Title

Full title: Epigenetic and evolutionary mechanisms uniquely contribute to rescue from global change

Short Title: Epigenetics facilitate resilience to global change

Authors

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Abstract

Both adaptation and plasticity are critical for species persistence during rapid anthropogenic global change. However, we know little about how these mechanisms of resilience interact, particularly the role of epigenetic variation in long-term adaptation. We used a 25-generation selection experiment simulating future acidification, warming, and their combination to reveal how epigenetic and genetic mechanisms interact to promote resilience in a foundational copepod at the base of the marine food web. Epigenetic divergence was positively linked to gene expression divergence, indicating that epigenetic changes may facilitate phenotypic change. In contrast, genetic and epigenetic changes were negatively related, where genomic regions experiencing significant epigenetic changes had lower genetic divergence than those without. Thus, evolutionary and epigenetic changes acted in different regions of the genome during adaptation to global change conditions, due to either local inhibition of one another or distinct functional targets of selection. These results suggest that both genetic and epigenetic mechanisms underlie resilience to global change but have unique contributions.

Teaser

Epigenetic divergence is linked to both gene expression and genetic divergence during evolutionary rescue from global change.

MAIN TEXT

Introduction

As global conditions rapidly change, populations must adjust their physiologies to mitigate the stressful effects of novel conditions. Phenotypic plasticity is likely to play a role in this process as it can allow individual genotypes to shift their phenotypes to more closely match the environment. However, for most populations, plasticity alone will be insufficient to completely buffer the effects of global change (*1*) and rapid adaptation or evolutionary rescue (*2*) from standing genetic variation will be crucial (*3*). These two mechanisms interact in complex ways, facilitating and inhibiting one another during responses to changing environments (*4*). For example, plasticity can facilitate short-term population persistence; therefore, allowing time for selection to act and adaptation to proceed (*5*). Alternatively, by moving a phenotype closer to the environmental optimum a population may be shielded from selection, inhibiting adaptation (*6*). However, the mechanisms by which phenotypes can rapidly shift are only beginning to be understood.

Epigenetics is broadly defined as mechanisms unrelated to changes in DNA sequence that affect gene expression of a cell, including those that are not heritable (*7*). As epigenetic mechanisms impact gene expression regulation, modifications at the epigenetic level can have phenotypic effects (*8*). For instance, methylation in marine invertebrates correlates with gene expression, where genes with stable expression tend to have high methylation and environmentally responsive genes have low methylation (*9*). Further, epigenetic patterns are generally responsive to environmental conditions (*10*) and have been linked to phenotypic plasticity during acclimation (*11*). Therefore, changes at the epigenetic level can impact the ability of populations to respond to changing environmental conditions.

While epigenetic changes can impact population phenotypes, it remains a major challenge to understand the interaction of genetic (evolutionary) and epigenetic (plastic) responses during rapid acclimation and adaptation to global change conditions (*12*). For example, how do epigenetic and genetic changes interact during multi-generation responses to global change? Do epigenetic changes facilitate or inhibit genetic changes? Are the same genomic regions targeted by epigenetic and genetic shifts? Answering these questions will shed light into the mechanisms that may facilitate population persistence under global change.

To address these questions, we leveraged genomic, epigenomic, and transcriptomic sequencing across 25 generations of experimental evolution under ambient (18°C; 400 µatm pCO_2) and simulated future acidification (18°C; 2,000 µatm pCO_2) , warming (22°C; 400 µatm pCO_2), and their combination (OWA; (22°C; 2,000 µatm pCO_2) in the marine copepod, *Acartia tonsa* (*13*). Replicates were each started with 160 female and 80 male wild-caught adults and each treatment consisted of four replicates and census sizes of at least 3,000. Copepods provide an ideal model as their small size (~1 mm), short generation times (~3 weeks), and genomic resources make multi-generation and complex experiments with high replication and census sizes tractable (*14*). Additionally, copepods are foundational to marine ecosystems

worldwide as they are one of the most abundant metazoans (*15*) and provide critical links between higher level consumers and primary producers (*16*). *Acartia tonsa* specifically is a cosmopolitan estuarine species that is dominant in the Atlantic ocean (*17*) and serves as a main prey item for fishes (*18*). Therefore, the resilience of copepods and *A. tonsa* under global change conditions has important implications for the stability of marine ecosystems across the globe.

Results

Genome-wide epigenetic changes

After 25 generations of evolution in ambient, acidification, warming, and combined warming and acidification (OWA), methylation frequencies of pools of 30 individuals from each of four replicates per treatment were quantified using reduced representation bisulfite sequencing (RRBS). This resulted in 96,207 methylated sites sequenced across all samples at a depth of at least 15x per sample. Mean methylation percentage was different across treatments (ANOVA p-value = 0.002) and was driven by a decrease in methylation from F0 to F25 (mean methylation ± SD: F0: 0.254 ± 0.003; F25: 0.229 ± 0.009; Tukey test: $p < 0.03$; Fig. S1; table S1) but did not differ between treatments at F25 ($p > 0.7$).

