Vol. 646: 45–68, 2020
https://doi.org/10.3354/meps13407

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published July 30[§]

Genetic differences in thermal tolerance among colonies of threatened coral *Acropora cervicornis*: potential for adaptation to increasing temperature

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ABSTRACT: Climate change is resulting in warmer temperatures that are negatively impacting corals. Understanding how much individuals within a population vary in their thermal tolerance and whether this variation is heritable is important in determining whether a species can adapt to climate change. To address this, *Acropora cervicornis* fragments from 20 genetically distinct colonies collected from the Coral Restoration Foundation Tavernier nursery (Florida, USA) were kept at either ambient (28 ± 1°C) or elevated (32 ± 1°C) temperatures, and mortality was monitored for 26 d. Both broad-sense (H^2) and narrow-sense (h^2) heritability of thermal tolerance were estimated to determine the amount of genetic variation underlying survival to elevated temperature. To understand the physiological basis of thermal tolerance, tissue from both treatments was taken 12 h after the start of the experiment to investigate gene expression at the mRNA and protein level between tolerant and susceptible colonies. Results revealed that this population has considerable total genetic variation in thermal tolerance (H^2 = 0.528), but low variance in relatedness among colonies prevented us from making any conclusions regarding h^2. Despite high transcriptomic variability among and within colonies, 40 genes were consistently and significantly different between tolerant and susceptible colonies, and could be potential biomarkers for thermal tolerance should they be verified in a larger sample. Overall, the results suggest that this population has substantial genetic variation for traits that directly impact thermal tolerance; however, their response to projected increases in temperature will depend on more precise estimates of the additive components of this variation (h^2).

KEY WORDS: Thermal tolerance · Heritability · *Acropora cervicornis* · Adaptation · Coral · Florida Keys

1. INTRODUCTION

Human activities have substantially increased the atmospheric concentrations of greenhouse gases, resulting in warmer air and ocean temperatures that are having a negative impact on marine ecosystems worldwide (IPCC 2013). This is a dire threat to reef corals and their algal endosymbionts, which can both suffer heat stress from changes as small as 1°C above long-term regional maximum temperatures (Goreau & Hayes 1994, Hoegh-Guldberg 1999, Coles & Brown 2003). Higher ocean temperatures and irradiance levels (Brown et al. 2002, Takahashi & Murata 2008) have repeatedly been reported to disrupt the symbiosis between corals and their algal endosymbionts, leading to higher occurrences of endosymbiont loss from coral tissue, resulting in coral bleaching and mortality (Glynn 1996, Hoegh-Guldberg 1999,

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[§]The spelling of the 5th author's name was corrected after publication. This corrected version: August 5, 2020

Douglas 2003). These endosymbionts supply up to 95% of the coral host's energy requirements (Muscatine 1990, Jones et al. 2008), so exposure to especially high or long-term elevated temperatures can result in increased mortality of the host.

There are a number of ways in which corals could respond to a changing thermal environment. Corals could respond to temperature changes within a single generation through acclimatization (Mayfield et al. 2013, Sawall et al. 2015, DeCarlo et al. 2019). An example of how corals can achieve this is through the shuffling of different concentrations of symbiont taxa within their tissues to better suit their immediate thermal environment (Baker 2003, Berkelmans & van Oppen 2006, Jones et al. 2008). Corals could also respond to temperature changes through short-term trans-generational processes in which individual colonies inherit greater tolerance from parents who have been previously exposed to a similar stress (Putnam & Gates 2015, Liew et al. 2018, Eirin-Lopez & Putnam 2019), which may include symbiont and microbiome shuffling in species capable of vertical transmission (Webster & Reusch 2017, Quigley et al. 2019). Finally, coral populations could adapt to increased sea surface temperatures if some genotypes have increased survival and reproductive success relative to others (Hoegh-Guldberg 2014). While acclimatization is a potential short-term solution, the long-term success of many coral species is likely dependent on their ability to adapt to the new thermal environment. Evolution by means of natural selection, however, is only possible if genetic variation exists within a population for traits that affect fitness (Fisher 1930). Therefore, to determine whether coral populations are likely to have the ability to adapt to predicted changes in climate, estimates of adaptive genetic variation in traits related to thermotolerance are needed.

While coral genotype can significantly impact coral thermal tolerance (Jin et al. 2016), only a few studies have focused on quantifying the genetic variation (heritability) present in the trait of thermal tolerance among different coral genotypes (Meyer et al. 2009, Császár et al. 2010, Dixon et al. 2015, Kenkel et al. 2015, Dziedzic et al. 2019). In other organisms, estimates of heritability are made through multiple generations of controlled breeding experiments, which are difficult to accomplish in many species of coral due to their long lifespans and delayed sexual maturity (Chamberland et al. 2016). Some studies have overcome these constraints by crossing adults of different genotypes and investigating genetic variance in the larvae produced (Polato et al. 2013, Quigley et al. 2017), but the phenotypes expressed in the planktonic larval stage may not translate well to a sessile adult stage. Fortunately, with the advent of molecular genotyping and the ability of corals to be fragmented to propagate clones, studies can be designed to estimate the heritability of thermal tolerance among coral genotypes and also measure the underlying differences in gene expression between tolerant and susceptible phenotypes (Császár et al. 2010, Carlon et al. 2011, Dziedzic et al. 2019).

With the threat of global warming, much research has focused on understanding the underlying physiological mechanisms of thermal tolerance in corals. Previous work has shown consistent changes in the expression of ubiquitin (Barshis et al. 2010, DeSalvo et al. 2010a, Dziedzic et al. 2019), ferritin (Császár et al. 2009), heat shock proteins (Császár et al. 2009, DeSalvo et al. 2010a), and genes regulating apoptosis, ribosomal RNA, and mRNA processing (DeSalvo et al. 2010a, Bay & Palumbi 2014, Seneca & Palumbi 2015, Dziedzic et al. 2019) in coral either from different thermal environments or from different experimental temperature treatments. While many of these studies have been able to connect temperature stress with patterns of gene expression, few studies have been able to connect patterns in gene expression to heritable traits that increase thermal tolerance (but see Dziedzic et al. 2019). This is truly important when trying to quantify the potential for adaptation, as increased thermal tolerance is likely to lead to differences in survival during bleaching events, and these differences in survival can have direct implications for reproductive success.

Coral thermal tolerance is not only affected by host genotype and host gene expression, but can also be influenced by their algal endosymbionts. Several studies on a number of coral species have provided support for corals hosting symbionts in the family Symbiodiniaceae of the genus *Durusdinium* (formerly clade D, see LaJeunesse et al. 2018) having higher thermal tolerance and lower bleaching susceptibility (Glynn et al. 2001, Rowan 2004, Császár et al. 2010). However, generalizations should be made cautiously, as this interaction between symbiont and temperature tolerance in the coral host may also be species-specific. For example, Abrego et al. (2008) found that *Acropora tenuis* harboring *Durusdinium* sp. were more susceptible to temperature and light stress than colonies harboring C1 symbionts (genus *Cladocopium*), illustrating that harboring *Durusdinium* sp. may not result in increased thermal tolerance in all coral hosts or life-stages. Corals have also been shown to change in Symbiodiniaceae compo-

sition following bleaching events (known as 'symbiont shuffling', Baker 2003) (van Oppen et al. 2005, Berkelmans & van Oppen 2006, Jones et al. 2008, Lewis et al. 2019), which can lead to changes in host gene expression (DeSalvo et al. 2010b) and may be a way for corals to respond to their changing environment in the short term. Therefore, it is important to consider symbiont composition as well as host genotype when investigating thermal tolerance in any coral species.

With the decline of many reefs globally over recent decades, coral restoration has become increasingly imperative. These restoration efforts typically include propagation of coral fragments in off-shore nurseries, followed by transplantation of these nursery-raised fragments onto degraded reefs (Rinkevich 1995, 2014). It has been suggested that these restoration efforts could be improved through 'assisted evolution,' where especially tolerant colonies are identified and used to 'seed' reefs with genotypes that are resistant to elevated temperature stress and bleaching (van Oppen et al. 2015). Efforts like these will require that tolerant genotypes are easily identifiable, and thus there is a need for understanding how tolerance is related to patterns of biomarker expression or specific genetic markers.

To understand the ability of coral populations to adapt to warming ocean temperatures, we estimated the amount of genetic variation in thermal tolerance among different genotypes from a nursery colony of the staghorn coral *Acropora cervicornis*, and patterns of gene expression at both the mRNA and protein level were measured and tested for correlations to survival under thermal stress. This species was selected for study due to its rapid population decline and threatened status under the United States Endangered Species Act (NMFS 2006). Populations of *A. cervicornis* have declined by up to 98% since 1980 throughout their range, and this has mainly been attributed to disease outbreaks as well as temperature and salinity variation, bleaching, human impacts, and low genetic diversity (NMFS 2015). While a recent study has revealed high genetic diversity at neutral genetic markers among *A. cervicornis* along the Florida Reef Tract (Drury et al. 2016), the amount of genetic variation in traits related to thermal tolerance as well as biomarkers correlated to increased tolerance have yet to be identified in this species. Determining whether adaptive genetic variation of thermal tolerance is present within these nursery populations will provide information on whether outplanting can be used to promote adaptation in wild populations (van Oppen et al. 2015).

2. MATERIALS AND METHODS

2.1. Collection of corals, heat-stress experiments, and tissue collection

A total of 13 fragments (~8–9 cm in length) were obtained for 20 genetically distinct colonies of the staghorn coral *Acropora cervicornis* from the Coral Restoration Foundation's (CRF) Tavernier (Florida, USA) offshore nursery (24° 58′ 55″ N, 80° 26′ 11″ W) in June 2016. These colonies were originally sourced from 13 different sites in the upper and middle Florida Keys, spanning a distance of approximately 63.84 km (Table S1 in the Supplement at www.int-res.com/articles/suppl/m646p045_supp.pdf). Colonies were fragmented and maintained at the nursery for a minimum of 30 d to heal before being transported to the Keys Marine Laboratory located in Layton, Florida. One fragment per genotype was stored immediately in RNAlater at 4°C for 24 h before being stored at −80°C until use for genotyping of the coral host and Symbiodiniaceae community. The remaining fragments were then moved to a single raceway and given 48 h to acclimate at ambient temperature (28 ± 1°C) to lab conditions. Fragments were then randomly assigned to positions within each of 6 separate tanks, such that each tank contained 2 fragments per genotype from all 20 genotypes. All tanks and raceways used were located under a shaded area with light levels ranging from approximately 233 µmol s^{-1} of photosynthetically active radiation (PAR) at 09:45 h EST to 863 µmol s^{-1} of PAR at 14:30 h EST. All tanks were outfitted with additional mesh shading over the top of each tank. All tanks were cycled on a flow-through system with an approximate flow rate of 1.25–2.00 l min^{-1}.

Following acclimation, temperature was increased over the course of 6 h to 32 ± 1°C in 3 of the tanks, while 3 of the tanks were kept as controls and were maintained at ambient temperature. While the bleaching threshold for the Florida Reef tract is >30.5°C (Manzello et al. 2007), many of the shallow-water reefs frequently reach maximum temperatures of 32°C during the summer months (M. Colella pers. comm.). Therefore, a treatment of 32°C should represent a sub-lethal temperature at short-term exposure, but will be high enough to elicit strong physiological, bleaching, and lethal responses in the long term. This temperature is also consistent with the wider coral thermal tolerance literature (Császár et al. 2010, Kenkel et al. 2013, Davies et al. 2016). Following 12 h of elevated temperature exposure, 1 fragment per genotype was removed from each tank

from both control and elevated temperature treatments, and this fragment was split into 2 pieces, one of which was used for RNA-Seq, and the other for protein and molecular assays. Both of these samples were used to analyze differences in early gene expression associated with greater thermal tolerance. For RNA-Seq experiments, the partial fragment was first stored in RNAlater at 4°C for 24 h before being stored at −80°C until use. For protein and other molecular assays, the partial fragment was immediately frozen at −80°C until use. The survival of the remaining fragments was measured for the remainder of the experiment for a total of 26 d. Pulse amplitude modulated (PAM) fluorometry using a diving PAM with gain and intensity set at 3 was used to measure the maximum photochemical efficiency of photosystem II (F_v/F_m) for each fragment at both dawn (~07:30 h EST) and dusk (~19:30 h EST). Mortality was determined once F_v/F_m scores for individual fragments reached <0.2 for 2 consecutive time periods and was confirmed by total loss of coral tissue. Previous studies have shown that these scores correspond to severe bleaching and mortality (Rasher & Hay 2010).

2.2. Heritability estimation

Two different measures for the heritability of thermal tolerance in *A. cervicornis* were estimated in this study: broad-sense (H^2) and narrow-sense (h^2) heritability. Broad-sense heritability is a measure of the total variation in a trait (phenotypic variance, V_P) that is due to variation in genotype (total genotypic variance, V_G). However, to determine whether a trait can potentially respond to selection, the estimation of narrow-sense heritability is more informative (Lande & Shannon 1996). This is because narrow-sense heritability is based on the proportion of additive genetic variance (V_A) in a trait, which is the only portion of the total genetic variance that can respond to selection (Lande & Shannon 1996).

