

# Does DNA Methylation Facilitate Phenotypic Plasticity?

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University of Washington  
School of Aquatic and Fishery Sciences

**robertslab.info**  
**@sr320**

2 February 2016



# Open Science

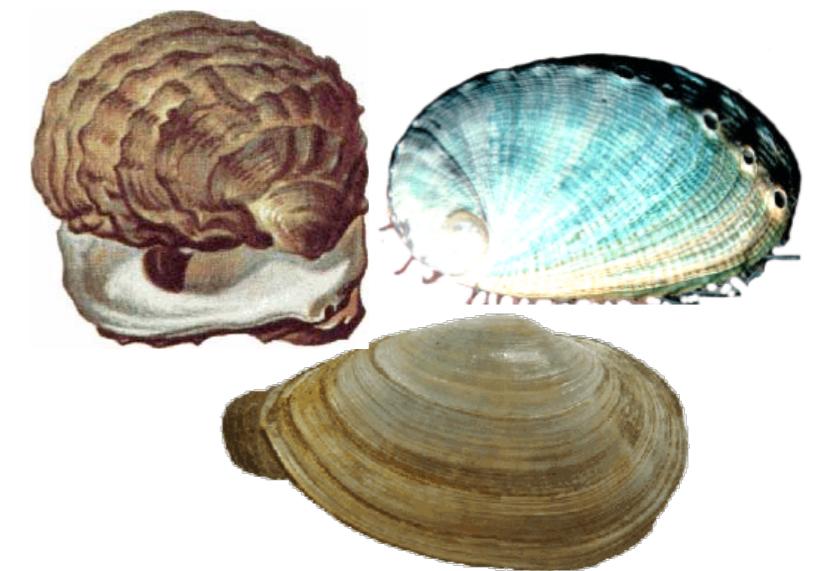