Genome-wide methylation frequencies were summarized for all samples using multidimensional scaling (MDS) and generally showed clustering by treatment groups (Fig. 1A). The F0 samples were largely distinct from F25 samples and within F25 samples, OWA and warming clustered together while ambient and acidification clustered together. Differential methylation analysis showed that OWA had the largest number of loci changing methylation frequency relative to ambient controls (753 loci; methylation change > 10%; FDR < 0.05) followed by warming (161 loci) and acidification (128 loci; Fig. 2). There was limited shared change in methylation between treatments with 48 loci overlapping between OWA and warming, 50 between OWA and acidification, 16 between warming and acidification, and 8 shared across all three treatments (Fig. 1B).

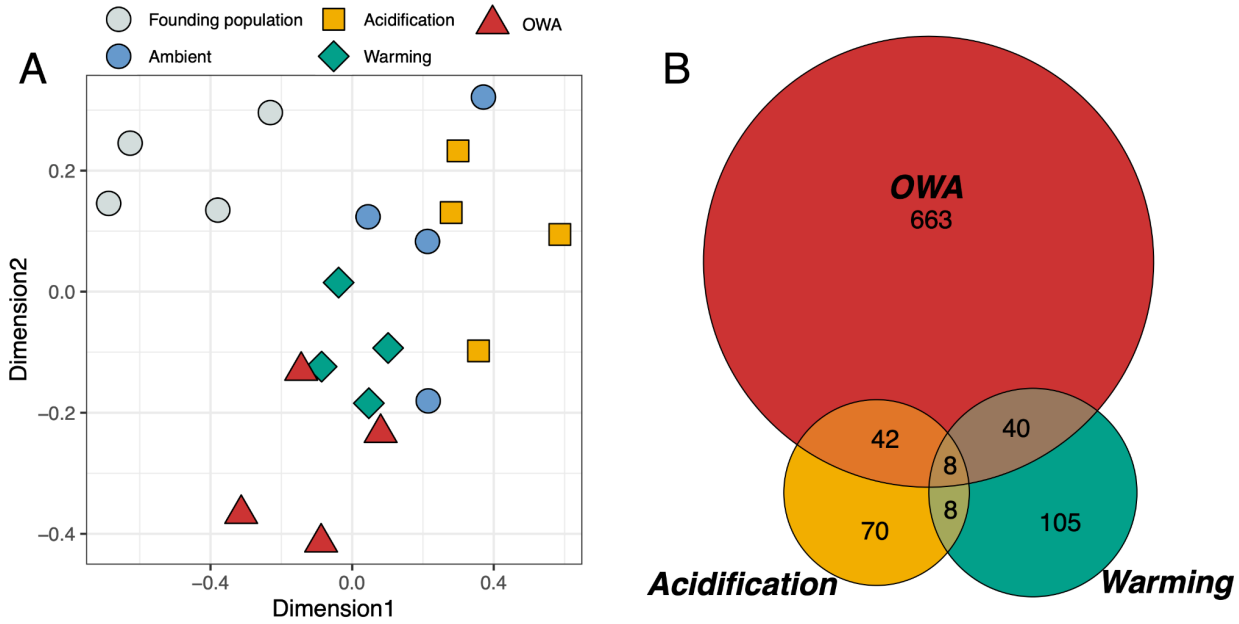

Figure 1: **Overview of epigenetic changes.** **(A)** Multidimensional scaling of methylation frequencies of 96,207 loci across founding and replicate selection populations. **(B)** UpSet plot of significant methylation changes relative to ambient F0 and F25 (10% change in frequency, FDR < 0.05).

