response in corals to maintain homeostasis. *Science Advances*, **3**, 1–10.

Rumble SM, Lacroute P, Dalca A V., Fiume M, Sidow A, Brudno M (2009) SHRiMP: Accurate mapping of short color-space reads. *PLoS Computational Biology*, **5**, 1–11.

Seneca FO, Palumbi SR (2015) The role of transcriptome resilience in resistance of corals to bleaching. *Molecular Ecology*, **24**, 1467–1484.

Seneca FO, Forêt S, Ball EE, Smith-Keune C, Miller DJ, Oppen MJH (2010) Patterns of Gene Expression in a Scleractinian Coral Undergoing Natural Bleaching. *Marine Biotechnology*, **12**, 594–604.

Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, Fujie M (2011) Using the Acropora digitifera genome to understand coral responses to environmental change. *Nature*, **476**, 320–323.

Shinzato C, Inoue M, Kusakabe M (2014) A snapshot of a coral "holobiont": A transcriptome assembly of the scleractinian coral, Porites, captures a wide variety of genes from both the host and symbiotic zooxanthellae. *PLoS ONE*, **9**.

Silverstein RN, Cunning R, Baker AC (2015) Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Global Change Biology*, **21**, 236–249.

Smith EG, Hume BCC, Delaney P, Wiedenmann J, Burt JA (2017) Genetic structure of coral-Symbiodinium symbioses on the world's warmest reefs. *PLoS ONE*, **12**, 1–12.

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**, 1312–1313.

Stamatakis A (2016) RAxML. *Manual/tutorial*, 1–5.

Stewart ZK, Pavasovic A, Hock DH, Prentis PJ (2017) Transcriptomic investigation of wound healing and regeneration in the cnidarian Calliactis polypus. *Scientific Reports*, **7**, 41458.

Sully S, Burkepile DE, Donovan MK, Hodgson G, van Woesik R (2019) A global analysis of coral bleaching over the past two decades. *Nature Communications*, **10**, 1–5.

Thomas L, Rose NH, Bay RA, López EH, Morikawa MK, Ruiz-Jones L, Palumbi SR (2018) Mechanisms of Thermal Tolerance in Reef-Building Corals across a Fine-Grained Environmental Mosaic: Lessons from Ofu, American Samoa. *Frontiers in Marine Science*, **4**, 1–14.

Tomanek L, Somero GN (2002) Interspecific- and acclimation-induced variation in levels of heat-shock proteins 70 (hsp70) and 90 (hsp90) and heat-shock transcription factor-1 (HSF1) in congeneric marine snails (genus Tegula): implications for regulation of hsp gene expression. *The Journal of experimental biology*, **205**, 677–85.

Traylor-Knowles N, Granger BR, Lubinski TJ et al. (2011) Production of a reference transcriptome and transcriptomic database (PocilloporaBase) for the cauliflower coral, Pocillopora damicornis. *BMC Genomics*, **12**, 585.

Ulstrup KE, Berkelmans R, Ralph PJ, Oppen MJH Van (2006) Variation in bleaching sensitivity of two coral species across a latitudinal gradient on the Great Barrier Reef: the role of zooxanthellae. *Marine Ecology Progress Series*, **314**, 135–138.

Vidal-Dupiol J, Zoccola D, Tambutte E et al. (2013) Genes related to ion-transport and energy production are upregulated in response to CO2-driven pH decrease in corals: new insights from transcriptome analysis. *PLoS ONE*, **8**, e58652.

Voolstra CR, Schnetzer J, Peshkin L, Randall CJ, Szmant AM, Medina M (2009) Effects of temperature on gene expression in embryos of the coral Montastraea faveolata. *BMC genomics*, **10**, 627.

Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews: Genetics*, **10**, 57–63.

Wham DC, Ning G, LaJeunesse TC (2017) Symbiodinium glynnii sp. nov ., a species of stress-tolerant symbiotic dinoflagellates from pocilloporid and montiporid corals in the Pacific Ocean . *Phycologia*, **56**, 396–409.

Van Woesik R, Sakai K, Ganase A, Loya Y (2011) Revisiting the winners and the losers a decade after coral bleaching. *Marine Ecology Progress Series*, **434**, 67–76.

CHAPTER 5 – Conclusion

The research presented here provides new information about how the coral host is responding to stress and what mechanisms might be used to become tolerant and adapt to the changing climate. With global mass bleaching events taking place on a global scale, we need to understand whether the host has the capacity to acclimate and potentially adapt to these changing conditions. This dissertations describes potential for adaptation, a change that causes an organism to become better suited for their environment, to occur in natural populations of Cnidarians (Chapter 2 and 3), and also explores the mechanisms of acclimation, a phenotypic change to an environmental factor, that could feed into thermal tolerance within a coral's lifetime (Chapter 4). These three data chapters offer insights into whether genetic variation exists in natural populations of Cnidarians, if this genetic variation is heritable, and what genes may be playing a more mechanistic role before and after stress. I use a collection of reef-building corals from the Caribbean and Indo-Pacific regions, as well as a temperate anemone to explore the genetic basis of thermal tolerance and possible mechanisms of thermal acclimation. These studies identify substantial heritable variation in thermal tolerance and shared genetic processes that will help further our understanding of how the cnidarian host may be able to acclimate and adapt to changing ocean conditions.

Heritable variation in thermal tolerance across natural populations of Cnidarians

Variation in bleaching susceptibility across natural populations has been documented for decades, but investigations of whether this variation is heritable have remained unexplored until recently. In Chapter 2, I described results from a genome wide association study and transcriptomic investigation of thermal tolerance in a natural population of corals found in Panama (Dziedzic *et al.*, 2019). We found genetic markers significantly associated with thermal tolerance, and put them into a genomic context to determine what genes these markers may be linked. This study demonstrates the benefits of integrating genomic resources, such as a genetic linkage map and a genome sequence assembly, to provide a more functional context for thermal tolerance differences across

coral genotypes. While genomic resources have historically been limited for coral species, genome and transcriptome assemblies are becoming increasingly available, enabling studies like this one (Meyer *et al.*, 2009a; Medina *et al.*, 2011; Polato *et al.*, 2011; Shinzato *et al.*, 2011; Traylor-Knowles *et al.*, 2011; Shinzato *et al.*, 2014; Kitchen *et al.*, 2015; Anderson *et al.*, 2016; Mansour *et al.*, 2016; Kenkel & Bay, 2017; ReFuGe 2020 Consortium, 2017; Voolstra *et al.*, 2017; Cunning *et al.*, 2018; Ying *et al.*, 2018).

In addition, we provide one of the first quantitative estimates for heritability of bleaching responses in a natural population, providing evidence that this population of corals may be able to adapt to the changing climate. While other studies have estimated heritability in corals, these studies have focused on coral larvae and recruits due to the inherent ease of controlled genetic crosses (Meyer *et al.*, 2009b; Dixon *et al.*, 2015; Kenkel *et al.*, 2015; Kirk *et al.*, 2018). In our study, sequencing-based genotyping identified thousands of SNPs across our coral genotypes and use these to infer genetic relatedness and provide reliable estimates of heritability. This approach enabled us to sample multiple individuals within a species simultaneously, a tool that can be used to continue asking these questions in different environmental conditions, across different populations, and across multiple coral species. Overall, these findings provide crucial data for models aiming to predict the adaptive capacity of coral populations to ocean warming, and identify genetic markers and genes that may be useful for future studies on the genetic basis of coral thermal tolerance.

Adaptation through genetic change can play an important role in allowing organisms to become better suited for their environment and persist during ongoing change. By estimating heritability, both broad and narrow-sense, we can better predict evolutionary changes in host phenotypes, such as bleaching responses. In Chapter 3, I used *Anthopleura elegantissima*, a temperate anemone, as a model system to explore genetic variation and heritability of thermal tolerance in a natural population of anemones. Using similar sequencing approaches in Chapter 2, I estimated both clonal repeatability (proxy for broad-sense heritability; H^2) and narrow-sense heritability (h^2) in a natural population of anemones from the Oregon coast. Again, we found high additive genetic variance in bleaching responses across anemone aggregations (colonies). More

specifically, narrow-sense heritability was more than half of the clonal repeatability measurement, indicating that majority of the genetic variation can be explained by additive genetic variance. This finding demonstrates the genetic potential for this population to respond to selection for increased thermal tolerance. We found four specific genetic markers associated with thermal tolerance, two of which were directly on stress-relevant genes, a heat shock protein and a methyltransferase. This pattern suggests a conserved mechanism for dealing with acute stress during intertidal fluxes. For instance, other intertidal invertebrates have been shown to increase heat shock proteins extensively to survive periods of extreme stress (Tomanek & Sanford, 2003; Snyder & Rossi, 2004). Methyltransferases have been described in other cnidarians, indicating they are more highly expressed in response to acute heat stress (Dixon *et al.*, 2016; Li *et al.*, 2018). Interestingly, these markers show evidence for heterozygote advantage, when a heterozygous genotype shows greater fitness than either homozygous genotype, at these significant loci. Heterozygote advantages have been widely documented in other systems, but our knowledge have not been previously reported in Cnidarian stress responses. If similar effects occurred in stress tolerance in corals, this would become an important consideration for restoration and management (Mitton, 1997; Bellis *et al.*, 2016; Sellis *et al.*, 2016).

Together, Chapter 2 and 3 highlight the potential for adaptation in natural populations of Cnidarians. I uniquely pinpoint particular regions in a coral and anemone genome that may be linked to thermal tolerance traits and also find high heritability in both populations. Thermal tolerance of corals is most likely a quantitative trait that results from the interaction of many loci (Bay & Palumbi, 2014). Therefore, examining multiple allelic variations may provide insights into genomic regions under selection. Combining SNP data and phenotypic information within or across populations is a powerful tool for assessing functional genomics and examining genetic and phenotypic variation (Reitzel *et al.*, 2013). These chapters outline novel perspectives of host adaptation and demonstrate new genomic tools that can be used to answer similar questions in other populations of corals.

Acclimation capacity across multiple reef-building coral species

While adaptation is important for long-term responses, acclimation may allow individuals to persist within the short term. Acclimation may be an important but underestimated role in allowing coral reefs to become robust to rapid environmental changes, such as changes in temperature. In Chapter 4, I characterized the acclimation capacity of eight reef-building corals found in the Indo-Pacific as well as highlight some potential mechanisms that may allow corals to acclimate in the short term. In quantifying the effect of acclimation, we found corals differed wildly in their capacities, with some taxa showing strong acclimation effects, while other species had very little or negative responses over time. Comparing these responses to overall thermal tolerance, we find that some species which are known to be thermally tolerant (i.e. *Porites*) had a very a poor capacity to acclimate to warmer temperatures, whereas other species which are known to be more thermally susceptible (i.e. *Acropora*) had a high capacity for acclimation (although not statistically significant). For *Acropora*, acclimation may be a way for this genus to remain tolerant in the short-term, and therefore allow adaptation to take place across populations (Barshis *et al.*, 2013; Bay & Palumbi, 2015).

By exploring the mechanisms that might facilitate acclimation, we found differences in gene expression patterns, the magnitude of expression across acclimation temperatures, and symbiont communities over time. Acclimation at lower temperatures (24.5°C) had a large impact on gene expression across all species, and also reduced tolerance during heat-stress. In contrast, we found dampened gene expression at high acclimation across all species, which may reflect lower stress levels after acclimation despite increased temperatures (Bellantuono *et al.*, 2012; Bay & Palumbi, 2015). While we only explored gene expression after acclimation, these genes could have been upregulated earlier. Previous studies on the magnitude and timing of expression show heat stress triggers upregulation of heat shock and other related proteins within the first couple of hours, and then return to normal within 24 hours (Gates & Edmunds, 1999; Tomanek & Somero, 2002; Dixon *et al.*, 2015; Kirk *et al.*, 2018). Therefore, early or dampened gene expression may be a mechanism for acclimation across coral species, but more studies need to explore these differences.

Lastly, symbiont communities may play a role in short-term responses to stress. A large number of studies have focused on physiological differences between various host-symbiont associations, showing that some symbiont species have a greater thermal tolerance capacity, while others are much more susceptible to environmental changes (Jones & Berkelmans, 2010; Silverstein *et al.*, 2015; Boulotte *et al.*, 2016). Characterizing the communities as well as the change in these communities may provide insights into how the coral host can survive and persist during periods of acute heat stress. In Chapter 4, I describe differences in symbiont species and their composition in the low, medium and high acclimation temperatures and document both subtle and extreme changes over time. While there is evidence for some shuffling of symbionts, these changes may be short lived, just providing the host with temporary thermal tolerance (Cunning *et al.*, 2015; Matthews *et al.*, 2017, 2018; Rouzé *et al.*, 2019).

Host transcriptomic variation across acclimation and heat stress

Gene expression analysis allows for simultaneous evaluation of expression patterns of thousands of genes, providing global insights into which genes may play a mechanistic role in thermal tolerance. In Chapter 2 and 3, we found considerable variation in transcriptomic responses across heat-susceptible and heat-tolerant phenotypes, and in Chapter 4 we found strong transcriptional changes associated with various acclimation treatments. Deciphering the overall role of certain genes and their correlation with tolerance traits can be challenging. However, we identified specific genes, or group of genes, associated with differences in bleaching responses as a function of phenotype, host colony (aggregations), or acclimation temperature. Genes found across all three Chapters included collagen proteins, heat shock proteins, glutathione s-transferase or peroxidase, methyltransferase, ubiquitin-ligases, all genes involved in either oxidative stress, the unfolded protein response, or immune function (Cyr *et al.*, 1994; Pickart, 2001; Moya *et al.*, 2012; Sabourault *et al.*, 2012; Barshis *et al.*, 2018). These repeated observations not only confirm the relative roles in oxidative stress and immune functions in response to heat stress, but also show potential mechanisms for heat tolerance differences across the taxa. Interestingly, these genes have been explored as

possible biomarkers to diagnose and predict coral stress (Louis *et al.*, 2017). These gene expression biomarkers may be able to help reef managers and restoration programs identify certain reefs or species under stress, help identify what the stress is, and offer a course of action to help mitigate impacts (Weis, 2010; Louis *et al.*, 2017; Wright *et al.*, 2017; Parkinson *et al.*, 2018). However, due to the extensive variation across individual corals and reefs, it may be challenging to provide a "one size fits all" approach to characterizing stress (Louis *et al.*, 2017; Parkinson *et al.*, 2018). As we continue to understand and characterize these dynamic transcriptional responses, we can more accurately pinpoint informative markers across coral species and reefs.

Specifically in Chapter 2, I found a larger number of genes differentially expressed as a function of treatment × phenotype, as well as in individual effects of treatment and phenotype. I also found a strong signal in the interaction between treatment × anemone aggregation and the individual effect of colony in heat stressed anemone in Chapter 3. Majority of the variation in expression was related to variation across anemone aggregations, confirming that differences in anemones may be due to genetically determined differences across aggregations. From the co-expression analysis in the same study, I found a module that was significantly correlated with thermal tolerance, with genes responsive to oxidative stress. In Chapter 4, we found genes uniquely up- or down-regulated in each of the four taxa we explored, but did find groups of genes with similar patterns of expression in the high acclimation temperature (30°C).

In order to explore expression patterns within and across multiple species, we need transcriptomic resources. With advances in sequencing technology over the last two decades, more coral transcriptomes are becoming available as well as studies using these resources to document similar gene expression responses across multiple cnidarian hosts (Voolstra *et al.*, 2009; Barshis *et al.*, 2013; Kenkel *et al.*, 2013; Palumbi *et al.*, 2014; Kenkel & Matz, 2016; Ruiz-Jones & Palumbi, 2017). Importantly, these studies are documenting a diverse set of possible mechanisms that could facilitate acclimation and adaptation within the coral host. As we continue to explore these responses, we are beginning to unravel more sequence-level details about host-specific responses to acclimation and heat stress.

Future Studies

The data presented in Chapters 2 and 3 provide evidence for sufficient standing genetic variation in natural populations of Cnidarians and therefore the potential for these populations to adapt to the changing climate. Corals already exhibit substantial variation in bleaching responses, which suggests genetic variation in bleaching thresholds (Smith-Keune & Van Oppen, 2006; Riegl *et al.*, 2011; Howells *et al.*, 2013). However, very few studies have quantified this genetic variation in natural populations and even fewer have used this variation to estimate of heritability of adaptive traits. Therefore, more studies need to focus on the heritable variation across populations by taking advantage of evolutionary quantitative genetics, similar to methods presented in Chapters 2 and 3. We currently lack enough empirical information needed to predict evolutionary responses for multiple host traits, such as growth, larval mortality, and bleaching responses (Donner *et al.*, 2005; Webster *et al.*, 2017). Through genomic studies and population genetic surveys, we can use these methods to determine which coral species have the capacity to adapt, and which populations globally and regionally have enough genetic variation for selection to act on. Additionally, more studies need to focus on whether variation in thermal tolerance is genetically determined across multiple coral species. Linking genes and molecular process to thermal tolerance can be challenging due to the diverse genetic repertoire, but Chapter 2 and 3 highlight genomewide association studies that pinpoint loci significantly associated with thermal tolerance. Using just a few thousand SNPs, we were able to find significant associations and begin to characterize the functional role of thermal tolerance. However, this is only one population and one species in the Caribbean, limiting our generalizations to other species and populations found in very different geographic locations. While other studies show genetic differentiation between populations of corals and possible candidate genes for adaptive responses in corals, we are only just beginning to understand selective responses across the coral genome and possible adaptive mechanisms that may increase bleaching thresholds (Kenkel *et al.*, 2013; Lundgren *et al.*, 2013; Bay & Palumbi, 2014).