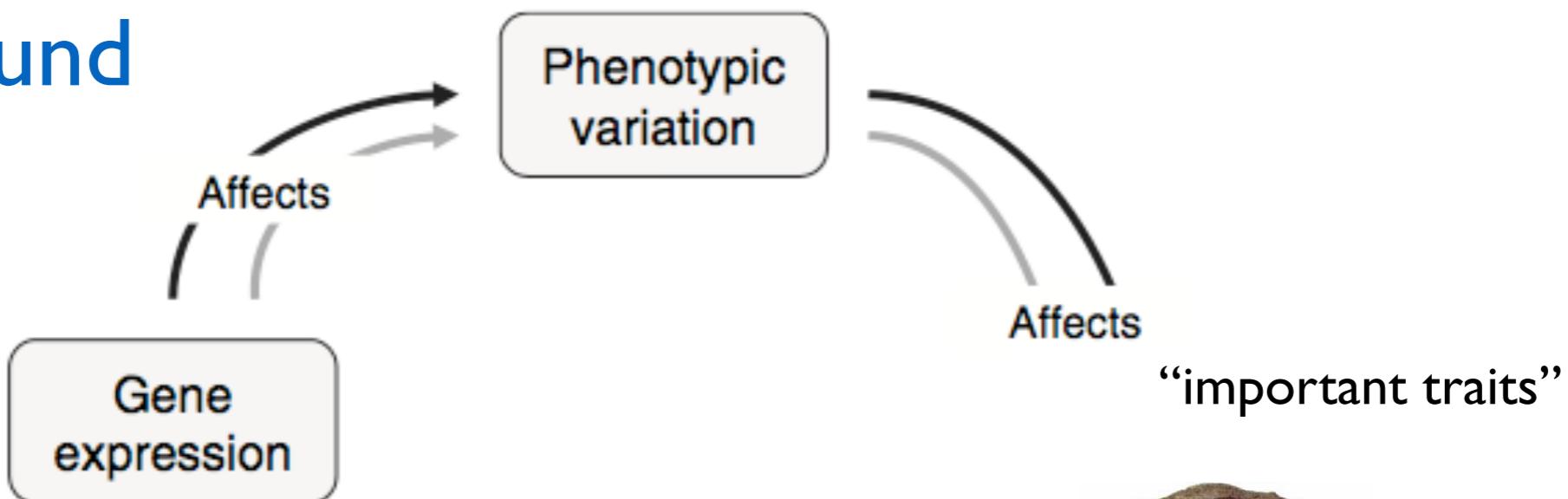
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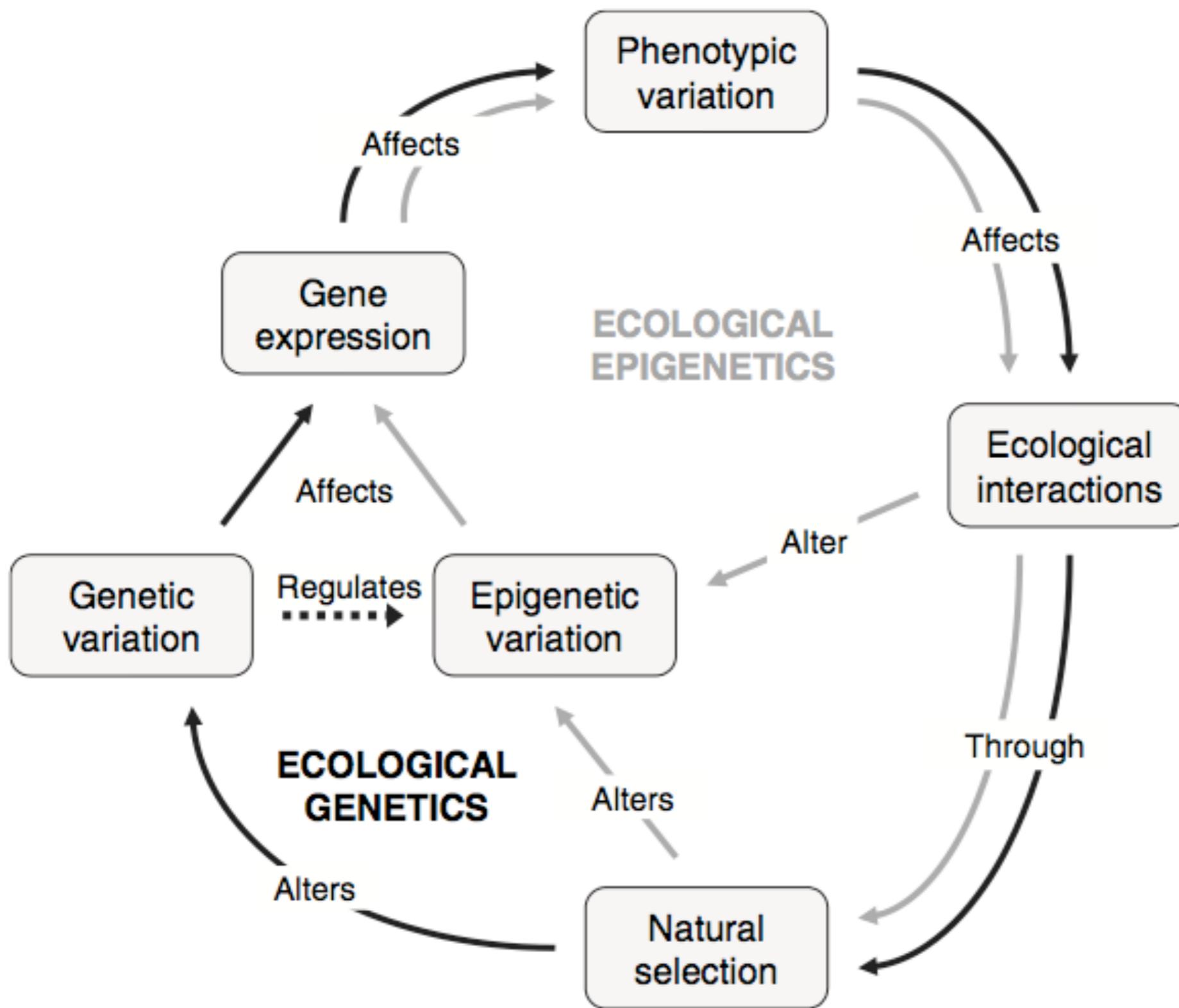
- You are free to Share!
- Our lab practices open notebook science
- Data, Preprints, Proposals, Lab Meetings, Web Cams, Slidedecks

