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EPIGENETIC STUDIES IN ECOLOGY AND EVOLUTION

Germline DNA methylation in reef corals: patterns and potential roles in response to environmental change

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Abstract

DNA methylation is an epigenetic mark that plays an inadequately understood role in gene regulation, particularly in nonmodel species. Because it can be influenced by the environment, DNA methylation may contribute to the ability of organisms to acclimatize and adapt to environmental change. We evaluated the distribution of gene body methylation in reef-building corals, a group of organisms facing significant environmental threats. Gene body methylation in six species of corals was inferred from in silico transcriptome analysis of CpG O/E, an estimate of germline DNA methylation that is highly correlated with patterns of methylation enrichment. Consistent with what has been documented in most other invertebrates, all corals exhibited bimodal distributions of germline methylation suggestive of distinct fractions of genes with high and low levels of methylation. The hypermethylated fractions were enriched with genes with housekeeping functions, while genes with inducible functions were highly represented in the hypomethylated fractions. High transcript abundance was associated with intermediate levels of methylation. In three of the coral species, we found that genes differentially expressed in response to thermal stress and ocean acidification exhibited significantly lower levels of methylation. These results support a link between gene body hypomethylation and transcriptional plasticity that may point to a role of DNA methylation in the response of corals to environmental change.

Keywords: climate change, coral, DNA methylation, epigenetics

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Introduction

As human influence on the planet expands, many organisms must acclimatize and adapt to rapid environmental change. Phenotypic plasticity facilitates a more rapid response to environmental change than is possible through natural selection and will likely be critical to the persistence of many species (Charmantier *et al.* 2008; Chevin *et al.* 2010). Phenotypic change often involves modifications in gene expression. Epigenetic mechanisms, involving alterations to the genome that do not affect the underlying DNA sequence, are increasingly recognized as some of the principal mediators of gene expression (Duncan *et al.* 2014). The most researched and best understood epigenetic process is DNA methylation, which most commonly involves the addition of a methyl group to a cytosine in a CpG dinucleotide pair. The role of DNA methylation is best understood in mammals, where methylation in promoter regions has a repressive effect on gene expression (Jones & Takai 2001). In plants and invertebrates, methylation of gene bodies prevails and is thought to be the ancestral pattern (Zemach *et al.* 2010). Gene body methylation appears to have a range of functions, including regulating alternative splicing, repressing intragenic promoter activity and reducing the efficiency of transcriptional elongation (Duncan *et al.* 2014). Methylation of gene bodies also varies according to gene function, and studies on invertebrates indicate that highly conserved genes with housekeeping functions tend to be more heavily methylated than those with inducible functions (Roberts & Gavery 2012; Sarda *et al.* 2012; Dixon *et al.* 2014; Gavery & Roberts 2014). This has led to speculation that gene body methylation may promote predictable expression of essential genes for

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basic biological processes, while an absence of methylation could allow for stochastic transcriptional opportunities in genes involved in phenotypic plasticity (Roberts & Gavery 2012; Dixon *et al.* 2014; Gavery & Roberts 2014).

Direct relationships between DNA methylation and phenotypic plasticity are increasingly being established. Some examples include caste structure in honeybees and ants (Kucharski *et al.* 2008; Bonasio *et al.* 2012), expression of the agouti gene in mice (Wolff *et al.* 1998) and the influence of prenatal maternal mood on newborn stress levels in humans (Oberlander *et al.* 2008). In many cases, changes in methylation patterns can be attributed to external cues such as temperature, stress or nutrition. A prime example is the honeybee *Apis mellifera*, where larval consumption of royal jelly induces changes in methylation that ultimately determine the developmental fate of an individual into a queen or a worker (Kucharski *et al.* 2008). Thus, DNA methylation has been established as a key link between environment and phenotype.

Reef-building corals, the organisms that form the trophic and structural foundation of coral reef ecosystems, are known to display a significant degree of phenotypic plasticity (Todd 2008; Forsman *et al.* 2009; Granados-Cifuentes *et al.* 2013). As long-lived, sessile organisms, corals are thought to be particularly reliant on phenotypic plasticity to cope with environmental heterogeneity, because they must be able to withstand whatever nature imposes on them over long periods of time (Bruno & Edmunds 1997). As phenotypically flexible as they may be, corals' longevity and immobility may also contribute to their vulnerability in a changing environment. Reef corals worldwide are experiencing severe declines due to a variety of anthropogenic effects, including climate change, ocean acidification and a host of local stressors (Hoegh-Guldberg *et al.* 2007). This has raised doubt concerning the ability of corals to survive coming decades. Yet there are also signs that, at least in some cases, corals possess sufficient resiliency to overcome their numerous challenges (Palumbi *et al.* 2014). Recent studies on gene expression variation, for example, support the view that phenotypic plasticity in corals is robust and may provide resilience in the face of ocean warming (Barshis *et al.* 2013; Granados-Cifuentes *et al.* 2013; Palumbi *et al.* 2014). However, the underlying basis of gene expression variation, and indeed phenotypic plasticity, remains largely unknown.