While acclimation may be beneficial in the short-term for some reef species, it is still unknown if there are any potential costs to the coral host or its associated symbiont partners. Phenotypic plasticity may be energetically costly and therefore may limit the ability of certain phenotypes to become fixed within a population (Dewitt *et al.*, 1998; Relyea, 2002). Therefore, more studies need to focus on the costs and limits to acclimation, specifically the development and metabolic changes that might take place, types of signaling between the host and its symbiotic partner, and the genetic costs. Determining these responses will not only help predict the acclimation capacity of coral species, but will also determine which species are at a clear disadvantage. In addition, we need to explore a range of acclimation temperatures as well as differentiate the impacts of timing and magnitude of acclimation periods. Differences in acclimation capacity could be due to differing periods of acclimation (10 days versus 12 months), as well as an effect of the coral species and location studied (*Acropora* versus *Porites* species; Caribbean versus Pacific Ocean) (Middlebrook *et al.*, 2008; Dimond *et al.*, 2012; Edmunds, 2014; Bay & Palumbi, 2015; Barshis *et al.*, 2018; Gibbin *et al.*, 2018). While there is evidence for acclimatization in the field and acclimation in the lab experiments, it is still uncertain whether short-term (1-2 week) or long-term (1-2 years) acclimation provides any benefit for coral thermal tolerance and whether this mechanism of bleaching resistance occurs in every coral species. Lastly, while acclimation is known to provide tolerance within a coral's lifetime, the ability for trans-generational acclimation has been relatively unexplored. Parental thermal history has been shown to alter offspring thermal tolerance in other invertebrates, and one study on *Pocillopora damicornis* brooding adults and larvae showed effects on growth and respiration across generations (Donelson *et al.*, 2012; Putnam & Gates, 2015). Further studies are needed to determine if these non-genetic changes through acclimation can facilitate rapid phenotypic changes across populations.

As coral reefs continue to decline, more comparative studies are needed to pinpoint corals that have mechanisms to combat warming temperatures and other anthropogenic stressors, as well as those that do not. We need to compare across morphologies, life history traits, geographic locations, and across multiple experimental

conditions in order to determine what conservation actions need to take place (i.e. assisted evolution, restoration of certain reefs, resilience-based management, etc.) (van Oppen *et al.*, 2015, 2017; Hoegh-Guldberg *et al.*, 2018; Mcleod *et al.*, 2019). Additionally, we need to systematically determine what scientific questions have yet to be explored in terms of bleaching mechanisms and recovery/resistance processes and which should be a priority for management and restoration efforts around the world (Webster *et al.*, 2017; Hoegh-Guldberg *et al.*, 2018). Coral reefs provide a wealth of ecosystem services, providing more than $11 billion annually, so it is imperative we find solutions to preserve their diversity as well as overall ecosystem function. The data presented in this dissertation provide novel insights into the adaptive and acclimatory capacity of coral reef species, information that can help find species and reef sites to prioritize in conservation efforts. Specifically, if we continue to find populations that exhibit enough genetic variation, conservation actions can be put in place to protect and preserve the diversity of reef habitats and thus the genetic variation in thermal tolerance. Genetic and genomic data can uniquely help influence policies and management decisions for coral reef ecosystems, but this data needs to be communicated effectively so that urgent action can take place.

References

Anderson DA, Walz ME, Weil E, Smith MC (2016) RNA-Seq of the Caribbean reef-building coral Orbicella faveolata (Scleractinia- Merulinidae) under bleaching and disease stress expands models of coral innate immunity. *PeerJ*, **4:e1616**, DOI 10.7717/peerj.1616.

Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR (2013) Genomic basis for coral resilience to climate change. *PNAS*, **110**, 1387–1392.

Barshis DJ, Birkeland C, Toonen RJ, Gates RD, Stillman JH (2018) High-frequency temperature variability mirrors fixed differences in thermal limits of the massive coral Porites lobata . *The Journal of Experimental Biology*, **221**, jeb188581.

Bay RA, Palumbi SR (2014) Multilocus adaptation associated with heat resistance in reef-building corals. *Current Biology*, **24**, 2952–2956.

Bay RA, Palumbi SR (2015) Rapid acclimation ability mediated by transcriptome changes in reef-building corals. *Genome Biology and Evolution*, **7**, 1602–1612.

Bellantuono AJ, Granados-Cifuentes C, Miller DJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2012) Coral thermal tolerance: tuning gene expression to resist thermal stress. **7**, e50685.

Bellis ES, Howe DK, Denver DR (2016) Genome-wide polymorphism and signatures of selection in the symbiotic sea anemone Aiptasia. *BMC Genomics*, **17**, 1–14.

Boulotte NM, Dalton SJ, Carroll AG, Harrison PL, Putnam HM, Peplow LM, van Oppen MJ (2016) Exploring the Symbiodinium rare biosphere provides evidence for symbiont switching in reef-building corals. *The ISME Journal*, **10**, 1–9.

Cunning R, Silverstein RN, Baker AC (2015) Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20141725.

Cunning R, Bay RA, Gillette P, Baker AC, Traylor-Knowles N (2018) Comparative analysis of the Pocillopora damicornis genome highlights role of immune system in coral evolution. *Scientific Reports*, **8**, 1–10.

Cyr DM, Langer T, Douglas MG (1994) DnaJ-like proteins: molecular chaperones and specific regulators of Hsp70. *Trends in Biochemical Science*, **19**, 176–181.

Dewitt TJ, Sih A, Wilson DS (1998) DeWitt TREE 1998.pdf. *TREE*, **13**, 77–81.

Dimond JL, Holzman BJ, Bingham BL (2012) Thicker host tissues moderate light stress

in a cnidarian endosymbiont. *Journal of Experimental Biology*, **215**, 2247–2254.

Dixon GB, Davies SW, Aglyamova GV, Meyer E, Bay LK, Matz MV (2015) Genomic determinants of coral heat tolerance across latitudes. **348**, 2014–2016.

Dixon GB, Bay LK, Matz M V (2016) Evolutionary Consequences of DNA Methylation in a Basal Metazoan. *Molecular Biology and Evolution*, **33**, 2285–2293.

Donelson JM, Munday PL, McCormick MI, Pitcher CR (2012) Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change*, **2**, 30–32.

Donner SD, Skirving WJ, Little CM, Oppenheimer M, Hoegh-Gulberg O (2005) Global assessment of coral bleaching and required rates of adaptation under climate change. *Global Change Biology*, **11**, 2251–2265.

Dziedzic K, Elder H, Tavalire H, Meyer E (2019) Heritable variation in bleaching responses and its functional genomic basis in reef-building corals (Orbicella faveolata. *Molecular Ecology*, 1–16.

Edmunds PJ (2014) Is acclimation beneficial to scleractinian corals, Porites spp.? *Marine Biology*, **161**, 1531–1542.

Gates RD, Edmunds PJ (1999) The Physiological Mechanisms of Acclimatization in Tropical Reef Corals. *American Zoologist*, **39**, 30–43.

Gibbin EM, Krueger T, Putnam HM, Barott KL, Bodin J, Gates RD, Meibom A (2018) Short-Term Thermal Acclimation Modifies the Metabolic Condition of the Coral Holobiont. *Frontiers in Marine Science*, **5**, 1–11.

Hoegh-Guldberg O, Kennedy E V., Beyer HL, McClennen C, Possingham HP (2018) Securing a Long-term Future for Coral Reefs. *Trends in Ecology and Evolution*, **33**, 936–944.

Howells EJ, Berkelmans R, van Oppen MJH, Willis BL, Bay LK (2013) Historical thermal regimes define limits to coral acclimatization. *Ecology*, **94**, 1078–1088.

Jones A, Berkelmans R (2010) Potential costs of acclimatization to a warmer climate: growth of a reef coral with heat tolerant vs. sensitive symbiont types. *PLoS ONE*, **5**, e10437.

Kenkel CD, Bay LK (2017) Novel transcriptome resources for three scleractinian coral species from the Indo-Pacific. *GigaScience*, **6**, 1–4.

Kenkel C, Matz M V (2016) Enhanced gene expression plasticity as a mechanism of adaptation to a variable environment in a reef-building coral. *bioRxiv*, **1**, 059667.

Kenkel CD, Meyer E, Matz M V. (2013) Gene expression under chronic heat stress in populations of the mustard hill coral (Porites astreoides) from different thermal environments. *Molecular Ecology*, **22**, 4322–4334.

Kenkel CD, Setta SP, Matz M V (2015) Heritable differences in fitness-related traits among populations of the mustard hill coral, Porites astreoides. *Heredity*, **115**, 509–516.

Kirk NL, Howells EJ, Abrego D, Burt JA, Meyer E (2018) Genomic and transcriptomic signals of thermal tolerance in heat-tolerant corals (Platygyra daedalea) of the Arabian/Persian Gulf. *Molecular Ecology*, **27**, 5180–5194.

Kitchen SA, Crowder CM, Poole AZ, Weis VM, Meyer E (2015) De Novo Assembly and Characterization of Four Anthozoan (Phylum Cnidaria) Transcriptomes. *G3: Genes, Genomes, Genetics*, **5**, 2441–2452.

Li Y, Liew YJ, Cui G et al. (2018) DNA methylation regulates transcriptional homeostasis of algal endosymbiosis in the coral model Aiptasia. *Science Advances*, **4**, 1–11.

Louis YD, Bhagooli R, Kenkel CD, Baker AC, Dyall SD (2017) Gene expression biomarkers of heat stress in scleractinian corals: Promises and limitations. *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology*, **191**, 63–77.

Lundgren P, Vera JC, Peplow L, Manel S, van Oppen MJH (2013) Genotype environment correlations in corals from the Great Barrier Reef. *BMC Genetics*, **14**, 1.

Mansour TA, Rosenthal JJC, Brown CT, Roberson LM (2016) Transcriptome of the Caribbean stony coral Porites astreoides from three developmental stages. *GigaScience*, **5**, 1–6.

Matthews JL, Crowder CM, Oakley CA et al. (2017) Optimal nutrient exchange and immune responses operate in partner specificity in the cnidarian-dinoflagellate symbiosis. *Proceedings of the National Academy of Sciences*, **114**, 201710733.

Matthews JL, Oakley CA, Lutz A et al. (2018) Partner switching and metabolic flux in a model cnidarian–dinoflagellate symbiosis. *Proceedings of the Royal Society B: Biological Sciences*, **285**, 1–10.

Mcleod E, Anthony KRN, Mumby PJ et al. (2019) The future of resilience-based management in coral reef ecosystems. *Journal of Environmental Management*, **233**, 291–301.

Medina M, Hannah B, Morrison C et al. (2011) Orbicella faveolata Genome Project. *http://montastraea.psu.edu/genome/*.

Meyer E, Aglyamova G V, Wang S et al. (2009a) Sequencing and de novo analysis of a coral larval transcriptome using 454 GSFlx. *BMC genomics*, **10**, 219.

Meyer E, Davies S, Wang S, Willis BL, Abrego D (2009b) Genetic variation in responses to a settlement cue and elevated temperature in the reef-building coral Acropora millepora. *Mar Ecol Prog Ser*, **392**, 81–92.

Middlebrook R, Hoegh-Guldberg O, Leggat W (2008) The effect of thermal history on the susceptibility of reef-building corals to thermal stress. *Journal of Experimental Biology*, **211**, 1050–1056.

Mitton JB (1997) *Selection in Natural Populations*.

Moya A, Ganot P, Furla P, Sabourault C (2012) The transcriptomic response to thermal stress is immediate, transient and potentiated by ultraviolet radiation in the sea anemone Anemonia viridis. *Molecular Ecology*, **21**, 1158–1174.

van Oppen MJH, Oliver JK, Putnam HM, Gates RD (2015) Building coral reef resilience through assisted evolution. *Proceedings of the National Academy of Sciences*, **112**, 1–7.

van Oppen MJH, Gates RD, Blackall LL et al. (2017) Shifting paradigms in restoration of the world's coral reefs. *Global Change Biology*, **23**, 3437–3448.

Palumbi SR, Barshis DJ, Traylor-Knowles N, Bay RA (2014) Mechanisms of reef coral resistance to future climate change. *Science*, **344**, 895–898.

Parkinson JE, Devlin-durante EBMK, Lustic C et al. (2018) Extensive transcriptional variation poses a challenge to thermal stress biomarker development for endangered corals. *Molecular Ecology*, 1103–1119.

Pickart CM (2001) Mechanisms underlying ubiquitination. *Annual Review of Biochemistry*, **70**, 503–33.

Polato NR, Vera JC, Baums IB (2011) Gene discovery in the threatened elkhorn coral: 454 sequencing of the Acropora palmata transcriptome. *PLoS ONE*, **6**, e28634–e28634.

Putnam HM, Gates RD (2015) Preconditioning in the reef-building coral Pocillopora damicornis and the potential for trans-generational acclimatization in coral larvae under future climate change conditions. *Journal of Experimental Biology*, **218**, 2365–2372.

ReFuGe 2020 Consortium (2017) The ReFuGe 2020 Consortium—using "omics" approaches to explore the adaptability and resilience of coral holobionts to environmental change. *Frontiers in Marine Science*, **2**.

Reitzel AM, Herrera S, Layden MJ, Martindale MQ, Shank TM (2013) Going where traditional markers have not gone before: utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics. *Molecular Ecology*, **22**, 2953–2970.

Relyea RA (2002) Costs of Phenotypic Plasticity. *The American Naturalist*, **159**, 272–282.

Riegl BM, Purkis SJ, Al-Cibahy AS, Abdel-Moati MA, Hoegh-Guldberg O (2011) Present limits to heat-adaptability in corals and population-level responses to climate extremes. *PLoS ONE*, **6**, e24802–e24802.

Rouzé H, Lecellier GJ, Pochon X, Torda G, Berteaux-Lecellier V (2019) Unique Symbiodiniaceae clade signature of coral colonies revealed through spatio-temporal survey of three coral species in Moorea (French Polynesia). *Submitted*, 1–11.

Ruiz-Jones LJ, Palumbi SR (2017) Tidal heat pulses on a reef trigger a fine-tuned transcriptional response in corals to maintain homeostasis. *Science Advances*, **3**, 1–10.

Sabourault C, Ganot P, Moya A, Furla P (2012) Endosymbiosis drives transcriptomic adjustments and genomic adaptations in cnidarians. In: *Proceedings of the 12th International Coral Reef Symposium*.

Sellis D, Kvitek DJ, Dunn B, Sherlock G, Petrov DA (2016) Heterozygote advantage is a common outcome of adaptation in Saccharomyces cerevisiae. *Genetics*, **203**, 1401–1413.

Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, Fujie M (2011) Using the Acropora digitifera genome to understand coral responses to environmental change. *Nature*, **476**, 320–323.

Shinzato C, Inoue M, Kusakabe M (2014) A snapshot of a coral "holobiont": A transcriptome assembly of the scleractinian coral, Porites, captures a wide variety of genes from both the host and symbiotic zooxanthellae. *PLoS ONE*, **9**.

Silverstein RN, Cunning R, Baker AC (2015) Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Global Change Biology*, **21**, 236–249.

Smith-Keune C, Van Oppen M (2006) Genetic structure of a reef-building coral from thermally distinct environments on the Great Barrier Reef. *Coral reefs*, **25**, 493–502.

Snyder MJ, Rossi S (2004) Stress protein (HSP70 family) expression in intertidal benthic organisms: the example of Anthopleura elegantissima (Cnidaria: Anthozoa)*. *Scientia Marina*, **68**, 155–162.

Tomanek L, Sanford E (2003) Heat-Shock Protein 70 (Hsp70) as a Biochemical Stress Indicator : an Experimental Field Test in Two Congeneric Intertidal Gastropods (Genus: Tegula). *Biological Bulletin*, **205**, 276–284.

Tomanek L, Somero GN (2002) Interspecific- and acclimation-induced variation in levels of heat-shock proteins 70 (hsp70) and 90 (hsp90) and heat-shock transcription factor-1 (HSF1) in congeneric marine snails (genus Tegula): implications for regulation of hsp gene expression. *The Journal of experimental biology*, **205**, 677–85.

Traylor-Knowles N, Granger BR, Lubinski TJ et al. (2011) Production of a reference transcriptome and transcriptomic database (PocilloporaBase) for the cauliflower coral, Pocillopora damicornis. *BMC Genomics*, **12**, 585.

Voolstra CR, Schnetzer J, Peshkin L, Randall CJ, Szmant AM, Medina M (2009) Effects of temperature on gene expression in embryos of the coral Montastraea faveolata. *BMC genomics*, **10**, 627.

Voolstra CR, Li Y, Liew YJ et al. (2017) Comparative analysis of the genomes of Stylophora pistillata and Acropora digitifera provides evidence for extensive differences between species of corals. 1–14.

Webster MS, Colton MA, Darling ES, Armstrong J, Pinsky ML, Knowlton N, Schindler DE (2017) Who Should Pick the Winners of Climate Change? *Trends in Ecology and Evolution*, **32**, 167–173.

Weis VM (2010) The susceptibility and resilience of corals to thermal stress: adaptation, acclimatization or both? *Molecular Ecology*, **19**, 1515–1517.

Wright RM, Kenkel CD, Dunn CE, Shilling EN, Bay LK, 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**, 1–13.

Ying H, Cooke I, Sprungala S et al. (2018) Comparative genomics reveals the distinct evolutionary trajectories of the robust and complex coral lineages. *Genome biology*, **19**, 175.

APPENDIX A: A universal Symbiodiniaceae primer for quantitative PCR (qPCR) and its application for symbiont quantification

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Abstract

To determine how coral reefs may respond to the changing climate, we need to monitor and assess the abundance of algal endosymbionts in response to a variety of environmental stressors. Current quantitative PCR techniques use species (or lineage)-specific Symbiodiniaceae primers to evaluate presence/absence and quantity of algal symbionts. While this approach highlights specific associations of symbionts and hosts, it may miss additional background symbionts and mixed symbiont communities. To address this issue, we describe a new quantitative PCR (qPCR) primer set that estimates the total amount of symbiont cells present in tissue samples or in culture, regardless of Symbiodiniaceae species or strains. Our universal primer was developed based on multiple sequence alignments of the cp23S-rDNA locus from multiple Symbiodiniaceae species. We identified regions that were sufficiently conserved to design primers suitable for qPCR. The primer set was highly efficient (>98%) with cultured Symbiodiniaceae representing 7 distinct species (*Symbiodinium pilosum, Symbiodinium tridacnidorum, Brevicolum minutum, Cladocopium goreaui, Durusdinium trenchii, Effrenium voratum, Fugacium kawagutii*) and accurately quantified total symbiont cells present with known concentrations of mixed symbiont cultures. Our primer is a precise, high-throughput tool for quantifying total amounts of Symbiodiniaceae species. Our method improves upon existing quantitative measurements of Symbiodiniaceae communities and may offer insights into how endosymbiont communities change over time.

