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

Katherine E. Dziedzic[1], Holland Elder[1], & Eli Meyer[1*]

[1]Department of Integrative Biology, Oregon State University, Corvallis OR

Keywords: *heritability, genomic, thermal tolerance, coral bleaching*

[*]Author for Correspondence:

email: eli.meyer@oregonstate.edu

Article Type: Primary Research Article

Abstract

Reef-building corals are highly sensitive to rising ocean temperatures, and substantial adaptation will be required for animals 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 this variation was highly heritable (h^2=0.91). This estimate suggests substantial potential for adaptive responses to warming by natural populations of *O. faveolata* in this region. To investigate the functional basis for this variation, we applied genomic and transcriptomic approaches enabled by expanding sequence resources for this species. We used a genetic linkage map we have recently developed to map associations between SNP genotypes and bleaching responses, and identified four genetic markers associated with bleaching. We also profiled gene expression in corals with contrasting bleaching phenotypes, uncovering substantial variation in responses to thermal stress between heat-tolerant and heat-susceptible corals. Integrating these genomic and transcriptomic data with quantitative genetic analysis provides a new perspective on the mechanistic basis for thermal tolerance phenotypes and the potential for adaptation 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 ocean temperatures 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 (*Symbiodinium spp.*) 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 a population allows individuals to adapt to environmental conditions (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.

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. Since the heritability of a trait is specific to a particular population and environment, it remains unclear whether the estimates of h^2 from Indo-Pacific Acroporids can be generalized to evaluate adaptive potential in other regions and species.

The Caribbean has seen considerable loss in coral cover over the last thirty years (Hughes & Tanner, 2000; Gardner, 2003) and questions still remain whether existing populations can adapt to the changing climate. 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. We focus on the contribution of genetic factors to variation in thermal tolerance of corals, providing new insights into the potential for corals' thermal tolerance to respond to selection during ongoing climate change. Thermal stress experiments were conducted at the Smithsonian Tropical Research Institute in Bocas del Toro, Panama, using coral fragments collected from natural populations.

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* genotypes. We collected coral colonies from seven reef sites around the Bocas del Toro, Panama archipelago in 2015 (Figure 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). Colonies were maintained at ambient temperature in aquaria at the Smithsonian Tropical Research Institute (STRI) on Isla Colon, Bocas del Toro. Prior to thermal experiments, each colony was cut into nine smaller uniform fragments with approximately 15-20 polyps per fragment. 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 genotype 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, with daily pH and salinity measurements taken. 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 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. 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 is required to eliminate any 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, then aligned reads to the reference 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). Genotypes were called with a permissive threshold of ≥ 5x 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 dominant symbiont type in each colony. We amplified ITS2 using PCR primers previously described for studies of *Symbiodinium* diversity (LaJeunesse, 2002), and sequenced the resulting amplicons using Sanger Sequencing. The resulting sequences were compared with multiple known ITS2 sequences from all clades A-H (Hunter *et al.*, 2007). 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. While these data lack resolution to describe quantitative mixtures of algal symbiont 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. We included this information 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.*, 2015). We quantified collected samples after stress experiments in qPCR reactions. DNA from all six fragments post heat stress 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 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, 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.*, 2015). To quantify Symbiodinium in each sample we used a pair of universal primers developed based on multiple sequence alignments of the cp23S-rDNA locus from multiple Symbiodinium clades (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. Host cell quantifications (C_T values) were subtracted from symbiont cell quantifications to calculate the dC_T value in each fragment, a measure of the ratio of symbiont cells to host cells. These dC_T values were then compared to a reference control sample to generate ddC_T values, representing the symbiont density in all samples relative to a

reference sample. 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 (Tavalire *et al. in prep.*; Queller & Goodnight, 1989; Muir & Frasier, 2015). 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 genotype and symbiont type as random effects and population source as a fixed effect (Tavalire *et al. in prep.*). To estimate heritability and the associated standard error, we used 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 genotype as a random effect and population source as a fixed effect. To account for errors rising 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 in rrBLUP to conduct association tests, requiring minor allele frequencies > 0.08, and included source population as a fixed effect. We identified genes linked to significant markers by comparing their relative positions in the integrated map our group has recently developed by combining the linkage map with the draft genome assembly (Snelling *et al.*, 2017), focusing on genes within 10 cM of bleaching-associated markers.