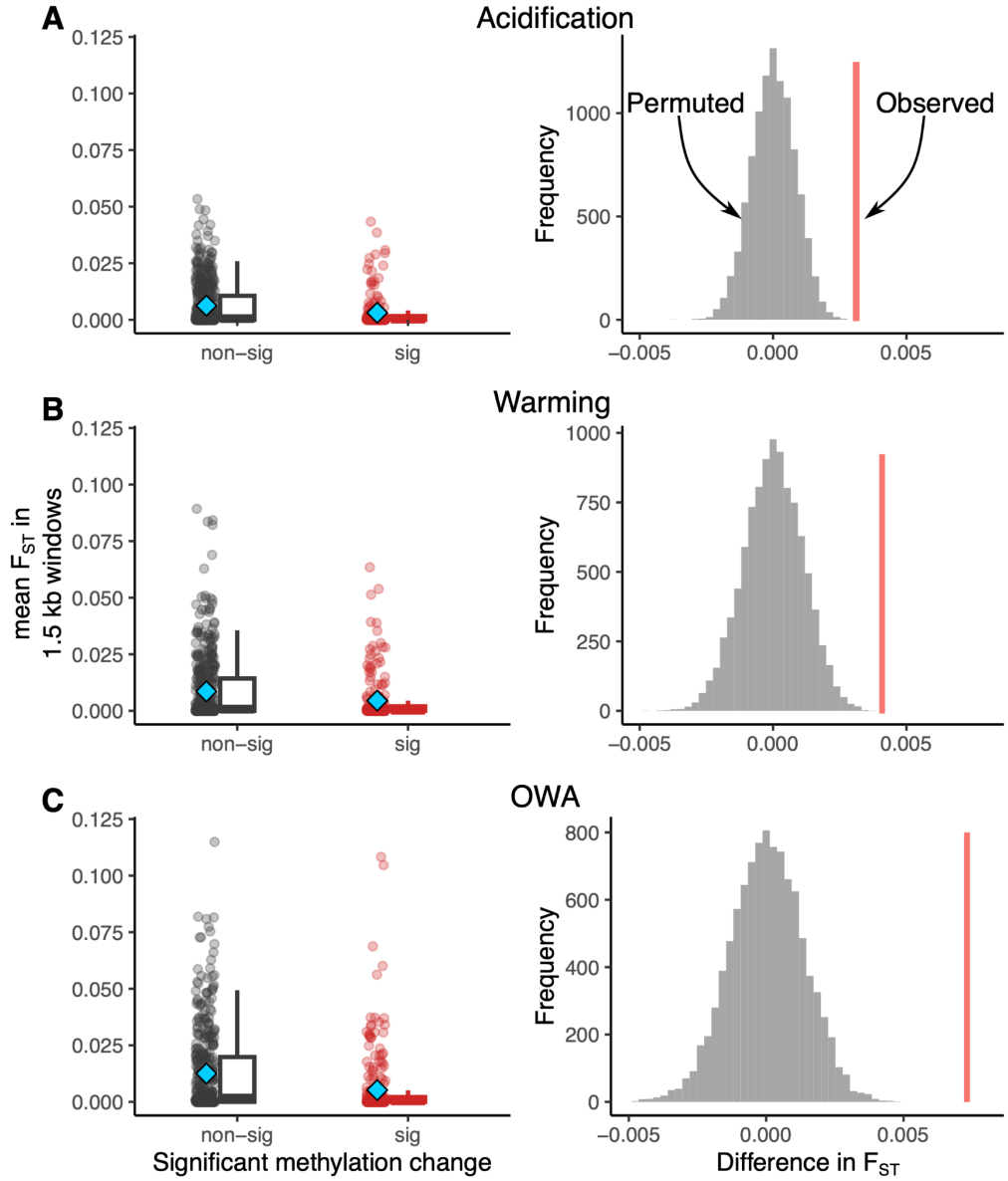

Figure 2: Relationship between F_{ST} and methylation change. Panels refer to acidification **(A)**, warming **(B)**, and OWA **(C)**. For the left side of the plot, the genome was broken into 1.5 kb windows that contained at least 5 SNPs and 5 methylation sites resulting in 665 windows across the genome. If a window contained a significant change in methylation from the ambient treatment it was considered significant ("sig"). Mean F_{ST} was calculated for each window and is shown as a blue diamond with Tukey box plots representing the distribution for each group. The right shows the permutation test for the difference in F_{ST} observed for each treatment. See methods for full details.

Interaction between epigenomic and genomic changes

Allele frequencies for 394,667 loci across all samples were reported in Brennan et al. (*19*). Comparing allele frequency change from F0 to F25 to methylation changes summarized at the gene level showed a significant negative relationship where higher allele frequency changes corresponded to lower

methylation changes (Fig. S2; $p < 0.001$). To more directly quantify the relationship between the epigenetic and genomic changes, we compared changes in 1.5 kb windows across the genome for windows that had at least 5 SNPs and 5 epi-loci, resulting in 665 windows. There was again a negative relationship between methylation and allelic changes where windows containing significant methylation changes had lower allele frequency divergence (Fig. 2). For OWA, windows without a significant change in methylation had 2.6-fold higher F_{ST} (0.013) than those with (0.005; $p = 0.0001$); similar patterns were observed for warming (Non-significant methylation change window $F_{ST} = 0.009$, Significant methylation change window $F_{ST} = 0.005$; $p = 0.0005$) and acidification (Non-significant methylation change window $F_{ST} = 0.006$, Significant methylation change window $F_{ST} = 0.003$; $p = 0.0002$). This relationship was robust to changes in window size (Fig. S3) and was present for warming and OWA, but not acidification, when calculating the reverse: the mean methylation changes for windows with significant allele frequency divergence (Fig. S4). Thus, epigenetic and genetic changes were inversely related after 25 generations.

To determine if there was a relationship between genetic variation and methylation change we quantified Tajima's π between genomic regions with and without significant methylation changes. For the OWA line, genetic variation was higher in regions with significant methylation change than those with non-significant changes (Tajima's π: 0.0196 ± 0.0007 vs. 0.0171 ± 0.0001; $p = 0.0001$; Kolmogorov-Smirnov Test; Fig. S5). There was no difference between these regions for acidification (0.0165 ± 0.0001 vs. 0.0169 ± 0.003; $p = 0.2$) or warming (0.0169 ± 0.0001 vs. 0.0205 ± 0.002; $p = 0.7$). However, the total number of windows overlapping with significant methylation changes was much lower for these two treatments than for OWA, which may make estimates unreliable (acidification = 30; warming = 90; OWA = 456).