Introduction

Coral reef communities are productive and successful due to an important symbiosis with marine algae. This symbiosis between corals (Scleractinia) and their photosynthetic endosymbionts (Symbiodiniaceae spp.) provide the foundation for coral reef ecosystems, contributing to the diversity and complexity of these ecosystems (Davy *et al.*, 2012; Muller-Parker *et al.*, 2015). Symbiodiniaceae provide their host with energy and food that fuels calcification and reef formation as well as reproduction while the host protects and provides essential nutrients for photosynthesis (Muscatine *et al.*, 1981; Muller-Parker *et al.*, 2015). In return, the coral host protects the algal symbiont and

provides essential inorganic nutrients for photosynthesis. Specific combinations of coral hosts and symbionts have been documented for a variety of coral species, but the dynamics that make up this relationship are still poorly understood (Rowan & Powers, 1991; Baker, 2003; LaJeunesse *et al.*, 2004; Thornhill *et al.*, 2011). Recent evidence shows that coral harbor diverse species of symbionts (Baker, 2003; Silverstein *et al.*, 2012; Cunning *et al.*, 2015a). In addition, in some species these communities can change over time due to seasonal influences of temperature, salinity, and light exposure (Jones *et al.*, 2008; Bellantuono *et al.*, 2012; Cunning *et al.*, 2015a; Silverstein *et al.*, 2015). Varying associations may allow for differences in thermal tolerance (Rowan *et al.*, 1997; Baker *et al.*, 2004; Rowan, 2004; Berkelmans & van Oppen, 2006; Jones & Berkelmans, 2010; Cunning *et al.*, 2015a; Silverstein *et al.*, 2015, 2017) and growth rates (Fitt, 2000; Cunning *et al.*, 2015b). Nevertheless, we are just beginning to appreciate the complexity of these relationships, particularly with how they are affected by environmental stressors such as ocean acidification, increased nutrient levels, and temperature as well their overall diversity (Parkinson *et al.*, 2015, 2016; LaJeunesse *et al.*, 2018).

Climate change has dramatically affected the health of these coral reef ecosystems worldwide. Temperatures in the ocean have been rising for decades, endangering the future of these ecosystems (Hoey et al., 2016; Hoegh-Guldberg, 2010; Hughes et al., 2019). As temperatures rise, the symbiosis between corals and their endosymbionts begin to break down (Brown, 1997; Weis, 2008; Davy *et al.*, 2012; Oakley & Davy, 2018). Coral bleaching, defined as the breakdown of symbiotic relationships between corals and dinoflagellates (Symbiodiniaceae *spp.*), has increased in both frequency and severity over the past few decades (Hughes *et al.*, 2017; McClanahan, 2017). Bleaching susceptibility has been documented as an effect of differences in symbiont types, as well as abundance (Rowan *et al.*, 1997; Jones *et al.*, 2008; Sampayo *et al.*, 2008). It is important to note that these communities can change over time, specifically following a natural or induced bleaching event. These changes may include symbiont shuffling, adjusting the abundance of major species, or symbiont switching, changing symbiont species to readily available or favorable types (Baker *et al.*, 2004; Jones *et al.*, 2008; Cunning *et al.*, 2015a; Silverstein *et al.*, 2015). In fact, studies have shown that switching symbiont types after a

bleaching event can increase their tolerance to successive events to an extent (Berkelmans & van Oppen, 2006; Silverstein *et al.*, 2015). However, these changes may not take place in every scleractinian coral species, and therefore it is imperative to understand how this relationship may adapt to future conditions, especially in more specific host-symbiont associations. To predict the fate of corals in a warming climate, scientists aim to understand how their thermal capacity changes over time, specifically examining how the symbiotic relationship is maintained overtime.

To assess how corals are responding to stress and how their symbiotic partner is affected, methods of symbiont quantification have been developed. Currently, individual cell counts, symbiont auto-fluorescence, direct sequencing, and quantitative PCR (qPCR) methods are used to evaluate symbiont species as well as overall abundance. Manual cell counts using a hemocytometer have been a historic method in determining the density of cells within cultures or tissue samples (e.g. Fitt *et al.*, 2000; Guillard & Sieracki, 2005). In recent years, more detailed and precise approaches have been developed, based on fluorescence and DNA sequencing. Fluorescence microscopy, using blue light excitation-emission to evaluate cell abundance within a sample, demonstrates direct correlation between fluorescence intensity and number of symbiont cells (Bellis & Denver, 2017). Additionally, flow cytometry and automated cell counters have allowed for rapid counting of symbiont cells in culture and within the host (Krediet *et al.*, 2015; Takahashi, 2018). Fluorescence in-situ hybridization (FISH) has also been used to quantify mixed populations of symbionts and has been shown to match measurements obtained from qPCR (Loram *et al.*, 2007). A more recent study combined FISH with flow cytometry for more precise quantification and targeting of specific symbiont genotypes (McIlroy *et al.*, 2014). Conventional PCR methods, including denaturing gradient gel electrophoresis (DGGE), restriction fragment length polymorphism (RFLP), and amplicon deep sequencing, have also been used to identify cultured and *in-hospite* Symbiodiniaceae (Rowan & Powers, 1991; Santos *et al.*, 2002; Mieog *et al.*, 2009; LaJeunesse *et al.*, 2010; Quigley *et al.*, 2014; Parkinson *et al.*, 2016; Cunning *et al.*, 2017). While these techniques identify specific symbiont species, they provide no quantitative estimates of symbiont cells within the host. However, with the introduction of qPCR technology and

specific markers for symbiont identification, studies have been able to identify and quantify densities of major species as well as background symbiont communities within coral species (Correa *et al.*, 2009; Mieog *et al.*, 2009; Silverstein *et al.*, 2012). These quantitative methods provide useful information about the specific types of symbionts present within a sample, but not all studies require such detailed insight.

Despite advances in the ability to detect and report Symbiodiniaceae abundances, there is no universal method for quantifying most to all Symbiodiniaceae species present within a sample. This information can be beneficial in understanding how communities change over time with respect to various environmental stressors regardless of complement type. Here, we describe a universal qPCR primer set that amplifies the chloroplast 23S rDNA gene across all tested species of Symbiodiniaceae. To ensure our primer adequately amplified all major Symbiodiniaceae species, we tested the primer on cultured Symbiodiniaceae in serial dilutions. We also mixed species in known quantities to test the ability of the primer to amplify varying densities of symbiont cells.

Materials and Methods

Sample Collection and Symbiodiniaceae Sources

Cultured Symbiodiniaceae species (*Symbiodinium pilosum, Symbiodinium tridacnidorum, Brevicolum minutum, Cladocopium goreaui, Durusdinium trenchii, Effrenium voratum, Fugacium kawagutii*) were collected from stock cultures at Oregon State University (Table A1). All cultures were Sanger sequenced using the ITS2 marker to validate species identity prior to quantitative PCR (Hume *et al.*, 2018). For cultured and isolated Symbiodiniaceae, cells were first counted using a hemocytometer. Mixes of Symbiodiniaceae cell cultures were made based on manual cell counts and 50:50, 10:90 and 90:10 concentrations were made up in following combinations: species ITS2 types A3 and B1; B1 and A3 for all three concentrations. For all samples, we standardized the amount of symbiont cells in each sample to ensure similar concentrations. Genomic DNA was extracted using the Omega bio-tek E.Z.N.A. Tissue DNA Kit (Omega bio-tek, Norcross, GA) with addition of glass beads to disrupt cell walls. We quantified DNA

concentrations with fluorescence measurements using a Spectrofluorometer.

Quantitative PCR

The chloroplast 23S rDNA locus was used to generate a universal primer sequence. We developed our primers using multiple sequence alignments of the cp23S-rDNA locus from multiple Symbiodiniaceae species (https://www.auburn.edu/~santosr/sequencedatasets.htm). We aligned sequences using CLUSTAL version 2.1 to identify regions that were sufficiently conserved to design primers suitable for qPCR (53-76 and 169-189 in that alignment) (Larkin *et al.*, 2007). We conducted qPCR with forward (5'- CTACCTGCATGAAACATAGAACG -3') and reverse (5'- CCCTATAAAGCTTCATAGGG -3') primer pairs.

Primer efficiency was tested on all seven species (Table A1). Genomic DNA from cultured Symbiodiniaceae was prepared in serial dilutions (100, 50, 10, 5, 1, 0.5, and 0.1 ng/μL). The primer was also tested on mixed species combinations of species ITS2 types A3:B1 and B1:A3 (as described above) to ensure detection of mixed communities at varying densities. Ratios between each set of symbionts were calculated in the following equation: symbiont 1 DNA : symbiont 2 DNA = $(2^{Ct(2)-Ct(1)})$.

Samples were amplified in duplicate qPCR reactions on an Eppendorf Realplex 4 machine using the SYBR and ROX filters. Amplifications were achieved using SensiFAST SYBR Hi-ROX master mix (Bioline, Taunton, MA), forward and reverse primers at a final concentration of 0.4 μM, and 2 μL of genomic DNA in a final volume of 15 μL. The thermal profile for each reaction consisted of an initial denaturing step of 95°C for 2 min, followed by 40 cycles of: 95°C for 5 s, annealing temperature of 60°C for 30 s, and then 72°C for 30 sec. A melt curve was used on all reactions with the following profile: 95°C for 15 s to dissociate all primers, an annealing temperature of 60°C for 15 s, followed by 40 cycles of 30 s, in which the temperature was incrementally increased 1 °C per cycle (60°C to 95°C). All samples were run using the same reaction parameters and were analyzed together.

For mixtures of Symbiodiniaceae samples, species specific primers previously developed were used to compare universal primer efficiency (Mieog *et al.*, 2007; Correa

et al., 2009). For duplicate samples, C_T values were checked to ensure they did not vary by more than one unit. If C_T values varied, reactions were re-run to ensure efficient replication.

Results

Primer efficiency on Symbiodiniaceae cultures

Using known cultures of Symbiodiniaceae, we found that our universal primer amplified symbionts of all species ITS2 types (A-F). The universal primer set was >98% efficient for each of the seven species tested demonstrating its sensitivity to multiple species (Table A2).

Mixtures of DNA from symbionts in ITS2 types A3 and B1 confirmed that the primer set was able to capture mixed communities (Table A3). These mixes indicate that the presence of an additional clade did not significantly affect the efficiency of the primer set in amplifying the target DNA. Species-specific primers previously developed by Loram *et al.*, 2007 were compared with our universal primer set. Species-specific primers detected the expected Symbiodiniaceae species concentration compared to the universal primer set which quantified a larger amount of cells, indicating its ability to capture the major clade and the secondary species added to the tube.

Discussion

The purpose of this study was to create a universal method for quantifying the entire complement of Symbiodiniaceae in cnidarian species. The qPCR assay and primer set presented here demonstrate a well-optimized and highly efficient detection of multiple symbiont species living within a mixed community. This method is highly efficient for algal species and is successful in quantifying both major and background levels of Symbiodiniaceae in cultured ratios. Our quantifications with multiple species in mixed ratios show that this primer set is not species-specific, but universal in detecting all seven Symbiodiniaceae species tested in six different genera. In addition, mixed species ratios validate the practicality of this primer set. Not only does this primer set amplify symbionts in high abundance, but also is able to detect lower quantities of symbionts due

to bleaching events as previously shown (Dziedzic *et al.* 2019, Dziedzic & Meyer *in prep*). These studies use this primer set on the coral reef species *Orbicella faveolata* (known to harbor *Brevicolum minutum* and *Durusdinium trenchii* as major symbiont types, and *Fugacium kawagutii* in background types) as well as a temperate anemone *Anthopleuera elegantissima* (known to harbor *Symbiodinium muscatinei*) to quantify changes in bleaching during thermal stress (Dziedzic *et al.* 2019, Dziedzic & Meyer *in prep*). The studies show the practical use of our primer set to identify changes in symbiont communities over time in relation to various stressors like increased temperature or irradiance.

Although quantitative PCR is a high-resolution tool that has been used to detect endosymbiotic microbial communities in corals, anemones and other organisms that form symbioses with the genus Symbiodiniaceae. Strain-specific qPCR primers have previously demonstrated high levels of sensitivity, efficiency, and specificity for particular species (Mieog *et al.*, 2007, 2009; Correa *et al.*, 2009; Cunning *et al.*, 2015b; Silverstein *et al.*, 2015). However, these primers may underestimate the total Symbiodiniaceae present within the host due to this specificity. Additionally, these estimates based on strain-specific primers can include false positives for the type of symbionts present, misleading researchers on the type and density of certain species within their hosts (Quigley *et al.*, 2014). While understanding the specific associations of symbiont types within hosts is important for addressing questions of specificity and onset and maintenance of the relationship, estimating total abundance of Symbiodiniaceae allows researchers to determine how environmental factors influence the density of host communities. Our method enables more accurate estimates of entire endosymbiont communities and captures the overall density of Symbiodiniaceae species universally within a sample, making it a reliable method for examining changes in community abundance over time.

Our method of quantifying Symbiodiniaceae should enhance our understanding of mixed communities and how these communities change over time. Previous studies have asked questions about mechanisms of symbiont switching and shuffling, focusing on which symbiont type is present and how that relates to the fitness of the host (Jones *et al.*,

2008; Cunning *et al.*, 2015a; Boulotte *et al.*, 2016). While these studies have provided insights into specific partner associations and the benefits and tradeoffs, a universal primer will allow us to determine if density of the symbiont in host tissue is a factor in thermal tolerance, growth, bleaching recovery, among others (Cunning & Baker, 2012; Wiedenmann *et al.*, 2012). This method is an efficient technique for quantifying Symbiodiniaceae isolated from a wide variety of hosts from different environments and geographic locations. The primer set developed here will help our understanding of prominent and background Symbiodiniaceae communities and how the density of the symbiont community changes with respect to various environmental stressors.

Table A1. Symbiodiniaceae species and ITS2 type screened for species-specific efficiency of the universal primer using qPCR.

Species	ITS2 Type	Culture	Host	Source
Symbiodinium pilosum	A2	ZS	*Zoanthus sociatus*	Jamaica
Symbiodinium tridacnidorum	A3-Pacific	T	*Tridacna gigas*	Unknown
Symbiodinium tridacnidorum	A3-Pacific	CassE	*Cassiopeia sp.*	Unknown
Brevicolum minutum	B1	Ap2	*Aiptasia sp.*	Unknown
Brevicolum minutum	B1	CCMP2	*Aiptasia sp.*	Sargasso Sea
Cladocopium goreaui	C1	rt152	*Rhodactis osculifera*	Unknown
Durusdinium trenchii	D1a	CCMP 2556	*Orbicella faveolata*	Florida
Effrenium voratum	E1	CCMP 421 Davy	Water column	Wellington, New Zealand
Fugacium kawagutii	F1	Mv	*Montipora verrucosa*	Unknown
Fugacium kawagutii	F1	Mf8.03b	*Orbicella faveolata*	Florida
Fugacium kawagutii	F1	Pd	Unknown	Unknown

Table A2. Primer set efficiency for all cultured Symbiodiniaceae. The * indicates species used for mixed clade calculations.

Species	ITS2 Type	Culture	Primer Efficiency (%)	Slope
Symbiodinium pilosum	A2	ZS	99.55	-1.0065
Symbiodinium tridacnidorum	A3-Pacific	T	99.8	-1.0035
Symbiodinium tridacnidorum	A3-Pacific*	CassE	114.8	-0.8342
Brevicolum minutum	B1	Ap2	98.7	-1.0195
Brevicolum minutum	B1*	CCMP2	105.4	-0.929
Cladocopium goreaui	C1	rt152	112.4	-0.8552
Durusdinium trenchii	D1a	CCMP 2556	108.3	-0.8972
Effrenium voratum	E1	CCMP 421 Davy	120.1	-0.7907
Fugacium kawagutii	F1	Mv	99.7	-1.0045
Fugacium kawagutii	F1	Mf8.03b	100.4	-0.9937
Fugacium kawagutii	F1	Pd	104.5	-0.9397

Table A3. Mixed species ratios calculated from C_T values for species type specific primers and our universal primer. Ratios were calculated using the following equation: $2^{(Ct(B)-Ct(A))}$ and $2^{(Ct(A)-Ct(B))}$ for each set of mixes. Using the 1:1 ratio measurement, we corrected each set of ratios (the Relative Difference) and compared them to the expected ratio for each mix.

Type A3 Specific Primers	**Mixture**	**Species A : Species B ratio**	**Relative Difference**	**Expected ratio**
	9A : 1B	39.06	19.53	9
	1A : 1B	2	1	1
	1A : 9B	0.27	0.14	0.1
Type B1 Specific Primers	**Mixture**	**Species B : Species A ratio**	**Relative Difference**	**Expected ratio**
	9B : 1A	3.66	7.32	9
	1B : 1A	0.5	1	1
	1B : 9A	0.03	0.05	0.1
Universal Primer	**Mixture**	**Universal : Species A+B ratio**	**Relative Difference**	**Expected ratio**
	9A : 1B	15.63	1.41	1
	1A : 1B	11.08	1	1
	1A : 9B	21.20	1.91	1

References

Baker AC (2003) Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of Symbiodinium. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 661–689.

Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Corals' adaptive response to climate change. *Nature*, **430**, 741–741.

Bellantuono AJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2012) Resistance to thermal stress in corals without changes in symbiont composition. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 1100–1107.

Bellis ES, Denver DEER (2017) Natural Variation in Responses to Acute Heat and Cold Stress in a Sea Anemone Model System for Coral Bleaching. *Biological Bulletin*, **233**, 168–181.

Berkelmans R, van Oppen MJH (2006) The Role of Zooxanthellae in the Thermal Tolerance of Corals: A "Nugget of Hope" for Coral Reefs in an Era of Climate Change. *Proceedings: Biological Sciences*, **273**, 2305–2312.

