# 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 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 then 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 ($h^2$=0.89). 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 growing sequence resources for this species. We used a genetic linkage map we've recently developed to map associations between SNP genotypes and bleaching responses, and identified two 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 ecosystems 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 hjave 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 corals' thermal tolerance, 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

155 microbial symbionts) (Abrego *et al.*, 2008; Howells *et al.*, 2011), we sequenced the dominant
156 symbiont type in each colony. We amplified ITS2 using PCR primers previously described for
157 studies of *Symbiodinium* diversity (LaJeunesse, 2002), and sequenced the resulting amplicons
158 using Sanger Sequencing. The resulting sequences were compared with multiple known ITS2
159 sequences from all clades A-H (Hunter *et al.*, 2007). Using our symbiont sequences and these
160 reference ITS2 sequences, we created an alignment in the program MEGA (Kumar *et al.*, 2017).
161 A maximum likelihood phylogenetic tree was created with all known and unknown sequences to
162 determine which clades our coral samples fell into. The dominant symbiont type was assigned
163 for each sample by comparing the phylogenetic tree of unknown and known samples. While
164 these data lack resolution to describe quantitative mixtures of algal symbiont types, we interpret
165 these sequences as the dominant symbiont types in each sample based on the presence of a single
166 dominant haplotype in sequencing chromatograms. We included this information in quantitative
167 models of bleaching responses to evaluate the contribution of variation in the dominant symbiont
168 type to variation in thermal tolerance.
169
170 *Quantifying bleaching responses*
171 To quantify bleaching in each fragment, we used qPCR to estimate the abundance of
172 algal symbionts relative to host cells (Cunning *et al.*, 2015). We quantified collected samples
173 after stress experiments in qPCR reactions. DNA from all six fragments post heat stress was
174 extracted using an organic phase extraction. All qPCR reactions were run on an Eppendorf
175 Realplex 4 machine using the SYBR and ROX filters. Each reaction consisted of 7.5 µL
176 SensiFAST SYBR Hi-ROX master mix (Bioline, Taunton, MA), 4.3 µl NFW, 0.6 µl each of
177 forward and reverse 10-µM primers, and 2 µl of genomic DNA in a final volume of 15 µl. The
178 thermal profile for each reaction consisted of an initial denaturing step of 95°C for 2 min,
179 followed by 40 cycles of: 95°C for 5 s, annealing temperature of 60°C for 30 s, and then 72°C
180 for 30 sec. All samples were run using the same reaction parameters and were analyzed together.
181 In addition, one sample was included on every plate as an inter-plate calibrator. We quantified
182 host cells using host actin loci using the forward (5'-CGCTGACAGAATGCAGAAAGAA-3')
183 and reverse (5'-CACATCTGTTGGAAGGTGGACA-3') primers, as previously described
184 (Cunning *et al.*, 2015). To quantify Symbiodinium in each sample we used a pair of universal
185 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 (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. We used a linear mixed model with genotype and symbiont type as random effects and population source as a fixed effect.

*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 to identify loci associated with bleaching. 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 searched 10 cM upstream and downstream of each significant SNP 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 chosen for 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 counts 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 produced clear ITS2 amplicon reads for each colony, suggesting that a single type was dominant in these colonies. 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 that information on the dominant type was 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 0.89. 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 association between bleaching responses and coral genotypes. For this analysis, we conducted a series of linear mixed models with genotype as a random factor and population source as a fixed factor. We conducted tests at each locus to determine if the bleaching responses depended on the genotype at that locus. 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 two loci significantly associated with bleaching with q-values $\leq 0.05$ (Storey, 2003).

To further characterize where these SNPs were located and their biological functions, we used the integrated map (Snelling *et al.*, 2017) to search 10cM up and downstream of each SNP. Our SNPs fell onto linkage groups 11 and 5, with the SNP on linkage group 5 falling directly onto a gene, Scm-like protein involved in histone-binding. Within the 20 cM windows around our SNPs, we searched across scaffolds and located annotated and unannotated genes within

gene neighborhoods. All genes identified in this analysis are shown in Supplementary Table S2. On linkage group 5, genes of interest included a universal stress protein, poly (ADP-ribose) polymerase, a protein involved in oxidative stress response, a potassium voltage-gated channel protein, and proteins involved in translation. Genes linked to the marker on LG 11 included a hypopyruvate reductase, cytoskeletal proteins, and proteins involved in organelle organization and biosynthesis.