Correlations between epigenetic changes and gene expression

Potential phenotypic effects of the epigenetic changes were assessed by quantifying the relationship of these changes with gene expression. We previously quantified gene expression changes in the ambient and OWA lines in their treatment conditions and following a reciprocal transplant (*20*); we integrate these data with the current methylation data. At the gene level, divergence in expression between OWA and ambient lines was significantly associated with changes in methylation. For genes that had at least five methylation sites and one of these sites with significant methylation change, larger changes in methylation for OWA were correlated with larger divergence in gene expression (Fig. 3A; R-squared = 0.025, $p = 0.015$). There was also a significant relationship between methylation percentage and the degree of gene expression plasticity. Genes that showed significant expression change following transplant of the OWA line to ambient conditions had significantly lower methylation than those with no change in gene expression (Fig. 3B; $p= 0.02$; mean methylation ± se: Non-plastic: 0.115 ± 0.005; Plastic: 0.026 ± 0.004). This result was also present for the ambient line when transplanted to OWA conditions (Fig. S6; $p= 8.6e-8$; mean methylation ± se: Non-plastic: 0.133 ± 0.006; Plastic: 0.068 ± 0.0070). Taken together, these results suggest that fixed differences in gene expression as populations adapted to global change were reinforced with changes in methylation, while genes that were plastically responsive to sudden changes in environment remained lowly methylated.

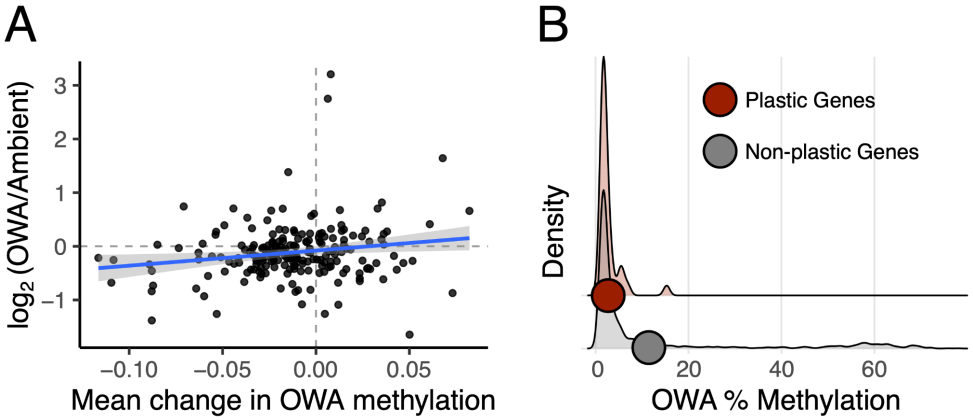

Figure 3: Methylation and gene expression changes. (A) Divergence in gene expression between OWA and ambient lines versus the mean change in OWA methylation from the ambient line. Genes were required to have at least 5 methylation sites with at least one significantly diverged in OWA. Solid blue line is the regression between these factors (R-squared = 0.025, p = 0.015). **(B)** The percent methylation of plastic vs. non-plastic genes following transplant of the OWA line from OWA to ambient at generation F21. Solid points show the mean methylation percent for each group (Non-plastic: 0.115 ± 0.005; Plastic: 0.026 ± 0.004; mean ± se). Non-plastic genes had significantly higher methylation percent than plastic genes (p= 0.019, Kolmogorov-Smirnov Test)

Discussion

The interaction between plasticity and adaptation is an integral component underlying resilience, or lack thereof, of populations to rapidly changing environments. While authors have studied how these components interact during adaptation across natural environmental gradients (*10*, *21*, *22*), limited work has focused on how epigenetic and genetic changes might drive phenotypic responses during evolutionary rescue from global change. Our results show that epigenetic and genetic changes are negatively related and affect unique regions of the genome (Fig. 2). Further, epigenetic divergence was positively correlated with gene expression divergence in global change adapted lines (Fig. 3). Thus, both genetic and epigenetic changes facilitate physiological resilience during evolutionary rescue from global change.

The negative relationship between epigenetic and genetic changes suggests that these mechanisms function in unique regions of the genome or may inhibit one another locally (Fig. 2). Similar results have been found in three-spined stickleback, *Gasterosteus aculeatus*, where adaptive changes in methylation were more likely to occur in regions of low genetic divergence, which the authors suggest is due to the epigenetic modifications achieving the phenotypic effect in place of genetic changes (*23*). That is, if functional changes are achieved via epigenetic shifts, this might preclude selection from acting on genetic variation and thus reduce genetic divergence in these regions. In addition, we found that regions with methylation divergence also harbored higher levels of nucleotide diversity relative to regions without methylation divergence (Fig. S5). Similar patterns of increased genetic diversity associated with methylation divergence have been found in *G. aculeatus* that have adapted from marine to freshwater environments (*21*), though these changes occurred over much longer timescales, around 700 years, ~230-700 generations (*24*), relative to the 25 generations in the current study. The authors suggest that environmentally responsive methylation sites might be under relaxed selection at the genetic level, thus increasing genetic diversity in these regions. Alternatively, higher levels of methylation can influence