Broad-sense heritability was estimated using a 1-way ANOVA (IBM SPSS Statistics 25) with thermal tolerance as the dependent variable and coral genotype as a random effect, following the clonal method described by Császár et al. (2010). Thermal tolerance in this study was defined as the difference between the lifespan of fragments maintained at elevated temperatures and the mean lifespan of fragments from the same genotype maintained at ambient temperatures (note that all fragments of all genotypes survived the duration of the experiment at ambient temperature, see Fig. 1A). Therefore, colonies with a smaller difference in lifespan between elevated temperature and control were considered to have a higher thermal tolerance, while colonies with larger differences were considered to have lower thermal tolerance. The ANOVA allows the estimation of the amount of variance in thermal tolerance among fragments from the same colony (environmental variance, V_E), as well as the amount of variance in tolerance among colonies of different genotypes (V_G). The summation of these variance components provides an estimate of V_P and allows H^2 to be calculated using the equation (Falconer & MacKay 1996):

$$H^2 = \frac{V_G}{V_P} \quad (1)$$

Due to the small sample sizes utilized in this experiment, the standard error (SE) of H^2 was evaluated by the parametric bootstrapping method using the R statistical environment (v 3.4.4, R Foundation for Statistical Computing). Bootstrap samples were simulated from a Gaussian distribution, whose parameters are estimated based on the real sample (Nakagawa & Schielzeth 2010, Stoffel et al. 2017) (R code is included in the Supplement [Code S1]).

Narrow-sense heritability was estimated using 2 different methods. The first method involved the marker-based computer program MARK (Ritland 1996), which uses a mathematical regression model developed by Lynch & Ritland (1999) to calculate the relatedness between individual fragments based on their multi-locus genotype. The program then compares the relatedness coefficient to an estimate of phenotypic similarity (in this case thermal tolerance) to calculate h^2 of the trait in question. High heritability is indicated by more related individuals possessing similar phenotypes and less related individuals possessing dissimilar phenotypes. One hundred bootstraps were performed on the estimate of h^2, and a bootstrap percentile test was used to determine if the estimate of h^2 was significantly different from bootstrapped values at $\alpha = 0.05$, as well as the SE of h^2.

We also estimated narrow-sense heritability (h^2) utilizing other estimates of relatedness than those available in MARK. Pairwise relatedness of all fragments was calculated with the COANCESTRY program (Wang 2011), which implements 7 different algorithms to estimate the pairwise relatedness between individuals using those individuals' multi-locus genotypes. These pairwise relatedness (r) estimates were then regressed against the pairwise differences in thermal tolerance for each unique pair of frag-

ments (n = 780) (IBM SPSS Statistics 25), in which the R^2 and SE from the model provided is equal to h^2 ± SE. Pairwise r was set as the dependent variable, while the difference in thermal tolerance was set as the independent variable. Some of the algorithms implemented by COANCESTRY for estimating relatedness are only appropriate for completely outbred populations (moment estimator described by Wang 2002; moment estimator described by Lynch 1988 and Li et al. 1993; moment estimator described by Lynch & Ritland 1999; moment estimator described by Ritland 1996; and moment estimator described by Queller & Goodnight 1989), while others (the triadic likelihood estimator described by Wang 2007 and the dyadic likelihood estimator described by Milligan 2003) can be used effectively with inbred populations. Since *A. cervicornis* reproduces both asexually and sexually (Tunnicliffe 1981, Neigel & Avise 1983, Vargas-Angel et al. 2006), and since we had known clones in our data set, we calculated h^2 using all 7 available estimators provided by COANCESTRY and included those results for comparison to the estimate of h^2 derived from MARK.

For h^2 estimates to be reliable, these methods are highly dependent on the markers utilized and require significant variation in actual relatedness to be present among the samples (Ritland 1996). MARK was used to calculate the variance in relatedness by using the pairwise relatedness summary function and the regression-based estimator described by Lynch & Ritland (1999). We calculated the mean r and variance in r for each estimation algorithm with the 'summary statistics' function in COANCESTRY.

To estimate relatedness among coral colonies, a total of 15 microsatellite loci were amplified in each colony by using primers developed for *A. palmata* by Baums et al. (2005a, 2009), which also amplify the same loci in *A. cervicornis* (van Oppen et al. 2000, Vollmer & Palumbi 2002, Baums et al. 2005a, 2009, 2010). Fragments of approximately 2–3 cm^2 of tissue with skeleton from each colony were digested in CHAOS solution (4 M guanidine thiocyanate, 0.1% N-lauroyl sarcosine sodium, 10 mM Tris pH 8, 0.1 M 2-mercaptoethanol; Fukami et al. 2004) for 7 d at room temperature and then stored at −80°C until extraction. The DNA extractions were performed following the protocol outlined by Levitan et al. (2011), and the resulting DNA was then stored at −20°C. PCR reaction mixtures consisted of 2.5 µl of 10× PCR buffer (Invitrogen), 1.5 µl of 50 mM $MgCl_2$, 0.5 µl of 10 mM dNTPs, 0.2 µl of *Taq* (Invitrogen), 0.2 µl of forward primer (10 µM), 0.4 µl of reverse primer (10 µM), 1.5 µl of DNA template, and nuclease free water to bring to a total volume of 26.5 µl. All PCR amplifications were carried out using the annealing temperatures described by Baums et al. (2005a, 2009) on a BioRad C1000 Touch Thermal Cycler. The PCR products were sent to the University of Florida ICBR Gene Expression & Genotyping facility for sequencing using an AB 3730 with an internal size standard (LIZ600) for determining fragment size. Electropherograms were analyzed with GeneMarker software V2.7.0 (SoftGenetics). Allele frequencies for each locus and mean heterozygosity are included in Table S2.

To assess the potential effects of different Symbiodiniaceae taxa on thermal tolerance and therefore our calculations of heritability, the Symbiodiniaceae community within each coral genotype was characterized. While the measurement of narrow-sense heritability considers only the effect of the coral's multi-locus microsatellite genotype on thermal tolerance, measurements of broad-sense heritability utilizing the clonal method used here are really estimates of the degree to which genetic variation in the holobiont underlies variation in thermal tolerance. If there is a relationship between host genotype and symbiont taxa, then the variance in thermal tolerance attributed to symbiont identity would be included in the estimation of V_G, inflating our broad-sense heritability estimate.

To determine the relative abundance and identity of the symbiont taxa hosted by the corals used here, the chloroplast 23S (cp23S) gene for each coral colony was sequenced using an Illumina MiSeq. An approximately 480 base region of this gene was amplified with the primers F (AAT AAC GAC CTG CAT GAA AC) and R (GCC TGT TAT CCG TAG AGT AGC) using GoTaq polymerase. Initial PCR reactions were composed of 10 µl of 2× GoTaq polymerase master mix, 1 µl of DNA template, 0.5 µl of each primer, 1.2 µl of 25 mM $MgCl_2$, and 6.8 µl of nuclease-free water. The thermal regime of the PCR was 3 min at 95°C followed by 35 cycles of 95°C, 55°C annealing, and 72°C extension each for 30 s, terminating with a 5 min extension at 72°C. Amplicons were then indexed according to the Illumina 16S amplicon sequencing protocol using KAPA *Taq* DNA polymerase and following a similar program: 3 min at 95°C and 10 cycles of 95°C, 55°C annealing, and 72°C extension each for 30 s. After the 10 cycles, an additional 5 min at 72°C facilitated a final extension. After both the initial and indexing PCRs, DNA was post-PCR purified with magnetic beads and several ethanol washes following the Illumina 16S amplicon

sequencing kit protocol. Indexed DNA samples were then paired-end, 300 cycle sequenced on an Illumina Mi-seq.

The QIIME2 pipeline was employed in order to control quality and assign taxonomic identity to cp23S amplicons. Sequencing errors were filtered out using the DADA2 plug-in for QIIME2. Primers were trimmed and chimeras were purged (Caporaso et al. 2010, Callahan et al. 2016). All reads were truncated to 250 bp, and then forward and reverse paired end reads were merged into 1 continuous read. Singletons were removed, and representative sequences were clustered based upon 99% similarity in order to collapse polymerase errors. Polymerase manufacturer error rates indicated that 17.5% of amplicons should have a single nucleotide error and 3% of amplicons should have 2 single nucleotide errors due to polymerase infidelity. These 2 errors would be responsible for a 0.4% deviance from true sequence identity which was corrected by our 99% operational taxonomic unit (OTU) clustering into 12 OTUs (Table S3). Representative sequences of each of these 12 OTUs were then used to assign taxonomic identity to their respective OTUs through the National Center for Biotechnology Information (NCBI) BLAST search. Spearman's rho correlation analyses were then performed to determine if any of the proportions of identified symbiont variants were linearly related to thermal tolerance for the coral genotypes used in this study.

2.3. Gene expression using RNA-Seq

To analyze gene expression associated with increased thermal tolerance in this species, samples taken after 12 h from both control and heated treatments were used to evaluate mRNA expression using RNA-Seq. First, cDNA libraries were generated for fragments from both treatments for the 3 most thermal tolerant genotypes and the 3 least thermal tolerant genotypes, as determined by the time of mortality following heat stress (Section 3, see Fig. 1B) (n = 36). Total RNA was first extracted from a small portion (<1 cm) of each fragment using TRI Reagent (ThermoFisher Scientific), then purified using RNeasy columns (Qiagen) following the methods of Bay et al. (2009). Quality and concentration of total RNA were determined via spectrophotometer readings at 260 and 280 nm.

The total RNA was then fragmented by incubating at 95°C for 15 min and used to synthesize first-strand cDNA with adapters at both 5′ and 3′ ends (Matz et al. 1999, Meyer et al. 2011) using a PrimeScript First Strand cDNA synthesis kit (Clontech) per the manufacturer's instructions. The second strand of cDNA was synthesized and amplified using the following PCR reaction: 0.5 µl of dNTPs (2.5 mM each), 2.5 µl of 10× PCR buffer, 0.5 µl each of 10 µM of both adapter sequences, 0.5 µl of Klentaq1 polymerase (DNA Polymerase Technology), and 10 µl of first strand cDNA in a total volume of 30 µl. The PCR amplification was carried out on a BioRad C1000 Touch Thermal Cycler and carried out as follows: an initial denaturation step at 94°C for 2 min followed by 15 cycles of 94°C for 30 s, 63°C for 1 min, and 68°C for 2 min. Each cDNA sample was labeled with a specific combination of 2 barcodes and gel-extracted using a MinElute Gel Extraction kit (Qiagen) to purify the size fraction (400–500 bp) for sequencing.

Barcoded samples were pooled for sequencing on the Illumina HiSeq 2500 at the Case Western Reserve University School of Medicine Genomics Core, and the libraries were sequenced with single-end 50 bp read lengths, which resulted in 1690 3791 ± 793 620 raw reads per sample (mean ± SE, n = 36). Raw sequence data were deposited in the NCBI Sequence Read Archive (SRA) under the associated BioProject accession number PRJNA555587. Raw sequences were filtered to remove rRNA sequences from Bacteria, Archaea, and Eukarya, using the 'sortmeRNA' package (Kopylova et al. 2012). A custom Perl script provided by Dr. Mikhail Matz was used to discard reads sharing the same sequence as the read and degenerate adaptor (PCR duplicates) and to trim the leader sequence from remaining reads (available for download at https://github.com/z0on/tag-based_RNAseq/blob/master/tagseq_clipper.pl) (Kenkel & Matz 2016). The header specified in our pipeline was modified from the default header in the script to '[ATGC][ATGC][AC][AT]GGG+|[ATGC][ATGC]AC ATGGG+'. The 'fastx_toolkit' (http://hannonlab.cshl.edu/fastx_toolkit/) was then used following the parameters of Kenkel & Matz (2016) to trim reads after a homopolymer run of 'A' ≥8 bases was encountered, to retain reads with a minimum sequence length of 20 bases, and to quality filter, requiring PHRED of at least 20 over 90% of the read. After the removal of PCR duplicates and quality trimming, 1 744 729 ± 126 387 reads per sample remained.

An *A. cervicornis* draft genome was kindly provided by Dr. Iliana Baums (Penn State University) and this was used as a reference for alignments. Trimmed reads were aligned to this reference with 'Bowtie2' (Langmead & Salzberg 2012), and read

counts were assembled using 'htseq-count' in the 'HTSeq' package (Anders et al. 2015). On average, 39.03% of the reads within our samples aligned to the provided *A. cervicornis* reference with 'Bowtie2.' Sequences that did not align were compared against the NCBI nucleotide database using 'megablast,' and these non-aligning sequences contained mainly coral mitochondrial sequences, coral rRNA sequences, and Symbiodiniaceae sequences. For sequences that successfully aligned to the *A. cervicornis* genome, these unique reads mapped to 200411 unique genes, with an average mapping rate of 35%.

Differential expression analysis was carried out in the R statistical environment (v 3.4.4) using the quasi-likelihood algorithm within the software package 'edgeR' (v 3.20.9) (Robinson et al. 2010, McCarthy et al. 2012, Chen et al. 2016). Samples were first grouped by tolerance (tolerant, susceptible) and treatment (control, heated) and low-count genes were removed (counts per million [CPM] <0.5), leaving 16381 genes for the analysis. The trimmed mean of M-values normalization method was then used to calculate effective library size. A multi-dimensional scaling (MDS) plot was generated to explore overall differences between the expression profiles of samples based on both thermotolerance and temperature treatment. To model the read counts for each gene, we used the quasi-likelihood extension for the negative binomial distribution (Chen et al. 2016). Differential expression analysis was then conducted on the following pairwise comparisons: heated vs. control (n = 18 per group), tolerant vs. susceptible (n = 18 per group), tolerant heated vs. susceptible heated (n = 9 per group), and tolerant control vs. susceptible control (2 groups, n = 9 per group), in which the first group listed in each comparison was considered the treatment, and the second group was the reference or control group. A false discovery rate (FDR) of 5% as well as a $\log_2$ fold-change (FC) >1.5 and <–1.5 were used as cutoffs to determine significantly differentially expressed (DE) genes using the 'glmTreat' function in R. The results were then plotted in fitted model mean-difference (MD) plots in which the $\log_2$ FC for each gene was plotted against the average abundance in $\log_2$ CPM. The 'topGO' package (Alexa & Rahnenfuhrer 2019) was used to determine what gene ontology (GO) 'biological process' (BP) terms were significantly enriched in gene sets significantly upregulated or significantly downregulated in thermotolerant genotypes. The enrichment score for each GO term was determined by taking the $-\log_{10}$ of the p-value provided by the Fisher's exact test when using the 'classic' algorithm in 'topGO.'

