



Genotype structures DNA methylation variation while acute thermal exposure induces transient changes in the stony coral *Acropora cervicornis*

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ABSTRACT

Rapid ocean warming and increasingly frequent marine heatwaves pose a major threat to reef-building corals, yet some thermally sensitive taxa exhibit pronounced phenotypic plasticity and recovery following acute stress. Epigenetic regulation, particularly DNA methylation, has been implicated in coral acclimatization, but its role during the earliest stages of thermal stress remains poorly understood. Here, we examined fine-scale temporal dynamics of DNA methylation in the Caribbean reef-building coral *Acropora cervicornis* during an acute thermal challenge. Using methylation-sensitive amplified polymorphism (MSAP), we characterized MSAP-detected DNA methylation variation in three coral genotypes exposed to a 13-h thermal pulse peaking 7 °C above ambient temperature. DNA methylation profiles were strongly structured by genotype, with significant genotype-by-treatment interactions, indicating strong genotype-associated differences in MSAP profiles. Although global methylation patterns remained largely stable, heated corals exhibited rapid, time-dependent shifts in methylation state composition. Temporal changes in methylation-state composition were observed during thermal exposure, although the magnitude and direction of responses varied among coral lines. Analyses of methylation-sensitive MSAP fragments identified a subset of fragments consistently contributing to treatment differentiation, exhibiting treatment-associated methylation changes during early stress exposure. Collectively, these findings demonstrate that DNA methylation variation detected by MSAP in *A. cervicornis* can change within hours of acute thermal stress despite strong genotypic structuring, revealing previously unresolved early epigenetic dynamics and suggesting that rapid changes in a subset of MSAP fragments may be associated with early responses to thermal stress.

1. Introduction

As global climate change accelerates, coral reef ecosystems are increasingly exposed to rapid and pronounced environmental shifts, particularly in temperature, pH, and salinity (Hoey et al., 2016; Carpenter et al., 2008). Rising sea surface temperatures and the increasing frequency, intensity, and duration of marine heatwaves pose especially acute threats to reef-building corals, many of which exhibit narrow thermal tolerances. These thermal anomalies frequently precipitate mass bleaching events and other manifestations of physiological stress, contributing to widespread reef degradation (Krämer et al., 2022; Hoegh-Guldberg, 1999; Hughes et al., 2017; Yu et al., 2025; McClanahan et al., 2009).

Bleaching susceptibility varies substantially among coral taxa. Genera such as *Acropora*, *Pocillopora*, and *Millepora* often experience disproportionately high bleaching incidence relative to more thermally tolerant taxa (Baird and Marshall, 2002; Marshall and Baird, 2000). Despite this vulnerability, several of these highly susceptible corals are capable of rapid recovery following acute bleaching events (Hoey et al., 2016; Linares et al., 2011; Palumbi et al., 2014; Oliver and Palumbi, 2011). This apparent paradox has been attributed to high growth rates and pronounced phenotypic plasticity, traits that may enhance resilience to episodic environmental stress.

Phenotypic plasticity allows corals with identical genotypes to modify morphological, physiological, or metabolic traits in response to environmental change, thereby enhancing acclimatization capacity and

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promoting fitness under stressful conditions (Todd, 2008; Pigliucci, 2001; Hoey et al., 2016). At the molecular level, phenotypic plasticity is largely mediated by environmentally responsive regulation of gene expression (Aubin-Horth and Renn, 2009). Epigenetic mechanisms play a central role in this regulatory flexibility, particularly in marine invertebrates and corals inhabiting highly variable environments (Eirin-Lopez and Putnam, 2019; Kim et al., 2024).

Epigenetic modifications (including DNA methylation, histone modifications, and non-coding RNAs) can alter gene expression without altering the underlying DNA sequence and can be maintained across cell divisions (Deans and Maggert, 2015). Among these processes, DNA methylation is the most extensively characterized in marine organisms. This modification involves the addition of a methyl group to cytosine residues, most commonly at CpG dinucleotides, and can influence transcriptional activity, gene body regulation, and genomic stability (Suzuki and Bird, 2008; Kim et al., 2024; Eirin-Lopez and Putnam, 2019). In marine organisms exposed to fluctuating environmental conditions, DNA methylation has been repeatedly implicated in regulating genes involved in metabolism, apoptosis, stress response pathways, and thermal tolerance (Eirin-Lopez and Putnam, 2019; Martell et al., 2025; Lopez-Jimenez et al., 2026).

Empirical evidence across marine invertebrates demonstrates that prolonged and chronic exposure to elevated temperatures induces genome-wide and gene-specific DNA methylation changes. These responses have been documented in taxa such as sea urchins (Rahman et al., 2024) and Pacific oysters (*Crassostrea gigas*) (Arredondo-Espinoza et al., 2021; Wang et al., 2023), highlighting the evolutionary conservation of epigenetic regulation in thermal acclimatization. In corals specifically, DNA methylation has been linked to environmental stress tolerance, phenotypic plasticity, and acclimatization across seasonal and spatial gradients (Liew et al., 2018; Durante et al., 2019; Dixon et al., 2018; Hackerott et al., 2023).