203 mutation rates with a higher probability of spontaneous deamination of methylated cytosines to thymines
204 (*25*, *26*). In our 25-generation study, the combination of reduced genetic divergence in the epigenetically
205 responsive regions of the genome supports the hypothesis that epigenetic changes can functionally replace
206 genetic changes locally within the genome during short-term responses to the environment (*27*), leading
207 to increased genetic variation and decreased genetic responses to selection in these regions.
208 Positive correlation between changes in methylation and gene expression suggests that epigenetic
209 divergence is linked to phenotypic divergence between the selection regimes (Fig. 3). Indeed, methylation
210 levels have been consistently linked to gene expression in diverse marine invertebrates, including during
211 responses to global change conditions (*10*, *11*, *28–31*). Together, these results suggest that epigenetic
212 responses may help to facilitate physiological resilience to global change conditions. We previously found
213 a rapid recovery of fitness in the OWA line after only three generations (*13*) coupled with a loss of
214 plasticity and fitness with transplantation at the 21st generation and beyond (*20*). While there was a strong
215 signal of adaptation in later generations (*19*), the genetic and epigenetic changes at early generations were
216 not quantified. We hypothesize that plastic epigenetic shifts may in part underlie the immediate fitness
217 recovery and allow population persistence and time for adaptation to proceed, reminiscent of the Baldwin
218 Effect (*5*); additional data focusing on short-term responses to OWA conditions would be required to test
219 this hypothesis.
220 The fate of the epigenetic changes observed here over longer timescales remains unclear. In
221 studies focused on longer-term divergence and adaptation between environments, it is common that
222 genetic and epigenetic divergence are positively associated (*10*, *32*). For example, in the coral, *Acropora*
223 *millepora*, methylation patterns that are fixed between populations (i.e., not responsive to the
224 environment) occur in regions with elevated genetic divergence (*10*). This suggests that under stable
225 conditions, plastic epigenetic changes may become fixed due to underlying genetic changes, i.e. genetic
226 assimilation (*33*, *34*). Over the 25 generations in the current experiment, it is likely that there has been
227 insufficient time for genetic divergence to fix the previously plastic epigenetic response. Future studies
228 should focus on short and long-term to determine the pace at which epigenetic variation becomes
229 assimilated due to genetic divergence.
230 While there were observed links between methylation, genetic, and transcriptomic changes, there
231 were limitations to the study that should be taken into account. First, the method used to quantify
232 epigenetic variation was a reduced representation approach (RRBS), which covers only a portion of the
233 genome. Full genome quantification of methylation markers would provide a higher resolution view of
234 the relationship between genetic and epigenetic changes. Second, we do not know if the epigenetic
235 changes between lines are responsive to the environment or fixed between populations. To understand
236 this aspect of the data, it would be necessary to conduct transplant experiments and quantify methylation
237 levels following transplant across generations. Similarly, it is unknown the order in which genetic and
238 epigenetic changes arose in the lines, which would require fine-scale quantification of both factors across
239 generations at the early stage of the experimental evolution, which was not done here. Though we found
240 consistent, directional epigenetic change after 25 generations of selection, it is unknown if these changes
241 are due to transgenerational inheritance of epigenetic states or re-establishment of epigenetic marks at
242 each generation in response to the developmental environment. Importantly, due to the negative
243 relationship between genetic and epigenetic changes, epigenetic differences among groups are unlikely to
244 be due to linked selection on genetic loci. Understanding the fine-scale interactions between epigenetic
245 and genetic changes during the early stages of adaptation to global change conditions will require

additional experiments and is critical to understand how these factors promote resilience to ongoing environmental change.

Materials and Methods

Data generation

The details of the experiment have been reported previously in Dam et al., (*13*). Adult copepods (n=1000) were collected from Esker Point Beach in Groton, Connecticut, USA (41.320725° N, 72.001643° W) in June 2016 and common gardened for three generations. For the selection experiment, replicate cultures were started with 160 females and 80 males in each of the following conditions: ambient (18 °C, 400 µatm CO_2, pH ~8.2); acidification (18 °C, 2,000 µatm CO_2, pH ~7.5); warming (22 °C, 400 µatm CO_2, pH~8.2); OWA (22 °C, 2,000 µatm CO_2, pH~7.5). All cultures were held at a salinity of 32.75 (CI[32.4, 33.46]) Practical Salinity Units and 12 h light, 12 h dark and fed a combination of *Tetraselmis sp*., *Rhodomonas sp.*, and *Thalassiosira weissflogii* every 2 to 3 d at food-replete condition (≥ 800 µg carbon L^{-1}) (*35*). Cultures were held in these conditions for 25, non-overlapping, generations and populations sizes were > 3,000 per replicate.

Epigenetics library prep/sequencing

Reduced representation bisulfite sequencing was used to quantify methylation frequencies for each sample following vonHoldt et al. (*36*). This approach is based on a modified NEBNext Ultra library preparation that includes a restriction digest and bisulfite conversion. For each sample, 250ng of high quality genomic DNA was digested with the restriction enzyme *Msp1*, 3' ends were repaired with adenine and Illumina methylated sequence adapters for NEBNext Ultra library preparation were ligated (New England Biolabs, Ipswich, MA, USA). To quantify bisulfite conversion efficiency, 1ng unmethylated lambda DNA (Promega) was added to each sample. Libraries were size-selected to retain fragments between 100 and 500bp. Following size selection, libraries were bisulfite converted using a Qiagen Epitech kit with the protocol for low DNA concentrations and an extended incubation period of 10 hours to ensure high conversion efficiency of unmethylated cytosines to uracils. Libraries were then PCR amplified for 15 cycles where uracil was converted to thymine through use of EpiMark Hot Start Taq (New England Biolabs, Ipswich, MA, USA) and a unique barcode was added to each sample. Libraries were pooled at equimolar concentrations and sequenced on 2 lanes of an Illumina HiSeq 4000 with a 20% phiX spike in to increase complexity and improve data quality.