*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 our libraries twice, once on Illumina HiSeq 3000 and once on HiSeq 4000, and all sequenced reads from both runs were combined. In total, 56.7 million raw reads were produced, with 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 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 1,097, 62, and 210 differentially expressed genes (DEGs) when testing for type, treatment, and interaction effects, 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 by 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. 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. Gene expression profiles could be categorized into two dominant patterns. In the first patterns, 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 cluster with similar patterns as 6b but with more variation across genes (not shown).

## Discussion

Our study provides some of the first quantitative estimates for heritability of variation in adult corals' bleaching responses. 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 genetic linkage map and draft sequence assemblies to identify of some of the genes linked to these markers. We found

372 that transcriptional responses of heat-tolerant corals to thermal stress are markedly different from
373 those of heat-susceptible colonies. We identified over 200 genes differentially expressed as a
374 function of type × treatment interactions, which were generally expressed at higher levels in
375 tolerant corals and regulated in opposite directions by tolerant and susceptible corals in response
376 to thermal stress.

377 This study builds on growing evidence that coral populations harbor genetic variation that
378 may support adaptation to ocean warming. These questions are especially pressing for Caribbean
379 corals, where reefs have declined severely over the last few decades. Since the genetic variation
380 supporting heritable variation in traits under selection is species- and population-specific,
381 measuring these parameters in Caribbean populations is vital for understanding the future of
382 these ecosystems. Our study documents considerable genetic variation in thermal tolerance for a
383 population of the mountainous star coral, *Orbicella faveolata*, an important reef-builder
384 throughout the Caribbean.

385 This suggests the genetic potential for substantial adaptive responses to selection for
386 thermal tolerance in this population. Responses to selection can be modeled with the univariate
387 breeder's equation to estimate the expected rate of adaptation within a single generation
388 (Falconer and Mackay, 1996). These predictions require empirical estimates for the narrow-sense
389 heritability of the trait under selection, the proportion of phenotypic variation attributable to
390 additive genetic variation (Falconer and Mackay, 1996). While it has been clear for some time
391 that corals possess substantial variation in thermal tolerance, in part resulting from
392 acclimatization or association with different algal symbionts (Fitt *et al.*, 2001; Howells *et al.*,
393 2011; Oliver & Palumbi, 2011; Silverstein *et al.*, 2012), the variation attributable to genetic
394 factors in the coral host has remained understudied. This genetic variation will determine the
395 adaptive responses of corals in the immediate future, since rapid adaptation relies on standing
396 genetic variation in natural populations (Barrett & Schluter, 2008). Our study contributes novel
397 information on this potential for adaptation to ocean warming, confirming that heritability of
398 bleaching responses in adult corals can be comparable to the high heritability of thermal
399 tolerance observed in some previous larval studies (Dixon *et al.*, 2015).

400 Importantly, these estimates of $h^2$ express genetic potential for adaptation, and other
401 factors may constrain the adaptive responses that are actually realized in nature. The breeder's
402 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 (even switching signs) 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 approached 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's 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