Boulotte NM, Dalton SJ, Carroll AG, Harrison PL, Putnam HM, Peplow LM, van Oppen MJ (2016) Exploring the Symbiodinium rare biosphere provides evidence for symbiont switching in reef-building corals. *The ISME Journal*, **10**, 1–9.

Brown BE (1997) Coral bleaching: causes and consequences. *Coral Reefs*, **16**, S129–S138.

Correa AMS, McDonald MD, Baker AC (2009) Development of clade-specific Symbiodinium primers for quantitative PCR (qPCR) and their application to detecting clade D symbionts in Caribbean corals. *Marine biology*, **156**, 2403–2411.

Cunning R, Baker AC (2012) Excess algal symbionts increase the susceptibility of reef corals to bleaching. *Nature Climate Change*, **2**, 1–4.

Cunning R, Silverstein RN, Baker AC (2015a) Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20141725.

Cunning R, Gillette P, Capo T, Galvez K, Baker AC (2015b) Growth tradeoffs associated with thermotolerant symbionts in the coral Pocillopora damicornis are lost in warmer oceans. *Coral Reefs*, **34**, 155–160.

Cunning R, Gates RD, Edmunds PJ (2017) Using high-throughput sequencing of ITS2 to describe Symbiodinium metacommunities in St. John, US Virgin Islands . *PeerJ*, **5**,

e3472.

Davy SK, Allemand D, Weis VM (2012) Cell Biology of Cnidarian-Dinoflagellate Symbiosis. *Microbiology and Molecular Biology Reviews*, **76**, 229–261.

Fitt WK (2000) Cellular growth of host and symbiont in a cnidarian-zooxanthellar symbiosis. *The Biological Bulletin*, **198**, 110–120.

Fitt WK, McFarland FK, Warner ME (2000) Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnology and Oceanography*, **45**, 677–685.

Guillard R, Sieracki M (2005) *Counting cells in cultures with the light microscope In: Andersen R, editor. Algal culturing techniques*. 239–267 pp.

Hughes TP, Kerry JT, Álvarez-Noriega M et al. (2017) Global warming and recurrent mass bleaching of corals. *Nature*, **543**, 373–377.

Hume BCC, Ziegler M, Poulain J et al. (2018) An improved primer set and amplification protocol with increased specificity and sensitivity targeting the Symbiodinium ITS2 region. *PeerJ*, **6**, e4816.

Jones A, Berkelmans R (2010) Potential costs of acclimatization to a warmer climate: growth of a reef coral with heat tolerant vs. sensitive symbiont types. *PLoS ONE*, **5**, e10437.

Jones AM, Berkelmans R, van Oppen MJ., Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 1359–1365.

Krediet CJ, DeNofrio JC, Caruso C, Burriesci MS, Cella K, Pringle JR (2015) Rapid, precise, and accurate counts of symbiodinium cells using the guava flow cytometer, and a comparison to other methods. *PLoS ONE*, **10**, 1–19.

LaJeunesse TC, Thornhill DJ, Cox EF, Stanton FG (2004) High diversity and host specificity observed among symbiotic dinoflagellates in reef coral communities from Hawaii. *Coral reefs*, **23**, 596–603.

LaJeunesse TC, Pettay DT, Sampayo EM et al. (2010) Long-standing environmental conditions, geographic isolation and host-symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus Symbiodinium. *Journal of Biogeography*, **37**, 785–800.

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. *Current Biology*, **28**, 2570-2580.e6.

Larkin MA, Blackshields G, Brown NP et al. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, **23**, 2947–2948.

Loram JE, Boonham N, O'Toole P, Trapido-Rosenthal HG, Douglas AE (2007) Molecular Quantification of Symbiotic Dinoflagellate Algae of the Genus Symbiodinium. *The Biological Bulletin*, **212**, 259–268.

McClanahan TR (2017) Changes in coral sensitivity to thermal anomalies. *Marine Ecology Progress Series*, **570**, 71–85.

McIlroy S, Smith G, Geller J (2014) FISH-Flow : a quantitative molecular approach for describing mixed clade communities of Symbiodinium. *Coral Reefs*, **33**, 157–167.

Mieog JC, Oppen MJH, Cantin NE, Stam WT, Olsen JL (2007) Real-time PCR reveals a high incidence of Symbiodinium clade D at low levels in four scleractinian corals across the Great Barrier Reef: implications for symbiont shuffling. *Coral reefs*, **26**, 449–457.

Mieog JC, van Oppen MJH, Berkelmans R, Stam WT, Olsen JL (2009) Quantification of algal endosymbionts (Symbiodinium) in coral tissue using real-time PCR. *Molecular Ecology Resources*, **9**, 74–82.

Muller-Parker G, D'Elia CF, Cook CB (2015) Interactions Between Corals and Their Symbiotic Algae. In: *Coral Reefs in the Anthropocene*, In: Birkel edn, pp. 99–116. Springer, Dordrecht.

Muscatine L, McCloskey LR, Marian RE (1981) Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. *Limnology and OceanographyOceanography*, **26**, 601–611.

Oakley CA, Davy SK (2018) Cell Biology of Coral Bleaching. In: *Coral Bleaching*, pp. 189–211.

Parkinson JE, Banaszak AT, Altman NS, LaJeunesse TC, Baums IB (2015) Intraspecific diversity among partners drives functional variation in coral symbioses. *Scientific reports*, **5**, 12.

Parkinson JE, Baumgarten S, Michell CT, Baums IB, LaJeunesse TC, Voolstra CR (2016) Gene Expression Variation Resolves Species and Individual Strains among Coral-Associated Dinoflagellates within the Genus Symbiodinium. *Genome biology and evolution*, **8**, 665–680.

Quigley KM, Davies SW, Kenkel CD, Willis BL, Matz M V., Bay LK (2014) Deep-sequencing method for quantifying background abundances of Symbiodinium types: Exploring the rare Symbiodinium biosphere in reef-building corals. *PLoS ONE*, **9**.

Rowan R (2004) Coral bleaching: Thermal adaptation in reef coral symbionts. *Nature*,

430, 742.

Rowan R, Powers DA (1991) Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae). *Marine ecology progress series Oldendorf*.

Rowan R, Knowlton N, Baker A, Jara J (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature*, **388**, 265–269.

Sampayo EM, Ridgway T, Bongaerts P, Hoegh-Guldberg O (2008) Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. *PNAS*, **105**, 10444–10449.

Santos SR, Taylor DJ, Kinzie Robert A III, Sakaj K, Coffroth MA (2002) Evolution of length variation and heteroplasmy in the chloroplast rDNA of symbiotic dinoflagellates (Symbiodinium, Dinophyta) and a novel insertion in the universal core region of the large subunit rDNA. *Phycologia*, **41**, 311–318.

Silverstein RN, Correa AMS, Baker AC (2012) Specificity is rarely absolute in coral-algal symbiosis: implications for coral response to climate change. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 2609–2618.

Silverstein RN, Cunning R, Baker AC (2015) Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Global Change Biology*, **21**, 236–249.

Silverstein RN, Cunning R, Baker AC (2017) Tenacious D: Symbiodinium in clade D remain in reef corals at both high and low temperature extremes despite impairment. *The Journal of Experimental Biology*, **220**, 1192–1196.

Takahashi T (2018) Applicability of Automated Cell Counter with a Chlorophyll Detector in Routine Management of Microalgae. *Scientific Reports*, **8**, 1–12.

Thornhill DJ, Rotjan RD, Todd BD et al. (2011) A connection between colony biomass and death in Caribbean reef-building corals. *PLoS ONE*, **6**, e29535–e29535.

Weis VM (2008) Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *The Journal of experimental biology*, **211**, 3059–3066.

Wiedenmann J, D'Angelo C, Smith EG, Hunt AN, Legiret F-E, Postle AD, Achterberg EP (2012) Nutrient enrichment can increase the susceptibility of reef corals to bleaching. *Nature Climate Change*, **2**, 1–5.

APPENDIX B - Supplementary Data for Chapter 3

Table B1. Genes differentially expressed when testing for the interaction effect (anemone aggregation × treatment), aggregation effect only in heat stress samples, and genes showing varying patterns of expression in heat-susceptible (HS) vs. heat-tolerant (HT) anemones. Genes presented here are unannotated, which is a subset of all 588 DEGs. Annotated genes are presented in Table 3.3.

Effect	Transcript Name	Gene Description	pvalue
Interaction	comp101753_c0	Unknown	1.55E-04
	comp106516_c0	Unknown	1.93E-09
	comp11364_c0	Unknown	4.15E-04
	comp12280_c0	Unknown	4.96E-05
	comp12693_c0	Unknown	6.36E-05
	comp13356_c0	Unknown	1.29E-03
	comp1403_c0	Unknown	1.34E-04
	comp147050_c0	Unknown	7.24E-06
	comp1535_c0	Unknown	3.89E-04
	comp15705_c0	Unknown	1.81E-03
	comp165892_c0	Unknown	6.69E-04
	comp1670_c0	Unknown	2.11E-07
	comp17422_c0	Unknown	3.85E-07
	comp20297_c0	Unknown	6.44E-04
	comp205875_c0	Unknown	5.29E-05
	comp20805_c0	Unknown	4.44E-06
	comp21165_c0	Unknown	1.75E-04
	comp22024_c0	Unknown	6.24E-04
	comp2335_c0	Unknown	4.58E-04
	comp2568_c0	Unknown	6.77E-05
	comp27464_c0	Unknown	2.53E-05
	comp274677_c0	Unknown	1.06E-04
	comp277089_c0	Unknown	3.83E-04
	comp30702_c0	Unknown	1.51E-06
	comp31676_c0	Unknown	4.38E-04
	comp3232_c0	Unknown	1.77E-07
	comp32375_c0	Unknown	2.36E-07
	comp3766_c0	Unknown	8.45E-04
	comp3959_c0	Unknown	2.44E-08
	comp41801_c0	Unknown	2.38E-09
	comp47787_c0	Unknown	1.30E-07
	comp50793_c0	Unknown	6.05E-04
	comp51517_c0	Unknown	4.20E-03
	comp5151_c0	Unknown	7.51E-04

	comp51699_c0	Unknown	2.08E-04
	comp5235_c0	Unknown	1.71E-05
	comp55100_c0	Unknown	3.06E-04
	comp5511_c0	Unknown	1.33E-04
	comp55535_c0	Unknown	6.34E-07
	comp559_c0	Unknown	4.92E-05
	comp5705_c1	Unknown	2.70E-04
	comp6185_c0	Unknown	4.35E-04
	comp64946_c0	Unknown	1.40E-04
	comp72146_c0	Unknown	3.11E-04
	comp72193_c0	Unknown	1.45E-04
	comp75173_c0	Unknown	3.52E-01
	comp75389_c0	Unknown	4.06E-05
	comp7550_c1	Unknown	7.89E-05
	comp75734_c0	Unknown	1.31E-05
	comp781_c0	Unknown	7.43E-05
	comp79307_c0	Unknown	1.04E-04
	comp9455_c0	Unknown	1.19E-03
	comp95614_c0	Unknown	2.91E-07
	comp96018_c0	Unknown	8.35E-05
Colony only	comp10249_c0	Unknown	1.26E-05
	comp103_c1	Unknown	7.64E-04
	comp1047_c0	Unknown	1.61E-03
	comp104_c0	Unknown	2.06E-07
	comp10529_c0	Unknown	2.05E-04
	comp106837_c0	Unknown	2.80E-04
	comp107449_c0	Unknown	3.65E-06
	comp108678_c0	Unknown	1.91E-03
	comp113163_c0	Unknown	7.33E-04
	comp11658_c0	Unknown	1.48E-05
	comp117517_c0	Unknown	6.71E-04
	comp119073_c0	Unknown	2.68E-07
	comp122028_c0	Unknown	2.94E-07
	comp122_c0	Unknown	9.65E-04
	comp124334_c0	Unknown	1.60E-22
	comp126736_c0	Unknown	8.54E-04
	comp127087_c0	Unknown	9.44E-06
	comp127542_c0	Unknown	3.37E-06
	comp128136_c0	Unknown	3.32E-05
	comp1307_c1	Unknown	2.05E-03

	comp130801_c0	Unknown	2.06E-04
	comp131058_c0	Unknown	1.43E-05
	comp132313_c0	Unknown	5.00E-05
	comp132392_c0	Unknown	4.88E-04
	comp132673_c0	Unknown	2.00E-03
	comp133205_c0	Unknown	2.76E-04
	comp13356_c0	Unknown	1.29E-03
	comp1336_c0	Unknown	1.37E-05
	comp134485_c0	Unknown	2.27E-05
	comp135728_c0	Unknown	2.18E-04
	comp135923_c0	Unknown	1.13E-03
	comp13618_c0	Unknown	8.54E-04
	comp136441_c0	Unknown	1.86E-04
	comp137340_c0	Unknown	2.57E-09
	comp139983_c0	Unknown	1.29E-04
	comp1403_c0	Unknown	1.34E-04
	comp140874_c0	Unknown	9.18E-05
	comp141574_c0	Unknown	9.00E-04
	comp142187_c0	Unknown	9.08E-05
	comp14261_c0	Unknown	6.25E-04
	comp145125_c0	Unknown	1.00E-04
	comp147050_c0	Unknown	7.24E-06
	comp147050_c0	Unknown	7.24E-06
	comp149170_c0	Unknown	9.27E-04
	comp1509_c0	Unknown	1.57E-03
	comp15249_c0	Unknown	4.83E-05
	comp153789_c0	Unknown	3.84E-04
	comp155046_c0	Unknown	3.22E-07
	comp155385_c0	Unknown	1.16E-03
	comp1554_c0	Unknown	2.87E-04
	comp156591_c0	Unknown	1.35E-03
	comp15705_c0	Unknown	1.81E-03
	comp157141_c0	Unknown	7.76E-06
	comp161727_c0	Unknown	5.13E-04
	comp166649_c0	Unknown	3.58E-05
	comp167098_c0	Unknown	1.74E-03
	comp1670_c0	Unknown	2.11E-07
	comp170756_c0	Unknown	4.91E-05
	comp173772_c0	Unknown	1.40E-03
	comp173816_c0	Unknown	2.14E-06

	comp17479_c0	Unknown	8.90E-07
	comp175076_c0	Unknown	7.99E-05
	comp1765_c0	Unknown	2.96E-05
	comp177068_c0	Unknown	4.80E-05
	comp17727_c0	Unknown	1.23E-04
	comp178251_c0	Unknown	1.94E-03
	comp18080_c0	Unknown	2.51E-12
	comp185856_c0	Unknown	1.28E-03
	comp185925_c0	Unknown	1.08E-04
	comp1878_c0	Unknown	6.30E-04
	comp19043_c0	Unknown	4.45E-07
	comp190790_c0	Unknown	1.78E-04
	comp191102_c0	Unknown	5.61E-04
	comp194797_c0	Unknown	4.61E-04
	comp196184_c0	Unknown	1.03E-06
	comp1975_c0	Unknown	1.78E-04
	comp1985_c0	Unknown	1.71E-04
	comp20561_c0	Unknown	1.86E-04
	comp20757_c0	Unknown	4.43E-05
	comp20793_c0	Unknown	2.10E-02
	comp208135_c0	Unknown	1.77E-03
	comp209845_c0	Unknown	2.02E-04
	comp210734_c0	Unknown	2.51E-05
	comp211380_c0	Unknown	6.65E-04
	comp212151_c0	Unknown	4.27E-04
	comp212451_c0	Unknown	6.75E-06
	comp212791_c0	Unknown	9.32E-07
	comp216420_c0	Unknown	9.74E-05
	comp218173_c0	Unknown	7.61E-04
	comp2194_c0	Unknown	1.86E-04
	comp2197_c0	Unknown	8.20E-05
	comp219930_c0	Unknown	6.75E-04
	comp21998_c0	Unknown	1.98E-04
	comp22016_c0	Unknown	1.42E-03
	comp221_c0	Unknown	6.76E-04
	comp222_c0	Unknown	3.91E-10
	comp2232_c0	Unknown	1.10E-03
	comp22944_c0	Unknown	4.81E-04
	comp230_c0	Unknown	1.63E-05
	comp231711_c0	Unknown	1.86E-03

	comp2353_c0	Unknown	1.08E-04
	comp236867_c0	Unknown	1.34E-03
	comp23735_c0	Unknown	1.64E-04
	comp238267_c0	Unknown	6.73E-05
	comp238387_c0	Unknown	1.52E-03
	comp240017_c0	Unknown	7.26E-04
	comp244747_c0	Unknown	5.29E-04
	comp24506_c0	Unknown	1.02E-12
	comp246320_c0	Unknown	4.88E-04
	comp247463_c0	Unknown	2.75E-11
	comp24759_c0	Unknown	1.88E-03
	comp248185_c0	Unknown	6.04E-06
	comp2507_c0	Unknown	9.87E-04
	comp251815_c0	Unknown	5.52E-05
	comp26140_c0	Unknown	9.31E-04
	comp262264_c0	Unknown	6.38E-04
	comp262951_c0	Unknown	9.07E-06
	comp2637_c0	Unknown	2.50E-04
	comp2637_c0	Unknown	2.50E-04
	comp2647_c0	Unknown	1.60E-04
	comp26652_c0	Unknown	1.96E-04
	comp272119_c0	Unknown	3.70E-06
	comp273869_c0	Unknown	1.41E-03
	comp273960_c0	Unknown	1.38E-05
	comp27464_c0	Unknown	2.53E-05
	comp27469_c0	Unknown	7.61E-05
	comp275116_c0	Unknown	3.28E-05
	comp277184_c0	Unknown	1.22E-05
	comp28135_c0	Unknown	4.63E-08
	comp285527_c0	Unknown	4.35E-06
	comp2865_c0	Unknown	1.33E-03
	comp289296_c0	Unknown	2.34E-06
	comp29208_c0	Unknown	6.08E-04
	comp29230_c0	Unknown	5.78E-05
	comp2945_c0	Unknown	1.54E-05
	comp315085_c0	Unknown	2.81E-06
	comp315260_c0	Unknown	2.88E-02
	comp31582_c0	Unknown	3.92E-08
	comp316149_c0	Unknown	6.90E-04
	comp31676_c0	Unknown	4.38E-04