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 and 3 heat-susceptible) (Figure 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 on two runs, one run on the HiSeq 3000 platform at OSU's CGRB and the second run on HiSeq 4000 at the University of Oregon's Genomic and Cell Characterization Core Facility. We first processed the raw sequences 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 remaining 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 both constitutive and stress-induced expression across samples. We first tested for the interaction effect between treatment (control versus heat stress treatment) and bleaching response (susceptible versus tolerant). Next, we tested for each main effect, treatment and bleaching response. We extracted differentially expressed genes (DEGs) to compare expression across samples, then searched for patterns of expression across all DEGs for each of the three models tested, and categorized DEGs based on similarity in expression patterns.

Results

SNP genotyping and genetic analysis of corals

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 (Supplementary Table S1), including a large number of

putative polymorphisms. We further filtered genotypes to minimize missing data and genotyping errors, identifying a set of 5,512 high-quality SNPs that we used for all subsequent analyses.

Symbiodinium *communities in host colonies and bleaching responses*

Sanger sequencing of PCR products produced clean sequences, suggesting that a single type was dominant in each colony. To identify the dominant symbiont type in each sample we constructed a maximum likelihood tree using our sequences and multiple representatives from Symbiodinium clades A-H (Hunter *et al.*, 2007). According to our maximum likelihood phylogenetic tree, clades A-D were present across all colonies as a dominant type (Figure 1b), but symbiont type varied across sites. It is likely that there are mixed communities in these samples, but we judged the information on the dominant type to be sufficient to control for confounding effects of symbiont type.

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. While there was variation between colonies, there was little to no variation in bleaching among fragments from the same colony (Figure 2). We quantified symbiont densities in each fragment using qPCR, and compared stress and control phenotypes to determine the average reduction in symbiont abundance in each colony. We estimated the bleaching response of each colony as the difference between average ddC_T for stressed and control fragments. 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 and 3). Most colonies bleached in response to thermal stress, but the extent of these bleaching responses varied considerably (Figure 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 genotype and population source had significant effects on thermal tolerance (p=0.0002 and p=0.025, respectively), symbiont type had no effect (p=0.84). 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 based on this genetic relatedness matrix. This analysis revealed that variation in bleaching responses was highly heritable, with a narrow-sense heritability (h^2) of 0.91. 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 variation in thermal tolerance, we used our SNP genotypes to test for associations between bleaching responses and coral 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

4). After multiple test corrections, we found four markers significantly associated with bleaching (FDR ≤ 0.05).

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 (≤10 cM) with each marker. Our SNPs fell onto linkage groups 3 (two SNPs), 5, and 16. We used the integrated map to identify genes linked to each of these markers (within 10 cM). All genes identified in this analysis are shown in Supplementary Table S2. We found several groups of genes previously implicated in stress responses of corals or other Cnidarians. These included mucins, ubiquitin protein ligases, and cellular tumor antigen p53, a gene associated with apoptosis, on LG 3. Genes linked to the bleaching-associated LG 5 included carbonic anhydrase, an important protein for carbonate transport in symbiotic cnidarians; glutathione peroxidase, a gene involved in oxidative stress response; and sphingosine 1-phosphate receptor associated with innate immunity. Genes linked to the marker on LG 16 included catalase, genes associated with apoptosis (protein NLRC3 and ADP-ribosyl cyclase), a potassium voltage-gated channel protein; the chaperone DnaJ homolog involved in preventing inappropriate unfolding of proteins; two peptidyl-prolyl cis-trans isomerases involved in protein folding; and thioredoxin, a redox regulation protein.

We also found several 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 (9 genes altogether, linked to markers on LG 3, 5, and 16. Similarly, we repeatedly found that galanin receptors were linked to bleaching associated markers (27 galanin receptor genes linked to bleaching-associated markers on LG 3 and 16).

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 transient receptor potential channels (10 genes linked to bleaching-associated markers on LG 3 and 16), and collagen proteins (15 collagen genes linked to bleaching-associated markers on LG 3, 5, and 16). 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 contributes 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). 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 these libraries twice (on HiSeq3000 and HiSeq 4000), because of unexpectedly low yields in these sequencing runs, then combined all reads for analysis. In total, 56.7 million raw reads were produced, approximately 1.58 million reads per sample. The majority of these passed quality and adaptor filtering (95.6%) leaving 54.2 million HQ reads for expression analysis.

