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Coral host transcriptomic states are correlated with *Symbiodinium* genotypes

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Abstract

A mutualistic relationship between reef-building corals and endosymbiotic dinoflagellates (*Symbiodinium* spp.) forms the basis for the existence of coral reefs. Genotyping tools for *Symbiodinium* spp. have added a new level of complexity to studies concerning cnidarian growth, nutrient acquisition, and stress. For example, the response of the coral holobiont to thermal stress is connected to the host-*Symbiodinium* genotypic combination, as different partnerships can have different bleaching susceptibilities. In this study, we monitored *Symbiodinium* physiological parameters and profiled the coral host transcriptional responses in acclimated, thermally stressed, and recovered fragments of the coral *Montastraea faveolata* using a custom cDNA gene expression microarray. Interestingly, gene expression was more similar among samples with the same *Symbiodinium* content rather than the same experimental condition. In order to discount for host-genotypic effects, we sampled fragments from a single colony of *M. faveolata* containing different symbiont types, and found that the host transcriptomic states grouped according to *Symbiodinium* genotype rather than thermal stress. As the first study that links coral host transcriptomic patterns to the clade content of their *Symbiodinium* community, our results provide a critical step to elucidating the molecular basis of the apparent variability seen among different coral-*Symbiodinium* partnerships.

Keywords: coral bleaching, gene expression, microarray, *Symbiodinium*, thermal stress

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Introduction

Reef-building corals are critically important to the functioning of tropical coral reefs, the most biologically diverse and complex marine ecosystems. A mutualistic partnership between coral hosts and photosynthetic dinoflagellates in the diverse genus *Symbiodinium* is a well-documented driving force of the trophic and structural integrity of coral reef ecosystems. By trapping solar energy and nutrients, *Symbiodinium* provide up to 95% of the energy requirements of the coral hosts, which precipitate calcium carbonate skeletons at high rates (Muscatine 1990).

In recent decades, coral mortality and extinction risk have increased dramatically, predominantly owing to mass 'bleaching' events that have become more intense and more frequent (Hughes *et al.* 2003; Carpenter *et al.* 2008). The term 'bleaching' describes the paling of the coral tissue due to a disruption of the symbiosis between coral hosts and their obligate dinoflagellate endosymbionts resulting in a loss of endosymbiont cells and/or their photosynthetic pigments. Although bleaching can be triggered by a number of factors, high temperature and light stress are commonly considered as the ecologically most significant (Hoegh-Guldberg 1999). Under short or mild thermal stress conditions, corals may recover; although, adverse effects such as

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reduced growth and fecundity, as well as higher disease susceptibility, have been observed in subsequent periods (Harvell *et al.* 2002; Bruno *et al.* 2007; Sunagawa *et al.* 2009).

Our knowledge of the complexity underlying coral-dinoflagellate symbioses has increased with the advent of molecular genotyping tools for *Symbiodinium* spp. (reviewed by Baker 2003; Coffroth & Santos 2005; Pochon *et al.* 2006; Stat *et al.* 2006). Clade genotyping via restriction fragment length polymorphism (RFLP) of the 18S rRNA gene (Rowan & Powers 1991) and subclade genotyping via differential gradient gel electrophoresis (DGGE) of the internal transcribed spacer 2 (ITS2) region are now in common practice (LaJeunesse & Trench 2000). Many coral species house only one clade (Goulet 2006), some species house many different clades (Rowan & Knowlton 1995; Toller *et al.* 2001; Mieog *et al.* 2007; Frade *et al.* 2008), and some species can shuffle their *Symbiodinium* clade content following bleaching events (Toller *et al.* 2001; Berkelmans & van Oppen 2006; Rodriguez-Roman *et al.* 2006; Thornhill *et al.* 2006; Jones *et al.* 2008). The composition of coral-*Symbiodinium* partnerships has been shown to affect a number of important properties, including (i) adaptation to different environmental optima (Rowan & Knowlton 1995; Iglesias-Prieto *et al.* 2004), (ii) differences in growth rates (Little *et al.* 2004; Mieog *et al.* 2009), (iii) photosynthate transfer and carbon fixation efficiencies (Loram *et al.* 2007; Stat *et al.* 2008; Cantin *et al.* 2009), and (iv) susceptibility to thermal stress (Rowan *et al.* 1997; Baker *et al.* 2004; Middlebrook *et al.* 2008; Sampayo *et al.* 2008; Mieog *et al.* 2009).

Given the evidence that different symbiont genotypes can affect these processes, correlations between the *Symbiodinium* clade content and coral gene expression have the potential to inform us on the molecular basis of differences in coral-*Symbiodinium* partnerships. Gene expression microarrays have been widely used to investigate changes in the expression of all or a subset of transcribed genes (i.e. the transcriptome) under varying conditions. Gene expression differences in cnidarian holobionts have thus far been studied in symbiotic vs. aposymbiotic anemones (Rodriguez-Lanetty *et al.* 2006), under thermal and UV stress (DeSalvo *et al.* 2008; Richier *et al.* 2008), during acclimatization of different source populations to different environmental conditions (Bay *et al.* 2009), during the onset of symbiosis (Voolstra *et al.* 2009), and across distinct developmental stages (Grasso *et al.* 2008; Reyes-Bermudez *et al.* 2009).

In this study, we measured *Symbiodinium* physiological parameters and profiled host gene expression in acclimated, thermally stressed, and recovered fragments from different colonies of *Montastraea faveolata*—a coral known to associate with multiple clades of *Symbiodinium* (Rowan & Knowlton 1995; Toller *et al.* 2001; Iglesias-Prieto *et al.* 2004; Thornhill *et al.* 2006). In a parallel experiment, fragments harbouring different *Symbiodinium* clades were sampled from a single host colony in order to control for the host genotypic background. Based on photochemical efficiency and host gene expression measurements in fragments containing different *Symbiodinium* clades, we demonstrate that the coral host transcriptional state, as well as the magnitude of the thermal stress response, is correlated with the symbiont genotype.

Materials and methods

Sample collection and tank experiment

On 31 July 2007, six replicate fragments were collected using a hammer and chisel from the top sun-exposed surface of five different, healthy-looking *Montastraea faveolata* colonies near Puerto Morelos, Quintana Roo, México (20°52′28.77″N and 86°51′04.53″W). These 30 fragments were used in a time-series experiment of control, thermal stress, and recovery (referred to as the 'time-series experiment'). In addition, six replicate fragments were sampled from the top (2.7 m), middle (3.7 m), and bottom (5.2 m) of a single colony of *M. faveolata*. These 18 fragments were used in a single time point experiment of control vs. thermal stress (referred to as the 'single host genotype experiment'). All fragments were transported to the UNAM—Instituto de Ciencias del Mar y Limnología field station within 1 h and divided evenly between two 50 L aquaria (i.e. three fragments from each colony and three fragments from top, middle, and bottom parts of one single colony were placed into each tank) that received a constant flow of seawater (0.6 L/min) from the same source.