	comp319176_c0	Unknown	2.45E-04
	comp31927_c0	Unknown	1.33E-03
	comp3216_c0	Unknown	6.22E-04
	comp322309_c0	Unknown	8.13E-04
	comp32765_c0	Unknown	9.32E-04
	comp32909_c0	Unknown	7.41E-04
	comp330547_c0	Unknown	2.52E-04
	comp34001_c0	Unknown	1.04E-05
	comp340805_c0	Unknown	3.19E-04
	comp343111_c0	Unknown	2.23E-07
	comp34686_c0	Unknown	3.94E-05
	comp3482_c0	Unknown	3.44E-06
	comp348966_c0	Unknown	7.63E-05
	comp352069_c0	Unknown	8.83E-06
	comp35347_c0	Unknown	1.70E-03
	comp36071_c0	Unknown	1.15E-03
	comp36281_c0	Unknown	1.78E-03
	comp36558_c0	Unknown	1.10E-03
	comp36645_c0	Unknown	7.68E-05
	comp368_c1	Unknown	8.69E-10
	comp371102_c0	Unknown	1.16E-04
	comp372104_c0	Unknown	6.67E-05
	comp373645_c0	Unknown	4.80E-04
	comp3736_c0	Unknown	2.09E-04
	comp37402_c0	Unknown	1.01E-03
	comp38132_c0	Unknown	9.85E-04
	comp3813_c0	Unknown	6.50E-09
	comp3821_c0	Unknown	1.35E-06
	comp383982_c0	Unknown	3.80E-05
	comp38710_c0	Unknown	1.63E-03
	comp38737_c0	Unknown	5.77E-05
	comp388_c0	Unknown	8.35E-06
	comp38932_c0	Unknown	6.57E-04
	comp38988_c0	Unknown	4.28E-04
	comp39591_c0	Unknown	7.17E-04
	comp3959_c0	Unknown	2.44E-08
	comp40364_c0	Unknown	7.59E-04
	comp406664_c0	Unknown	9.58E-05
	comp41801_c0	Unknown	2.38E-09
	comp4202_c0	Unknown	1.43E-04

	comp4206_c0	Unknown	4.99E-08
	comp424169_c0	Unknown	6.20E-04
	comp4264_c0	Unknown	1.38E-05
	comp42803_c0	Unknown	3.85E-05
	comp43547_c0	Unknown	1.35E-07
	comp436_c0	Unknown	8.26E-05
	comp44173_c0	Unknown	8.00E-05
	comp469_c0	Unknown	5.59E-07
	comp470_c0	Unknown	6.88E-05
	comp47_c0	Unknown	1.86E-04
	comp4898_c0	Unknown	8.61E-10
	comp49959_c0	Unknown	7.42E-12
	comp50253_c0	Unknown	2.00E-04
	comp50265_c0	Unknown	1.82E-04
	comp51126_c0	Unknown	5.54E-04
	comp5128_c0	Unknown	3.22E-08
	comp5134_c0	Unknown	4.27E-04
	comp5267_c0	Unknown	5.09E-04
	comp53129_c0	Unknown	2.80E-05
	comp53610_c0	Unknown	1.92E-05
	comp54631_c0	Unknown	1.26E-04
	comp54_c0	Unknown	5.00E-04
	comp5511_c0	Unknown	1.33E-04
	comp555_c0	Unknown	3.93E-04
	comp55706_c0	Unknown	2.28E-04
	comp56006_c0	Unknown	6.90E-04
	comp56526_c0	Unknown	2.66E-04
	comp57199_c0	Unknown	4.36E-04
	comp57440_c0	Unknown	7.76E-09
	comp57568_c0	Unknown	4.14E-05
	comp600484_c0	Unknown	1.65E-03
	comp61282_c0	Unknown	9.08E-04
	comp6194_c1	Unknown	3.67E-06
	comp6215_c0	Unknown	1.42E-04
	comp62546_c0	Unknown	1.08E-04
	comp6258_c2	Unknown	1.35E-04
	comp64722_c0	Unknown	6.12E-04
	comp647_c0	Unknown	7.16E-14
	comp647_c1	Unknown	4.57E-04
	comp65029_c0	Unknown	5.47E-04

	comp65613_c0	Unknown	1.69E-06
	comp66389_c0	Unknown	8.84E-07
	comp692_c0	Unknown	3.90E-05
	comp69518_c0	Unknown	1.31E-05
	comp697_c0	Unknown	4.74E-04
	comp70162_c0	Unknown	9.95E-04
	comp7020_c0	Unknown	2.10E-04
	comp70641_c0	Unknown	1.67E-06
	comp71348_c0	Unknown	2.73E-05
	comp71991_c0	Unknown	1.71E-04
	comp72193_c0	Unknown	1.45E-04
	comp73091_c0	Unknown	2.04E-04
	comp73958_c0	Unknown	1.58E-05
	comp74235_c0	Unknown	1.26E-03
	comp74812_c0	Unknown	1.58E-04
	comp75153_c0	Unknown	8.70E-07
	comp75389_c0	Unknown	4.06E-05
	comp7550_c1	Unknown	7.89E-05
	comp75734_c0	Unknown	1.31E-05
	comp76062_c0	Unknown	8.42E-05
	comp76591_c0	Unknown	5.41E-04
	comp773_c0	Unknown	1.89E-06
	comp78705_c0	Unknown	7.39E-04
	comp79073_c0	Unknown	4.64E-08
	comp80779_c0	Unknown	4.35E-04
	comp8142_c0	Unknown	4.89E-07
	comp81927_c0	Unknown	1.74E-05
	comp8200_c0	Unknown	3.82E-04
	comp838_c0	Unknown	9.89E-11
	comp89697_c0	Unknown	5.67E-04
	comp9008_c0	Unknown	6.48E-04
	comp9156_c0	Unknown	1.15E-03
	comp92651_c0	Unknown	7.61E-18
	comp94390_c0	Unknown	5.04E-06
	comp9455_c0	Unknown	1.19E-03
	comp963_c0	Unknown	4.67E-04
	comp97598_c0	Unknown	3.08E-05
	comp99475_c0	Unknown	3.41E-04
	comp994_c0	Unknown	1.40E-03
HS vs HT	comp315260_c0	Unknown	6.71E-32

	comp445723_c0	Unknown	2.29E-03
	comp28409_c0	Unknown	3.40E-03
	comp20793_c0	Unknown	3.41E-02
	comp184609_c0	Unknown	3.12E-04
	comp235470_c0	Unknown	3.15E-04
	comp318254_c0	Unknown	3.36E-04
	comp282725_c0	Unknown	3.36E-04
	comp69338_c0	Unknown	4.03E-03
	comp533_c0	Unknown	5.33E-03
	comp277089_c0	Unknown	3.83E-04
	comp40305_c0	Unknown	1.37E-03
	comp8157_c0	Unknown	3.81E-03
	comp65492_c0	Unknown	3.75E-03
	comp10231_c0	Unknown	4.38E-04
	comp402111_c0	Unknown	3.77E-04
	comp28335_c0	Unknown	5.23E-03
	comp51517_c0	Unknown	4.20E-03
	comp23878_c0	Unknown	1.60E-03
	comp45760_c0	Unknown	1.77E-04
	comp13483_c0	Unknown	6.34E-04
	comp3099_c0	Unknown	1.85E-03
	comp23732_c0	Unknown	2.91E-03
	comp18749_c0	Unknown	2.82E-04
	comp312870_c0	Unknown	4.53E-03
	comp3133_c0	Unknown	4.24E-03
	comp268457_c0	Unknown	3.36E-04
	comp2309_c0	Unknown	3.15E-04
	comp170649_c0	Unknown	3.12E-04
	comp190790_c0	Unknown	1.78E-04
	comp229357_c0	Unknown	3.15E-04
	comp216420_c0	Unknown	9.74E-05
	comp197707_c0	Unknown	3.12E-04
	comp291925_c0	Unknown	3.36E-04
	comp293070_c0	Unknown	3.36E-04
	comp178251_c0	Unknown	1.94E-03
	comp44173_c0	Unknown	8.00E-05
	comp70182_c0	Unknown	6.09E-04
	comp756_c0	Unknown	8.66E-04

Table B2. Genes found highly correlated with thermal tolerance using WGCNA analysis. Genes presented here are unannotated, which is a subset of all 162 genes. Annotated genes are presented in Table 3.4.

Transcript Name	Gene Description	pvalue
comp4458_c0	Unknown	3.72E-03
comp93906_c0	Unknown	5.94E-03
comp6600_c0	Unknown	1.20E-02
comp7689_c0	Unknown	3.37E-02
comp32168_c0	Unknown	1.44E-02
comp52646_c0	Unknown	2.02E-01
comp107311_c0	Unknown	2.99E-02
comp73587_c0	Unknown	3.64E-02
comp69323_c0	Unknown	5.78E-02
comp5230_c0	Unknown	3.01E-02
comp6185_c0	Unknown	6.82E-02
comp48467_c0	Unknown	6.13E-02
comp12192_c0	Unknown	2.06E-01
comp4253_c0	Unknown	2.83E-03
comp8719_c0	Unknown	2.17E-02
comp690_c0	Unknown	4.14E-02
comp10991_c0	Unknown	5.08E-04
comp65119_c0	Unknown	7.40E-04
comp17144_c0	Unknown	2.64E-03
comp6733_c0	Unknown	4.01E-06
comp434_c0	Unknown	9.63E-04
comp34093_c0	Unknown	5.44E-04
comp9912_c0	Unknown	3.48E-02
comp127420_c0	Unknown	1.01E-02
comp191692_c0	Unknown	1.45E-02
comp77149_c0	Unknown	5.15E-03
comp71991_c0	Unknown	3.42E-03
comp26910_c0	Unknown	5.93E-02
comp108128_c0	Unknown	2.08E-02
comp5071_c0	Unknown	1.28E-04
comp109857_c0	Unknown	8.41E-03
comp160701_c0	Unknown	1.22E-02
comp5852_c0	Unknown	7.73E-03
comp153436_c0	Unknown	8.78E-02
comp16123_c0	Unknown	1.75E-03
comp49252_c0	Unknown	1.05E-02

comp17564_c0	Unknown	7.26E-03
comp43139_c0	Unknown	4.91E-02
comp138909_c0	Unknown	5.78E-03
comp24113_c0	Unknown	2.54E-03
comp13031_c0	Unknown	3.94E-02
comp187102_c0	Unknown	8.64E-04
comp6358_c0	Unknown	1.01E-03
comp414061_c0	Unknown	3.58E-02
comp153274_c0	Unknown	6.31E-02
comp22675_c0	Unknown	4.46E-03
comp4169_c0	Unknown	3.60E-03
comp164919_c0	Unknown	1.20E-04
comp4980_c0	Unknown	4.86E-04
comp7020_c0	Unknown	1.79E-06
comp77157_c0	Unknown	6.90E-05
comp40920_c0	Unknown	1.24E-03
comp18249_c0	Unknown	1.38E-03
comp111973_c0	Unknown	2.21E-04
comp43751_c0	Unknown	8.28E-03
comp30505_c0	Unknown	7.77E-03
comp221_c0	Unknown	1.99E-05
comp101628_c0	Unknown	9.27E-05

Combined References

Abrego D, Ulstrup KE, Willis BL, van Oppen MJH (2008) Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 2273–2282.

Agrawal AF, Stinchcombe JR (2009) How much do genetic covariances alter the rate of adaptation? *Proceedings of the Royal Society B: Biological Sciences*, **276**, 1183–1191.

Ainsworth TD, Heron SF, Ortiz JC et al. (2016) Climate change disables coral bleaching protection on the Great Barrier Reef. *Science*, **352**, 338–342.

Al-horani FA, Al-moghrabi SM, Beer D De (2003) Microsensor study of photosynthesis and calcification in the scleractinian coral, Galaxea fascicularis: active internal carbon cycle. *Journal of Experimental Marine Biology and Ecology*, **288**, 1–15.

Anderson DA, Walz ME, Weil E, Smith MC (2016) RNA-Seq of the Caribbean reef-building coral Orbicella faveolata (Scleractinia- Merulinidae) under bleaching and disease stress expands models of coral innate immunity. *PeerJ*, **4:e1616**, DOI 10.7717/peerj.1616.

Aranda M, Li Y, Liew YJ et al. (2016) Genomes of coral dinoflagellate symbionts highlight evolutionary adaptations conducive to a symbiotic lifestyle. *Scientific Reports*, **6**, 1–15.

Ayre DJ, Hughes TP, Standish RJ (1997) Genetic differentiation, reproductive mode, and gene flow in the brooding coral Pocillopora damicornis along the Great Barrier Reef, Australia. *Mar Ecol Prog Ser*, **159**, 175–187.

Babcock RC (1991) Comparative demograpgy of three species of scleractinian corals using age- and size-dependant classifications. *Ecological Monographs*, **61**, 225–244.

Baird AH, Guest JR, Willis BL (2009a) Systematic and Biogeographical Patterns in the Reproductive Biology of Scleractinian Corals. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 551–571.

Baird AH, Bhagooli R, Ralph PJ, Takahashi S (2009b) Coral bleaching: the role of the host. *Trends in ecology & evolution*, **24**, 16–20.

Baker A (2001) Reef corals bleach to survive change. *Nature*, **411**, 765–766.

Baker AC (2003) Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of Symbiodinium. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 661–689.

Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Corals' adaptive response to climate change. *Nature*, **430**, 741–741.

Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, Silliman BR (2011) The value of estuarine and coastal ecosystem services. *Ecological Monographs*, **81**, 169–193.

Barrett RDH, Schluter D (2008) Adaptation from standing genetic variation. *Trends in Ecology and Evolution*, **23**, 38–44.

Barshis DJ (2015) Genomic Potential for Coral Survival of Climate Change. In: *Coral Reefs in the Anthropocene*, pp. 133–146.

Barshis DJ, Stillman JH, Gates RD, Toonen RJ, Smith LW, Birkeland C (2010) Protein expression and genetic structure of the coral Porites lobatain an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity? *Molecular Ecology*, **19**, 1705–1720.

Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR (2013) Genomic basis for coral resilience to climate change. *PNAS*, **110**, 1387–1392.

Barshis DJ, Birkeland C, Toonen RJ, Gates RD, Stillman JH (2018) High-frequency temperature variability mirrors fixed differences in thermal limits of the massive coral Porites lobata . *The Journal of Experimental Biology*, **221**, jeb188581.

Baumgarten S, Simakov O, Esherick LY et al. (2015) The genome of Aiptasia , a sea anemone model for coral symbiosis. **112**, 11893–11898.

Baums IB, Polato NR, Xu D et al. (2013) Genotypic variation influences reproductive success and thermal stress tolerance in the reef building coral , Acropora palmata. *Coral Reefs*, **32**, 703–717.

Bay RA, Palumbi SR (2014) Multilocus adaptation associated with heat resistance in reef-building corals. *Current Biology*, **24**, 2952–2956.

Bay RA, Palumbi SR (2015) Rapid acclimation ability mediated by transcriptome changes in reef-building corals. *Genome Biology and Evolution*, **7**, 1602–1612.

Bay LK, Guérécheau A, Andreakis N, Ulstrup KE, Matz M V (2013) Gene Expression Signatures of Energetic Acclimatisation in the Reef Building Coral Acropora millepora. *PLoS ONE*, **8**, 1–10.

Bay RA, Rose NH, Logan CA, Palumbi SR (2017) Genomic models predict successful coral adaptation if future ocean warming rates are reduced. *Science Advances*, **3**, e1701413.

Bayer TT, Aranda MM, Sunagawa SS et al. (2012) Symbiodinium transcriptomes: genome insights into the dinoflagellate symbionts of reef-building corals. *PLoS*

ONE, **7**, e35269–e35269.

Bellantuono AJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2012a) Resistance to thermal stress in corals without changes in symbiont composition. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 1100–1107.

Bellantuono AJ, Granados-Cifuentes C, Miller DJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2012b) Coral thermal tolerance: tuning gene expression to resist thermal stress. **7**, e50685.

Bellis ES, Denver DEER (2017) Natural Variation in Responses to Acute Heat and Cold Stress in a Sea Anemone Model System for Coral Bleaching. *Biological Bulletin*, **233**, 168–181.

Bellis ES, Howe DK, Denver DR (2016) Genome-wide polymorphism and signatures of selection in the symbiotic sea anemone Aiptasia. *BMC Genomics*, **17**, 1–14.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society B*, **57**, 289–300.

Berkelmans R, van Oppen MJH (2006) The Role of Zooxanthellae in the Thermal Tolerance of Corals: A "Nugget of Hope" for Coral Reefs in an Era of Climate Change. *Proceedings: Biological Sciences*, **273**, 2305–2312.

Bertero E, Maack C (2018) Calcium signaling and reactive oxygen species in Mitochondria. *Circulation Research*, **122**, 1460–1478.

Bertucci A, Tambutté S (2011) A New Coral Carbonic Anhydrase in Stylophora pistillata. *Marine Biotechnology*, **13**, 992–1002.

Bertucci A, Moya A, Tambutté S, Allemand D, Supuran CT, Zoccola D (2013) Carbonic anhydrases in anthozoan corals — A review. *Bioorganic & Medicinal Chemistry*, **21**, 1437–1450.

Bhattacharya D, Agrawal S, Aranda M et al. (2016) Comparative genomics explains the evolutionary success of reef-forming corals. *eLife*, **5**, 1–26.

Bingham BL, Freytes I, Emery M, Dimond J, Muller-Parker G (2011) Aerial exposure and body temperature of the intertidal sea anemone Anthopleura elegantissima. *Invertebrate Biology*, **130**, 291–301.

Bingham BL, Dimond JL, Bingham BL (2014) Symbiotic state influences life-history strategy of a clonal cnidarian. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 1–8.

Bouchard JN, Yamasaki H (2008) Heat stress stimulates nitric oxide production in

Symbiodinium microadriaticum: A possible linkage between nitric oxide and the coral bleaching phenomenon. *Plant and Cell Physiology*, **49**, 641–652.

Boulotte NM, Dalton SJ, Carroll AG, Harrison PL, Putnam HM, Peplow LM, van Oppen MJ (2016) Exploring the Symbiodinium rare biosphere provides evidence for symbiont switching in reef-building corals. *The ISME Journal*, **10**, 1–9.

