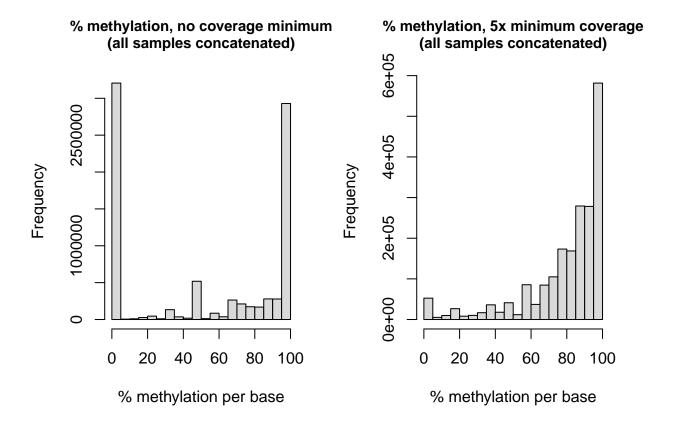
Supplementary

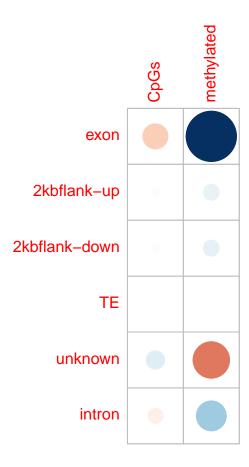
Epigenetic and genetic population structure is coupled in a marine invertebrate Katherine Silliman, Laura H. Spencer, Samuel J. White, Steven B. Roberts

1. General O. lurida methylation characteristics



Supplemental Figure 1: Frequency distribution of % methylation in *O. lurida* prior to filtering (left) and after filtering for loci with at minimum 5x coverage (right).

Feature	% of all CpGs in genome	% of all methylated loci	Ratio of % meth:%CpG
Exon	3.99%	14.71%	3.69
Intron	13.94%	19.76%	1.42
5' flanking region (-2kb)	3.91%	4.64%	1.19
3' flanking region (+2kb)	3.92%	4.66%	1.19
Transposable Elements	14.93%	13.83%	0.93
Unknown genome regions	56.01%	32.25%	0.58



Supplemental Figure 2: Top: Table showing the % of all CpG loci and methylated loci that overlap with each genomic feature. Bottom: A correlation plot, showing residuals from chi-squared tests of homogeneity on the distribution of methylated loci and CpG loci that intersect with features. Blue=positive associations, red=negative associations, and size of circles represents the absolute value of each correlation coefficient.

 $\label{lem:supplemental} \textbf{Table 1:} \ Biological functions that are enriched in the \textit{Ostrea lurida} genome. Also available in .csv format: https://github.com/sr320/paper-oly-mbdbs-gen/blob/master/analyses/methylation-characteristics/methylated-loci-enriched-BP.csv$

GO Term	Biological Process	PValue	FDR	GO slim
GO:0007067	mitotic nuclear division	0	0.37	cell cycle and proliferation
GO:0007049	cell cycle	0	0.66	cell cycle and proliferation
GO:0051321	meiotic cell cycle	0.05	1	cell cycle and proliferation
GO:0000082	G1/S transition of mitotic cell cycle	0.07	1	cell cycle and proliferation
GO:0000086	G2/M transition of mitotic cell cycle	0.09	1	cell cycle and proliferation
GO:0007067	mitotic nuclear division	0	0.37	cell organization and biogenesis