While these studies provide strong evidence that DNA methylation contributes to long-term acclimatization, far less is known about its role during the earliest stages of stress exposure. Experimental work has shown that short-term thermal stress elicits rapid transcriptional responses in corals, suggesting that regulatory reprogramming can occur on the scale of hours (Thomas et al., 2018; Stick et al., 2025; Savary et al., 2021). Such rapid molecular responses may be critical for modulating the severity of stress impacts, facilitating return to homeostasis, or initiating acclimatization trajectories (Manalili et al., 2025; Savary et al., 2021; Stick et al., 2025). However, the epigenetic mechanisms underlying these early responses—particularly the pace and extent of DNA methylation dynamics—remain poorly resolved.

Elucidating the molecular foundations of plastic responses to acute thermal stress is therefore essential for understanding coral persistence in an era of increasing climate variability, identifying early indicators of stress sensitivity, and clarifying mechanisms that support acclimatization (Rodriguez-Casariago et al., 2024; Aubin-Horth and Renn, 2009; Barshis et al., 2013). Although multiple epigenetic processes may contribute, DNA methylation provides a tractable and biologically meaningful entry point due to its established functional relevance and the availability of robust analytical approaches.

Here, we examine fine-scale DNA methylation dynamics in the Caribbean reef-building coral *Acropora cervicornis* during a short-term thermal pulse, capturing hour-scale epigenetic shifts that may be masked in longer-term studies. By characterizing methylation changes across multiple time points during a 13-h thermal challenge, this study assesses the timing and magnitude of early epigenetic responses across the onset, peak, and recovery phases of thermal stress. Our results document rapid changes in DNA methylation variation detected by MSAP, including transient shifts in global methylation-state composition and changes in a subset of MSAP fragments in *Acropora cervicornis* within hours of acute thermal exposure, indicating that DNA methylation variation detected by MSAP is dynamic during early stress and suggesting a potential, but yet unresolved, association between DNA

methylation variation and short-term responses to thermal stress.

2. Methods

2.1. Experimental design and sampling

Acropora cervicornis is a fast-growing, branching scleractinian coral endemic to Caribbean reefs and was historically a dominant reef-building species. Anthropogenic stressors including disease outbreaks (Aronson and Precht, 2001; Williams and Miller, 2005), elevated temperature stress, water pollution, and ocean acidification (Mercado-Molina et al., 2015; Hoegh-Guldberg et al., 2007; Bieler-Fraser et al., 2018; Ladd et al., 2017), have driven severe population declines, resulting in its designation as a highly threatened (Hogarth, 2006). Its ecological importance and sensitivity to environmental stressors make *A. cervicornis* a suitable model for investigating early epigenetic responses to acute thermal stress.

This study was based on a controlled thermal exposure experiment described in [(Hackerott et al., 2026), Table S1]. Briefly, coral fragments (ramets; ~10 cm) representing three distinct genotypes (genets) were collected from the Coral Restoration Foundation Nursery in Tavernier, Florida Keys (24.982715° N, 80.436286° W) and acclimated to experimental aquaria at the Keys Marine Laboratory. The three coral lines analyzed in this study (AC032, AC062, and AC112) correspond to Coral Restoration Foundation (CRF) nursery stock identifiers that were maintained as distinct individuals throughout the experiment. These designations were retained for consistency with the original experimental design described by Hackerott et al. (2026). Control corals were maintained at ambient temperature (26 °C), while heated corals were exposed to a 13-h acute thermal challenge in which temperature increased from 26 °C to a peak of approximately 33 °C over 7 h (approximately 1 °C h⁻¹) before returning to ambient conditions (Fig. 1). Coral tissue samples were collected at five time points during the thermal challenge (hours 1, 4, 6, 10, and 13; Table 1; Fig. 1). At each time point, three biological replicate ramets were sampled for each genotype and treatment combination (3 genotypes × 2 treatments × 3 replicate ramets = 18 samples per time point). Across all five time points, this design resulted in a total of 90 coral samples (5 time points × 2 treatments × 3 genotypes × 3 biological replicates). Samples were flash-frozen in liquid nitrogen and stored at -80 °C until DNA

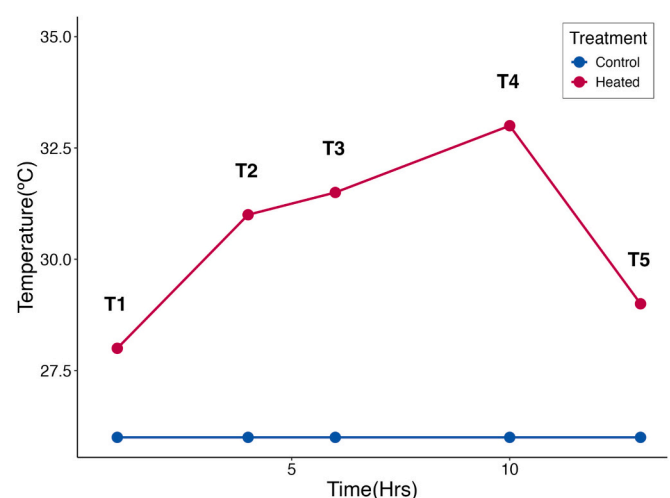


Fig. 1. *Acropora cervicornis* corals were exposed to two treatment conditions as described in (Hackerott et al., 2026), see also Table S1. Heated corals were exposed to increasing, then decreasing temperatures for 13 h, rising from 26 °C to a peak of 33 °C over approximately 7 h before returning to ambient conditions. Corals were sampled at five time points during the thermal challenge (T1–T5), with three biological replicate ramets collected per genotype and treatment at each time point.