Bradley JR, Pober JS (2001) Tumor necrosis factor receptor-associated factors (TRAFs). *Oncogene*, **29**, 6482–6491.

Brown BE (1997) Coral bleaching: causes and consequences. *Coral Reefs*, **16**, S129–S138.

Brown BE, Downs CA, Dunne RP, Gibb SW (2002) Exploring the basis of thermotolerance in the reef coral Goniastrea aspera. *Mar Ecol Prog Ser*, **242**, 119–129.

Bruckner A (2002) Life-Saving Products from Coral Reefs. *Issues in Science and Technology*, **18**, 39–44.

Callahan BJ, Mcmurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2 : High-resolution sample inference from Illumina amplicon data. **13**.

Cantin NE, Oppen MJH, Willis BL, Mieog JC, Negri AP (2009) Juvenile corals can acquire more carbon from high-performance algal symbionts. *Coral reefs*, **28**, 405–414.

Carpenter LW, Patterson MR, Bromage ES (2010) Water flow influences the spatiotemporal distribution of heat shock protein 70 within colonies of the scleractinian coral Montastrea annularis (Ellis and Solander, 1786) following heat stress: Implications for coral bleaching. *Journal of Experimental Marine Biology and Ecology*, **387**, 52–59.

Chang SW, Flynn BP, Ruberti JW, Buehler MJ (2012) Molecular mechanism of force induced stabilization of collagen against enzymatic breakdown. *Biomaterials*, **33**, 3852–3859.

Chapman RW, Mancia A, Beal M et al. (2011) The transcriptomic responses of the eastern oyster, Crassostrea virginica, to environmental conditions. *Molecular Ecology*, **20**, 1431–1449.

Chen B, Feder ME, Kang L (2018) Evolution of heat-shock protein expression underlying adaptive responses to environmental stress. *Molecular Ecology*, **27**, 3040–3054.

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry*,

162, 156–159.

Chomczynski P, Sacchi N (2006) The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. *Nature Protocols*, **1**, 581–585.

Chong KY, Lai CC, Lille S, Chang C, Su CY (1998) Stable overexpression of the constitutive form of heat shock protein 70 confers oxidative protection. *Journal of Molecular and Cellular Cardiology*, **30**, 599–608.

Cleves PA, Strader ME, Bay LK, Pringle JR, Matz M V. (2018) CRISPR/Cas9-mediated genome editing in a reef-building coral. *Proceedings of the National Academy of Sciences*, **115**, 5235–5240.

Coles SL, Riegl BM (2013) Thermal tolerances of reef corals in the Gulf: A review of the potential for increasing coral survival and adaptation to climate change through assisted translocation. *Marine pollution bulletin*, **72**, 323–332.

Colton MA, Bellis ES, Logan CA et al. Evolutionary Pathways to Coral Persistence if We Act Soon. *In prep*.

Conaco C, Neveu P, Zhou H, Arcila ML, Degnan SM, Degnan BM, Kosik KS (2012) Transcriptome profiling of the demosponge Amphimedon queenslandica reveals genome-wide events that accompany major life cycle transitions. *BMC Genomics*, **13**, 1–19.

Conner JK, Hartl DL (2004) *A Primer of Ecological Genetics*.

Cooper EL, Hirabayashi K, Strychar KB, Sammarco PW (2014) Corals and Their Potential Applications to Integrative Medicine. *Evidence-Based Complementary and Alternative Medicine*, 1–9.

Coulson T, Clegg S (2015) Selection on heritable heterozygosity but no response to selection. Why? *bioRxiv*, 1–8.

Császár NBM, Ralph PJ, Frankham R, Berkelmans R, van Oppen MJH (2010) Estimating the potential for adaptation of corals to climate warming. *PLoS ONE*, **5**, e9751–e9751.

Cunning R, Silverstein RN, Baker AC (2015a) Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20141725.

Cunning R, Gillette P, Capo T, Galvez K, Baker AC (2015b) Growth tradeoffs associated with thermotolerant symbionts in the coral Pocillopora damicornis are lost in warmer oceans. *Coral Reefs*, **34**, 155–160.

Cunning R, Bay RA, Gillette P, Baker AC, Traylor-Knowles N (2018) Comparative analysis of the Pocillopora damicornis genome highlights role of immune system in coral evolution. *Scientific Reports*, **8**, 1–10.

Cyr DM, Langer T, Douglas MG (1994) DnaJ-like proteins: molecular chaperones and specific regulators of Hsp70. *Trends in Biochemical Science*, **19**, 176–181.

D'Angelo C, Hume BCC, Burt J, Smith EG, Achterberg EP, Wiedenmann J (2015) Local adaptation constrains the distribution potential of heat-tolerant Symbiodinium from the Persian/Arabian Gulf. *The ISME Journal*, **9**, 2551–2560.

Damiano JS, Oliveira V, Welsh K, Reed JC (2004) Heterotypic interactions among NACHT domains: implications for regulation of innate immune responses. *Biochemical Journal*, **381**, 213–219.

Darling ES, Alvarez-Filip L, Oliver TA, McClanahan TR, Côté IM, Bellwood D (2012) Evaluating life-history strategies of reef corals from species traits. *Ecology Letters*, **15**, 1378–1386.

Davies SW, Scarpino S V, Pongwarin T (2015) The design and analysis of binary variable traits in common garden genetic experiments of highly fecund species to assess heritability.

Davy SK, Allemand D, Weis VM (2012) Cell Biology of Cnidarian-Dinoflagellate Symbiosis. *Microbiology and Molecular Biology Reviews*, **76**, 229–261.

Dergham P, Anctil M (1998) Distribution of serotonin uptake and binding sites in the cnidarian Renilla koellikeri: An autoradiographic study. *Tissue and Cell*, **30**, 205–215.

DeSalvo MK, Voolstra CR, Sunagawa S et al. (2008) Differential gene expression during thermal stress and bleaching in the Caribbean coral Montastraea faveolata. *Molecular Ecology*, **17**, 3952–3971.

DeSalvo MK, Sunagawa S, Voolstra CR (2010) Transcriptomic responses to heat stress and bleaching in the elkhorn coral Acropora palmata. *Marine Ecology Progress Series*, **402**, 97–113.

Dimond JL, Bingham BL, Muller-Parker G (2011) Seasonal stability of a flexible algal-cnidarian symbiosis in a highly variable temperate environment. *Limnology and Oceanography*, **56**, 2233–2242.

Dixon GB, Bay LK, Matz M V (2014) Bimodal signatures of germline methylation are linked with gene expression plasticity in the coral Acropora millepora. *BMC Genomics*, **15**, 1–11.

Dixon GB, Davies SW, Aglyamova GV, Meyer E, Bay LK, Matz MV (2015) Genomic

determinants of coral heat tolerance across latitudes. **348**, 2014–2016.

Dixon GB, Bay LK, Matz M V (2016) Evolutionary Consequences of DNA Methylation in a Basal Metazoan. *Molecular Biology and Evolution*, **33**, 2285–2293.

Donner SD, Skirving WJ, Little CM, Oppenheimer M, Hoegh-Gulberg O (2005) Global assessment of coral bleaching and required rates of adaptation under climate change. *Global Change Biology*, **11**, 2251–2265.

Douglas AE (2003) Coral bleaching - How and why? *Marine Pollution Bulletin*, **46**, 385–392.

Downs CA, Fauth JE, Halas JC, Dustan P, Bemiss J, Woodley CM (2002) Oxidative stress and seasonal coral bleaching. *Free Radical Biology and Medicine*, **33**, 533–554.

Dunn SR, Thomason JC, Le Tissier MDA, Bythell JC (2004) Heat stress induces different forms of cell death in sea anemones and their endosymbiotic algae depending on temperature and duration. *Cell Death and Differentiation*, **11**, 1213–1222.

Dunn SR, Phillips WS, Green DR, Weis VM (2007) Knockdown of actin and caspase gene expression by RNA interference in the symbiotic anemone Aiptasia pallida. *Biological Bulletin*, **212**, 250–258.

Dunn SR, Pernice M, Green K, Hoegh-Guldberg O, Dove SG (2012) Thermal stress promotes host mitochondrial degradation in symbiotic cnidarians: Are the batteries of the reef going to run out? *PLoS ONE*, **7**.

Dziedzic KE, Kirk NL, Meyer E A universal Symbiodiniaceae primer for quantitative PCR (qPCR) and its application for symbiont detection. *In prep*.

Dziedzic K, Elder H, Tavalire H, Meyer E (2019) Heritable variation in bleaching responses and its functional genomic basis in reef-building corals (Orbicella faveolata. *Molecular Ecology*, 1–16.

Eakin CM, Lough JM, Heron SF, Stednick JD (2009) Climate variability and change: Monitoring data and evidence for increased coral bleaching stress. *Coral Bleaching*, **205**, 41–67.

Edmunds PJ (2014) Is acclimation beneficial to scleractinian corals, Porites spp.? *Marine Biology*, **161**, 1531–1542.

Etterson JR, Shaw RG (2001) Constraint to Adaptive Evolution in Response to Global Warming. *Science*, **151**, 151–154.

Falconer DS, Mackay TFC (1996) *Introduction to Quantitative Genetics*, 4th edn.

Pearson Education.

Fernie AR, Carrari F, Sweetlove LJ (2004) Respiratory metabolism: glycolysis, the TCA cycle and mitochondrial electron transport. *Current Opinion in Plant Biology*, **7**, 254–261.

Ferrario F, Beck MW, Storlazzi CD, Micheli F, Shepard CC, Airoldi L (2014) The effectiveness of coral reefs for coastal hazard risk reduction and adaptation. *Nature Communications*, **5**, 1–9.

Finley D, Ozkaynak E, Varshavsky A (1987) The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation, and other stresses. *Cell*, **48**, 1035–1046.

Fitt WK (2000) Cellular growth of host and symbiont in a cnidarian-zooxanthellar symbiosis. *The Biological Bulletin*, **198**, 110–120.

Fitt W, Brown B, Warner M, Dunne R (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral reefs*, **20**, 51–65.

Franklin EC, Stat M, Pochon X, Putnam HM, Gates RD (2012) GeoSymbio : a hybrid , cloud-based web application of global geospatial bioinformatics and ecoinformatics for Symbiodinium – host symbioses. 369–373.

Fukami H, Chen CA, Budd AF et al. (2008) Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (Order Scleractinia, Class anthozoa, phylum cnidaria). *PLoS ONE*, **3**, e3222.

Ganot P, Moya A, Magnone V, Allemand D, Furla P, Sabourault C (2011) Adaptations to endosymbiosis in a Cnidarian-Dinoflagellate association: Differential gene expression and specific gene duplications. *PLoS Genetics*, **7**.

Gardner TA (2003) Long-Term Region-Wide Declines in Caribbean Corals. *Science*, **301**, 958–960.

Gates RD, Edmunds PJ (1999) The Physiological Mechanisms of Acclimatization in Tropical Reef Corals. *American Zoologist*, **39**, 30–43.

Geller J, Meyer C, Parker M, Hawk H (2013) Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources*, **13**, 851–861.

Ghosh J, Lun CM, Majeske AJ, Sacchi S, Schrankel CS, Smith LC (2011) Invertebrate immune diversity. *Developmental and Comparative Immunology*, **35**, 959–974.

Gibbin EM, Krueger T, Putnam HM, Barott KL, Bodin J, Gates RD, Meibom A (2018) Short-Term Thermal Acclimation Modifies the Metabolic Condition of the Coral Holobiont. *Frontiers in Marine Science*, **5**, 1–11.

Glynn PW, Enochs IC (2011) Invertebrates and Their Roles in Coral Reef Ecosystems. In: *Coral Reefs: An Ecosystem in Transition*, pp. 273–325.

Görlach A, Bertram K, Hudecova S, Krizanova O (2015) Calcium and ROS: A mutual interplay. *Redox Biology*, **6**, 260–271.

Grabherr M, Haas BJ, Yassour M et al. (2013) Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nature Biotechnology*, **29**, 644–652.

Granados-Cifuentes C, Bellantuono AJ, Ridgway T, Hoegh-Guldberg O, Rodriguez-Lanetty M (2013) High natural gene expression variation in the reef-building coral Acropora millepora: potential for acclimative and adaptive plasticity. *BMC Genomics*, **14**, 228.

Grottoli AG, Warner ME, Levas SJ et al. (2014) The cumulative impact of annual coral bleaching can turn some coral species winners into losers. *Global Change Biology*, **20**, 3823–3833.

Guest JR, Baird AH, Maynard JA et al. (2012) Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. *PLoS ONE*, **7**, 1–8.

Haas BJ, Papanicolaou A, Yassour M et al. (2013) De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature protocols*, **8**, 1494–512.

Hajj-Ali I, Anctil M (1997) Characterization of a serotonin receptor in the cnidarian Renilla koellikeri: A radiobinding analysis. *Neurochemistry International*, **31**, 83–93.

Hamdoun AM, Cheney DP, Cherr GN (2003) Phenotypic Plasticity of HSP70 and HSP70 Gene Expression in the Pacific Oyster (Crassostrea gigas): Implications for Thermal Limits and Induction of Thermal Tolerance. *The Biological Bulletin*, **205**, 160–169.

Hansson B, Westerberg L (2002) On the correlation between heterozygosity and fitness. *Molecular Ecology*, **11**, 2467–2474.

Harrison PL (2011) Sexual Reproduction of Sclerctinian Corals. In: *Coral Reefs: An Ecosystem in Transition*, pp. 59–85.

Hawkins TD, Bradley BJ, Davy SK (2013) Nitric oxide mediates coral bleaching through an apoptotic-like cell death pathway: evidence from a model sea anemone-dinoflagellate symbiosis. *Federation of American Socities for Experimental Biology*, **28**, 2737.

Helmuth B, Harley CDG, Halpin PM, Donnell MO, Hofmann GE, Blanchette CA (2002)

Climate Change and Latitudinal Patterns of Intertidal Thermal Stress. **298**, 1015–1018.

Heron SF, Maynard JA, van Hooidonk R, Eakin CM (2016) Warming Trends and Bleaching Stress of the World's Coral Reefs 1985–2012. *Scientific Reports*, **6**, 38402.

Hiebert TC, Bingham BL (2012) The effects of symbiotic state on heterotrophic feeding in the temperate sea anemone Anthopleura elegantissima. *Marine Biology*, **159**, 939–950.

Hillyer KE, Tumanov S, Villas-bo S, Davy SK (2016) Metabolite profiling of symbiont and host during thermal stress and bleaching in a model cnidarian – dinoflagellate symbiosis. *Journal of Experimental Biology*, **219**, 516–527.

Hoegh-guldberg O (2014) Coral reef sustainability through adaptation: glimmer of hope or persistent mirage? *Current Opinion in Environmental Sustainability*, **7**, 127–133.

Hoegh-Guldberg O, Jones RJ (1999) Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. *Mar Ecol Prog Ser*, **183**, 73–86.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ et al. (2007) Coral Reefs Under Rapid Climate Change and Ocean Acidification. *Science*, **318**, 1737–1742.

Hoegh-Guldberg O, Eakin CM, Hodgson G, Sale PF, Veron JEN (2018) Climate Change Threatens the Survival of Coral Reefs Only 12 years to Avoid the Worst Damage. **2015**, 1–4.

Hoey AS, Howells E, Johansen JL et al. (2016) Recent advances in understanding the effects of climate change on coral reefs. *Diversity*, **8**.

Hofmann GE, Somero GN (1995) Evidence for protein damage at environmental temperatures: seasonal changes in levels of ubiquitin conjugates and HSP70 in the intertidal mussel Mytilus trossulus. *The Journal of Experimental Biology*, **198**, 1509–1518.

Holcomb M, Allemand D, Tambutte S, Venn A, Tambutte E (2011) Live Tissue Imaging Shows Reef Corals Elevate pH under Their Calcifying Tissue Relative to Seawater. *PLoS ONE*, **6**, 1–9.

van Hooidonk R (2013) Temporary refugia for coral reefs in a warming world. *Nature Climate Change*, **3**, 508–511.

Houle D (1991) Genetic Covariance of Fitness Correlates : What Genetic Correlations are Made of and Why it Matters. *Evolution*, **45**, 630–648.

Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL, van Oppen MJH (2011) Coral

thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change*, **2**, 116–120.

Howells EJ, Berkelmans R, van Oppen MJH, Willis BL, Bay LK (2013) Historical thermal regimes define limits to coral acclimatization. *Ecology*, **94**, 1078–1088.

Howells EJ, Abrego D, Meyer E, Kirk NL, Burt JA (2016) Host adaptation and unexpected symbiont partners enable reef-building corals to tolerate extreme temperatures. *Global Change Biology*, **22**, 2702–2714.

Hughes TP (2003) Climate Change, Human Impacts, and the Resilience of Coral Reefs. *Science*, **301**, 929–933.

Hughes TP, Tanner JE (2000) Recruitment Failure, Life Histories, and Long-Term Decline of Caribbean Corals. *Ecology*, **81**, 2250–2263.

Hughes TP, Kerry JT, Álvarez-Noriega M et al. (2017) Global warming and recurrent mass bleaching of corals. *Nature*, **543**, 373–377.

Hughes TP, Kerry JT, Baird AH et al. (2018a) Global warming transforms coral reef assemblages. *Nature*, **556**, 492–496.

Hughes TP, Anderson KD, Connolly SR et al. (2018b) Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science*, **359**, 80–83.

Hume B, D'Angelo C, Burt J, Baker AC, Riegl B, Wiedenmann J (2013) Corals from the Persian/Arabian Gulf as models for thermotolerant reef-builders: Prevalence of clade C3Symbiodinium, host fluorescence andex situtemperature tolerance. *Marine pollution bulletin*, **72**, 313–322.

Hunter CL (1993) Genotypic Variation and Clonal Structure in Coral Populations with Different Disturbance Histories. *Evolution*, **47**, 1213–1228.

Hunter RL, LaJeunesse TC, Santos SR (2007) Structure and evolution of the rDNA internal transcribed spacer (ITS) region 2 in the symbiotic dinoflagellates (Symbiodinium, Dinophyta). *Journal of Phycology*, **43**, 120–128.