433 sampling design, we succeeded in identifying genetic markers associated with variation in
434 bleaching responses.
435 These significant markers are linked to biologically interesting genes that could
436 contribute to host thermal tolerance. For example, we found genes functions involved in
437 oxidative stress, neural responses, and cytoskeletal organization. Poly (ADP-ribose) polymerase
438 has been previously described in the sea anemone *Nematostella vectensis* as a mechanism for
439 activating TRP channels involved in producing a cellular response to oxidative stress (Kühn *et*
440 *al.*, 2016). Linkage with this gene could indicate a role for this gene in cellular responses to
441 thermal stress. Voltage-gated proteins have also been characterized in *Nematostella vectensis* and
442 have shown the importance of these proteins for maintaining cellular homeostasis, regulation of
443 movement, and feeding. Cnidarians have an extensive set of potassium voltage-gated channels
444 and the functional diversity is profound, but their true cellular role is still unknown (Jegla *et al.*,
445 2012). Perhaps the most interesting is the universal stress protein found in close proximity with
446 our SNP on linkage group 5. This intriguing protein, originally discovered in prokaryotes and
447 subsequently documented through metazoans (Jong *et al.*, 2011) has been previously reported in
448 studies of thermal stress responses in Cnidarians (Bellantuono *et al.*, 2012b). The close proximity
449 to our SNPs suggests a possible role for this gene in determining variation in thermal tolerance
450 among colonies of *O. faveolata*.
451 High-throughput sequencing has also enabled widespread application of RNA-Seq
452 approaches to profile gene expression (Wang *et al.*, 2009). These methods have been widely
453 adopted to study transcriptional responses to thermal stress in corals (DeSalvo *et al.*, 2008;
454 Voolstra *et al.*, 2009; Leggat *et al.*, 2011; Meyer *et al.*, 2011; Oliver & Palumbi, 2011;
455 Bellantuono *et al.*, 2012a, 2012b; Barshis *et al.*, 2013; Kenkel *et al.*, 2013; Palumbi *et al.*, 2014).
456 One finding that has emerged consistently from these studies is the observation that corals vary
457 widely in their transcriptional and phenotypic responses to thermal stress (Hunter, 1993; Ayre *et*
458 *al.*, 1997; Marshall & Baird, 2000; Baums *et al.*, 2013). Many studies have demonstrated
459 variation in gene expression among coral phenotypes, both in natural populations and in
460 controlled studies (López-Maury *et al.*, 2008; DeSalvo *et al.*, 2010; Meyer *et al.*, 2011;
461 Granados-Cifuentes *et al.*, 2013).
462 Here, we built upon these studies by quantifying variation in transcriptional responses to
463 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). 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' adaptation 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 after further study to evaluate the generalizability of these associations. 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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**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. HD contributed to sample collection and fieldwork assistance. All authors have read and approved the manuscript.

