Patterns of gene body methylation predict coral fitness in new environments

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Abstract

Despite widespread taxonomic representation, the function of gene body methylation (GBM) remains uncertain. One hypothesis is that GBM mediates phenotypic plasticity. To investigate this hypothesis, we performed whole-genome methylation and transcriptome profiling on reciprocally transplanted colony fragments of the reef-building coral *Acropora millepora*. GBM was only slightly affected by transplantation but these small changes correlated with coral fitness in the new environment. Specifically, for transplanted corals, similarity in GBM patterns to native corals positively correlated with growth rate, as well as carbohydrate, protein, lipid and endosymbiont content. Between populations, elevated GBM positively correlated with transcription, supporting previous findings that GBM is associated with stable and active transcription. Contrary to expectations however, changes in transcription as a result of transplantation did not correlate with changes in GBM and did not predict fitness. This indicates that on physiological time scales GBM is not directly coupled to transcription, leaving open the question about the mechanism linking GBM to fitness during acclimatization.

Introduction

DNA methylation is a covalent chromatin modification that influences transcription in plants, animals, and fungi. The relative stability of this modification gives it unique potential as an adaptive mechanism. Whereas genetic adaptation must be sculpted by natural selection within populations, DNA methylation can change throughout individual life-histories (1), and in response to environmental stimuli (2). Compared to transcription however, methylation is stable, and has much greater potential for transgenerational inheritance (3,4). DNA methylation therefore represents a middle ground between the rigidity of genotype and the transience of gene expression. These characteristics are the basis for hypotheses that DNA methylation mediates phenotypic plasticity and facilitates adaptation (5-9). Evidence for these hypotheses in marine invertebrates however, remains scarce.

In this study, we investigate the role of DNA methylation in acclimatization. Our study system is the reef-building coral, *Acropora millepora*: a basal metazoan uniquely amenable to ecological epigenetics because individuals can be fragmented into genetically identical replicates. Understanding acclimatization in this system is also of special importance because of corals' high vulnerability to

climate change (10). Using a reciprocal transplantation experiment, we evaluate the role of a particular form of DNA methylation, gene body methylation, as corals acclimatize to novel conditions.

Gene body methylation (GBM) refers to DNA methylation within transcribed regions of coding genes, most often on cytosines within CG dinucleotides (CpGs) (11). Although GBM occurs in both plants and animals (12,13), its adaptive function, if any, remains uncertain. In both plants and animals, GBM correlates with transcriptional activity and gene responsiveness (14-17). Constitutively expressed genes (i.e., housekeeping genes) tend to be strongly methylated and inducible genes tend to be weakly methylated, suggesting that GBM may be involved in modulating phenotypic plasticity (6).

To better understand the role of GBM in phenotypic plasticity, we assayed genome-wide patterns of DNA methylation in coral fragments transplanted to different sites on the Great Barrier Reef. Thirty colonies of *A. millepora* were divided into fragments and reciprocally transplanted between a warmer site near Orpheus Island, and a cooler site near Keppel Island (Fig. 1A-B). In this way, 30 genotypes were simultaneously exposed to distinct natural reef conditions. We refer to corals replaced at their home sites as 'natives' (coded KK and OO samples), and corals placed at the alternative site as 'transplants' (KO and OK samples, with the order of letters representing native then transplanted location)(Fig. 1A). Following a 3-month acclimatization period, tissues were collected from each sample and assayed for gene expression using Tag-seq (18), and DNA methylation using MBD-seq (17). These data were analyzed in the context of fitness-related traits to assess the relationship between GBM and acclimatization. Specifically, we tested three predictions: 1) GBM changes in response to environmental conditions, 2) GBM co-varies with fitness-related traits, and 3) changes in GBM co-vary with changes in gene expression. The first two predictions were supported, but the third was not.

Results

Absolute levels of GBM

For a subset of 12 samples, we sequenced both the captured and flow-through fractions from the MBD-seq library preparation. Log_2 fold differences between these samples were used to estimate absolute levels of methylation. As shown previously (17), this measure was bimodally distributed across genes and correlated with normalized CpG content (CpGo/e)(Fig 1 D-E). Targeted bisulfite sequencing of 13 loci further confirmed that MBD-seq accurately measures methylation in our system (Fig. 1F).