Epigenetic data analysis

Raw reads were trimmed with Trimmomatic v0.36 for quality and adapter contamination. Mapping was conducted using Bismark v0.22.3 (*37*) and Bowtie2 v2.2.6 with local alignments (*38*). Each of the two lanes were mapped separately to the *A. tonsa* genome (*39*) and reads with no alignment were re-mapped as single end reads to improve coverage. These data were checked for methylation bias and this bias removed when extracting CpG methylation calls with Bismark's methylation extractor; bias was present on the first and last 2 basepairs of reads, a common pattern in RRBS data. Following methylation extraction, extracted methylation data was concatenated by sample and output to bedGraph format with the bismark2bedGraph command.

Methylation conversion efficiency

We used the rate of methylation conversion in the spiked-in 1ng unmethylated lambda DNA to determine

methylation conversion efficiency. During bisulfite conversion, unmethylated cytosine is converted to thymine while methylated cytosine remains cytosine. Because the lambda DNA is completely unmethylated, all cytosine bases should be converted to thymine during bisulfite conversion. The proportion of thymine to cytosine represents the conversion efficiency. Raw reads were aligned to the lambda genome (NCBI accession: NC_001422.1) in the same manner as to the *A. tonsa* genome and methylation calls were extracted. From this, one minus the methylation rate is the bisulfite conversion efficiency.

Differential methylation

Bisulfite sequencing produced an average of 27.8 million paired end reads per sample. Overall mean mapping rate of trimmed sequence reads was 54 ± 14% (mean ± SD) and bisulfite conversion efficiency calculated from the lambda dna spike-in was 98.4 ± 0.3%; see table S1 for a summary of sequencing results.

Differentially methylated regions were identified in R version 3.6.0 (*40*) using edgeR following the methods described in Chen et al. (*41*). The approach treats the counts of methylated and unmethylated reads as separate observations and allows for the use of edgeR's generalized linear model framework to incorporate complex experimental designs. We required a minimum coverage of 15x for all samples and sites with coverage greater than the 97.5% quantile (974x) were removed to control for multi-mapping regions. This filtering resulted in 96,207 methylation sites with a mean coverage of 124x and median of 77x. We fit a negative binomial generalized linear model to detect differentially methylated regions between each treatment and the ambient control, considering methylation frequencies at (F0 and F25). A Benjamini-Hochberg correction was applied to control for multiple testing and differential methylation was considered significant at $p < 0.05$ and with methylation changes > 10 %.

DNA data collection and analysis

We have previously reported results from DNA sequencing of all lines and replicates at generation F0 and F25 (*19*). Pools of 50 individuals were sequenced from each replicate using a sequence capture approach targeting genetic and regulatory regions (32,413 probes). After mapping to the reference genome (*39*), this approach resulted in 394,667 variant sites; see Brennan et al. (*19*) for full details. F_{ST} was estimated on a per-site level using the R package *poolfstat* (*42*), mean F_{ST} from the ambient line was calculated in 1.5 kb windows and the intersection between methylation sites and F_{ST} windows was identified using BEDTools (*43*), keeping only windows with at least 5 SNPs and 5 methylation sites. The F_{ST} distributions were then compared between windows containing a significant change in methylation, as identified in the edgeR analysis above, versus those with no change in methylation. The results were robust to both window size (1.5 vs. 5 kb) and the number of SNP or methylation sites required (3 vs. 4 vs. 5). The significance of the F_{ST} differences between groups was calculated using a randomization test with 10,000 permutations, where window significance was randomly assigned and the observed difference in mean F_{ST} was calculated. P-values were calculated as the proportion of permuted values greater than the observed value. This analysis was also run for mean methylation in 1.5 kb windows and comparing the change in methylation from F0 to F25 in windows with or without significant allele frequency divergence (from (*19*)).

Finally, Tajima's π was estimated in 100bp windows using Popoolation2 (*44*) and the overlap between

these windows and methylation sites was identified using BEDTools. Distributions of Tajima's π were compared between windows with significant methylation change versus those without using Kolmogorov-Smirnov Tests with a Bonferroni correction in R.

Gene expression data collection and analysis

We previously reported gene expression results from a reciprocal transplant of ambient to OWA and OWA to ambient at generation F21 (*20*). Gene expression was analyzed at the gene level using Salmon v0.10.2 (*45*) and filtered for abundance (fewer than 10 counts in >90% of the samples), resulting in 23,324 genes. Expression levels and differences were analyzed using DESeq2 (*46*) and plastic genes were identified as those changing expression (adjusted p-value < 0.05) within a line following transplant to the opposite environment. Differential expression between OWA and ambient lines in their home environments was also identified (adjusted p-value < 0.05).

To compare gene expression with methylation, only genes with at least five methylation sites were used. We first regressed mean methylation change with gene expression divergence to test if genes with significant methylation change in OWA relative to ambient were correlated with gene expression divergence between the lines. We next compared the mean methylation percentage for genes that were plastic versus non-plastic in their expression and compared these distributions using Kolmogorov-Smirnov Tests.