GO Term	Biological Process	PValue	FDR	GO slim
GO:0016569	covalent chromatin modification	0.01	1	cell organization and biogenesis
GO:0060271	cilium morphogenesis	0.01	1	cell organization and biogenesis
GO:0042384	cilium assembly	0.01	1	cell organization and biogenesis
GO:0007030	Golgi organization	0.02	1	cell organization and biogenesis
GO:0030030	cell projection organization	0.05	1	cell organization and biogenesis
GO:0007005	mitochondrion organization	0.05	1	cell organization
GO:0001822	kidney	0.02	1	and biogenesis developmental
GO:0001843	development neural tube closure	0.07	1	processes developmental
GO:0006281	DNA repair	0	0.08	$\frac{1}{1}$ processes DNA metabolism
GO:0006260	DNA replication	0	0.37	DNA metabolism
GO:0006310	DNA replication DNA	0.04	1	DNA metabolism
GO.0000310	recombination	0.04	1	DIVA metabonsm
GO:0006302	double-strand break repair	0.08	1	DNA metabolism
GO:0006284	base-excision repair	0.09	1	DNA metabolism
GO:0051301	cell division	0	0.12	other biological processes
GO:0007018	microtubule-based movement	0.02	1	other biological processes
GO:0042254	ribosome biogenesis	0.05	1	other biological processes
GO:0016032	viral process	0.05	1	other biological processes
GO:0043547	positive regulation of GTPase activity	0.05	1	other biological processes
GO:0000910	cytokinesis	0.06	1	other biological
GO:0032092	positive regulation of protein binding	0.08	1	processes other biological
GO:0008104	protein localization	0.09	1	processes other biological
GO:0031047	gene silencing by RNA	0.01	1	processes other metabolic processes
GO:0006511	ubiquitin- dependent protein catabolic process	0	0.12	protein metabolism
GO:0006412	translation	0	0.37	$\begin{array}{c} \text{protein} \\ \text{metabolism} \end{array}$
GO:0050821	$rac{ ext{protein}}{ ext{stabilization}}$	0.01	1	protein metabolism
GO:0006468	protein phosphorylation	0.01	1	protein metabolism

GO Term	Biological Process	PValue	FDR	GO slim
GO:0006413	translational initiation	0.03	1	protein metabolism
GO:0000209	protein polyubiquitination	0.06	1	$rac{ ext{protein}}{ ext{metabolism}}$
GO:0042787	protein ubiquitination	0.08	1	protein metabolism
	involved in ubiquitin- dependent protein catabolic process			metabolism
GO:0016567	$\operatorname{protein}$ ubiquitination	0.09	1	$\operatorname{protein}$ $\operatorname{metabolism}$
GO:0000398	mRNA splicing, via spliceosome	0	0.53	RNA metabolism
GO:0006397	mRNA processing	0	1	RNA metabolism
GO:0006364	rRNA processing	0.01	1	RNA metabolism
GO:0006396	RNA processing	0.01	1	RNA metabolism
GO:0000330 GO:0008380				RNA metabolism
	RNA splicing	0.02	1	
GO:0006366	transcription from RNA polymerase II promoter	0.05	1	RNA metabolism
GO:0008033	tRNA processing	0.06	1	RNA metabolism
GO:0006355	regulation of transcription, DNA-templated	0.09	1	RNA metabolism
GO:0006974	cellular response to DNA damage stimulus	0	0.05	stress response
GO:0006281	DNA repair	0	0.08	stress response
GO:0006302	double-strand	0.08		
GO:0000502		0.08	1	stress response
GO:0006284	break repair base-excision	0.09	1	stross rosponso
	repair			stress response
GO:0015031	protein transport	0	0.23	$\operatorname{transport}$
GO:0006886	intracellular protein transport	0	0.81	transport
GO:0006888	ER to Golgi vesicle-mediated transport	0	0.96	${ m transport}$
GO:0016192	vesicle-mediated transport	0.02	1	transport
GO:0006406	mRNA export from nucleus	0.04	1	transport
GO:0006606	protein import into nucleus	0.09	1	transport
GO:0042147	retrograde transport, endosome to Golgi	0.1	1	transport
GO:0098609	cell-cell adhesion	0.04	1	NA
GO:0070936	protein K48-linked ubiquitination	0.07	1	NA
GO:0003341	cilium movement	0.07	1	NA