Table 1

Adapters and primer sequences used for MSAP applications. Primers for selective PCRs were fluorescently labeled.

PCR Step	Sequence Name	Sequence (5' to 3')	Fluorescent Label
Digestion-Ligation	EcoRI Adaptor Fwd	CTCGTAGACTGCGTACC	
	EcoRI Adaptor Rv	AATTGGTACGAGTCTAC	
	HpaII/MspI Adaptor Fwd	CGTTCTAGACTCATC	
	HpaII/MspI Adaptor Rv	GACGATGAGTCTAGAA	
	EcoRI + C	GACTGCGTACCAATTCC	
Pre-selective A	MspI-HpaII + A	GATGAGTCTAGAACGGA	
Pre-selective B	EcoRI + A	GACTGCGTACCAATTCA	
Selective A1	MspI-HpaII + T	GATGAGTCTAGAACGGT	
	MspI-HpaII-ATC	GATGAGTCTAGAACGGATC	HEX
Selective A2	MspI-HpaII-ACA	GATGAGTCTAGAACGGACA	HEX
	MspI-HpaII-ACT	GATGAGTCTAGAACGGACT	FAM
	MspI-HpaII-AAG	GATGAGTCTAGAACGGAAG	FAM
Selective B1	MspI-HpaII-TCA	GATGAGTCTAGAACGGTCA	HEX
	MspI-HpaII-TAG	GATGAGTCTAGAACGGTAG	HEX
Selective B2	MspI-HpaII-TTG	GATGAGTCTAGAACGGTTG	FAM
	MspI-HpaII-TCT	GATGAGTCTAGAACGGTCT	FAM

extraction.

2.2. Genomic DNA isolation

Genomic DNA was extracted from powdered coral tissue using a modified protocol from the Quick-DNA/RNA Miniprep Plus kit (Zymo Research, Irvine, CA, United States) as in (Rodríguez-Casariago et al., 2020). Approximately 200 mg of powdered tissue was homogenized with zirconia/silica beads, centrifuged, and processed according to the manufacturer's protocol with an added vacuum-drying step prior to elution. DNA integrity was verified by agarose gel electrophoresis, purity assessed spectrophotometrically (NanoVue, GE Healthcare), and concentrations quantified using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific).

2.3. DNA methylation analysis

DNA methylation patterns were assessed using the methylation-sensitive amplified polymorphism (MSAP) technique following established protocols for *A. cervicornis* (Reyna-López et al., 1997; Hackerott et al., 2023; Rodríguez-Casariago et al., 2020; Martell et al., 2025). MSAP uses methylation-sensitive restriction enzymes to generate anonymous methylation profiles across the genome and is well-suited for non-model species lacking fully annotated reference genomes (Reyna-López et al., 1997; Hackerott et al., 2023; Rodríguez-Casariago et al., 2020; Martell et al., 2025).

Standardized DNA (5.5 µL; 36.4 ng/µL) was digested in parallel using *EcoRI/HpaII* and *EcoRI/MspI* restriction enzyme combinations, ligated to adapters (Table 1), and amplified via pre-selective and selective PCR using previously described conditions (Table S2) (Reyna-López et al., 1997). Selective amplification products labeled with fluorescent primers (A1, A2, B1, B2) were submitted for fragment analysis at Florida International University's DNA Core Facility. MSAP profiles were used to assess global and relative methylation dynamics; genomic positions and functional annotations cannot be resolved without sequencing-based approaches.

2.4. DNA methylation variation analysis

Fragment profiles were generated using GeneMapper v.3.7 (Applied Biosystems), retaining peaks ≥ 50 RFU and fragment sizes between 50 and 1500 bp. Presence-absence matrices for *HpaII* and *MspI* digestions were used to assign methylation states with the R package (Perez-Figueroa, 2013): non-methylated (1/1), hemimethylated (1/0), internally methylated (0/1), or hypermethylated/lack of target (0/0). Loci exhibiting 0/0 across all samples within a genotype were removed as uninformative, while polymorphic loci were retained and classified as methylation-susceptible loci (MSL). Because MSAP profiles are based on anonymous restriction fragments and do not provide sequence identity information, the term methylation-sensitive MSAP fragments is used throughout this study to describe the polymorphic markers retained for downstream analyses. Hereafter, these are referred to as MSAP fragments. Downstream analyses were restricted to MSLs. Because band absence (0/0) may arise from either hypermethylation or restriction-site polymorphisms, MSAP profiles cannot completely distinguish between epigenetic and underlying sequence variation. Therefore, fragments showing 0/0 across all samples within a genotype were removed as uninformative, while polymorphic fragments were retained for downstream analyses (Martell et al., 2025). This conservative filtering step reduces, but does not eliminate, the potential influence of restriction-site polymorphisms on interpretation.