Evaluation of epigenetic processes therefore represents a logical next step in understanding coral gene expression and phenotypic variation. While recent annotation of the *Acropora digitifera* genome revealed a broad repertoire of genes involved in DNA methylation and other epigenetic processes (Dunlap *et al.* 2013), to date, only one study has investigated possible roles of epigenetic processes in corals (Dixon *et al.* 2014). Germline DNA methylation patterns in the transcriptome of *Acropora millepora* corroborated findings reported in studies of other invertebrate species (Dixon *et al.* 2014). Most interestingly, genes that were differentially expressed in response to a common garden transplantation experiment were among the genes exhibiting lower levels of germline methylation (Dixon *et al.* 2014), suggesting a link between hypomethylation and gene expression plasticity.

Coral gene expression studies continue to expand, providing rich data sets to further probe the relationship between DNA methylation and gene function. In this study, we performed a comprehensive evaluation of germline methylation patterns in reef corals by examining the transcriptomes of six scleractinian coral species. Germline methylation levels in these data were inferred based on the hypermutability of methylated cytosines, which leads to a reduction in CpG dinucleotides over evolutionary time (Sved & Bird 1990). These data were then matched with gene ontology information, permitting evaluation of methylation patterns associated with broad categories of biological processes. Lastly, in three of the six species, we evaluated germline methylation patterns in genes involved in response to thermal stress and ocean acidification.

Methods

Transcriptome data sources

The transcriptomes of six scleractinian coral species were evaluated to determine germline methylation patterns in relation to gene function and activity. Species examined included *Acropora hyacinthus*, *A. millepora*, *A. palmata*, *Pocillopora damicornis*, *Porites astreoides* and *Stylophora pistillata* (Table 1). These transcriptomes reflect a range of life history stages. Some transcriptomes were developed from life history stages that had not yet been infected with symbiotic dinoflagellates (*Symbiodinium* spp.), while others used bioinformatic techniques to filter out putative *Symbiodinium* sequences. However, two of the transcriptomes (*P. damicornis* and *S. pistillata*) were developed from adult corals and did not remove putative symbiont sequences. We therefore applied a filtering step to these transcriptomes by comparing them to *Symbiodinium* clade A and B transcriptomes from Bayer *et al.* (2012) using BLASTN (version 2.2.29). An *e*-value threshold of 10^{-5} was used for these queries, and all matched sequences were removed from further analyses. Details of the BLASTN

Table 1 Transcriptomes analysed in this study

Organism	Life history stage	Method	No. contigs	References
Acropora hyacinthus	Adult	Sequencing	33 496	Barshis *et al.* (2013)
Acropora millepora	Embryo to adult	Sequencing	52 963	Moya *et al.* (2012)
Acropora palmata	Larval	Sequencing	88 020	Polato *et al.* (2011)
Pocillopora damicornis	Adult	Sequencing	72 890	Vidal-Dupiol *et al.* (2013)
Porites astreoides	Adult	Sequencing	30 740	Kenkel *et al.* (2013)
Stylophora pistillata	Adult	Sequencing	15 052	Karako-Lampert *et al.* (2014)

query and filtering procedures are provided online (https://github.com/jldimond/Coral-CpG).

Differentially expressed gene data sets

In addition to analysing the whole transcriptomes, we also examined the genes differentially expressed in response to environmental stressors for the three acroporid species. For *A. hyacinthus* and *A. millepora,* these gene sets were derived from the same studies that developed the reference transcriptomes (Moya *et al.* 2012; Barshis *et al.* 2013), and for *A. palmata,* differentially expressed genes sets were reported in Polato *et al.* (2013a,b) (Table 2).

Annotation

To maintain consistency in comparing data sets, all transcriptomes and differentially expressed gene sets were compared to the UNIPROT/SWISS-PROT PROTEIN database (version 2/17/2015) using BLASTX (version 2.2.29) with an *e*-value threshold of 10^{-5}. To further annotate genes with functional categories, corresponding Gene Ontology Slim (GOSlim) biological process terms were joined to the database using SQLSHARE. Annotation workflows are provided in Jupyter notebooks in an online repository (https://github.com/jldimond/Coral-CpG).