Using a negative binomial model, we tested for changes in gene expression, evaluating differences in both constitutive and stress-induced expression. Our model tested for the effect of type (susceptible or tolerant, based on observed bleaching responses), the effect of treatment,

(control or heat treatment), and the interaction effect between type and treatment. We found 1,097, 62, and 210 differentially expressed genes (DEGs) when testing for main effects of type and treatment, and their interaction, respectively. The effects of type on gene expression can be visualized in a heatmap of expression for these DEGs (Figure 5), where heat tolerant colonies (red bars in figure 5) generally express these genes at higher levels than heat susceptible colonies (light blue bars in figure 5) regardless of treatment. A complete list of differentially expressed genes in each category is provided in Supplementary Table S3.

A substantial number of genes showed significant type × treatment effects, where the effects of treatment on expression differed between tolerant and susceptible corals. To categorize gene expression patterns in these interactions we conducted hierarchical clustering of expression patterns, subdividing the tree into clusters of correlated genes using the *cutree* function in R. This analysis revealed two dominant patterns. In the first pattern, genes were expressed at overall higher levels in heat-tolerant corals and were downregulated during thermal stress, and expressed at lower levels overall in heat-susceptible corals but upregulated during thermal stress. We found 159 genes in this category (Figure 6a). The second pattern was the opposite: genes that were expressed at higher levels overall and upregulated during thermal stress in heat-tolerant corals, and down-regulated in susceptible corals (Figure 6b). The remaining 33 genes formed a third weak 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 on our four linkage groups. These included many genes classically associated with thermal stress responses, and included substantial overlap with the genes identified in the association study (above). 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. Building on the findings from our association study, this analysis of gene expression provides an independent line of evidence associating these genes with genetically determined variation in corals' thermal tolerance.

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 of 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 over 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 peroxidase functions as an antioxidant enzyme in response to reactive oxygen species and has been shown to increase during thermal stress (Downs *et al.*, 2002; Anithajothi *et al.*, 2014). Linkage with this gene could indicate an important role for oxidative stress response to ROS production during stress (Downs *et al.*, 2002; Ros *et al.*, 2016). Voltage-gated proteins have also been characterized in *Nematostella vectensis* and have shown the importance of these proteins for maintaining cellular homeostasis, regulation of movement, and feeding. Cnidarians have an extensive set of potassium voltage-gated channels and the functional diversity is profound, but their true cellular role is still unknown (Jegla *et al.*, 2012). 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) (Maor-Landaw & Levy, 2016; Ruiz-Jones & Palumbi, 2017). 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). Peptidyl-prolyl cis-trans isomerase is also involved in proper folding and assembly of new proteins and has been expressed in response to heat shock (Galat, 1993; Sykes *et al.*, 1993). Studies have shown this

gene to be highly expressed in symbiotic anemones and suppressing its activity leads to the production of reactive oxygen species (Kuo *et al.*, 2004; Perez & Weis, 2008). The close proximity 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, transient receptor potential channels, 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). While the role of galanin in invertebrates has not been studied, galanin receptor proteins may have evolved from homologous genes found in invertebrates (Liu *et al.*, 2010). 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). Transient receptor potential (TRP) channels function as ion channels embedded within cell membranes and play a major role in sensory perception that allow organisms to sense their external environment (Peng *et al.*, 2015; Malafoglia *et al.*, 2016). In fact, some TRPs have been lost in Bilaterians but conserved in Cnidarians, which may be a function of their environment (Peng *et al.*, 2015). In vertebrates, TRP channels are induced by the expression of heat shock proteins as well as nitro-oxidative species and superoxide dismutase (Vriens *et al.*, 2011; Leonelli *et al.*, 2013). A similar study in *Hydra* pinpointed a particular TRP channel, TRPM3 that may play a role in thermosensation and oxidative stress responses in cnidarians (Malafoglia *et al.*, 2016). 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 suggests they contribute to variation in corals' thermal tolerance.

In addition to genetic analysis, high-throughput sequencing has also enabled widespread application of RNA-Seq approaches to profile gene expression (Wang *et al.*, 2009). 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 & Palumbi, 2011; Bellantuono *et al.*, 2012a, 2012b; 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. Focusing on the genes differentially expressed as a function of the type × treatment interaction, we identified a cluster of genes that were constitutively 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 (Fig 6a). These included genes associated with protein metabolism (eight 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). Differentially expressed genes that overlapped with our association study included collagen genes, mucins, DnaJ proteins, glutathione peroxidase, and peptidyl-prolyl cis-trans isomerases. 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. 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 suggest that differential regulation of these genes may play a role in variation among corals' thermal tolerances.

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.

Acknowledgements

The authors are grateful to the Smithsonian Tropical Research Institute and the Institute for Tropical Ecology and Conservation for use of their facilities and for assistance with sample collection and fieldwork logistics. We thank Rachel Vasta and Chelsea Eckhart for assistance with sample processing. We thank Oregon State University's College of Science for travel assistance through a travel grant to KD.

