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The role of transcriptome resilience in resistance of corals to bleaching

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Abstract

Wild populations increasingly experience extreme conditions as climate change amplifies environmental variability. How individuals respond to environmental extremes determines the impact of climate change overall. The variability of response from

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individual to individual can represent the opportunity for natural selection to occur as a result of extreme conditions. Here, we experimentally replicated the natural exposure to extreme temperatures of the reef lagoon at Ofu Island (American Samoa), where corals can experience severe heat stress during midday low tide. We investigated the bleaching and transcriptome response of 20 *Acropora hyacinthus* colonies five and twenty hours after exposure to control (29 °C) or heated (35 °C) conditions. We found a highly dynamic transcriptome response: twenty-seven percent of the coral transcriptome was significantly regulated one hour post-heat exposure. Yet fifteen hours later when heat-induced coral bleaching became apparent, only 12% of the transcriptome was differentially regulated. A large proportion of responsive genes at the first time point returned to control levels, others remained differentially expressed over time, while an entirely different subset of genes was successively regulated at the second time point. However, a noteworthy variability in gene expression was observed among individual coral colonies. Among the genes of which expression lingered over time, fast return to normal levels was associated with low bleaching. Colonies that maintained higher expression levels of these genes bleached severely. Return to normal levels of gene expression after stress has been termed transcriptome resilience, and in the case of some specific genes may signal the physiological health and response ability of individuals to environmental stress.

Introduction

Global warming due to climate change involves shifts in mean annual temperatures but also an increased frequency of extremes (Seneviratne *et al.* 2014). Exposure to transient extreme temperatures can cause mass mortality and dramatic transformation of

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an ecosystem (Dudgeon *et al.* 2010). Essentially, the response of organisms to normal fluctuation in temperature is overwhelmed during extreme conditions. As a consequence, the impact of climate change on natural assemblages depends on the rate of exposure to environmental extremes, the rate of response and recovery, and the ability of individuals to acclimatize or populations to adapt (Palumbi *et al.* 2014).

Recent availability of transcriptome data from a wide variety of species makes it possible to more finely study the molecular stress response of organisms to extreme temperatures (Runcie *et al.* 2012; Kenkel *et al.* 2013; Xie *et al.* 2013), and can help us evaluate the capacity for acclimatization and adaptation in populations of key species (Hoffmann & Sgrò 2011). For reef building corals, environmental data from around the tropics suggests that ocean temperatures one or two degrees above average generate physiological stress (Jokiel & Coles 1990). Prolonged low or acute level of temperature stress cause coral bleaching, the expulsion of essential endosymbiotic photosynthetic algae (reviewed by Weis 2008). The combination of a defined temperature trigger (Tolleter *et al.* 2013), a disadvantageous physiological response and a battery of transcriptome tools makes the coral holobiont system adequate to describe the rates, magnitudes, and duration of reaction to extreme climate events.

To date, twelve studies have investigated the transcriptomic changes in corals and anemones responding to heat stress. These studies have targeted the partial or near complete transcriptomes of symbiotic cnidarians including temperate anemones (Richier *et al.* 2008; Moya *et al.* 2012), adult reef-building corals (DeSalvo *et al.* 2008; 2010; Bellantuono *et al.* 2012; Barshis *et al.* 2013), and coral embryos (Voolstra *et al.* 2009; Portune *et al.* 2010; Polato *et al.* 2010; Meyer *et al.* 2011). Significantly regulated genes

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differ widely across these studies, (DeSalvo *et al.* 2008; 2010; Voolstra *et al.* 2009; Rodriguez-Lanetty *et al.* 2009; Meyer *et al.* 2011; Bellantuono *et al.* 2012; Barshis *et al.* 2013). Although change in apoptosis, antioxidant, and heat shock protein genes has been repeatedly observed after high temperature treatment, many other gene families are revealed in only one or a handful of studies. Moreover, these studies typically follow transcriptional changes that occur when bleaching begins, well after heat treatments commence. They show changes in transcription that occur after repeated daily heat stress, as water temperatures increase during the day, but have not focused on fast responses to extreme temperature changes. Because the onset of bleaching occurs one or several days after strong heat stress, gene expression during bleaching may be very different from gene expression a few hours post acute heat exposure. In addition, because of high variability in bleaching sensitivity among colonies, it is possible that the reaction of gene expression during acute heat stress, and the recovery of expression post-stress, sets the stage for later bleaching.

Transcriptome tools are also being used to document acclimatization and adaptation among coral populations. For example, some corals in Ofu Island, American Samoa, living in warm water back reef pools exhibit high heat tolerance by constitutively expressing more of certain stress-response genes (Barshis *et al.* 2013). Corals living in cooler parts of this reef can acquire higher heat tolerance after transplantation, and show acclimatization of gene expression patterns as well (Palumbi *et al.* 2014). However, about half the difference in heat tolerance among populations is not due to acclimatization. Furthermore, genetic differences at about 100 loci have been implicated in adaptation of these populations to repeated temperature extremes (Bay & Palumbi 2014). Bellantuono *et*

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al. (2012) showed that corals could acquire higher heat tolerance in as little as 2 weeks – but showed no constitutive transcriptional changes associated with this physiological shift.

In this study, we conducted a whole-transcriptome controlled heat stress experiment on corals from the Ofu Island (American Samoa) lagoon, which can experience extreme daily change in temperatures during summer low tides (+6 ˚C above the 29 ˚C summer average) that can lead to bleaching. Despite such high temperatures, there is a high abundance and diversity of live corals within the lagoon (Craig *et al.* 2001), some of which have shown relative resistance to those challenging temperature events (Oliver & Palumbi 2011) as well as evidence of post-bleaching resilience (pers. obs.). The response of Ofu corals to such drastic change in temperature is relevant to corals elsewhere, e.g., populations from the Arabian/Persian gulf can survive even more extreme temperature regime (Riegl *et al.* 2011). Furthermore, it is becoming more evident that some corals can gain thermotolerance or become dominant with increasing exposure to bleaching temperatures (Guest *et al.* 2012, Pratchett *et al.* 2013, Kemp *et al.* 2014), which provides opportunities to study the mechanisms of acclimatization and/or adaptation to climate change. We exposed 20 *Acropora hyacinthus* colonies to controlled bleaching conditions that mimic natural daily cycles of high heat stress during extreme temperature events. We compared changes in gene expression at the onset of heat stress (1 hour post heat exposure corresponding to the 5 hr time point) and 15 hours later at the onset of bleaching (*i.e.*, at the 20 hr time point) after colonies had been returned to normal temperatures. The results show a surprisingly wide transcriptome response before bleaching begins, and rapid return to normal expression levels for many genes. The return to normal gene expression levels varies from gene to gene and colony to colony, and may signal the

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resilience of the transcriptome to environmental stress. This variation in return to normal expression may underlie some of the variation in stress response seen in previous studies and adds to other components of the stress response in corals and other species.

Materials and Methods

Sample preparation and experimental design

Twelve small nubbins (~3 cm^3) of *Acropora hyacinthus* (cryptic species E in (Ladner & Palumbi 2012) were cut from each of 20 colonies from the south lagoon of Ofu Island, American Samoa (14°11'S, 169°36'W), and glued underwater onto nylon bolts using two-part underwater epoxy stick (Loctite® 82093). The nubbins were evenly distributed among and screwed onto 12 egg crate platforms, which were then secured to the substratum randomly across the area of sampling for a period of acclimatization and growth.

After 17 months, eight nubbins from each of 18 colonies and four nubbins from each of 2 colonies were collected and used in a controlled heat stress experiment as in the following design: (18 colonies in duplicate + 2 colonies in singleton) x 2 conditions x 2 time points (SI Materials and Methods). Control subjects were kept at a constant 29°C, while the heat stress treatment mimicked a natural bleaching temperature regime observed in the lagoon: heated corals were ramped from 29°C to 35°C over 3h, held at 35°C for 1h, and then freely returned to 29°C in ~2h. To coincide with the natural circadian rhythm of corals, the peak in heat stress was timed to occur in the early afternoon, when corals would normally experience temperature highs. Samples were collected after 5 hr and 20 hr from the start of the experiment. The 5 hr time point occurred 1h after the peak in heat stress and was

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chosen to capture the changes taking place at the onset of heat stress. Based on pilot experiments, the 20 hr time point was chosen to investigate the changes taking place at the onset of visually detected bleaching. At that time heated colonies were graded on a five-point scale of bleaching (1-normal=same as control, 2-slight= a little pale, 3-moderate=half as colored as control, 4-severe=very pale but still a tinge, 5-total=bone white) in relation to the control nubbins. One half of each nubbin was preserved in RNA stabilizing buffer and stored at -80°C until molecular analysis, and the other half was preserved in 100% ethanol for chlorophyll a pigment extraction.