Predicted germline methylation

Germline methylation levels were inferred based on the hypermutability of methylated cytosines, which tend towards conversion to thymines over evolutionary time. This results in a reduction in CpG dinucleotides, meaning that heavily methylated genomic regions are associated with reduced numbers of CpGs. Thus, methylation patterns that have been inherited through the germline over evolutionary time can be estimated using the ratio of observed to expected CpG, known as CpG O/E. Germline DNA methylation estimated by analysis of CpG O/E is highly correlated with direct assays of methylation (Suzuki *et al.* 2007; Sarda *et al.* 2012; Gavery & Roberts 2013). CpG O/E was defined as:

$$\text{CpG O/E} = \frac{\text{number of CpG}}{\text{number of C} \times \text{number of G}} \times \frac{l^2}{l} - 1$$

where l is the number of nucleotides in the contig. Only annotated sequences were used for calculation of CpG O/E to increase the likelihood that sequences were oriented in the 5′ to 3′ direction. For subsequent analyses, we set minimum and maximum limits for CpG O/E at 0.001 and 1.5, respectively, to exclude outliers. Details of germline methylation prediction methods are provided in Jupyter notebooks (https://github.com/jldimond/Coral-CpG).

Gene expression levels

For the three acroporid species, we also obtained data on gene expression levels (Moya *et al.* 2012; Barshis *et al.* 2013; Polato *et al.* 2013a,b). Gene expression for a given contig was averaged across treatments. These data were then joined with CpG O/E data, and contigs were sorted into 30 CpG O/E bins of equal sample size. Gene expression levels in each of these bins were then averaged. A log transformation was applied to *A. hyacinthus* and *A. millepora* expression data.

Table 2 Differentially expressed gene sets examined

Organism	Life history stage	Method	No. contigs	Environmental factor	References
Acropora hyacinthus	Adult	Sequencing	484	Thermal stress	Barshis *et al.* (2013)
Acropora millepora	Juvenile	Sequencing	234	Ocean acidification	Moya *et al.* (2012)
Acropora palmata	Larval	Microarray	2002	Thermal stress	Polato *et al.* (2013a,b)

Statistical analyses

Transcriptome CpG O/E patterns were fitted with the *normalmixEM* function in the mixtools package in the R statistical platform. Mixture models were evaluated against the null single-component model by comparison of log-likelihood statistics. High- and low-CpG O/E components were delineated in mixture models using the intersection point of component density curves. For each GOSlim term, enrichment in the high- and low-CpG O/E components identified in mixture models was evaluated with Fisher's exact test. For gene expression counts in the acroporid corals, mean expression level was plotted at the midpoint of each of the 30 bins sorted by CpG O/E. Relationships between expression and CpG O/E were assessed by fitting up to a sixth order polynomial function and selecting the best fit model based on the Akaike information criterion output provided by the *step* function in R. Whole transcriptome and differentially expressed gene CpG O/E distributions were compared with the Kolmogorov–Smirnov test. To compare representation of GOSlim terms in the differentially expressed genes relative to whole transcriptomes, the relative abundance of each GOSlim term in each data set was calculated as a percentage, and these values for whole transcriptomes were subtracted from those for differentially expressed genes. Data files generated for these analyses are available in a repository at doi:10.5061/dryad.pq827, and the analysis workflow is available at https://github.com/jldimond/Coral-CpG.

Results

Annotation

For *P. damicornis* and *Stylophora pistillata* transcriptomes, 2892 and 138 putative *Symbiodinium* sequences were removed from the transcriptomes, respectively. Comparisons of the six coral transcriptomes to the UNIPROT/SWISS-PROT database resulted in annotation of 26–47% of the contigs in each transcriptome (Table 3).

Predicted gene methylation

Whole transcriptome patterns of predicted germline DNA methylation were similar for all coral species, suggesting bimodal distributions of CpG O/E with relatively large high-CpG O/E components (Fig. 1). This was confirmed by mixture model analyses indicating that a 2-component Gaussian model provided better fit than a single-component model in all cases (Table 4). While there was some variability in models between species, all models were characterized by a low-CpG O/E component with a mean of 0.24–0.38 and weighted at 14–28% of the distribution. Conversely, high-CpG O/E components had means of 0.69–0.75 and weights of 72–86%.

When CpG O/E was evaluated according to gene function, we observed similar patterns among species in relation to broad classes of biological processes. For all six species, the top four biological processes with the highest mean CpG O/E were cell–cell signalling, cell adhesion, signal transduction and developmental processes (Fig. 2). Low-ranked biological processes with the lowest mean CpG O/E were more variable between species. However, DNA metabolism was consistently ranked lowest, while protein metabolism and other metabolic processes were also typically among the lowest categories in terms of CpG O/E. Relatively high- and low-ranked biological processes were also more likely to be significantly enriched in the high- and low-CpG O/E components identified in the mixture model.

Relationship between gene expression levels and CpG O/E

In *Acropora hyacinthus*, *A. millepora* and *A. palmata*, we observed similar patterns of gene expression with respect to CpG O/E (Fig. 3). Gene expression in all species was characterized by bell-shaped distributions, with the highest levels of gene expression occurring at intermediate levels of CpG O/E. These curves were skewed to the left in *A. hyacinthus* and to the right in *A. millepora* and *A. palmata*.