Jeno K, Brokordt K (2014) Nutritional status affects the capacity of the snail Concholepas concholepas to synthesize Hsp70 when exposed to stressors associated with tidal regimes in the intertidal zone. *Marine Biology*, **161**, 1039–1049.

Joint Genome Institute (1997) BBTools. http://jgi.doe.gov/data-and-tools/bbtools/.

Jokiel PL, Brown EK (2004) Global warming, regional trends and inshore environmental conditions influence coral bleaching in Hawaii. *Global Change Biology*, **10**, 1627–1641.

Jones A, Berkelmans R (2010) Potential costs of acclimatization to a warmer climate: growth of a reef coral with heat tolerant vs. sensitive symbiont types. *PLoS ONE*, **5**, e10437.

Jones AM, Berkelmans R (2011) Tradeoffs to Thermal Acclimation: Energetics and Reproduction of a Reef Coral with Heat Tolerant Symbiodinium Type-D . *Journal of Marine Biology*, **2011**, 1–12.

Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U (1998) Temperature-induced bleaching of corals begins with impairment of the CO2 fixation mechanism in zooxanthellae. *Plant, Cell and Environment*, **21**, 1219–1230.

Jones AM, Berkelmans R, van Oppen MJ., Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 1359–1365.

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic acids research*, **30**, 3059–66.

Kayal E, Roure B, Philippe H, Collins AG, Lavrov D V. (2013) Cnidarian phylogenetic relationships as revealed by mitogenomics. *BMC Evolutionary Biology*, **13**, 1–18.

Kenkel CD, Bay LK (2017) Novel transcriptome resources for three scleractinian coral species from the Indo-Pacific. *GigaScience*, **6**, 1–4.

Kenkel CD, Matz M V (2016a) Gene expression plasticity as a mechanism of coral adaptation to a variable environment. *Nature Publishing Group*, **1**, 1–6.

Kenkel C, Matz M V (2016b) Enhanced gene expression plasticity as a mechanism of adaptation to a variable environment in a reef-building coral. *bioRxiv*, **1**, 059667.

Kenkel CD, Meyer E, Matz M V. (2013) Gene expression under chronic heat stress in populations of the mustard hill coral (Porites astreoides) from different thermal environments. *Molecular Ecology*, **22**, 4322–4334.

Kenkel CD, Setta SP, Matz M V (2015) Heritable differences in fitness-related traits among populations of the mustard hill coral, Porites astreoides. *Heredity*, **115**, 509–516.

Kim B, Rhee J, Jeong C, Soo J, Soo G, Lee Y, Lee J (2014) Heavy metals induce oxidative stress and trigger oxidative stress-mediated heat shock protein (hsp) modulation in the intertidal copepod Tigriopus japonicus. *Comparative Biochemistry and Physiology, Part C*, **166**, 65–74.

Kirk NL, Howells EJ, Abrego D, Burt JA, Meyer E (2018) Genomic and transcriptomic

signals of thermal tolerance in heat-tolerant corals (Platygyra daedalea) of the Arabian/Persian Gulf. *Molecular Ecology*, **27**, 5180–5194.

Kitahara M V, Cairns SD, Stolarski J, Blair D, Miller DJ (2010) A Comprehensive Phylogenetic Analysis of the Scleractinia (Cnidaria, Anthozoa) Based on Mitochondrial CO1 Sequence Data (ed DeSalle R). *PLoS ONE*, **5**, e11490.

Kitchen SA, Crowder CM, Poole AZ, Weis VM, Meyer E (2015) De Novo Assembly and Characterization of Four Anthozoan (Phylum Cnidaria) Transcriptomes. *G3: Genes, Genomes, Genetics*, **5**, 2441–2452.

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**, 223–224.

Kress WJ, García-Robledo C, Uriarte M, Erickson DL (2015) DNA barcodes for ecology, evolution, and conservation. *Trends in Ecology and Evolution*, **30**, 25–35.

Kumar S, Stecher G, Tamura K (2017) MEGA7 : Molecular Evolutionary Genetics Analysis Version 7 . 0 for Bigger Datasets. **33**, 1870–1874.

Ladner JT, Barshis DJ, Palumbi SR (2012) Protein evolution in two co-occurring types of Symbiodinium: an exploration into the genetic basis of thermal tolerance in Symbiodinium clade D. *BMC Evolutionary Biology*, **12**, 217.

LaJeunesse TC, Thornhill DJ, Cox EF, Stanton FG (2004) High diversity and host specificity observed among symbiotic dinoflagellates in reef coral communities from Hawaii. *Coral reefs*, **23**, 596–603.

LaJeunesse TC, Pettay DT, Sampayo EM et al. (2010) Long-standing environmental conditions, geographic isolation and host-symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus Symbiodinium. *Journal of Biogeography*, **37**, 785–800.

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. *Current Biology*, **28**, 2570-2580.e6.

Lande R, Arnold SJ (1983) The Measurement of Selection on Correlated Characters. *Evolution*, **37**, 1210–1226.

Lang R, Gundlach AL, Holmes FE, Hobson SA, Wynick D, Hokfelt T, Kofler B (2014) Physiology, Signaling, and Pharmacology of Galanin Peptides and Receptors: Three Decades of Emerging Diversity. *Pharmacological Reviews*, **67**, 118–175.

Langfelder P, Horvath S (2008) WGCNA : an R package for weighted correlation network analysis. *BMC Bioinformatics*, **9**, 1–13.

Langfelder P, Horvath S (2014) Tutorials for the WGCNA Package. *UCLA*.

Lee HK, Braynen W, Keshav K, Pavlidis P (2005) ErmineJ : Tool for functional analysis of gene expression data sets. *BMC Bioinformatics*, **6**, 1–8.

Leggat WW, Seneca FF, Wasmund KK, Ukani LL, Yellowlees DD, Ainsworth TDTD (2011) Differential responses of the coral host and their algal symbiont to thermal stress. *PLoS ONE*, **6**, e26687.

Lesser MP (2011) Coral Bleaching: Causes and Mechanisms. In: *Coral Reefs: An Ecosystem in Transition*, pp. 405–419.

Li Y, Liew YJ, Cui G et al. (2018) DNA methylation regulates transcriptional homeostasis of algal endosymbiosis in the coral model Aiptasia. *Science Advances*, **4**, 1–11.

Liu G, Rauenzahn JL, Heron SF et al. (2013) *NOAA Technical Report NESDIS 143 - NOAA Coral Reef Watch 50 km Satellite Sea Surface Temperature-Based Decision Support System for Coral Bleaching Management*.

Lohman BK, Weber JN, Bolnick DI (2016) Evaluation of TagSeq, a reliable low-cost alternative for RNAseq. *Molecular Ecology Resources*, **16**, 1315–1321.

Lohr KE, Patterson JT (2017) Intraspecific variation in phenotype among nursery-reared staghorn coral Acropora cervicornis (Lamarck, 1816). *Journal of Experimental Marine Biology and Ecology*, **486**, 87–92.

López-Maury L, Marguerat S, Bähler J (2008) Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. *Nature Reviews: Genetics*, **9**, 583–593.

Louis YD, Bhagooli R, Kenkel CD, Baker AC, Dyall SD (2017) Gene expression biomarkers of heat stress in scleractinian corals: Promises and limitations. *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology*, **191**, 63–77.

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology*, **15**, 1–21.

Loya Y, Sakai K, Yamazato K, Nakano Y, Sambali H, van Woesik R (2001) Coral bleaching: the winners and the losers. *Ecology Letters*, **4**, 122–131.

Lundgren P, Vera JC, Peplow L, Manel S, van Oppen MJH (2013) Genotype environment correlations in corals from the Great Barrier Reef. *BMC Genetics*, **14**, 1.

Lynch M, Walsh B (1998) *Genetics and Analysis of Quantitative Traits*. Sinauer

Sunderland, MA, 980 pp.

Macrander JC, Dimond JL, Bingham BL, Reitzel AM (2018) Marine Genomics Transcriptome sequencing and characterization of Symbiodinium muscatinei and Elliptochloris marina , symbionts found within the aggregating sea anemone Anthopleura elegantissima. *Marine Genomics*, **37**, 82–91.

Mansfield KM, Carter NM, Nguyen L et al. (2017) Transcription factor NF-κB is modulated by symbiotic status in a sea anemone model of cnidarian bleaching. *Scientific Reports*, **7**, 1–14.

Mansour TA, Rosenthal JJC, Brown CT, Roberson LM (2016) Transcriptome of the Caribbean stony coral Porites astreoides from three developmental stages. *GigaScience*, **5**, 1–6.

Maor-Landaw K, Levy O (2016) Gene expression profiles during short-term heat stress; branching *vs.* massive Scleractinian corals of the Red Sea. *PeerJ*, **4**, e1814.

Maor-Landaw K, Karako-Lampert S, Ben-Asher HW, Goffredo S, Falini G, Dubinsky Z, Levy O (2014) Gene expression profiles during short-term heat stress in the red sea coral Stylophora pistillata. *Global Change Biology*, **20**, 3026–3035.

Marshall PA, Baird AH (2000) Bleaching of corals on the Great Barrier Reef:differential susceptibilities among taxa. *Coral reefs*, **19**, 155–163.

Matthews JL, Crowder CM, Oakley CA et al. (2017) Optimal nutrient exchange and immune responses operate in partner specificity in the cnidarian-dinoflagellate symbiosis. *Proceedings of the National Academy of Sciences*, **114**, 201710733.

Matthews JL, Oakley CA, Lutz A et al. (2018) Partner switching and metabolic flux in a model cnidarian–dinoflagellate symbiosis. *Proceedings of the Royal Society B: Biological Sciences*, **285**, 1–10.

Matz M V, Treml EA, Aglyamova G V, Bay LK (2018) Potential for rapid genetic adaptation to warming in a Great Barrier Reef coral. *PLoS Genetics*, 1–19.

McClanahan TR (2017) Changes in coral sensitivity to thermal anomalies. *Marine Ecology Progress Series*, **570**, 71–85.

Medina M, Hannah B, Morrison C et al. (2011) Orbicella faveolata Genome Project. *http://montastraea.psu.edu/genome/*.

Mehvar S, Filatova T, Dastgheib A, Steveninck EDR Van, Ranasinghe R (2018) Quantifying Economic Value of Coastal Ecosystem Services : A Review. *Journal of Marine Science and Engineering*, **6**, 1–18.

Messina FJ, Fry JD (2003) Environment-dependent reversal of a life history trade-off in

the seed beetle Callosobruchus maculatus. *Journal of Evolutionary Biology*, **16**, 501–509.

Meyer EE, Manahan DTDT (2010) Gene expression profiling of genetically determined growth variation in bivalve larvae (Crassostrea gigas). *The Journal of experimental biology*, **213**, 749–758.

Meyer E, Davies S, Wang S, Willis BL, Abrego D (2009a) Genetic variation in responses to a settlement cue and elevated temperature in the reef-building coral Acropora millepora. *Mar Ecol Prog Ser*, **392**, 81–92.

Meyer E, Aglyamova G V, Wang S et al. (2009b) Sequencing and de novo analysis of a coral larval transcriptome using 454 GSFlx. *BMC genomics*, **10**, 219.

Meyer E, Aglyamova G V, Matz M V (2011) Profiling gene expression responses of coral larvae (Acropora millepora) to elevated temperature and settlement inducers using a novel RNA‐Seq procedure. *Molecular Ecology*, **20**, 3599–3616.

Mieog JC, Oppen MJH, Cantin NE, Stam WT, Olsen JL (2007) Real-time PCR reveals a high incidence of Symbiodinium clade D at low levels in four scleractinian corals across the Great Barrier Reef: implications for symbiont shuffling. *Coral reefs*, **26**, 449–457.

Mitsukawa K, Lu X, Bartfai T (2009) Bidirectional regulation of stress responses by galanin in mice: Involvement of galanin receptor subtype 1. *Neuroscience*, **160**, 837–846.

Mitton JB (1993) Theory and Data Pertinent to the Relationship between Heterozygosity and Fitness. In: *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives*, pp. 17–41.

Mitton JB (1997) *Selection in Natural Populations*.

Moran Y, Barzilai MG, Liebeskind BJ, Zakon HH (2015) Evolution of voltage-gated ion channels at the emergence of Metazoa. *Journal of Experimental Biology*, **218**, 515–525.

Morrissey MB, Parker DJ, Korsten P, Pemberton JM, Kruuk LEB, Wilson AJ (2012) The prediction of adaptive evolution: Empirical application of the secondary theorem of selection and comparison to the breeder's equation. *Evolution*, **66**, 2399–2410.

Mousseau TA, Ritland K, Heath DD (1998) A novel method for estimating heritability using molecular markers. **80**, 218–224.

Moya A, Ganot P, Furla P, Sabourault C (2012) The transcriptomic response to thermal stress is immediate, transient and potentiated by ultraviolet radiation in the sea anemone Anemonia viridis. *Molecular Ecology*, **21**, 1158–1174.

Muir P, Frasier T (2015) Related : an R package for analysing pairwise relatedness from codominant molecular markers related : an R package for analysing pairwise relatedness from codominant molecular markers. *Molecular Ecology Resources*, **15**, 557–561.

Muller-Parker G, Davy SK (2001) Temperate and tropical algal-sea anemone symbioses. *Invertebrate Biology*, **120**, 104–123.

Muller-Parker G, Pierce-Cravens J, Bingham BL (2007) Broad thermal Tolerance of the Symbiotic Dinoflagellate Symbiodinium muscatinei (Dinophyta) in the Sea Anemone Anthopleura elegantissima (Cnidaria) from Northern Latitudes. *Journal of Phycology*, **43**, 25–31.

Muller-Parker G, D'Elia CF, Cook CB (2015) Interactions Between Corals and Their Symbiotic Algae. In: *Coral Reefs in the Anthropocene*, In: Birkel edn, pp. 99–116. Springer, Dordrecht.

Muscatine L, Falkowski PG, Porter JW, Dubinsky Z (1984) Fate of Photosynthetic Fixed Carbon in Light- and Shade-Adapted Colonies of the Symbiotic Coral Stylophora pistillata. *Proceedings. Biological sciences / The Royal Society*, **222**, 181–202.

Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews*, **85**, 935–956.

Nicholas H. Putnam, Mansi Srivastava, Uffe Hellsten et al. (2007) Sea Anemone Genome Reveals Ancestral Eumetazoan Gene Repertoire and Genomic Organization. *Science*, **317**, 86.

O'Neil ST, Dzurisin JDK, Carmichael RD, Lobo NF, Emrich SJ, Hellmann JJ (2010) Population-level transcriptome sequencing of nonmodel organisms Erynnis propertius and Papilio zelicaon. *BMC Genomics*, **11**, 1–15.

Oakley CA, Davy SK (2018) Cell Biology of Coral Bleaching. In: *Coral Bleaching*, pp. 189–211.

Oakley CA, Durand E, Wilkinson SP, Peng L, Weis VM, Grossman AR, Davy SK (2017) Thermal Shock Induces Host Proteostasis Disruption and Endoplasmic Reticulum Stress in the Model Symbiotic Cnidarian Aiptasia. *Journal of Proteome Research*, **16**, 2121–2134.

Oksanen J (2010) Cluster analysis: tutorial with R. *University of Oulu, Oulu*, 1–8.

Oliver TA, Palumbi SR (2011) Do fluctuating temperature environments elevate coral thermal tolerance? *Coral reefs*, **30**, 429–440.

van Oppen MJH, Catmull J, Mcdonald BJ, Hislop NR, Hagerman PJ, Miller DJ (2001) The Mitochondrial Genome of Acropora tenuis (Cnidaria ; Scleractinia) Contains a

Large Group I Intron and a Candidate Control Region. **55**, 1–13.

van Oppen MJH, Oliver JK, Putnam HM, Gates RD (2015) Building coral reef resilience through assisted evolution. *Proceedings of the National Academy of Sciences*, **112**, 1–7.

van Oppen MJH, Gates RD, Blackall LL et al. (2017) Shifting paradigms in restoration of the world's coral reefs. *Global Change Biology*, **23**, 3437–3448.

Van Oppen MJH, Mahiny AJ, Done TJ (2005) Geographic distribution of zooxanthella types in three coral species on the Great Barrier Reef sampled after the 2002 bleaching event. *Coral Reefs*, **24**, 482–487.

Palumbi SR, Barshis DJ, Traylor-Knowles N, Bay RA (2014) Mechanisms of reef coral resistance to future climate change. *Science*, **344**, 895–898.

Pandolfi JM (2003) Global Trajectories of the Long-Term Decline of Coral Reef. *Science*, **301**, 955–958.

Parkinson JE, Banaszak AT, Altman NS, LaJeunesse TC, Baums IB (2015) Intraspecific diversity among partners drives functional variation in coral symbioses. *Scientific reports*, **5**, 12.

Parkinson JE, Baumgarten S, Michell CT, Baums IB, LaJeunesse TC, Voolstra CR (2016) Gene Expression Variation Resolves Species and Individual Strains among Coral-Associated Dinoflagellates within the Genus Symbiodinium. *Genome biology and evolution*, **8**, 665–680.

Parra G, Bradnam K, Korf I (2007) Genome analysis CEGMA : a pipeline to accurately annotate core genes in eukaryotic genomes. **23**, 1061–1067.

Perez S, Weis VM (2006) Nitric oxide and cnidarian bleaching: an eviction notice mediates breakdown of a symbiosis. *Journal of Experimental Biology*, **209**, 2804–2810.

Pickart CM (2001) Mechanisms underlying ubiquitination. *Annual Review of Biochemistry*, **70**, 503–33.

Pigliucci M (2006) Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology*, **209**, 2362–2367.

Pinzón JH, Kamel B, Burge CA, Harvell CD, Medina M, Weil E, Mydlarz LD (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**, 140214.

Polato NR, Voolstra CR, Schnetzer J et al. (2010) Location-specific responses to thermal

stress in larvae of the reef-building coral Montastraea faveolata. *PLoS ONE*, **5**, e11221–e11221.