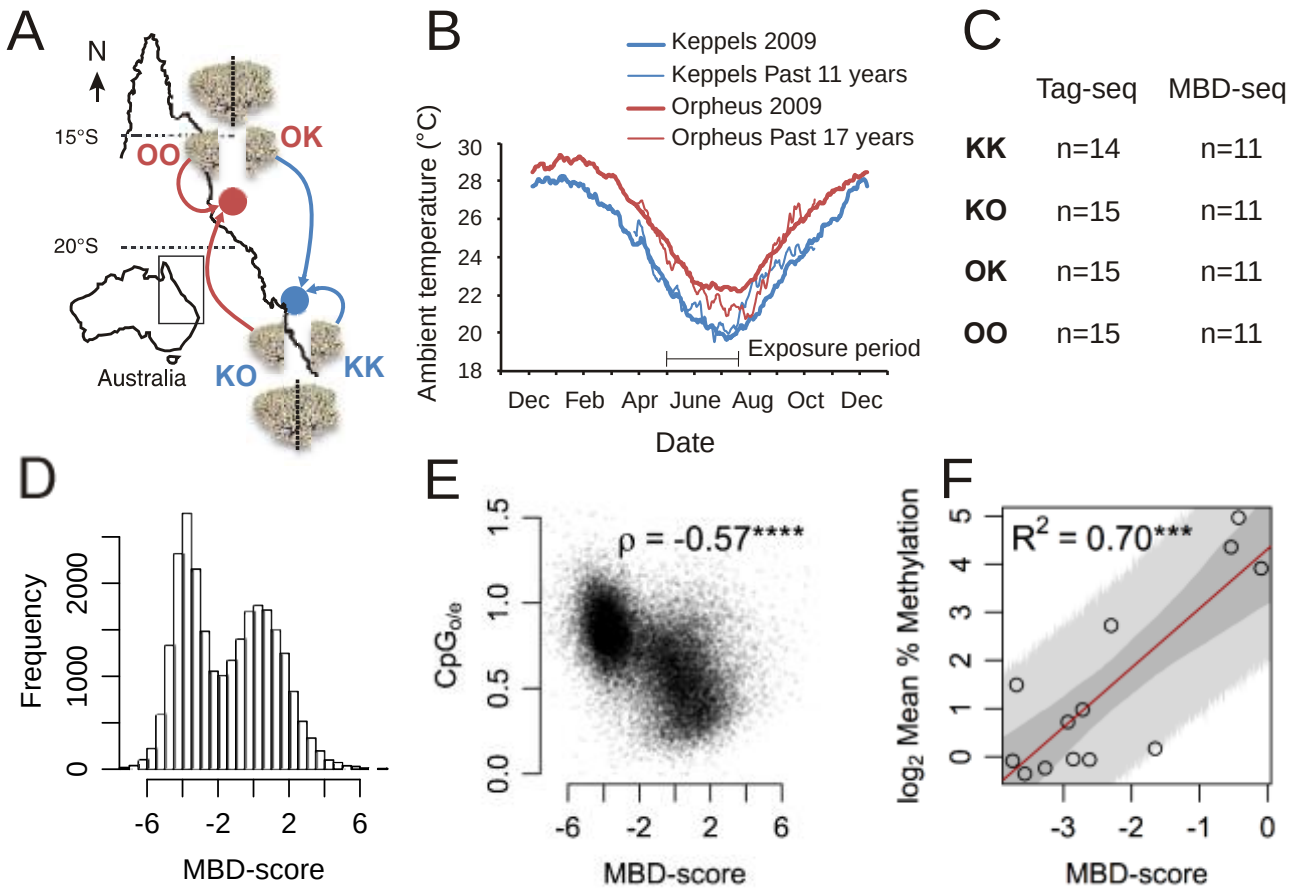

Fig. 1. Experimental design and validation of MBD-seq. (A) Map of experiment location in the Great Barrier Reef, Australia. Colonies were divided into fragments and reciprocally transplanted between two sites, a northern site, Orpheus (red), and a southern site, Keppel (blue). Sample groups are labeled with first letter indicating origin and second letter indicating transplant location (eg KO samples originated from Keppel and were transplanted to Orpheus). (B) Ambient temperatures differ between the two sites, providing distinct environmental pressures. (C) Table of sample sizes for transcription (Tag-seq) and methylation (MBD-seq) assays. (D) Distribution of methylation level (MBD-score) for all genes. MBD-score was calculated as the $\log_2$ fold difference between paired captured and flow-through libraries (n=12 pairs; see methods). Bimodal distribution of these values is consistent with expectations for GBM in invertebrate species. (E) Correlation between methylation score and normalized CpG content (CpGo/e), a metric that reflects historical germline methylation known to correlate with somatic methylation in diverse invertebrates (25). (F) Correlation between methylation estimates based on MBD-seq and targeted bisulfite sequencing. Mean percent methylation was calculated as the proportion methylated CpG sites within each gene averaged across all samples. Red line traces the expectation for a linear model. Grey shading indicates 90% posterior probability intervals for the mean (darker), and sample distribution (lighter).

GBM and transcription remain highly consistent among fragments of the same colony

Overall, patterns of GBM showed a strong dependence on colony identity (i.e., genotype). In spite of transplantation, all except one of the 22 clone-pairs showed greatest similarity to one another (Fig. S1).

Similar results were found for transcription (Fig. S2), highlighting the importance of genotype in shaping both methylation and gene expression patterns. Partitioning of variance between colony identity, origin, and transplantation site further confirmed these results, indicating an overwhelming effect of colony identity with only modest effects of origin and transplantation on both GBM and transcription (Fig. 3C,G).

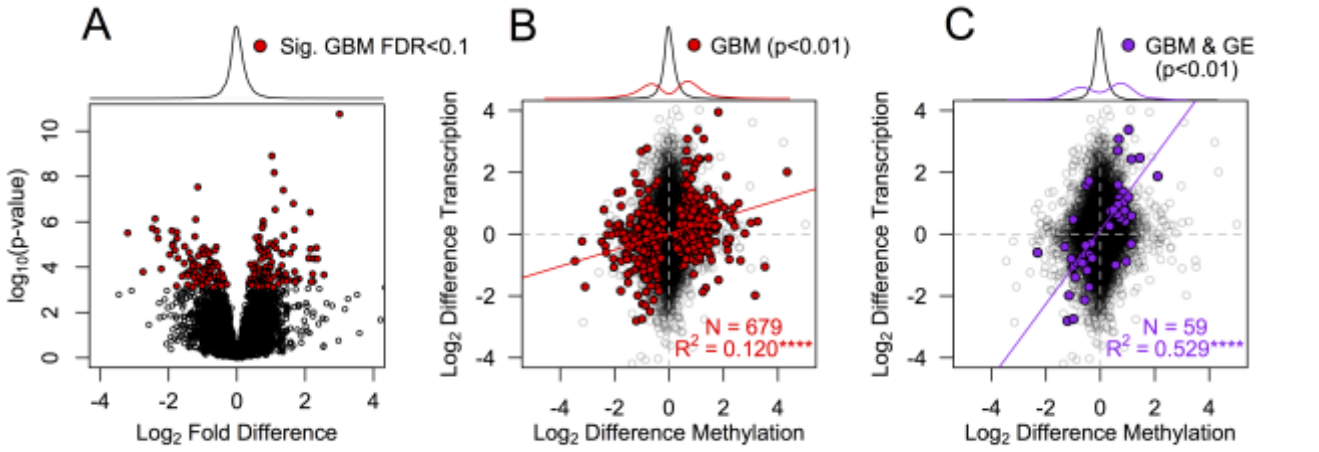

Fig. 2. Origin-specific GBM and transcription are positively correlated. (A) Differential GBM between all fragments originating from Orpheus and all fragments originating from Keppel. Significant genes (FDR < 0.1) are shown in red. (B) Scatterplot of log_2 fold differences in transcription and GBM. Log_2 fold differences are based comparisons of all fragments from Orpheus to all fragments from Keppel (OO and OK vs KK and KO). All genes are shown in black. Genes showing tendency (raw p<0.01) toward origin-based differences in GBM are shown in red. The red line traces least squares regression for only these genes. (C) The same scatterplot illustrating the correlation of log_2 fold differences for genes showing tendency (raw p<0.01) for origin-based differences in both GBM and transcription (purple). Purple line traces least squares regression for these genes. Traces above each scatterplot indicate x-axis density for all points (black) and overlaid points as indicated by color. Asterisks indicate significance of traced linear regressions (**** p < 0.0001).

GBM linked with stable transcription

Tests for differences in GBM depending on site of origin (irrespective of the site of transplantation) identified 197 differentially methylated genes (DMGs)(Fig. 2A). Correlations between origin and methylation were validated using targeted bisulfite sequencing (Fig. S3-S4). In terms of absolute methylation level, origin-specific DMGs tended to be intermediately or highly-methylated (Fig. S5). Differential methylation by origin (raw $p < 0.01$) correlated positively with variation in transcription (Fig. 2B): genes with higher GBM in one population tended to be more highly expressed in that population. This relationship was especially pronounced for genes that also tended toward differential transcription by origin (raw $p < 0.01$) (Fig. 2C). Moreover, differential GBM between native fragments (OO vs KK) correlated with transcription even among their transplanted clonal counterparts

(OK vs KO) (Fig. S6). Hence population level differences in GBM correlate with canalized transcription differences.

GBM patterns predict fitness in novel environments

The effect of transplantation on GBM was subtle. Although many genes, (2167), showed significant differences in transcription (FDR < 0.1), only two genes passed false discovery correction for GBM (Figure 3 A-B, E-F). Validation of transplant effects using targeted bisulfite-seq were not conclusive (Fig. S4B), but differences were generally in the same direction (Fig. S7). In terms of absolute methylation, genes that tended toward site-specific methylation (raw P < 0.01) tended to be weakly methylated (Fig. S5). Seventeen of these genes also showed a tendency toward origin-specific methylation.

