MOLECULAR ECOLOGY

Molecular Ecology (2013) **22**, 4322–4334 doi: 10.1111/mec.12390

Gene expression under chronic heat stress in populations of the mustard hill coral (*Porites astreoides*) from different thermal environments

C. D. KENKEL,* E. MEYER† and M. V. MATZ*

*Section of Integrative Biology, The University of Texas at Austin, 1 University Station C0990, Austin, TX 78712, USA, †Department of Zoology, Oregon State University, 3029 Cordley Hall, Corvallis, OR 97331, USA

Abstract

Recent evidence suggests that corals can acclimatize or adapt to local stress factors through differential regulation of their gene expression. Profiling gene expression in corals from diverse environments can elucidate the physiological processes that may be responsible for maximizing coral fitness in their natural habitat and lead to a better understanding of the coral's capacity to survive the effects of global climate change. In an accompanying paper, we show that *Porites astreoides* from thermally different reef habitats exhibit distinct physiological responses when exposed to 6 weeks of chronic temperature stress in a common garden experiment. Here, we describe expression profiles obtained from the same corals for a panel of 9 previously reported and 10 novel candidate stress response genes identified in a pilot RNA-Seq experiment. The strongest expression change was observed in a novel candidate gene potentially involved in calcification, SLC26, a member of the solute carrier family 26 anion exchangers, which was down-regulated by 92-fold in bleached corals relative to controls. The most notable signature of divergence between coral populations was constitutive up-regulation of metabolic genes in corals from the warmer inshore location, including the gluconeogenesis enzymes pyruvate carboxylase and phosphoenolpyruvate carboxykinase and the lipid beta-oxidation enzyme acyl-CoA dehydrogenase. Our observations highlight several molecular pathways that were not previously implicated in the coral stress response and suggest that host management of energy budgets might play an adaptive role in holobiont thermotolerance.

Keywords: coral, gene expression, metabolism, *Porites astreoides*, thermotolerance

Received 19 February 2013; revision received 6 May 2013; accepted 8 May 2013

Introduction

Gene expression analyses have emerged as a powerful means of assessing variation between individuals and populations (Oleksiak *et al.* 2002; Whitehead & Crawford 2006), evaluating organismal responses to both biotic and abiotic environments (Gasch *et al.* 2000; Evans & Hofmann 2012) and generating new hypotheses (Becker & Feijo 2007; Cui & Paules 2010). This method has proven particularly useful for nontraditional model organisms as it enables investigation of molecular pathways in ecological and evolutionary contexts without the need for traditional genetics manipulations (Aubin-Horth & Renn 2009; Wong & Hofmann 2010; Ekblom & Galindo 2011). Researchers studying reef-building corals have capitalized on this era of transcriptomics, employing gene expression analyses to improve our understanding of how these organisms respond to environmental stress (DeSalvo *et al.* 2008; Bay *et al.* 2009; Rodriguez-Lanetty *et al.* 2009; Polato *et al.* 2010; Seneca *et al.* 2010; Meyer *et al.* 2011).

Reef-building corals are obligate symbiotic organisms, consisting of a Cnidarian host and a dinoflagellate endosymbiont of the genus *Symbiodinium*. The ultimate phenotypic manifestation of a stress response in corals

Correspondence: Carly D Kenkel, Fax: 512 471 3878; E-mail: carly.kenkel@gmail.com

is the breakdown of this symbiotic relationship in the process known as coral bleaching (Glynn 1993). While bleaching can occur in response to a multitude of environmental stressors (Brown & Howard 1985), it has recently received the most attention in the context of thermal stress because of mass mortality following thermal bleaching events and the expected rise of sea surface temperatures under climate change (Brown 1997; Hoegh-Guldberg 1999). Conserved host transcriptomic responses to thermal stress–induced bleaching include up-regulation of heat shock protein (Hsp) activity and antioxidant enzymes; down-regulation of Ca2+ homoeostasis and ribosomal proteins; and changes to cytoskeleton and extracellular matrix proteins (DeSalvo *et al.* 2008, 2010a,b; Csaszar *et al.* 2009; Kenkel *et al.* 2011). Additional investigations into thermal stress responses in aposymbiotic juvenile life history stages also identified up-regulation of Hsp and antioxidant genes as well as cytoskeletal components, further supporting the idea of a conserved thermal stress response (Meyer *et al.* 2009b, 2011; Rodriguez-Lanetty *et al.* 2009; Voolstra *et al.* 2009; Polato *et al.* 2010). In addition to these evolutionarily conserved pathways, two stress responses apparently specific to the coral lineage have been described: differential regulation of GFP-like fluorescent proteins (Dove *et al.* 2006; DeSalvo *et al.* 2008; Smith-Keune & Dove 2008; Bay *et al.* 2009; Kenkel *et al.* 2011) and up-regulation of small cysteine-rich proteins (SCRiPs) (Sunagawa *et al.* 2009).

Characterizing molecular changes that occur in the coral host under thermal stress is critical for understanding how that response is manifested in a symbiotic system; however, it is important to note that corals also exhibit substantial variation in bleaching responses (Marshall & Baird 2000). Some coral species are characterized as thermotolerant, such as *Porites* spp. while others, such as *Acropora* and *Pocillopora* spp., are considered more susceptible to bleaching (Glynn 1984, 1993; Gleason 1993; Hoegh-Guldberg 1999), although recent work suggests that these identities may not be fixed through evolutionary time (van Woesik *et al.* 2011; Guest *et al.* 2012). Stress responses can also vary within a species (Jokiel & Coles 1990; Edmunds 1994). Conspecifics exhibit thermal tolerance limits that vary according to the latitudinal ranges they inhabit (Hughes *et al.* 2003), and even neighbouring individuals can display different bleaching responses to what is apparently the same thermal environment (Ogden & Wicklund 1988). Furthermore, the identity of *Symbiodinium* hosted can affect holobiont stress responses (Baker 2003; Mieog *et al.* 2009; van Oppen *et al.* 2009). However, not all of the variation observed between individuals and species can be attributed to flexibility in the symbiotic partnership. Host–symbiont interactions can also alter the ultimate holobiont phenotype. Different host species colonized by the same symbiont type can develop different holobiont thermotolerance physiologies (Abrego *et al.* 2008), suggesting a host role that warrants further study (Baird *et al.* 2009). Identifying transcriptomic variation between individuals and populations may help shed light on how host responses can affect the variation seen in holobiont thermal stress response phenotypes.

While considerable progress has been made in characterizing the molecular response to thermal stress in coral hosts, transcriptomic variation potentially underlying phenotypic variation observed among coral populations has not received much attention, but see Bay *et al.* (2009) and Morgan *et al.* (2005). To our knowledge, only two studies exploring population-level expression variation in corals in response to thermal stress have been published. The first was undertaken in an aposymbiotic larval system (Polato *et al.* 2010). The authors compared thermal stress gene expression responses between *Montastraea faveolata* larvae reared in either Florida or Mexico, obtained by cross-fertilizing gametes from 3 to 4 local adult corals (Polato *et al.* 2010). Early expression patterns in the larvae largely varied by site, with differential regulation of stress response and metabolic genes, although the later differences were primarily driven by temperature treatment (Polato *et al.* 2010). Given that adult *M. faveolata* are known to host a diversity of symbiont genotypes, sometimes within a single host coral, and that the identity of the symbiont can potentially influence expression patterns of the host (DeSalvo *et al.* 2010a), it is prudent to examine host expression in this species in an aposymbiotic state. However, it prevents identification of responses that may arise during the process of bleaching. Most recently, Barshis *et al.* (2013) used global gene expression profiling to evaluate molecular responses to short-term temperature stress in adult symbiotic corals from tidal pools that experience different variability in daily temperature regimes. Previous work showed that adult corals from more thermally variable pools exhibit greater thermotolerance (Oliver & Palumbi 2011). Barshis *et al.* (2013) found that these thermotolerant corals also exhibit constitutively elevated expression of stress response genes such as Hsps, antioxidant enzymes, apoptosis and tumour suppression factors, and innate immune components. They suggested that this mechanism of 'frontloading' transcription might facilitate increased thermotolerance in reef-building corals.