RNA isolation, sequencing and raw data processing

Total RNA was extracted from each sample following the modified TRIzol® Reagent (Life Technologies) protocol in Barshis *et al.* (2013; SI Materials and Methods). A total of 152 libraries were constructed using 1 µg of total RNA following the TruSeq RNA Sample Prep v2 (LS) protocol (Illumina). cDNA was synthesized using the SuperScript® III reverse transcriptase (Life Technologies). Purification of cDNAs throughout the TruSeq protocol was performed using the Agencourt AMPure XP system (Beckman Coulter). Sequencing was performed on the Illumina HiSeq 2000 sequencer at the Huntsman Cancer Institute of the University of Utah. All libraries were sequenced with 50 bp single-end sequencing length. TruSeq raw sequences were processed following the pipeline methodology described in DeWit *et al.* (2012). Reads were mapped to the *de novo* assembly constructed by Barshis *et al.* (2013) to produce the raw gene expression count data for further analysis.

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Gene expression and statistical analysis

Gene expression analysis was performed with the DESeq package (Anders & Huber 2010) in R (R Foundation for Statistical Computing 2013). After normalization for variation in library size, the data variance (i.e., dispersion) was estimated and the significance of heat stress at two different time points evaluated using log ratio tests of nested negative binomial generalized linear models (GLMs), taking into account fixed expression differences between individual coral colonies regardless of heat stress (SI Materials and Methods). P-values were adjusted using the Benjamini-Hochberg (BH) method controlling the false discovery rate at a 0.01 level.

Differences in expression levels were calculated in four comparisons: 1) control versus heated at 5 hr, 2) control vs. heated at 20 hr, 3) control at 5 hr vs. control at 20 hr, and 4) heated at 5 hr vs. heated at 20 hr, corresponding to the onset of heat stress, the onset of bleaching, the effect of time/acclimation, and the change from heat stress to bleaching, respectively. We applied two filters to exclude contiguous sequences (contigs) for which we had little power to detect expression differences from multiple test correction: 1) low contig-average in normalized expression across samples with a cutoff of 3 reads (i.e., the sample-average median for normalized expression level across all contigs) and 2) high inter-colony variability contigs (within-group mean <1 standard deviation). PCAs were computed in R using the entire normalized expression data in the pcaGoPromoter package (Hansen *et al.* 2012).

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Functional analysis

The 33,496 *A. hyacinthus* contigs were used to retrieve homologous gene identification codes (IDs) from UniProt (The UniProt Consortium 2014), KEGG (Kyoto Encyclopedia of Genes and Genomes, (Kanehisa *et al.* 2004), and GO (The Gene Ontology Consortium 2000) databases using the Basic Local Alignment Search Tool (NCBI). The homologous gene IDs for the filtered Differentially Expressed Contigs (DECs) subsets from the gene expression analyses were used in comparative and functional enrichment analyses using three methods: UniProt IDs with the Database for Annotation, Visualization and Integrated Discovery (DAVID 6.7; (Huang *et al.* 2008), KEGG Orthology (KO) codes with KEGG mapper (Kanehisa *et al.* 2014), and GO terms with keywords to compute hypergeometric probabilities (SI Materials and Methods).

Results

Our samples from 20 genetically distinct colonies exposed to control and heated conditions at two time points (5 hr and 20 hr) constitute a total of 152 sequenced transcriptomes, which to our knowledge, is one of the largest and most replicated genomics datasets to study heat stress in a marine organism. The reference transcriptome of *Acropora hyacinthus* used in this study was previously assembled *de novo* (Barshis *et al.* 2013) and consisted of 33,496 unique contigs including 24,980 (75 %) matching Nucleotide collection (nr/nt) entries (e-value < 10^{-4}, The National Center for Biotechnology Information). Based on the 27,000 predicted transcripts from the genome of the congener *Acropora digitifera* (Shinzato *et al.* 2012), the data generated in the present study likely represents a large fraction of the transcriptome of *Acropora hyacinthus*.

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On average over one million reads were analyzed per sample, with 50% of the contigs represented by ≥ 3 reads on average across all samples. Further comparison to curated functional databases led to the additional annotation of the transcriptome with 24,394 UniProt, 10,168 KEGG, 16,117 GO-Cellular Component, 18,297 GO-Biological Process, and 17,448 GO-Molecular Function homologous gene IDs (e-value < 10^{-4}). Overall, the lists of DECs detected at both time points were significantly enriched for 71 functional annotation clusters (using EASE-score < 0.05 and high classification stringency in DAVID 6.7; Huang *et al.* 2008) as well as 36 molecular pathways supported both by the mapping to KEGG Orthology and the representation of pathway keywords across GO terminologies (hypergeometric probability < 0.01).

Overall transcriptome changes

At the onset of heat stress, 1 hour post heat exposure (5 hr time point), a total of 8,913 DECs (27% of all contigs, p-adj < 0.01; Fig. 1) were found between heated and control samples (negative binomial GLM with a paired design by genotypes). Among these DECs, 4,283 and 4,630 were up- and down-regulated, respectively (-19.2 > fold change (FC) < 36.9, FC medians at -1.9 and 1.8). Fifteen hours later, when bleaching became obvious (Figure 3B), 3,846 DECs (12%) were detected between heated and control samples (1,975 up- and 1,871 down-regulated, -18.6 > FC < 19.1, FC medians at -1.6 and 1.6), representing a drop of 15% in overall transcriptome activity.

Comparing expression in heated samples, 8,372 DECs (25%) differed with time after heat stress (4,247 up- and 4,125 down-regulated, -45.2 > FC < 19.0, FC medians at -1.8 and 1.9). Some of these were genes that returned to normal expression after changing at 5 hr.

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Others altered expression only after the 5hr time point. A similar analysis across time points using control samples revealed 3,358 DECs (10%) that changed between 5 and 20 hr in the control tanks. These differences were influenced by circadian rhythm and acclimation to tank conditions (1,517 up- and 1,841 down-regulated, -10.5 > FC < 14.4, FC medians at -1.5 and 1.4).

Principal component analyses (PCAs) corroborated the major gene expression patterns. The PCA comparing controls between time points reveals some influence of time and acclimation, but the overall difference between time points is small when considering the large overlap in the 95% confidence intervals of each treatment (Fig. 2a). Conversely, there was a clear separation of the 95% confidence intervals between the control and heated samples at 5 hr (Fig. 2c) and between the heated samples at each time point (Fig. 2b). In both analyses, the treatment groups distinctively separated along principal component 2, which explains 14% of the variance for both analyses (Fig. 2c and 2b). By the time bleaching was observed (20 hr), a portion of the heat stress-responsive transcripts had already returned to control activity level as shown by (Fig. 2d): 1) the overlap in the 95% confidence intervals between the heated and control samples, and 2) only 9% of the variance explained by principal component 2.

A detailed analysis of the gene expression profiles among all differentially expressed contigs detected in our experiments revealed 4 main subsets of contigs co-expressed over time. We refer to these expression profiles as Transient, Slow return, Late, and Lingering

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responses for clarity. Figure 3A shows the absolute mean fold change representative of these main gene expression profiles and the number of genes exemplifying them.

Transient response – genes that were different at 5 hr but not at 20 hr: Among the differentially expressed contigs detected at 5 hr, 5,042 DECs (Fig. 1) showed different expression levels at 5 hr in heated versus control corals, but subsequently returned to control level by the time bleaching was observed at the 20 hr time point (Fig. 1), except for 8 contigs. These changes in expression occurred in 57% of the contigs that showed a heat stress response at 5 hr and thus represent the transient component in pre-bleaching gene expression activity.

This transient response was divided into 2,311 up- and 2,731 down-regulated contigs at 5 hr. The genes that were up-regulated at 5 hr but returned to control level at 20 hr were significantly enriched for: 1) the GO-BP including apoptosis, protein transport/localization/processing, regulation of phosphorylation, and lymphocyte activation, 2) the GO-MF including GTPase regulator and threonine-type endopeptidase activity, GTP and HSP binding, and 3) the GO-CC including cytoplasmic vesicle, membrane fraction, and proteasome (Table 1). Among the down-regulated genes at 5 hr that returned to control level at 20 hr, two main cellular activities were identified from the functional annotation clusters (DAVID): 1) ribosomal RNA processing and protein import into nucleus, and 2) messenger RNA processing/transport (Table 1).

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Slow return response – genes that were different at 5 hr and were returning toward normal at 20 hr: Another smaller set of genes (1,215 DECs) also showed expression changes in 5 hr but not 20 hr heated samples. However, they did not show significant differences between 5 hr and 20 hr in heated samples. For 178 of these contigs this trend resulted from similar expression values seen in 5 hr and 20 hr heated samples, and a change in expression in 20 hr controls instead. The remaining 1,037 contigs showed a tendency of heated samples to be more like control levels at 20 hr, which resulted in no longer being significantly different from controls at 20 hr, but not enough change had occurred to be statistically different from 5 hr heated samples. These genes can be considered to be at an intermediate stage of the return to control level exemplified by the genes of the transient response.