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Methodology: RSB, JAD, MF, HB, HGD, MHP
Investigation: RSB, JAD
Visualization: RSB
Writing—original draft: RSB, MHP
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Competing Interests: Authors declare that they have no competing interests.

Data and materials availability: Sequencing data are available on NCBI under BioProject accession PRJNA590963. Data files and supplemental tables can be found on Zenodo: https://doi.org/10.5281/zenodo.10797734. Code to run all analysis and produce all figures can be found on Zenodo: https://doi.org/10.5281/zenodo.10829107.

References

1. A. R. Gunderson, J. H. Stillman, Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc. Biol. Sci.* **282**, 20150401 (2015).

2. G. Bell, Evolutionary Rescue. *Annu. Rev. Ecol. Evol. Syst.* **48**, 605–627 (2017).

3. D. N. Reznick, J. Losos, J. Travis, From low to high gear: there has been a paradigm shift in our understanding of evolution. *Ecol. Lett.* **22**, 233–244 (2019).

4. R. J. Fox, J. M. Donelson, C. Schunter, T. Ravasi, J. D. Gaitán-Espitia, Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **374**, 20180174 (2019).

5. G. G. Simpson, The Baldwin Effect. *Evolution* **7**, 110–117 (1953).

6. S. C. Stearns, The evolutionary significance of phenotypic plasticity. *Bioscience* (1989).

7. D. C. H. Metzger, P. M. Schulte, Epigenomics in marine fishes. *Mar. Genomics* **30**, 43–54 (2016).

8. K. McGuigan, A. A. Hoffmann, C. M. Sgrò, How is epigenetics predicted to contribute to climate change adaptation? What evidence do we need? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **376**, 20200119 (2021).

9. J. L. Dimond, S. B. Roberts, Germline DNA methylation in reef corals: patterns and potential roles in response to environmental change. *Mol. Ecol.* **25**, 1895–1904 (2016).

10. G. Dixon, Y. Liao, L. K. Bay, M. V. Matz, Role of gene body methylation in acclimatization and adaptation in a basal metazoan. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 13342–13346 (2018).

11. Y. J. Liew, D. Zoccola, Y. Li, E. Tambutté, A. A. Venn, C. T. Michell, G. Cui, E. S. Deutekom, J. A. Kaandorp, C. R. Voolstra, S. Forêt, D. Allemand, S. Tambutté, M. Aranda, Epigenome-associated phenotypic acclimatization to ocean acidification in a reef-building coral. *Sci Adv* **4**, eaar8028 (2018).

12. J. M. Eirin-Lopez, H. M. Putnam, Marine Environmental Epigenetics. *Ann. Rev. Mar. Sci.* **11**, 335–368 (2019).

13. H. G. Dam, J. A. deMayo, G. Park, L. Norton, X. He, M. B. Finiguerra, H. Baumann, R. S. Brennan, M. H. Pespeni, Rapid, but limited, zooplankton adaptation to simultaneous warming and acidification. *Nat. Clim. Chang.* **11**, 780–786 (2021).

14. H. G. Dam, Evolutionary adaptation of marine zooplankton to global change. *Ann. Rev. Mar. Sci.* **5**, 349–370 (2013).

15. A. G. Humes, How many copepods? *Hydrobiologia* **292**, 1–7 (1994).

16. J. Mauchline, *The Biology of Calanoid Copepods* (Academic Press, 1998).

17. W. Yuh Lee, B. J. McAlice, Seasonal succession and breeding cycles of three species of Acartia (Copepoda: Calanoida) in a maine estuary. *Estuaries* **2**, 228–235 (1979).

18. J. T. Turner, “The feeding ecology of some zooplankters that are important prey items of larval fish”

(7, NOAA Technical Report NMFS, 1984); https://repository.library.noaa.gov/view/noaa/5581/noaa_5581_DS1.pdf.

19. R. S. Brennan, J. A. deMayo, H. G. Dam, M. Finiguerra, H. Baumann, V. Buffalo, M. H. Pespeni, Experimental evolution reveals the synergistic genomic mechanisms of adaptation to ocean warming and acidification in a marine copepod. *Proc. Natl. Acad. Sci. U. S. A.* **119**, e2201521119 (2022).

20. R. S. Brennan, J. A. deMayo, H. G. Dam, M. B. Finiguerra, H. Baumann, M. H. Pespeni, Loss of transcriptional plasticity but sustained adaptive capacity after adaptation to global change conditions in a marine copepod. *Nat. Commun.* **13**, 1147 (2022).

21. J. Ord, T. I. Gossmann, I. Adrian-Kalchhauser, High Nucleotide Diversity Accompanies Differential DNA Methylation in Naturally Diverging Populations. *Mol. Biol. Evol.* **40** (2023).

22. C. F. de Carvalho, J. Slate, R. Villoutreix, V. Soria-Carrasco, R. Riesch, J. L. Feder, Z. Gompert, P. Nosil, DNA methylation differences between stick insect ecotypes. *Mol. Ecol.*, doi: 10.1111/mec.17165 (2023).