## Figures and Tables

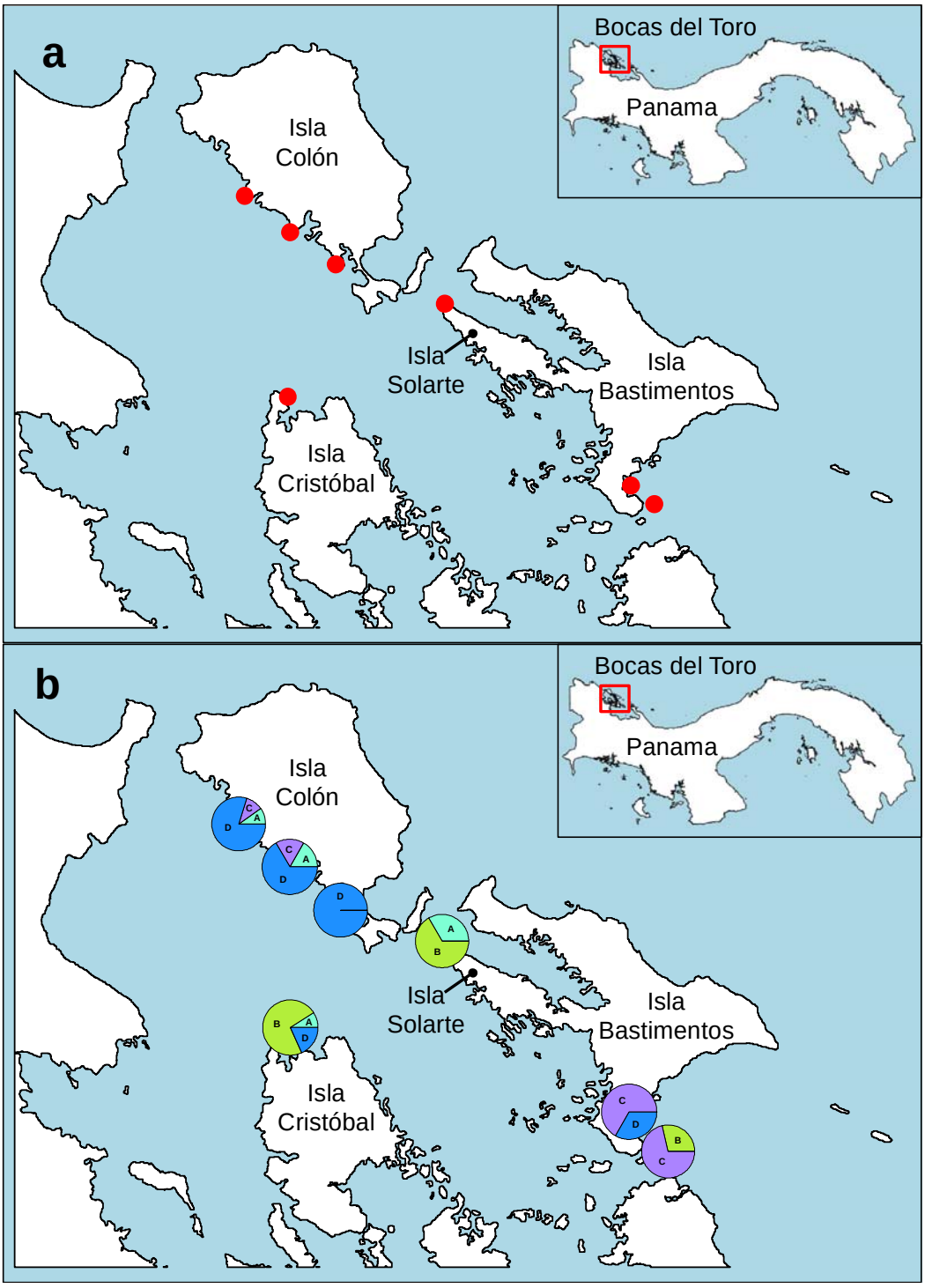


**Figure 1.** Map of collection sites around Bocas del Toro Archipelago, Panama. (a) Map of the seven locations where coral genotypes were collected around the archipelago. (b) Proportion of dominant symbiont types found at each site across colonies collected.