Methylation-state composition was analyzed separately for each coral line (Blue, Green, and Yellow; Fig. 4), and treatment-associated changes in methylation-state frequencies were evaluated within each coral line. Multivariate patterns of DNA methylation were visualized using discriminant analysis of principal components (DAPC) implemented in adegenet (Jombart et al., 2010). Permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) was used to test the effects of treatment, genotype, and time on methylation profiles using Gower distances and the adonis2 function in vegan (Oksanen et al., 2022). Gower dissimilarity matrices were calculated using the cluster package (Maechler et al., 2008), because they accommodate categorical data, such as discrete methylation states, without imposing assumptions of Euclidean structure. Additional PERMANOVAs were conducted within individual time points with genotype as a stratifying factor, and post hoc pairwise comparisons were performed using pairwiseAdonis (Martínez Arbizu, 2020).

To assess methylation-state dynamics, proportions of each methylation state were calculated separately for each coral line and visualized across treatments and time points. MSAP fragment contributions to treatment separation were evaluated by examining loadings on the first discriminant axis (LD1); MSAP fragments within the top 95th percentile of LD1 contributions were retained, and temporal changes in their methylation states were visualized for heated and control samples.

3. Results

3.1. Patterns of DNA methylation variation detected by MSAP

Methylation-sensitive amplified polymorphism (MSAP) were used to characterize DNA methylation variation in *Acropora cervicornis* during acute thermal exposure. Across the four selective primer combinations, a total of 2058 loci were detected after classification of hypermethylated profiles and removal of uninformative loci. Of these, 2057 loci (99.9%) were classified as methylation-susceptible loci (MSL), while a single locus (0.01%) was classified as non-methylated (NML), indicating widespread epigenetic variability across the sampled genome. Primer combinations A1, A2, B1, and B2 yielded 671, 687, 539, and 160 MSL, respectively, demonstrating broad genomic coverage.

Overall epigenetic diversity was high, as reflected by Shannon's Diversity Index (SDI) across MSL (mean $\pm$ SD: 0.29 ± 0.21), indicating substantial variability in DNA methylation states among samples. Of the 2057 MSL, 537 loci exhibited polymorphism across samples; following

removal of loci lacking target sites across genotypes, 533 polymorphic MSL were retained for downstream analyses. These polymorphic loci formed the basis for investigating temporal and treatment-related variation in DNA methylation during acute thermal stress.

3.2. Effects of genotype and thermal treatment on global DNA methylation patterns

Multivariate analyses revealed that DNA methylation patterns were strongly structured by genotype. PERMANOVA analyses indicated a significant main effect of genotype ($p = 0.001$) and a significant treatment $\times$ genotype interaction ($p = 0.025$; Table S3), demonstrating that baseline DNA methylation profiles differed among the three previously characterized genotypes (AC032, AC062, and AC112) and that genotypic background influenced methylation responses to thermal exposure. Discriminant analysis of principal components (DAPC) further supported this pattern, with genotypes forming well-defined and distinct clusters along the first two discriminant axes (LD1 and LD2; Fig. 2), indicating clear differentiation in MSAP profiles among genotypes.

Pairwise comparisons corroborated these findings, revealing significant differences in DNA methylation profiles across all genotype pairs ($\alpha \leq 0.05$; Table S4). In contrast, comparisons between heated and control samples within the same genotype were not significant, indicating that genotypic variation accounted for the largest proportion of genome-wide methylation differences. Consistent with this observation, PERMANOVA analyses assessing treatment effects within genotypes showed no significant effect of heating on global DNA methylation profiles ($p = 0.643$) or treatment $\times$ timepoint interactions ($p = 0.742$; Table S3). Despite the absence of statistically significant treatment effects at the genome-wide level, moderate separation between heated and control samples was observable in multivariate space (Fig. 2), suggesting more subtle treatment-dependent methylation shifts.

3.3. Temporal dynamics of DNA methylation during acute thermal stress

To further explore treatment effects over time, DAPC analyses were conducted separately for each time point to visualize temporal changes in DNA methylation patterns between heated and control samples (Fig. 3). These analyses revealed progressive separation of treatment

clusters over the course of the thermal pulse, with increasing divergence from time points T1 through T3 and maximum separation observed at time point T4, immediately prior to the peak temperature exposure. This pattern suggests that DNA methylation differences between treatments emerged rapidly and intensified during the early and mid-phases of thermal stress.

To account for the strong influence of genotype on MSAP profiles, methylation-state composition was analyzed separately for each coral line (Fig. 4). Across genotypes, temporal changes in methylation-state frequencies were observed during thermal exposure, although the magnitude and direction of these responses varied among coral lines. Although global methylation profiles did not differ significantly between treatments at the multivariate level, examination of methylation-state composition within individual coral lines revealed temporal variation in methylation-state frequencies during thermal exposure (Fig. 4). Although genotype-specific comparisons were conducted, no consistent statistically significant treatment-associated differences in methylation-state composition were detected across coral lines. The magnitude and direction of these shifts differed among coral lines, consistent with the strong influence of genotype on MSAP profiles identified in the multivariate analyses.

3.4. Methylation variation in informative MSAP fragments

To identify DNA methylation changes potentially driving the observed separation between treatments, MSAP fragment contributions to variation along the first discriminant axis (LD1) were examined for each time point. Loci within the top 95th percentile of LD1 contributions were extracted, representing loci most strongly associated with treatment-related differences (Fig. 3). Four MSAP fragments were selected for detailed examination because they consistently contributed strongly to treatment differentiation across sampling time points. Temporal methylation trajectories for these fragments were subsequently examined separately within each coral line (Fig. 5).