We examined transcriptomic responses to long-term thermal stress in adult populations of the mustard hill coral, *Porites astreoides*, from thermally distinct reef habitats in the Florida Keys. In the accompanying paper (C. Kenkel *et al.*, 2013), we show that inshore origin *P. astreoides* holobionts (the combination of host and symbiont) are more heat-tolerant than conspecifics from

an offshore reef. Over the course of a six-week heat stress experiment, inshore corals maintained elevated symbiont photosynthetic function, bleached less and tended to grow better. These results indicate the presence of local thermal acclimatization or adaptation, which is probably induced by more frequent temperature extremes experienced at inshore reefs. In highly dispersive broadcast-spawning coral species that acquire their symbionts upon recruitment to the local environment, such location-specific thermotolerances can often be attributed to differences in the acquired symbiont strains (van Oppen *et al.* 2001; Ulstrup & van Oppen 2003; Fabricius *et al.* 2004). However, variation in symbiont ITS types was not observed for *P. astreoides* populations used in the present experiment (C. Kenkel *et al.*, 2013). Instead, coral host populations showed significant genetic divergence at neutral loci between locations (Fig. 1), which led us to hypothesize that location-specific thermotolerances in *P. astreoides* may be due to divergence in host-specific traits (C. Kenkel *et al.*, 2013). The present study explores this hypothesis by analysing expression of a number of host genes implicated in stress response, energy metabolism, growth and calcification, in corals from the same two-population six-week heat stress experiment.

Methods

Heat stress experiment

The experiment is described in detail in the accompanying paper (C. Kenkel *et al.*, 2013). Briefly, fifteen colonies of *Porites astreoides* were collected from an inshore patch reef (N 24°35.142, W 81°34.957) and fifteen from an offshore reef (N 24°31.303, W 81°34.605). Colonies were halved and allowed to acclimate in a shaded raceway for 10 days. One half of each colony was then subjected to heat stress conditions, while the other half was left at ambient temperature conditions. The experiment continued for 6 weeks. On average, temperatures were 27.2 °C (± 0.43 °C) in the control tanks and 30.9 °C (± 1.1 °C) in the heated tanks. By the end of the experiment, all coral fragments in the stress treatment (especially those of offshore origin) paled or completely bleached and exhibited significantly diminished growth and symbiont photosystem function, while control fragments remained healthy (C. Kenkel *et al.*, 2013). Tissue samples for RNA isolation were removed from the skeleton using a razor blade, snap-frozen in liquid nitrogen at the end of the six-week temperature treatment and stored at −20 °C for 3 days followed by storage at −80 °C until extraction. Total RNA was extracted using an RNAqueous kit (Ambion). RNA quality was assessed through gel electrophoresis and evaluated based on the presence of the ribosomal RNA bands. Of the 60 original paired samples, 11 inshore/control, 11 inshore/heat, 12 offshore/control and 12 offshore/heat samples were of sufficient quality for gene expression analysis.

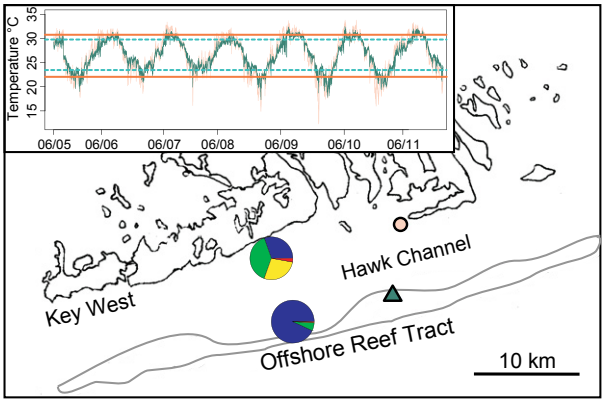

Fig. 1 Map of the lower Florida Keys showing locations of the two sampled coral populations represented as pie charts illustrating genetic structure for the coral host, following C. Kenkel *et al.*, 2013. Offshore reef tract shown in grey. Inset shows hourly temperature data for a representative inshore site and offshore site, marked by the red circle and blue triangle, respectively. Upper and lower solid lines indicate mean June–August and December–February temperatures for the inshore site, while dashed lines indicate means for the offshore site.

Porites astreoides transcriptome

To facilitate gene expression profiling in *P. astreoides*, we constructed a reference transcriptome assembly by sequencing (454 Titanium) cDNA prepared from a single colony unrelated to those used in the stress experiments. Library preparation, assembly and annotation were conducted as previously described (Meyer *et al.* 2009a). Because *P. astreoides* transmits symbionts vertically and therefore does not naturally occur in an aposymbiotic state, some fraction of cDNA sequences was probably derived from the symbionts. To screen for these contaminants, assembled sequences were compared against the Symbiodinium clade A transcriptome (Bayer *et al.* 2012) using BLASTN, and sequences with *e*-values 10^{-5} or lower (2915 total) were excluded. To verify the completeness of the assembly, sequences were further classified by pathway using the KEGG KAAS tool (http://www.genome.jp/tools/kaas/), and the representation of annotated metabolic pathways was compared graphically to the metabolic map inferred from the complete genome sequence of the sea anemone *Nematostella vectensis* using iPath2 (Moriya *et al.* 2007; Letunic *et al.* 2008) (http://pathways.embl.de/, Fig. S1, Supporting information). The annotated transcriptome data were released for unrestricted use prior

to this publication (http://www.bio.utexas.edu/research/matz_lab/matzlab/Data.html).

Gene expression profiling

Expression of candidate stress response genes was measured using qRT–PCR assays designed using Primer3 (Rozen & Skaletsky 2000) based on the annotated transcriptome assembly. Nine genes regulated in response to short-term heat stress (24–48 h) have been described previously (Kenkel *et al.* 2011). Ten additional genes were chosen based on a preliminary RNA-Seq experiment using five stress/control paired samples in inshore and offshore corals. The fragments used for RNA-Seq came from the same common garden experiment but were different from those used in the qRT–PCR part of this study (Table 1). The identified candidate genes were considered long-term stress response candidates given the six-week duration of treatment used in this study. Although RNA-Seq data were unsuitable for rigorous systems-level analysis due to high sampling variance most likely stemming from the insufficient amount of RNA that was available (300 ng of total RNA per sample on average), they were useful for identifying candidate genes for further in-depth validation. The unpublished RNA-Seq data are available on DRYAD (doi:10.5061/dryad.1kn38). RNA samples for qPCR were DNAse-treated and reverse-transcribed into cDNA as in the study by Kenkel *et al.* (2011). qPCRs were performed in the Roche LightCycler 480 (Roche) in 15 μL volumes using 2× LightCycler 480 SYBR Green I Master Mix (Roche), 0.1 μM forward and reverse primers and 1ng of cDNA template. For amplification detection, we diluted SYBR Green 1 Nucleic Acid Gel Stain (Invitrogen) 200-fold and used this dilution as a 100× concentrate, using 1× in each reaction. Preliminary analyses (Cp calling and melting curve analysis) were performed using the GeneScan software (Roche). Each cDNA sample was assayed in duplicate in independent qPCR runs, and these technical replicates were averaged.