Acclimation of collected coral fragments

Control and experimental aquaria were placed in a large common fibreglass tank with constantly flowing water to equilibrate the temperature of incoming seawater and to buffer temperature fluctuations. Both aquaria were exposed to the same shaded ambient light condition in order to control for the light-related factors that determine the ecological zonation of *Symbiodinium* spp. within *M. faveolata*. Each aquarium was fit with a water pump connected to a spray-bar to provide constant water movement and aeration. All coral fragments were mounted on plasticene and kept at a depth of 7 cm. From 10 to 19 August 2007, both aquaria received an average water temperature of 27.9 ± 0.6 °C (as recorded by HOBO Light/Temperature Data Loggers, Onset Corp.). Beginning on 11 August, dark-adapted

maximum quantum yields for charge separation (*Fv/Fm*) were measured at dusk for all coral fragments using a DIVING-Pulse Amplitude Modulated (PAM) fluorometer (Walz, Germany). Photosynthetically active radiation (PAR) was measured at noon and averaged 318 ± 129 μmol m^{-2} s^{-1}. From 20 to 21 August 2007, all coral fragments were brought inside during the passage of Hurricane Dean. On 22 August, the experiment was reconstituted.

During acclimation of the coral fragments, top fragments in the single host genotype experiment did not show any significant variation in *Fv/Fm* (0.575 ± 0.030) relative to the values observed in the field (0.550 ± 0.028). Likewise, none of the 30 fragments in the time-series experiment displayed a significant decrease in *Fv/Fm* throughout the acclimation period. In contrast, middle fragments in the single host genotype experiment experienced a significant reduction in *Fv/Fm* upon transfer to the experimental tanks from 0.582 ± 0.023 in the field to 0.481 ± 0.054 after 48 h in the tanks. Similarly, bottom fragments experienced a dramatic reduction of *Fv/Fm* after transfer to the experimental tanks from 0.610 ± 0.030 in the field to 0.348 ± 0.042 after 48 h of exposure to the experimental tank conditions. However, at the end of the control period (see below), all coral fragments were completely acclimated to the experimental tank conditions (Fig. S1 in the online Supporting Information).

The control period of the experiments started on 23 August (day 1) and lasted until 1 September (day 10). During this time, both aquaria received a mean water temperature of 28.5 ± 0.8°C, and mean PAR of 371 ± 169 μmol m^{-2} s^{-1}. On the night of day 1, control time point samples from five different colonies were collected from each tank. Afterward, one 200-W aquarium heater was turned on in the treatment aquarium (rate: 0.35 °C/h). A second heater was turned on 3 days later. During the thermal stress period, the control aquarium received mean water temperature of 28.8 ± 1.2 °C, the heated aquarium 31.5 ± 1.1 °C, and both tanks received mean PAR of 420 ± 152 μmol m^{-2} s^{-1}. On the night of day 16, all fragments in the single host genotype experiment were flash-frozen in liquid nitrogen. On day 20, and day 58, one sample each from the five colonies used in the time-series experiment was collected from both tanks for the thermal stress and recovery time point, respectively. All samples were exported to the USA through a CITES permit (MX-HR-007-MEX). See SI Text for methods related to statistical analysis of *Fv/Fm* data.

Symbiodinium and coral host genotyping

Genomic DNA was isolated from approximately 100–200 mg of frozen coral powder using the Power Plant DNA Isolation kit (MoBio). The *Symbiodinium* 18S ribosomal RNA gene was amplified using the primers ss5 and ss3Z (Rowan & Powers 1991) and digested with TaqI restriction enzyme. The resulting fragments were compared to *Symbiodinium* clade standards (Rowan & Knowlton 1995). The internal transcribed spacer region 2 (ITS2) was PCR amplified using cycling conditions and primers ITSintfor2 and ITS2CLAMP (without the clamp sequence) reported in LaJeunesse & Trench (2000). ITS2 amplicons were cloned, sequenced, and assigned to *Symbiodinium* clades based on both BLASTn results and phylogenetic reconstruction using reference sequences (see SI Text for details). All ITS2 sequences were deposited in GenBank with the accession numbers FJ223886-FJ224080 and FJ811907–FJ811960. Coral hosts were genotyped (see SI Text for details) using five microsatellite loci (Severance *et al.* 2004).

Microarray analysis of coral host gene expression

Total RNA from all samples was isolated and checked for quality as previously described (DeSalvo *et al.* 2008). Contamination of total RNA with *Symbiodinium* RNA is expected to be low as determined by microscopic analysis of re-suspended coral powder, which revealed *Symbiodinium* cells to be intact. For the time-series experiment, a pool of amplified RNA from all control tank fragments was used as a reference RNA sample, against which each of the 15 amplified RNA samples from the treatment tank (five control, five stressed, five recovered) was competitively hybridized. For the single genotype experiment, the reference sample constituted a pool of total RNA from all 18 samples. Fifteen micrograms of total RNA from control (three replicates per region) and heat-stressed (three replicates per region) samples were competitively hybridized against an equal amount of reference RNA resulting in a total of 18 hybridizations. Dye swaps were not performed, as any dye bias present is equal in all comparisons to the reference.

Samples were hybridized to *M. faveolata* microarrays containing 2620 features (1310 genes spotted in duplicate) that were annotated based on BLASTx hits (E-value cutoff: 1e–5) to the UniProt Knowledgebase database SwissProt (The_UniProt_Consortium 2008) and its GO-term associations (UniProt GOA, March 2008). All sequences/clone IDs are searchable at: http://sequoia.ucmerced.edu/SymBioSys/index.php. Based on hybridization of *Symbiodinium* RNA directly to the *M. faveolata* microarray, we determined the potential for cross-hybridization with *Symbiodinium* transcripts to be below 8% (data not shown). All microarrays were scanned using an Axon 4000B scanner (Molecular Devices) where care was taken to manually balance

photomultiplier tube (PMT) settings. TIFF images were generated with GenePix Pro 6.0, and gridding was performed using TIGR Spotfinder 3.1.1 (Saeed *et al.* 2003) with the *Otsu* segmentation method. The top 25% of background pixels were discarded prior to the estimation of the median local background intensity, which was subsequently subtracted from the median foreground intensity. Using TIGR MIDAS 2.19 (Saeed *et al.* 2003), background-corrected data were LOWESS normalized, and in-slide duplicates were averaged. Both PMT balancing and LOWESS normalization equalize for differing amounts of host RNA input (a potential issue when processing tissue where symbiont densities can vary). Genes were included in statistical analyses only if at least 60% of representative spots were called positive in each condition tested. This corresponds to three out of five (time-series experiment), or two out of three (single host genotype experiment) hybridizations for a given category (i.e. control, thermal stress, recovery, or top-control, top-treatment, etc., respectively). After this filtration step, 1012 (time-series experiment) and 1236 (single host genotype experiment) genes were used for subsequent analyses. Microarray hybridization data (both raw and normalized) along with methodological details are deposited in GEO with the series record number GSE15262.

