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 remains uncertain. One hypothesis is that GBM mediates phenotypic plasticity. To investigate this hypothesis, we performed whole-genome methylation and transcriptomic assays on reciprocally transplanted colony fragments of the reef-building coral *Acropora millepora*. We found that variation in gene body methylation (GBM) predicted coral fitness following transplantation. 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. Differences in GBM between populations also correlated with differences in transcription, with stronger GBM associated with elevated transcription. Our results further confirm GBM as a signature of stably active transcription and indicate that GBM tracks physiologically important genome-environment interactions.

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 expression level and gene responsiveness (14). Constitutively expressed genes (i.e., housekeeping genes) tend to be strongly methylated and inducible genes tend to be weakly methylated. This association extends to environmentally driven expression (15-17), 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 role of GBM in 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.

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).

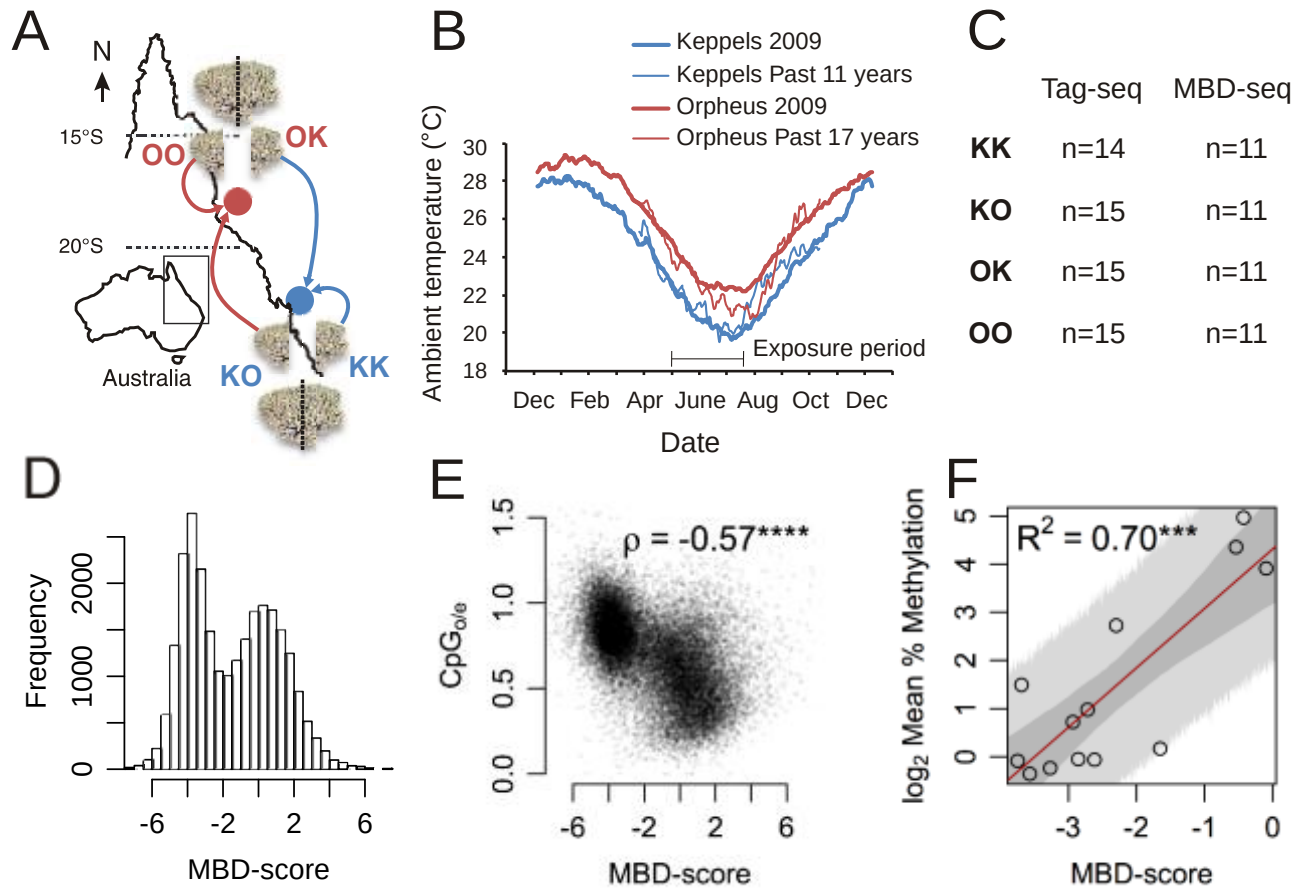

Fig. 1. Experimental design and validation of MBD-seq. (A) Map of experiment location in the Great Barrier Reef, Australia. Colonies were divided into fragments and reciprocally transplanted between two sites, a northern site, Orpheus (red), and a southern site, Keppel (blue). Sample groups are labeled 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 remains 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).

