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
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 coraldinoflagellate 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) photosynthetic 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 fiberglass 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 \((F_{o}/F_{m})\) 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 \(\mu\text{mol m}^{-2} \text{s}^{-1}\). 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 \(F_{o}/F_{m}\) (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 \(F_{o}/F_{m}\) throughout the acclimation period. In contrast, middle fragments in the single host genotype experiment experienced a significant reduction in \(F_{o}/F_{m}\) 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 \(F_{o}/F_{m}\) 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 \(\mu\text{mol m}^{-2} \text{s}^{-1}\). 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 \(\mu\text{mol m}^{-2} \text{s}^{-1}\). 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 \(F_{o}/F_{m}\) 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 ITSInfor2 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...
trees were created according to average linkage and
and B>T.
terns, i.e.: B>M>T, B<M<T, B = M>T, B = M<T,
further grouped into 12 possible gene expression pat-
differentially expressed under these conditions were
both control and stressed conditions were tested: T>M,
statistical tests were performed: control>stressed, con-
correlations to non-overlapping 95
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 proba-
bility 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 conserva-
tive 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, con-
trol<stressed, control<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
infinitely differentially expressed under these conditions
were further grouped into 12 possible gene expression pat-
terns, 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 per-
formed using TIGR TMVEV 4.0 (Saed et al. 2003). Array
trees were created according to average linkage and
Euclidean distance metric on log2 ratios of signal intens-
ties, 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 hierar-
chical clustering. The stability of clusters was tested
using the R-package pvclust by calculating approxi-
mately unbiased p-values via multiscale bootstrap re-
sampling: a measure that has been demonstrated to
provide a better indicator for cluster support than regu-
lar 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 signific-
cance cutoff was set to α = 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 experi-
ment (Fig. 1A, Table S1). A significant decrease in
Fv/Fm in previously acclimated coral fragments was
observed four days after increasing the water tempera-
ture. The total duration of the thermal stress treatment
was 10 days and the difference in Fv/Fm became insig-
nificant 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. 1B, 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 aver-
ages in colonies C1 and C2 were significantly different
from those in colonies C3, C4, and C5 during the con-
trol 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 geno-
types (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

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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. Fo/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 Fo/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 RFLP 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

Fig. 1 Relative Fo/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. Fo/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).
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.

<table>
<thead>
<tr>
<th>Fragment(s)</th>
<th>18S rRNA RFLP</th>
<th>ITS2 sequencing</th>
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<tbody>
<tr>
<td></td>
<td>1°</td>
<td>2°</td>
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<tr>
<td>C1</td>
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<td>C2</td>
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<tr>
<td>C3</td>
<td>C</td>
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<td>C4</td>
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<tr>
<td>C5</td>
<td>B</td>
<td>—</td>
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<td>B1–3</td>
<td>A</td>
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<tr>
<td>B4</td>
<td>B</td>
<td>A</td>
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<tr>
<td>B5</td>
<td>B</td>
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<tr>
<td>R1–5</td>
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<td>Top 1,3,6</td>
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<td>Top 4</td>
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<td>Middle 6</td>
<td>C</td>
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<td>Bottom 1–6</td>
<td>C</td>
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</table>

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...
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 \textit{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 \textit{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 \textit{Symbiodinium} factors affect dark-adapted maximum quantum yields (\(F_{v}/F_{m}\))

Maximum quantum efficiencies in \textit{Symbiodinium} sp. type C7-associated with \textit{Montastraea faveolata} were lower compared to the control tree (Fig. 3B top panel). This pattern is consistent with the PAM data showing that \textit{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.
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 housed 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 colonies containing different *Symbiodinium* types. We used hierarchical clustering and pattern analysis to identify genes that were differentially expressed between top, middle, and bottom control and stressed fragments. The results showed that gene expression changes were linked to *Symbiodinium* type and thermal stress conditions. The discrepancies between our study and previous studies suggest that host contributions and previous conditions play a significant role in the response to thermal stress.
The symbiont genotype had a substantial influence on regime, etc. However, we provide strong evidence that B=M<T and B=M>T. Cluster Clone ID vs. 0.35

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 yielded a low number (about 2% of all assayed genes

**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).

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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. favobata* 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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M.K.D. works on the molecular basis of coral bleaching, S.S. is interested in the diversity and variability of coral-associated microbes. P.L.F. and R.I.P. investigate the physiology of *Symbiodinium*. C.R.V. is interested in evolutionary genomics, adaptation and symbiosis, and M.M. works on a wide range of topics regarding the ecology and evolution of marine organisms.

**Supporting information**

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

**Fig. S1** Recovery of relative $F_v/F_m$ values of Montastraea fragments during acclimation to the experimental tanks.

**Fig. S2** Reflectance measurements and best statistical $F_v/F_m$ 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 $F_v/F_m$ 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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