The ratio between the fluorescence intensity of the two channels was used as input for BAGEL (Bayesian Analysis of Gene Expression Levels) (Townsend & Hartl 2002). The BAGEL software uses Bayesian probability to infer a relative expression level of each gene. An estimated mean and 95% credible interval of the relative level of expression of each gene is computed in each treatment and time point. We used the conservative gene-by-gene criterion of non-overlapping 95% credible intervals to regard a gene as significantly differentially expressed. The following pair-wise statistical tests were performed: control>stressed, control<stressed, control>recovered, control<recovered, stressed>recovered, and stressed<recovered (time-series experiment) and control>stressed, control<stressed (single genotype experiment). Similarly, gene expression in top (T), middle (M), and bottom (B) fragments under both control and stressed conditions were tested: T>M, T<M, T>B, T<B, M>B, and M<B. The genes found to be differentially expressed under these conditions were further grouped into 12 possible gene expression patterns, i.e.: B>M>T, B<M<T, B = M>T, B = M<T, B>M =T, B<M = T, B = T>M, B = T<M, B>M<T and B>T, B<M>T and B<T, B>M<T and B<T, and B<M>T and B>T.

Hierarchical clustering of gene expression was performed using TIGR TMEV 4.0 (Saeed *et al.* 2003). Array trees were created according to average linkage and Euclidean distance metric on $\log_2$ ratios of signal intensities, which were normalized across arrays in the case of the time-series experiment. Signal intensities from control and stressed replicate fragments ($n = 3$) from top, middle, and bottom were averaged prior to hierarchical clustering. The stability of clusters was tested using the R-package pvclust by calculating approximately unbiased p-values via multiscale bootstrap resampling; a measure that has been demonstrated to provide a better indicator for cluster support than regular bootstrapping procedures (Suzuki & Shimodaira 2006). To assess over-representation of Gene Ontology (GO) terms in the lists of significant genes, we used default statistical tests and multiple-testing adjustments in GOEAST (Zheng & Wang 2008) except the significance cutoff was set to $\alpha = 0.001$.

Results

Experimental thermal stress and recovery

We measured dark-adapted maximum quantum yields for charge separation (*Fv/Fm*) in fragments sampled from five biological replicates of the coral *M. faveolata* throughout the time course of a thermal stress experiment (Fig. 1A, Table S1). A significant decrease in *Fv/Fm* in previously acclimated coral fragments was observed four days after increasing the water temperature. The total duration of the thermal stress treatment was 10 days and the difference in *Fv/Fm* became insignificant two days after termination of the treatment (Fig. 1A). The initial decrease in *Fv/Fm* was linear, whereas its increase during the recovery period was explained best by a logarithmic function (Fig. S2, Table S2).

Differences in *Fv/Fm* among the sampled colonies were observed in all experimental periods ($P_{adj} < 0.001$; Table S1). Post-hoc analyses revealed that *Fv/Fm* averages in colonies C1 and C2 were significantly different from those in colonies C3, C4, and C5 during the control period. Colony C2 showed very little reduction in *Fv/Fm* upon heat treatment, and *Fv/Fm* values were significantly higher than in all other colonies during the stress and recovery periods. The differences between colonies were most likely due to a combinatorial effect of differences in *Symbiodinium* (Table 1) and host genotypes (Fig. S3).

Restriction fragment length polymorphism (RFLP) analysis and sequencing of internal transcribed spacer 2 (ITS2) loci were used to genotype the *Symbiodinium* clades hosted by the experimental coral fragments. In the control samples, the dominant *Symbiodinium* clades were clade A in colonies one and two, clade B in colonies four and five, and clade C in colony three. In

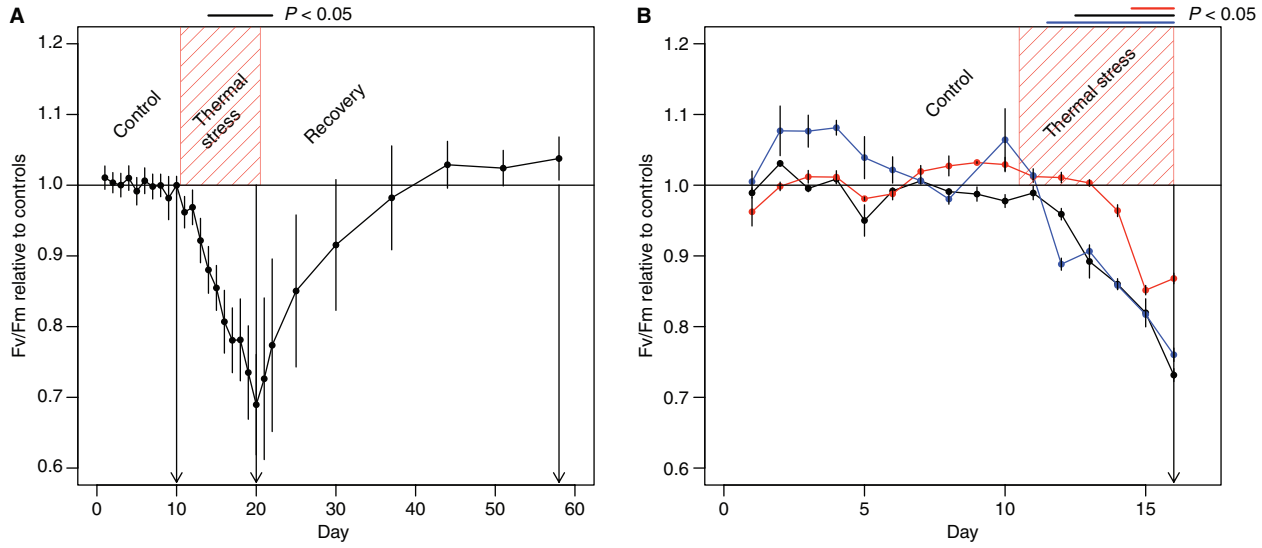

Fig. 1 Relative *Fv/Fm* changes before, during, and after exposure of coral fragments to thermal stress. Dark-adapted maximum quantum yields relative to control samples were measured in five biological replicates during an experimental heat stress experiment (A) and in top (black), middle (red), and bottom fragments (blue) collected from a single coral colony (B). After acclimation, experimental coral fragments were exposed to elevated temperatures (shown by shaded areas) before being collected for microarray analyses at time points indicated by arrows. *Fv/Fm* data are averages (± standard error) of treatment tank replicates relative to the average of coral fragments maintained at control temperature. Horizontal bars above the plot indicate periods during which the difference between control and heat-stressed fragments were significant ($P < 0.05$).

thermally stressed samples, clade A was the dominant type in colony three, whereas clade A and clade B remained the dominant clade types in colonies one and two, and colonies four and five, respectively (Table 1). While the five replicate colonies initially harbored a range of symbiont clades (A, B, and C), following recovery from bleaching all colonies harbored clade A, i.e., the dominant *Symbiodinium* clade type residing in colonies three, four and five changed from clade C/B to clade A. ITS2 sequencing results were generally congruent with RFLP analyses in control fragments, and additionally allowed for both the determination of relative contributions of different *Symbiodinium* subtypes in fragments hosting more than one clade type and an increase in resolution of *Symbiodinium* clades to the subclade level. Based on both BLASTn results and phylogenetic reconstruction (Fig. S4), we found the dominant haplotype of clade A, clade B, and clade C ITS2 sequences to be type A3 (most similar to EU074857), type B1 (EU074875), and type C7 (AF499797), respectively.