To better examine these subtle environmental effects, we used discriminate analysis of principal components (DAPC). DAPC is designed to find the axis in multivariate space that best discriminates samples into predefined groups (19). The function that describes this axis can then be applied to values from additional samples to assess their variation in the context of the pre-specified contrast (20). We used DAPC to discriminate between native samples (KK and OO; Fig 1A) based on genes that showed evidence of GBM plasticity (n=560 genes with raw p < 0.01; see methods). We then applied the discriminant function to the transplants (Figure 3D). The same analysis was performed using transcriptional data (Fig. 3H), and SNP data (Fig. S8A). Based on both the number of significant genes (FDR < 0.1), and the magnitude of shift along with discriminant axis (Fig. 3), transcription was much more plastic than GBM.

Projection of our transplanted samples onto the discriminant axis allowed us to quantify the extent to which the transplants' GBM patterns matched those of native corals. Initially, we found that daily weight gain correlated with DAPC coordinates, but only of transplanted samples (Fig 4B). The nearly orthogonal relationships for the two transplant groups suggested that greater similarity to native GBM patterns predicted greater fitness. To further investigate this trend, we calculated a 'similarity' value expressing the proximity of each transplant along the discriminant axis to the mean for natives of the site (Fig. 4A; see methods). We regressed these similarity values against each fitness-related trait. Strikingly, five different traits (percent daily weight gain, lipid, carbohydrate, protein and zoxanthellae content) correlated positively with GBM similarity (Fig. S9C-G). The same analyses were performed using transcription data (Fig. S10), and SNP data (Fig. S8), but did not detect significant relationships. To provide a summary index for coral fitness, we took the first principal component (explaining 44% of variation) for four of the fitness proxies (weight gain, lipids, carbohydrates, and protein) among the

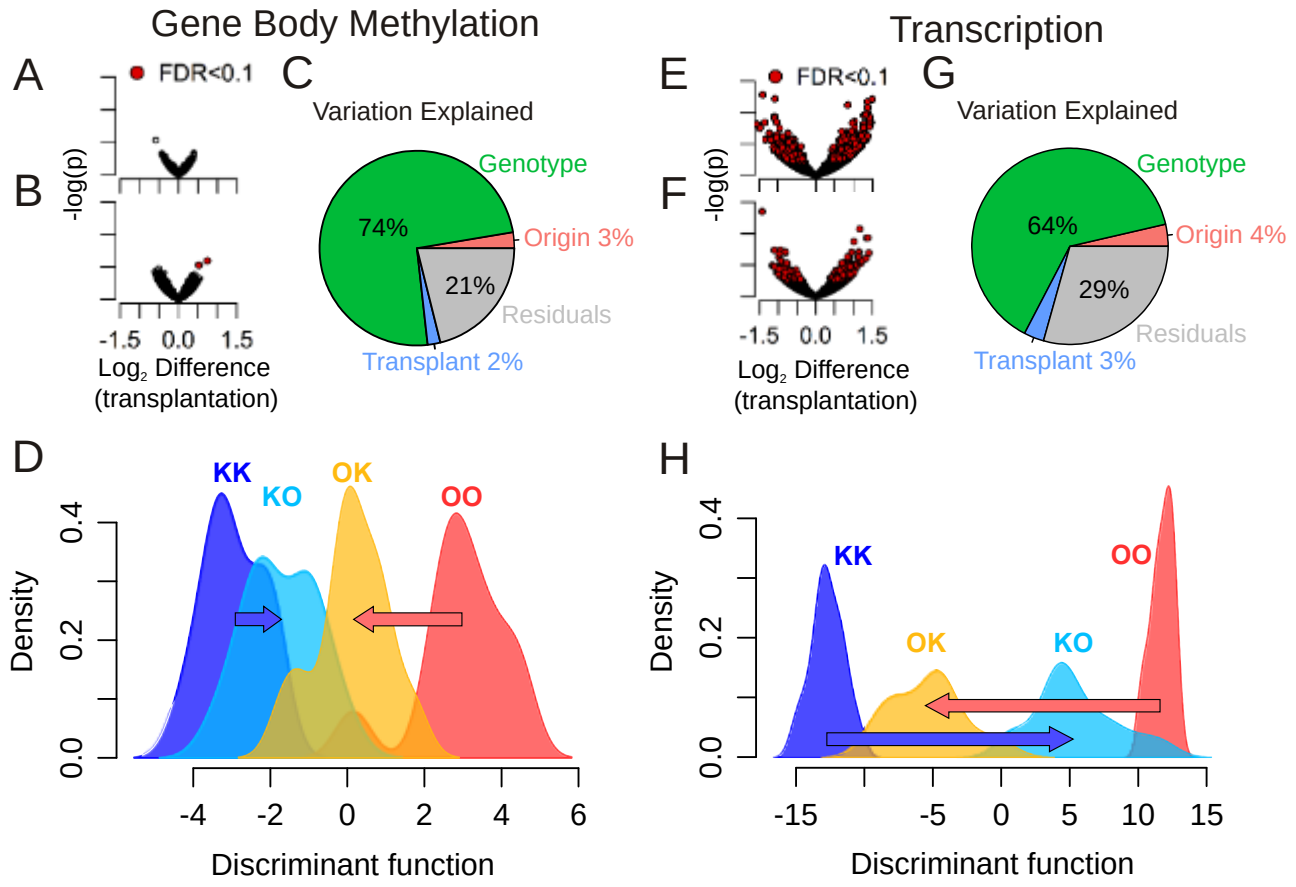

Fig. 3. Effects of transplantation on GBM and transcription. (A) Summary of transplantation effects on GBM for all genes (n= 24853) in corals originating from Keppel (KK vs KO). (B) Summary of transplantation effects on GBM in corals originating from Orpheus (OO vs OK). Significant genes (FDR < 0.1) are shown in red. (C) Pie chart illustrating partitioning of variance in GBM based on distance matrices. (D) Density plot of sample loading values for discriminant analysis of principal components (DAPC). Normalized read counts for genes showing evidence of GBM plasticity (raw p < 0.01 in either of the transplantation tests summarized in A and B) were input into DAPC to discriminate between the native groups (KK and OO). The function was then applied to the transplanted groups (KO and OK). Loading values for the transplanted fragments summarize the shift in their GBM patterns that occurred during the acclimatization period (3-months). Arrows indicate the change in mean loading values from each native group to their transplanted clonal counterparts. (E-H) Equivalent figures generated based on transcription (Tag-seq). Greater 'shift' along discriminant axis observed for transcription (H) compared to GBM (D) is consistent with higher levels of differential transcription based on transplantation (E,F) compared to GBM (A,B).

transplanted samples (Fig. S11). This fitness index also correlated with GBM similarity (Fig. 4H).