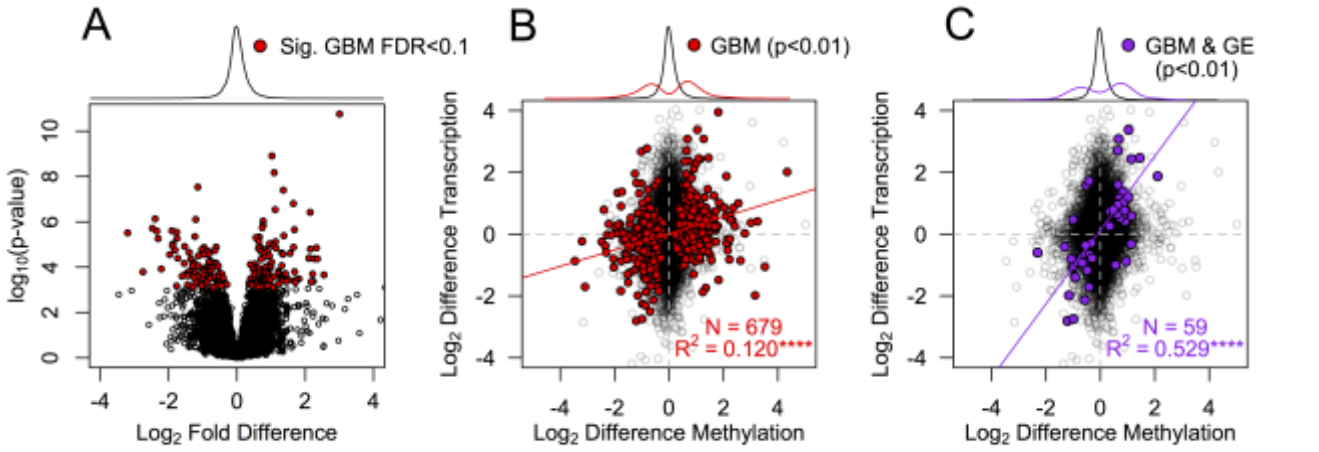

Fig. 2. Origin-specific GBM and transcription are positively correlated. (A) Differential GBM between all fragments originating from Orpheus and all fragments originating from Keppel. Significant genes (FDR < 0.1) are shown in red. (B) Scatterplot of log_2 fold differences in transcription and GBM. Log_2 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 for 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