Analysis of a single host genotype harbouring different Symbiodinium clades

In a parallel experiment, we sampled replicate fragments from top, middle and bottom parts of a single coral colony to obtain samples containing different *Symbiodinium* clade types in one host genetic background. *Fv/Fm* values began to decline following exposure to elevated temperature (Fig. 1B). The bottom fragments showed a significant decrease relative to their controls two days after heating. Top fragments also showed a significant decrease after two days albeit less than the bottom fragments. The middle fragments showed a significant decrease after four days. By the end of the experiment, all heat-stressed fragments had significantly lower *Fv/Fm* values compared to their controls, and the top and bottom heat-stressed fragments had significantly lower maximum quantum yields than the middle heat-stressed fragments (Fig. 1B). RFLP analyses revealed that middle and bottom fragments harboured mainly clade C; top fragments, mainly clade B (Table 1). Again, ITS2 sequencing results were largely consistent with RFLPs analyses (Table 1). After sequence alignment and phylogenetic analyses (Fig. S4), we found that the dominant haplotype of clade C sequences was identical to type C7 (AF499797), and the dominant haplotype of clade B sequences was identical to type B17 (EU449083).

Microarray gene expression analysis

After the generation of *Symbiodinium* physiological and genotypic data, we were interested in differentially expressed genes in control, thermally stressed, and

Table 1 Results of *Symbiodinium* genotyping assays. The top half corresponds to the time series experiment, the bottom half to the single host colony experiment. When more than one sample appears on the same row, they contained the same *Symbiodinium* type and proportions. Abbreviations used: C, B, R followed by a number denote control, bleached, and recovered fragments, respectively

	18S rRNA RFLP		ITS2 sequencing				
Fragment(s)	1°	2°	1°	%	2°	%	n
C1	A	—	A3	73	C7	27	11
C2	A	—	A3	90	C7	10	10
C3	C	B	C7	82	B1	18	11
C4	B	—	B1	91	C7	9	11
C5	B	—	B1	100	—	—	11
B1–3	A	—	n/a				
B4	B	A	n/a				
B5	B	—	n/a				
R1–5	A	—	n/a				
Top 1,3,6	B	—	B17	100	—		11,11,12
Top 2	B	C	B17	83	C7	17	12
Top 4	B	C	B17	80	C7	20	10
Top 5	B	C	B17	73	C7	27	11
Middle 1,4	C	—	C7	100	—		14,15
Middle 2	C	—	C7	69	B17	31	13
Middle 3	C	—	C7	82	B17	18	11
Middle 5	C	B	B17	75	C7	25	12
Middle 6	C	—	C7	67	B17	33	12
Bottom 1–6	C	—	C7	100	—		8–9

recovered coral fragments from different coral colonies (Fig. 2A). In addition, control and thermally stressed coral fragments from different regions of a single host colony were analysed for differentially expressed genes (Fig. 2B), a design that eliminated potential differences due to host genotypic background effects.

Pair-wise comparisons between control, stressed, and recovered samples resulted in a relatively small number (n = 57–84) of differentially expressed genes, which is most likely attributable to the different *Symbiodinium* genotypes that were hosted by the control and stressed fragments (Table 1). Nevertheless, a gene ontology (GO) enrichment analysis revealed that genes related to regulation of transcription and response to stress were significantly enriched ($P < 0.001$) among the annotated, differentially expressed genes (Fig. 2A, Table S3). A direct comparison between control and thermally stressed samples from a single host colony yielded only 28 differentially expressed genes with low fold-changes ranging from 1.08 to 1.53 (Fig. 2B, Table S3).

Next, we performed hierarchical cluster analyses (using Euclidean distance) on all assayed (1000+) genes to group together samples with similar transcriptomic activity, and mapped *Symbiodinium* physiological and genotypic data onto the resulting array trees (Fig. 3). With respect to the time-series experiment, the array tree revealed four major groups, which displayed congruency with *Symbiodinium* clade content and possibly with host genotypic differences. All recovered samples, which exclusively harbored *Symbiodinium* clade A, clustered together (Group 1, Fig. 3A). In contrast, neither control nor thermally stressed samples were grouped together, i.e. according to experimental conditions. Instead, they were divided into groups that suggest the influence of both host and *Symbiodinium* genotype effects. Control and stressed samples from colony 2, which was determined to be genetically distinct from all other colonies (Fig. S3), contained mainly symbionts of clade A (Group 2, Fig. 3A). Group 3 was formed by control and stressed fragments from colonies one and three, which mainly contained clade A symbionts. The only exception to this observation was that the control fragment of colony 3 was dominated by clade C. These two colonies were genetically different from each other as well as different from colonies two, four and five (Fig. S3). Finally, different to all other control and stressed fragments, colonies four and five could not be genetically differentiated (Fig. S3) and contained mainly clade B (Group 4, Fig. 3A).

Another aspect of our analyses revealed an apparent trend between the magnitude of the transcriptomic response to thermal stress (determined by the distance between the control and stressed samples in Fig. 3) and the symbiont genotype. In clade A (colonies one and two) or clade C (colony three) hosting fragments, the difference was relatively small (branch length distances between control and stressed samples: 37.02, 36.08, and 31.56, respectively) compared to those dominated by clade B (44.55 and 61.86 for colonies 4 and 5, respectively).

Unlike in the time-series experiment, differences in gene expression between samples originating from a single host genotype were not biased by host genotypic background, thus strengthening the observed correlation between host transcriptomic states and *Symbiodinium* clade contents (Fig. 3B). We found that middle and bottom control fragments (both dominated by clade C symbionts) exhibited gene expression patterns more similar to each other than to clade B-dominated top fragments (Fig. 3B top panel). This association was highly significant according to cluster support. However, the correlation between host transcriptomic states and *Symbiodinium* clade contents noticeably diminished in the top, middle, and bottom stressed fragments (Fig. 3B lower panel). Not only were the transcriptomic states of the top and bottom stressed fragments more similar to each other than to the middle stressed fragments, but the branch lengths and cluster support were