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.

Anithajothi R, Duraikannu K, Umagowsalya G, Ramakritinan CM (2014) The presence of biomarker enzymes of selected scleractinian corals of Palk Bay, southeast coast of India. *BioMed Research International*, **2014**.

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) Coral reefs: 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 AJAJ, Granados-Cifuentes CC, Miller DJDJ, Hoegh-Guldberg OO, Rodriguez-Lanetty MM (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.

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.

Galat A (1993) Peptidylproline cis-trans-isomerases□: immunophilins. **707**, 689–707.

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.

Jegla T, Marlow HQ, Chen B, Simmons DK, Jacobo SM, Martindale MQ (2012) Expanded Functional Diversity of Shaker K + Channels in Cnidarians Is Driven by Gene Expansion. *PLoS ONE*, **7**, e51366.

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 MJH, 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. Biological sciences / The Royal Society*, **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.

Kuo J, Chen M-C, Lin C-H, Fang L-S (2004) Comparative gene expression in the symbiotic and aposymbiotic Aiptasia pulchella by expressed sequence tag analysis. *Biochemical and Biophysical Research Communications*, **318**, 176–186.

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.

Leonelli M, Martins DO, Britto LRG (2013) Retinal cell death induced by TRPV1 activation involves NMDA signaling and upregulation of nitric oxide synthases. *Cellular and Molecular Neurobiology*, **33**, 379–392.

Liu Z, Xu Y, Wu L, Zhang S (2010) Evolution of galanin receptor genes: Insights from the deuterostome genomes. *Journal of Biomolecular Structure and Dynamics*, **28**, 97–106.

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.

Malafoglia V, Traversetti L, Del Grosso F et al. (2016) Transient receptor potential melastatin-3 (TRPM3) mediates nociceptive-like responses in Hydra vulgaris. *PLoS ONE*, **11**, 1–15.

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.

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.

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.

Peng G, Shi X, Kadowaki T (2015) Evolution of TRP channels inferred by their classification in diverse animal species. *Molecular Phylogenetics and Evolution*, **84**, 145–157.

Perez S, Weis V (2008) Cyclophilin and the regulation of symbiosis in Aiptasia pallida. *Biological Bulletin*, **215**, 63–72.

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

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.

Ros M, Pernice M, Le Guillou S, Doblin MA, Schrameyer V, Laczka O (2016) Colorimetric detection of caspase 3 activity and reactive oxygen derivatives: Potential early indicators of thermal stress in corals. *Journal of Marine Biology*, **2016**.

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.
Sykes K, Gething MJ, Sambrook J (1993) Proline isomerases function during heat shock. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 5853–7.
T L (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine biology*, **141**, 387–400.
Tavalire HF, Beechler BR, Gorsich EE et al. There is no cure that does not cost: fitness trade-offs and multiple forms of resistance to bovine tuberculosis in a wild mammal. *Manuscript in preparation*.
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.
Vriens J, Owsianik G, Hofmann T et al. (2011) TRPM3 Is a Nociceptor Channel Involved in the Detection of Noxious Heat. *Neuron*, **70**, 482–494.
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.
Zhao JH (2007) gap: Genetic Analysis Package. *Journal of Statistical Software*, **23**, 1–18.

Supplementary Information

Supplementary Table S1. Excel spreadsheet. Summary of sequencing yields, processing, and mapping efficiencies for RNASeq and 2bRAD sequencing libraries.

Supplementary Table S2. Excel spreadsheet. Complete list of genes linked to SNP markers significantly associated with variation in bleaching responses (within 10 cM of the markers).

Supplementary Table S3. Excel spreadsheet. Complete list of genes differentially expressed as a function of temperature, thermal tolerance type, or their interactions.

Data Accessibility

Reference numbers for data in public repositories: sequence data archived at NCBI's Sequence. Read Archive under accession SUB3108676.

Scripts used for analysis can be found at https://github.com/Eli-Meyer.

Authors' Contributions

KD and EM conceived the study and conducted analyses. KD collected samples, conducted thermal stress experiments, prepared sequencing libraries, and wrote the manuscript. HE contributed to sample collection and fieldwork assistance. All authors have read and approved the manuscript.