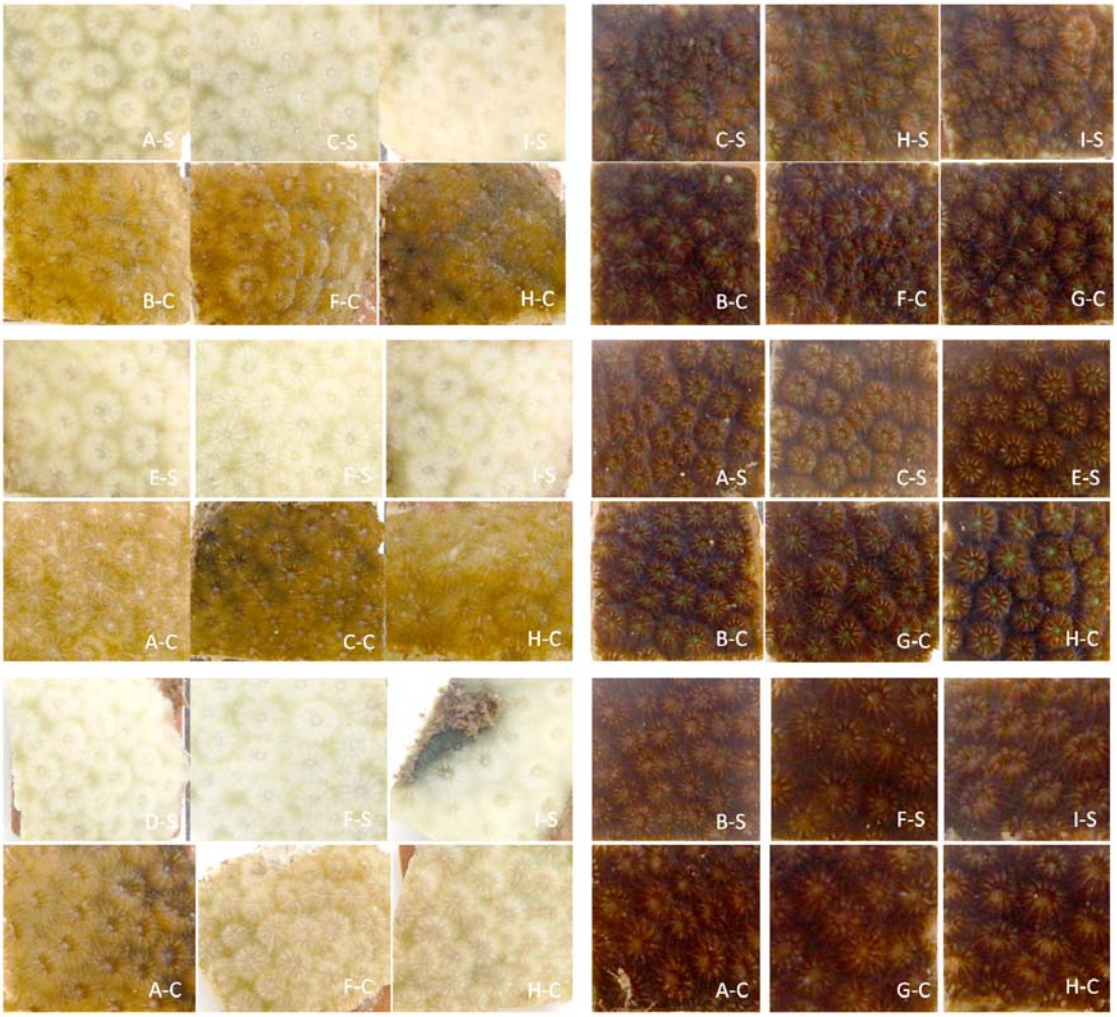


**Figure 2.** An example of the striking contrast between bleaching phenotypes of heat-susceptible and -tolerant corals sampled for this study. Each panel of six images represents fragments from a single colony, with control fragments indicated with "-C" (bottom of each panel) and heat-stressed fragments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

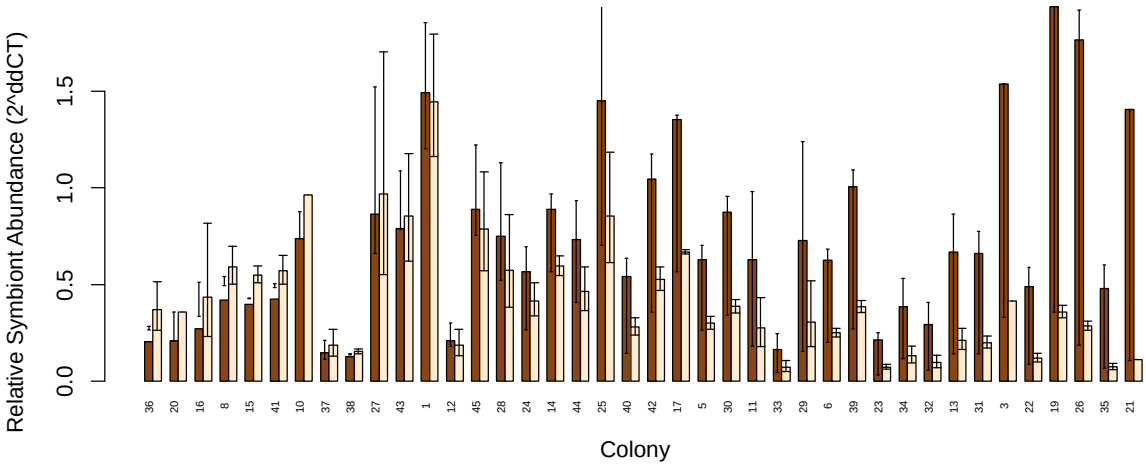


**Figure 3.** Quantification of algal symbiont densities using qPCR reveals variation in bleaching phenotypes. Control (brown) and stress (white) bars represent relative symbiont abundance after four weeks in experimental conditions. Bars are paired for each colony and colonies are ordered by their relative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