To further dissect the nature of these relationships, we examined two contributing components of GBM similarity: *Pre-Similarity* and *Shift*. *Pre-Similarity* was calculated just like Similarity, only based on the native clone mate for each transplant rather than the transplant itself (Fig. 4A). *Pre-Similarity* reflected how similar a colony's GBM profile was to the transplantation site's native mean regardless of transplantation. *Shift* was calculated as the proportional distance along the discriminant axis between

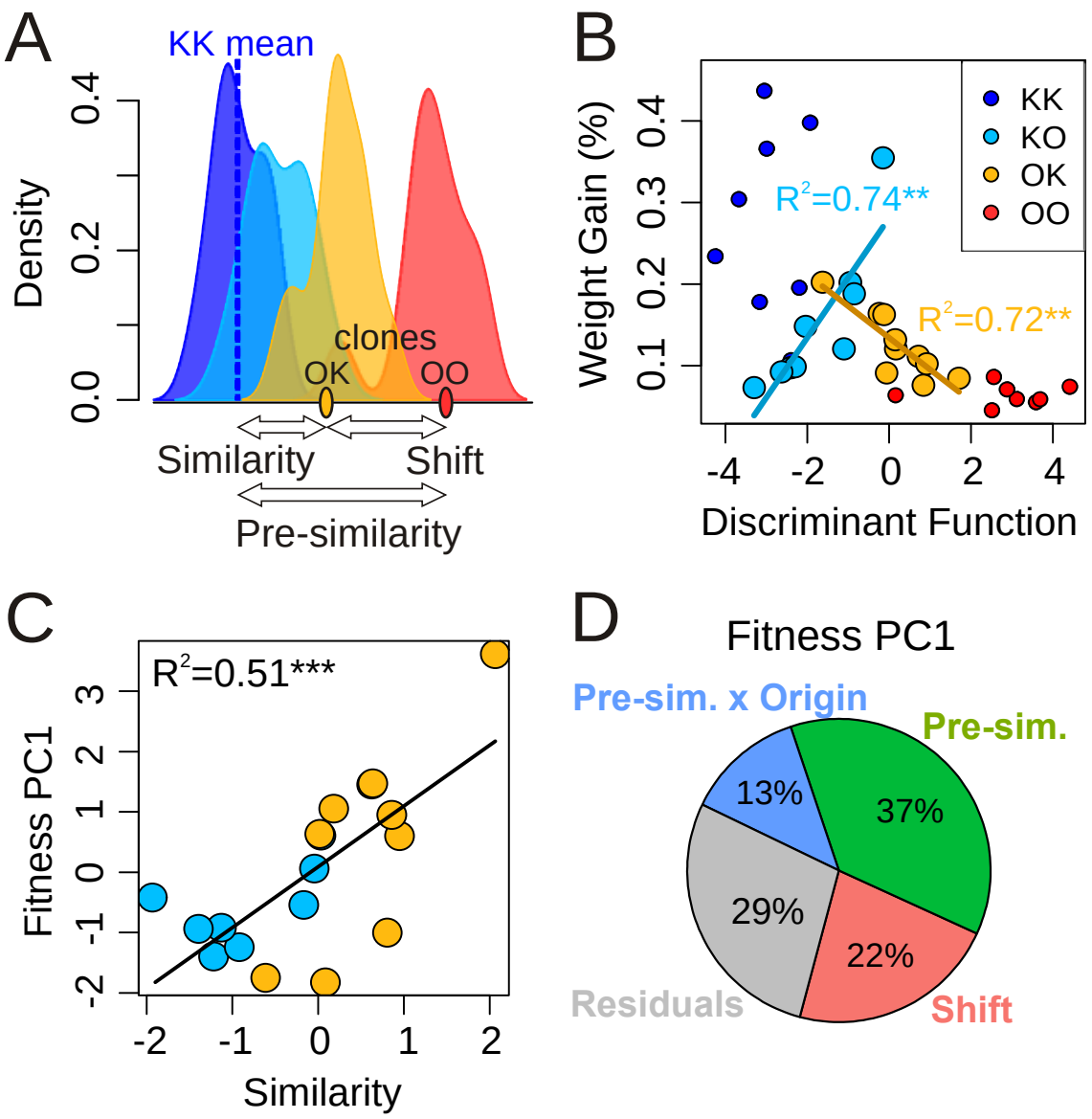

Fig. 4. Correlation between gene body methylation (GBM) and fitness-related traits. (A) Projection of transplanted samples onto the discriminant axis allowed us to quantify their similarity in GBM patterns to natives of the transplant site. Similarity was quantified as the inverse distance of a transplanted sample from the mean value for corals native to the site (arrow labeled 'Similarity'; see methods). This similarity value could be described as two separate components: 'Shift' which describes how much the transplanted sample's GBM patterns shifted from its native clonal counterpart, and 'Pre-Similarity', which describes the genotype's innate similarity to the mean for the alternative site (see methods). (B) Scatterplot showing correlation between transplanted samples' discriminant axis coordinates and daily percent weight gain, an important fitness proxy for stony corals. The nearly orthogonal relationships seen for the two transplant groups (blue and golden lines) illustrate how similarity in GBM patterns to natives of their respective transplantation sites was associated with higher growth rate. (C) Correlation between 'Similarity' (as indicated in A) and a summary fitness index: the first principal component (44% of variance explained) for daily weight gain, lipid, carbohydrate, and protein content. (D) Pie chart showing partitioning of variance among three predictors of the optimal linear model of the fitness index (Pre-Similarity, Shift, and Pre-similarity by Origin interaction).

each transplanted sample and its native clone mate. *Shift* was intended to describe the extent of plastic change in GBM in response to transplantation. Based on Akaike information criterion (AIC), the linear model that included both *Pre-Similarity* and *Shift* provided better prediction of fitness than Similarity alone (AIC = 0.75 and 1.87 respectively). Comparing a diversity of linear models, including predictors from the SNP and transcription discriminant axes, we found that the optimal linear model for fitness included *Pre-Similarity*, *Shift*, and an interaction between *Pre-Similarity* and Origin (AIC = -2.05). Of these predictors, *Pre-Similarity* explained greatest amount of fitness variation (Fig. 4D).

Discussion

GBM is a signature for canalized transcription

Within plant and animal genomes, correlations between GBM and transcription are generally weak (17, 21-23), and evidence that GBM directly regulates transcription in a general context remains scarce (14). Some associations however, are consistent. Across plant and animal taxa, GBM is often bimodally distributed, separating genes into strongly and weakly methylated classes (24-25). In both groups, strongly methylated genes tend toward active transcription across broad cellular, developmental, and ecological contexts, whereas weakly methylated genes tend toward context specificity. Here we show that in a basal metazoan, variation in GBM between populations is predictive of variation in transcription. Genes with elevated GBM in one population tend to show higher transcription in that population, even when the individuals are transplanted to alternative environments. These results further establish GBM as a signature for stable active transcription, and suggest that variation in GBM between populations may be of adaptive importance.

GBM and acclimatization

In response to transplantation, patterns of GBM changed only subtly, were considerably less responsive than transcription, and continued to be predominantly attributable to genotype (broad-sense heritability). Despite this subtle response, analysis of genes showing trends toward GBM plasticity (raw $p = 0.01$) revealed that for transplanted corals, similarity in GBM to native corals positively correlated with all measured fitness-related traits (Fig. 4 and Fig. S9). It is plausible that, due to either selection or plasticity, differences in GBM between native corals reflect relatively higher fitness to their local conditions. If this is the case, then similarity of GBM could be interpreted as a measure of the degree to which a transplant matched an optimum profile for the local environment. This similarity could be divided into two components: the degree to which the colony already matched the natives (*Pre-Similarity*; Fig. 4A), and the extent to which its GBM patterns changed during the experiment (*Shift*;

Fig. 4A). *Shift* explained roughly 20% of variation in fitness, with roughly 50% explained by *Pre-Similarity* and *Pre-Similarity* by Origin interactions (Fig. 4D). Because of the high correlation in GBM between clone mates (Fig. S1-2), we suggest that *Pre-Similarity* largely reflects genetic makeup of the individual, while *Shift* is the actual measure of GBM plasticity. With this in mind, roughly 20% of fitness variation was explained by GBM plasticity, with an additional 50% by innate GBM variation (Fig. 4D). These results are consistent with previous work on another species of *Acropora* emphasizing the importance of both acclimatization and genetic adaptation for thermal tolerance (26). Moreover, they illustrate that comparative methylation assays can shed light on the extent to which corals are pre-adapted for particular environments.