Figures and Tables

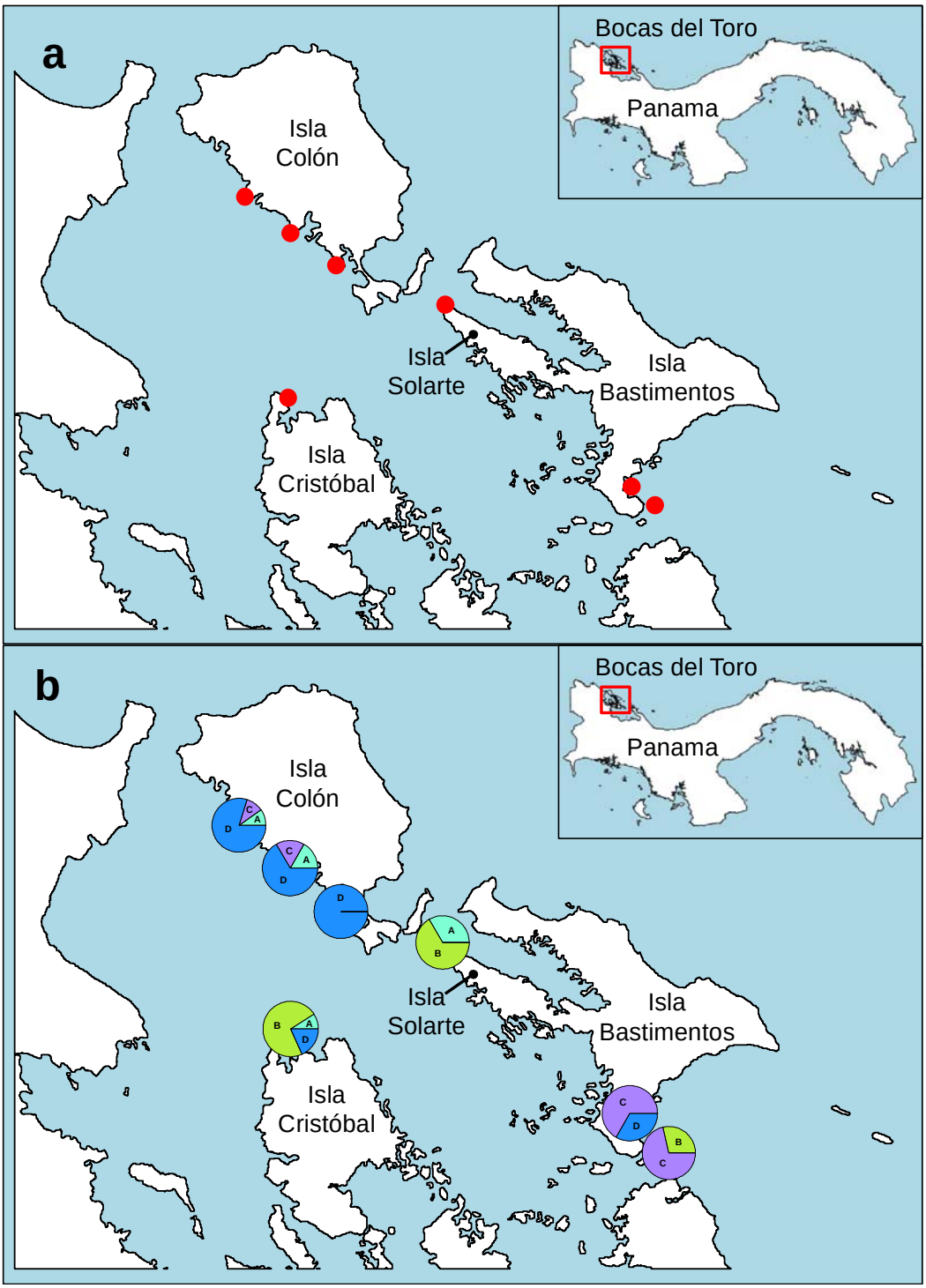

Figure 1. Map of Collection Sites around Bocas del Toro Archipelago, Panama. (a) Map of the seven locations where coral genotypes were collected around the archipelago. (b) Proportion of dominant symbiont types found at each site across colonies collected.