These 1,215 DECs were divided into 611 up- and 604 down-regulated contigs. Among the genes that were up-regulated at 5 hr, the GO-BP negative regulation of cell migration and the regulation of phospholipase/lyase activity were significantly represented. The down-regulated contigs were enriched for the GO-BP antibiotic transport and mRNA splicing/processing (Table 1).

Late response – genes that were different at 20 hr but not at 5 hr: At the 20 hr time point, 417 differentially expressed contigs that were at control level at 5 hr, showed different expression levels in heated versus control samples (Fig. 1), representing a late response relative to the heat stress. Another larger set of 773 contigs showed a similar trend, however like the Slow return genes, these genes (773 DECs) did not show different expression levels between 5 hr and 20 hr in heated samples. For 344 of the 773 DECs, the

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difference in expression levels at 20 hr resulted from changes in the control samples over time. The remaining 429 DECs were excluded from the 5 hr comparison by the statistical filters.

Among the genes that were unresponsive to heat stress at 5 hr but showed a significant change in expression at 20 hr (417 and 773 DECs), 485 up-regulated contigs were significantly enriched for proteinaceous extracellular matrix (ECM). The 705 down-regulated contigs at 20 hr were enriched for regulation of actin, and tissue morphogenesis (Table 1).

Lingering response – genes that were detected at both 5h and 20h: There were 2,656 contigs (2,040 and 616 DECs) with different expression levels in heated versus controls at both 5 hr and 20 hr time points (Fig.1). The 616 contigs that were differentially expressed in heated versus control samples at both time points remained the same in heated samples from 5 to 20 hr, representing a lingering response to heat stress until the onset of bleaching. The other 2,040 contigs showed significant changes in expression levels at 5 and 20 hr but also differed in heated samples between 5 and 20 hr. Similarly to the 1,215 and 5,042 DECs above, most of the genes represented in the 2,040 DECs showed an attenuation towards control levels over time. In other words, expression levels in heated samples were closer to control levels at 20 hr than at 5 hr.

Of the combined 2,656 DECs, 1,235 and 1,040 were up- and down-regulated at both time points, respectively. The remaining 381 DECs showed opposite fold changes at the two time points: 126 DECs up-regulated at 5h were down-regulated at 20 hr and 255 DECs exhibited the reverse pattern. The consistently up-regulated DECs (1,235) represented the

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functional annotation clusters for regulation of immune system, apoptosis, and transcription. Other functional clusters included in the up-regulated genes were G protein coupled receptor protein signaling, myosin filament assembly, protein phosphorylation, and vesicle targeting (Table 1). The consistently down-regulated genes (1,040) were significantly enriched for: 1) the collagens, ECM, and lysosome GO-CCs, 2) the cation/ion transport, antioxidant activity, ubiquinone and steroid metabolic processes, nucleosome assembly, and negative regulation of transcription GO-BPs, and 3) the ECM structural constituent, carbonate dehydratase activity, sodium and selenium ion binding, and serine hydrolase activity GO-MFs (Table 1).

Molecular pathways involved in heat stress and bleaching responses

Using the KEGG maps as backgrounds, 15 molecular pathways were significantly represented at 5 hr (control vs. heated) and 24 pathways were identified at 20 hr (control vs. heated; Table S1). Four of these pathways were common to both time points. Mapping against the KEGG Orthology system provides an informative and immediate visualization of the representation of genes potentially involved in the same molecular pathway(s) over a background of all genes present in the transcriptome. Additionally, a complementary analysis using GO terms and keywords produced results based on much more exhaustive functional annotation information. The GO analysis identified 24 and 22 enriched molecular pathways among DECs at 5 hr and 20 hr, respectively (Table S1). Sixteen pathways were in common to both time points. Combining the results from the KEGG and GO analyses allowed identifying the five and eight most robust pathways for the 5 hr and 20 hr comparisons, respectively (Table S1). At 5 hr, the pathways influenced by heat stress

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were protein processing in the endoplasmic reticulum, cell cycle, metabolism and pyruvate metabolism. On the other hand, RNA transport, spliceosome, DNA replication, extracellular matrix receptor interaction, osteoclast differentiation, glutathione metabolism, and mismatch repair were implicated during the onset of bleaching at 20 hr. Only one pathway was found to be in common to both analyses and both time points: ribosome biogenesis in eukaryotes.

Transcriptome resilience and bleaching

The return of gene expression values to control levels after acute stress has been called transcriptome resilience, and is a key feature of the gene expression patterns we observe. For each colony we correlated gene expression levels at 20 hr against bleaching score (1=none, 5=total, see methods) for all contigs. Overall, only about 5.3% of contigs had individually significant correlation coefficients (RSQ (18)> 0.444, $p < 0.05$), but contigs in the Lingering response category showed much higher levels (i.e., 18.4% for Lingering compared to 5.7% for Transient, 8.7% for Late, and 4.8% for Slow return response categories, 2x4 contingency table, $p < 0.001$, Figure 4).

For example, expression at 20 hr of the Tumor Necrosis Factor Receptor-associated Factor 3 gene (TRAF3) shows a significant positive correlation with bleaching, indicating that low expression correlates with low bleaching and high expression to high bleaching at 20 hr (Figure 4 inset). For this gene, the low expression at 20 hr in more resistant colonies resulted from a faster decline from high levels at 5 hr. In this case a more rapid return to control levels (i.e., transcriptome resilience) is associated with thermal tolerance. This pattern appears many times among Lingering genes: out of the 100 contigs with the best

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correlation between 20 hr expression levels and bleaching, 91 showed more rapid return to control levels in more resistant colonies. For these 100 genes, expression in colonies showing none or slight bleaching returned to 98% of control values whereas for colonies showing severe or complete bleaching, expression remained 2.5 fold higher than controls. These data show that although Lingering genes remain differentially expressed on average across all colonies and throughout the experiment, in some colonies transcriptome resilience for those same genes tends to be most associated with bleaching resistance.

Discussion

Our results show that corals mount a large and rapid transcriptional response to acute thermal stress that leads to bleaching. Over ¼ of the transcriptome of *A. hyacinthus* was quickly influenced by the bleaching temperatures used here, but 57% of those genes showed clear transcriptome resilience (i.e., the Transient response) over 15 hours. In addition, among the genes still regulated after 15h, some exhibit a fast return to normal expression in colonies showing bleaching resistance. Our data also show that heat stress broadly affects protein processing, cell cycle and metabolism at first, while the later bleaching response correlated with activity in RNA transport, extracellular matrix, calcification, and DNA replication and repair.

Immediate effects of heat stress on the coral transcriptome.

Previous heat and bleaching stress experiments on corals and other cnidarians have generally found fewer genes being regulated and lesser overlap between successive time points in response to thermal challenges than that documented here (Table S2). The

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primary difference between our experimental design and previous studies is the use of an immediate post-stress time point followed by a time point during bleaching within 24 hours of the acute heat exposure. Previous studies exposed adult corals for at least one day before collecting samples for gene expression. Here, we took advantage of the natural system found in the backreef lagoon of Ofu Island, where corals can experience conditions that can trigger bleaching over a single tidal cycle during summer. Those acute temperature anomalies capable of triggering bleaching over a shorter time period than what was observed in the past are likely to become more common and to occur in many other places across the tropics as climate change progresses (Cai *et al.* 2014).

In this study, we documented a much higher response to heat stress at earlier sampling time points. The consistent response to heat stress across 20 coral colonies consisted of 27% of the transcriptome being influenced. Other transcriptome-wide studies on marine organisms including copepods, mussels, oysters, and fish have tended to find less gene expression activity (Lang *et al.* 2009; Lewis *et al.* 2010; Lockwood *et al.* 2010; Schoville *et al.* 2012; Liu *et al.* 2013). For example, the molecular heat shock response of copepods one hour after exposure to 15˚C above ambient temperature (for 1h) only involved 0.88-1.49% of the transcriptome (Schoville *et al.* 2012). Similarly, mussels exposed to ramping temperature (1˚C/1h) and held at 11, 15, and 19˚C above ambient for 1 hour, showed change in only 3.4% of the genes investigated (Lockwood *et al.* 2010). This difference between our study and others is partially the result of our large dataset and differential gene expression detection methods. However, even after applying a cutoff of ± 2 fold change on our list of DECs (FDR corrected p-value <0.01), which represents an even

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more stringent detection level than in the studies aforementioned, 11% of the coral trancriptome is still engaged at this level.

The substantial gene expression changes detected immediately after heat stress suggests that corals may suffer a large energetic cost from their heat response due to declines in genes involved in metabolism and cell cycle. In addition, the main source of energy for the host – their symbiotic algae – decreases as a result of bleaching. It is therefore conceivable that the fate of the colony depends on a balance between the cost of the early molecular response and the source of energy still available for recovery after bleaching. However, another aspect of the stress response demonstrated here in corals is likely to play a part in the colony's fate: its capacity to quickly return to homeostasis or transcriptome resilience.