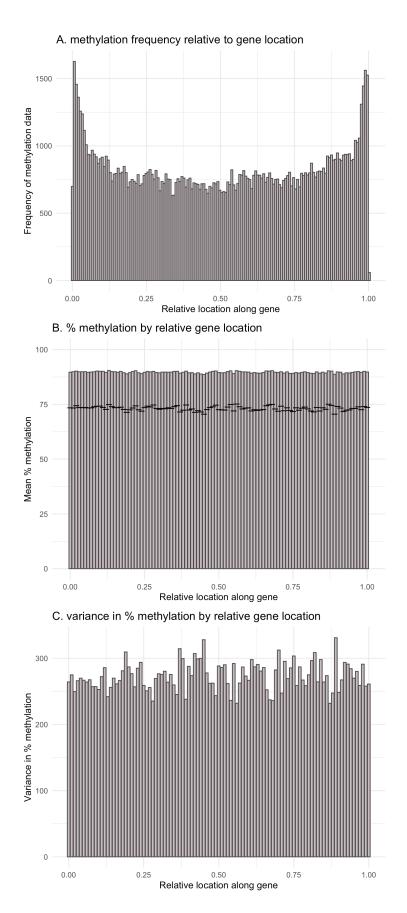
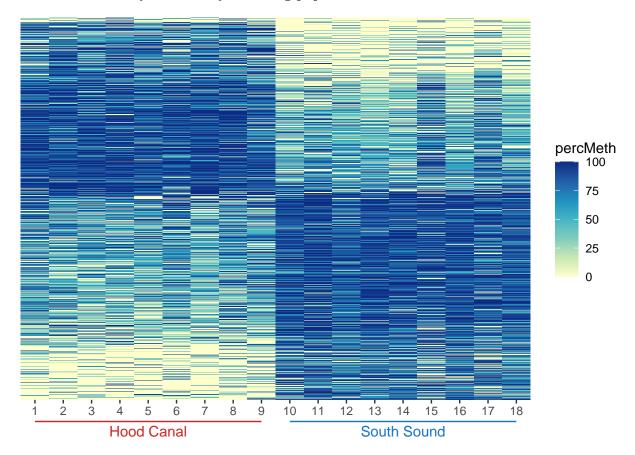
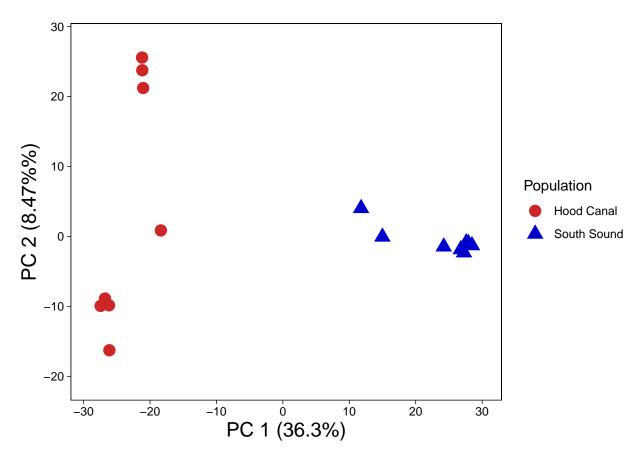


Figure 1: Supplemental Figure 3: Gene body methylation patterns. A) relative location of methylation within gene bodies in $Ostrea\ lurida$. For this analysis methylated loci were designated as those with >50% methylation when averaged across all MBD18 samples, B) percent methylation across a gene body averaged across all MBD18 samples, and C) variation in percent methylation across a gene body, across all MBD18 samples.

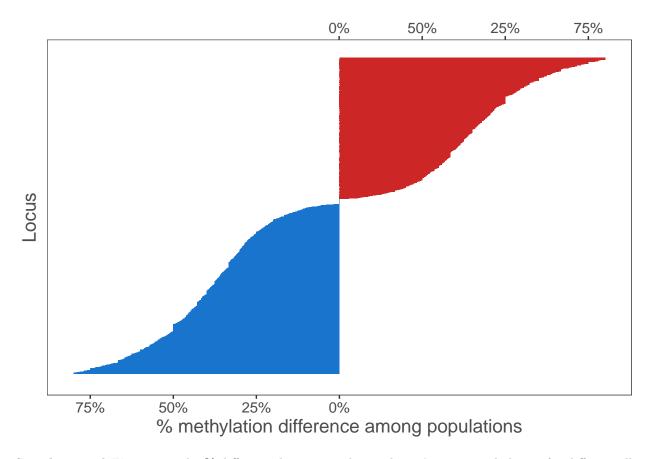
2. Differential methylation analysis among populations



Supplemental Figure 4: Heat map of loci that are differentially methylated (DMLs) between the two populations.



 ${\bf Supplemental\ Figure\ 5:\ PCA\ of\ methylation\ data\ using\ DMLs\ only}$



Supplemental Figure 6: The % difference between each population's mean methylation for differentially methylated loci, which highlights that both populations have a similar number of hyper/hypo-methylated loci. Loci are sorted by % methylation difference.