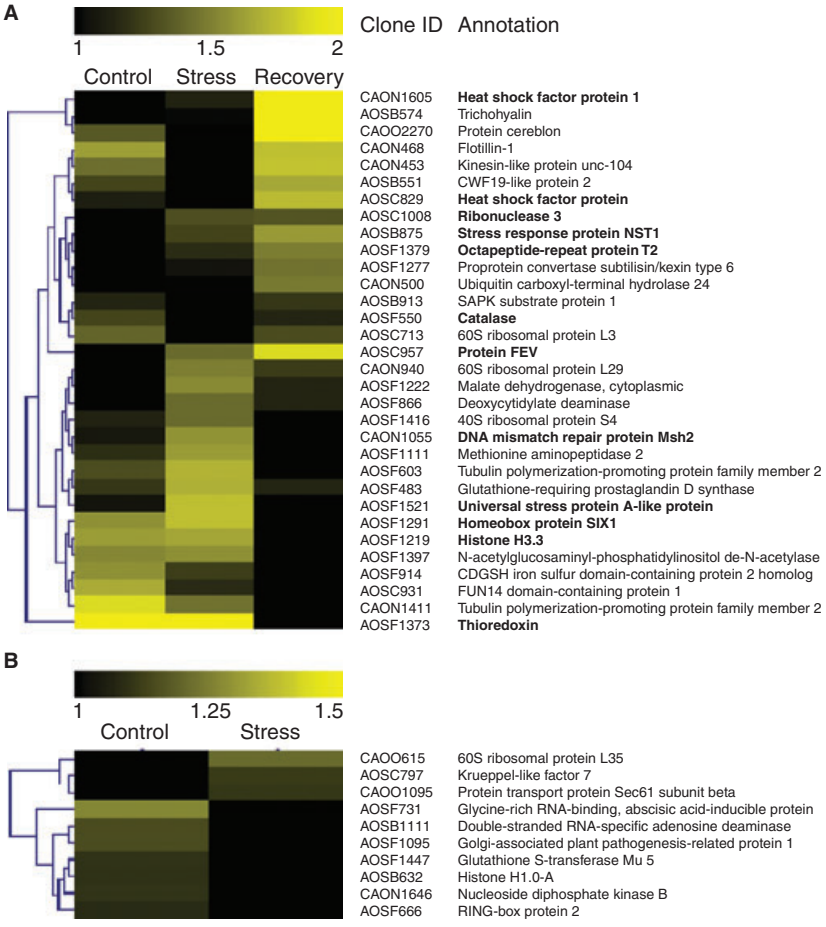

Fig. 2 Heat map of stress-responsive differentially expressed genes. Annotated differentially expressed genes identified in the time-series (A) and single host genotype experiment (B) are shown with clone IDs and annotations. Colour scales correspond to BAGEL-computed gene expression estimates where a value of one is assigned to the class with the lowest expression. Bold-faced genes are involved in transcription regulation and stress response according to GO enrichment analysis.

lower compared to the control tree (Fig. 3B top panel). This pattern is consistent with the PAM data showing that *Symbiodinium* within top and bottom fragments experienced more stress than those within middle fragments (Fig. 1B).

Expression patterns of differentially expressed genes

Testing for pair-wise differences between colony locations showed that among the control samples, many genes were differentially expressed between top and middle (n = 204) and top and bottom (n = 312); however, a very small number of genes were differentially expressed between middle and bottom (n = 24). These numbers were substantially lower for the corresponding analysis of thermally stressed samples: top vs. middle (n = 51); top vs. bottom (n = 96); and middle vs. bottom (n = 49) (see Table S3 for all gene expression results).

We further analysed differentially expressed genes between top, middle, and bottom samples by grouping them into 12 major expression patterns. Among control samples, 173 genes were sorted into 8 different patterns. The vast majority (90%) of these genes were either upregulated (B = M < T, n = 105) or downregulated (B = M > T, n = 50) in top vs. middle/bottom samples (Fig. 4, Table S3). The distribution into distinct patterns for stressed samples was more even, further supporting a diminishing correlation between transcriptomic states and *Symbiodinium* genotypes during thermal stress as previously mentioned (Fig. 3B). Nevertheless, similar to the control samples, the majority (60%) of differentially expressed genes were either upregulated (B = M < T, n = 20) or downregulated (B = M>T, n = 6) in top vs. middle/bottom samples (Fig. 4, Table S3).

GO enrichment analysis of the most populated patterns, i.e. those with *Symbiodinium* genotype differences among the control samples (B = M < T and B = M > T), showed genes involved in protein metabolism (e.g. translation, protein folding/degradation) to be significantly (P < 0.001) enriched (Fig. 5).

Discussion

Host and Symbiodinium factors affect dark-adapted maximum quantum yields (Fv/Fm)

Maximum quantum efficiencies in *Symbiodinium* sp. type C7-associated with *Montastraea faveolata* were

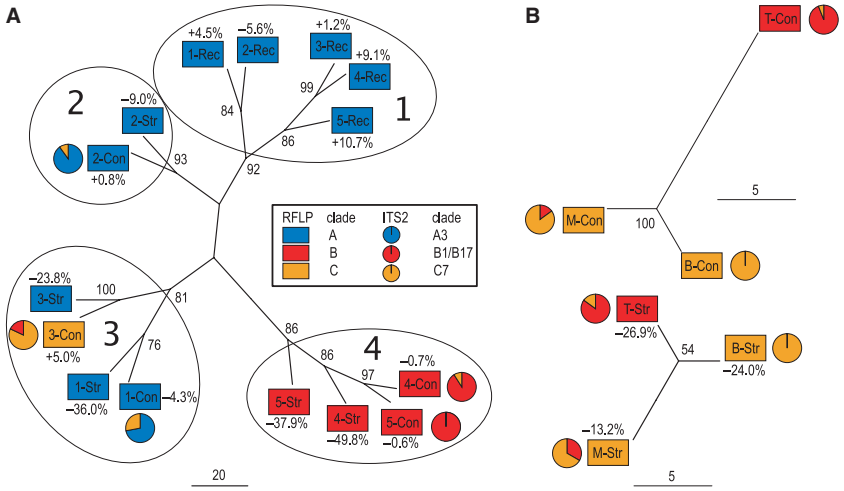

Fig. 3 Hierarchical clustering of gene expression intensities shows that samples group according to differences in *Symbiodinium* clade content. (A) Samples from a thermal stress and recovery experiment using replicates from five different coral colonies were clustered into the following groups: (i) all recovered fragments, containing exclusively *Symbiodinium* clade A, (ii) fragments from colony two containing predominantly clade A, (iii) fragments from colonies one and three containing mainly clade A, and (iv) fragments from colonies four and five containing predominantly clade B. (B) Averaged samples (n = 3) from top, middle, and bottom parts of a single coral host colony group according to *Symbiodinium* genotypes in control (top panel), but not thermally stressed samples (bottom panel). Legend: sample names (one—five: sampled colony; T: top, M: middle, B: bottom; Con: control, Str: thermally stressed, Rec: recovered) and RFLP analysis results are shown as colour-coded rectangles. Pie charts illustrate proportions of *Symbiodinium* clades based on ITS2 sequence analysis in selected fragments (note: red indicates type B1 in panel A and type B17 in panel B). Numbers next to rectangles represent the percent differences in *Fv/Fm* between treated and untreated fragments. Approximately unbiased p-values are shown next to tree nodes.

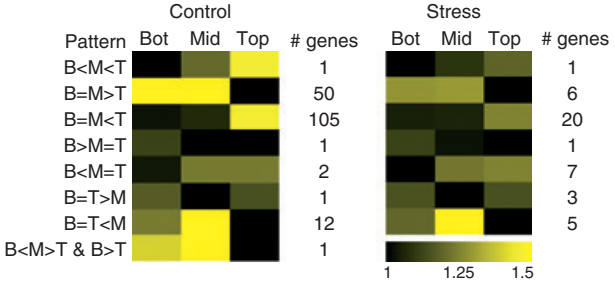

Fig. 4 Pattern analysis of differentially expressed genes. Genes that were differentially expressed between top, middle, and bottom control (left panel) and stressed (right panel) fragments were sorted into groups of distinct expression patterns. Heat map intensities were calculated by averaging the BAGEL-estimated expression levels for all genes within each pattern. Of the 12 patterns tested, only those containing at least one gene are depicted.

previously shown to decrease more in response to experimental thermal stress compared to type B1-associated with *M. faveolata* (Warner *et al.* 2006). In our study, however, type B1-hosting colonies actually decreased more in *Fv/Fm* than type A3- and type C7-containing colonies (Fig. 3A). Similarly, type B17-containing top fragments experienced a greater decrease in *Fv/Fm* than type C7-containing middle fragments. However, the response of B17-containing top fragments was similar to C7-containing bottom fragments. Marked differences were also found in the degree of photoinactivation of PSII between different colonies that hosted the same *Symbiodinium* type. For example, colony two experienced hardly any reduction in *Fv/Fm* while colony one experienced a sharp decrease in *Fv/Fm*, yet both housed clade A.