Transcriptome resilience post heat stress.

The experimental design used here revealed the capacity of corals to respond to improving environmental conditions quickly, a phenomenon described as transcriptome resilience by Franssen *et al.* (2011). Over the 15 hour time interval separating the early heat stress response from the onset of bleaching, 57% of the genes detected at the first time point returned to control expression levels. This resilience may have reduced the number of genes seen to be involved in the heat stress response in some previous studies (Table S2). Two prior studies used earlier time points, yet detected slight gene expression activity differences between 12, 24.5 and 48 hours (Voolstra *et al.* 2009; Portune *et al.* 2010).

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Here, the transient genes group showed evidence for protein degradation, transport, catabolic process, phosphorylation and chaperone activity being positively regulated as a result of heat stress and recovering quickly as conditions improved. This provides evidence for the importance of maintenance of protein conformation and activity as well as the recycling of damage molecules shortly to minimize energetic cost. On the other hand, the down-regulation of ribosome and mRNA processing suggest a trade-off limiting post-transcriptional activity. This may point to essential cellular mechanisms taking place at the forefront of the cellular heat stress response but which also have to be under tight regulatory control in order to allow for recovery and survival of the organism once the conditions improved.

Transcriptome resilience may therefore be important to the resistance of corals to climate change. The capacity to rapidly return to homeostasis (normal cellular function) following stress is crucial to survival (de Nadal *et al.* 2011). A study looking at congeners of a temperate seagrass species with different thermal backgrounds showed that they have similar molecular heat stress responses, but differ in the speed of their recovery post heat treatment (Franssen *et al.* 2011). Such transcriptome resilience was proposed to be analogous to ecological resilience (Franssen *et al.* 2011). In our study, transcriptome resilience linked to bleaching performance was discovered among genes that overall respond early to heat stress and remain differentially expressed at the onset of bleaching. For these Lingering response genes, a return to control levels at the onset of bleaching correlates with resistance to bleaching. In other words, colonies showing no or slight bleaching response had a subset of the Lingering response genes returned to control levels, whereas colonies severely to totally bleached were still expressing these genes at 2.5 fold

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higher levels than controls. Whether these genes mediate bleaching when expressed at high or low levels for longer, or are symptoms of physiological stress levels is not yet known.

Gene expression changes occurring with the onset of bleaching.

At the 20 hr time point, 31% of the genes that changed in expression had not changed by the 5hr time point (the Late genes). These late genes include extra cellular matrix proteins, and genes involved in regulation of actin and morphogenesis of the epithelium, suggesting that structural events of cells and tissues, such as exocytosis and cell detachment (Weis 2008), are important at this stage.

On the other hand, 25% of the genes that responded early were still differentially expressed when bleaching became apparent (The Lingering genes). For example, the positive regulation of the G-protein coupled receptor protein signaling pathway, kinase/kinase inhibitor activity, T-helper type immune response, and apoptosis strongly suggest that the breakdown of symbiosis begins with the sensing of extracellular molecules and stress signal transduction leading to apoptosis. In light of recent findings on the role of the TNF pathway in apoptosis of corals (Quistad *et al.* 2014), we hypothesize that stress molecule receptors such as TNFR are involved in signaling. Many other immune related genes such as Toll-like receptor, C-type lectin, and FC receptor homologs are in this Lingering category, supporting the role of an immune response in the initiation of bleaching.

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Gene expression changes occurring with time in controls.

Natural light phases synchronize behavioral and physiological cycles over varying time periods in both plants and animals (Beckwith & Yanovsky 2014). Corals like plants are sessile organisms depending on sunlight for energy and not surprisingly exhibit diel cycles of physiology and gene expression (Ruiz-Jones and Palumbi, submitted). In this study, we timed the peak in heat stress to occur in the early afternoon (i.e., 14:00-15:00), an ecologically relevant time for corals to experience elevated temperature in the native field environment. The same period also represents a significant stage in coral circadian rhythm, as demonstrated by the common peak in expression of a suite of genes independent of daylight or temperature (Levy *et al.* 2011). Among those genes evident in the control comparison that fluctuated with time only, cryptochromes were some of the most regulated genes, corroborating the suggestion that this group follows a circadian rhythm (Levy *et al.* 2007). In our experiment, cryptochrome-2 is notably down-regulated over time in controls but not in heated samples. Such an expression profile implies that heat stress inhibits normal circadian gene regulation. This result highlights the importance of accounting for the natural temporal regulation of genes in the interpretation of coral bleaching gene expression.

Evidence for TNF mediated apoptosis during coral bleaching.

The importance of the coral TNF pathway in the bleaching response was emphasized by the involvement of many genes and several pathways with ties to the immune and apoptosis response. In addition to its obvious function in disease response (Pinzon *et al.* 2014), coral immunity is attracting increasing attention for its possible role in

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adaptation to climate change (Hayes *et al.* 2010; Iguchi *et al.* 2011) through its likely involvement in symbiosis (Kvennefors *et al.* 2010). Recent genome-wide studies of bacterial cell wall exposure and the NOD-like receptor genes in corals have revealed an extraordinary complexity in immune-related genes compared to human and ecdysozoans (Hamada *et al.* 2013; Weiss *et al.* 2013), implicating the importance of coral immunity in the evolution of symbiosis with both diverse bacterial communities (Sharp & Ritchie 2012) and *Symbiodinium* types (Kvennefors *et al.* 2010).

We hypothesize that dysfunctional *Symbiodinium* cells caused by heat stress might cease to be accepted; as a result, the now foreign body triggers the TNF signaling pathway to lead to apoptosis, an immune response, or cell survival (Traylor-Knowles & Palumbi 2014). The significance of coral TNF family genes was unmistakable in our results through several enriched biological processes and molecular functions, including apoptosis, lymphocyte activation, and immune system development. In a recent report, Quistad *et al.* (2014) showed that apoptosis and bleaching in corals could be triggered via the activation of the TNF pathway using human TNF-alpha and that corals possess more TNF family members than any organisms sequenced thus far. In a subset of the corals from this experiment, we recently identified members of the TNF superfamily as components of thermo-tolerance (Barshis *et al.* 2013) acquired through acclimatization (Palumbi *et al.* 2014).

Despite these results, it remains to be shown however that TNF mediated cellular events play a role in temperature driven coral bleaching. We identified two distinct groups of TNFR genes in the present dataset showing divergent regulation, and a potential connection to different downstream gene pathways (Traylor-Knowles *et al.* submitted).

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The intra-cellular, TNFR-linked TRAF genes also play a significant role: in particular expression of TRAF3 at 20 hr is linked to colony bleaching performance (Fig. 4 inset). Altogether these results emphasize the central involvement of the TNF family and immunity in the coral response to climate-driven temperature anomalies. We encourage future studies to investigate the role of the TNF signaling pathway during bleaching and in the fate of corals through climate change.

Multiple aspects of the response of corals to climate stress.

Future climate change is likely to increase the intensity and the frequency of ocean warming events and storms, the rate of ocean acidification, and sea level rise. Exposure to new environmental extremes constitutes a strong selective pressure, and populations that harbor adaptive polymorphisms in their response to extreme conditions may subsequently evolve. Here we studied the transcriptome-wide response to a simulated summer peak in temperature in a population of corals living across a marked temperature mosaic, in order to understand the effects of such anomalies on the molecular machinery of stress responses.

Previous work in this region has identified several mechanisms that are associated with coral bleaching. Corals living at warmer conditions constitutively up-regulate a set of genes that are otherwise induced only during heat stress. By frontloading these gene products, these colonies may be better prepared to circumvent stress, or respond to it (Barshis *et al.* 2013). In addition, these colonies also show a combination of acclimatization and genetic adaptation. Reciprocal transplants between warmer and cooler environments show that corals can acquire heat resistance, and that acclimatization is associated with

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location-specific gene expression differences (Palumbi *et al.* 2014). However, acclimatization accounts for roughly 50% of the enhanced resistance to warm water with the balance probably due to fixed effects such as evolutionary adaptation or epigenetics. Bay and Palumbi (2014) showed that 114 loci were strong candidates for genes under selection for heat resistance in these corals. These results suggested that heat resistance was a polygenic trait contributed to by many loci with diverse cellular functions.

To this palette of heat response mechanisms – frontloading, acclimatization, and adaptation – the current study adds a fourth, transcriptome resilience. By studying the short-term dynamics of gene expression response across many colonies for the first time, we found that many more genes than previously thought respond to acute heat stress. The pace of return of some of these genes to normal levels within individual colonies is strongly associated with bleaching resistance among colonies showing a range of bleaching responses. We do not know if this fast return to homeostasis of these genes in resistant colonies signals low potential for bleaching. Alternatively, prolonged unusual gene expression level may cause bleaching.