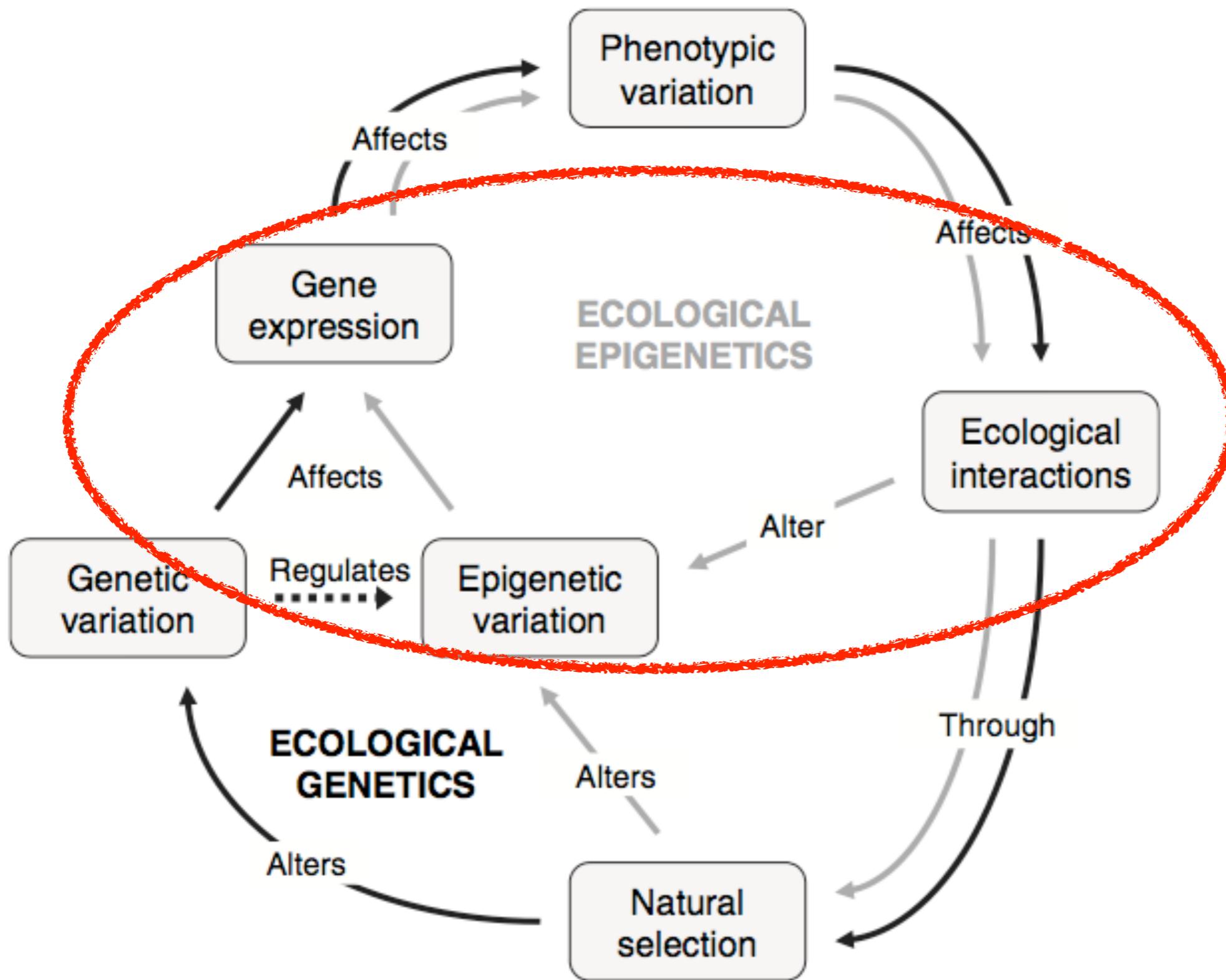


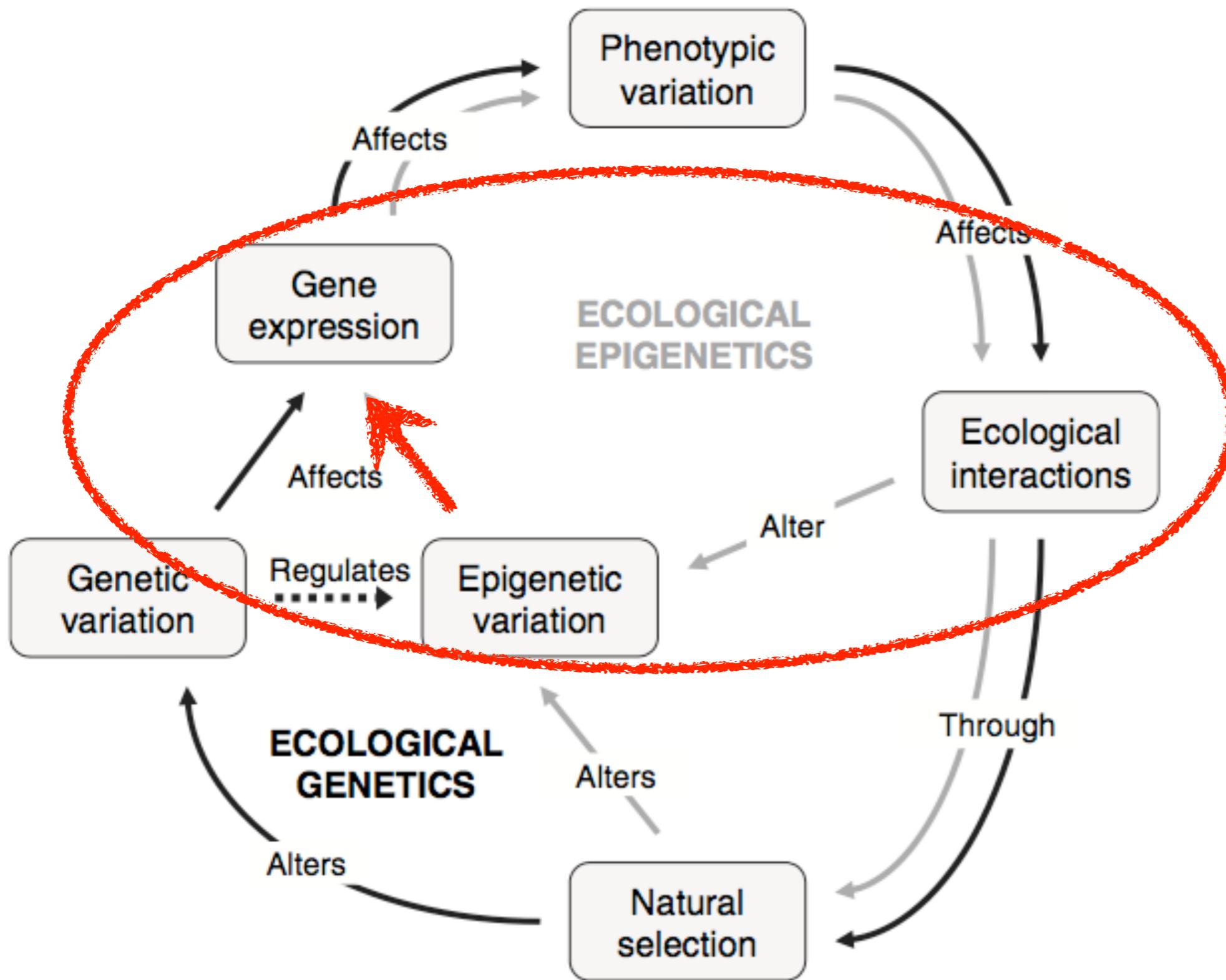
These slides plus links @  
[github.com/sr320/talk-univ-perp-2016](https://github.com/sr320/talk-univ-perp-2016)