Double-gene assays

A double-gene qRT–PCR assay, as introduced by Kenkel *et al.* (2011), measures the fold difference in abundances of two indicator genes exhibiting opposite responses to the factor being measured. The assay does not require any control genes because the template loading factor (sample-specific deviation due to template amount and quality) cancels out when computing the difference in expression of two genes from the same sample (Livak & Schmittgen 2001). The correction for amplification efficiency (Pfaffl 2001) is performed prior

Table 1 Novel long-term stress candidate genes suggested from the pilot RNA-Seq data set of the six-week temperature stress experiment described in this study. Short-term stress (24–48 h) candidate genes were previously described in the study by Kenkel *et al.* (2011)

Gene name	Abbreviation	Primer sequence (5′–3′)	Size (bp)	Efficiency
Acyl-CoA dehydrogenase	ACAD	F: ATGGCCATATCAGCCAACAT R: GCATTAGAAGAGGCGACCAA	101	1.96
Alanine–glyoxylate aminotransferase	AGXT	F: GTGACATGCCTTGACGACAC R: GAAGAGCCGATCAAGATTGC	132	2.04
Apolipophorin	ALP	F: ATTTGGTGGCCTTGTACTCG R: GAAGAGCATTAGCGCCAAAC	116	2.10
Carbonic anhydrase	CA	F: AAAACAGGCCAGTGGTCATC R: ATCGTCCACCATTGGAACTC	138	2.14
Collagen type V1 alpha 1		F: TCACCTTCTGCTCCCTCAGT R: CAAGACGGGTTTAAGGGTGA	137	1.98
Exocyst complex component 4	EXOC4	F: CTCCAGTCCATCCATCCAGT R: TCCACAAGAATTGCAGCATC	123	2.03
Malate synthase		F: TCTGGCAAAACACAGTCAGG R: GCTGCAGTGAACATGGAAGA	131	2.00
Phosphoenolpyruvate carboxykinase	PEPCK	F: CTTTCGCAGGGATTCACATT R: CAGGCACCATCAACACTGAC	104	1.93
Pyruvate carboxylase	PC	F: TGCCGCTCCAAAGTCTTAGT R: TTGAACATCAAGGTGCTTGC	131	1.95
Solute carrier family 26 member 6	SLC26	F: TCTAGTTTGGCTGCGTCCTT R: ATTTGTCTGATGGTGGCACA	149	2.06

Abbreviations as listed in text, oligonucleotide sequences, target amplicon sizes and primer efficiencies as used for qPCR analyses and efficiency correction of raw Cp values for the double-gene assays.

to this computation using the formula $Ca = -Cp \times \log_2(E)$ (Kenkel *et al.* 2011), where Ca is the corrected value corresponding to $\log_2$ of the starting target amount, Cp is the raw expression measurement and E is the amplification factor per PCR cycle measured using a dilution series as in the study by Pfaffl (2001). The anticorrelated responses of the two indicator genes also serve to increase the power and the dynamic range of the assay, improving detection capability (Kenkel *et al.* 2011). We evaluated two pairs of genes that showed opposing expression patterns with respect to bleaching phenotype. Exocyst complex component 4 and a ubiquitin-like protein showed trends of up- and down-regulation, respectively, in pale samples. Their difference values are referred to as the bleaching-in-progress double-gene assay. Phosphoenolpyruvate carboxykinase and a member of solute carrier family 26 showed respective patterns of up- and down-regulation in bleached samples, and their difference values are referred to as the long-term stress double-gene assay.

Statistical analysis

The analysis followed the procedures outlined in the study by Kenkel *et al.* (2011). Briefly, after initial correction of Cp values for amplification efficiency and normalization using three control genes (ND5, RPL11 and EIF3H, Kenkel *et al.* 2011), the data were analysed using linear mixed models on a gene-by-gene basis. Bleaching status, origin and their interaction were modelled as fixed factors. Bleaching status had three levels (normal, pale or bleached), assigned based on coral fragment brightness in the red channel of intensity-normalized photographs (Winters *et al.* 2009). The distribution of coral samples among bleaching status categories (Fig. 2) reflected the result presented in the accompanying paper (C. Kenkel *et al.*, 2013): offshore corals were more susceptible to heat-induced bleaching than inshore corals. Of the samples analysed for gene expression, heat-treated fragments were classified as either 'bleached' (top 50% brightness quantile, $n = 12$) or 'pale' (bottom 50% brightness quantile, $n = 11$, Fig. S2, Supporting information). All fragments that were maintained under control conditions were classified as 'normal' ($n = 23$). The origin factor had two levels, 'inshore' ($n = 22$) and 'offshore' ($n = 24$). The number of biological replicates for each combination of factors ranged from 5 ('bleached inshore') to 12 ('normal offshore'). In all models, colony identity was included as a scalar random factor. An additional random effect of tank was included if model fit was significantly improved with its addition (there were 10 heat-treated and 10 control tanks, to which the fragments were assigned randomly, $n = 3$ fragments per tank). Significance of fixed and random factors was evaluated using likelihood ratio tests (LRT). If the effect of bleaching status was found to be significant at the $P < 0.1$ level following false discovery rate correction using the method of Benjamini & Hochberg (1995), a post hoc Tukey's test was used to evaluate the significance of each pairwise comparison using the function *ghlt* of the *multcomp* package (Bretz *et al.* 2010). All statistical analyses were carried out using R software (R Development Core Team 2013).

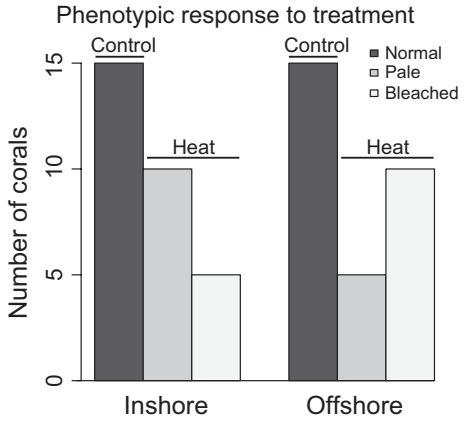

Fig. 2 Bleaching phenotypes at the end of the six-week experimental period. All control fragments were designated 'normal'. Heat-treated fragments were divided into 'pale' and 'bleached' categories based on coral fragment brightness in the red channel of intensity-normalized photographs. Of these 60 samples, 22 inshore (11 normal, 6 pale, 5 bleached) and 24 offshore (12 normal, 5 pale, 7 bleached) yielded a sufficient quality and quantity of RNA for qPCR analyses.

Results

P. astreoides transcriptome

Sequencing yielded 291 044 total reads of which 258 482 were assembled into contigs comprising 31 663 isogroups (an isogroup is a collection of sequences originating from the same gene). Most isogroups (30 393) included a single contig. The total length of the assembly, not including singletons, was 16.3 million bases (Mb). Large contigs (≥500 bp) accounted for more than half (54%) of the assembly; the average length of large contigs was 840 bp, and 90.3% of their consensus quality scores were at least 40 (i.e. an error rate of less than one in 10 000 bases). Symbiont-derived isogroups (2915 total) were excluded from further analysis based on a BLAST match to the *Symbiodinium* clade A transcriptome (Bayer *et al.* 2012).

Pathway annotation of this data set using the KEGG KAAS tool resulted in 3378 unique annotations of which 2156 could be mapped to KEGG reference pathways. Visualization using iPath2 revealed that these

account for the overwhelming majority of core metabolic functions that are represented in a fully sequenced genome of the sea anemone *Nematostella vectensis*, the only other representative of class Anthozoa currently available in the KEGG database (Fig. S1, Supporting information). This suggests the transcriptome produced in this study provides a relatively complete reference for the analysis of metabolic changes in the coral host.

The *P. astreoides* transcriptome contained two enzymes involved in the glyoxylate cycle: isocitrate lyase (IL) and malate synthase (MS). This pathway, which is present in prokaryotes, protists, plants and fungi (but was secondarily lost in most multicellular animals Kondrashov *et al.* 2006), allows for the synthesis of carbohydrates from the products of lipid beta-oxidation (Voet & Voet 2004). Expression of these genes has been reported in aposymbiotic coral larvae of *Acropora millepora* (Meyer *et al.* 2009a) as well as in adult and larval *Acropora palmata* in response to thermal stress (DeSalvo *et al.* 2010b; Polato *et al.* 2013), in addition to being present in the *Acropora digitifera* genome (Shinzato *et al.* 2011). IL and MS activity is considered a signature of the glyoxylate cycle as these enzymes are specific to this pathway and essential for its function (Voet & Voet 2004). Expression of these genes in two families of scleractinian corals suggests that these animals have retained this functional pathway.