Temporal analyses of methylation states revealed coral line-specific responses to thermal exposure (Fig. 5). Although treatment-associated methylation changes were observed in multiple coral lines, the magnitude and timing of these responses varied among coral lines, consistent with the strong genotype-associated variation identified in the multivariate analyses.

4. Discussion

In this study, we used MSAP to characterize DNA methylation variation and temporal changes in methylation-sensitive MSAP fragments in the reef-building coral *Acropora cervicornis* during acute thermal stress. Across all samples and time points, approximately two-thirds of methylation-sensitive MSAP fragments were classified as hyper-methylated in both heated and control treatments (Fig. 4), consistent with previous MSAP-based studies in *A. cervicornis* and other Caribbean corals (Hackerott et al., 2023; Rodríguez-Casariello et al., 2020; Martell et al., 2025). This consistency suggests that MSAP-detected DNA methylation variation was broadly stable across treatments, even under thermal stress. However, despite this overall stability, we detected rapid and localized decreases in DNA methylation during the early phases of thermal exposure, highlighting previously unresolved short-term dynamics of the coral epigenome.

Global DNA methylation patterns were primarily structured by genotype, indicating that host genetic background represents a major source of variation in MSAP profiles (Table S3; Fig. 2). Against this strong genotypic signal, we detected more subtle and transient changes in DNA methylation variation associated with acute thermal exposure (Figs. 3–5).

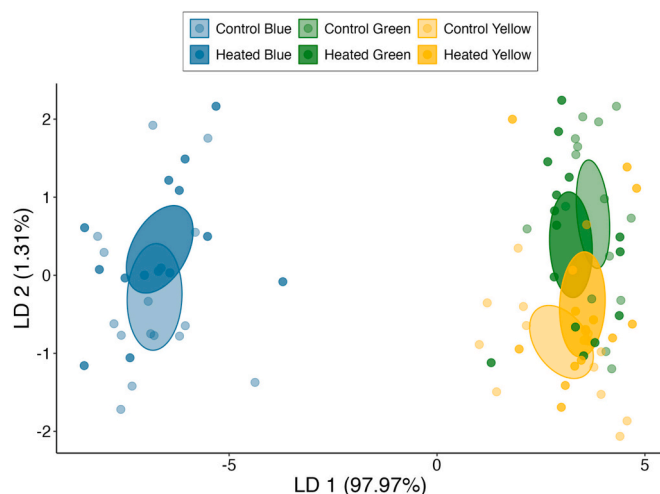


Fig. 2. Discriminant Analysis of Principal Components (DAPC) showing significant differences between the three *A. cervicornis* genotypes studied during the acute thermal stress episode. Each genotype was assigned a different colour (Blue, Green, Yellow). Within each genotype, transparent circles represent the control treatment and opaque circles represent the heated treatment (Hackerott et al., 2023). Ellipses represent the 95% confidence interval of each genotype by treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

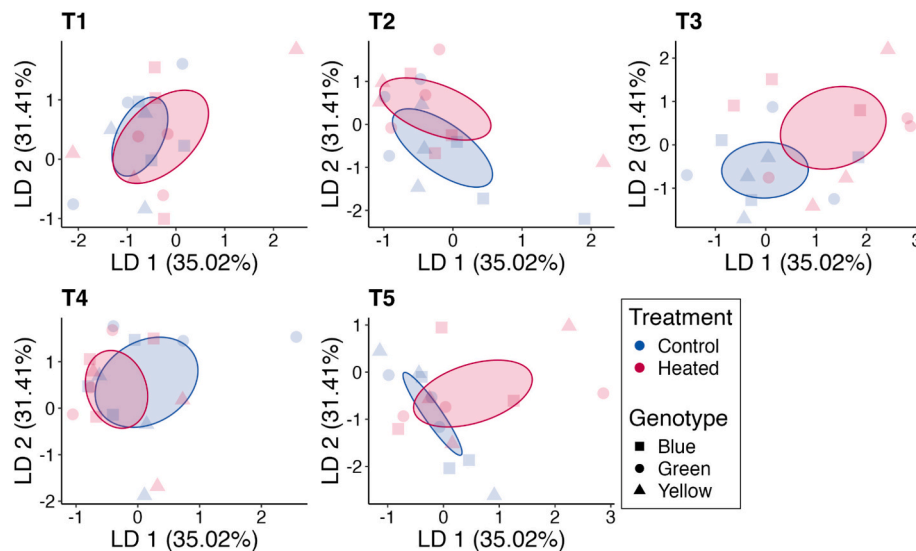


Fig. 3. Discriminant Analysis of Principal Components (DAPC) showing heated and control *A. cervicornis* corals over time points (T1: 1 h, T2: 4 h, T3: 6 h, T4: 10 h, and T5: 13 h). Ellipses represent the 95% confidence interval of each treatment by genotype.