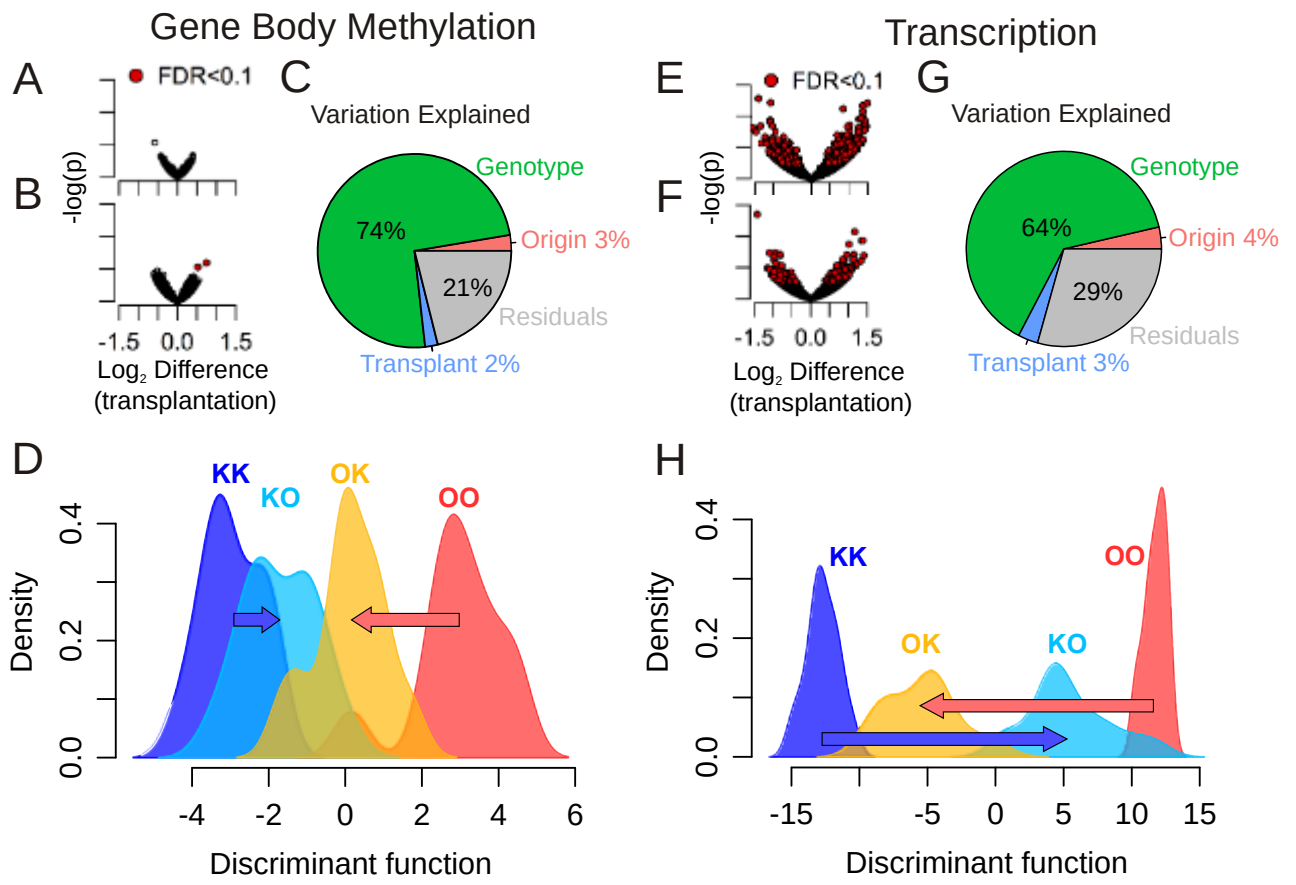

Fig. 3. Effects of transplantation on GBM and transcription. (A) Summary of transplantation effects on GBM for all genes (n= 24853) in corals originating from Keppel (KK vs KO). (B) Summary of transplantation effects on GBM in corals originating from Orpheus (OO vs OK). Significant genes (FDR < 0.1) 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 is intended to describe how similar a colony's GBM patterns were regardless of transplantation. Shift was calculated as the proportional distance along the discriminant axis between each transplanted sample