Table 3 Transcriptome annotation results

Organism	No. contigs	Contigs annotated (UniProt/Swiss-Prot)	Contigs annotated (GOslim)
Acropora hyacinthus	33 496	11 593	9935
Acropora millepora	52 963	21 026	17 274
Acropora palmata	88 020	35 303	29 450
Pocillopora damicornis	72 890	19 133	16 150
Porites astreoides	30 740	13 788	11 621
Stylophora pistillata	15 052	7061	5812

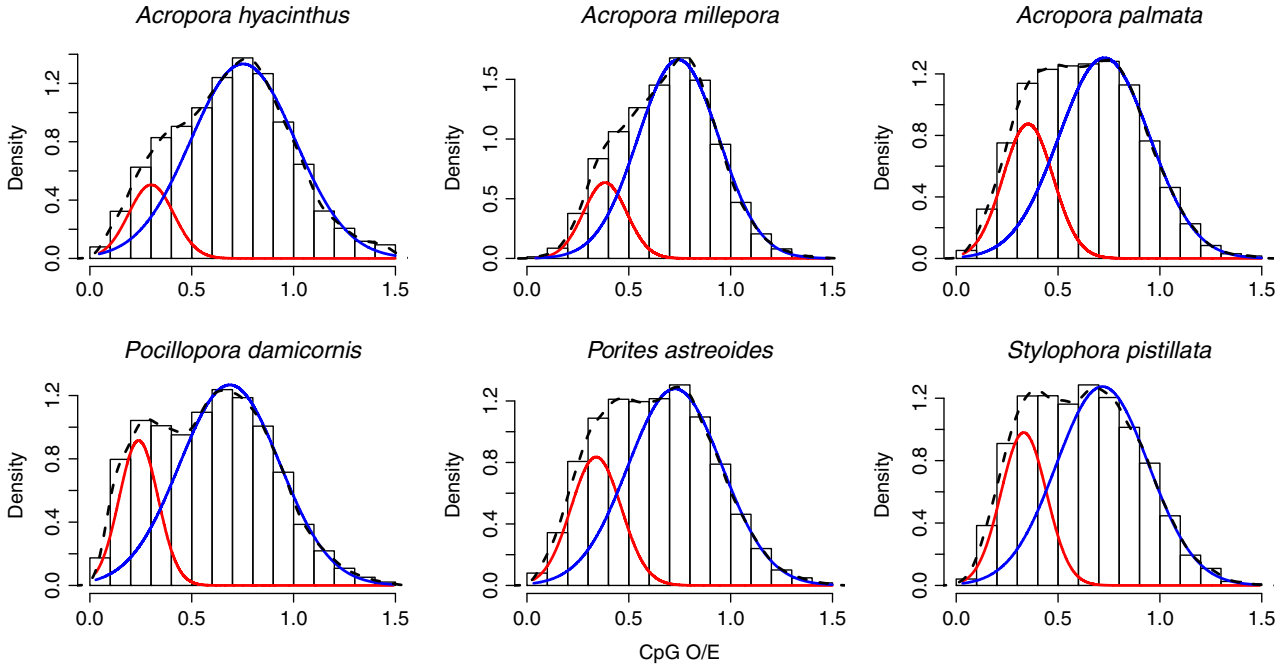

Fig. 1 Transcriptome-wide CpG O/E in the six coral species. The component density curves of two-component mixture models are superimposed over histograms and density curves.

Table 4 Results of mixture model analyses of CpG O/E in the six coral transcriptomes

Organism	Lambda	Mu	Sigma	Log-likelihood (k = 1)	Log-likelihood (k = 2)
Acropora hyacinthus	0.14, 0.86	0.30, 0.75	0.11, 0.26	−1950	−1771
Acropora millepora	0.17, 0.83	0.38, 0.74	0.11, 0.20	1023	1307
Acropora palmata	0.27, 0.73	0.35, 0.73	0.12, 0.22	−2664	−1824
Pocillopora damicornis	0.22, 0.78	0.24, 0.69	0.10, 0.25	−3324	−2464
Porites astreoides	0.26, 0.74	0.34, 0.73	0.12, 0.23	−1450	−1115
Stylophora pistillata	0.28, 0.72	0.33, 0.72	0.11, 0.23	−660	−413

Model statistics are reported for a 2-component mixture model, which provided better fit than a single-component model as indicated by log-likelihood statistics. Numbers separated by commas represent statistics for the first and second components, respectively.