4.1. DNA methylation is structured by genotype and genotype-by-environment interactions

Global DNA methylation patterns differed significantly among genotypes and were influenced by genotype $\times$ treatment interactions (Table S3), consistent with previous studies in *Acropora cervicornis*, *A. palmata*, and *Montastraea cavernosa* (Rodríguez-Casarié et al., 2022; Hackerott et al., 2023; Rodríguez-Casarié et al., 2024; Durante et al., 2019; Drury and Lirman, 2021; Drury et al., 2017; Martell et al., 2025). In multivariate space, genotypes formed well-defined and distinct clusters along discriminant axes (LD1 and LD2; Fig. 2), indicating stable genotype-associated differences in MSAP profiles. Pairwise comparisons further demonstrated that DNA methylation profiles differed significantly among genotypes (Table S4), while heated and control treatments were not significantly different within genotypes at the global level. These patterns underscore that host genetic background explains a substantial proportion of epigenetic variation and can modulate the magnitude and direction of environmentally induced methylation changes.

Importantly, although our filtering strategy reduced the influence of genotype-invariant restriction-site polymorphisms on downstream analyses, the contribution of underlying sequence variation to genotype-specific MSAP profiles cannot be completely excluded. Therefore, the observed differences among genotypes may reflect a combination of genetic background and DNA methylation variation detected by MSAP. In marine invertebrates, DNA methylation variation can be influenced by environmentally responsive regulatory networks and has been associated with shifts that occur alongside genetic and environmental variation (Hackerott et al., 2023; Chang et al., 2023; Zhang et al., 2017; Zhao et al., 2021). In *Acropora* corals, stress-induced changes in enzymes responsible for DNA methylation and chromatin remodeling further support this link between environment and epigenomic regulation (Rodríguez-Casarié et al., 2018; Abbott et al., 2024). Genomic variation has been widely linked to coral stress tolerance, reproductive success, symbiont associations, and bleaching susceptibility (Drury et al., 2019; Durante et al., 2019; Parkinson and Baums, 2014; Baums et al., 2013; Drury and Lirman, 2021). Collectively, these results identify genotype-associated variation as the dominant source of structure in MSAP profiles and emphasize the importance of considering host genotype when evaluating environmentally associated DNA methylation variation (Fig. 2). Given that the magnitude of environmentally induced epigenetic change varies among genotypes, incorporating multiple

genets remains critical for understanding how genetic and epigenetic processes jointly contribute to coral plasticity and acclimatization.

4.1.1. Rapid hypomethylation during acute thermal stress

Despite the strong influence of genotype on MSAP variation, temporal changes in methylation-state composition were observed during thermal exposure (Fig. 4). However, the magnitude and direction of these responses varied among coral lines, indicating substantial genotype-associated heterogeneity in DNA methylation dynamics. Unlike vertebrates, invertebrate genomes typically exhibit mosaic gene body methylation (GBM), characterized by interspersed methylated and unmethylated regions across coding sequences (Keller et al., 2016; Suzuki et al., 2007). In corals, highly methylated gene bodies tend to be associated with constitutively expressed housekeeping genes, whereas genes involved in cellular signaling, stress response, and environmental sensing are typically less methylated and exhibit higher regulatory plasticity (Dixon et al., 2014; Dimond and Roberts, 2016; Dixon et al., 2018; Wang et al., 2014). Reduced methylation at these loci has been linked to increased transcriptional responsiveness and plasticity (Putnam et al., 2016; Liew et al., 2018).

The transient hypomethylation observed here occurred during a time frame in which rapid transcriptional responses to thermal stress have been reported in corals, although direct links between these processes cannot be established from the present data. Rapid transcriptional responses to short-term thermal exposure have been reported across coral taxa, including upregulation of heat-shock proteins, oxidative stress pathways, and unfolded protein response genes (Hou et al., 2018; Ruiz-Jones and Palumbi, 2017; Traylor-Knowles et al., 2017; Savary et al., 2021; Stick et al., 2025). In *Acropora hyacinthus*, natural tidal heat pulses ($\sim 31^\circ\text{C}$) produced temporary transcriptional activation that reverted within 24 h (Ruiz-Jones and Palumbi, 2017), while similar transient responses were observed in *Stylophora pistillata*, *Galaxea fascicularis*, and *A. tenuis* (Hou et al., 2018; Savary et al., 2021).

Consistent with these findings, the four MSAP fragments contributing most strongly to treatment separation along LD1 exhibited treatment-associated changes in methylation state at T3 (Fig. 5), although the magnitude and direction of these responses varied among coral lines. These changes in MSAP fragments occurred despite relatively modest global shifts, suggesting that thermal-associated DNA methylation variation may be transient and confined to a subset of MSAP fragments. A recent study in *Acropora nana* demonstrated that gene-specific methylation changes occur following acute heat stress and