Responses of individual genes

Of the nine candidate genes chosen based on previous studies of short-term thermal stress in *P. astreoides* (24–48 h, Kenkel *et al.* 2011), two showed significant down-regulation under heat treatment, irrespective of bleaching status: adenosine kinase (ADK, $P < 0.05$, LRT) and Hsp90 ($P < 0.001$, LRT, Fig. 3). Samples that paled under heat treatment exhibited 2-fold reduced expression of ADK ($P < 0.05$, Tukey's HSD) and a 2.4-fold reduction in Hsp90 ($P < 0.001$, Tukey's HSD). Samples that bleached reduced the expression of ADK by 2.1-fold ($P < 0.01$, Tukey's HSD) and Hsp90 by 1.6-fold ($P < 0.01$, Tukey's HSD). Expression of these two genes did not differ significantly between pale and bleached samples (Fig. 3). No differences were observed with respect to origin or the status by origin interaction for these short-term stress candidates.

The ten long-term stress candidates selected from the exploratory RNA-seq analysis exhibited differential regulation with respect to both origin and treatment when tested in remaining experimental samples using qPCR. Three candidate genes exhibited differential regulation in response to heat treatment that varied with respect to bleaching status of the sample. Expression of a carbonic anhydrase decreased under heat stress ($P < 0.05$, LRT) with a down-regulation of 5.3-fold on average ($P < 0.05$, Tukey's HSD) in bleached samples relative to controls ('normal', Fig. 3). This result appears to be primarily driven by expression in offshore samples, although no significant interaction terms were detected for any of these long-term stress candidates. Phosphoenolpyruvate carboxykinase (PEPCK) showed a pattern of up-regulation, with a 4.8-fold difference in expression between control and bleached samples ($P < 0.01$, Tukey's HSD, Fig. 3). A member of solute carrier family 26 (SLC26) showed the strongest response of any gene. Pale samples were distinguishable from controls by an 8.5-fold down-regulation ($P < 0.01$, Tukey's HSD, Fig. 3). Bleached samples were also differentiated from pale samples by an additional 10.9-fold down-regulation ($P < 0.01$, Tukey's HSD). The full extent of SLC26 down-regulation in bleached samples relative to normal samples was 92-fold ($P < 0.001$, Tukey's HSD).

Three genes showed significant up-regulation in inshore origin samples in comparison with offshore origin samples: collagen type IV by 2.3-fold ($P < 0.1$, LRT), pyruvate carboxylase by 1.4-fold ($P < 0.1$, LRT) and acyl-CoA dehydrogenase by 4.9-fold (ACAD, $P < 0.05$, LRT, Fig. 3). Five candidate genes were selected based on their putative involvement in lipid oxidation and gluconeogenesis: ACAD, malate synthase, alanine–glyoxylate aminotransferase (AGXT), pyruvate carboxylase and PEPCK. Notably, all of these metabolic genes, except for malate synthase, showed a trend of elevated expression in inshore samples ($P < 0.05$ prior to FDR correction), even under control conditions ('normal', Fig. 3). An additional metabolism-related trend in the same direction is demonstrated by apolipophorin (ALP), which was also expressed at a higher level in inshore corals under control conditions (Fig. 3). However, it is important to note that none of the long-term stress candidates exhibited significant differences with respect to the origin by bleaching status interaction.

Diagnostic double-gene assays

The inverse regulation observed for some pairs of candidates suggested that these genes might be used in diagnostic double-gene assays indicative of chronic thermal stress. We previously described such an assay to measure acute stress in *P. astreoides* based on the consistent up-regulation of Hsp16 and down-regulation of actin (Kenkel *et al.* 2011). For a bleaching-in-progress assay, we selected the ubiquitin-like protein and exocyst complex component 4, as these genes showed a trend of anticorrelated regulation only in pale samples. The large dynamic range of SLC26 renders it an ideal candidate for a diagnostic assay for long-term stress. As its partner, we selected PEPCK, as it was the only gene in our

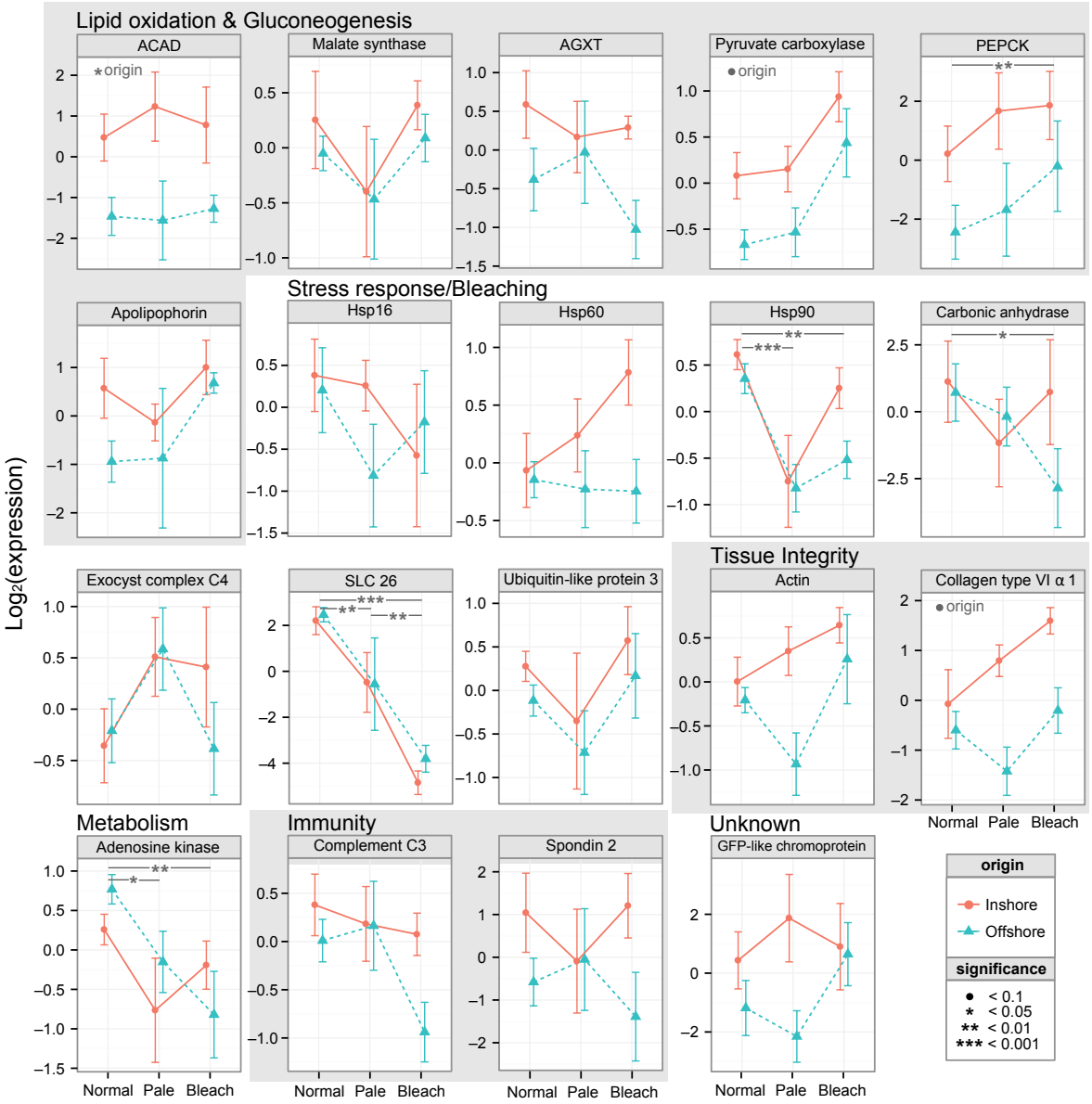

Fig. 3 Gene expression in response to chronic heat stress in populations of *Porites astreoides* from different thermal environments. Normalized $\log_2$-transformed expression values (± SEM) of candidate genes with respect to origin (red circles = inshore, blue triangles = offshore) and bleaching status. Significance of post hoc Tukey's HSD comparisons between bleaching phenotypes is shown for genes with $P < 0.1$ after false discovery rate correction (Benjamini & Hochberg 1995). Candidates with significant origin terms following FDR correction have an 'origin' designation in the panel. ACAD: Acyl-CoA dehydrogenase; AGXT: Alanine–glyoxylate aminotransferase; PEPCK: Phosphoenolpyruvate carboxykinase; Hsp: Heat shock protein; SLC26: member of solute carrier family 26.