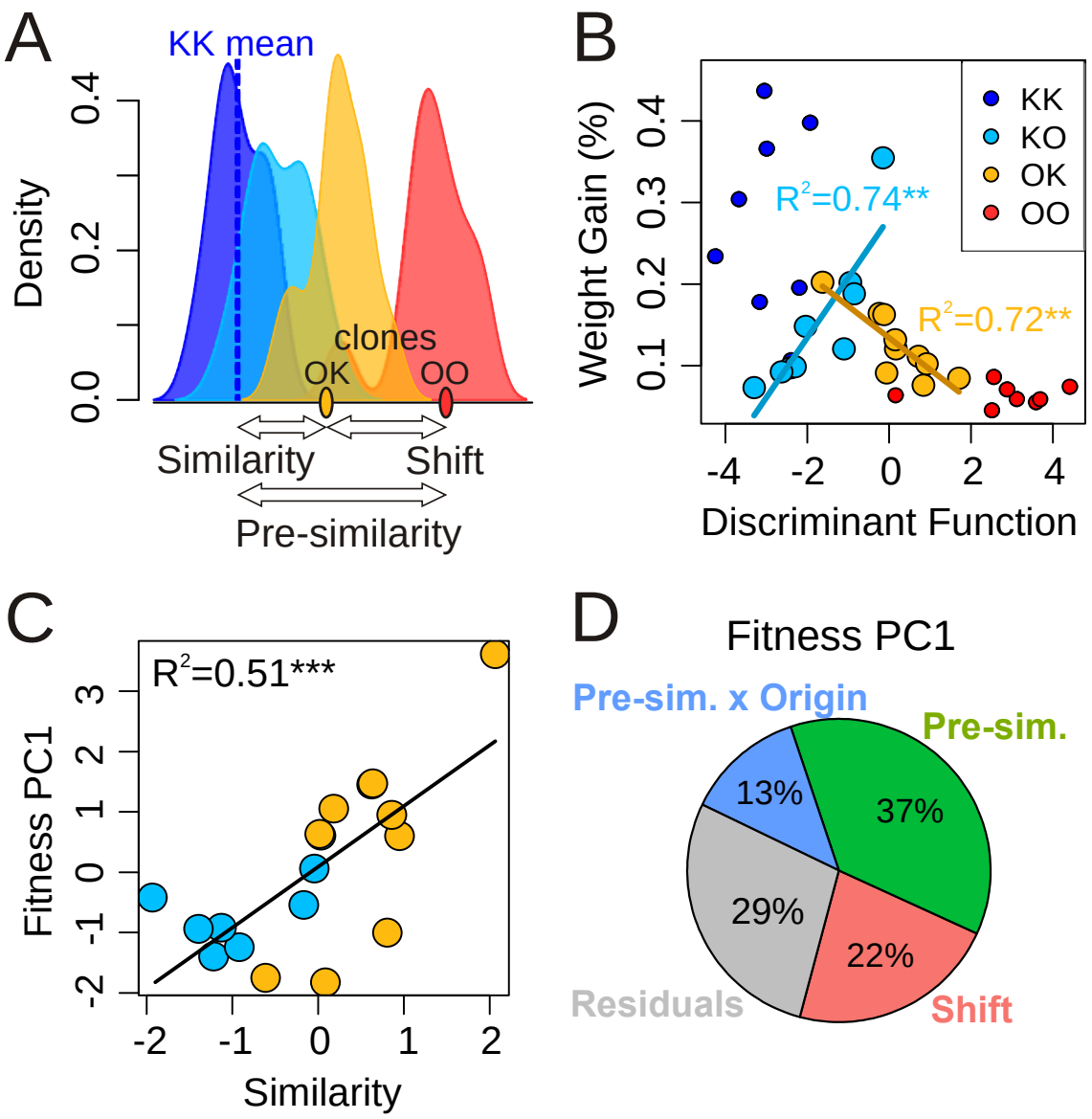

Fig. 4. Correlation between gene body methylation (GBM) and fitness-related traits. (A) Projection of transplanted samples onto the discriminant axis allowed us to quantify their similarity in GBM patterns to natives of the transplant site. Similarity was quantified as the inverse distance of a transplanted 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 how similar the genotype already was 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, and 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).

and its native clone mate. Shift was intended to describe the extent of plastic change in GBM in response to transplantation. Based on 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 stable 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 demonstrate that variation in GBM between populations may be of functional 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 patterns to native corals positively correlated with fitness-related traits (Fig. 4). It is plausible that, due to either selection or plasticity, GBM patterns of native corals reflect relatively higher fitness to their local conditions. If this is the case, then similarity of GBM patterns could be interpreted as a measure of the degree to which a transplant matched a 'target' genomic profile well-suited to the local environment. This similarity could be divided into two components: the degree to which the colony already matched its target ('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 patterns between clone mates (Fig. S1-2), we suggest that Pre-Similarity largely reflects genetic diversity, and that Shift is the better 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). They further illustrate that comparative methylation assays can shed light on the extent to which corals are pre-adapted for particular environments.