In order to determine whether all genotypes within tolerant or susceptible groups were consistent and showed the same expression patterns, the significant DE genes that were identified due to tolerance were examined further. The gene counts for each sample were first normalized by sequencing depth and gene length using reads per kilobase of transcript, per million mapped reads. The average expression of each DE gene for each genotype in either the control or heated treatment was compared to the average expression for the tolerant group (if a susceptible genotype) or susceptible group (if a tolerant genotype) to determine if it matched the upregulation or downregulation of that gene based on the 'edgeR' 'glmTreat' results. If one genotype within tolerant or susceptible groups differed in expected expression, the gene was considered 'inconsistent;' otherwise, the gene was considered 'consistent.'

2.4. Protein biomarker concentration and activity

The concentration or activity of a selective panel of protein biomarkers was analyzed to determine if they were associated with increased thermal tolerance in *A. cervicornis*. The 4 molecules selected were: catalase, heat shock protein 70 (HSP70), ubiquitin, and 4-hydroxynonenal (4HNE). These biomarkers were chosen due to their responses under elevated temperature stress in other coral species (Barshis et al. 2010, Ross et al. 2013). Elevation in reactive oxygen species due to thermal stress often results in an upregulation of catalase, an enzyme that breaks down H_2O_2 into O_2 and H_2O, and a greater abundance of 4HNE, a molecule produced by reactive lipid peroxides (Halliwell & Gutteridge 1999, Halliwell 2006, Barshis et al. 2010, Ross et al. 2013). Ubiquitin is a cellular protein tag that marks proteins for degradation, and higher levels can be indicative of elevated levels of stress (Hawkins 1991, Barshis et al. 2010, Jin et al. 2016). HSP70 is a molecular chaperone that is often found to be upregulated in response to thermal stress and aids in maintaining protein structure and function following heat-related denaturation (Feder & Hofmann 1999, Barshis et al. 2010, 2013, DeSalvo et al. 2010a). Biomarker relative concentration and activity were examined for samples removed after 12 h for all genotypes used in this study and in both treatments (n = 120).

Coral tissue was removed from each fragment by airbrushing with a 50 mmol sodium phosphate buffer containing 0.05 mol l^{-1} dithiothreitol and lyophilized. The lyophilized tissue was resuspended in sodium

phosphate buffer before use. Catalase activity within coral tissue was measured using the Amplex® Red Catalase Assay Kit (ThermoFisher Scientific) per the manufacturer's instructions, and then adjusted for the total amount of protein (U activity mg^{-1} total protein) as measured using the Pierce™ BCA Protein Assay Kit (ThermoFisher Scientific) according to the manufacturer's instructions (Ross et al. 2013). HSP70, ubiquitin, and 4HNE levels were first detected in samples through immunoblot assays following the methods of Barshis et al. (2010), and then quantified through ELISA.

For immunoblot assays, 50 µg of total protein per sample were added to 10 µl of 2× Laemmli SDS-PAGE sample buffer (Bio-Rad). Positive controls consisted of 5–10 µg of heat-shocked HeLa cell lysate (ADI-LYC-HL101-F, Enzo Life Sciences) for HSP70 and 4HNE-conjugates, and rat brain tissue extract for ubiquitin-conjugates (ADI-LYT-RB100-F, Enzo Life Sciences) following the methods of Barshis et al. (2010). All samples, standards, and positive controls were loaded into Mini PROTEAN TGX precast gels (4–20%) (Bio-Rad), and run for approximately 30 min at 200 V on a Mini-PROTEAN Tetra Vertical Electrophoresis Cell (Bio-Rad). Protein transfer was performed in a Mini TransBlot Cell (Bio-Rad) onto an Immuno-Blot PVDF membrane (Bio-Rad) at 100 V for 1 h.

Membranes were blocked overnight in 50 ml of 10% nonfat dry milk in Tris buffer saline (TBS, 0.05 M TrisBase, 0.15 M NaCl, pH 7.6) at 4°C and then incubated at 4°C overnight with primary antibody diluted 1:2000 in 1% nonfat dry milk in TBS (HSP70: cat. no. ADI-SPA-822, Enzo Life Sciences; ubiquitin: cat. no. ADI-SPA-200, Enzo Life Sciences; 4HNE: cat. no. AB5605, EMD Millepore) (Barshis et al. 2010). Following the primary antibody incubation, membranes were incubated at room temperature for 1 h with secondary antibody diluted 1:10000 in 1% nonfat dry milk in TBS (anti-mouse alkaline phosphatase [AP] conjugated: cat no. A3562; anti-rabbit AP conjugated: cat. no. A0418; anti-goat AP conjugated: cat. no. A7650, all Sigma-Aldrich) (Barshis et al. 2010). Afterwards, a BCIP/NBT Alkaline Phosphatase Substrate Kit (Vector Laboratories) was used to visualize detection of the protein biomarkers by the primary antibody, per the manufacturer's instructions. All blot images were recorded using an Amersham Imager 600 (GE Healthcare Life Sciences).

For ELISA, 25 µg total protein per sample were added to 100 µl of 0.2 M sodium carbonate-bicarbonate buffer (pH 9.4) per well on a 96-well plate; 100 µl of phosphate buffer saline (PBS, 0.01 M monobasic NaH_2PO_4, 0.15 M NaCl, pH 7.2–7.4) were used as a blank. Both primary and secondary antibody dilutions were 1:10000 in 1% nonfat dry milk in PBS. A TMB Substrate Kit (ThermoFisher Scientific) was used for the detection of HSP70, ubiquitin, and 4HNE. Absorbance was measured at 450 nm using a Synergy HT plate reader (BioTek) and analyzed using Gen5 (BioTek). All samples and blanks were run in triplicate and averaged together.

To determine whether the expression of biomarkers at the protein level significantly differed among tolerant and susceptible colonies or among treatments, the mean relative concentrations or the mean activity of each biomarker were compared among the 3 most thermal tolerant and the 3 least tolerant genotypes and for both treatments using a 2-way ANOVA or Scheirer-Ray-Hare extension for Kruskal-Wallis tests if assumptions for normality were not met (IBM SPSS Statistics 25) (n = 18 per tolerance group, and per treatment). In order to determine whether the expression of these protein biomarkers had a significant effect on thermal tolerance, an ANCOVA was used in which difference in lifespan was provided as the dependent variable, coral genotype as a fixed effect, and each biomarker concentration or activity in the heated treatment as a covariate in separate analyses. A second series of ANCOVA analyses was performed on each biomarker utilizing all 20 genotypes. Finally, a Pearson correlation or Spearman's rho (if data deviated significantly from normality) (IBM SPSS Statistics 25) was used to determine if mean relative concentrations or mean activity of each biomarker in the heated treatment correlated with thermal tolerance for either the 6 genotypes that differed most in thermal tolerance, or all 20 genotypes used in this study.

3. RESULTS

3.1. Heritability estimation

During the long-term temperature stress, it was discovered that one of the heated tanks had elevated temperatures of 1°C higher than the other 2 heated tanks for a period of approximately 12 h. At the conclusion of the experiment, a 2-way ANOVA was performed with treatment and tank as main effects and thermal tolerance as the dependent variable, and a significant tank effect was observed ($F_{2,38} = 3.963$, p = 0.027). This effect disappeared once the aberrant tank was removed from the data set ($F_{1,19} = 0.416$, p = 0.527), suggesting that this short-term elevation was likely responsible. Therefore, all subsequent analy-

ses involving difference in lifespan were conducted without the inclusion of the aberrant tank. Since this temperature anomaly occurred 16 d into the experiment, it did not affect samples collected for gene expression analysis, so samples from all tanks were retained for those experiments.

The lifespan of *Acropora cervicornis* coral fragments was significantly affected by temperature. While all of the control fragments held at ambient temperature survived the entire length of the experiment (612 h) and showed no decrease in health either visually or according to F_v/F_m scores, all fragments maintained at elevated temperature showed signs of decline in F_v/F_m after 204 h and had a significantly reduced lifespan compared to the controls ($F_{1,98}$ = 795.821, p < 0.001) (Fig. 1A). While fragments under thermal stress survived 474.9 h on average, this reduction in lifespan relative to the controls was not consistent across all coral genotypes ($F_{1,19}$ = 3.15, p = 0.008) (Fig. 1B). The average difference in lifespan between control and treated fragments varied from 72 to 216 h among genotypes, with genotype U33 and M7 (p = 0.041 for both) having significantly lower thermal tolerance than genotype M5 according to a Tukey's HSD post hoc test. The significant differences in lifespan among genotypes resulted in a significant broad-sense heritability of thermal tolerance of H^2 = 0.528 ± 0.173 (mean ± SE) (Table 1). The narrow-sense heritability estimates were consistently low, ranging from h^2 = 0.000 ± 31.194 to 0.032 ± 0.121, depending on the relatedness estimator utilized, and the majority were not significantly different from 0 (Table 1). The variance in relatedness (V_r) for these genotypes was also low, with MARK calculating V_r = −0.144 ± 0.003 based on the provided multi-locus genotypes, whereas COANCESTRY calculated V_r ranging from 0.029–0.102 (lower limit = triadic assuming no inbreeding [Wang 2007], upper limit = Lynch and Li [Lynch 1988, Li et al. 1993]) depending on the relatedness estimation algorithm used.

Symbiodiniaceae were successfully genotyped in 13 of the 20 colonies used in the experiment. We found that the Symbiodiniaceae community differed slightly among the colonies used in this study (Fig. 1B, Table S2), with 10 colonies harboring predominately *Symbiodinium fitti* (type A3) variants and the remaining 3 colonies harboring predominately *Breviolum* sp. variants (either type B1 or B2). Only 1 colony (U46), harbored both *Breviolum* sp. and *S. fitti*. While these colonies had been maintained in the same environment at the CRF Tavernier nursery for 5–10 yr prior to this experiment, these community

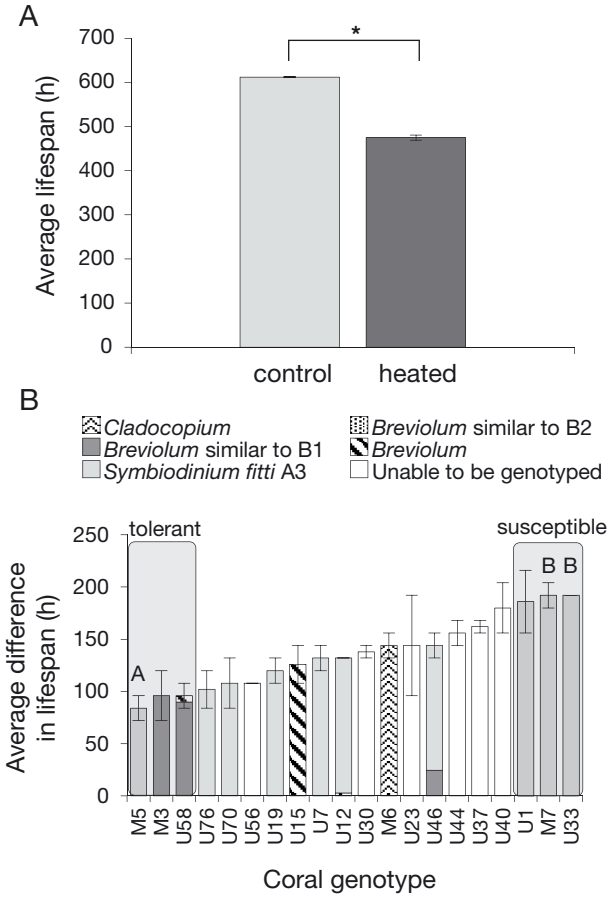

Fig. 1. (A) Average lifespan for *Acropora cervicornis* coral fragments maintained at either ambient (control: n = 60) or elevated temperatures (heated: n = 40). Significant differences (p < 0.05) are denoted by an asterisk (*) and error bars show SE. All control fragments survived for the duration of the experiment, so there was no difference in lifespan among the control fragments (SE = 0). (B) Average difference in lifespan between heated and control fragments over 26 d for 20 *A. cervicornis* genotypes (n = 2 per genotype). Significantly different genotypes (p < 0.05) are indicated by different letters as determined by a Tukey's HSD post hoc test. Error bars show SE. The boxes at either end of the *x*-axis indicate the 3 most thermal tolerant and the 3 most thermal susceptible genotypes that were selected for comparisons of gene expression. Patterns of each bar indicate the proportion of cp23S reads attributed to different Symbiodiniaceae operational taxonomic units (OTUs) within each coral colony. Representatives were assigned to OTUs using NCBI BLAST. Variants of the same taxa were combined per coral genotype (see Table S2 for complete list of variants per genotype and total cp23S reads). The cp23S region could not be amplified for genotypes U23, U30, U37, U40, U44, and U56 ('unable to be genotyped'). Note: *Breviolum* similar to B2 and *Cladocopium* make up less than 5% of the total reads for genotypes M3, U12, and U1. While *Cladocopium* cp23S reads make up 100% of the reads from M6, only 9 reads could be successfully identified compared to >1000 reads for all other samples, and this sample was therefore removed from further Symbiodiniaceae analyses

Table 1. Estimates of the broad-sense (H^2) and narrow-sense (h^2) heritability of thermal tolerance in *Acropora cervicornis*, ±SE. H^2 does not require an estimate of relatedness (NA, not applicable). Significant estimates are indicated by an asterisk (*p < 0.05) and **bold** font (p < 0.01). Significance of the MARK model was determined using a bootstrap percentile test to determine if the estimate of h^2 was significantly different from the 100 bootstrapped values at α = 0.05. Significance of the remaining h^2 estimates was determined using linear regression of the pairwise r estimates to pairwise measurements of thermal tolerance (SPSS)