panel significantly up-regulated in bleached samples. Both double-gene assays were able to discriminate between samples of different bleaching status (Fig. 4). For the bleaching-in-progress assay, pale samples showed an approximately 3.6-fold increase in the assay's response in comparison with controls and a 4.1-fold increase in comparison with bleached corals (Fig. 4). In the long-term stress assay, pale samples were also differentiated from controls by a 13.5-fold increase in response. Bleached samples showed an additional 31-fold increase relative to pale samples and were distinguishable from controls by a 415-fold increase (Fig. 4).

Discussion

Coral condition had the strongest effect on gene expression patterns reported in this study. In addition, accounting for phenotypic variation of corals (pale or bleached) in response to heat treatment revealed patterns of expression that would have been masked by

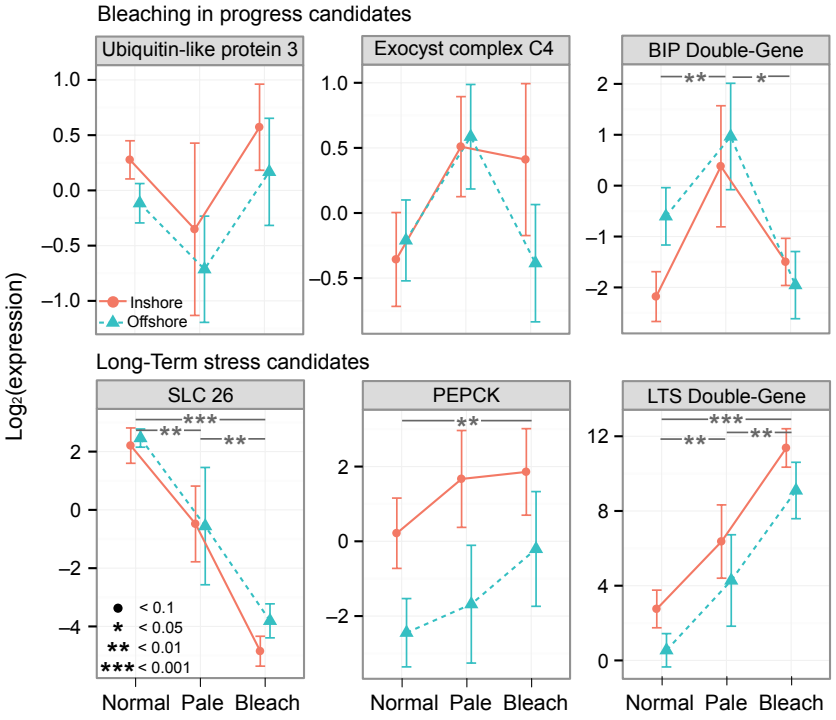

Fig. 4 Development of diagnostic double-gene assays indicative of bleaching-in-progress (top row) and long-term stress (bottom row). The first two panels in each row show log_2-transformed normalized expression values (± SEM) of candidate indicator genes with respect to origin (red circles = inshore, blue triangles = offshore) and bleaching status. The response of a double-gene assay (last panel in each row) is the log2-transformed fold difference in expression between the two indicator genes, which can be computed without the use of additional control genes for normalization.

comparing treatment levels alone (Fig. S3, Supporting information), providing additional insight into differential regulation during the bleaching process. We also observed significant differences in expression between inshore and offshore coral populations, suggesting that these populations have developed differential responses to thermal stress, likely because of differing temperature regimes that occur at these reef sites. Below, we discuss patterns of gene regulation in the context of candidate gene function and relate these functions to coral phenotype and population-level differentiation.

Direct effects of heat stress

Two genes, Hsp90 and adenosine kinase (ADK), showed significant down-regulation in both pale and bleached individuals relative to controls, suggesting that they are directly modulated by thermal stress rather than by bleaching status. Although Hsp90 is a chaperone involved in maintenance of protein structure under stress, it is costly to sustain elevated Hsp expression under chronic stress (Sørensen *et al.* 2003). Its down-regulation under long-term heat stress has been observed in aposymbiotic larvae (Meyer *et al.* 2011), but to our knowledge, this is the first report of a similar pattern of down-regulation of Hsp90 in an adult coral. However, another chaperone, Hsp70, did show a similar pattern of down-regulation in bleached individuals of *Goniopora djiboutiensis* following long-term heat stress (Sharp *et al.* 1997).

Adenosine kinase supports transmethylation reactions involving s-adenosine-methionine: ADK removes free adenosine, the inhibitory by-product of these reactions (Boison *et al.* 2002; Moffatt *et al.* 2002). Methylation of histones facilitates chromatin remodelling during cellular differentiation (reviewed in Mohn & Schübeler 2009), and indeed methylation appears to play a role in the control of metamorphosis and pattern formation in hydrozoa (Berking 1986). If this is the case, ADK could be a marker of new polyp formation associated with normal growth. Therefore, its down-regulation could indicate suppression of host growth under heat stress, although further research is necessary to ascertain the function of this enzyme in corals.

Calcification

A carbonic anhydrase (CA) showed significant down-regulation only in bleached individuals relative to controls. A member of solute carrier family 26 (SLC26) showed the most significant down-regulation both in pale and bleached corals, with pale individuals being intermediate between bleached and control samples. These patterns suggest that these genes do not respond to heat stress directly, but are regulated in response to symbiont loss during bleaching. Their down-regulation most likely represents a signature of decreased calcification. CAs catalyse the reversible hydration of carbon dioxide into bicarbonate and are known to be directly involved in skeletal deposition in scleractinian corals

(Moya *et al.* 2008). Bleaching incurs substantial metabolic costs in corals due to the loss of symbiont-derived nutrition (Muscatine 1990). Down-regulation of calcification could reflect this metabolic deficit, as less energy is available to support calcification. Alternatively, slowing down bicarbonate production may be a means of concentrating CO_2 to support photosynthesis by the symbionts (Weis *et al.* 1989). Whatever the proximate cause, CAs are down-regulated in both adult corals (Edge *et al.* 2005) and aposymbiotic larvae (Meyer *et al.* 2011) in response to temperature stress. As growth was depressed in experimentally heat-treated individuals (C. Kenkel *et al.*, 2013) and most pronounced in fully bleached individuals (data not shown), we hypothesize that expression of CA is reflective of reduced energetic input into skeletal growth.

Strong down-regulation of SLC26 in pale and bleached corals lends further support to this conjecture. Like the carbonic anhydrase family, SLC26 isoforms constitute a large, conserved family of anion exchangers, mediating transport of chloride for bicarbonate, hydroxyl, sulphate, formate, iodide or oxalate with variable specificity (Soleimani & Xu 2006). The best match for the SLC26 used in this study is SLC26-A6 (UniProt, Q0VA12), which functions as a bicarbonate/chloride exchanger in vertebrates (Gray 2004). Notably, the existence of such an exchanger has been postulated in pharmacological studies of coral calcification (Allemand & Grillo 1992), with the role of excreting metabolically generated CO_2 (converted into bicarbonate by CA activity) to the outside of the host cells to make it available for calcification. If SLC26 plays this role, its down-regulation could be linked to a reduced production of metabolic CO_2 during bleaching and/or a reduced supply of CA-generated bicarbonate. In contrast to other metabolic enzymes discussed below, we did not detect constitutive differences in the expression of CA or SLC26 between inshore and offshore populations.