Methylation of differentially expressed genes

In the acroporids, genes expressed differentially in response to environmental stress showed distinct CpG O/E distributions from those of the whole transcriptomes (Fig. 4, upper panel). In all cases, mean CpG O/E of differentially expressed genes was higher than that of the whole transcriptome. This was especially true for differentially expressed genes in response to thermal stress in *A. hyacinthus* and *A. palmata* (both $P < 0.001$), but CpG O/E distributions of ocean acidification differentially expressed genes and the whole transcriptome of *A. millepora* were also significantly different ($P = 0.005$).

We also evaluated the contribution of different biological processes (GOslim terms) to differentially expressed gene profiles by comparing their abundances in differentially expressed genes relative to whole transcriptomes (Fig. 4, lower panel). In *A. hyacinthus*, differentially expressed genes were overrepresented by biological processes associated with the high-CpG O/E components identified in the mixture model and underrepresented by biological processes associated with the low-CpG O/E component. In *A. millepora*, differentially expressed genes were characterized by a striking increase in transport processes, which were not significantly enriched in either the high- or low-CpG O/E components of the mixture model. To a lesser extent, cell adhesion and developmental processes were overrepresented in differentially expressed genes, and these processes were enriched in the high-CpG O/E component. Biological processes underrepresented in differentially

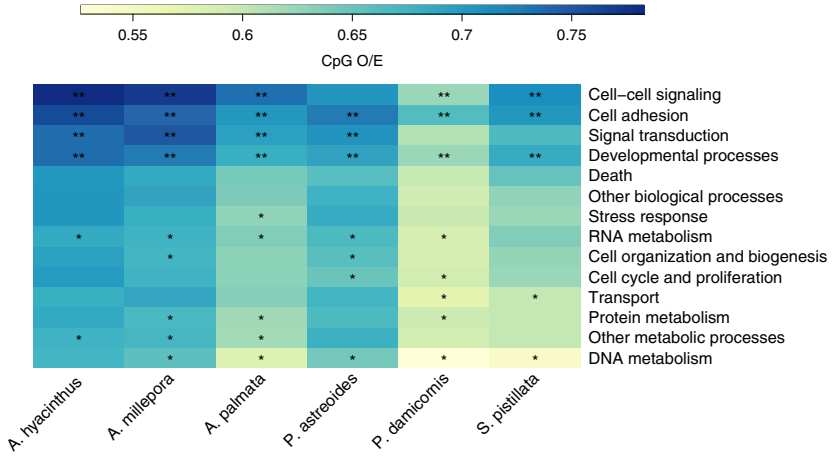

Fig. 2 Patterns of CpG O/E in relation to gene function (GOSlim biological processes terms) in the six coral species. Mean CpG O/E for each term is shown, with terms ordered by the grand mean across species. Asterisks denote results of significant Fisher's exact tests ($\alpha = 0.05$); double asterisks indicate terms that were significantly enriched in the high-CpG O/E component, and single asterisks indicate terms significantly enriched in the low-CpG O/E component.

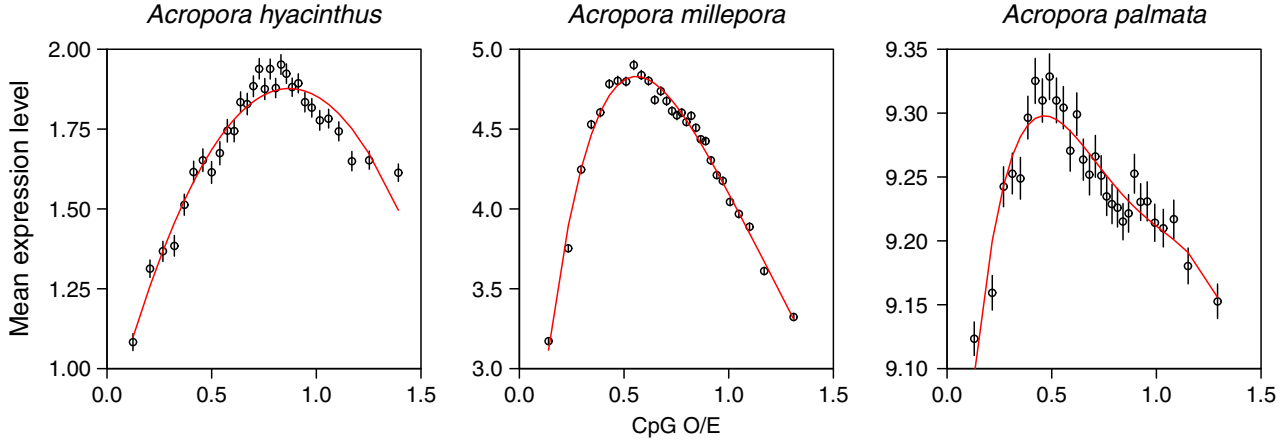

Fig. 3 CpG O/E vs. mean gene expression level. Means (±SE) for 30 bins of equal sample size are shown. Data were fitted with 2nd (*Acropora hyacinthus*) and 4th (*Acropora millepora* and *Acropora palmata*) order polynomial functions (red lines).

expressed genes tended to be associated with the low-CpG O/E component. Finally, in *A. palmata*, a mixture of processes enriched in both the high- and low-CpG O/E components were overrepresented in the differentially expressed genes. Processes underrepresented in differentially expressed genes were largely associated with the low-CpG O/E component.