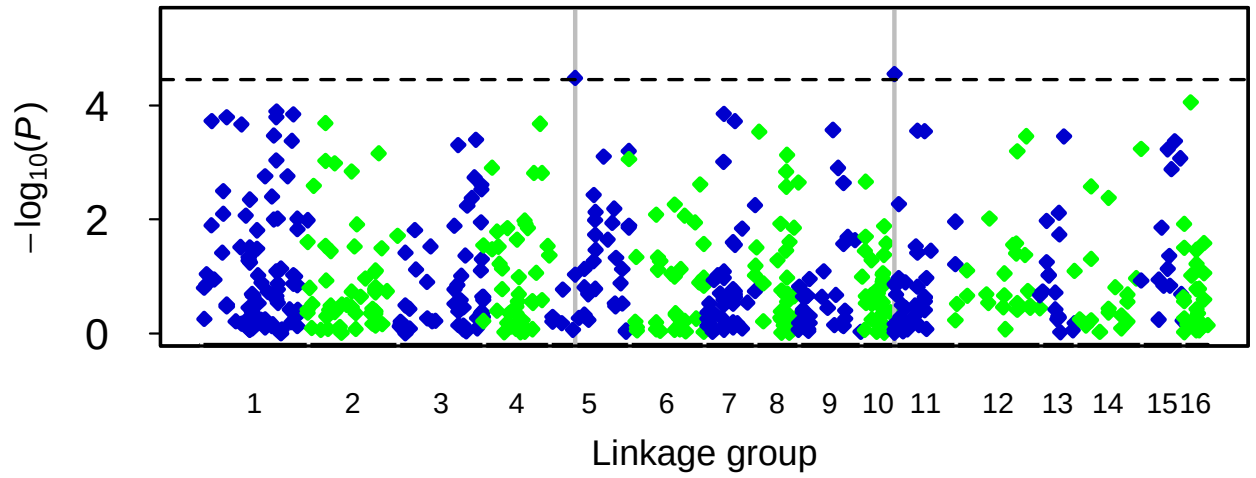


**Figure 4.** Mapping statistical associations between SNP genotypes and bleaching responses onto the linkage map identifies genomic regions associated with thermal tolerance in *O. faveolata*. Genetic markers are mapped against the linkage groups, indicated by alternating colors. Two markers, on linkage group 5 and 6, were significantly associated (gray lines) with variation in bleaching responses during thermal stress (FDR<0.05).