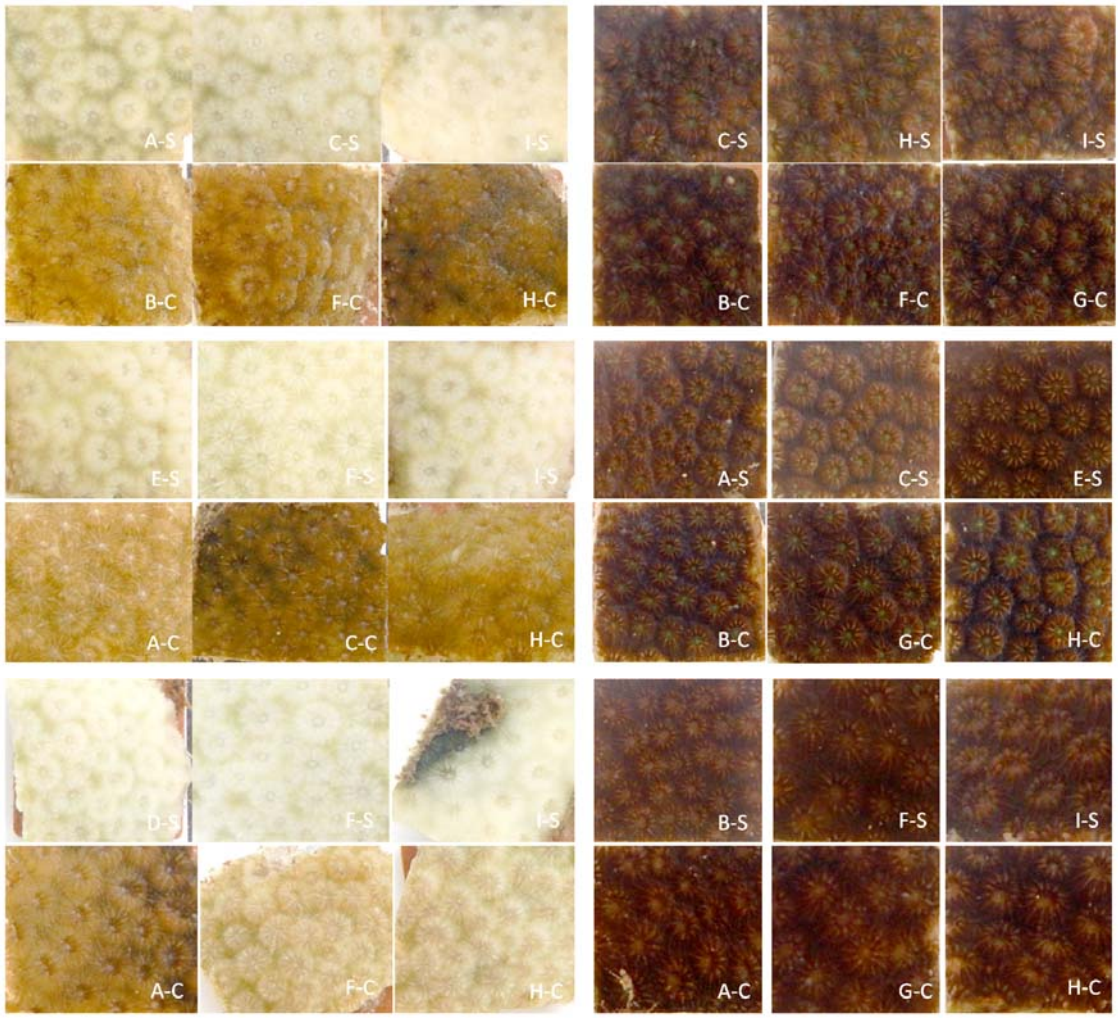

Figure 2. An example of the striking contrast between bleaching phenotypes of heat-susceptible and -tolerant corals sampled for this study. Each panel of six images represents fragments from a single colony, with control fragments indicated with "-C" (bottom of each panel) and heat-stressed fragments indicated with "-S" (top of each panel). Bleaching responses varied widely among colonies, but very little among fragments prepared from each colony.