Missing mechanism

While our results demonstrate a clear association between GBM and fitness, the actual mechanism linking these traits remains unclear. The third prediction of our hypothesis was that environmentally induced changes in GBM would co-vary with transcription. Although population-specific GBM and transcription were correlated (Fig. 2), GBM patterns associated with transplantation showed either no correlation, or a *negative* correlation with transcription (Fig S12-13). Hence, in this dataset, the relationship between environmentally dynamic GBM and transcription was qualitatively different from that observed for origin-based differences, and across genes within plant (12) and animal (13) genomes. In short, GBM appears to be positively correlated with stably elevated transcription, but not with plastically elevated transcription. One possible explanation for these observations is that the dynamics of GBM and transcription operate on distinct timescales. We suggest that GBM changes slowly, only in response to sustained changes in transcription. If this is the case, GBM patterns could provide a more integrated picture of transcription across long time periods, in contrast to the temporally localized '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). This explanation is also consistent with the observation that GBM correlates with stable, origin-based transcription (Fig. 2) but not with environmental dynamic transcription (Fig. S12). To clarify, the observed differences in transcription based on transplantation likely included not only responses to conditions *characteristic* of the two sites, but also to transient conditions, such as the weather or components of water quality immediately preceding collection. Such transient conditions could produce acute differences in transcription disproportionate to their actual ecological importance over a 3-month timescale. If, on the other hand, accumulation or depletion of GBM results only from persistent changes in transcription, it would better reflect sustained genome-

environment interactions characteristic of the contrasting environments, and hence showed stronger associations with fitness-related traits. This 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 four major results using an 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, differences in GBM between populations correlated with similar differences in transcription, demonstrating that variation GBM not only correlates with transcriptional activity within genomes, but also between populations. Third, GBM is considerably less plastic than transcription. As a result, large sample population sizes are necessary to detect environmental effects on GBM. Finally, patterns of GBM correlate with coral fitness under ecologically realistic novel conditions. This result demonstrates the potential for methylomics to elucidate complex ecological traits such as local adaptation and acclimatization, possibly with greater precision than transcriptomes or SNPs.

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 (27). Protein, carbohydrate and lipid content were standardized to coral surface area measured using a twice dip paraffin wax method (28). 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 (29). Lipids were extracted using a modified version of protocol described in (30) 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). Raw reads were trimmed of non-template sequence using Cutadapt (31) 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) (32) using Bowtie2 (33). Reads mapping to annotated

coding sequences were counted using HTseq (34). Fold coverage across transcription start sites was assessed using BEDTools (35). Transcription was assayed using Tag-seq (18,36). Tag-seq reads were mapped to the *A. digitifera* reference genome using SHRiMP (37) and counted using HTseq. Normalization and statistical analyses of fold coverage for MBD-seq and Tag-seq were performed with DESeq2 (38). SNPs were called from MBD-seq reads using mpileup (39). DAPC was implemented in the R package adegenet (19). We quantified this 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:

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

Where D_x is its distance along the discriminant axis from the mean for natives of the site it was transplanted to, $\overline{D}$ 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 peformed using Bismark (40). Unless otherwise noted error bars reflect standard error of the mean. Adjustments for multiple test correction were performed using Benjamini-Hochberg correction (41). Adjusted p - values are reported using 'FDR' (eg FDR < 0.1). In many figures significance is indicated symbolically: ($^{n/s}$ not significant; $^{\&}$ $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

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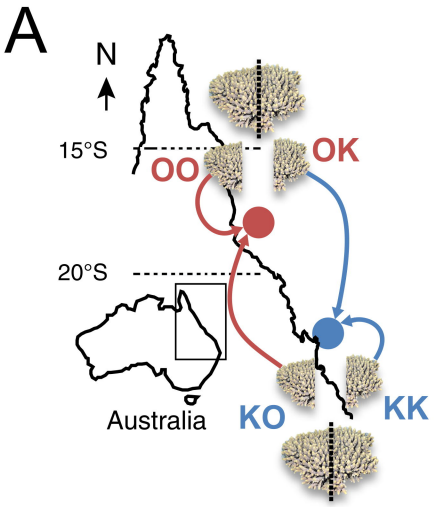