Nevertheless, our data suggest two kinds of important areas for future research on stress resistance: short term expression dynamics and inter-individual variation in gene expression. In the current study, both have been important for discovery of transcriptome resilience as a stress-mitigating factor. The exposure of populations to extreme conditions can spark diverse physiological responses and gene expression profiles in different individuals. These different responses are the raw material for natural selection – either for increased acclimatory ability, increased stress resistance or both. As climate change exerts

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a higher level of environmental extremes on populations, the basis for their ability to evolve in the future is an important area of research.

Conclusion

Our study reveals the surprising capacity of corals to mount a large and rapid molecular stress response to heat stress many hours prior to bleaching. The later response shows important additional activity of the animal host transcriptome coinciding with the observation of bleaching. Moreover, between the heat and bleaching stress responses, a large portion of the genes returned to control level by the time bleaching started, suggesting transcriptome resilience. Importantly, the resilience of a subset of genes correlates with bleaching resistance. Among the many molecular pathways detected, the TNF signaling pathway possibly linked to apoptosis stands out as the most likely path leading to the breakdown of the symbiosis between the host and the algae, and may also play a role in the resistance to bleaching. This study greatly improves the current state of knowledge on the gene expression activity in a key organism under heat and bleaching stress. Further characterization of the immunity/apoptosis genes combined with population genetics surveys and detailed studies of naturally occurring variation in thermo-tolerance will shed light on the capacity of corals to acclimatize and/or adapt to future climate change.

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References

Anders S, Huber W (2010) Differential expression analysis for sequence count data. *Genome Biology*, **11**, R106.

Barshis DJ, Ladner JT, Oliver TA, Palumbi SR (2014) Lineage-specific transcriptional profiles of Symbiodinium spp. unaltered by heat stress in a coral host. *Molecular Biology and Evolution*, **31**, 1343–1352.

Barshis DJ, Ladner JT, Oliver TA *et al.* (2013) Genomic basis for coral resilience to climate change. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 1387–1392.

Bay RA, Palumbi SR (2014) Multilocus Adaptation Associated with Heat Resistance in Reef-Building Corals. *Current Biology*, 1–5.

Beckwith EJ, Yanovsky MJ (2014) Circadian regulation of gene expression: at the crossroads of transcriptional and post-transcriptional regulatory networks. *Current opinion in genetics & development*, **27**, 35–42.

Bellantuono AJ, Granados-Cifuentes C, Miller DJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2012) Coral Thermal Tolerance: Tuning Gene Expression to Resist Thermal Stress (SCA Ferse, Ed,). *PLoS ONE*, **7**, e50685.

Cai W, Borlace S, Lengaigne M *et al.* (2014) Increasing frequency of extreme El Niño events due to greenhouse warming. *Nature Climate Change*, **5**, 1–6.

Craig P, Birkeland C, Belliveau S (2001) High temperatures tolerated by a diverse assemblage of shallow-water corals in American Samoa. *Coral Reefs*, **20**, 185–189.

de Nadal E, Ammerer G, Posas F (2011) Controlling gene expression in response to stress. *Nature Publishing Group*, **12**, 833–845.

De Wit P, Pespeni MH, Ladner JT *et al.* (2012) The simple fool's guide to population genomics via RNA-Seq: an introduction to high-throughput sequencing data analysis. *Molecular Ecology Resources*, **12**, 1058–1067.

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

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

Dudgeon SR, Aronson RB, Bruno JF, Precht WF (2010) Phase shifts and stable states on coral reefs. *Marine Ecology Progress Series*, **413**, 201–216.

Franssen SU, Gu J, Bergmann N *et al.* (2011) Transcriptomic resilience to global warming in

This article is protected by copyright. All rights reserved.

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the seagrass Zostera marina, a marine foundation species. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 19276–19281.
Guest JR, Baird AH, Maynard JA *et al.* (2012) Contrasting Patterns of Coral Bleaching Susceptibility in 2010 Suggest an Adaptive Response to Thermal Stress (MV Matz, Ed,). *PLoS ONE*, **7**, e33353.
Hamada M, Shoguchi E, Shinzato C *et al.* (2013) The complex NOD-like receptor repertoire of the coral Acropora digitifera includes novel domain combinations. *Molecular Biology and Evolution*, **30**, 167–176.
Hansen M, Gerds TA, Nielsen OH *et al.* (2012) pcaGoPromoter - An R Package for Biological and Regulatory Interpretation of Principal Components in Genome-Wide Gene Expression Data (AB Khodursky, Ed,). *PLoS ONE*, **7**, e32394.
Hayes ML, Eytan RI, Hellberg ME (2010) High amino acid diversity and positive selection at a putative coral immunity gene (tachylectin-2). *BMC Evolutionary Biology*, **10**, 150.
Hoffmann AA, Sgrò CM (2011) Climate change and evolutionary adaptation. *Nature*, **470**, 479–485.
Huang DW, Sherman BT, Lempicki RA (2008) Systematic and Integrative Analysis of Large Gene Lists Using DAVID Bioinformatics Resources. *Nature Protocols*, **4**, 44–57.
Iguchi A, Shinzato C, Forêt S, Miller DJ (2011) Identification of Fast-Evolving Genes in the Scleractinian Coral Acropora Using Comparative EST Analysis (JH Badger, Ed,). *PLoS ONE*, **6**, e20140.
Jokiel PL, Coles SL (1990) Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. *Coral Reefs*.
Kanehisa M, Goto S, Kawashima S, Okuno Y, Hattori M (2004) The KEGG resource for deciphering the genome. *Nucleic Acids Research*, **32**, D277–80.
Kanehisa M, Goto S, Sato Y *et al.* (2014) Data, information, knowledge and principle: back to metabolism in KEGG. *Nucleic Acids Research*, **42**, D199–205.
Kemp DW, Hernandez-Pech X, Iglesias-Prieto R, Fitt WK, Schmidt GW (2014) Community dynamics and physiology of Symbiodiniumspp. before, during, and after a coral bleaching event. *Limnology and Oceanography*, **59**, 788–797.
Kenkel CD, Goodbody-Gringley G, Caillaud D *et al.* (2013) Evidence for a host role in thermotolerance divergence between populations of the mustard hill coral (Porites astreoides) from different reef environments. *Molecular Ecology*, **22**, 4335–4348.
Kvennefors ECE, Leggat W, Kerr CC *et al.* (2010) Analysis of evolutionarily conserved innate immune components in coral links immunity and symbiosis. *Developmental & Comparative Immunology*, 1–11.
Ladner JT, Palumbi SR (2012) Extensive sympatry, cryptic diversity and introgression throughout the geographic distribution of two coral species complexes. *Molecular Ecology*, **21**, 2224–2238.
Lang RP, Bayne CJ, Camara MD *et al.* (2009) Transcriptome Profiling of Selectively Bred Pacific Oyster Crassostrea gigas Families that Differ in Tolerance of Heat Shock. *Marine Biotechnology*, **11**, 650–668.
Levy O, Appelbaum L, Leggat W *et al.* (2007) Light-Responsive Cryptochromes from a Simple Multicellular Animal, the Coral Acropora millepora. *Science*, **318**, 467–470.
Levy O, Kaniewska P, Alon S *et al.* (2011) Complex diel cycles of gene expression in coral-algal symbiosis. *Science*, **331**, 175–175.
Lewis JM, Hori TS, Rise ML, Walsh PJ, Currie S (2010) Transcriptome responses to heat

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stress in the nucleated red blood cells of the rainbow trout (Oncorhynchus mykiss). *Physiological Genomics*, **42**, 361–373.

Liu S, Wang X, Sun F *et al.* (2013) RNA-Seq reveals expression signatures of genes involved in oxygen transport, protein synthesis, folding, and degradation in response to heat stress in catfish. *Physiological Genomics*, **45**, 462–476.

Lockwood BL, Sanders JG, Somero GN (2010) Transcriptomic responses to heat stress in invasive and native blue mussels (genus Mytilus): molecular correlates of invasive success. *Journal of Experimental Biology*, **213**, 3548–3558.

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. *Molecular Ecology*, **20**, 3599–3616.

Moya A, Huisman L, Ball EE *et al.* (2012) Whole Transcriptome Analysis of the Coral Acropora millepora Reveals Complex Responses to CO2-driven Acidification during the Initiation of Calcification. *Molecular Ecology*, **21**, 2440–2454.

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

Pinzon C JH, Beach-Letendre J, Weil E, Mydlarz LD (2014) Relationship between Phylogeny and Immunity Suggests Older Caribbean Coral Lineages Are More Resistant to Disease (CR Voolstra, Ed,). *PLoS ONE*, **9**, e104787.

Polato NR, Voolstra CR, Schnetzer J *et al.* (2010) Location-Specific Responses to Thermal Stress in Larvae of the Reef-Building Coral Montastraea faveolata (DA Carter, Ed,). *PLoS ONE*, **5**, e11221.