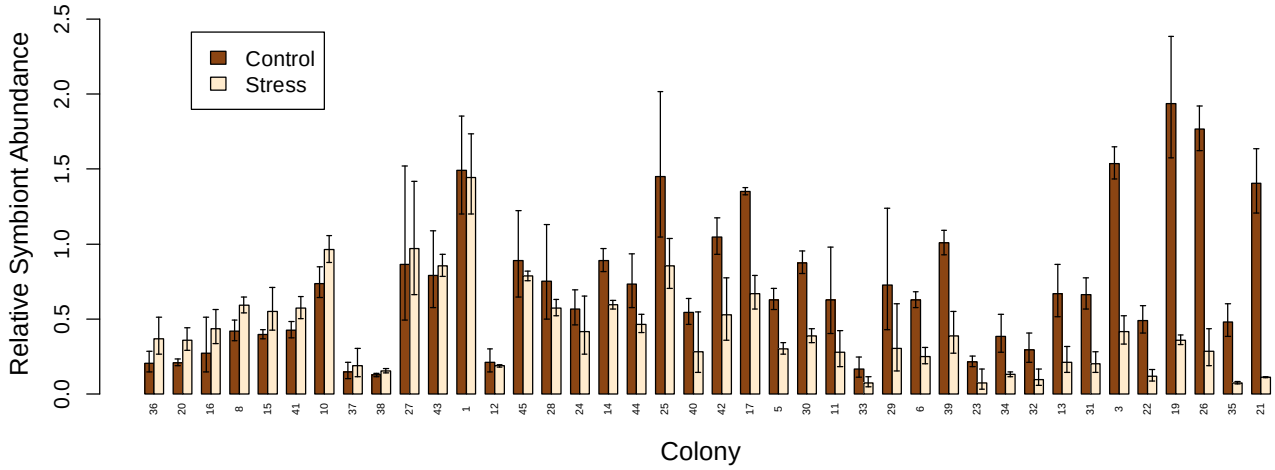

Figure 3. Quantification of algal symbiont densities using qPCR reveals variation in bleaching phenotypes. Control (brown) and stress (white) bars represent relative symbiont abundance after four weeks in experimental conditions. Bars are paired for each colony and colonies are ordered by their relative change in symbiont abundance (bleaching response) between control and stress treatments.

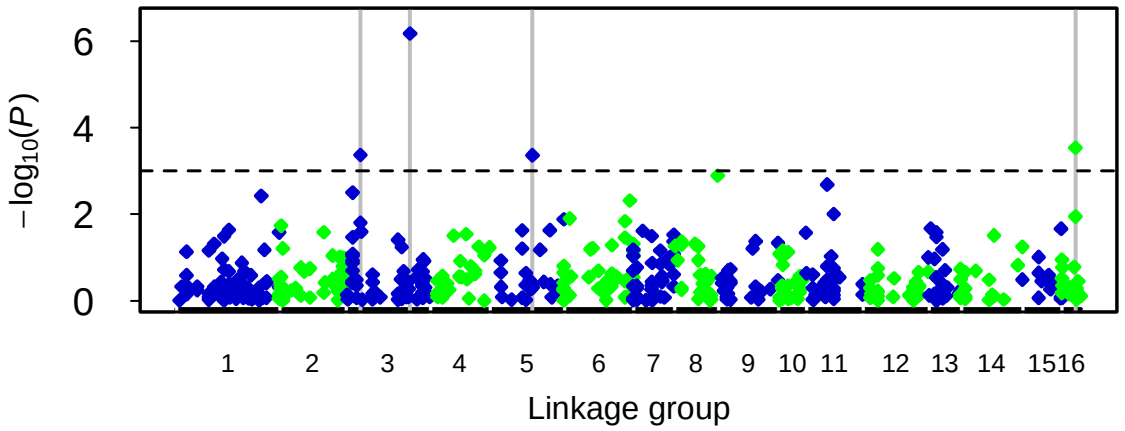

Figure 4. Mapping statistical associations between SNP genotypes and bleaching responses onto the linkage map identifies genomic regions associated with thermal tolerance in *O. faveolata*. Genetic markers are mapped against the linkage groups, indicated by alternating colors. Four markers, on linkage groups 3, 5 and 16, were significantly associated (gray lines) with variation in bleaching responses during thermal stress (FDR<0.05).