Discussion

Research on DNA methylation to date has revealed that its extent and function varies considerably among organisms. While several studies have compared DNA methylation patterns between distantly related taxa (Tweedie *et al.* 1997; Zemach *et al.* 2010; Sarda *et al.* 2012), our analysis focused on a single taxonomic group, the reef-building scleractinian corals. Within this group, we took a comparative approach of six species to provide a comprehensive evaluation of germline methylation patterns. In three of these species, representing three independent studies, we found that genes expressed differentially in response to environmental stressors exhibited significantly lower levels of methylation. This work adds to a small but growing body of evidence supporting an association between hypomethylation and gene expression plasticity (Roberts & Gavery 2012; Dixon *et al.* 2014).

As in most other invertebrate taxa surveyed (Gavery & Roberts 2010; Sarda *et al.* 2012), we observed patterns of CpG O/E that were indicative of bimodal distributions in all of the coral transcriptomes. All distributions were dominated by a relatively high-CpG O/E fraction, suggesting that the majority of genes in reef corals experience relatively low levels of methylation. A similar pattern was observed in a genomewide analysis of the sea anemone *Nematostella vectensis* (Zemach *et al.* 2010; Sarda *et al.* 2012), and in an analysis of exons in *A. millepora* (Dixon *et al.* 2014). In contrast, CpG O/E profiles in other invertebrates, such as the oyster *Crassostrea gigas* and the sea squirt *Ciona intestinalis*, suggest

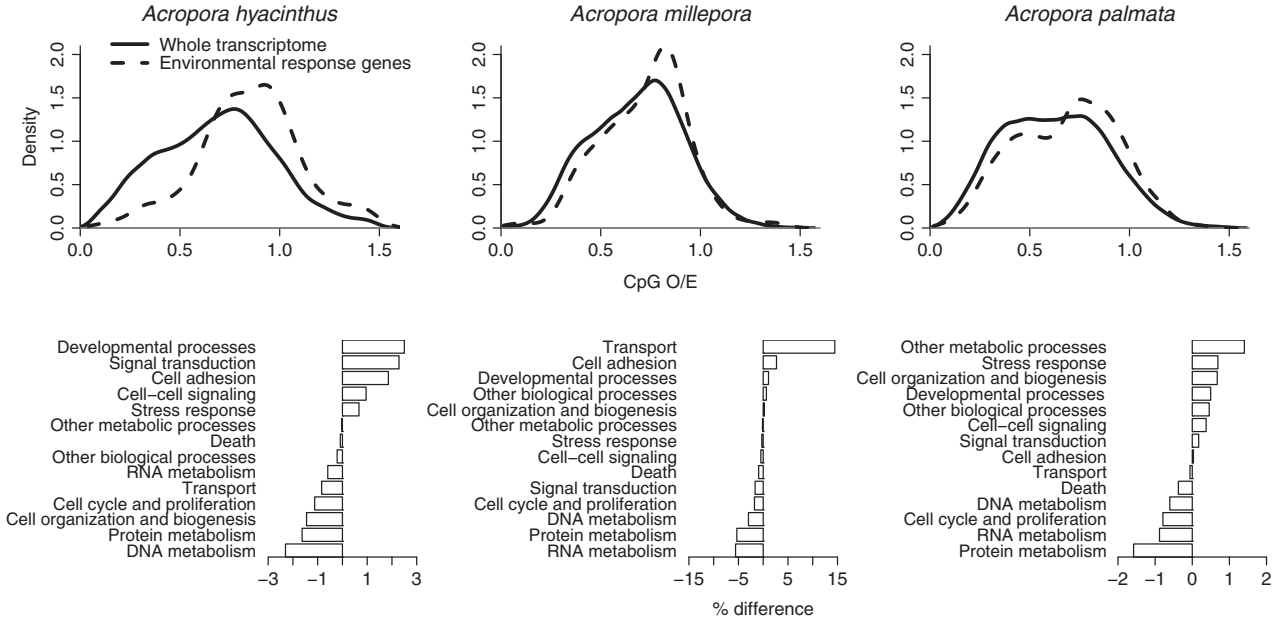

Fig. 4 Comparison of transcriptome-wide and differentially expressed gene CpG O/E in the acroporid corals. Upper panel: density curves of whole transcriptomes (solid lines) and differentially expressed genes (dashed lines). Lower panel: representation of different gene classes in differentially expressed genes relative to whole transcriptome levels. These values were obtained by determining the per cent representation of each of the 14 GOSlim classes in whole transcriptomes and differentially expressed genes, then taking the difference between the two.