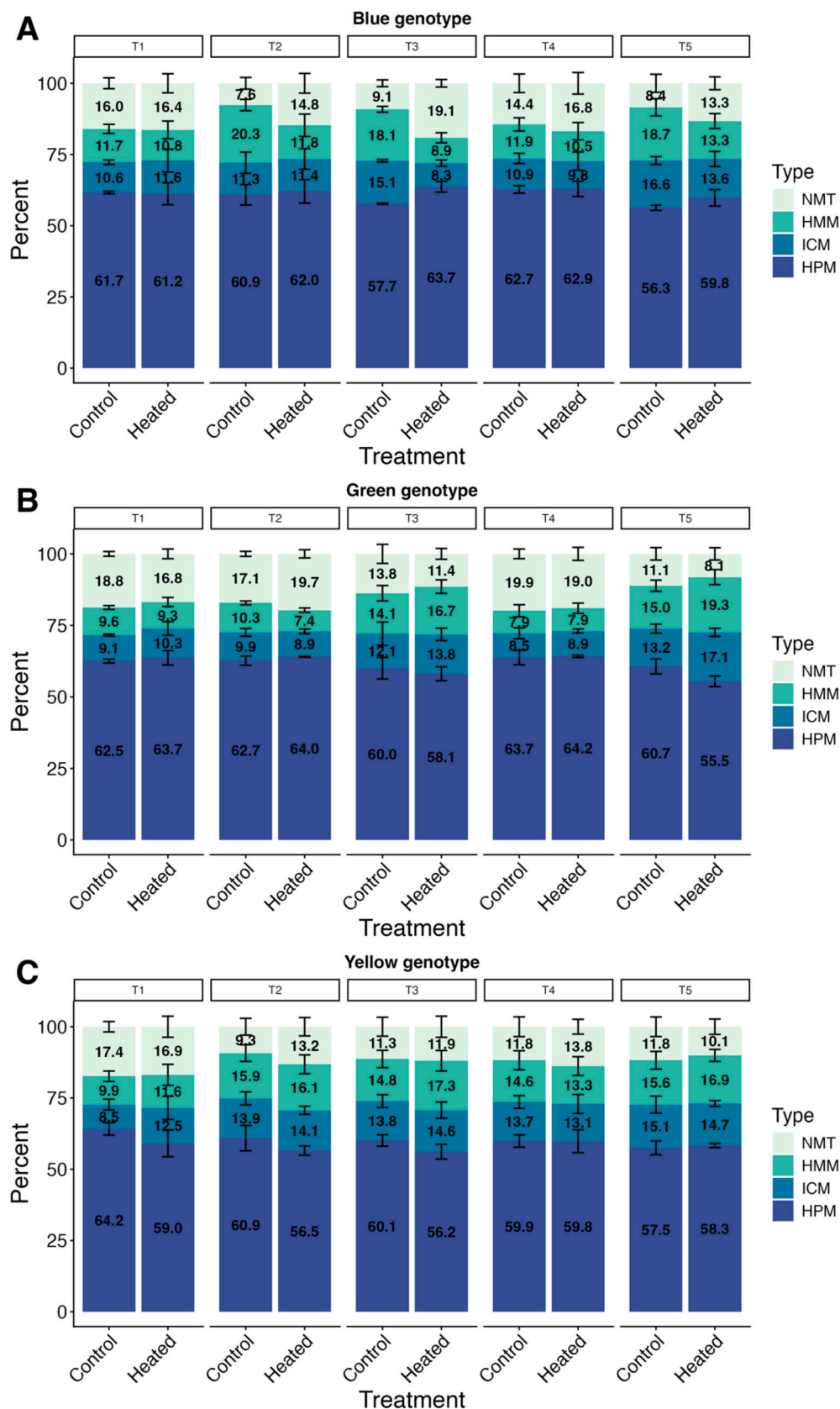


Fig. 4. Percentage of non-methylated (NMT), hemimethylated (HMM), internal-C methylated (ICM), and hypermethylated (HPM) target loci calculated for heated and control groups of *A. cervicornis* corals exposed to a thermal challenge. Methylation-state proportions are shown separately for each coral line (Blue, Green, and Yellow genotypes) across treatments and time points. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