Portune KJ, Voolstra CR, Medina M, Szmant AM (2010) Development and heat stress-induced transcriptomic changes during embryogenesis of the scleractinian coral Acropora palmata. *Marine Genomics*, **3**, 51–62.

Pratchett MS, McCowan D, Maynard JA, Heron SF (2013) Changes in Bleaching Susceptibility among Corals Subject to Ocean Warming and Recurrent Bleaching in Moorea, French Polynesia (RMH Merks, Ed,). *PLoS ONE*, **8**, e70443.

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

Richier S, Rodriguez-Lanetty M, Schnitzler CE, Weis VM (2008) Response of the symbiotic cnidarian Anthopleura elegantissima transcriptome to temperature and UV increase. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics*, **3**, 283–289.

Riegl BM, Purkis SJ, Al-Cibahy AS, Abdel-Moati MA, Hoegh-Guldberg O (2011) Present Limits to Heat-Adaptability in Corals and Population-Level Responses to Climate Extremes (CR Voolstra, Ed,). *PLoS ONE*, **6**, e24802.

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

Runcie DE, Garfield DA, Babbitt CC *et al.* (2012) Genetics of gene expression responses to temperature stress in a sea urchin gene network. *Molecular Ecology*, **21**, 4547–4562.

Schoville SD, Barreto FS, Moy GW, Wolff A, Burton RS (2012) Investigating the molecular basis of local adaptation to thermal stress: population differences in gene expression across the transcriptome of the copepod Tigriopus californicus. *BMC Evolutionary Biology*, **12**, 170.

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Seneviratne SI, Donat MG, Mueller B, Alexander LV (2014) No pause in the increase of hot temperature extremes. *Nature Climate Change*, **4**, 1–4.
Sharp KH, Ritchie KB (2012) Multi-partner interactions in corals in the face of climate change. *The Biological bulletin*, **223**, 66–77.
Shinzato C, Shoguchi E, Kawashima T *et al.* (2012) Using the Acropora digitifera genome to understand coral responses to environmental change. *Nature*, **476**, 320–323.
Stat M, Gates RD (2011) Clade D Symbiodinium in Scleractinian Corals: A "Nugget" of Hope, a Selfish Opportunist, an Ominous Sign, or All of the Above? *Journal of Marine Biology*, **2011**, 1–9.
Tolleter D, Seneca FO, DeNofrio JC *et al.* (2013) Coral Bleaching Independent of Photosynthetic Activity. *Current Biology*, **23**, 1782–1786.
Traylor-Knowles N, Palumbi SR (2014) Translational environmental biology: cell biology informing conservation. *Trends in cell biology*, **24**, 265–267.
Voolstra CR, Schnetzer J, Peshkin L *et al.* (2009) Effects of temperature on gene expression in embryos of the coral Montastraea faveolata. *BMC Genomics*, **10**, 627.
Weis VM (2008) Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *The Journal of experimental biology*, **211**, 3059–3066.
Weiss Y, Forêt S, Hayward DC *et al.* (2013) The acute transcriptional response of the coral Acropora millepora to immune challenge: expression of GiMAP/IAN genes links the innate immune responses of corals with those of mammals and plants. *BMC Genomics*, **14**, 400.
Xie F, Stewart CN Jr, Taki FA *et al.* (2013) High-throughput deep sequencing shows that microRNAs play important roles in switchgrass responses to drought and salinity stress. *Plant Biotechnology Journal*, **12**, 354–366.

Data Accessibility

- Final transcriptome assembly, raw counts data files, R scripts and input files can be accessed on Dryad: doi:10.5061/dryad.hd922
- Raw sequence data can be found on NCBI-SRA, Accession: PRJNA274410

Author Contributions

F.O.S. designed and performed research. F.O.S and S.R.P. analyzed data and wrote the manuscript.

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Figures and Tables

Figure 1. Venn diagram showing the number of differentially expressed contigs detected in analyses addressing the change in gene expression level at the onset of heat stress, during the time interval leading to bleaching, and at the onset of bleaching.

Figure 2. PCAs of normalized expression values for all 33,496 contigs of each sample in all four comparisons: (A) 5h vs. 20h in controls, (B) 5h vs. 20h heated (C) control vs. heated at 5h, and (D) control vs. heated at 20h. Axes labels show the proportion of variance explained by each principal component. Symbols (time points) and colors (treatments) explained in key at bottom. The colored ellipses show the 95% confidence intervals for each treatment and the dotted line ellipse corresponds to the overall 95% confidence interval.

Figure 3. A) Mean absolute fold change for DECs belonging to the four main gene expression profiles identified during the bleaching experiments. B) The average loss of chlorophyll a pigments in heated samples across colonies at the 20 hr time point. C) Summer temperature anomaly logged *in situ* in the Ofu Island southern lagoon.

Figure 4. Distribution of Pearson product moment correlation coefficients (RSQ) relating expression at 20 hr after acute heat stress to bleaching score. The Lingering category of genes has a larger set of high-correlation genes. Inset: An example from TRAF3, which shows lower bleaching with lower expression at 20 hr. Low bleaching and low expression occur when colonies return TRAF3 expression to near normal by 20 hr. High bleaching and high expression occur when high levels are maintained over the time interval.

Table 1. Broad biological processes, molecular functions and cellular components for which the Transient, Slow return, Late and Lingering DEC groups were enriched using UniProt IDs (DAVID high classification stringency, EASE score < 0.05).