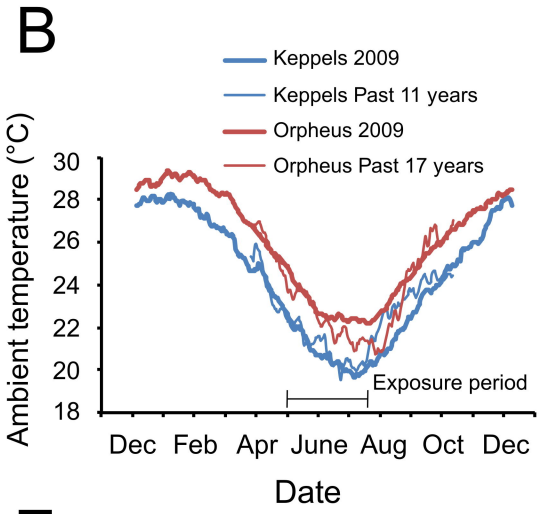

C

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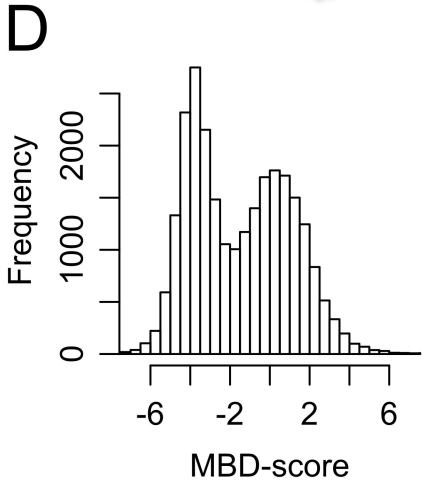
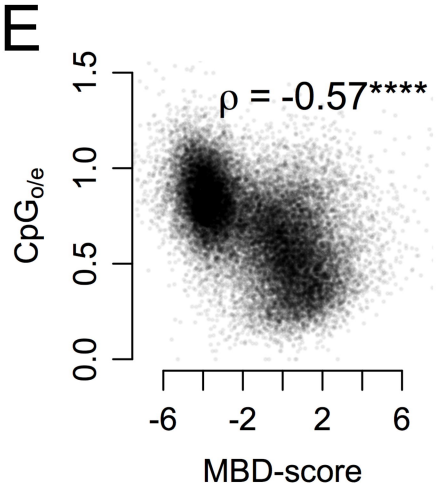