# Background

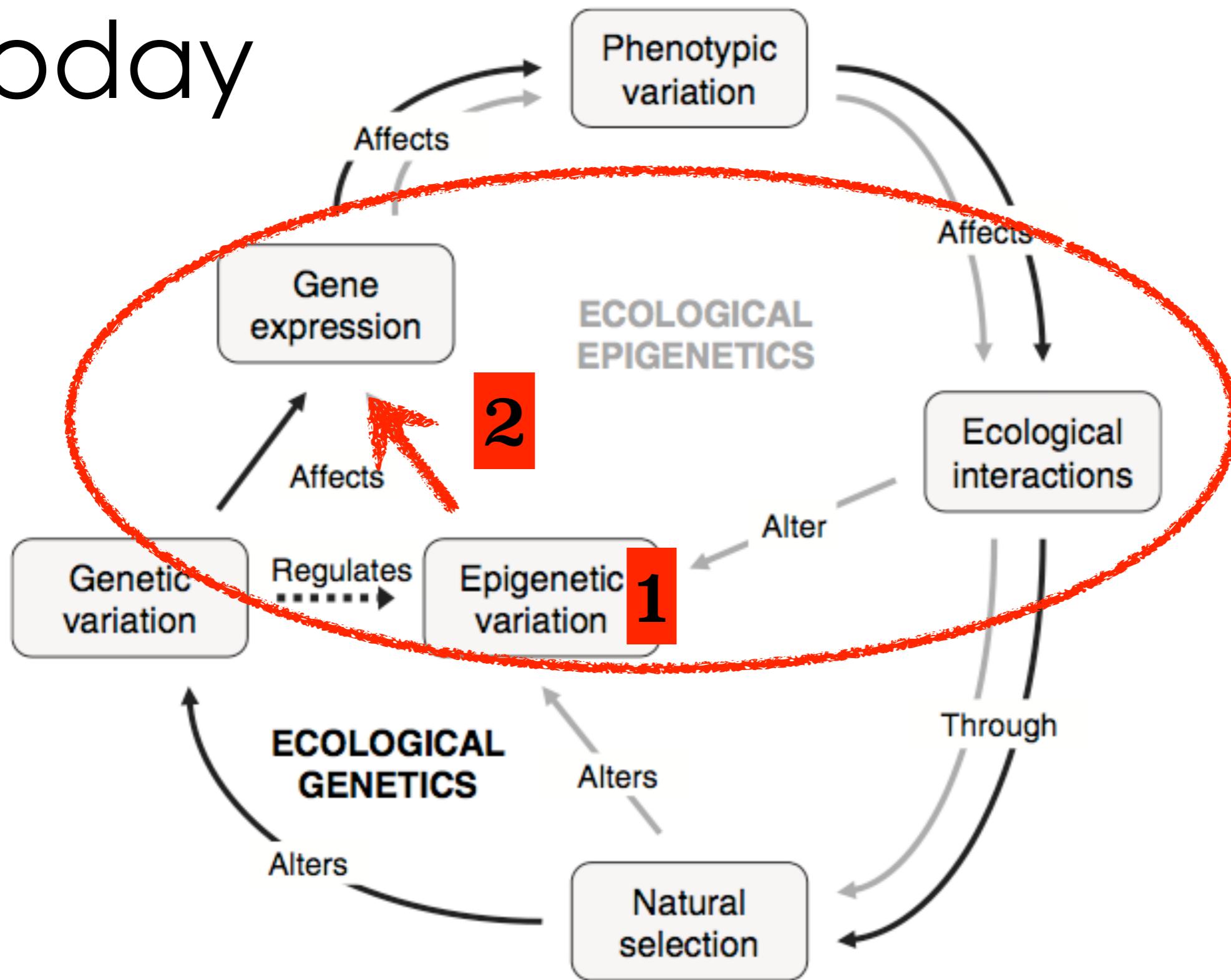








# Today



*Ecology Letters*, (2008) 11: 106–115

doi: 10.1111/j.1461-0248.2007.01130.x

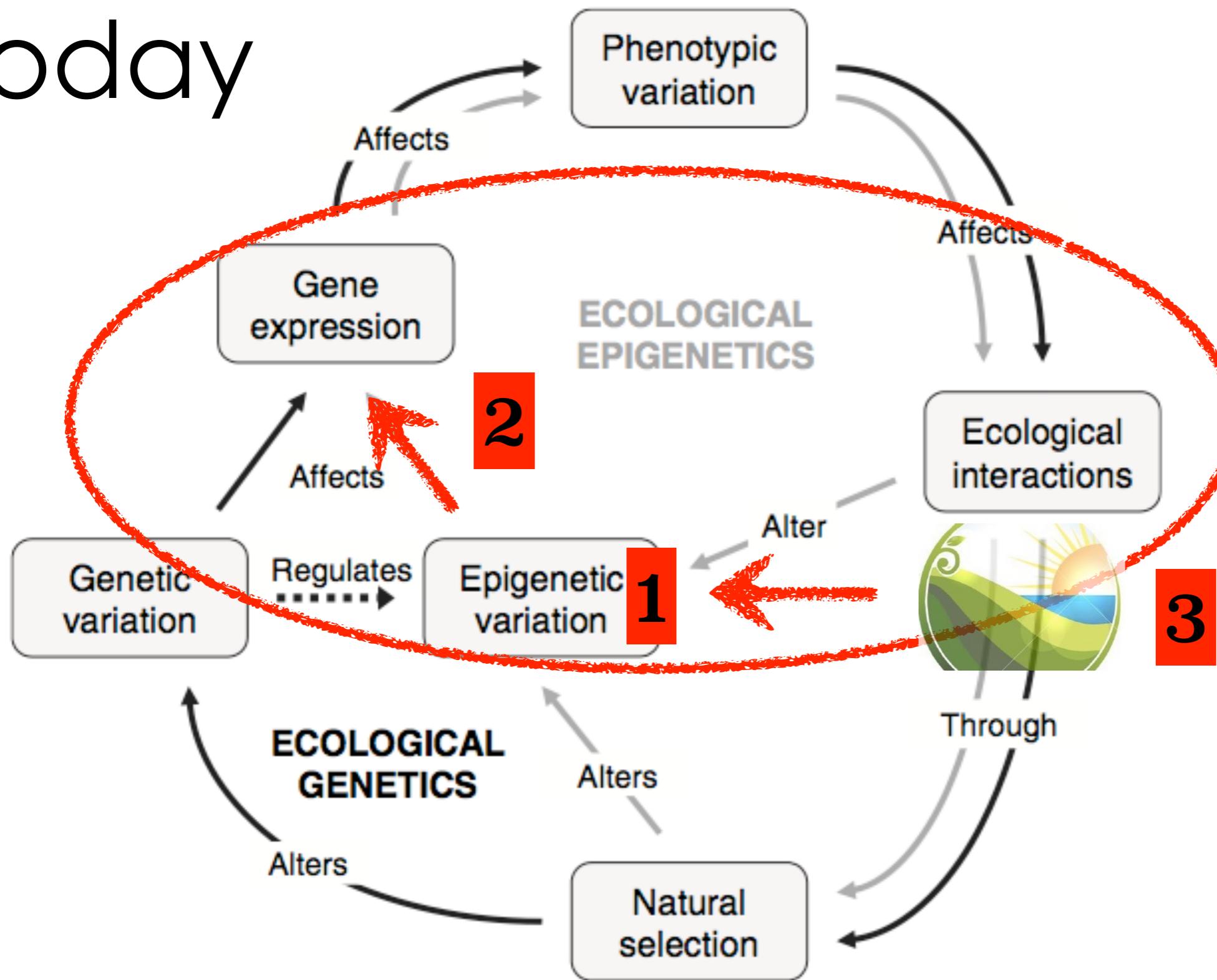
modified from

IDEA AND  
PERSPECTIVE

Epigenetics for ecologists

Oliver Bossdorf,<sup>1,\*</sup> Christina L.  
Richards<sup>2</sup> and Massimo Pigliucci<sup>3</sup>