Signatures of bleaching-in-progress

The combined expression patterns of a ubiquitin-like protein (UBL) and exocyst complex component 4 (EXOC4) showed a pattern of differential regulation only in pale individuals. UBLs are part of the eukaryotic ubiquitin family of proteins that are involved in post-translational modification of macromolecules (van der Veen & Ploegh 2012). There are almost 20 members in this protein family and they are involved in a wide range of cellular processes, the most well known being proteasomal degradation. However, ubiquitination does not always lead to degradation; additional roles for this family have been described in endocytosis, cell signalling, membrane-protein trafficking and DNA repair (Hochstrasser 2009). The UBL in this study most closely resembles a membrane-anchored ubiquitin-fold protein, UBL3 (UniProt, Q7ZXN0). Such proteins have been shown to move specific components of the ubiquitination system to the plasma membrane (Dowil *et al.* 2011). Their down-regulation in pale individuals might therefore be indicative of a change in the plasma membrane associated with expulsion of the symbionts or membrane damage from oxidative stress. Conversely, EXOC4 showed a trend of up-regulation in pale individuals. EXOC4 is part of the multiprotein exocyst complex, which is essential for targeting exocytic vesicles to specific docking sites on the plasma membrane (Terbush *et al.* 1996) and therefore has an even more direct logical connection to the process of symbionts' expulsion. Differential expression of exocytosis factors has been previously observed during light-induced bleaching in *Acropora microphthalma* (Starcevic *et al.* 2010).

Structural proteins

Collagen, the main component of the extracellular matrix, and actin, a major cytoskeleton protein, demonstrated very similar regulation patterns: they were both up-regulated in inshore corals. In addition, they both have a tendency towards elevated expression in bleached corals from both reef sites, although this trend was not statistically significant. Their up-regulation during stress is generally unexpected: actin is substantially down-regulated under short-term thermal stress in this species (Kenkel *et al.* 2011), and collagen genes have been previously shown to be down-regulated during UV-induced bleaching in sea anemones (Moya *et al.* 2012). Although the trends of up-regulation of these two structural proteins are in contrast to previous reports, divergence of these responses in corals from different populations warrants their future study.

Up-regulation of gluconeogenesis in bleached corals

The breakdown of symbiosis can incur substantial metabolic costs (Muscatine 1990). Corals in this experiment were subject to 6 weeks of elevated temperature stress in 20 micron filtered seawater without supplemental feeding. In addition, the first signs of bleaching occurred after only 2 weeks of treatment, rendering nutritional deprivation likely, especially in fully bleached individuals.

Phosphoenolpyruvate carboxykinase (PEPCK) showed significant up-regulation only in bleached individuals, although a trend of up-regulation in pale individuals was also observed. PEPCK catalyses the irreversible conversion of oxaloacetate to phosphoenolpyruvate and is a rate-limiting enzyme in gluconeogenesis (Pilkis &

Granner 1992). Pyruvate carboxylase (PC), another gluconeogenesis enzyme, also showed a trend towards up-regulation in bleached individuals. PC catalyses the conversion of pyruvate to oxaloacetate directly preceding the step catalysed by PEPCK in the process of gluconeogenesis. Both PEPCK and PC are known to be up-regulated during starvation in mammals, to facilitate enhanced gluconeogenesis (Tilghman *et al.* 1974; Jitrapakdee & Wallace 1999). Increased expression of these genes in bleached individuals suggests that coral hosts make up for the loss of symbiont-derived nutritional products by converting their internal energy stores to carbohydrates.

The role of the glyoxylate cycle

In contrast to most multicellular animals (Kondrashov *et al.* 2006), corals appear to possess a full complement of glyoxylate cycle enzymes (Meyer *et al.* 2009a; DeSalvo *et al.* 2010b; Polato *et al.* 2013), which is further evidenced by the presence of the two enzymes necessary to complete this pathway, isocitrate lyase and malate synthase, in the *P. astreoides* transcriptome. Malate synthase generates a four-carbon compound (malate) from a pair of two-carbon compounds (glyoxylate and acetyl-CoA), making it possible to channel the product of lipid beta-oxidation (acetyl-CoA) into the gluconeogenesis pathway (Voet & Voet 2004). This gives the organism an ability to synthesize carbohydrates from lipids, which is especially critical for corals because lipids comprise their main energetic stores (Pearse & Muscatine 1971; Davies 1991). The up-regulation of lipid beta-oxidation and regulation of the glyoxylate cycle have been previously proposed as mechanisms of nutritional stress mitigation in coral larvae (Polato *et al.* 2013). Our data suggest that adult corals may also employ this mechanism and that the products of these reactions might be channelled towards gluconeogenesis. Glyoxylate cycle enzymes are induced by starvation in both yeast and plants (Dduntze *et al.* 1969; Graham *et al.* 1994); however, we did not observe up-regulation of malate synthase in response to bleaching, nor did we see an up-regulation of one of the key enzymes in beta-oxidation, acyl-CoA dehydrogenase (ACAD). If our hypothesis is correct, the rate-limiting regulatory step of the chain of events from lipid breakdown to carbohydrate synthesis must lie elsewhere, one probable candidate being PEPCK.

Metabolic differences between populations

Although the strongest gene expression responses observed in our study were with respect to bleaching condition, there were some notable differences with respect to coral origin. All of the metabolic enzymes mentioned in the previous two sections, with the exception of malate synthase, are either constitutively up-regulated or exhibit trends towards up-regulation during bleaching in inshore corals but not in offshore corals. It is tempting to speculate that this pattern is reflective of population-level differences in the utilization of symbiont products. Perhaps inshore corals assimilate symbiont-derived photosynthetic products into long-term energy stores (e.g. lipids) and generally operate on a storage budget involving higher lipid transport, lipid oxidation and gluconeogenesis. Offshore corals, on the other hand, may rely more directly on symbiont-supplied carbohydrates, glycerol and other products. This might render offshore corals more energy efficient under benign conditions but more vulnerable to environmental perturbations that affect symbiont performance (such as heat stress).

Concluding remarks

In an accompanying paper (C. Kenkel *et al.*, 2013), we show that inshore and offshore populations of *P. astreoides* exhibit different holobiont thermotolerances. In addition, coral host populations showed significant genetic differentiation between inshore and offshore reefs, while the genetic composition of resident *Symbiodinium* communities remained constant between inshore and offshore populations and showed no change in the relative frequencies of individual haplotypes ('shuffling', Berkelmans & van Oppen 2006) in response to heat stress (C. Kenkel *et al.*, 2013). Combined with the population-specific host gene expression patterns reported here, these results suggest that the coral host may play a substantial role in maximizing holobiont fitness under local conditions in *P. astreoides*. In particular, constitutive up-regulation of metabolic genes in inshore corals could be indicative of transcriptional 'frontloading', a mechanism by which corals may elevate their thermotolerance in response to periodic stress events (Barshis *et al.* 2013). However, as the genetic marker currently used for symbiont genotyping does not fully reflect variation in their thermal physiology (e.g. Howells *et al.* 2011), we cannot exclude the possibility that symbionts also play a role in holobiont thermotolerance. Additional studies that involve high-resolution genotyping and global gene expression profiling of both symbiotic partners will help clarify the relative contribution of host and symbiont to population-level differences in the holobiont.

Acknowledgements

We extend our thanks to R Wright for completing expression analysis of ACAD and PEPCK. G Aglyamova completed 454

library preparation for sequencing the *P. astreoides* transcriptome. RM Harris and three anonymous reviewers provided valuable commentary on earlier versions of this manuscript. Funding for this study was provided by the National Science Foundation grant DEB-1054766 to MVM and UT Austin's Carl Gottfried Hartmann Fellowship to CDK.