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	GO_Cellular components	GO_Biological processes	GO_Molecular functions
Transient: up-regulated at 5 hr and recover over time	GO:0031410~cytoplasmic vesicle GO:0016023~cytoplasmic membrane-bounded vesicle GO:0031988~membrane-bounded vesicle GO:0044433~cytoplasmic vesicle part GO:0030662~coated vesicle membrane GO:0030659~cytoplasmic vesicle membrane GO:0012506~vesicle membrane GO:0005839~proteasome core complex GO:0005626~insoluble fraction GO:0000267~cell fraction GO:0005624~membrane fraction	GO:0012501~programmed cell death GO:0006915~apoptosis GO:0016265~death GO:0008219~cell death GO:0015031~protein transport GO:0045184~establishment of protein localization GO:0008104~protein localization GO:0030163~protein catabolic process GO:0009057~macromolecule catabolic process GO:0051603~proteolysis involved in cellular protein catabolic process GO:0044257~cellular protein catabolic process GO:0044265~cellular macromolecule catabolic process GO:0019941~modification-dependent protein catabolic process GO:0043632~modification-dependent macromolecule catabolic process GO:0045321~leukocyte activation GO:0046649~lymphocyte activation GO:0001775~cell activation GO:0006886~intracellular protein transport GO:0034613~cellular protein localization GO:0070727~cellular macromolecule localization GO:0042325~regulation of phosphorylation GO:0045859~regulation of protein kinase activity GO:0019220~regulation of phosphate metabolic process GO:0051174~regulation of phosphorus metabolic process GO:0043549~regulation of kinase activity	GO:0060589~nucleoside-triphosphatase regulator activity GO:0005083~small GTPase regulator activity GO:0030695~GTPase regulator activity GO:0005525~GTP binding GO:0019001~guanyl nucleotide binding GO:0032561~guanyl ribonucleotide binding GO:0031072~heat shock protein binding GO:0004298~threonine-type endopeptidase activity GO:0070003~threonine-type peptidase activity
Transient: down-regulated at 5 hr and recover over time	GO:0031974~membrane-enclosed lumen GO:0043233~organelle lumen GO:0070013~intracellular organelle lumen GO:0031981~nuclear lumen GO:0005840~ribosome	GO:0042254~ribosome biogenesis GO:0016072~rRNA metabolic process GO:0006364~rRNA processing GO:0008380~RNA splicing GO:0006397~mRNA processing GO:0016071~mRNA metabolic process GO:0051028~mRNA transport GO:0050657~nucleic acid transport GO:0050658~RNA transport GO:0051236~establishment of RNA localization GO:0015931~nucleobase, nucleoside, nucleotide and nucleic acid transport GO:0006403~RNA localization GO:0017038~protein import GO:0033365~protein localization in organelle GO:0006605~protein targeting GO:0006606~protein import into nucleus GO:0051170~nuclear import GO:0034504~protein localization in nucleus GO:0000375~RNA splicing, via transesterification reactions GO:0000377~RNA splicing, via transesterification reactions with bulged adenosine as nucleophile GO:0000398~nuclear mRNA splicing, via spliceosome	GO:0016859~cis-trans isomerase activity GO:0003755~peptidyl-prolyl cis-trans isomerase activity GO:0003735~structural constituent of ribosome
Slow return: up-regulated at 5 hr	GO:0005840~ribosome GO:0030127~COPII vesicle coat GO:0012507~ER to Golgi transport vesicle membrane GO:0030134~ER to Golgi transport vesicle	GO:0030336~negative regulation of cell migration GO:0040013~negative regulation of locomotion GO:0051271~negative regulation of cell motion GO:0007200~activation of phospholipase C activity by G-protein coupled receptor protein signaling pathway coupled to IP3 second messenger GO:0007202~activation of phospholipase C activity GO:0010518~positive regulation of phospholipase activity GO:0010863~positive regulation of phospholipase C activity GO:0045761~regulation of adenylate cyclase activity GO:0051339~regulation of lyase activity GO:0031279~regulation of cyclase activity	GO:0003735~structural constituent of ribosome
Slow return: down-regulated at 5 hr	GO:0008021~synaptic vesicle GO:0030135~coated vesicle GO:0044433~cytoplasmic vesicle part	GO:0042891~antibiotic transport GO:0015904~tetracycline transport GO:0015893~drug transport GO:0008380~RNA splicing GO:0016071~mRNA metabolic process GO:0006397~mRNA processing	GO:0015307~drug/hydrogen antiporter activity GO:0008493~tetracycline transporter activity GO:0015520~tetracycline:hydrogen antiporter activity GO:0042895~antibiotic transporter activity
Late: up- over time and up-regulaed at 20 hr	GO:0031012~extracellular matrix GO:0005578~proteinaceous extracellular matrix GO:0044421~extracellular region part		
Late: down- over time and down-regulated at 20 hr	GO:0031224~intrinsic to membrane GO:0016021~integral to membrane	GO:0008064~regulation of actin polymerization or depolymerization GO:0030832~regulation of actin filament length GO:0032956~regulation of actin cytoskeleton organization GO:0032970~regulation of actin filament-based process GO:0030837~negative regulation of actin filament polymerization GO:0032272~negative regulation of protein polymerization GO:0051693~actin filament capping GO:0031333~negative regulation of protein complex assembly GO:0030834~regulation of actin filament depolymerization GO:0030835~negative regulation of actin filament depolymerization GO:0051493~regulation of cytoskeleton organization GO:0043242~negative regulation of protein complex disassembly GO:0051494~negative regulation of cytoskeleton organization GO:0043244~regulation of protein complex disassembly GO:0044087~regulation of cellular component biogenesis GO:0010639~negative regulation of organelle organization GO:0030833~regulation of actin filament polymerization GO:0002009~morphogenesis of an epithelium GO:0016331~morphogenesis of embryonic epithelium GO:0060429~epithelium development GO:0048729~tissue morphogenesis GO:0016331~morphogenesis of embryonic epithelium GO:0001838~embryonic epithelial tube formation GO:0035148~tube lumen formation	
Lingering: up-regulated at 5 hr and 20 hr		GO:0002520~immune system development GO:0048534~hemopoietic or lymphoid organ development GO:0030097~hemopoiesis GO:0010557~positive regulation of macromolecule biosynthetic process GO:0031328~positive regulation of cellular biosynthetic process GO:0010604~positive regulation of macromolecule metabolic process GO:0009891~positive regulation of biosynthetic process GO:0045941~positive regulation of transcription GO:0010628~positive regulation of gene expression GO:0045893~positive regulation of transcription, DNA-dependent GO:0045935~positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process GO:0051254~positive regulation of RNA metabolic process GO:0051173~positive regulation of nitrogen compound metabolic process GO:0007186~G-protein coupled receptor protein signaling pathway GO:0012501~programmed cell death GO:0006915~apoptosis GO:0008219~cell death GO:0016265~death GO:0042088~T-helper 1 type immune response GO:0042108~positive regulation of cytokine biosynthetic process GO:0045410~positive regulation of interleukin-6 biosynthetic process GO:0042981~regulation of apoptosis GO:0010941~regulation of cell death GO:0043067~regulation of programmed cell death GO:0030241~muscle thick filament assembly GO:0031034~myosin filament assembly GO:0031033~myosin filament assembly or disassembly GO:0014866~skeletal myofibril assembly GO:0006468~protein amino acid phosphorylation GO:0016310~phosphorylation GO:0006796~phosphate metabolic process GO:0006793~phosphorus metabolic process GO:0051650~establishment of vesicle localization GO:0006903~vesicle targeting GO:0051648~vesicle localization	GO:0004713~protein tyrosine kinase activity GO:0004868~protein kinase inhibitor activity GO:0019210~kinase inhibitor activity
Lingering: down-regulated at 5 hr and 20 hr	GO:0005581~collagen GO:0005578~proteinaceous extracellular matrix GO:0031012~extracellular matrix GO:0005586~collagen type III GO:0000323~lytic vacuole GO:0005764~lysosome GO:0005773~vacuole	GO:0006814~sodium ion transport GO:0016209~antioxidant activity GO:0016684~oxidoreductase activity, acting on peroxide as acceptor GO:0004601~peroxidase activity GO:0042375~quinone cofactor metabolic process GO:0006743~ubiquinone metabolic process GO:0006744~ubiquinone biosynthetic process GO:0045426~quinone cofactor biosynthetic process GO:0008203~cholesterol metabolic process GO:0016125~sterol metabolic process GO:0008202~steroid metabolic process GO:0065004~protein-DNA complex assembly GO:0006334~nucleosome assembly GO:0031497~chromatin assembly GO:0034728~nucleosome organization GO:0006333~chromatin assembly or disassembly GO:0045892~negative regulation of transcription, DNA-dependent GO:0051253~negative regulation of RNA metabolic process GO:0016481~negative regulation of transcription	GO:0005201~extracellular matrix structural constituent GO:0004089~carbonate dehydratase activity GO:0031402~sodium ion binding GO:0031420~alkali metal ion binding GO:0008236~serine-type peptidase activity GO:0017171~serine hydrolase activity GO:0004252~serine-type endopeptidase activity GO:0008430~selenium binding

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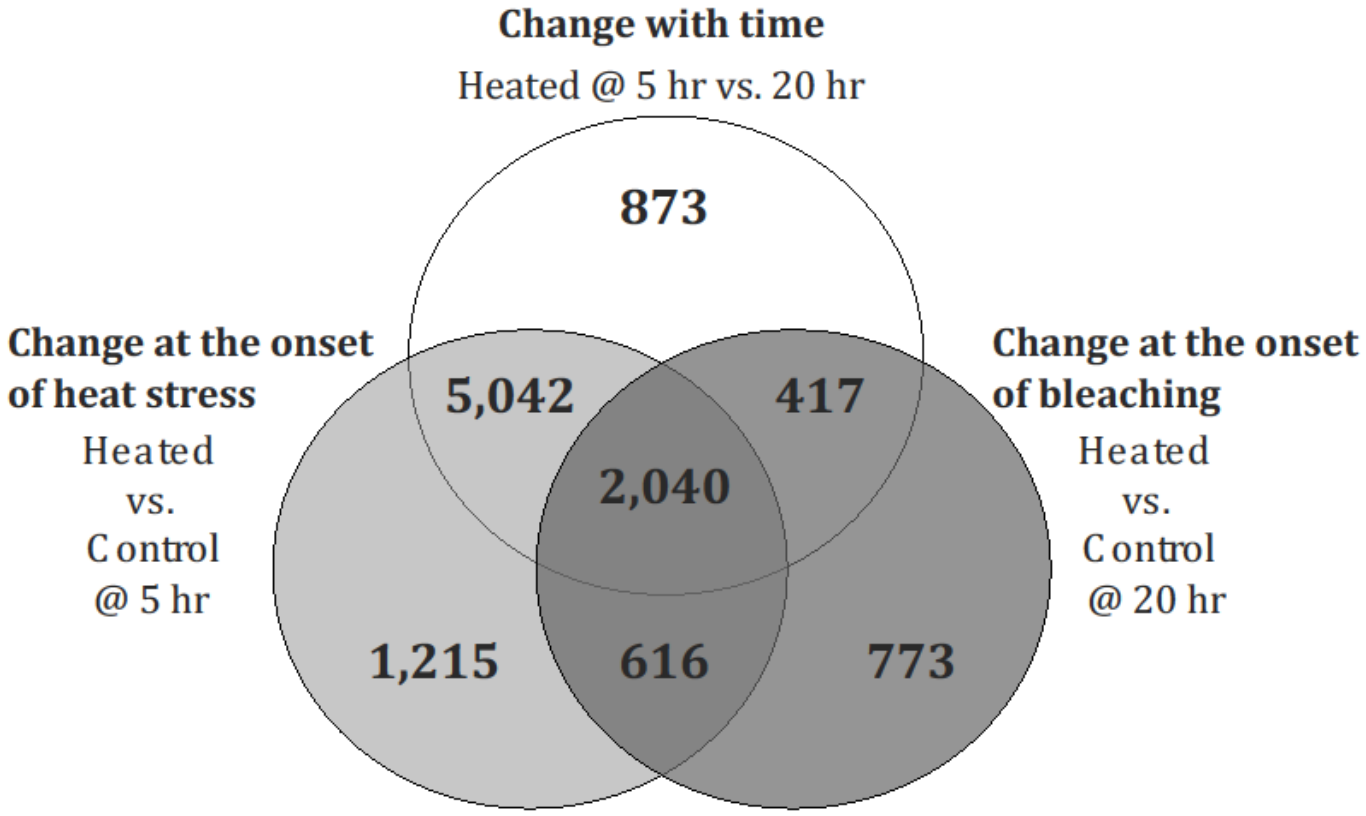