# Today



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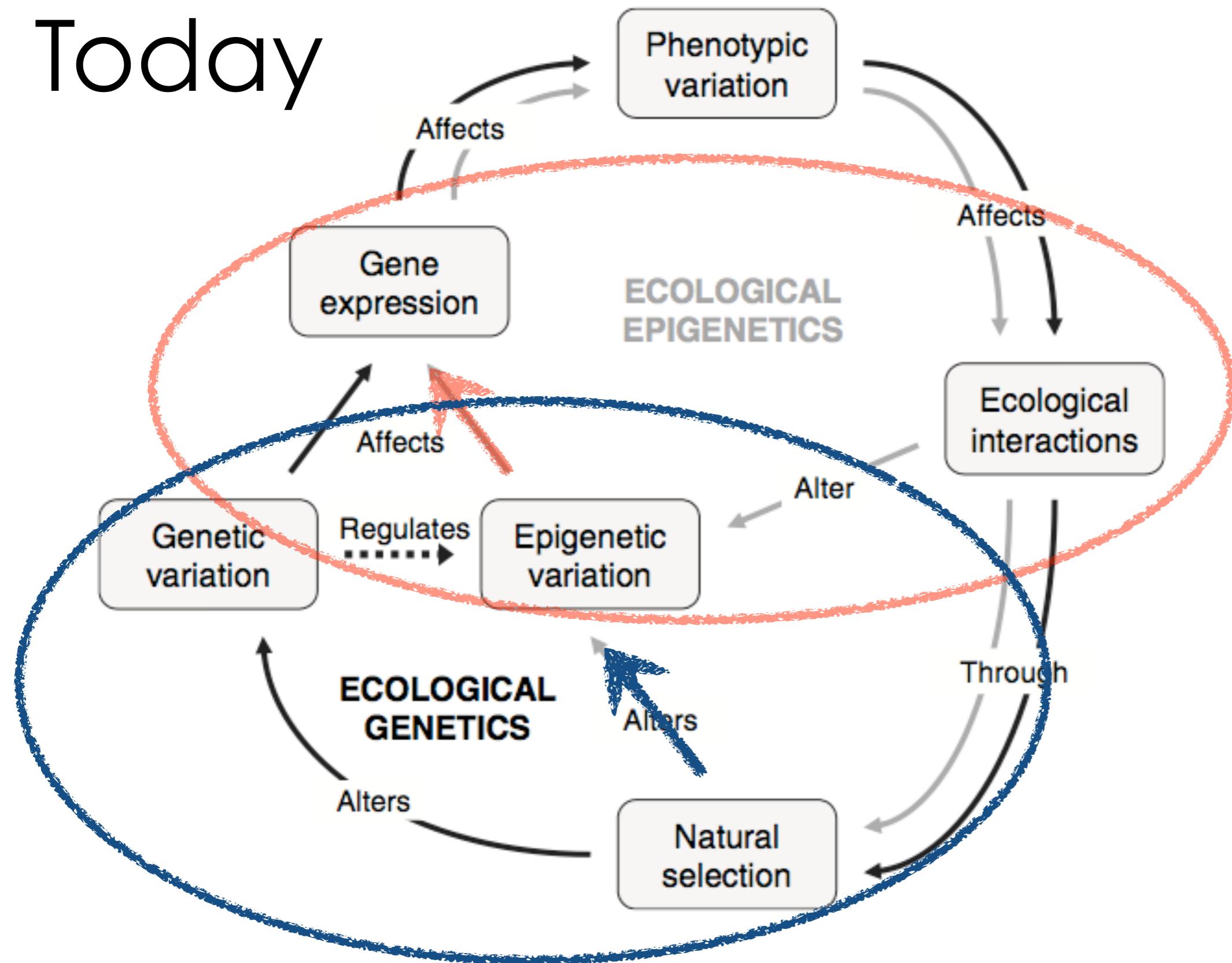
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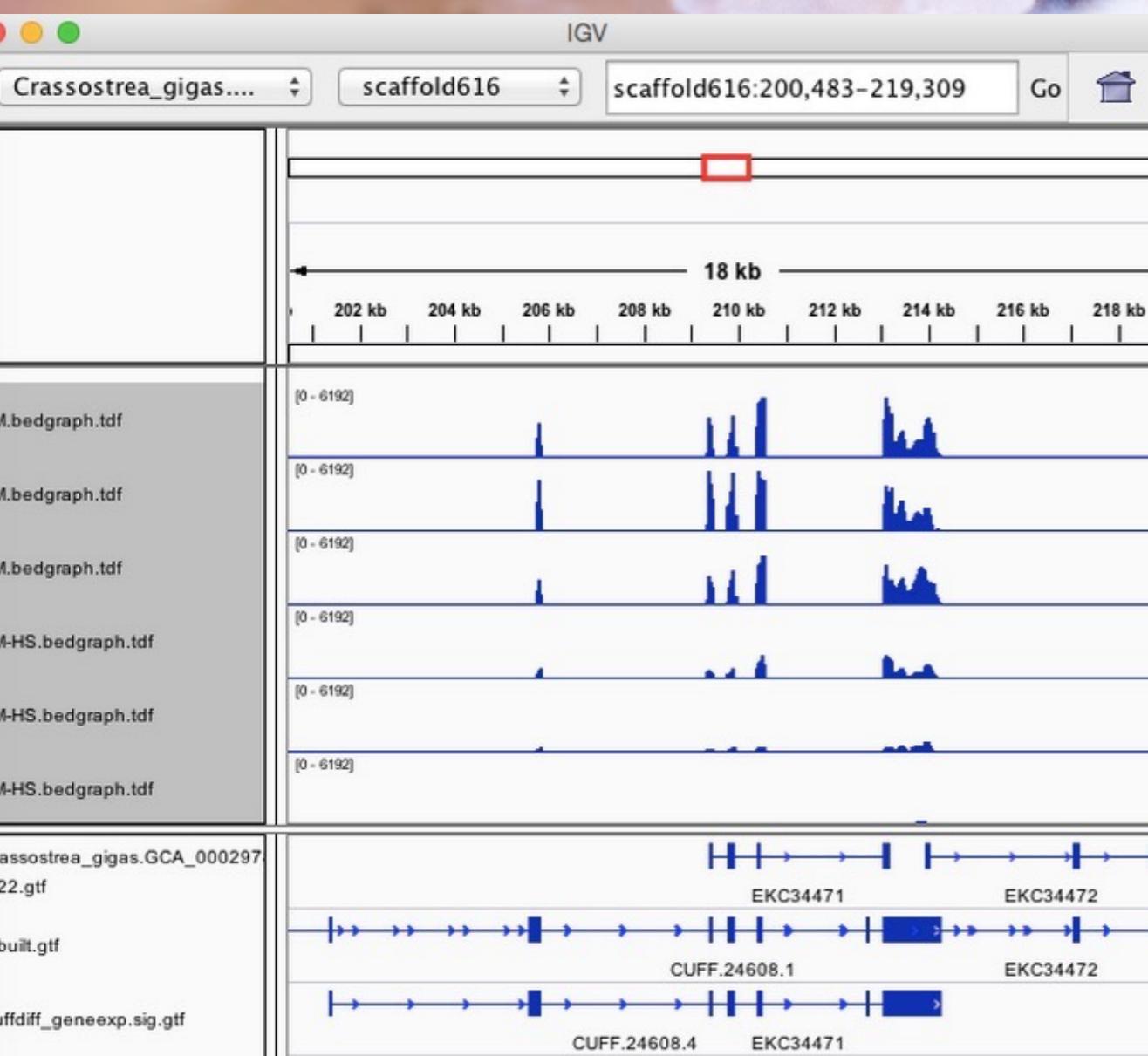
IDEA AND  
PERSPECTIVE