	Method	Relatedness estimator	Heritability
H^2			
	One-way ANOVA[a]	NA	**0.529 ± 0.173***
h^2			
	MARK[b]	Lynch & Ritland (1999)	0.032 ± 0.121
	COANCESTRY with linear regression[c]	Dyadic, assuming no inbreeding (Milligan 2003)	**0.010 ± 0.174***
	COANCESTRY with linear regression[c]	Dyadic, assuming inbreeding (Milligan 2003)	**0.012 ± 0.188***
	COANCESTRY with linear regression[c]	Triadic, assuming no inbreeding (Wang 2007)	**0.009 ± 0.171***
	COANCESTRY with linear regression[c]	Triadic, assuming inbreeding (Wang 2007)	0.013 ± 0.182*
	COANCESTRY with linear regression[c]	Wang (2002)	0.000 ± 0.296
	COANCESTRY with linear regression[c]	Lynch (1988) & Li et al. (1993)	0.004 ± 0.318
	COANCESTRY with linear regression[c]	Lynch & Ritland (1999)	0.006 ± 0.201*
	COANCESTRY with linear regression[c]	Ritland 1996	0.003 ± 0.251
	COANCESTRY with linear regression[c]	Queller & Goodnight 1989	0.000 ± 0.260

[a]Clonal method of Császár et al. (2010) and Falconer & MacKay (1996)
[b]Genetic marker-based program developed by Ritland (1996)
[c]Pairwise relatedness values calculated using COANCESTRY developed by Wang (2011), with h^2 estimated from a linear regression of relatedness and difference in lifespan

differences are possibly due to different reef origins (Table S1 in the Supplement). Different colonies from the same sites of origin, however, also had different dominant symbiont types in some cases. For example, M3, M5, and M7 were all originally sourced from East Turtle Shoal near Grassy Key, but M3 hosted predominately *Breviolum* sp. while M5 and M7 hosted predominately *S. fitti*. Two genotypes (U1 and U12) were also found to contain a very small number of reads identified as *Cladocopium* sp. (clade C). Genotype M6 was also found to contain *Cladocopium* sp., but since this sample had a very low number of reads (9 compared to >1000 for all other samples), this sample was removed from further symbiont community analyses.

Colonies that harbored predominately *Breviolum* sp. variants tended to have higher thermal tolerance (Fig. 1B), and this resulted in a significant linear correlation with thermal tolerance for 1 specific variant of *Breviolum* similar to B1 (Spearman rank correlation, $R^2 = 0.1464$, n = 26, p = 0.033). However, like the other variants identified in this study, the proportions of these variants were not continuous (were either close to 0 or close to 1), and no other variants had a significant linear correlation with thermal tolerance (see Fig. S1). Therefore, symbiont composition was not considered to have a significant impact on thermal tolerance for these genotypes and was not used as a covariate in our estimates of H^2.

3.2. RNA-Seq results

While the 3 genotypes that had the highest thermal tolerance (M3, M5, and U58) differed from the 3 genotypes that had the lowest thermal tolerance (M7, U1, and U33) by at least 90 h of lifespan differential (Fig. 1B), the MDS plot, which clusters samples based on gene expression similarity, showed no clear clustering of samples by either shared thermotolerance or shared temperature treatment (Fig. 2A). In fact, only 2 genes were identified as being significantly differentially expressed (1 upregulated, 1 downregulated) between heated and control samples, based on an FDR <0.05 and logFC >1.5 and <–1.5 (Fig. S2). On the other hand, despite the high degree of overlap among samples in the MDS plot, there were 129 significant DE genes that were identified between tolerant and susceptible genotypes (FDR <0.05, logFC <–1.5 and >1.5) (Fig. 3). The expression profiles for these 129 significant DE genes resulted in clear clusters by similarity on an MDS plot, both by tolerance group as well as by individual genotypes (Fig. 2B). Out of this gene set, 60 genes were significantly downregulated in tolerant genotypes relative to susceptible genotypes, while 69 were significantly upregulated (Fig. 3). The 69 genes that were significantly upregulated in tolerant genotypes were significantly enriched for biological pathway GO terms related to cellular component synthesis and as-

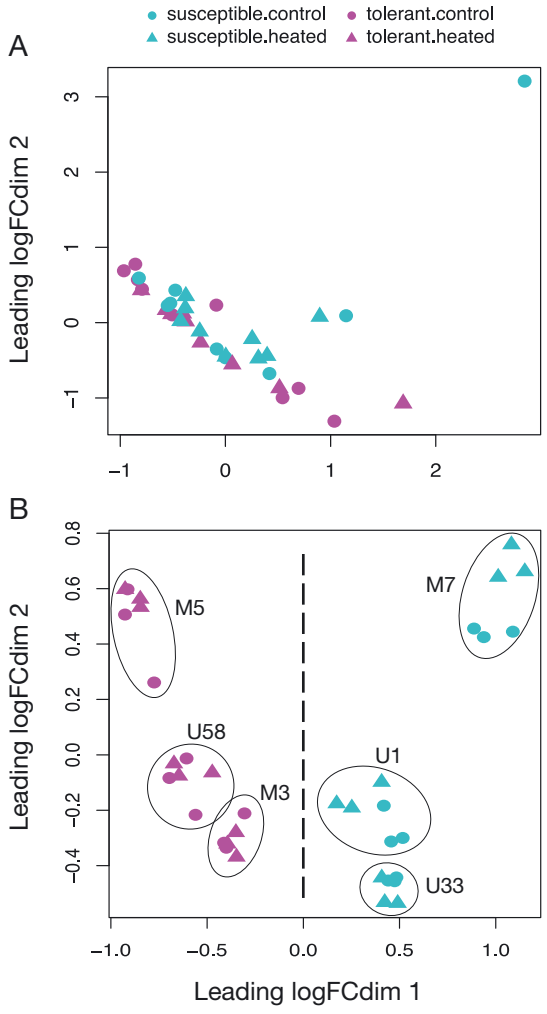

Fig. 2. Multidimensional scaling plot showing: (A) the overall differences between mRNA expression profiles of susceptible *Acropora* colonies at ambient temperature (28 ± 1°C), susceptible colonies at elevated temperature (32 ± 1°C), tolerant colonies at ambient temperature, and tolerant colonies at elevated temperature (n = 9 per group), and (B) the differences in the mRNA expression profiles of the 129 significantly different differentially expressed (DE) genes between tolerant and susceptible colonies among the same 4 groups. Significant DE genes were determined using the 'glmTreat' function in R to specify both a $\log_2$ fold-change (FC) of 1.5 as well as a false discovery rate of 5%

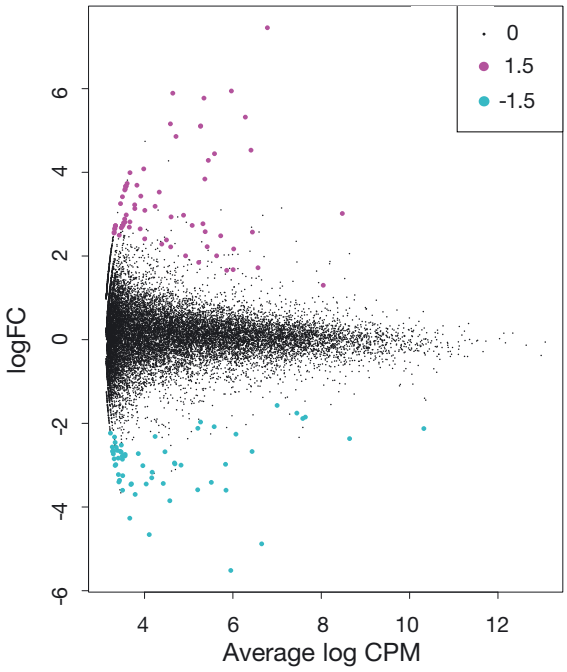

Fig. 3. Fitted model mean-difference plot showing expression changes between tolerant and susceptible *Acropora* colonies (n = 18 per group). The $\log_2$ fold-change (FC) for each gene is plotted against the average abundance in $\log_2$ counts per million (CPM). Pink (blue) points show genes that are significantly upregulated (downregulated) in tolerant colonies relative to susceptible colonies. Significant differentially expressed genes were determined using the 'glmTreat' function in R to specify both a $\log_2$ FC cut-off of 1.5 as well as a false discovery rate of 5%

sembly, inflammatory response, innate immune response, and cell adhesion (Fisher's exact test, $p < 0.05$) (Fig. 4A). The 60 genes that were significantly downregulated in tolerant genotypes were annotated with biological pathway GO terms related to responses to nitrogen compounds, lipid biosynthesis and metabolism, regulation of NIK/NF-kappaB signaling, regulation of protein serine/threonine kinase activity, and DNA recombination; however, none of these terms was considered significantly enriched (Fisher's exact test, $p > 0.05$) (Fig. 4A).

While average expression levels of these 129 genes were significantly different between tolerant and susceptible genotypes, their expression patterns were not always consistent among all genotypes within each group. Within this gene set, 40 genes were identified that were consistent in their average expression across each genotype within both tolerant and susceptible groups (Fig. 5). Out of this gene set, 14 genes were significantly downregulated in tolerant genotypes compared to susceptible genotypes, while 26 genes were significantly upregulated. The GO term enrichment analysis was rerun for the 40 DE genes that showed consistent expression patterns, and the results are shown in Fig. 4B. The 26 genes that were consistently upregulated in tolerant genotypes were significantly enriched for cell adhesion, embryonic morphogenesis and development, cell and apical junctions, and inflammatory response BP GO terms (Fisher's exact test, $p < 0.05$). The 14 genes that were consistently downregulated in tolerant genotypes were annotated with GO terms related to transmembrane transport, ion transport, and

cellular ion homeostasis, but only inorganic ion transmembrane transport was significantly enriched (Fisher's exact test $p < 0.05$) (Fig. 4B). Based on the dendrogram clustering, we also saw that these genes were highly consistent in terms of expression among different samples within genotypes, except for genotypes M7 and U1, which clustered closely with one another (Fig. 5).

Of the 129 significant DE genes based on tolerance, 89 were inconsistent in expression patterns among genotypes within tolerance groups (Fig. S3). Tolerant genotypes M3 and M5 were most similar to each other in terms of expression for these 89 genes, and so were susceptible genotypes U1 and U33 (Fig. S3). However, U58 differed greatly from the other tolerant genotypes in terms of expression, mainly through lower expression of genes such as Codanin-1 (Fig. S3). This was also seen with M7, which differed greatly from the other 2 susceptible genotypes, mainly through the much higher expression of a subset of genes, including tetratricopeptide repeat protein 28 and tumor necrosis factor (TNF) receptor-associated factor 6-B (Fig. S3). These 89 DE genes also showed the most inconsistency among samples within a single genotype, as shown by the dendrogram clustering (Fig. S3). While samples from M3, M5, U33, and M7 clustered closely with samples within their own genotypes, samples from U1 and U58 were more separated from each other, with some U58 samples (tolerant) clustering closely with U1 samples (susceptible).

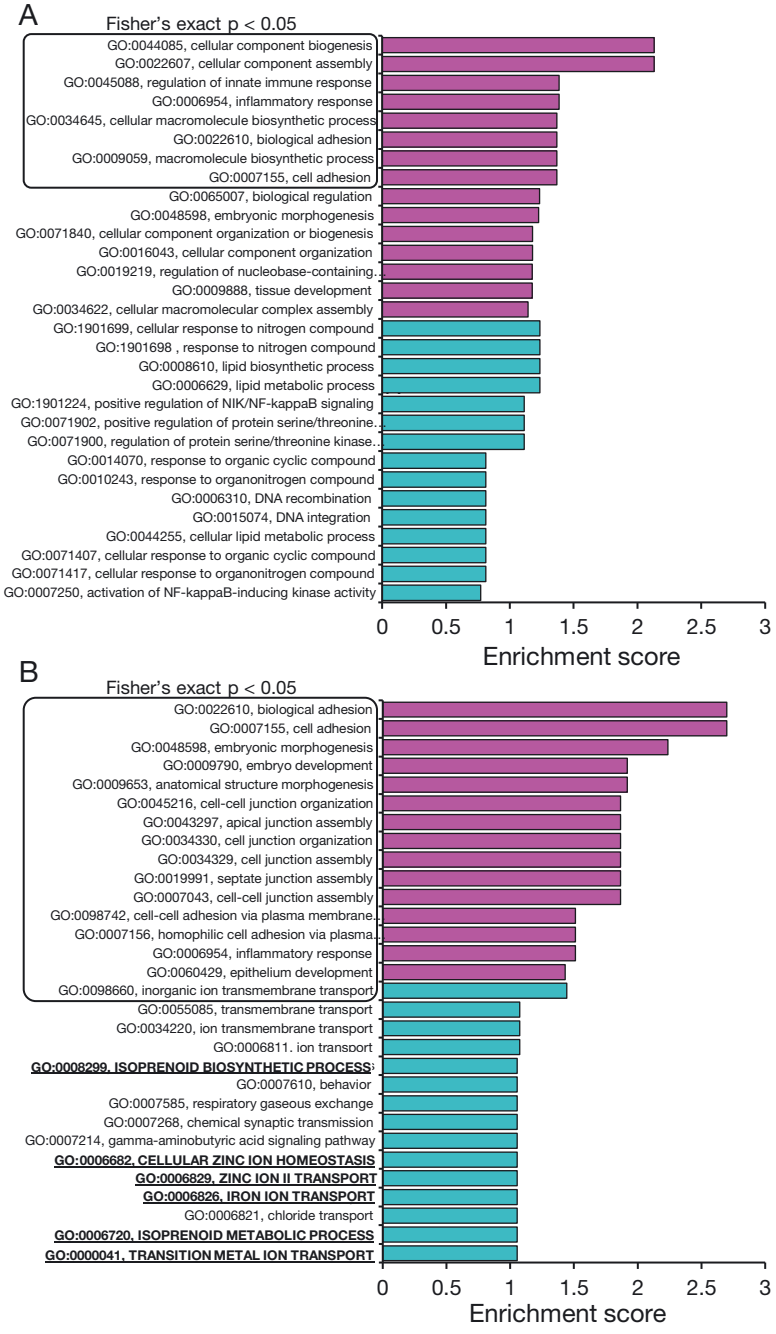

Fig. 4. (A) Gene ontology (GO) term enrichment analysis for the 93 significant differentially expressed (DE) genes among tolerant and susceptible *Acropora* genotypes (n = 18 per group) that had GO term annotations. (B) GO term enrichment analysis for the 30 DE genes that had GO term annotation and were significant between tolerant and susceptible genotypes (n = 18 per group) but were also considered consistent in expression among the genotypes within tolerance groups. (A,B) For GO term enrichment analysis, biological process ontology was used. The enrichment score for each GO term was determined by taking the –log10 of the p-value from the Fisher's exact test using the classic algorithm in 'topGO.' The top 15 enriched GO terms in genes that were significantly upregulated in tolerant colonies relative to susceptible colonies (pink), as well as in genes that were significantly downregulated in tolerant colonies (blue) are shown. A box is used around GO terms that were significantly enriched in these gene sets based on the Fisher's exact test ($p < 0.05$). Terms in **bold and underlined** are GO terms that were also significantly enriched in significant DE genes downregulated in either tolerant vs. susceptible colonies in the heated treatment (cellular zinc homeostasis, zinc II ion transport, iron ion transport, transition metal ion transport) or tolerant vs. susceptible colonies in the control treatment (isoprenoid biosynthetic process, isoprenoid metabolic process)