References

Abrego D, Ulstrup KE, Willis BL, van Oppen MJH (2008) Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proceedings of the Royal Society B-Biological Sciences*, **275**, 2273–2282.

Allemand D, Grillo M-C (1992) Biocalcification mechanism in gorgonians: 45Ca uptake and deposition by the Mediterranean red coral Corallium rubrum. *Journal of Experimental Zoology*, **262**, 237–246.

Aubin-Horth N, Renn SC (2009) Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. *Molecular Ecology*, **18**, 3763–3780.

Baird AH, Bhagooli R, Ralph PJ, Takahashi S (2009) Coral bleaching: the role of the host. *Trends in Ecology & Evolution*, **24**, 16–20.

Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology and biogeography of Symbiodinium. *Annual Review of Ecology, Evolution, and Systematics*, **66**, 1–689.

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

Bay 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.

Bayer T, Aranda M, Sunagawa S *et al.* (2012) Symbiodinium transcriptomes: genome insights into the dinoflagellate symbionts of reef-building corals. *PLoS ONE*, **7**, e35269. doi:10.1371/journal.pone.0035269.

Becker JD, Feijo JA (2007) How many genes are needed to make a pollen tube? Lessons from transcriptomics. *Annals of Botany*, **100**, 1117–1123.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate - a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Methodological*, **57**, 289–300.

Berkelmans R, van Oppen MJH (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 the Royal Society B-Biological Sciences*, **273**, 2305–2312.

Berking S (1986) Transmethylation and control of pattern formation in hydrozoa. *Differentiation*, **32**, 10–16.

Boison D, Scheurer L, Zumsteg V *et al.* (2002) Neonatal hepatic steatosis by disruption of the adenosine kinase gene. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 6985–6990.

Bretz F, Hothorn T, Westfall P (2010) *Multiple Comparisons Using R*. CRC Press, Boca Raton.

Brown BE (1997) Coral bleaching: causes and consequences. *Coral Reefs*, **16**, S129–S138.

Brown BE, Howard LS (1985) Assessing the effects of "stress" on reef corals. *Advances in Marine Biology*, **22**, 1–63.

Csaszar NBM, Seneca FO, van Oppen MJH (2009) Variation in antioxidant gene expression in the scleractinian coral *Acropora millepora* under laboratory thermal stress. *Marine Ecology Progress Series*, **392**, 93–102.

Cui Y, Paules RS (2010) Use of transcriptomics in understanding mechanisms of drug-induced toxicity. *Pharmacogenomics*, **11**, 573–585.

Davies PS (1991) Effect of daylight variations on the energy budgets of shallow water corals. *Marine Biology*, **108**, 137–144.

Dduntze W, Neumann W, Atzpodien D, Holzer H, Gancedo JM (1969) Studies on the regulation and localization of the glyoxylate cycle enzymes in Saccharomyces cerevisiae. *European Journal of Biochemistry*, **10**, 83–89.

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.

DeSalvo MK, Sunagawa S, Fisher PL *et al.* (2010a) Coral host transcriptomic states are correlated with Symbiodinium genotypes. *Molecular Ecology*, **19**, 1174–1186.

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

Dove S, Ortiz JC, Enriquez S *et al.* (2006) Response of holosymbiont pigments from the scleractinian coral Montipora monasteriata to short-term heat stress. *Limnology and Oceanography*, **51**, 1149–1158.

Dowil RT, Lu X, Saracco SA, Vierstra RD, Downes BP (2011) Arabidopsis membrane-anchored ubiquitin-fold (MUB) proteins localize a specific subset of ubiquitin-conjugating (E2) enzymes to the plasma membrane. *Journal of Biological Chemistry*, **286**, 14913–14921.

Edge SE, Morgan MB, Gleason DF, Snell TW (2005) Development of a coral cDNA array to examine gene expression profiles in *Montastraea faveolata* exposed to environmental stress. *Marine Pollution Bulletin*, **51**, 507–523.

Edmunds PJ (1994) Evidence that reef-wide patterns of coral bleaching may be the result of the distribution of bleaching-susceptible clones. *Marine Biology*, **121**, 137–142.

Ekblom R, Galindo J (2011) Applications of next generation sequencing in molecular ecology of non-model organisms. *Heredity*, **107**, 1–15.

Evans TG, Hofmann GE (2012) Defining the limits of physiological plasticity: how gene expression can assess and predict the consequences of ocean change. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **367**, 1733–1745.

Fabricius K, Mieog JC, Colin PL, Idip D, van Oppen MJH (2004) Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. *Molecular Ecology*, **13**, 2445–2458.

Gasch AP, Spellman PT, Kao CM *et al.* (2000) Genomic expression programs in the response of yeast cells to environmental changes. *Molecular Biology of the Cell*, **11**, 4241–4257.

Gleason MG (1993) Effects of disturbance on coral communities: bleaching in Moorea, French Polynesia. *Coral Reefs*, **12**, 193–201.

Glynn PW (1984) Widespread coral mortality and the 1982/83 El Nino warming event. *Environmental Conservation*, **11**, 133–146.

Glynn PW (1993) Coral-reef bleaching - ecological perspectives. *Coral Reefs*, **12**, 1–17.

Graham IA, Denby KJ, Leaver CJ (1994) Carbon catabolite repression regulates glyoxylate cycle gene expression in cucumber. *The Plant Cell*, **6**, 761–772.

Gray MA (2004) Bicarbonate secretion: it takes two to tango. *Nature Cell Biology*, **6**, 292–294.

Guest JR, Baird AH, Maynard JA *et al.* (2012) Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. *PLoS ONE*, **7**, e33353. doi:10.1371/journal.pone.0033353.

Hochstrasser M (2009) Origin and function of ubiquitin-like proteins. *Nature*, **458**, 422–429.

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

Howells EJ, Beltran VH, Larsen NW *et al.* (2011) Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change*, **2**, 116–120.

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

Jitrapakdee S, Wallace JC (1999) Structure, function and regulation of pyruvate carboxylase. *Biochemical Journal*, **340**, 1–16.

Jokiel P, Coles SL (1990) Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. *Coral Reefs*, **8**, 155–162.

Kenkel C, Aglyamova G, Alamaru A *et al.* (2011) Development of gene expression markers of acute heat-light stress in reef-building corals of the genus Porites. *PLoS ONE*, **6**, e26914.

Kenkel CD, Goodbody-Gringley G, Caillaud D, Davies SW, Matz MV (2013) Evidence for a host role in thermotolerance divergence between populations of the mustard hill coral (*Porites astreoides*) from different reef environments. *Molecular Ecology*, doi:10.1111/mec.12391.

Kondrashov FA, Koonin EV, Morgunov IG, Finogenova TV, Kondrashova MN (2006) Evolution of glyoxylate cycle enzymes in Metazoa: evidence of multiple horizontal transfer events and pseudogene formation. *Biology Direct*, **1**, 31.

Letunic I, Yamada T, Kanehisa M, Bork P (2008) iPath: interactive exploration of biochemical pathways and networks. *Trends in Biochemical Sciences*, **33**, 101–103.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2 (T)(-Delta Delta C) method. *Methods*, **25**, 402–408.

Marshall PA, Baird AH (2000) Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. *Coral Reefs*, **19**, 155–163.

Meyer E, Aglyamova G, Wang S *et al.* (2009a) Sequencing and de novo analysis of a coral larval transcriptome using 454 GSFlx. *BMC Genomics*, **10**, 219, doi:10.1186/1471-2164-1110-1219.

Meyer E, Davies S, Wang S *et al.* (2009b) Genetic variation in responses to a settlement cue and elevated temperature in the reef-building coral *Acropora millepora*. *Marine Ecology Progress Series*, **392**, 81–92.

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

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. doi:10.1371/journal.pone.0006364.

Moffatt BA, Stevens YY, Allen MS *et al.* (2002) Adenosine kinase deficiency is associated with developmental abnormalities and reduced transmethylation. *Plant Physiology*, **128**, 812–821.