Missing mechanism

Although we found strong associations between GBM and fitness, the mechanism linking these measurements remains unclear. The third prediction of our hypothesis was that environmentally induced changes in GBM would co-vary with transcription, but we find no support for this prediction. This presents a confounding inconsistency in the associations between GBM and transcription. On the one hand, within both plant (12) and animal (13) genomes, elevated GBM is associated with constitutively active transcription. Similarly, here we found that higher GBM between populations correlated with higher transcription (Fig 2). In contrast, *changes* in GBM due to transplantation showed either no correlation, or a *negative* correlation with transcription (Fig S12-13). Hence the relationship between environmentally induced GBM and transcription was qualitatively distinct from that observed within genomes and between populations. One possible explanation for this inconsistency is that the dynamics of GBM and transcription operate on distinct timescales. Possibly, GBM changes slowly, only in response to sustained changes in transcription. This hypothesis is consistent with observations of mutant lines for *met1* methyltransferase in *Arabidopsis*, where genome-wide loss of GBM returns only slowly following reintroduction of the wild-type gene (27-28). If this were the case, GBM would integrate over acute transcriptional fluctuations and might better represent the developing acclimatization response compared to the one-time 'snapshot' provided by transcriptomic assays. This could potentially explain the surprising result that GBM similarity predicted fitness (Fig. 4; Fig. S9) when the equivalent measure based on transcription did not (Fig. S10). Clearly more work is needed to resolve the interplay between transcription, GBM and phenotype. Our hypothesis could be tested with time series of concurrent GBM and transcriptomic assays, especially under experimental conditions that cause persistent changes in transcription.

Conclusions and outlook

Here we present five major conclusions from ecological experiment with a reef-building coral. First, patterns of GBM and transcription depend predominantly on genotype. This result highlights the need to carefully consider genotypic effects in interpretations of ecological transcriptomic and methylomic data. Second, between our two sample populations, differences in GBM correlate with differences in transcription, demonstrating that variation GBM correlates with transcriptional activity not only across genes in a genome, but also between populations. Third, GBM is considerably less plastic than transcription. As a result, large sample population sizes will be necessary to detect environmental effects on GBM. Fourth, variation in GBM predicts fitness under novel ecological conditions. This result demonstrates the potential for comparative methylomics to elucidate complex ecological traits such as local adaptation and acclimatization, possibly with greater precision than transcriptomes or genotypes. Finally, there seems to be no direct link between environmentally responsive GBM and transcription, which prompts further inquiries into proximal mechanisms linking GBM to phenotype and fitness.

Methods

For full methods, please see supplementary methods file. The reciprocal transplantation experiment was performed as described in (15). Daily weight gain was measured as described in (29). Protein, carbohydrate and lipid content were standardized to coral surface area measured using a twice dip paraffin wax method (30). Protein concentration was quantified in three technical replicates of 50ul of coral protein extract using a microplate Peterson – Lowry assay following the manufacturer's recommendations (Sigma: TP0300). Carbohydrate content estimates were obtained from the average of three technical replicates of 50 uL coral slurry using D-glucose as a standard (31). Lipids were extracted using a modified version of protocol described in (32) and concentration was determined gravimetrically from dried samples (60^{o}C over night) in pre-weighed acetone washed aluminum trays. *Symbiodinium* cell numbers were determined using homogenized formalin preserved samples from six KI and seven OI source colonies in their native and transplant locations at three time points (n = 75). Enrichment reactions for MBD-seq were performed using the MethylCap kit (Diagenode Cat. No. C02020010). For a subset of 12 samples, both the enriched and the flow-through fractions were sequenced. Fold differences between these flow-through libraries and their methylation enriched counterparts allowed us to assess absolute levels of methylation across genes. For the rest of the samples only the captured fractions were sequenced. We did this for only a subset of samples because we were primarily interested in relative differences in GBM between groups. As relative differences could be assessed without

sequencing the flowthrough, we chose to focus our sequencing resources on increasing sample size rather than more thorough estimates of absolute methylation levels. This choice did not appear to greatly influence detection of differential GBM (Fig. S14). Raw reads were trimmed of non-template sequence using Cutadapt (32) and quality filtered using Fastx toolkit (http://cancan.cshl.edu/labmembers/gordon/fastx_toolkit/). Reads were mapped to the reference genome for *Acropora digitifera* (version 1.1) (34) using Bowtie2 (35). Reads mapping to annotated coding sequences were counted using HTseq (36). Fold coverage across transcription start sites was assessed using BEDTools (37). Transcription was assayed using Tag-seq (18,38). Tag-seq reads were mapped to the *A. digitifera* reference genome using SHRiMP (39) and counted using HTseq. Normalization and statistical analyses of fold coverage for MBD-seq and Tag-seq were performed with DESeq2 (40). SNPs were called from MBD-seq reads using mpileup (41). DAPC was implemented in the R package adegenet (19). We quantified GBM similarity for each transplanted sample as the inverse distance between the its loading value and the mean value for natives of the transplantation site (Fig. 4A). Specifically, we took the absolute value of the difference between each transplant's DAPC loading value and the mean value for natives of the transplant site, converted these distances into z-scores, and multiplied the z-scores by -1 so that they reflect proximity to native patterns:

$$Similarity_x = -1 \times (|D_x| - \bar{D})/\sigma$$

Where D_x is its distance along the discriminant axis from the mean for natives of the site it was transplanted to, $\bar{D}$ is the mean distance for all transplants, and σ is the standard deviation of distance for all transplants. Validation of MBD-seq was performed using targeted bisulfite sequencing. Quantification of methylation for bisulfite sequencing data was performed using Bismark (42). Unless otherwise noted error bars reflect standard error of the mean. Adjustments for multiple testing were performed using Benjamini-Hochberg correction (43). Adjusted p - values are reported using 'FDR' (eg FDR < 0.1). In many figures significance is indicated symbolically: ($^{n/s}$ not significant; $^{\&}$ $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p <$ 1e-3; **** $p <$ 1e-4).