larger low-CpG O/E fractions (Gavery & Roberts 2010; Sarda *et al.* 2012). This is consistent with higher genomewide levels of CpG methylation in *C. intestinalis* and *C. gigas* than in *N. vectensis* (21.6%, 15%, and 9.4%, respectively; Zemach *et al.* 2010; Olson & Roberts 2014a). Levels of DNA methylation broadly reflect evolutionary relationships (Tweedie *et al.* 1997; Zemach *et al.* 2010), so it could be speculated that coral methylation is similar to that of *N. vectensis*. However, phylogenies derived from methylation patterns may differ considerably from those derived from protein sequences, suggesting lineage-specific changes in methylation (Sarda *et al.* 2012). Lineage-specific changes in methylation could reflect differences in life history strategies (Sarda *et al.* 2012).

Across reef coral species, ranking of biological processes according to mean CpG O/E was largely consistent, corroborating a trend reported in other studies of invertebrate gene body methylation (Gavery & Roberts 2010; Sarda *et al.* 2012; Dixon *et al.* 2014). With little variation between coral species, the biological processes with high-CpG O/E values (predicted to have low-CpG DNA methylation) reflect genes involved in inducible functions, while processes with low-CpG O/E values reflect genes for essential housekeeping functions. Housekeeping genes are ubiquitously expressed across tissue types and tend to be evolutionarily conserved. Indeed, higher levels of germline DNA methylation are associated with gene orthology among invertebrate taxa (Suzuki *et al.* 2007; Park *et al.* 2011; Sarda *et al.* 2012), suggesting a protective effect that contrasts with the tendency for methylated cytosines to experience higher mutation rates than nonmethylated nucleotides. However, it may be that DNA methylation has an overall protective influence despite the mutagenic effect on CpGs, or that CpG mutations have largely run their course over time in heavily methylated genes, with fewer methylated CpGs left for substitutions (Park *et al.* 2011). One hypothesis for the role of gene body methylation is that it may facilitate consistent expression of ubiquitously expressed core genes needed for essential biological functions (Bird 1995; Roberts & Gavery 2012; Gavery & Roberts 2014).

While there have been some reports of negative associations between gene body methylation and gene expression in invertebrates (Suzuki *et al.* 2007; Riviere *et al.* 2013), positive or bell-shaped relationships have been reported in several taxa, including corals (Zemach *et al.* 2010; Jjingo *et al.* 2012; Gavery & Roberts 2013; Dixon *et al.* 2014). In *A. millepora* (exons only), Dixon *et al.* (2014) reported that the most highly expressed genes in a reciprocal transplant experiment were those exhibiting higher levels of gene body methylation. These genes were enriched for housekeeping functions. A similar phenomenon of increased expression among hypermethylated genes was reported for *C. gigas*, in

addition to an inverse relationship between DNA methylation and variation in expression between tissues (Gavery & Roberts 2013). High transcript abundances among highly methylated genes may reflect their widespread expression across cell or tissue types (Gavery & Roberts 2013; Dixon *et al.* 2014). Our results contrast with these studies, but were surprisingly consistent across the three acroporid species, and suggest that the most highly expressed genes tend to have intermediate rather than high levels of methylation. This is not inconsistent with what has been reported in several invertebrate taxa, including *N. vectensis* (Zemach *et al.* 2010). It is also important to note that the studies from which these acroporid data sets were obtained specifically tested the effects of environmental change, which may have created an abundance of inducible genes with relatively low levels of methylation.

In contrast to hypermethylation and consistent expression, our finding of relatively low methylation among differentially expressed genes in response to thermal stress and ocean acidification in the three acroporid corals supports the hypothesis that hypomethylation is associated with transcriptional plasticity (Roberts & Gavery 2012). Sparse methylation of gene bodies, and the tendency for genes involved in inducible functions to have lower methylation levels, is thought to leave these genes open to a greater variety of transcriptional opportunities (Roberts & Gavery 2012). Potential sources of transcriptional variation could include access to alternative start sites, increased sequence mutations, exon skipping and transient methylation (Roberts & Gavery 2012). Such transcriptional variation could contribute to phenotypic plasticity, and it might be particularly beneficial among species that experience variable environments that require constant tuning of gene expression, such as corals and other sessile organisms. Dixon *et al.* (2014) found support for this theory in *A. millepora*, showing that genes differentially expressed in response to transplantation to new environments were significantly enriched in the low methylation (high-CpG O/E) component.