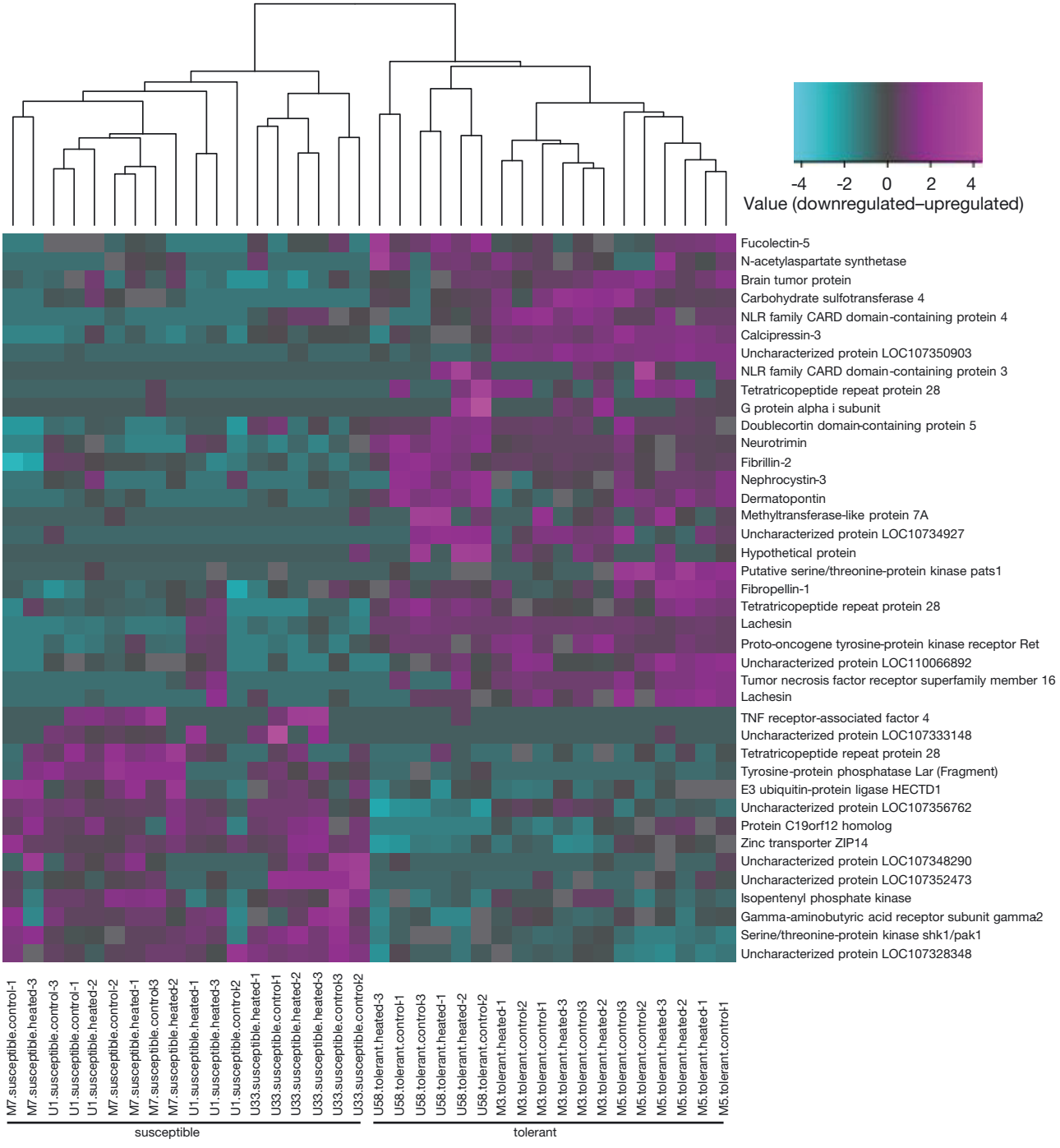

Fig. 5. Heat map across all samples using the 40 significant differentially expressed (DE) genes between tolerant and susceptible *Acropora* genotypes (n = 18 per group) that were the most consistent among genotypes within tolerant and susceptible groupings. Colors range from pink (significantly upregulated in tolerant colonies relative to susceptible colonies) to blue (significantly downregulated in tolerant colonies) and are based on the log counts per million. Significant DE genes were determined using the 'glmTreat' function in R to specify both a $\log_2$ fold-change cut-off of 1.5 as well as a false discovery rate of 5%. Consistent genes were determined by taking each DE gene and comparing the average control and heated reads per kilobase of transcript, per million mapped reads (RPKM) for each genotype to the average RPKM for that gene for tolerant or susceptible colonies and seeing whether they matched expectations (based on the 'glmTreat' results) for either upregulation or downregulation. Genes are ordered from top to bottom by ascending p-value (3.41×10^{-10} to 3.71×10^{-4})

In an effort to focus on gene expression differences between tolerant and susceptible genotypes that are most likely to be involved in tolerance to high temperatures, gene expression was compared between tolerant genotypes and susceptible genotypes using only heated treatment samples, and then compared again using only control samples. In the control treatment, 6 genes were significantly downregulated in tolerant genotypes relative to susceptible genotypes, while 10 genes were significantly upregulated (Fig. 6A). Three of these significant DE genes were not previously identified by comparing all tolerant to all susceptible samples: nephrocystin-3 (different transcript), tyrosine aminotransferase, and uncharacterized LOC107358729. Furthermore, the downregulated DE genes were significantly enriched for 2 BP GO terms: isoprenoid metabolic process and isoprenoid biosynthetic process (Fig. 4B). All 16 of these DE genes showed very consistent expression patterns among genotypes within each tolerance group; only 2 U1 (susceptible) heated samples grouped with tolerant genotypes (Fig. 6A).

In the heated treatment, 5 genes were significantly downregulated in tolerant genotypes relative to susceptible genotypes, while 4 genes were significantly upregulated (Fig. 6B). These DE genes were also identified in the analysis of all differentially expressed loci between tolerant and susceptible genotypes. The 5 downregulated DE genes were significantly enriched for the following BP GO terms: cellular zinc homeostasis, zinc II ion transport, iron ion transport, and transition metal ion transport. Expression patterns for these 9 genes were highly inconsistent among the tolerant genotypes, but all 3 susceptible genotypes had similar expression profiles (Fig. 6B).

3.3. Protein concentration and activity results

Antibody recognition of proteins from *A. cervicornis* tissues was observed in immunoblots for ubiquitin-conjugated and 4HNE-conjugated proteins, but not for HSP70; therefore, HSP70 was not analyzed further. When investigating the effect of treatment on protein expression differences among the 3 most thermal tolerant and the 3 least tolerant genotypes, elevated temperature was found to have no effect on either the activity of catalase (Scheirer-Ray-Hare, $F_{1,20} = 0.285$, $p = 0.598$) or the relative concentrations of ubiquitin (2-way ANOVA, $F_{1,29} = 0.615$, $p = 0.439$) or 4HNE (2-way ANOVA, $F_{1,29} = 0.281$, $p = 0.6$) (Fig. 7A–C). Genotype, on the other hand, was found to have a significant effect on the activity of catalase (Scheirer-Ray-Hare, $F_{5,29} = 10.6$, $p < 0.001$) and the relative concentration of 4HNE (2-way ANOVA, $F_{5,29} = 35.569$, $p < 0.001$), but not the relative concentration of ubiquitin, which varied little among samples (2-way ANOVA, $F_{5,29} = 0.629$, $p = 0.679$) (Fig. 7A–C).

None of the biomarkers tested, however, had a significant effect on the thermal tolerance of these 6 genotypes when included as a covariate in an ANCOVA with genotype as a fixed effect (catalase, $F_{1,5} = 0.932$, $p = 0.379$; ubiquitin, $F_{1,5} = 0.041$, $p = 0.848$; 4HNE, $F_{1,5} = 0.192$, $p = 0.680$). Nevertheless, catalase activity was significantly correlated with difference in lifespan (Pearson, $R^2 = 0.450$, $n = 12$, $p = 0.017$), and this model explained 45.02% of the variation in thermal tolerance observed among these 6 genotypes (Fig. 7D). This significant result disappeared, however, when all 20 genotypes were included in the correlation (catalase, $R^2 = 0.034$, $n = 40$, $p = 0.255$) and there were no significant correlations observed for the other 2 biomarkers (ubiquitin, $R^2 = 0.005$, $n = 40$, $p = 0.660$; 4HNE, $R^2 = 0.040$, $n = 40$, $p = 0.218$) (Fig. S4). Moreover, the addition of all 20 genotypes into the ANCOVAs for each biomarker did not change the result, and only coral genotype had a significant effect on thermal tolerance (catalase, $F_{1,19} = 0.598$, $p = 0.449$; ubiquitin, $F_{1,19} = 0.434$, $p = 0.518$; 4HNE, $F_{1,19} = 0.014$, $p = 0.906$).

4. DISCUSSION

4.1. Heritability of thermal tolerance in *Acropora cervicornis*

This population showed a significant effect of genotype with a relatively high broad-sense heritability of thermal tolerance ($H^2 = 0.528 \pm 0.173$). Despite this high broad-sense heritability estimate, our low narrow-sense heritability estimates (h^2) of thermal tolerance in this nursery population of *A. cervicornis* suggest that there is little additive genetic variation involved in this trait. Since additive genetic variance is the only component that can respond to selection, our low estimates of h^2 suggest that this nursery population of *A. cervicornis* may lack the genetic variation in thermal tolerance necessary to respond to predicted increases to ocean temperatures.

There are, however, several reasons to think there is more additive genetic variation for thermal tolerance than our h^2 estimates indicate. First, the estimation of h^2 is likely to reflect the low levels of variance