 $\begin{tabular}{ll} \bf Supplemental\ Table\ 2:\ GO\ terms\ and\ GO\ Slim\ terms\ of\ biological\ functions\ enriched\ in\ DMGs\ \&\ DMLs. \\ EASE\ scores\ shown.\ FDR\ for\ all\ terms\ is\ 1.0.\ Also\ available\ in\ .csv\ format:\ https://github.com/sr320/paper-oly-mbdbs-gen/blob/master/analyses/DMG-DML-EnrichedBP.csv \\ \end{tabular}$

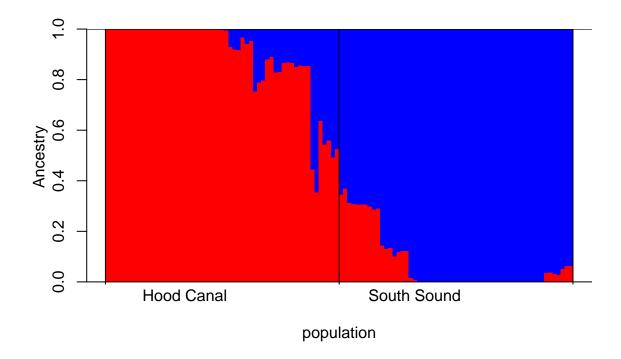
Go Slim	Term	Function	DMG	DML
cell adhesion	GO:0007155	cell adhesion	0.012	
cell adhesion	GO:0007156	homophilic cell adhesion via	0.026	0.00091
		plasma membrane adhesion molecules		
cell adhesion	GO:0016339	calcium-dependent cell-cell adhesion via plasma membrane cell adhesion molecules		0.083
cell organization and biogenesis	GO:0007015	actin filament organization	0.014	
cell organization and biogenesis	GO:0048675	axon extension	0.047	
cell organization and biogenesis	GO:0007411	axon guidance	0.063	

Go Slim	Term	Function	DMG	DML
cell organization and biogenesis	GO:0000904	cell morphogenesis involved in differentiation	0.095	
cell organization and biogenesis	GO:0030198	extracellular matrix organization	0.033	
cell organization and biogenesis	GO:0032836	glomerular basement membrane development	0.046	
cell organization and biogenesis	GO:0007040	lysosome organization	0.047	
cell organization and biogenesis	GO:0031023	microtubule organizing center organization	0.095	
cell organization and biogenesis	GO:0051259	protein oligomerization	0.098	
cell organization and biogenesis	GO:0048841	regulation of axon extension involved in axon guidance	0.033	
cell organization and biogenesis	GO:0045214	sarcomere organization	0.069	0.019
cell organization and biogenesis	GO:0055003	cardiac myofibril assembly		0.082
cell organization and biogenesis	GO:0032438	melanosome organization		0.021
cell organization and biogenesis	GO:0045332	phospholipid translocation		0.0058
death	GO:0008625	extrinsic apoptotic signaling pathway via death domain receptors	0.095	
developmental processes	GO:0048675	axon extension	0.047	
developmental processes	GO:0007411	axon guidance	0.063	
developmental processes	GO:0032836	glomerular basement membrane development	0.046	
developmental processes	GO:0048841	regulation of axon extension involved in axon guidance	0.033	
developmental processes	GO:0045214	sarcomere organization	0.069	0.019
developmental processes	GO:0007423	sensory organ development	0.095	
developmental processes	GO:0055003	cardiac myofibril assembly		0.082
developmental processes	GO:0060429	epithelium development		0.071

developmental GO:0040027 negative regulation Go:0021942 radial glia guided GO:0058 regulation of GO:0058 regulation GO:0058	Go Slim	Term	Function	DMG	DML
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December Color	developmental	GO:0021942			0.082
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other metabolic GO:0010923 negative regulation 0.028		GO:0010508	-	0.063	
	-	CO.0010022	1 00		0.029
processes or phosphatase		GO:0010925			0.028
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protein GO:0031398 positive regulation 0.098 0.093	protein	GO:0031398		0.098	0.093
metabolism of protein	-	5.5.5554500	-	3.000	0.000
ubiquitition			_		
protein GO:0016567 protein 0.0033	protein	GO:0016567	_		0.0033
metabolism ubiquitition	metabolism		ubiquitition		