In our analysis, lower methylation among differentially expressed genes was at least partially attributable to increased representation of genes involved in inducible functions relative to those with housekeeping functions. This was especially true in *A. hyacinthus*. While unsurprising, this highlights the caveat that our analysis is only correlative. Low methylation among differentially expressed genes could simply reflect the fact that environmental stressors promote expression of inducible genes and that these genes simply happen to exhibit different methylation patterns. Furthermore, it remains unclear whether gene body DNA methylation actively regulates genes, as opposed to the alternative hypothesis that it is simply a by-product of an open chromatin state (Jjingo *et al.* 2012). For example, gene body methylation may reflect exposure of DNA to DNA methyltransferases (DNMTs) as a consequence of unpacked chromatin during transcription (Jjingo *et al.* 2012). Further studies will be needed to evaluate causative relationships between DNA methylation and transcriptional activity, such as through inhibition or knockdown of DNMTs responsible for de novo methylation, or through assessment of chromatin state using methods such as ChIP-seq. Additionally, despite the utility of CpG O/E to infer germline methylation patterns, experimental work on transient methylation in somatic cells and how it might influence transcription is needed. However, experimental studies on social insects have already provided compelling evidence for links between differential DNA methylation, transcription and phenotypic plasticity (Kucharski *et al.* 2008; Elango *et al.* 2009). Studies of DNA methylation in plants have gone even further, having already incorporated epigenetics into ecological research (Bossdorf *et al.* 2008).

There is a great deal of interest in the adaptive potential of corals in the face of continued environmental change. Perhaps the most intriguing aspect of DNA methylation is that its effects may extend beyond an individual organism's lifetime and be transferred to successive generations. Although the epigenomes of some organisms, such as mammals, are extensively reprogrammed during meiosis and embryogenesis, in some cases DNA methylation can be passed on to offspring (Duncan *et al.* 2014). This is especially true in plants (Hauser *et al.* 2011; Heard & Martienssen 2014), but there is also evidence for inheritance of DNA methylation in oysters (Olson & Roberts 2014b). Transgenerational epigenetic inheritance is a potentially transformative biological concept, but its extent and significance is only just beginning to be understood, and remains largely unstudied in the vast majority of organisms (Grossniklaus *et al.* 2013; Heard & Martienssen 2014).

Our results illustrate patterns of gene body DNA methylation that are similar across coral species and largely consistent with what has been found in other invertebrates. This work serves as a basis with which to further characterize and compare DNA methylation in corals and related taxa. Within the context of environmental challenges faced by corals, our analysis found broad support for an inverse relationship between gene body methylation and differential gene expression. This suggests that the potential role of DNA methylation in the response of corals to environmental change warrants closer investigation.

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J.L.D. conceived and designed the study, performed the analyses and drafted the manuscript. S.B.R. conceived and designed the study, analysed the data and contributed analysis tools.

Data accessibility

This study made use of publicly available transcriptome data. Data files derived from the original data and generated for this study are available at Dryad under doi:10.5061/dryad.pq827. The analyses can be reproduced using a repository with the complete workflow at https://github.com/jldimond/Coral-CpG.

Original data sets were obtained from several sources. *Acropora hyacinthus* data were obtained from Barshis *et al.* (2013), available at http://palumbi.stanford.edu/data/33496_Ahyacinthus_CoralContigs.fasta.zip (transcriptome assembly) and http://palumbi.stanford.edu/data/Barshis_etal_33496contigs_deduped_all_scaledounts_Nov2012.txt (gene expression counts). *Acropora millepora* data were obtained from Moya *et al.* (2012), available at the NCBI TRANSCRIPTOME SHOTGUN ASSEMBLY Database under Accession nos JR970414–JR999999 and JT000001–JT023377 (transcriptome assembly) and the NCBI GENE EXPRESSION OMNIBUS (GEO) database under Accession no. GSE33016 (gene expression counts). *Acropora palmata* transcriptome data were obtained from Polato *et al.* (2011, 2013a,b), located at https://usegalaxy.org/datasets/cb51c4a06d7ae94e/display?to_ext=fasta (transcriptome assembly) and NCBI GEO database Accession no. GSE36983 (gene expression counts). *Pocillopora damicornis* data were obtained from Vidal-Dupiol *et al.* (2013) at http://2ei.univ-perp.fr/telechargement/transcriptomes/blast2go_fasta_Pdamv2.zip (transcriptome assembly). *Porites astreoides* data were obtained from Kenkel *et al.* (2013) and downloaded from http://www.bio.utexas.edu.offcampus.lib.washington.edu/research/matz_lab/matzlab/Data.html (transcriptome assembly). *Stylophora pistillata* data were obtained from Karako-Lampert *et al.* (2014) and downloaded from http://data.centrescientifique.mc/Data/454Isotigs.fas.zip (transcriptome assembly).