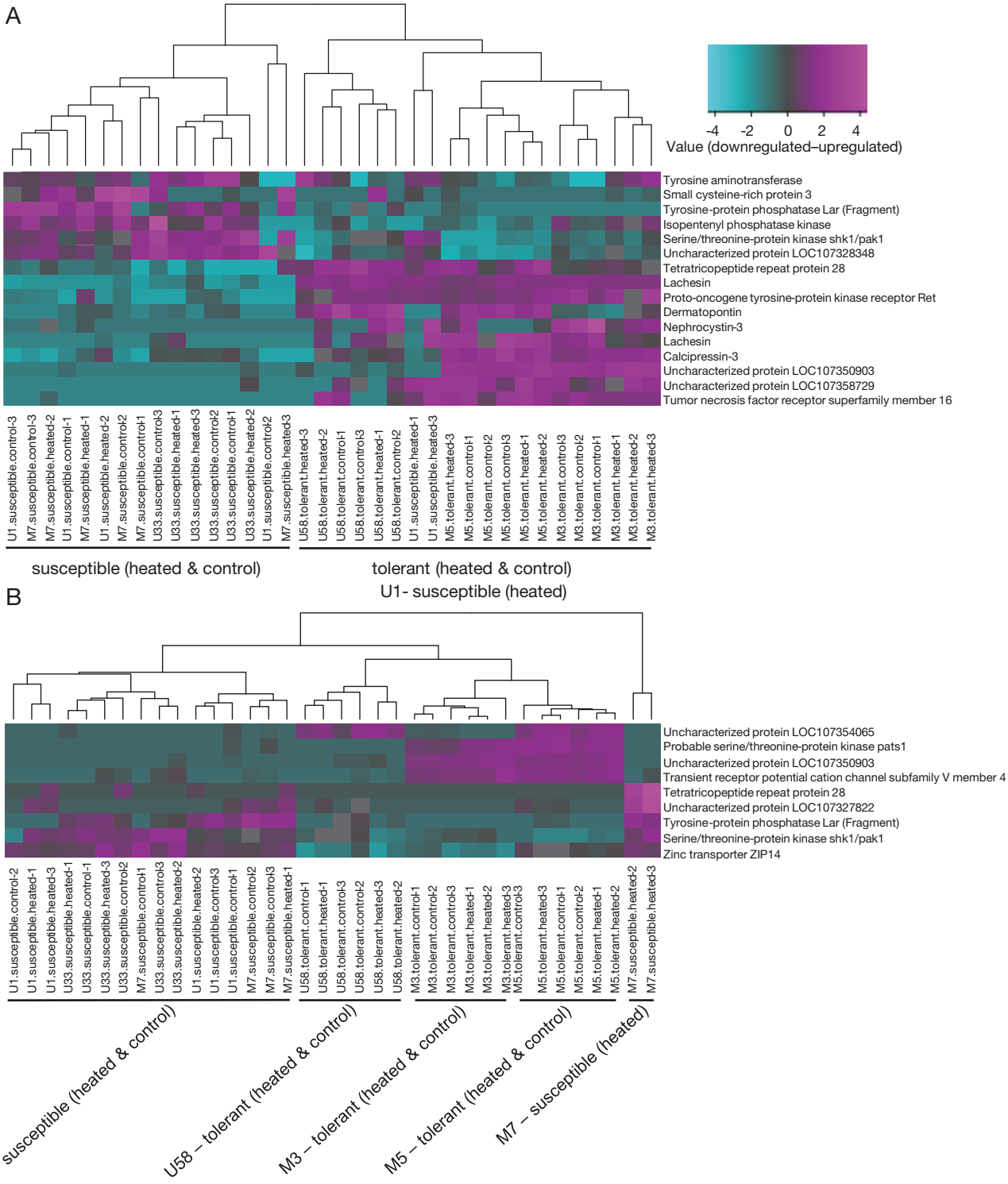

Fig. 6. Heat map across all samples using the (A) significant differentially expressed (DE) genes between tolerant and susceptible *Acropora* genotypes in the control treatment only (n = 9 per group), and (B) significant DE genes between tolerant and susceptible genotypes in the heated treatment only (n = 9 per group). Colors range from pink (significantly upregulated in tolerant colonies relative to susceptible colonies) to blue (significantly downregulated in tolerant colonies) and are based on the log counts per million. Significant DE genes were determined using the 'glmTreat' function in R to specify both a $\log_2$ fold-change cut-off of 1.5 as well as a false discover rate of 5%. Genes are ordered from top to bottom by ascending p-value: 8.10×10^{-8} to 4.46×10^{-5} (A); 4.11×10^{-7} to 2.58×10^{-5} (B)

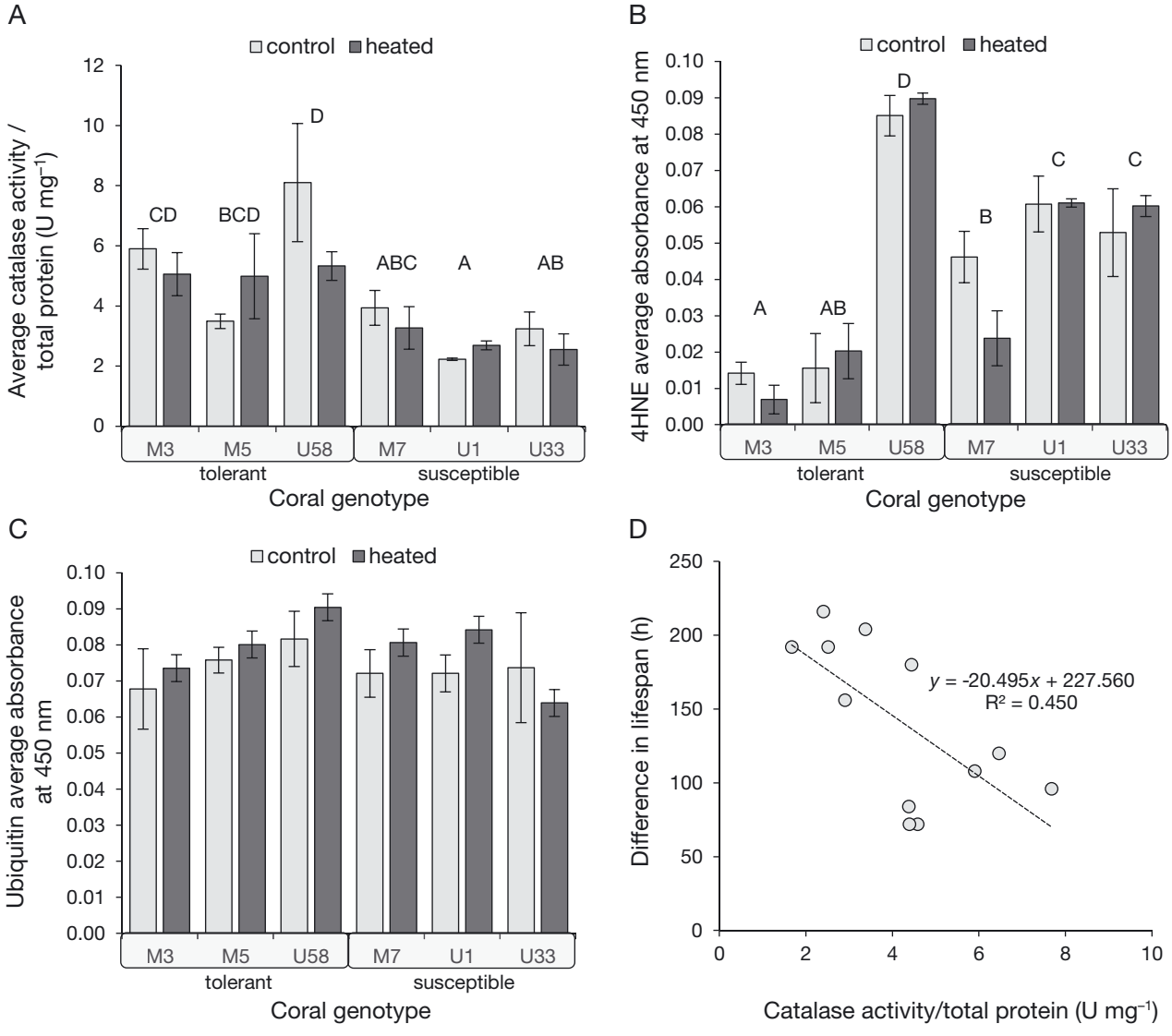

Fig. 7. Differences in average (A) catalase activity, (B) 4HNE concentration as measured by relative absorbance, and (C) ubiquitin concentration as measured by relative absorbance for the 3 most thermal tolerant and 3 most susceptible *Acropora* genotypes (n = 3 per bar). Different letters indicate significant pairwise differences between genotypes based on Tukey's HSD post hoc test. Error bars show standard error. (D) Model I linear regression showing the relationship of difference in lifespan of the 3 most tolerant and the 3 least tolerant genotypes to catalase activity in the heated treatment (n = 12)

in actual relatedness in our samples. Marker-based studies require high variance in relatedness in order to accurately estimate h^2. Variance in relatedness among the 20 genotypes tested in this study was consistently low and often negative, which implies that the h^2 estimates measured here are likely inaccurate and not representative of the true amount of additive genetic variance in this population. According to Ritland (1996), zero or negative values for variances in relatedness are either due to sampling individuals that are all similarly related, or to utilizing too few markers or markers with few alleles. The 15 microsatellite loci used in this study as well as the number of alleles per locus (mean ± SE = 6.733 ± 0.802) are well within the range recommended by Ritland (1996). Pairwise estimates of relatedness, on the other hand, were typically near zero for any fragments that were not clones, leading to the overall lack of variance. Ritland (1996) recommended that the number of pairwise comparisons should be as large as possible (10^4 or 10^5 if feasible), and in our study there were only 780 pairwise comparisons between fragments due to sampling constraints when working with a threatened species. This finding illu-

minates the need to consider the mating system and population structure of the focal species when using the marker-based approach. In contrast to the outcrossing mating system and potential for long-distance dispersal in *A. cervicornis* (Baums et al. 2005b, Drury et al. 2016), Carlon et al. (2011) used the same technique to estimate H^2 and h^2 in morphological traits among different populations of *Favia fragum*, a highly selfing coral with limited dispersal, and found significant variation in relatedness and significant h^2 for a number of traits related to corallite shape and structure. Therefore, the estimates of h^2 reported here should be interpreted with caution, and further studies will be needed utilizing a wider range of *A. cervicornis* colonies with a higher variance in actual relatedness to more accurately estimate h^2.

A second reason to think adaptation may be possible is that empirical data from a range of traits and species suggests that most genetic variance is in fact additive, typically accounting for 50–100% of the total broad sense heritability (H^2) (Hill et al. 2008). If we assume that additive genetic variance accounts for half of our H^2 estimate, then the real value of h^2 within this population may be closer to ~0.25. This is similar to the average h^2 reported for physiological traits across a variety of experimental organisms (h^2 = 0.33) (Mousseau & Roff 1987, Hill et al. 2008), but lower than values reported in other studies of coral traits in response to thermal stress, such as larval settlement propensity (h^2 = 0.49, Meyer et al. 2009) and bleaching response (h^2 = 0.58, Dziedzic et al. 2019). While it is likely that the h^2 of thermal tolerance in these nursery colonies of *A. cervicornis* is >0, and would therefore be able to respond to selection, there is still a level of uncertainty regarding the total amount of additive genetic variance for thermal tolerance present in these colonies. Therefore, additional research will be needed across more closely related colonies to obtain precise measurements of h^2 of thermal tolerance in this species.

Third, even if the total genetic variance for the trait of thermal tolerance in this population is largely non-additive, previous theoretical work has shown that epistatic variance can become additive genetic variance in populations following a bottleneck event due to decreases in heterozygosity (Cheverud & Routman 1995, 1996). This could potentially provide the necessary source of genetic variation for a population to be able to adapt, even following drastic population declines. Given the relatively recent population declines of *A. cervicornis* in the Florida Keys, conversion to additive genetic variance may be a real possibility.

Of course, it is also possible that our estimate of H^2 is inflated, and mostly due to variation in symbiont composition among colonies rather than to differences within the host genome. Previous work by Quigley et al. (2017) has shown that the initial symbiont community is highly dependent on host family and is highly heritable (h^2 = 0.3), even in a broadcast-spawning *Acropora* species. While an overestimation of H^2 is possible, it is unlikely to be the case. If there is a relationship between host genotype and symbiont taxa, then the variance in thermal tolerance attributed to symbiont identity would be included in the estimation of V_G, inflating our broad-sense heritability estimate. Our experimental design does not provide the ability to determine whether host genotype has a significant effect on the symbionts present (as only 1 fragment per colony was assessed for symbiont composition), but no significant association was detected between the symbionts present and thermal tolerance (which was related to genotype). This suggests that the symbionts can likely be considered part of the 'environmental' component of variance in the present study and that our estimate of H^2 predominately reflects the genetic variance in the host genome.

Few previous studies have estimated broad-sense heritability of thermal tolerance in corals, which limits the comparisons that can be made to the H^2 reported in our current study. Császár et al. (2010) estimated the broad-sense heritability of several traits related to thermal stress in a coral holobiont, the coral host (*A. millepora*) and its symbionts, utilizing the same clonal method as the present study. They found a similar estimate of genetic variation in the holobiont-derived trait of growth rate (H^2 = 0.59) compared to the holobiont-derived trait of survival under temperature stress shown here. Conversely, our broad-sense heritability estimate of thermal tolerance was much lower than the estimate for the heritability of thermal tolerance in *A. millepora* reported by Dixon et al. (2015), who estimated a broad-sense heritability of 0.87. However, their study differed from ours in that they measured the heritability of thermotolerance by subjecting coral larvae from multiple crosses to a thermal stress and measuring survival, which may or may not be consistent with how adult corals respond to a thermal stress. It is also important to note that heritability estimates (both broad and narrow) are dependent on a population's specific allele frequencies for the trait of interest (Falconer & MacKay 1996). This means that different species and populations will likely vary in their amount of genetic variation in thermal tolerance.

4.2. Gene expression patterns of thermal tolerance

Gene expression profiles for all *A. cervicornis* aligned genes overlapped significantly among the 6 colonies tested here (Fig. 2A), and only 2 DE genes were detected that were significantly different due to temperature treatment, 129 between tolerant and susceptible colonies, 9 between tolerant and susceptible colonies in the heated treatment, and 16 between tolerant and susceptible colonies in the control treatment. The number of DE genes detected in the present study is fairly low compared to the number identified in other studies of thermotolerance in corals, most of which have identified several hundred to several thousand DE genes (Meyer et al. 2011, Barshis et al. 2013, Rosic et al. 2014, Dixon et al. 2015, Davies et al. 2016). While samples within our study were highly consistent within a colony except for certain subsets of genes (see Fig. S3 and Figs. 5 & 6), there was a great deal of variability within tolerance groups among the different genotypes. This high degree of inter-colony transcriptional variability has been noted throughout the literature, both in the same species as the present study (Parkinson et al. 2018), and in others (Bay et al. 2009, Granados-Cifuentes et al. 2013, Bertucci et al. 2015), making this a potentially widespread phenomenon in corals in general.

The extremely low number of significant DE genes between control and elevated temperature treatments compared to previous studies is striking and unexpected, since studies with sample collection at later time points (20 h – 96 d) in other coral species have noted large gene expression differences due to thermal stress (DeSalvo et al. 2010a, Barshis et al. 2013, Davies et al. 2016, Dziedzic et al. 2019). This discrepancy could be due to several reasons. As mentioned previously, corals exhibit a high degree of natural gene expression variation, and it is possible that each colony is responding to temperature stress differently such that a significant difference among treatments cannot be detected (Bellantuono et al. 2012, Granados-Cifuentes et al. 2013). On the other hand, the thermal stress chosen for this experiment may not have been high enough to elicit a strong transcriptional response. Studies such as those by DeSalvo et al. (2010a), Seneca & Palumbi (2015), and Dziedzic et al. (2019) sampled tissues for mRNA expression at times when bleaching was already apparent for a subset of samples. Here, bleaching did not occur until at least 8 d post stress exposure in the elevated temperature treatment, or even longer for tolerant colonies. The 12 h sampling time point could have also been too early or too late to see divergent responses between treatments. It is also entirely possible that this species uses a more 'fixed' gene expression strategy to combat thermal stress and that the lack of differential expression observed here between temperature treatments is accurate, but this is unlikely to be the case. Parkinson et al. (2018) found several hundred DE genes significantly different between field-collected *A. cervicornis* fragments held at 35°C for 1 h compared to fragments held at ambient temperature (24–28°C). While their temperature treatment was much more extreme than the one used here, it does illustrate that this species is entirely capable of elucidating a strong transcriptomic response under thermal stress. Unfortunately, without additional time points for comparison, it is difficult to say whether the colonies used here underwent transcriptional changes immediately after thermal exposure and then returned to control conditions (similar to what was reported by Seneca & Palumbi 2015), or if they were not yet experiencing thermal stress and were therefore exhibiting similar transcriptional profiles to controls.