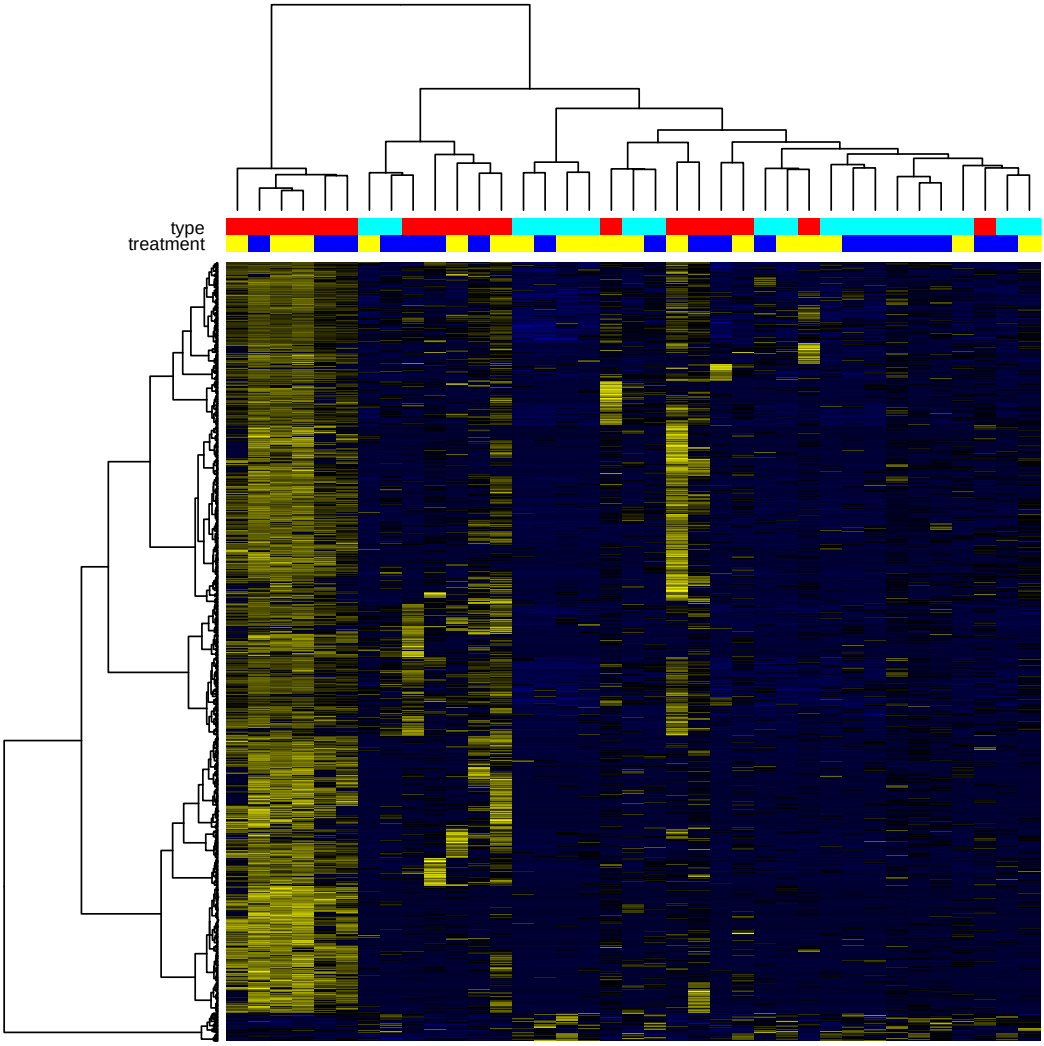

Figure 5. Heatmap showing relative expression of genes that were differentially expressed between heat-tolerant and heat-susceptible corals. The 1,097 DEG in this category are shown here, with samples and genes grouped with hierarchical clustering based on similarity in gene expression patterns. In the 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.

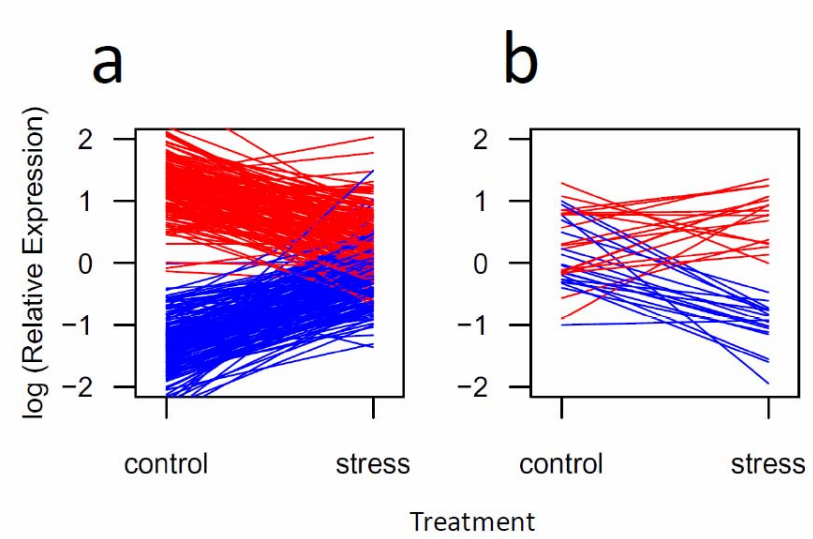

Figure 6. Genotype × temperature effects on gene expression fall into two general categories. a) 159 genes were downregulated in heat-tolerant corals (red) and upregulated in heat-susceptible corals (blue). b) 18 genes showed contrasting expression changes, but in the opposite directions: up-regulated 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).