The discrepancies between our study and Warner *et al.* (2006), in addition to the differences between middle and bottom fragments and the fact that colonies one and two differed in thermal susceptibility, argues against the idea that thermal stress-susceptibility of corals is solely linked to symbiont genotype (Iglesias-Prieto *et al.* 2004; Rowan 2004; Tchernov *et al.* 2004; LaJeunesse *et al.* 2007; Sampayo *et al.* 2008). Instead, our results and other studies (Brown *et al.* 2002; Bhagooli & Hidaka 2003; Goulet *et al.* 2005) support the notion that host contributions and/or effects of previous conditions (Brown *et al.* 2002; Middlebrook *et al.* 2008) must be taken into account when discussing differences in stress-induced *Fv/Fm* losses with respect to differences in *Symbiodinium* type.

Host gene expression profiling during thermal stress and recovery

In a previous coral microarray study on heat stress, we assayed gene expression changes in fragments from a single colony and fragments from different

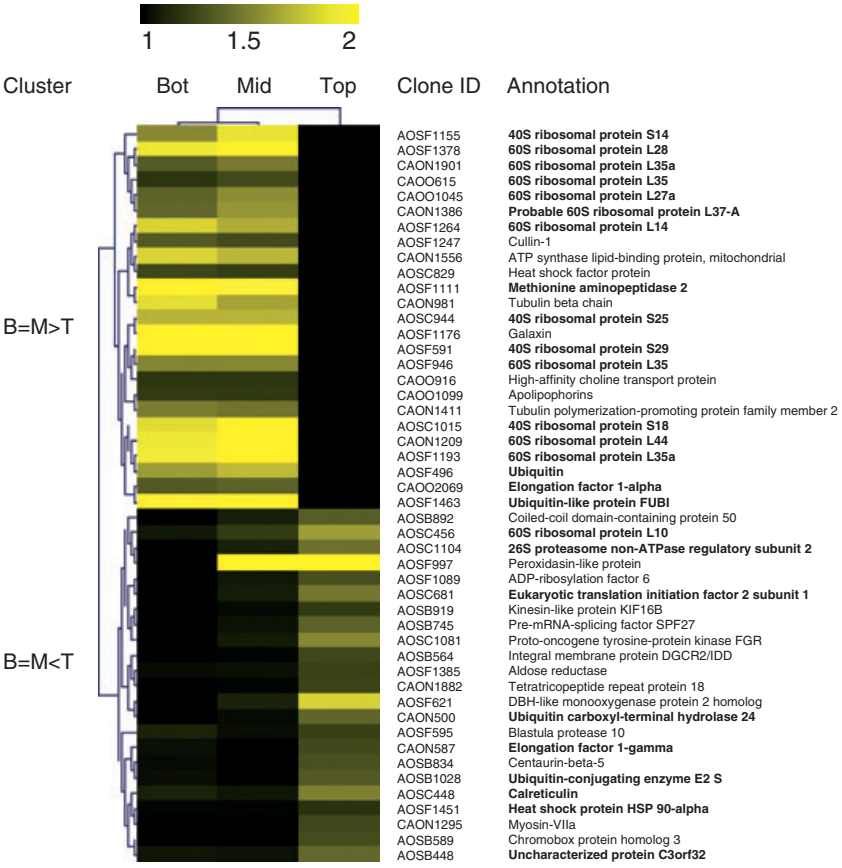

Fig. 5 Heat map of genes differentially expressed between top, middle, and bottom control fragments. Annotated differentially expressed genes identified in the single host genotype experiment are shown with clone IDs and annotations. Colour scales correspond to BAGEL-computed gene expression estimates where a value of one is assigned to the class with the lowest expression. Boldfaced genes are involved in protein metabolism (e.g. translation, protein folding/degradation) according to GO enrichment analysis.

colonies (DeSalvo *et al.* 2008). We found that while using biological replicates (i.e. different colonies) increased our ability to draw conclusions beyond the limits of a single genotype, it confounded our ability to detect significant changes in genes that show only a small difference in expression given a similar number of microarrays. Nevertheless, many genes identified in the single-genotype experiment were corroborated in the multi-colony experiment (DeSalvo *et al.* 2008). In the present study, we followed a similar two-pronged experimental design by using multiple replicates from a single host colony, in combination with sampling different colonies.

Unexpectedly, we found: (i) few genes differentially expressed in response to thermal stress and recovery, and (ii) low overlap with previous results (DeSalvo *et al.* 2008). In our previous experiment greater than 20% of genes were found differentially expressed upon induction of bleaching (DeSalvo *et al.* 2008). These discrepancies might be explained by differences in experimental procedures such as: acclimation time (4 days vs. 21 days), initial rate of temperature change (0.73 °C/h vs. 0.35 °C/h), sampling time points, water quality, light regime, etc. However, we provide strong evidence that the symbiont genotype had a substantial influence on host gene expression (see below), and thus diminished our ability to detect more genes differentially expressed during thermal stress and recovery. Nevertheless, we found an enrichment of genes related to transcription regulation and stress response (Fig. 2). Interestingly, a number of those genes are differentially expressed between control and recovered samples. These genes (e.g. heat shock factor protein) represent candidates for future studies given their possible involvement in the acquisition of experience-mediated thermal stress resistance (Brown *et al.* 2002).

The results from comparing control and stressed fragments from the same host genotype, an approach that allowed us to disentangle the contribution of host genetic background and the *Symbiodinium* type hosted, yielded a low number (about 2% of all assayed genes) of differentially expressed genes with fold-changes less than 1.53. This is in stark contrast to the single-genotype experiment described in DeSalvo *et al.* 2008, where 24% of all assayed genes were found differentially expressed. Intriguingly, all fragments in the 2008 study hosted the same *Symbiodinium* genotype (clade A), which, besides methodological differences, supports the notion that the host gene expression is correlated with the symbiont-genotype hosted.

Based on the results found in this study, i.e. an influence of *Symbiodinium* genotypes on coral transcriptomes, and the variability in transcriptomic responses seen in different experiments, the identification of a core set of stress-responsive genes represents a daunting challenge. Such an endeavour will require accounting for differences in both abiotic and biotic factors such as experimental procedures (acclimation conditions, rate and magnitude of temperature increase, stress exposure time, etc.) and host and symbiont genotypic backgrounds, respectively.