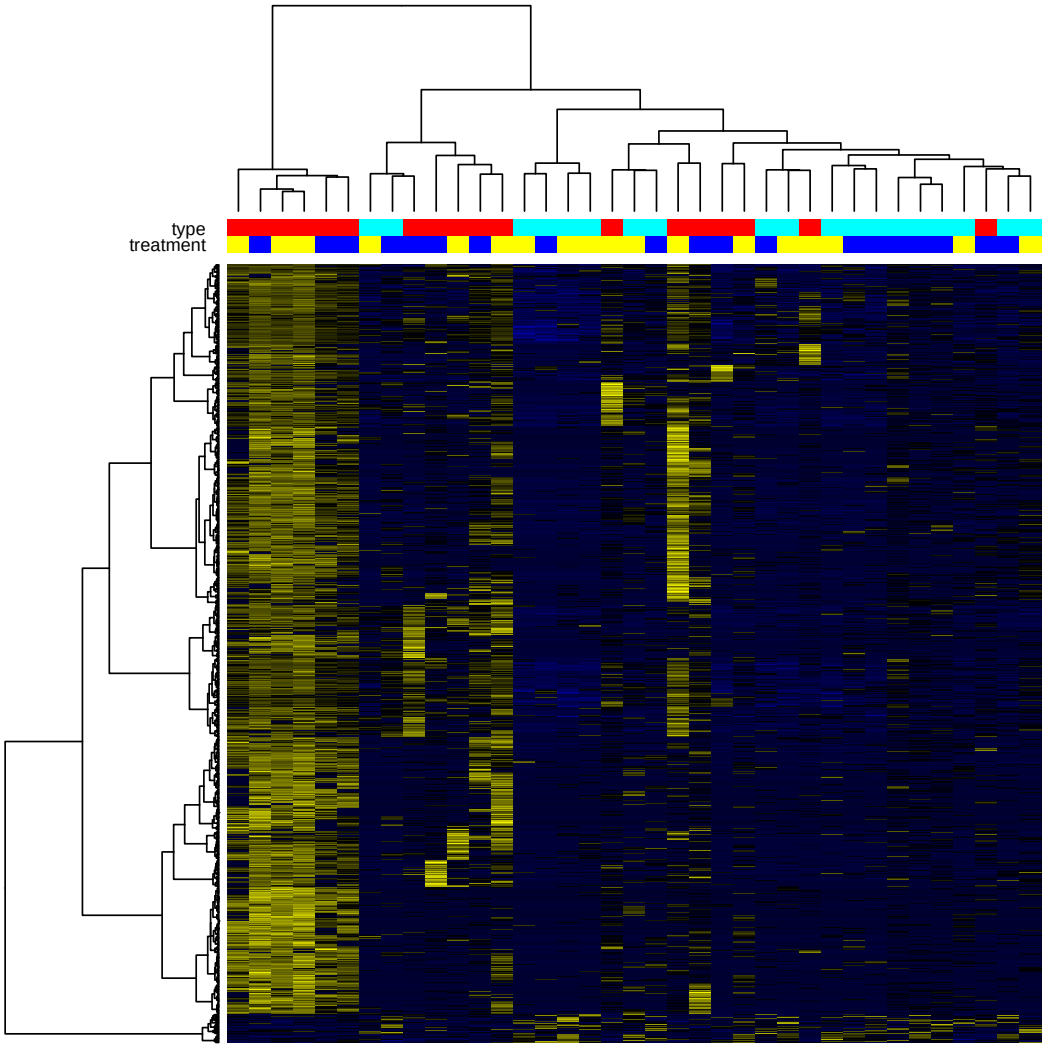


**Figure 5.** Heatmap showing relative expression of genes that were differentially expressed between heat-tolerant and heat-susceptible corals. The 1,097 DEG in this category are shown here, with samples and genes grouped with hierarchical clustering based on similarity in gene expression patterns.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

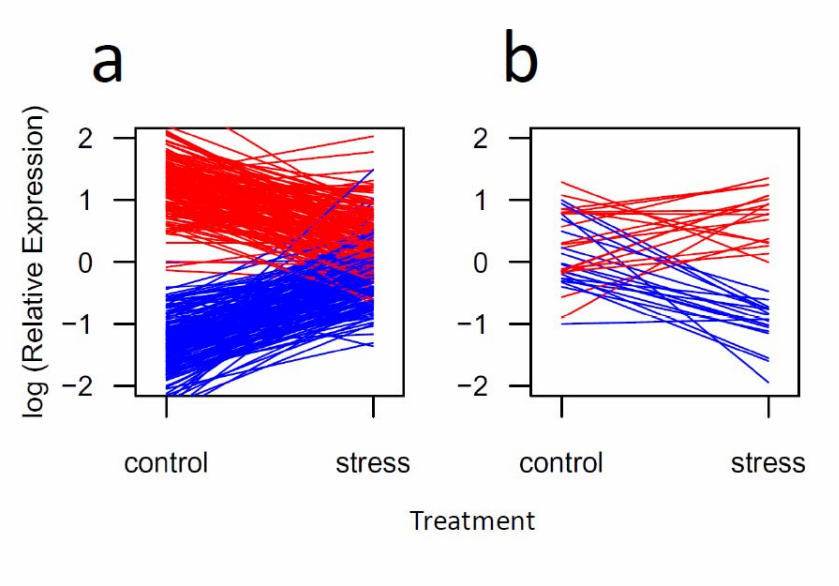


**Figure 6.** Type × treatment effects on gene expression fall into two general categories. a) 159 genes were downregulated in heat-tolerant corals (red) and upregulated in heat-susceptible corals (blue). b) 18 genes showed contrasting expression changes, but in the opposite directions: up-regulated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

584 **Supplementary Information**
585
586 Supplementary Table S1. Excel spreadsheet. Summary of sequencing yields, processing, and
587 mapping efficiencies for RNASeq and 2bRAD sequencing libraries.
588
589 Supplementary Table S2. Excel spreadsheet. Complete list of genes linked to SNP markers
590 significantly associated with variation in bleaching responses (within 10 cM of the markers).
591
592 Supplementary Table S3. Excel spreadsheet. Complete list of genes differentially expressed as a
593 function of temperature, thermal tolerance type, or their interactions.
594
595

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