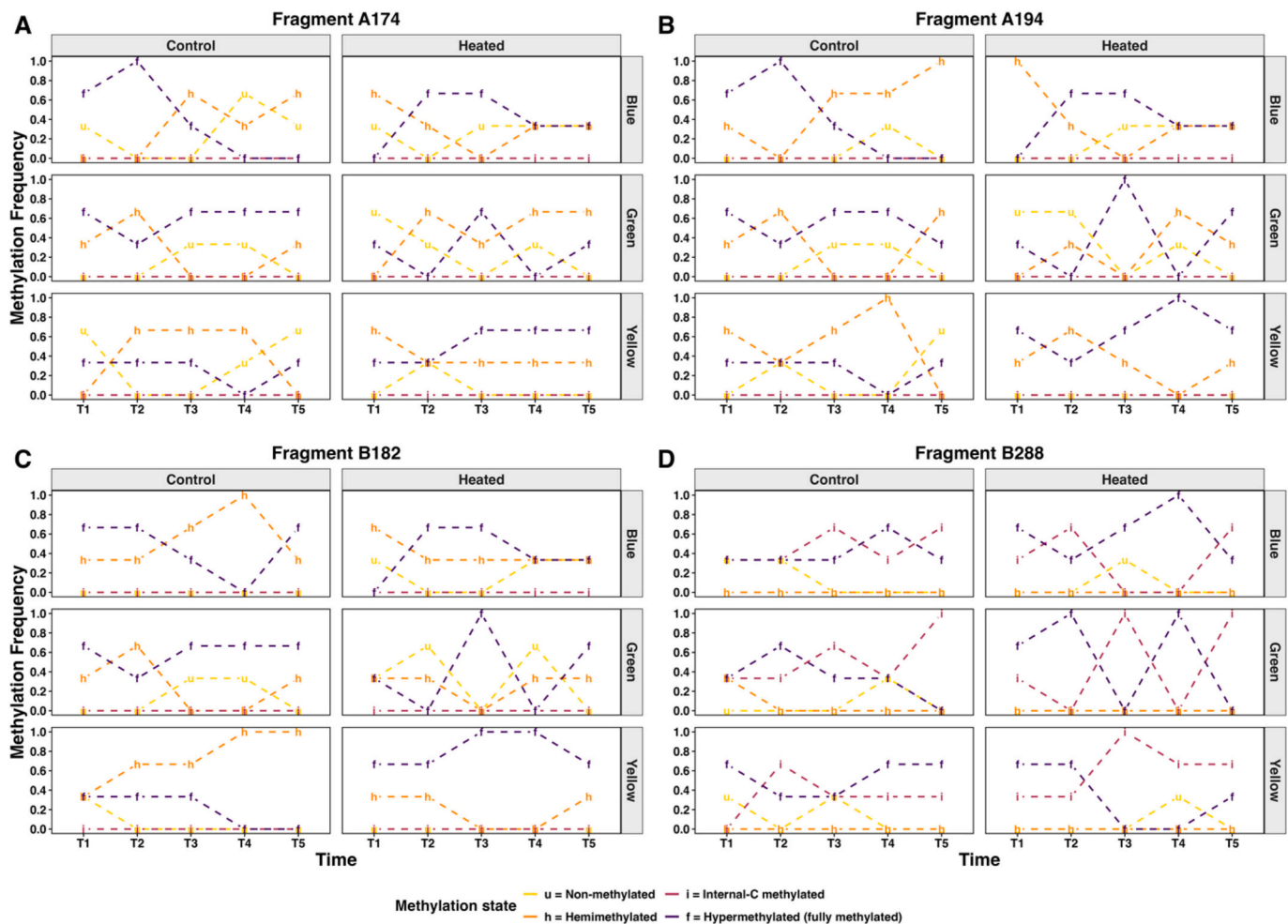


Fig. 5. Methylation variation in four influential MSAP fragments (>95th percentile) that contributed to the variation on LD1 for time points T1 through T5 in *A. cervicornis* corals exposed to an acute thermal episode. Methylation states are indicated as h (hypermethylated), i (internally methylated), f (hemimethylated), and u (unmethylated). Rows correspond to the Blue, Green, and Yellow coral genotypes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

correspond to transcriptional shifts, although methylation magnitude did not directly predict expression level (Guerrero and Bay, 2024). This decoupling highlights that DNA methylation operates within a broader regulatory network that includes chromatin structure, histone modifications, transcription factors, and signaling pathways (López-Maurý et al., 2008).

4.2. Methodological considerations and implications for future work

Although MSAP provides valuable insight into genome-wide methylation dynamics across many samples, it cannot resolve the genomic identity or functional context of differentially methylated loci. Importantly, even sequencing-based approaches such as whole-genome bisulfite sequencing have struggled to identify direct, causal relationships between DNA methylation and gene expression in corals (Rodríguez-Casariégo et al., 2020), suggesting that methylation effects may be context-dependent or temporally decoupled from transcription. Evidence from cnidarian models such as *Aiptasia* indicates extensive crosstalk between DNA methylation and histone modifications in regulating gene expression (Li et al., 2018; Nawaz et al., 2022). Thus, integrating multiple epigenetic layers—including histone modifications, chromatin accessibility, and non-coding RNAs—will be essential for fully resolving regulatory mechanisms underlying coral stress responses.

5. Conclusions

Together, this study demonstrates that DNA methylation variation detected by MSAP in *Acropora cervicornis* is primarily structured by genotype, while acute thermal exposure is associated with more subtle and transient changes in subsets of MSAP fragments within hours of acute thermal exposure. While global methylation patterns remained largely stable (Figs. 2 and 4; Table S3), early and transient shifts in methylation states and key loci (Figs. 3 and 5) point to a dynamic epigenetic response during stress onset. These findings provide new insight into the relative contributions of genotype-associated and thermal-associated variation in coral DNA methylation and establish a framework for future studies investigating short-term epigenetic responses to environmental stress.

Abbreviation list

MSAP – Methylation-Sensitive Amplified Polymorphism
 DNA – Deoxyribonucleic Acid
 CpG – Cytosine-phosphate-guanine dinucleotide
 DAPC – Discriminant Analysis of Principal Components
 PERMANOVA – Permutational Multivariate Analysis of Variance
 MSL – Methylation-Susceptible Loci
 NML / NMT – Non-Methylated Loci
 HMM – Hemimethylated Loci
 ICM – Internally Cytosine-Methylated Loci

HPM – Hypermethylated Loci
 GBM – Gene Body Methylation
 LD1 / LD2 – Linear Discriminant Axes 1 and 2
 SDI – Shannon's Diversity Index
 PCR – Polymerase Chain Reaction
 RFU – Relative Fluorescence Units
 bp – Base Pairs
 °C – Degrees Celsius

CRedit authorship contribution statement

Sabrina Mansoor: Writing – original draft, Investigation, Formal analysis. **Serena Hackerott:** Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Conceptualization. **Javier Rodriguez-Casariago:** Writing – review & editing, Visualization, Software, Investigation. **Daniel Garcia-Souto:** Writing – review & editing, Formal analysis, Data curation. **Jose Eirin-Lopez:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Given his role as Editor in Chief for the Journal Gene Reports, he had no involvement in the peer review of this article and had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to another journal editor. JEL. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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