Go Slim	Term	Function	$\overline{\mathrm{DMG}}$	DML
protein metabolism	GO:0042787	protein ubiquitition involved in ubiquitin- dependent protein		0.003
signal transduction	GO:0046426	catabolic process negative regulation of JAK-STAT cascade		0.071
stress response	GO:0042594	response to starvation		0.03
transport	GO:0006895	Golgi to endosome transport	0.043	0.019
transport	GO:0034220	ion transmembrane transport	0.086	0.00049
transport	GO:0008333	endosome to lysosome transport		0.083
transport	GO:0045332	phospholipid translocation		0.0058
NA	GO:0044331	cell-cell adhesion mediated by cadherin	0.033	0.071
NA	GO:0072015	glomerular visceral epithelial cell development	0.095	
NA	GO:0086010	membrane depolarization during action potential	0.095	
NA	GO:1903955	positive regulation of protein targeting to mitochondrion	0.0082	
NA	GO:0038061	NIK/NF-kappaB sigling		0.071
NA	GO:0090175	regulation of establishment of plar polarity		0.082

3. Genetic Structure



Supplemental Figure 7: Admixture plot for 114 individuals at K=2 (determined to be the best K using the Evanno method), based on 3,724 SNPs.

Supplemental Table 3: Annotations for outlier SNPs that fall within 2kb of a gene. Also available in .bed format, $HCSS_Afilt32m70_01_BS$ -gene2kb.bed

SNP Contig	SNP Position	Feature Contig	Feature Start	Feature End	Feature Type	Genome ID	Gene Name	GO Terms
Contig29033	27589	Contig29033	11407	32648	gene	OLUR_00003721	Note=Similar to G2/mitotic-specific cyclin-B (Hydra viridissima OX%3D6082);	Ontology_term=GO:0005634;
Contig77382	11621	Contig77382	1559	12918	gene	OLUR_00016056	Note=Similar to Socs5: Suppressor of cytokine signaling 5 (Mus musculus OX%3D10090);	NA

Supplemental Table 4: DAVID enrichment results for genes with FST > 0.3. Also available in .tsv format, fst_g03_david.tab

Category	Term	Count	Percent Genes	PValue	Fold Enrichment	FDR
UP_KEYWORDS	Zinc-finger	7	26.92	0.0198	2.97	1
GOTERM_BP_DIRECT	GO:0006897~endocytosis	3	11.54	0.0236	11.34	1
GOTERM_BP_DIRECT	GO:0006915~apoptotic process	4	15.38	0.0281	5.50	1
UP_SEQ_FEATURE	compositionally biased region:Ser-rich	4	15.38	0.0584	4.19	1
GOTERM_BP_DIRECT	GO:0043401~steroid hormone mediated signaling pathway	2	7.69	0.0620	30.24	1
UP_SEQ_FEATURE	region of interest:Ligand-binding	2	7.69	0.0693	27.21	1
GOTERM_BP_DIRECT	GO:0010506~regulation of autophagy	2	7.69	0.0917	20.16	1
UP_KEYWORDS	Zinc	7	26.92	0.0969	2.05	1
INTERPRO	IPR000536:Nuclear hormone receptor, ligand-binding, core	2	7.69	0.0972	19.12	1
INTERPRO	IPR001628:Zinc finger, nuclear hormone receptor-type	2	7.69	0.0972	19.12	1
INTERPRO	IPR001723:Steroid hormone receptor	2	7.69	0.0972	19.12	1

4. mQTL analysis

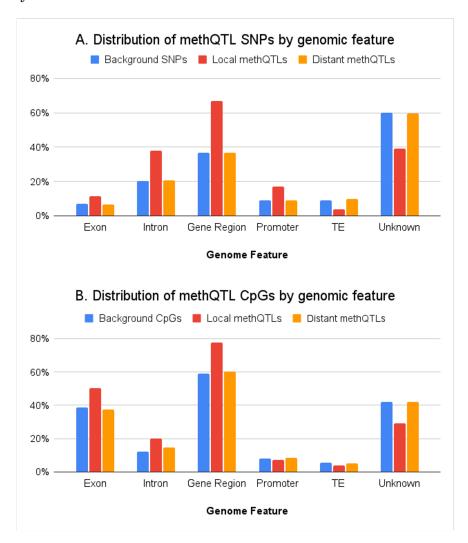


Figure 2: Supplemental Figure 9: Comparison of mQTL results that intersect with the following genomic features: exon, intron, promoter region (within 2kb of the 5' end of a gene), gene region (genes plus 2kb upstream and downstream), transposable element, and unknown region of genome. A) SNPs designated as either local (red, within 50kb) or distant (orange) mQTLs, compared with the background (blue) of all SNPs used in the mQTL analysis. B) CpGs associated with either local (red) or distant (orange) mQTLs, compared to the background (blue) of all CpGs used in the mQTL