Polato NR, Vera JC, Baums IB (2011) Gene discovery in the threatened elkhorn coral: 454 sequencing of the Acropora palmata transcriptome. *PLoS ONE*, **6**, e28634–e28634.

Putnam HM, Gates RD (2015) Preconditioning in the reef-building coral Pocillopora damicornis and the potential for trans-generational acclimatization in coral larvae under future climate change conditions. *Journal of Experimental Biology*, **218**, 2365–2372.

Putnam HM, Davidson JM, Gates RD (2016) Ocean acidification influences host DNA methylation and phenotypic plasticity in environmentally susceptible corals. *Evolutionary Applications*, **9**, 1165–1178.

Queller DC, Goodnight KF (1989) Estimating Relatedness Using Genetic Markers. *Evolution*, **43**, 258–275.

Quigley KM, Davies SW, Kenkel CD, Willis BL, Matz M V., Bay LK (2014) Deep-sequencing method for quantifying background abundances of Symbiodinium types: Exploring the rare Symbiodinium biosphere in reef-building corals. *PLoS ONE*, **9**.

Quistad SD, Stotland A, Barott KL et al. (2014) Evolution of TNF-induced apoptosis reveals 550 My of functional conservation. *Proceedings of the National Academy of Sciences*, **111**, 9567–9572.

Rast JP, Messier-Solek C (2008) Marine Invertebrate Genome Sequences and Our. *Biological Bulletin*, **214**, 274–283.

ReFuGe 2020 Consortium (2017) The ReFuGe 2020 Consortium—using "omics" approaches to explore the adaptability and resilience of coral holobionts to environmental change. *Frontiers in Marine Science*, **2**.

Reitzel AM, Sullivan JC, Traylor-knowles N, Finnerty JR (2010) Genomic Survey of Candidate Stress-Response Genes in the Estuarine Anemone Nematostella vectensis. *Genomics*, **214**, 233–254.

Reynolds WS, Schwarz JA, Weis VM (2000) Symbiosis-enhanced gene expression in cnidarian-algal associations: cloning and characterization of a cDNA, sym32, encoding a possible cell adhesion protein. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **126**, 33–44.

Richier S, Rodriguez-Lanetty M, Schnitzler CE, Weis VM (2008) Response of the symbiotic cnidarian Anthopleura elegantissima transcriptome to temperature and UV increase. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, **3**, 283–289.

Riegl BM, Purkis SJ, Al-Cibahy AS, Abdel-Moati MA, Hoegh-Guldberg O (2011) Present limits to heat-adaptability in corals and population-level responses to climate extremes. *PLoS ONE*, **6**, e24802–e24802.

Riesgo A, Andrade SCS, Sharma PP et al. (2012) Comparative description of ten transcriptomes of newly sequenced invertebrates and efficiency estimation of genomic sampling in non-model taxa. *Frontiers in Zoology*, **9**, 1–24.

Robbart ML, Peckol P, Scordilis SP, Curran HA, Brown-Saracino J (2004) Population recovery and differential heat shock protein expression for the corals Agaricia agaricites and A. tenuifolia in Belize. *Marine Ecology Progress Series*, **283**, 151–160.

Rodolfo-Metalpa R, Hoogenboom MO, Rottier CC, Ramos-Espla A, Baker AC, Fine M, Ferrier-Pagès CC (2014) Thermally tolerant corals have limited capacity to acclimatize to future warming. *Global Change Biology*, **20**, 3036–3049.

Rodriguez-Lanetty, Phillips WS, Weis VM (2006) Transcriptome analysis of a cnidarian-dinoflagellate mutualism reveals complex modulation of host gene expression. *BMC Genomics*, **7**, 23.

Rodriguez-Lanetty M, Harii S, Hoegh-Guldberg O (2009) Early molecular responses of coral larvae to hyperthermal stress. *Molecular Ecology*, **18**, 5101–5114.

Roth MS, Deheyn DD (2013) Effects of cold stress and heat stress on coral fluorescence in reef-building corals. *Scientific reports*, **3**, 1421.

Rowan R (2004) Coral bleaching: Thermal adaptation in reef coral symbionts. *Nature*, **430**, 742.

Rowan R, Knowlton N, Baker A, Jara J (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature*, **388**, 265–269.

Ruiz-Jones LJ, Palumbi SR (2017) Tidal heat pulses on a reef trigger a fine-tuned transcriptional response in corals to maintain homeostasis. *Science Advances*, **3**, 1–10.

Rumble SM, Lacroute P, Dalca A V., Fiume M, Sidow A, Brudno M (2009) SHRiMP: Accurate mapping of short color-space reads. *PLoS Computational Biology*, **5**, 1–11.

Schlötterer C, Kofler R, Versace E, Tobler R, Franssen SU (2015) Combining experimental evolution with next-generation sequencing: a powerful tool to study adaptation from standing genetic variation. *Heredity*, **114**, 431–440.

Schwarz JA, Weis VM (2003) Localization of a Symbiosis-Related Protein, Sym32, in the Anthopleura elegantissima-Symbiodinium muscatinei Association. *Biological Bulletin*, **205**, 339–350.

Schwarz JA, Brokstein PB, Voolstra CR et al. (2008) Coral Life History and Symbiosis: functional genomic resources for two reef building Caribbean corals, Acropora palmata and Montastraea faveolata. *BMC Genomics*, **9**, 97.

Sciolino NR, Smith JM, Stranahan AM, Freeman KG, Edwards GL, Weinshenker D, Holmes P V. (2015) Galanin mediates features of neural and behavioral stress resilience afforded by exercise. *Neuropharmacology*, **89**, 255–264.

Sellis D, Callahan BJ, Petrov DA, Messer PW (2011) Heterozygote advantage as a natural consequence of adaptation in diploids. *Proceedings of the National Academy of Sciences*, **108**, 20666–20671.

Sellis D, Kvitek DJ, Dunn B, Sherlock G, Petrov DA (2016) Heterozygote advantage is a common outcome of adaptation in Saccharomyces cerevisiae. *Genetics*, **203**, 1401–1413.

Seneca FO, Palumbi SR (2015) The role of transcriptome resilience in resistance of corals to bleaching. *Molecular Ecology*, **24**, 1467–1484.

Seneca FO, Forêt S, Ball EE, Smith-Keune C, Miller DJ, Oppen MJH (2010) Patterns of Gene Expression in a Scleractinian Coral Undergoing Natural Bleaching. *Marine Biotechnology*, **12**, 594–604.

Sgrò CM, Hoffmann AA (2004) Genetic correlations, tradeoffs and environmental variation. *Heredity*, **93**, 241–248.

Shahsavarani H, Sugiyama M, Kaneko Y, Chuenchit B, Harashima S (2012) Superior thermotolerance of Saccharomyces cerevisiae for efficient bioethanol fermentation can be achieved by overexpression of RSP5 ubiquitin ligase. *Biotechnology Advances*, **30**, 1289–1300.

Shen H-M, Pervaiz S (2006) TNF receptor superfamily-induced cell death: redox-dependent execution. *The FASEB Journal*, **20**, 1589–1598.

Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, Fujie M (2011) Using the Acropora digitifera genome to understand coral responses to environmental change. *Nature*, **476**, 320–323.

Shinzato C, Inoue M, Kusakabe M (2014) A snapshot of a coral "holobiont": A transcriptome assembly of the scleractinian coral, Porites, captures a wide variety of genes from both the host and symbiotic zooxanthellae. *PLoS ONE*, **9**.

Silverstein RN, Correa AMS, Baker AC (2012) Specificity is rarely absolute in coral-algal symbiosis: implications for coral response to climate change. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 2609–2618.

Silverstein RN, Cunning R, Baker AC (2015) Change in algal symbiont communities

after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Global Change Biology*, **21**, 236–249.

Silverstein RN, Cunning R, Baker AC (2017) Tenacious D: Symbiodinium in clade D remain in reef corals at both high and low temperature extremes despite impairment. *The Journal of Experimental Biology*, **220**, 1192–1196.

Smith-Keune CC, Dove SS (2008) Gene expression of a green fluorescent protein homolog as a host-specific biomarker of heat stress within a reef-building coral. *Marine Biotechnology*, **10**, 166–180.

Smith-Keune C, Van Oppen M (2006) Genetic structure of a reef-building coral from thermally distinct environments on the Great Barrier Reef. *Coral reefs*, **25**, 493–502.

Smith EG, Ketchum RN, Burt JA (2017a) Host specificity of Symbiodinium variants revealed by an ITS2 metahaplotype approach. *The ISME Journal*, **11**, 1500–1503.

Smith EG, Hume BCC, Delaney P, Wiedenmann J, Burt JA (2017b) Genetic structure of coral-Symbiodinium symbioses on the world's warmest reefs. *PLoS ONE*, **12**, 1–12.

Snelling J, Dziedzic K, Guermond S, Meyer E (2017) Integrating genomic resources for a threatened Caribbean coral (Orbicella faveolata) using a genetic linkage map developed from individual larval genotypes. *bioRxiv*, **2925759**, 1–40.

Snyder MJ, Rossi S (2004) Stress protein (HSP70 family) expression in intertidal benthic organisms: the example of Anthopleura elegantissima (Cnidaria: Anthozoa)*. *Scientia Marina*, **68**, 155–162.

Spalding M, Burke L, Wood SA, Ashpole J, Hutchison J (2017) Mapping the global value and distribution of coral reef tourism. *Marine Policy*, **82**, 104–113.

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**, 1312–1313.

Stamatakis A (2016) RAxML. *Manual/tutorial*, 1–5.

Stanton-Geddes J, Yoder JB, Briskine R, Young ND, Tiffin P (2013) Estimating heritability using genomic data. *Methods in Ecology and Evolution*, **4**, 1151–1158.

Stat M, Pochon X, Cowie ROM, Gates RD (2009) Specificity in communities of Symbiodinium in corals from Johnston Atoll. *Marine Ecology Progress Series*, **386**, 83–96.

Stewart ZK, Pavasovic A, Hock DH, Prentis PJ (2017) Transcriptomic investigation of wound healing and regeneration in the cnidarian Calliactis polypus. *Scientific Reports*, **7**, 41458.

Storey JD (2003) The positive false discovery rate: A Bayesian interpretation and the q-value. *Annals of Statistics*, **31**, 2013–2035.

Sully S, Burkepile DE, Donovan MK, Hodgson G, van Woesik R (2019) A global analysis of coral bleaching over the past two decades. *Nature Communications*, **10**, 1–5.

T L (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine biology*, **141**, 387–400.

Tavalire HF, Beechler BR, Buss PE et al. (2018) Context-dependent costs and benefits of tuberculosis resistance traits in a wild mammalian host. *Ecology and Evolution*, **8**, 12712–12726.

Tchernov D, Gorbunov MY, de Vargas C, Narayan Yadav S, Milligan AJ, Haggblom M, Falkowski PG (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings of the National Academy of Sciences*, **101**, 13531–13535.

Thomas L, Rose NH, Bay RA, López EH, Morikawa MK, Ruiz-Jones L, Palumbi SR (2018) Mechanisms of Thermal Tolerance in Reef-Building Corals across a Fine-Grained Environmental Mosaic: Lessons from Ofu, American Samoa. *Frontiers in Marine Science*, **4**, 1–14.

Thornhill DJ, Xiang Y, Fitt WK, Santos SR (2009) Reef Endemism , Host Specificity and Temporal Stability in Populations of Symbiotic Dinoflagellates from Two Ecologically Dominant Caribbean Corals. *PLoS ONE*, **4**, 1–12.

Tomanek L, Sanford E (2003) Heat-Shock Protein 70 (Hsp70) as a Biochemical Stress Indicator : an Experimental Field Test in Two Congeneric Intertidal Gastropods (Genus: Tegula). *Biological Bulletin*, **205**, 276–284.

Tomanek L, Somero GN (2002) Interspecific- and acclimation-induced variation in levels of heat-shock proteins 70 (hsp70) and 90 (hsp90) and heat-shock transcription factor-1 (HSF1) in congeneric marine snails (genus Tegula): implications for regulation of hsp gene expression. *The Journal of experimental biology*, **205**, 677–85.

Traylor-Knowles N, Granger BR, Lubinski TJ et al. (2011) Production of a reference transcriptome and transcriptomic database (PocilloporaBase) for the cauliflower coral, Pocillopora damicornis. *BMC Genomics*, **12**, 585.

Traylor-Knowles N, Rose NH, Sheets EA, Palumbi SR (2017a) Early transcriptional responses during heat stress in the coral Acropora hyacinthus. *Biological Bulletin*, **232**, 91–100.

Traylor-Knowles N, Rose NH, Palumbi SR (2017b) The cell specificity of gene

expression in the response to heat stress in corals. *The Journal of Experimental Biology*, **220**, 1837–1845.

Turrens JF (2003) Mitochondrial formation of reactive oxygen species. *Journal of Physiology*, **552**, 335–344.

Ulstrup KE, Berkelmans R, Ralph PJ, Oppen MJH Van (2006) Variation in bleaching sensitivity of two coral species across a latitudinal gradient on the Great Barrier Reef: the role of zooxanthellae. *Marine Ecology Progress Series*, **314**, 135–138.

Veron JEN (2011) Coral Taxonomy and Evolution. In: *Coral Reefs: An Ecosystem in Transition*, pp. 37–45.

Veron J (2013) Overview of the taxonomy of zooxanthellate Scleractinia. *Zoological Journal of the Linnean Society*, 1–24.

Vidal-Dupiol J, Zoccola D, Tambutte E et al. (2013) Genes related to ion-transport and energy production are upregulated in response to CO2-driven pH decrease in corals: new insights from transcriptome analysis. *PLoS ONE*, **8**, e58652.

Visscher PM, Hill WG, Wray NR (2008) Heritability in the genomics era — concepts and misconceptions. *Nature Reviews Genetics*, **9**, 255–266.

Voolstra CR (2013) A journey into the wild of the cnidarian model system Aiptasia and its symbionts. *Molecular Ecology*, **22**, 4366–4368.

Voolstra CR, Schnetzer J, Peshkin L, Randall CJ, Szmant AM, Medina M (2009) Effects of temperature on gene expression in embryos of the coral Montastraea faveolata. *BMC genomics*, **10**, 627.

Voolstra CR, Li Y, Liew YJ et al. (2017) Comparative analysis of the genomes of Stylophora pistillata and Acropora digitifera provides evidence for extensive differences between species of corals. 1–14.

Walter P, Ron D (2011) The Unfolded Protein Response: From Stress Pathway to Homeostatic Regulation. *Science*, **334**, 1081–1086.

Wang Z, Gerstein M, Snyder M (2009a) RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews: Genetics*, **10**, 57–63.

Wang S, Zhang L, Meyer E, Matz M V (2009b) Construction of a high-resolution genetic linkage map and comparative genome analysis for the reef-building coral Acropora millepora. *Genome biology*, **10**, R126.

Wang S, Meyer E, McKay JK, Matz M V (2012) 2b-RAD: a simple and flexible method for genome-wide genotyping. *Nature Methods*, **9**, 808–810.

Warner ME, Fitt WK, Schmidt GW (1999) Damage to photosystem II in symbiotic dinoflagellates: A determinant of coral bleaching. *PNAS*, **96**, 8007–8012.

Weis VM (1991) The Induction of Carbonic Anhydrase in the Symbiotic Sea Anemone Aiptasia pulchella. *Biological Bulletin*, **180**, 496–504.

Weis VM (2008) Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *The Journal of experimental biology*, **211**, 3059–3066.

Weis VM, Reynolds WS (1999) Carbonic Anhydrase Expression and Synthesis in the Sea Anemone Anthopleura elegantissima Are Enhanced by the Presence of Dinoflagellate Symbionts. *Physiological and Biochemical Zoology*, **72**, 307–316.

Weis VM, Davy SK, Hoegh-Guldberg O, Rodriguez-Lanetty M, Pringle JR (2008) Cell biology in model systems as the key to understanding corals. *Trends in Ecology and Evolution*, **23**, 369–376.

Welchman RL, Gordon C, Mayer RJ (2005) Ubiquitin and ubiquitin-like proteins as multifunctional signals. *Nature Reviews Molecular Cell Biology*, **6**, 599–609.

Westfall J a, Elliott SR, MohanKumar PS, Carlin RW (2000) Immunocytochemical evidence for biogenic amines and immunogold labeling of serotonergic synapses in tentacles of Aiptasia pallida (Cnidaria, Anthozoa). *Invertebrate Biology*, **119**, 370–378.

Weston AJ, Dunlap WC, Beltran VH, Starcevic A, Hranueli D, Ward M, Long PF (2015) Proteomics Links the Redox State to Calcium Signaling During Bleaching of the Scleractinian Coral Acropora microphthalma on Exposure to High Solar Irradiance and Thermal Stress . *Molecular & Cellular Proteomics*, **14**, 585–595.

Westram AM, Rafajlović M, Chaube P et al. (2018) Clines on the seashore: The genomic architecture underlying rapid divergence in the face of gene flow. *Evolution Letters*, **2**, 297–309.

Wham DC, Ning G, LaJeunesse TC (2017) Symbiodinium glynnii sp. nov ., a species of stress-tolerant symbiotic dinoflagellates from pocilloporid and montiporid corals in the Pacific Ocean . *Phycologia*, **56**, 396–409.

Wilson AJ, Réale D, Clements MN et al. (2010) An ecologist's guide to the animal model. *Journal of Animal Ecology*, **79**, 13–26.

Van Woesik R, Sakai K, Ganase A, Loya Y (2011) Revisiting the winners and the losers a decade after coral bleaching. *Marine Ecology Progress Series*, **434**, 67–76.

Wright RM, Kenkel CD, Dunn CE, Shilling EN, Bay LK, 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–

13.

Ying H, Cooke I, Sprungala S et al. (2018) Comparative genomics reveals the distinct evolutionary trajectories of the robust and complex coral lineages. *Genome biology*, **19**, 175.

Zhao JH (2007) gap: Genetic Analysis Package. *Journal of Statistical Software*, **23**, 1–18.

Zhou Z, Zhang G, Chen G et al. (2017) Elevated ammonium reduces the negative effect of heat stress on the stony coral Pocillopora damicornis. *Marine Pollution Bulletin*, **118**, 319–327.