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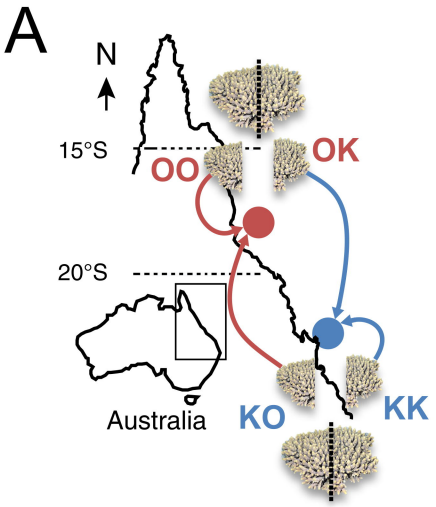
References

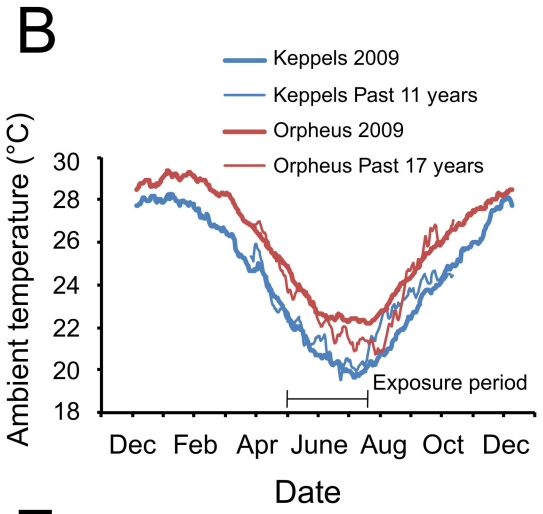
1. Law J a, Jacobsen SE (2010) Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat Rev Genet* 11(3):204–220.
2. Feil R, Fraga MF (2011) Epigenetics and the environment: emerging patterns and implications. *Nat Rev Genet* 13(2):97–109.
3. Herman JJ, Sultan SE, Herman JJ (2016) DNA methylation mediates genetic variation for adaptive transgenerational plasticity.
4. Wang X, Werren JH, Clark AG (2016) Allele-Specific Transcriptome and Methylome Analysis Reveals Stable Inheritance and Cis-Regulation of DNA Methylation in Nasonia. *PLOS Biol* 14(7):e1002500.
5. Angers B, Castonguay E, Massicotte R (2010) Environmentally induced phenotypes and DNA methylation: how to deal with unpredictable conditions until the next generation and after. *Mol Ecol* 19(7):1283–95.
6. Roberts SB, Gavery MR (2012) Is there a relationship between DNA methylation and phenotypic plasticity in invertebrates? *Front Physiol* 2(116):1–5.
7. Verhoeven KJF, VonHoldt BM, Sork VL (2016) Epigenetics in ecology and evolution: What we know and what we need to know. *Mol Ecol* 25(8):1631–1638.
8. Putnam HM, Davidson J, Gates RD (2016) Ocean acidification influences DNA methylation and phenotypic plasticity in environmentally susceptible corals. *Integr Comp Biol* 56:E177–E177.
9. Hofmann GE (2017) Ecological Epigenetics in Marine Metazoans. *Front Mar Sci* 4(January):1–7.
10. Hoegh-Guldberg O, et al. (2007) Coral Reefs Under Rapid Climate Change and Ocean Acidification. *Science (80-)* 318(5857):1737–1742.
11. Suzuki MM, Bird A (2008) DNA methylation landscapes: provocative insights from epigenomics. *Nat Rev Genet* 9(6):465–76.
12. Zilberman D, Gehring M, Tran RK, Ballinger T, Henikoff S (2007) Genome-wide analysis of Arabidopsis thaliana DNA methylation uncovers an interdependence between methylation and transcription. *Nat Genet* 39(1):61–69.
13. Zemach A, McDaniel IE, Silva P, Zilberman D (2010) Genome-wide evolutionary analysis of eukaryotic DNA methylation. *Science (80-)* 328(5980):916–9.
14. Zilberman D (2017) An evolutionary case for functional gene body methylation in plants and animals. *Genome Biol* 18(1):87.
15. Dixon GB, Bay LK, Matz M V (2014) Bimodal signatures of germline methylation are linked with gene expression plasticity in the coral Acropora millepora. *BMC Genomics* 15:1109.
16. Dimond JL, Roberts SB (2016) Germline DNA methylation in reef corals: patterns and potential roles in response to environmental change. *Mol Ecol* 25:1895–1904.
17. Dixon G, Bay LK, Matz M V (2016) Evolutionary consequences of DNA methylation in a basal metazoan. *Mol Biol Evol* 33(9):2285–2293.
18. Meyer E, Aglyamova G V, Matz M V (2011) Profiling gene expression responses of coral larvae (Acropora millepora) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. *Mol Ecol* 20(17):3599–616.

19. Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet* 11(1):94.
20. Kenkel C, Matz M V (2016) Enhanced gene expression plasticity as a mechanism of adaptation to a variable environment in a reef-building coral. *Nat Ecol Evol* 1:14.
21. Zhang X, et al. (2006) Genome-wide High-Resolution Mapping and Functional Analysis of DNA Methylation in Arabidopsis. *Cell* 126(6):1189–1201.
22. Ball MP, et al. (2009) Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. *Nat Biotechnol* 27(4):361–8.
23. Wang X, et al. (2014) Genome-wide and single-base resolution DNA methylomes of the Pacific oyster Crassostrea gigas provide insight into the evolution of invertebrate CpG methylation. *BMC Genomics* 15(1119). doi:10.1186/1471-2164-15-1119.
24. Takuno S, Gaut BS (2012) Body-methylated genes in arabidopsis thaliana are functionally important and evolve slowly. *Mol Biol Evol* 29(1):219–227.
25. Sarda S, Zeng J, Hunt BG, Yi S V. (2012) The evolution of invertebrate gene body methylation. *Mol Biol Evol* 29(8):1907–1916.
26. Palumbi SR, Barshis DJ, Traylor-Knowles N, Bay RA (2014) Mechanisms of Reef Coral Resistance to Future Climate Change. *Science (80-)* 344(6186):895–898.
27. Bewick AJ, et al. (2016) On the origin and evolutionary consequences of gene body DNA methylation. *Proc Natl Acad Sci U S A* 113(32):9111–6.
28. Bewick AJ, Schmitz RJ (2017) Gene body DNA methylation in plants. *Curr Opin Plant Biol* 36:103–110.
29. Jokiel P, Maragos J, Franzisket L (1978) Coral growth: buoyant weight technique. *Coral Reefs: Research Methods*, eds Stoddart DR, Johannes RE (UNESCO, Paris), pp 529–542. 5th Ed.
30. Stimson J, Kinzie RA (1991) The temporal pattern and rate of release of zooxanthellae from the reef coral Pocillopora damicornis (Linnaeus) under nitrogen-enrichment and control conditions. *J Exp Mar Bio Ecol* 153(1):63–74.
31. Masuko T, et al. (2005) Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. *Anal Biochem* 339(1):69–72.
32. Harland AD, Navarro JC, Spencer Davies P, Fixter LM (1993) Lipids of some Caribbean and Red Sea corals: total lipid, wax esters, triglycerides and fatty acids. *Mar Biol* 117(1):113–117.
33. Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnetjournal* 17(1):10–12.
34. Shinzato C, et al. (2011) Using the Acropora digitifera genome to understand coral responses to environmental change. *Nature* 476(7360):320–3.
35. Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9(4):357–9.
36. Anders S, Pyl PT, Huber W (2015) HTSeq-A Python framework to work with high-throughput sequencing data. *Bioinformatics* 31(2):166–169.
37. Quinlan AR, Hall IM (2010) BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics* 26(6):841–842.
38. Lohman BK, Weber JN, Bolnick DI (2016) Evaluation of TagSeq, a reliable low-cost alternative for RNAseq. Mol Ecol Resour 16(6):1315–1321.
39. Rumble SM, et al. (2009) SHRiMP: Accurate mapping of short color-space reads. PLoS Comput Biol 5(5):1–11.
40. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. Genome Biol 15(550):1–21.
41. Li H, et al. (2009) The Sequence Alignment/Map format and SAMtools. Bioinformatics 25(16):2078–2079.
42. Krueger F, Andrews SR (2011) Bismark: A flexible aligner and methylation caller for Bisulfite-

Seq applications. Bioinformatics 27(11):1571–1572.