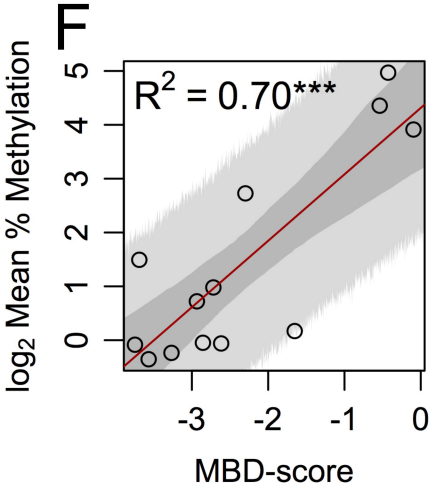
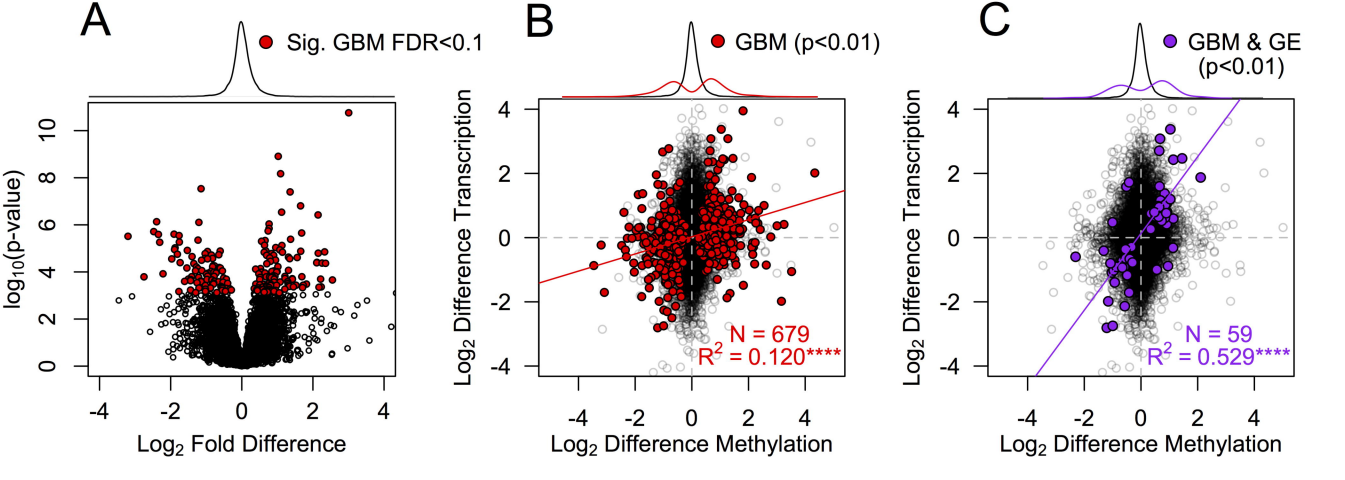
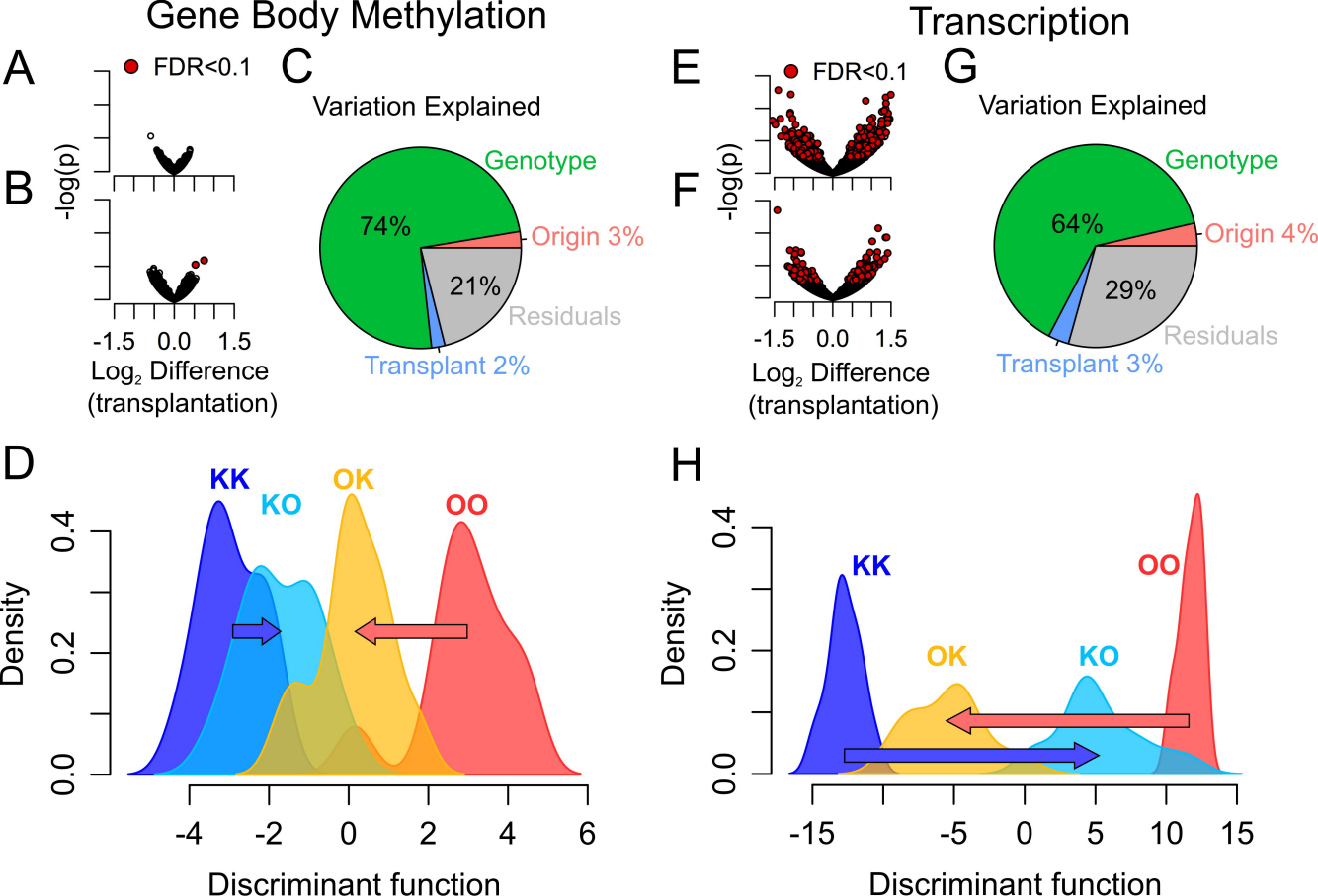
KO	n=15	n=11
OK	n=15	n=11
OO	n=15	n=11