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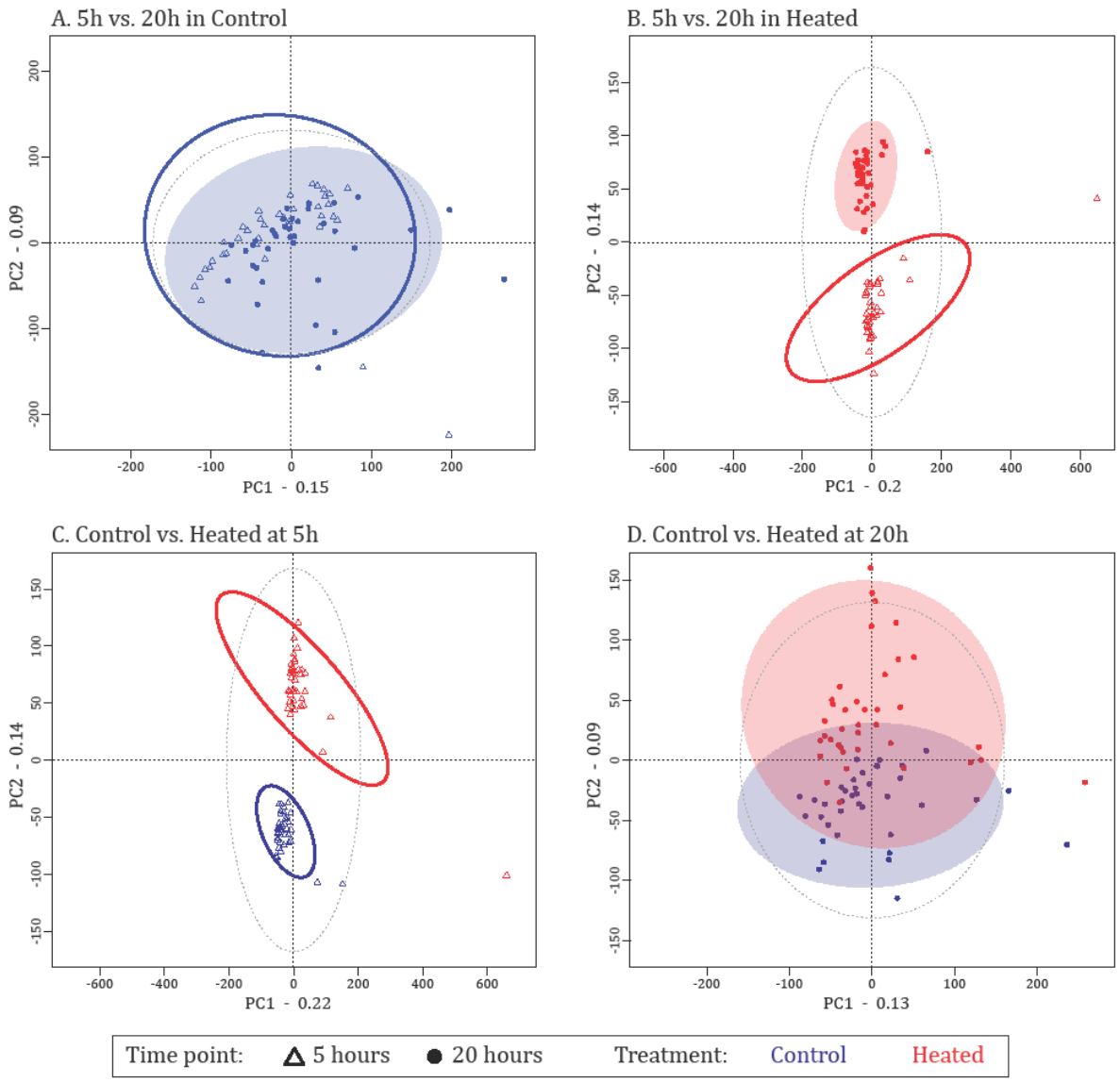

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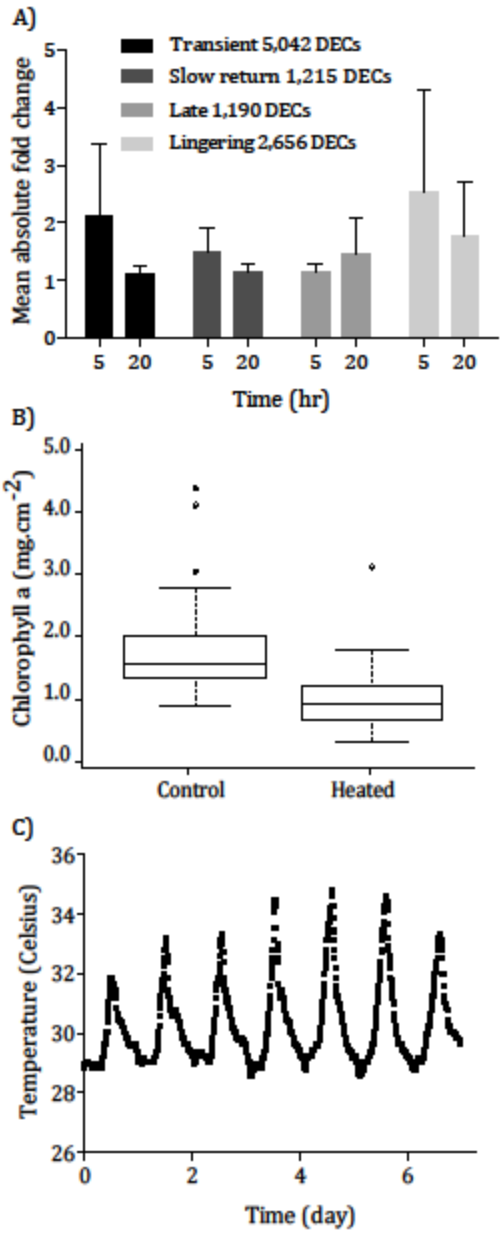

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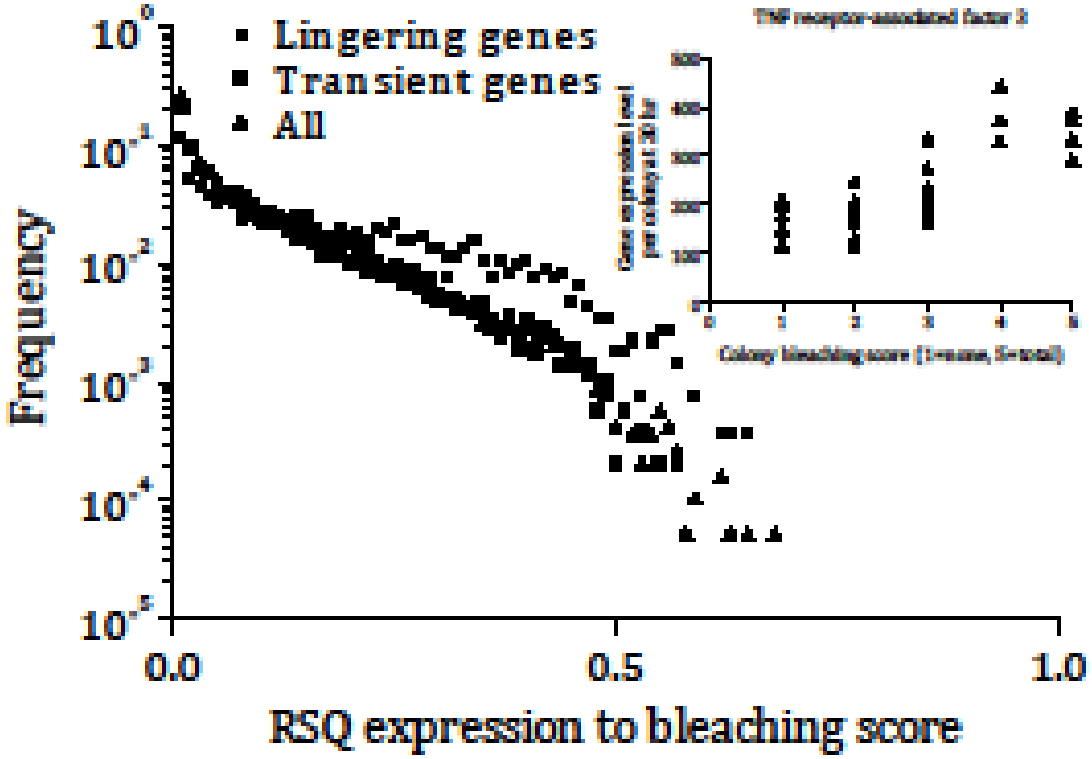

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