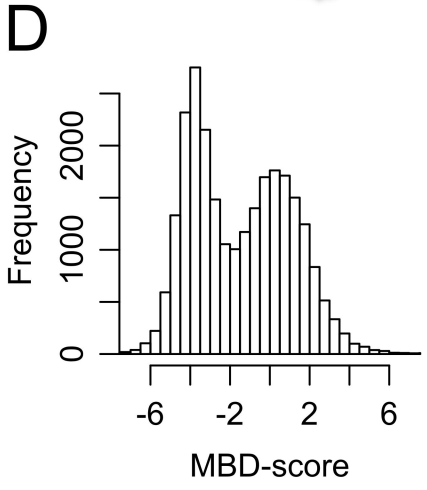
43. Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: a Practical and Powerful Approach to Multiple Testing. J R Stat Soc 57(1):289–300.

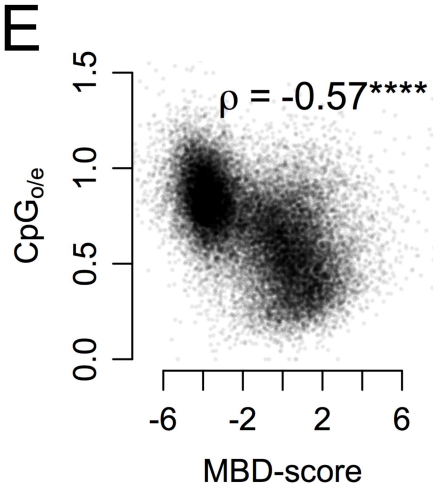


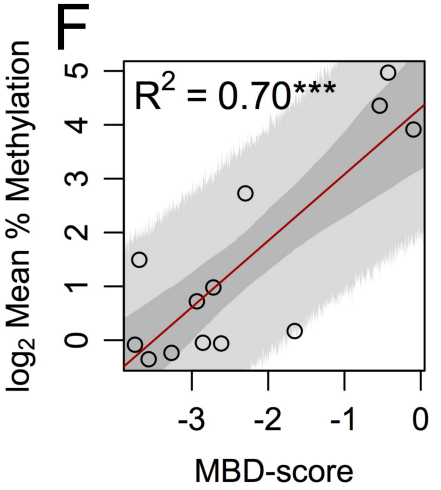


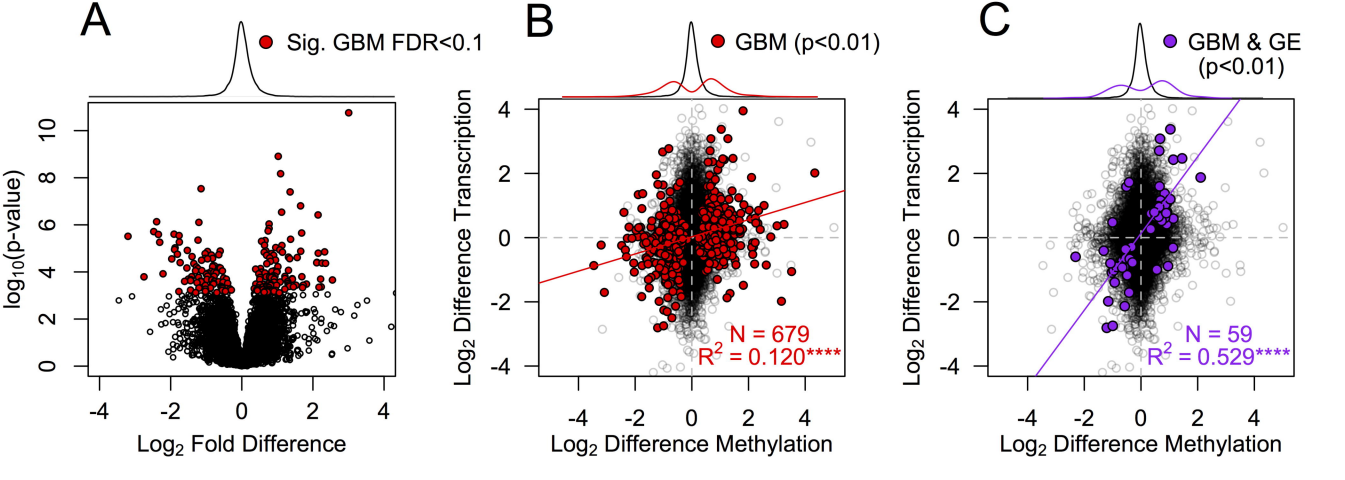
C

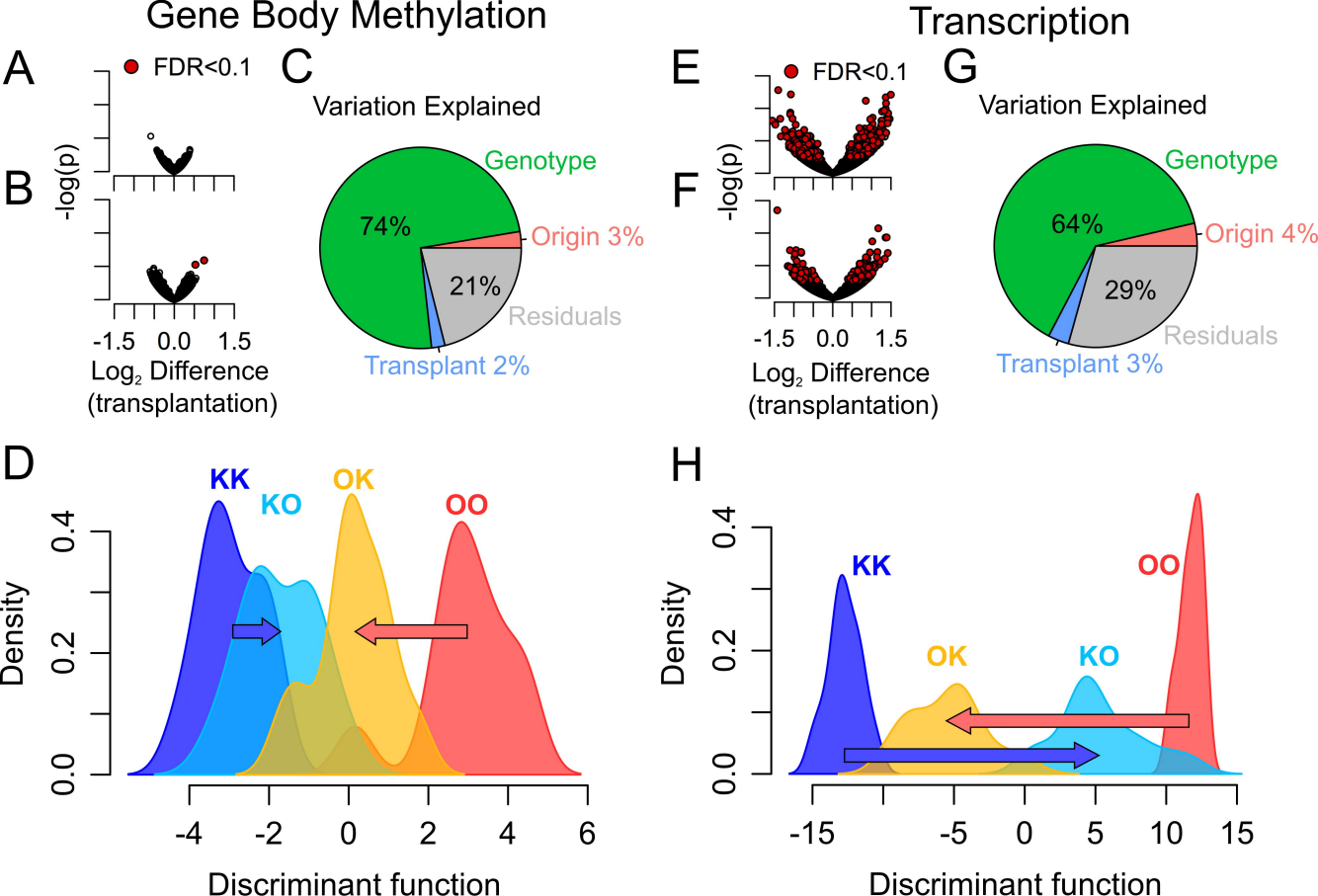
	Tag-seq	MBD-seq
KK	n=14	n=11
KO	n=15	n=11
OK	n=15	n=11
OO	n=15	n=11