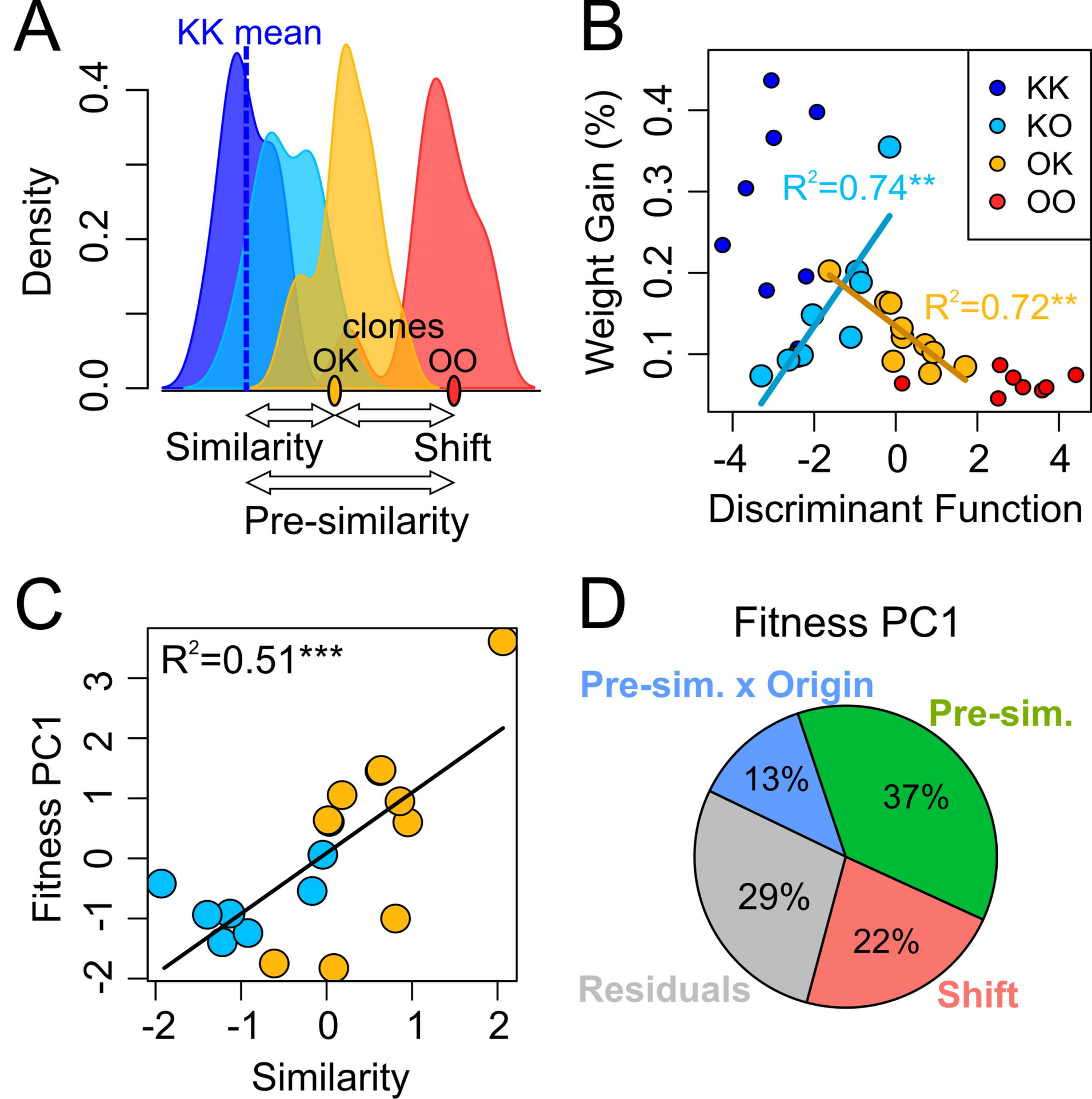







A
Sig. GBM FDR<0.1
log10(p-value)
Log2 Fold Difference
B
GBM (p<0.01)
Log2 Difference Transcription
Log2 Difference Methylation
N = 679
R2 = 0.120****
C
GBM & GE
(p<0.01)
Log2 Difference Transcription
Log2 Difference Methylation
N = 59
R2 = 0.529****


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