Integration of host gene expression and Symbiodinium genotyping analyses

Numerous studies support the notion that different symbionts have far-reaching effects on host physiology. Growth rates in *Aiptasia pulchella* (Kinzie & Chee 1979) and in juvenile *Acropora millepora* and *Acropora tenuis* (Little *et al.* 2004; Mieog *et al.* 2009) are dependent on the symbiont genotype. Host growth is likely dependent on nutrient availability; thus, differential growth as a result of symbiont genotype may be related to the type (Loram *et al.* 2007) and amount (Stat *et al.* 2008; Cantin *et al.* 2009) of nutrients translocated from the symbiont to the host. Furthermore, a recent study investigated growth, survival, and thermal tolerance in different host-*Symbiodinium* combinations and found that *Symbiodinium* type was a better predictor of holobiont fitness than host genetic effects (Mieog *et al.* 2009). Finally, during natural bleaching of *Montastraea* spp., clade A- and C-associated colony regions were less susceptible to thermal stress compared to clade B- and C-associated regions (Rowan *et al.* 1997).

In both of our experiments, acclimation of field-collected samples to the same light and temperature conditions for 4 weeks did not cause homogenization of *Symbiodinium* genotypes as seen in the control samples. Thus, it is possible that the observed patterns in host gene expression could have resulted from long-term acclimatization effects (months, years) to different environmental conditions in the field (e.g. light levels and temperature). The homogenization of *Symbiodinium* genotypes following thermal stress (all recovered fragments hosted *Symbiodinium* clade A) could also be interpreted as host physiology determining symbiont type dominance or alternatively, symbiont type content driving host gene expression. Taken together, we acknowledge that our experimental design did not allow disentangling a cause and effect relationship, i.e. whether a change in environmental conditions changed host physiology, and in turn a change in *Symbiodinium* type dominance, or that a change in environmental conditions caused a change in *Symbiodinium* type dominance and in turn a change host physiology (reflected in transcriptomic states). Nevertheless, the congruency between the array tree groupings and the *Symbiodinium* community compositions (Fig. 3), which is seen in both experiments presented here, constitutes strong evidence that there exists a correlation between different *Symbiodinium* genotypes and the state of the coral host transcriptome. Our statistical analyses corroborate these findings in that the greatest differences in gene expression were found between clade B hosting top fragments and clade C hosting middle/bottom fragments (Fig. 4). However, in thermally stressed top, middle, and bottom samples the correlation notably diminished as seen by the lower number of differentially expressed genes compared to the corresponding analysis in control samples (Fig. 4). The observed reduction was due to genes becoming more similar in their expression during stress rather than an increase in variation (Table S3).

The comparison between top, middle, and bottom fragments suggests that pathways involved in protein translation, folding, and degradation, in addition to other genes listed in Fig. 5, are differentially affected in coral fragments hosting different *Symbiodinium* genotypes. Since these results are based on a single host genotype and a relatively small microarray, they set the stage for an in-depth analysis of differentially regulated pathways involving a multi-colony experimental design. If we are to succeed in such an effort, then surveying host and symbiont genotypic diversity must precede and thus inform the design of future experiments. At the time of writing, a *M. faveolata* microarray containing greater than 10 000 features is under fabrication, which will be utilized in the future to address this important gap of knowledge.

Conclusions and outlook

While the order of events, i.e. host physiology driving symbiont content and/or symbiont content driving host physiology, requires further investigation, the convergent findings of the two experiments outlined in this study strongly argues for the existence of a correlation between host transcriptional states and the symbiont genotype. It would thus be interesting to hypothesize that different symbiont types may modulate the host transcriptome in more or less stress-responsive configurations, which would add a host transcriptomic perspective to a *Symbiodinium*-centric view of bleaching susceptibility.

Advancement in the understanding of the apparent differences observed in bleaching susceptibility of different coral-*Symbiodinium* partnerships are of particular relevance given the grim outlook of coral populations in the face of large-scale climate change-induced bleach-

ing events. The ability of some corals to multi-associate most likely arose because symbiont effectiveness changes with environmental conditions (Douglas 1998). This scenario is consistent with the idea that organisms gain robustness to environmental perturbation by extending their system boundary via the integration of foreign biological entities such as symbionts (Kitano & Oda 2006). This is exemplified by different coral-*Symbiodinium* genotypic combinations being locally adapted to irradiance (Rowan & Knowlton 1995; Iglesias-Prieto *et al.* 2004). So far, only a handful of studies have addressed the effects of mutualistic symbionts on animal host transcriptomes, e.g. human gut microbiome (Hooper *et al.* 2001), *Vibrio*-squid symbiosis (Chun *et al.* 2008), and *Symbiodinium*-coral larvae and *Symbiodinium*-anemone symbioses (Rodriguez-Lanetty *et al.* 2006; Voolstra *et al.* 2009). These examples illustrate effects on host gene expression upon infection by different symbionts, whereas our study extends this body of knowledge by describing a correlation between symbiont genotype and the host transcriptome in both established symbioses and responses to thermal stress.

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References

Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. *Annual Review of Ecology Evolution and Systematics*, **34**, 661–689.

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

Bay LK, Ulstrup KE, Nielsen HB *et al.* (2009) Microarray analysis reveals transcriptional plasticity in the reef building coral *Acropora millepora*. *Molecular Ecology*, **18**, 3062–3075.

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. *Proceedings of Biological Sciences*, **273**, 2305–2312.

Bhagooli R, Hidaka M (2003) Comparison of stress susceptibility of in hospite and isolated zooxanthellae among five coral species. *Journal of Experimental Marine Biology and Ecology*, **291**, 181–197.

Brown BE, Dunne RP, Goodson MS, Douglas AE (2002) Experience shapes the susceptibility of a reef coral to bleaching. *Coral Reefs*, **21**, 119–126.

Bruno JF, Selig ER, Casey KS *et al.* (2007) Thermal stress and coral cover as drivers of coral disease outbreaks. *PLoS Biology*, **5**, e124.

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

Carpenter KE, Abrar M, Aeby G *et al.* (2008) One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science*, **321**, 560–563.

Chun CK, Troll JV, Koroleva I *et al.* (2008) Effects of colonization, luminescence, and autoinducer on host transcription during development of the squid-vibrio association. *Proceedings of the National Academic Sciences, USA*, **105**, 11323–11328.

Coffroth MA, Santos SR (2005) Genetic diversity of symbiotic dinoflagellates in the genus Symbiodinium. *Protist*, **156**, 19–34.

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.

Douglas AE (1998) Host benefit and the evolution of specialization in symbiosis. *Heredity*, **81**, 599–603.

Frade PR, De Jongh F, Vermeulen F, Van Bleijswijk J, Bak RPM (2008) Variation in symbiont distribution between closely related coral species over large depth ranges. *Molecular Ecology*, **17**, 691–703.

Goulet TL (2006) Most corals may not change their symbionts. *Marine Ecology-Progress Series*, **321**, 1–7.

Goulet TL, Cook CB, Goulet D (2005) Effect of short-term exposure to elevated temperatures and light levels on photosynthesis of different host-symbiont combinations in the Aiptasia pallidal Symbiodinium symbiosis. *Limnology and Oceanography*, **50**, 1490–1498.