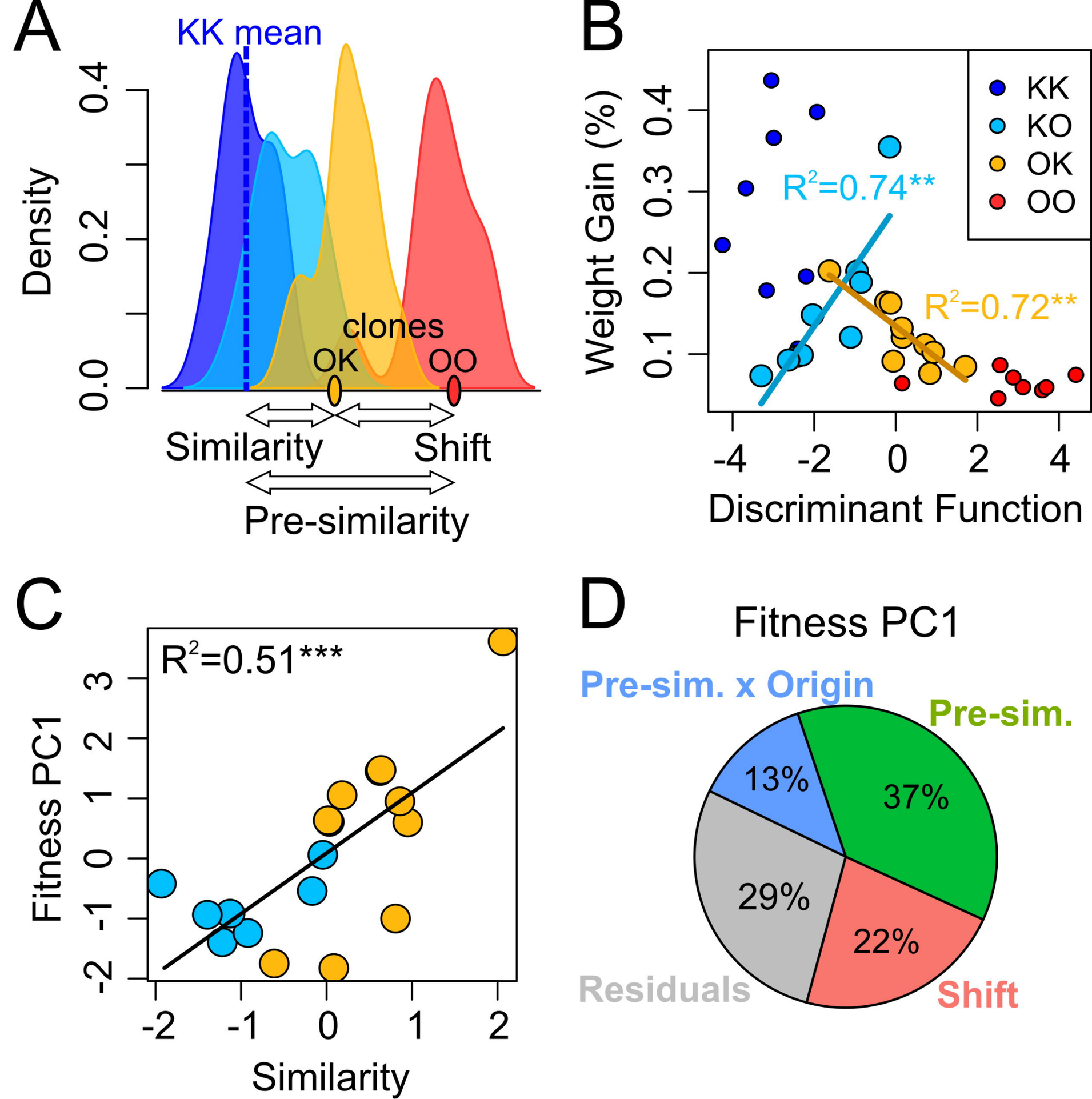







A
Sig. GBM FDR<0.1
log10(p-value)
Log2 Fold Difference
B
GBM (p<0.01)
Log2 Difference Transcription
Log2 Difference Methylation
N = 679
R2 = 0.120****
C
GBM & GE
(p<0.01)
Log2 Difference Transcription
Log2 Difference Methylation
N = 59
R2 = 0.529****


Gene Body Methylation
Transcription
A
B
C
D
E
F
G
H
FDR<0.1
-log(p)
-1.5 0.0 1.5
Log2 Difference
(transplantation)
Variation Explained
Genotype
74%
Origin 3%
21%
Residuals
Transplant 2%
64%
Origin 4%
29%
Residuals
Transplant 3%
KK
KO
OK
OO
Density
0.0
0.2
0.4
-4 -2 0 2 4 6
Discriminant function
-15 -5 0 5 10 15


A
KK mean
Density
0.0
0.2
0.4
clones
OK
OO
Similarity
Shift
Pre-similarity
B
Weight Gain (%)
0.1
0.2
0.3
0.4
KK
KO
OK
OO
R²=0.74**
R²=0.72**
-4
-2
0
2
4
Discriminant Function
C
R²=0.51***
Fitness PC1
-2
-1
0
1
2
3
Similarity
D
Fitness PC1
Pre-sim. x Origin
Pre-sim.
13%
37%
29%
22%
Residuals
Shift