23. M. J. Heckwolf, B. S. Meyer, R. Häsler, M. P. Höppner, C. Eizaguirre, T. B. H. Reusch, Two different epigenetic information channels in wild three-spined sticklebacks are involved in salinity adaptation. *Sci Adv* **6**, eaaz1138 (2020).

24. K. Reid, M. A. Bell, K. R. Veeramah, Threespine Stickleback: A Model System For Evolutionary Genomics. *Annu. Rev. Genomics Hum. Genet.* **22**, 357–383 (2021).

25. Y. Zhou, F. He, W. Pu, X. Gu, J. Wang, Z. Su, The Impact of DNA Methylation Dynamics on the Mutation Rate During Human Germline Development. *G3* **10**, 3337–3346 (2020).

26. J. Xia, L. Han, Z. Zhao, Investigating the relationship of DNA methylation with mutation rate and allele frequency in the human genome. *BMC Genomics* **13 Suppl 8**, S7 (2012).

27. F. D. Klironomos, J. Berg, S. Collins, How epigenetic mutations can affect genetic evolution: model and mechanism. *Bioessays* **35**, 571–578 (2013).

28. A. M. Downey-Wall, L. P. Cameron, B. M. Ford, E. M. McNally, Y. R. Venkataraman, S. B. Roberts, J. B. Ries, K. E. Lotterhos, Ocean Acidification Induces Subtle Shifts in Gene Expression and DNA Methylation in Mantle Tissue of the Eastern Oyster (Crassostrea virginica). *Frontiers in Marine Science* **7** (2020).

29. X. Dang, Y.-K. Lim, Y. Li, S. B. Roberts, L. Li, V. Thiyagarajan, Epigenetic-associated phenotypic plasticity of the ocean acidification-acclimated edible oyster in the mariculture environment. *Mol. Ecol.* **32**, 412–427 (2023).

30. G. E. Hofmann, Ecological Epigenetics in Marine Metazoans. *Frontiers in Marine Science* **4** (2017).

31. M. R. Gavery, S. B. Roberts, Predominant intragenic methylation is associated with gene expression characteristics in a bivalve mollusc. *PeerJ* **1**, e215 (2013).

32. K. Silliman, L. H. Spencer, S. J. White, S. B. Roberts, Epigenetic and Genetic Population Structure is Coupled in a Marine Invertebrate. *Genome Biol. Evol.* **15** (2023).

33. C. H. Waddington, Genetic assimilation of an acquired character. *Evolution* **7**, 118–126 (1953).

450 34. M. J. West-Eberhard, *Developmental Plasticity and Evolution* (Oxford University Press, 2003).

451 35. L. R. Feinberg, H. G. Dam, Effects of diet on dimensions, density and sinking rates of fecal pellets
452 of the copepod Acartia tonsa. *Mar. Ecol. Prog. Ser.* **175**, 87–96 (1998).

453 36. B. vonHoldt, E. Heppenheimer, V. Petrenko, P. Croonquist, L. Y. Rutledge, Ancestry-Specific
454 Methylation Patterns in Admixed Offspring from an Experimental Coyote and Gray Wolf Cross. *J.*
455 *Hered.* **108**, 341–348 (2017).

456 37. F. Krueger, S. R. Andrews, Bismark: a flexible aligner and methylation caller for Bisulfite-Seq
457 applications. *Bioinformatics* **27**, 1571–1572 (2011).

458 38. B. Langmead, S. L. Salzberg, Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **9**, 357–359
459 (2012).

460 39. T. S. Jørgensen, B. Petersen, H. C. B. Petersen, P. D. Browne, S. Prost, J. H. Stillman, L. H. Hansen,
461 B. W. Hansen, The Genome and mRNA transcriptome of the cosmopolitan calanoid copepod
462 *Acartia tonsa* Dana improve the understanding of copepod genome size evolution. *Genome Biol.*
463 *Evol.* **11**, 1440–1450 (2019).

464 40. R Core Team, R: A Language and Environment for Statistical Computing. R Foundation for
465 Statistical Computing [Preprint] (2022). https://www.R-project.org/.

466 41. Y. Chen, B. Pal, J. E. Visvader, G. K. Smyth, Differential methylation analysis of reduced
467 representation bisulfite sequencing experiments using edgeR. *F1000Res.* **6**, 2055 (2017).

468 42. M. Gautier, R. Vitalis, L. Flori, A. Estoup, f-statistics estimation and admixture graph construction
469 with Pool-Seq or allele count data using the R package poolfstat. *Mol. Ecol. Resour.* **22**, 1394–1416
470 (2022).

471 43. A. R. Quinlan, I. M. Hall, BEDTools: a flexible suite of utilities for comparing genomic features.
472 *Bioinformatics* **26**, 841–842 (2010).

473 44. R. Kofler, R. V. Pandey, C. Schlötterer, PoPoolation2: identifying differentiation between
474 populations using sequencing of pooled DNA samples (Pool-Seq). *Bioinformatics* **27**, 3435–3436
475 (2011).

476 45. R. Patro, G. Duggal, M. I. Love, R. A. Irizarry, C. Kingsford, Salmon provides fast and bias-aware
477 quantification of transcript expression. *Nat. Methods* **14**, 417–419 (2017).

478 46. M. I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq
479 data with DESeq2. *Genome Biol.* **15**, 550 (2014).