Grasso LC, Maindonald J, Rudd S *et al.* (2008) Microarray analysis identifies candidate genes for key roles in coral development. *BMC Genomics*, **9**, 540.

Harvell CD, Mitchell CE, Ward JR *et al.* (2002) Climate warming and disease risks for terrestrial and marine biota. *Science*, **296**, 2158–2162.

Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Journal of Marine and Freshwater Research*, **50**, 839–866.

Hooper LV, Wong MH, Thelin A *et al.* (2001) Molecular analysis of commensal host-microbial relationships in the intestine. *Science*, **291**, 881–884.

Hughes TP, Baird AH, Bellwood DR *et al.* (2003) Climate change, human impacts, and the resilience of coral reefs. *Science*, **301**, 929–933.

Iglesias-Prieto R, Beltran VH, LaJeunesse TC, Reyes-Bonilla H, Thome PE (2004) Different algal symbionts explain the

vertical distribution of dominant reef corals in the eastern Pacific. *Proceedings of Biological Science*, **271**, 1757–1763.

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

Kinzie RA, Chee GS (1979) Effect of different zooxanthellae on the growth of experimentally re-infected hosts. *Biological Bulletin*, **156**, 315–327.

Kitano H, Oda K (2006) Self-extending symbiosis: a mechanism for increasing robustness through evolution. *Biologial Theory*, **1**, 61–66.

LaJeunesse TC, Trench RK (2000) Biogeography of two species of *Symbiodinium* (Freudenthal) inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt). *Biological Bulletin*, **199**, 126–134.

LaJeunesse TC, Reyes-Bonilla H, Warner ME (2007) Spring ''bleaching'' among *Pocillopora* in the Sea of Cortez, Eastern Pacific. *Coral Reefs*, **26**, 265–270.

Little AF, van Oppen MJ, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. *Science*, **304**, 1492–1494.

Loram JE, Trapido-Rosenthal HG, Douglas AE (2007) Functional significance of genetically different symbiotic algae *Symbiodinium* in a coral reef symbiosis. *Molecular Ecology*, **16**, 4849–4857.

Middlebrook R, Hoegh-Guldberg O, Leggat W (2008) The effect of thermal history on the susceptibility of reef-building corals to thermal stress. *Journal of Experimental Biology*, **211**, 1050–1056.

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

Mieog JC, Olsen JL, Berkelmans R *et al.* (2009) The roles and interactions of symbiont, host and environment in defining coral fitness. *PLoS ONE*, **4**, e6364.

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

Pochon X, Montoya-Burgos JI, Stadelmann B, Pawlowski J (2006) Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus Symbiodinium. *Molecular Phylogenetics and Evolution*, **38**, 20–30.

Reyes-Bermudez A, DeSalvo MK, Voolstra CR *et al.* (2009) Gene expression microarray analysis encompassing metamorphosis and the onset of calcification in the scleractinian coral *Montastraea faveolata*. *Marine Genomics*, **2**, 149–159.

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 D: Genomics Proteomics*, **3**, 283–289.

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

Rodriguez-Roman A, Hernandez-Pech X, Thome PE, Enriquez S, Iglesias-Prieto R (2006) Photosynthesis and light utilization in the Caribbean coral Montastraea faveolata recovering from a bleaching event. *Limnology and Oceanography*, **51**, 2702–2710.

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

Rowan R, Knowlton N (1995) Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proceedings of National Academic Sciences, USA*, **92**, 2850–2853.

Rowan R, Powers DA (1991) Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae). *Marine Ecology-Progress Series*, **71**, 65–73.

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

Saeed AI, Sharov V, White J *et al.* (2003) TM4: a free, open-source system for microarray data management and analysis. *BioTechniques*, **34**, 374–378.

Sampayo EM, Ridgway T, Bongaerts P, Hoegh-Guldberg O (2008) Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. *Proceedings of the National Academic Sciences, USA*, **105**, 10444–10449.

Severance EG, Szmant AM, Karl SA (2004) Microsatellite loci isolated from the Caribbean coral, *Montastraea annularis*. *Molecular Ecology Notes*, **4**, 74–76.

Stat M, Carter D, Hoegh-Guldberg O (2006) The evolutionary history of Symbiodinium and scleractinian hosts—symbiosis, diversity, and the effect of climate change. *Perspectives in Plant Ecology Evolution and Systematics*, **8**, 23–43.

Stat M, Morris E, Gates RD (2008) Functional diversity in coral-dinoflagellate symbiosis. *Proceedings of the National Academuc Sciences of the USA*, **105**, 9256–9261.

Sunagawa S, Desantis TZ, Piceno YM *et al.* (2009) Bacterial diversity and White Plague Disease-associated community changes in the Caribbean coral *Montastraea faveolata*. *Isme Journal*, **3**, 512–521.

Suzuki R, Shimodaira H (2006) Pvclust: an R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics*, **22**, 1540–1542.

Tchernov D, Gorbunov MY, de Vargas C *et al.* (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings of the National Academic Sciences, USA*, **101**, 13531–13535.

The_UniProt_Consortium (2008) The Universal Protein Resource (UniProt). *Nucleic Acids Research*, **36**, D190–D195.

Thornhill DJ, Lajeunesse TC, Kemp DW, Fitt WK, Schmidt GW (2006) Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. *Marine Biology*, **148**, 711–722.

Toller WW, Rowan R, Knowlton N (2001) Zooxanthellae of the Montastraea annularis species complex: patterns of distribution of four taxa of Symbiodinium on different reefs and across depths. *Biology Bulletin*, **201**, 348–359.

Townsend JP, Hartl DL (2002) Bayesian analysis of gene expression levels: statistical quantification of relative mRNA level across multiple strains or treatments. *Genome Biology*, **3**, RESEARCH0071.

Voolstra CR, Schwarz JA, Schnetzer J *et al.* (2009) The host transcriptome remains unaltered during the establishment of coral-algal symbioses. *Molecular Ecology*, **18**, 1823–1833.

Warner ME, LaJeunesse TC, Robison JD, Thur RM (2006) The ecological distribution and comparative photobiology of symbiotic dinoflagellates from reef corals in Belize: Potential implications for coral bleaching. *Limnology and Oceanography*, **51**, 1887–1897.

Zheng Q, Wang XJ (2008) GOEAST: a web-based software toolkit for Gene Ontology enrichment analysis. *Nucleic Acids Research*, **36**, W358–W363.

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Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Recovery of relative *Fv/Fm* values of Montastraea fragments during acclimation to the experimental tanks.

Fig. S2 Reflectance measurements and best statistical *Fv/Fm* models for each period.

Fig. S3 Host genotyping using published microsatellite markers.

Fig. S4 Maximum-likelihood-based phylogenetic tree of *Symbiodinium* spp. based on ITS2 sequences.

Table S1 Linear models used to test for differences between colonies and tanks. Raw and adjusted p-values are shown for tanks and colonies or fragments.

Table S2 Parameters of statistical models for *Fv/Fm* during each experimental period are shown with the best model determined by a comparison of R^2 and p-values.

Table S3 Results of gene expression analyses.

Appendix S1 Supplementary material.

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