Mohn F, Schübeler D (2009) Genetics and epigenetics: stability and plasticity during cellular differentiation. *Trends in Genetics*, **25**, 129–136.

Morgan MB, Edge SE, Snell TW (2005) Profiling differential gene expression of corals along a transect of waters adjacent to the Bermuda municipal dump. *Marine Pollution Bulletin*, **51**, 524–533.

Moriya Y, Itoh M, Okuda S, Yoshizawa A, Kanehisa M (2007) KAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic Acids Research*, **35**, W182–W185.

Moya A, Tambutte S, Bertucci A *et al.* (2008) Carbonic anhydrase in the scleractinian coral stylophora pistillata: characterization, localization, and role in biomineralization. *The Journal of Biological Chemistry*, **283**, 25475–25484.

Moya A, Ganot P, Furla P, Sabourault C (2012) The transcriptomic response to thermal stress is immediate, transient and potentiated by ultraviolet radiation in the sea anemone Anemonia viridis. *Molecular Ecology*, **21**, 1158–1174.

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,), pp. 75–87. Elsevier, New York.

Ogden JC, Wicklund R (1988) Mass bleaching of coral reefs in the Caribbean: a research strategy. *National Undersea Research Program Research Report No. 88-2*. US Department of Commerce, National Oceanic and Atmospheric Administration, Washington.

Oleksiak MF, Churchill GA, Crawford DL (2002) Variation in gene expression within and among natural populations. *Nature Genetics*, **32**, 261–266.

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

van Oppen MJH, Palstra FP, Piquet AM, Miller DJ (2001) Patterns of coral-dinoflagellate associations in *Acropora*: significance of local availability and physiology of Symbiodinium strains and host-symbiont selectivity. *Proceedings of the Royal Society B-Biological Sciences*, **268**, 1759–1767.

van Oppen MJH, Baker AC, Coffroth MA, Willis BL (2009) Bleaching resistance and the role of algal endosymbionts. In: *Coral Bleaching: Patterns, Processes, Causes and Consequences (Ecological Studies 205)* (eds. van Oppen MJH, Lough JM). Springer-Verlag, Berlin.

Pearse V, Muscatine L (1971) Role of symbiotic algae (zooxanthellae) in coral calcification. *Biological Bulletin*, **141**, 350–363.

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, **29**, e45.

Pilkis SJ, Granner DK (1992) Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annual Review of Physiology*, **54**, 885–909.

Polato NR, Voolstra CR, Schnetzer J *et al.* (2010) Location-specific responses to thermal stress in larvae of the reef-building coral *Montastraea faveolata*. *PLoS ONE*, **5**, e11221. doi: 10.1371/journal.pone.0011221.

Polato NR, Altman NS, Baums IB (2013) Variation in the transcriptional response of threatened coral larvae to elevated temperatures. *Molecular Ecology*, doi:10.1111/mec.12163.

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

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S. & Misener S.), pp. 365–386. Humana Press, Totowa.

Seneca FO, Foret S, Ball EE *et al.* (2010) Patterns of gene expression in a scleractinian coral undergoing natural bleaching. *Marine Biotechnology*, **12**, 594–604.

Sharp V, Brown BE, Miller DJ (1997) Heat shock protein (hsp 70) expression in the tropical reef coral Goniopora djiboutiensis. *Journal of Thermal Biology*, **22**, 11–19.

Shinzato C, Shoguchi E, Kawashima T *et al.* (2011) Using the *Acropora digitifera* genome to understand coral responses to environmental change. *Nature*, **476**, 320–323.

Smith-Keune C, Dove S (2008) Gene expression of a green fluorescent protein homolog as a host-specific biomarker of heat stress within a reef-building coral. *Marine Biotechnology*, **10**, 166–180.

Soleimani M, Xu J (2006) SLC26 chloride/base exchangers in the kidney in health and disease. *Seminars in Nephrology*, **26**, 375–385.

Sørensen JG, Kristensen TN, Loeschcke V (2003) The evolutionary and ecological role of heat shock proteins. *Ecology Letters*, **6**, 1025–1037.

Starcevic A, Dunlap W, Cullum J *et al.* (2010) Gene expression in the scleractinian *Acropora microphthalma* exposed to high solar irradiance reveals elements of photoprotection and coral bleaching. *PLoS ONE*, **5**, e13975.

Sunagawa S, DeSalvo MK, Voolstra CR, Reyes-Bermudez A, Medina M (2009) Identification and gene expression analysis of a taxonomically restricted cysteine-rich protein family in reef-building corals. *PLoS ONE*, **4**, e4865.

R Development Core Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Terbush DR, Maurice T, Roth D, Novick P (1996) The exocyst is a multiprotein complex required for exocytosis in Saccharomyces cerevisiae. *The EMBO Journal*, **15**, 6483–6494.

Tilghman SM, Hanson RW, Reshef L, Hopgood MF, Ballard FJ (1974) Rapid loss of translatable messenger RNA of phosphoenolpyruvate carboxykinase during glucose repression in liver. *Proceedings of the National Academy of Sciences of the United States of America*, **71**, 1304–1308.

Ulstrup KE, van Oppen MJH (2003) Geographic and habitat partitioning of genetically distinct zooxanthellae (Symbiodinium) in *Acropora* corals on the Great Barrier Reef. *Molecular Ecology*, **12**, 3477–3484.

van der Veen AG, Ploegh HL (2012) Ubiquitin-like proteins. *Annual Review of Biochemistry*, **81**, 323–357.

Voet D, Voet JG (2004) *Biochemistry*, 3rd edn. John Wiley & Sons, Inc., Hoboken.

Voolstra CR, Schnetzer J, Peshkin L *et al.* (2009) Effects of temperature on gene expression in embryos of the coral *Montastraea faveolata*. *BMC Genomics*, **10**, 627, doi:10.1186/1471-2164-10-627

Weis VM, Smith GJ, Muscatine L (1989) A "CO2 supply" mechanism in zooxanthellae cnidarians: role of carbonic anhydrase. *Marine Biology*, **100**, 195–202.

Whitehead A, Crawford DL (2006) Variation within and among species in gene expression: raw material for evolution. *Molecular Ecology*, **15**, 1197–1211.

Winters G, Holzman R, Blekhman A, Beer S, Loya Y (2009) Photographic assessment of coral chlorophyll contents: implications for ecophysiological studies and coral monitoring. *Journal of Experimental Marine Biology and Ecology*, **380**, 25–35.

van Woesik R, Sakai K, Ganase A, Loya Y (2011) Revisiting the winners and losers a decade after coral bleaching. *Marine Ecology Progress Series*, **434**, 67–76.

Wong RY, Hofmann HA (2010) Behavioural Genomics: An Organismic Perspective. In: *Encyclopedia of Life Sciences (ELS)*, DOI: 10.1002/9780470015902.a9780470022554. John Wiley & Sons, Ltd, Chichester.

CDK and MVM conceived and designed the long-term stress experiment. CDK performed the experiments and analysed data. EM assembled and annotated the *P. astreoides* transcriptome. CDK, EM and MVM wrote the manuscript.

Data accessibility

P. astreoides transcriptome and pilot RNA-seq data: DRYAD doi:10.5061/dryad.1kn38. Raw qPCR Cp data: DRYAD doi:10.5061/dryad.1kn38. R scripts: DRYAD doi:10.5061/dryad.1kn38.

Supporting information

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

Fig. S1. Mapping of the KEGG-annotated metabolic pathways from the *P. astreoides* transcriptome to pathways annotated in the fully sequenced genome of the sea anemone *Nematostella vectensis*.

Fig. S2. Red channel brightness values in intensity-normalized photographs of heat-treated coral fragments illustrating their classification into "pale" and "bleached" categories.

Fig. S3. Gene expression in response to chronic heat stress in populations of *Porites astreoides* from different thermal environments.