The DE genes that were significantly upregulated in thermotolerant colonies are involved in pathways such as cellular component biogenesis, organization, and assembly, immune response, cellular macromolecule biosynthetic processes, adhesion, cell junction assembly and organization, and other metabolic processes. This is consistent with what has been identified in other studies of thermal stress and thermal tolerance in corals (DeSalvo et al. 2008, Barshis et al. 2013, Palumbi et al. 2014). Tolerant corals of the species *A. hyacinthus* have also been shown to upregulate immune-specific genes such as members of the TNF superfamily, TNF receptor-associated factors (TRAFs), and oncogenes or protooncogenes (Barshis et al. 2013, Palumbi et al. 2014). These pathways mediate the bleaching response of corals (Ainsworth et al. 2011, Tchernov et al. 2011, Pinzón et al. 2015), and may be involved in the thermal tolerance observed in the present study. Our dataset, however, shows some discrepancies in the expression patterns between different transcripts that produce similar protein products. For example, while a TNF superfamily transcript and 1 TRAF transcript were indeed upregulated in tolerant colonies in our data set, other TRAF transcripts showed the opposite pattern. Cell adhesion-related genes were also found to be frontloaded in more tolerant colonies of *A. hyacinthus* (Barshis et al. 2013), and regulation of cell adhesion has been proposed to be involved in

the coral bleaching process through the detachment of gastrodermal cells containing zooxanthellae (Gates et al. 1992, DeSalvo et al. 2008).

The DE genes that were significantly downregulated in thermotolerant colonies are involved in pathways such as response to nitrogen compounds, lipid synthesis and metabolism, positive regulation of NIK/NF-kappaB signaling, positive regulation of protein serine/threonine kinase activity, gamma-aminobutyric acid signaling pathway, and ion transmembrane transport. This is consistent with what has been identified in other studies of thermal tolerance in corals, with thermally tolerant *A. millepora* larvae downregulating genes related to ion transport (Dixon et al. 2015). Ion transport-related genes have also been implicated in the thermal stress response of corals, with *A. hyacinthus* also downregulating ion transport-related genes 5 and 20 h post elevated temperature exposure (Palumbi et al. 2014) and *Siderastrea siderea* downregulating ion transport biological processes 95 d post elevated temperature exposure (Davies et al. 2016). The downregulation of ion transport in more tolerant corals may reflect a diverting of energy away from calcification or other important mechanisms into processes that circumvent future thermal stress. Ladd et al. (2017) showed a tradeoff between growth and survival following thermal stress in *A. cervicornis*, and this could potentially be related to the downregulation of these mechanisms.

In addition, we saw downregulation of NIK/NF-kappa B (NF-kB) signaling regulation as well as protein serine/threonine kinase activity in tolerant corals, which both play a role in apoptosis (Cross et al. 2000, Perkins 2007). The observed downregulation of protein serine/threonine kinase activity is similar to what was found by DeSalvo et al. (2010) in *Acropora palmata* samples 2 d post heat stress, but they found the opposite pattern in NF-kB, which was upregulated in 1 d and 2 d post-thermal stress colonies relative to controls. Bellantuono et al. (2012), however, found that NF-kB inhibitor expression was lower in corals that were not pre-conditioned to a thermal stress when compared to preconditioned corals, supporting the role of lower NF-kB signaling in thermal tolerance. The downregulation of genes related to apoptosis in the tolerant colonies tested here may be correlated to a lower stressed state relative to susceptible colonies.

Based on our RNA-Seq results, 40 DE genes were identified that had highly consistent expression patterns among the 3 colonies in each tolerance group, with at least 3 of these DE genes being highly consistent among those groups in the heated temperature treatment. This consistency makes them potential targets for use as gene expression biomarkers of thermal tolerance, but will require verification with additional testing. While these genes showed stark differences in expression patterns between tolerant and susceptible colonies, the fact that many did not differ in expression according to treatment makes it questionable as to whether they are involved in differences in thermal tolerance between the genotypes and not merely indicative of other unrelated genetic differences. Given the limited sample size in the present study, it is difficult to test with enough power whether the observed gene expression patterns are actually related to differences in lifespan. Therefore, it would be necessary to test these putative DE genes in a larger number of tolerant and susceptible colonies, particularly the 9 genes that were differentially expressed due to tolerance following temperature stress.

It should also be noted that these expression profiles are only based on a single time point for control conditions and 12 h post elevated temperature exposure. For these genes to be reliable biomarkers for thermal tolerance, they would need to be consistent (between tolerant and susceptible colonies) at multiple time points within a single day and across seasons. Differences in gene expression in corals between day and night conditions have already been shown (Bertucci et al. 2015), but more research at multiple time points both within a single day and across seasons would be necessary in order to understand changes in expression of these genes either due to circadian rhythms or seasonality.

Finally, although there were 40 genes that showed consistent expression among the different genotypes within a tolerance group, there were more than double that number (89 genes) that showed inconsistent expression patterns. This suggests that different genotypes can possess similar thermal stress tolerance phenotypes while using different gene expression strategies to achieve this outcome. For example, while genotypes M3 and M5 both shared a tolerant phenotype and showed similar expression patterns at these 89 genes, the other tolerant genotype, U58, showed considerably different patterns. Since both M3 and M5 were originally from the same location, while U58 was from a different reef, it is possible to imagine there may be site-specific differences in gene expression pathways of thermal tolerance; however, with the limited number of samples tested thus far it is too early to

tell. At the very least, the differences in gene expression patterns imply that the genetic basis for thermal tolerance is not necessarily simple or limited to a single shared pathway.

4.3. Patterns of potential protein biomarkers with thermal tolerance

Out of the 4 potential protein biomarkers chosen due to their responses under elevated temperature stress in other species (Barshis et al. 2010, Ross et al. 2013), only catalase and 4HNE showed slight, but not significant, trends with thermal tolerance. Catalase activity was higher on average in the 3 most tolerant genotypes compared to the 3 most susceptible, and it was significantly correlated with greater differences in lifespan. 4HNE concentration showed the opposite pattern, with higher concentrations present in more susceptible colonies on average. Higher catalase activity has been found in corals immediately after exposure to a thermal stress, which is mainly due to oxidative stress from the increased production of reactive oxygen species (DeSalvo et al. 2008, Rodriguez-Lanetty et al. 2009, Polato et al. 2013, Ross et al. 2013, Rosic et al. 2014). This was not observed in the present study, as catalase did not differ significantly between treatments. However, Granados-Cifuentes et al. (2013) and Polato et al. (2013) found significant differences in catalase expression among different coral genotypes, and we argue these differences in catalase expression could be involved in differences in thermal tolerance among these colonies. Higher 4HNE concentrations were also found in a population of *Porites lobata* adapted to thermally extreme conditions rather than in colonies adapted to a less extreme environment, and did not significantly change after reciprocal transplantation between these environments (Barshis et al. 2010). While similar genotypic specificity of 4HNE was observed in this study, 4HNE was found in colonies with higher thermotolerance on average, suggesting that these colonies may be experiencing less oxidative stress compared to more susceptible colonies at the time of sampling.

Unfortunately, neither catalase nor 4HNE are likely to be good candidates for biomarkers of thermal tolerance in this species. While we noted the following trends as mentioned above, both of these protein products showed a high degree of variability among the 3 genotypes within each tolerance group. This variability is amplified when all 20 colonies are included in the analysis, as there were no trends seen among protein concentration and difference in lifespan (Fig. S4 in the Supplement). Finally, while both catalase and 4HNE are known to respond to thermal stress in corals, these genes and pathways are also associated with general and oxidative stress responses and therefore may not be good indicators of thermal stress specifically (Halliwell & Gutteridge 1999, DeSalvo et al. 2008, Voolstra et al. 2009, Barshis et al. 2010).

In summary, while we cannot say conclusively whether crucial additive genetic variation may be limited or not in this species, there is a high amount of total genetic variation in thermal tolerance in the 20 genotypes of *A. cervicornis* sampled here from a CRF nursery, which suggests that there may be potential for this species to respond to future warming conditions. Furthermore, while the gene expression results reported here show great variability both within and among genotypes, suggesting that the genetic basis for thermal tolerance is not simple, these results lend support for previously observed patterns in known stress biomarkers of thermal tolerance. Further research at multiple time points will be necessary to understand how this species responds at the onset of thermal stress and shortly afterward, as well as what is occurring at the onset of temperature-induced bleaching.

Acknowledgements. We thank the Florida 'Protect Our Reefs' fund (POR-2013-20), the PADI Foundation Grant, the American Museum of Natural History Richard Gilder Graduate School Lerner-Gray Memorial Fund for Marine Research, and the University of North Florida Graduate School and Coastal Biology Program for providing funding for this research. Thanks to the Coral Restoration Foundation for providing the coral fragments and the Keys Marine Laboratory for assistance with experimental setup. F. Heng, M.V. Matz, C. Schnitzler, N. Fogarty, S. Kitchen, I.B. Baums, C. Ross, J. Gelsleichter, and O. Ross all provided use of their facilities, protocols, and assistance throughout various aspects of this research. We also thank the 2 reviewers for their valuable comments on the manuscript. Funding was provided by NSF to M.R. (OCE-1560213) and M.R.L. (OCE-1503483).

LITERATURE CITED

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. Proc R Soc B 275:2273–2282

Ainsworth TD, Wasmund K, Ukani L, Seneca F, Yellowlees D, Miller D, Leggat W (2011) Defining the tipping point: a complex cellular life/death balance in corals in response to stress. Sci Rep 1:160

Alexa A, Rahnenfuhrer J (2019) topGO: enrichment analysis for gene onotology. R package version 2.38.1.

https://bioconductor.org/packages/release/bioc/html/topGO.html
Anders S, Pyl PT, Huber W (2015) HTSeq — a Python framework to work with high-throughput sequencing data. Bioinformatics 31:166–169
Baker AC (2003) Flexibility and specificity in coral–algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium.* Annu Rev Ecol Evol Syst 34:661–689
Barshis DJ, Stillman JH, Gates RD, Toonen RJ, Smith LW, Birkeland C (2010) Protein expression and genetic structure of the coral *Porites lobata* in an environmentally extreme Samoan back reef: Does host genotype limit phenotypic plasticity? Mol Ecol 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. Proc Natl Acad Sci USA 110:1387–1392
Baums IB, Hughes CR, Hellberg ME (2005a) Mendelian microsatellite loci for the Caribbean coral *Acropora palmata.* Mar Ecol Prog Ser 288:115–127
Baums IB, Miller MW, Hellberg ME (2005b) Regionally isolated populations of an imperiled Caribbean coral *Acropora palmata.* Mol Ecol 14:1377–1390
Baums IB, Devlin-Durante MK, Brown L, Pinzón JH (2009) Nine novel, polymorphic microsatellite markers for the study of threatened Caribbean acroporid corals. Mol Ecol Resour 9:1155–1158
Baums IB, Johnson ME, Devlin-Durante MK, Miller MW (2010) Host population genetic structure and zooxanthellae diversity of two reef-building coral species along the Florida reef tract and wider Caribbean. Coral Reefs 29: 835–842
Bay RA, Palumbi SR (2014) Multilocus adaptation associated with heat resistance in reef-building corals. Curr Biol 24: 2952–2956
Bay LK, Ulstrup KE, Nielsen HB, Jarmer H and others (2009) Microarray analysis reveals transcriptional plasticity in the reef building coral *Acropora millepora.* Mol Ecol 18: 3062–3075
Bellantuono AJ, Granados-Cifuentes C, Miller DJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2012) Coral thermal tolerance: tuning gene expression to resist thermal stress. PLOS ONE 7:e50685
Berkelmans R, van Oppen MJ (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. Proc R Soc B 273:2305–2312
Bertucci A, Foret S, Ball EE, Miller DJ (2015) Transcriptomic differences between day and night in *Acropora millepora* provide new insights into metabolite exchange and light-enhanced calcification in corals. Mol Ecol 24: 4489–4504
Brown BE, Dunne RP, Goodson MS, Douglas AE (2002) Experience shapes the susceptibility of a reef to coral bleaching. Coral Reefs 21:119–126.
Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13: 581–583
Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K and others (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336
Carlon DB, Budd AF, Lippe C, Andrew RL (2011) The quantitative genetics of incipient speciation: heritability and genetic correlations of skeletal traits in populations of diverging *Favia fragum* ecomorphs. Evolution 65: 3428–3447
Chamberland VF, Petersen D, Latijnhouwers KRW, Snowden S, Mueller B, Vermeij MJA (2016) Four-year-old Caribbean *Acropora* colonies reared from field-collected gametes are sexually mature. Bull Mar Sci 92: 263–264
Chen Y, Lun ATL, Smyth GK (2016) From reads to genes to pathways: differential expression analysis of RNA-Seq experiments using Rsubread and the edgeR quasi-likelihood pipeline. F1000Research 5:1438
Cheverud JM, Routman EJ (1995) Epistasis and its contribution to genetic variance components. Genetics 139: 1455–1461
Cheverud JM, Routman EJ (1996) Epistasis as a source of increased additive genetic variance at population bottlenecks. Evolution 50:1042–1051
Coles SL, Brown BE (2003) Coral bleaching-capacity for acclimatization and adaptation. Adv Mar Biol 46: 183–223
Cross TG, Scheel-Toellner D, Henriquez NV, Deacon E, Salmon M, Lord JM (2000) Serine/threonine protein kinases and apoptosis. Exp Cell Res 256:34–41
Császár NBM, Seneca FO, van Oppen MJH (2009) Variation in antioxidant gene expression in the scleractinian coral *Acropora millepora* under laboratory thermal stress. Mar Ecol Prog Ser 392:93–102
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
Davies SW, Marchetti A, Ries JB, Castillo KD (2016) Thermal and pCO_2 stress elicit divergent transcriptomic responses in a resilient coral. Front Mar Sci 3:112
DeCarlo TM, Harrison HB, Gajdzik L, Alaguarda D and others (2019) Acclimatization of massive reef-building corals to consecutive heatwaves. Proc Biol Sci 286: 20190235
DeSalvo MK, Voolstra CR, Sunagawa S, Schwarz JA and others (2008) Differential gene expression during thermal stress and bleaching in the Caribbean coral *Montastraea faveolata.* Mol Ecol 17:3952–3971
DeSalvo MK, Sunagawa S, Voolstra CR, Medina M (2010a) Transcriptomic responses to heat stress and bleaching in the elkhorn coral *Acropora palmata.* Mar Ecol Prog Ser 402:97–113
DeSalvo MK, Sunagawa S, Fisher PL, Voolstra CR, Iglesias-Prieto R, Medina M (2010b) Coral host transcriptomic states are correlated with *Symbiodinium* genotypes. Mol Ecol 19:1174–1186
Dixon GB, Davies SW, Aglyamova GV, Meyer E, Bay LK, Matz MV (2015) Genomic determinants of coral heat tolerance across latitudes. Science 348:1460–1462
Douglas AE (2003) Coral bleaching how and why? Mar Pollut Bull 46:385–392
Drury C, Dale KE, Panlilio JM, Miller SV and others (2016) Genomic variation among populations of threatened coral: *Acropora cervicornis.* BMC Genomics 17:286
Dziedzic KE, Elder H, Tavalire H, Meyer E (2019) Heritable variation in bleaching responses and its functional genomic basis in reef-building corals (*Orbicella faveolata*). Mol Ecol 28:2238–2253
Eirin-Lopez JM, Putnam HM (2019) Marine environmental epigenetics. Annu Rev Mar Sci 11:335–368
Falconer DS, MacKay TFC (1996) Introduction to quantitative genetics. Longman, Harlow

Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu Rev Physiol 61:243–282

Fisher RA (1930) The genetical theory of natural selection. Clarendon Press, Oxford

Fukami H, Budd AF, Levitan DR, Jara J, Kersanach R, Knowlton N (2004) Geographical differences in species boundaries among members of the *Montastraea annularis* complex based on molecular and morphological markers. Evolution 58:324–337

Gates RD, Baghdasarian G, Muscatine L (1992) Temperature stress causes host cell detachment in symbiotic cnidarians: implications for coral bleaching. Biol Bull (Woods Hole) 182:324–332

Glynn PW (1996) Coral reef bleaching: facts, hypotheses and implications. Glob Change Biol 2:495–509

Glynn PW, Mate JL, Baker AC, Calderon MO (2001) Coral bleaching and mortality in Panama and Ecuador during the 1997–1998 El Niño–Southern Oscillation event: spatial/temporal patterns and comparisons with the 1982–1983 event. Bull Mar Sci 69:79–109

Goreau TJ, Hayes RL (1994) Coral bleaching and ocean hotspots. Ambio 23:176–180

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

Halliwell B (2006) Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol 141:312–322

Halliwell B, Gutteridge JMC (1999) Free radicals in biology and medicine. Oxford University Press, Oxford

Hawkins AJS (1991) Protein turnover: a functional appraisal. Funct Ecol 5:222–233

Hill WG, Goddard ME, Visscher PM (2008) Data and theory point to mainly additive genetic variance for complex traits. PLOS Genet 4:e1000008

Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. Mar Freshw Res 50:839–866

Hoegh-Guldberg O (2014) Coral reef sustainability through adaptation: glimmer of hope or persistent mirage? Curr Opin Environ Sustain 7:127–133

IPCC (2013) Introduction. In: Stocker TF, Qin D, Plattner GK, Tignor M and others (eds) Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, p 119–158

Jin YK, Lundgren P, Lutz A, Raina JB and others (2016) Genetic markers for antioxidant capacity in a reef-building coral. Sci Adv 2:e1500842

Jones AM, Berkelmans R, van Oppen MJH, Mieog JC, Sinclair W (2008) A community shift in the symbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. Proc R Soc B 275: 1359–1365

Kenkel CD, Matz MV (2016) Gene expression plasticity as a mechanism of coral adaptation to a variable environment. Nat Ecol Evol 1:14

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

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

Kopylova E, Noé L, Touzet H (2012) SortMeRNA: Fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. Bioinformatics 28:3211–3217

Ladd MC, Shantz AA, Bartels E, Burkepile DE (2017) Thermal stress reveals a genotype-specific tradeoff between growth and tissue loss in restored *Acropora cervicornis.* Mar Ecol Prog Ser 572:129–139

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Curr Biol 28:2570–2580

Lande R, Shannon S (1996) The role of genetic variation in adaptation and population persistence in a changing environment. Evolution 50:434–437

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359

Levitan DR, Fogarty ND, Jara J, Lotterhos KE, Knowlton N (2011) Genetic, spatial, and temporal components of precise spawning synchrony in reef building corals of the *Montastraea annularis* species complex. Evolution 65: 1254–1270

Lewis C, Neely K, Rodriguez-Lanetty M (2019) Recurring episodes of thermal stress shift the balance from a dominant host-specialist to a background host-generalist zooxanthella in the threatened pillar coral, *Dendrogyra cylindrus.* Front Mar Sci 6:5

Li CC, Weeks DE, Chakravarti A (1993) Similarity of DNA fingerprints due to chance and relatedness. Hum Hered 43:45–52

Liew YJ, Zoccola D, Li Y, Tambutté E and others (2018) Epigenome-associated phenotypic acclimatization to ocean acidification in a reef-building coral. Sci Adv 4: eaar8028

Lynch M (1988) Estimation of relatedness by DNA fingerprinting. Mol Biol Evol 5:584–599

Lynch M, Ritland K (1999) Estimation of pairwise relatedness with molecular markers. Genetics 152:1753–1766

Manzello DP, Berkelmans R, Hendee JC (2007) Coral bleaching indices and thresholds for the Florida Reef Tract, Bahamas, and St. Croix, US Virgin Islands. Mar Pollut Bull 54:1923–1931

Matz M, Shagin D, Bogdanova E, Britanova O, Lukyanov S, Diatchenko L, Chenchik A (1999) Amplification of cDNA ends based on template-switching effect and step-out PCR. Nucleic Acids Res 27:1558–1560

Mayfield AB, Fan TY, Chen CS (2013) Physiological acclimation to elevated temperature in a reef-building coral from an upwelling environment. Coral Reefs 32:909–921

McCarthy DJ, Chen Y, Smyth GK (2012) Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Res 40: 4288–4297

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

Meyer E, Aglyamova GV, Matz MV (2011) Profiling gene expression responses of coral larvae (*Acropora millepora*) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. Mol Ecol 20: 3599–3616

Milligan BG (2003) Maximum-likelihood estimation of relatedness. Genetics 163:1153–1167

Mousseau TA, Roff DA (1987) Natural selection and the heritability of fitness components. Heredity 59:181–197

Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. In: Dubinsky Z (ed) Ecosystems of the world, Vol 25. Coral reefs. Elsevier, Amsterdam, p 75–87

Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol Rev Camb Philos Soc 85:935–956

Neigel JE, Avise JC (1983) Clonal diversity and population structure in a reef-building coral, *Acropora cervicornis*: self-recognition analysis and demographic interpretation. Evolution 37:437–453

NMFS (2006) Endangered and threatened species: final listing determinations for elkhorn coral and staghorn coral. Fed Regist 71:26852–26861

NMFS (2015) Recovery plan: elkhorn coral (*Acropora palmata*) and staghorn coral (*A. cervicornis*). Prepared by the *Acropora* Recovery Team for the National Marine Fisheries Service, Silver Spring, MD. https://repository.library.noaa.gov/view/noaa/8950

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

Parkinson JE, Bartels E, Devlin-Durante MK, Lustic C and others (2018) Extensive transcriptional variation poses a challenge to thermal stress biomarker development for endangered corals. Mol Ecol 27:1103–1119

Perkins ND (2007) Integrating cell-signalling pathways with NF-kB and IKK function. Nat Rev Mol Cell Biol 8:49–62

Pinzón JH, Kamel B, Burge CA, Harvell CD, Medina M, Weil E, Mydlarz LD (2015) Whole transcriptome analysis reveals changes in expression of immune-related genes during and after bleaching in a reef-building coral. R Soc Open Sci 2:140214

Polato NR, Altman N, Baums IB (2013) Variation in the transcriptional response of threatened coral larvae to elevated temperatures. Mol Ecol 22:1366–1382

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

Queller DC, Goodnight KF (1989) Estimating relatedness using molecular markers. Evolution 43:258–275

Quigley KM, Willis BL, Bay LK (2017) Heritability of the *Symbiodinium* community in vertically- and horizontally-transmitting broadcast spawning corals. Sci Rep 7: 8219

Quigley KM, Willis BL, Kenkel CD (2019) Transgenerational inheritance of shuffled symbiont communities in the coral *Montipora digitata.* Sci Rep 9:13328

Rasher DB, Hay ME (2010) Chemically rich seaweeds poison corals when not controlled by herbivores. Proc Natl Acad Sci USA 107:9683–9688

Rinkevich B (1995) Restoration strategies for coral reefs damaged by recreational activities: the use of sexual and asexual recruits. Restor Ecol 3:241–251

Rinkevich B (2014) Rebuilding coral reefs: Does active reef restoration lead to sustainable reefs? Curr Opin Environ Sustain 7:28–36

Ritland K (1996) A marker-based method for inferences about quantitative inheritance in natural populations. Evolution 50:1062–1073

Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26: 139–140

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

Rosic N, Kaniewska P, Chan CKK, Ling EYS, Edwards D, Dove S, Hoegh-Guldberg O (2014) Early transcriptional changes in the reef-building coral *Acropora aspera* in response to thermal and nutrient stress. BMC Genomics 15:1052

Ross C, Ritson-Williams R, Olsen K (2013) Short-term and latent post-settlement effects associated with elevated temperature and oxidative stress on larvae from the coral *Porites astreoides.* Coral Reefs 32:71–79

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

Sawall Y, Al-Sofyani A, Hohn S, Banguera-Hinestroza E, Voolstra CR, Wahl M (2015) Extensive phenotypic plasticity of a Red Sea coral over a strong latitudinal temperature gradient suggests limited acclimatization potential to warming. Sci Rep 5:8940

Seneca FO, Palumbi SR (2015) The role of transcriptome resilience in resistance of corals to bleaching. Mol Ecol 24:1467–1484

Seneca FO, Foret S, Ball EE, Smith-Keune C, Miller DJ, van Oppen MJH (2010) Patterns of gene expression in a scleractinian coral undergoing natural bleaching. Mar Biotechnol 12:594–604

Stoffel MA, Nakagawa S, Schielzeth H (2017) rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods Ecol Evol 8: 1639–1644

Takahashi S, Murata N (2008) How do environmental stresses accelerate photoinhibition? Trends Plant Sci 13: 178–182

Tchernov D, Kvitt H, Haramaty L, Bibby TS, Gorbunov MY, Rosenfield H, Falkowski PG (2011) Apoptosis and the selective survival of host animals following thermal bleaching in zooxanthellate corals. Proc Natl Acad Sci USA 108:9905–9909

Tunnicliffe V (1981) Breakage and propagation of the stony coral *Acropora cervicornis.* Proc Natl Acad Sci USA 78: 2427–2431

van Oppen MJH, Willis BL, Van Vugt HWJA, Miller DJ (2000) Examination of species boundaries in the *Acropora cervicornis* group (Scleractinia, Cnidaria) using nuclear DNA sequence analyses. Mol Ecol 9:1363–1373

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

van Oppen MJH, Oliver JK, Putnam HM, Gates RD (2015) Building coral reef resilience through assisted evolution. Proc Natl Acad Sci USA 112:2307–2313

Vargas-Angel B, Colley SB, Hoke SM, Thomas JD (2006) The reproductive seasonality and gametogenic cycle of *Acropora cervicornis* off Broward County, Florida, USA. Coral Reefs 25:110–122

Vollmer SV, Palumbi SR (2002) Hybridization and the evolution of reef coral diversity. Science 296:2023–2025

Voolstra CR, Schnetzer J, Peshkin L, Randall CJ, Szmant AM, Medina M (2009) Effects of temperature on gene

expression in embryos of the coral *Montastraea faveolata*. BMC Genomics 10:627

Wang J (2002) An estimator for pairwise relatedness using molecular markers. Genetics 160:1203–1215

Wang J (2007) Triadic IBD coefficients and applications to estimating pairwise relatedness. Genet Res 89: 135–153

Wang J (2011) COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Mol Ecol Resour 11:141–145

Webster NS, Reusch TBH (2017) Microbial contributions to the persistence of coral reefs. ISME J 11:2167–2174

Editorial responsibility: Karen Miller, Hobart, Tasmania, Australia

Submitted: October 3, 2019; Accepted: June 29, 2020
Proofs received from author(s): July 26, 2020