Epigenetics for ecologists

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# Genome Resources

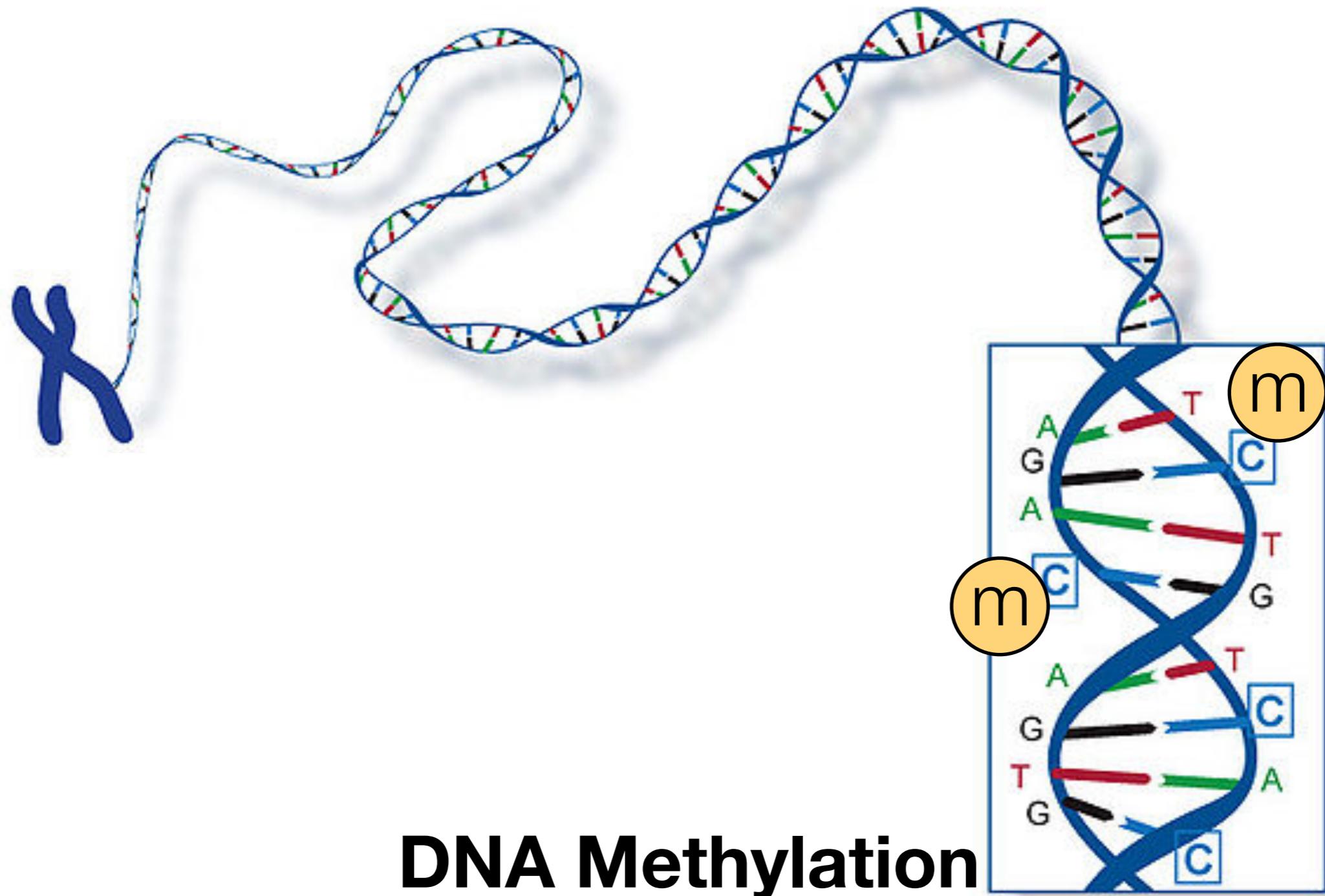


A background photograph showing several oysters being processed. Some oysters are open, revealing their meat, while others are closed. A person wearing blue gloves is visible, working on one of the oysters. The scene is set in a kitchen or laboratory environment with a green surface and various tools in the background.

**Genome Resources**

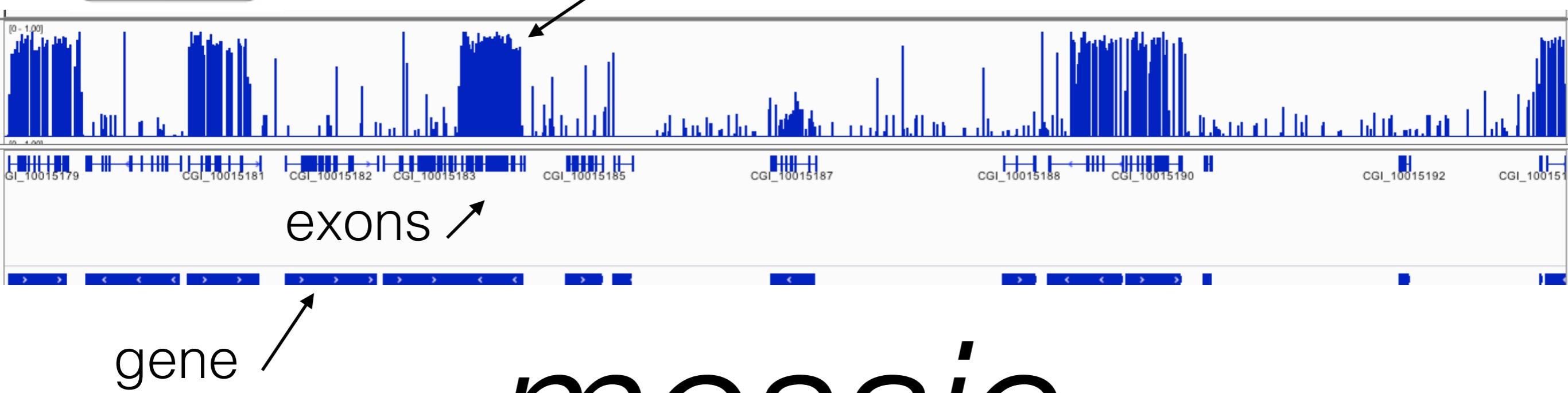
An inset photograph showing a close-up of a hand wearing a blue glove holding a partially open oyster shell. The shell is dark brown and textured. The background is a solid dark color.

**As of this week**



Epigenetic variation **1**

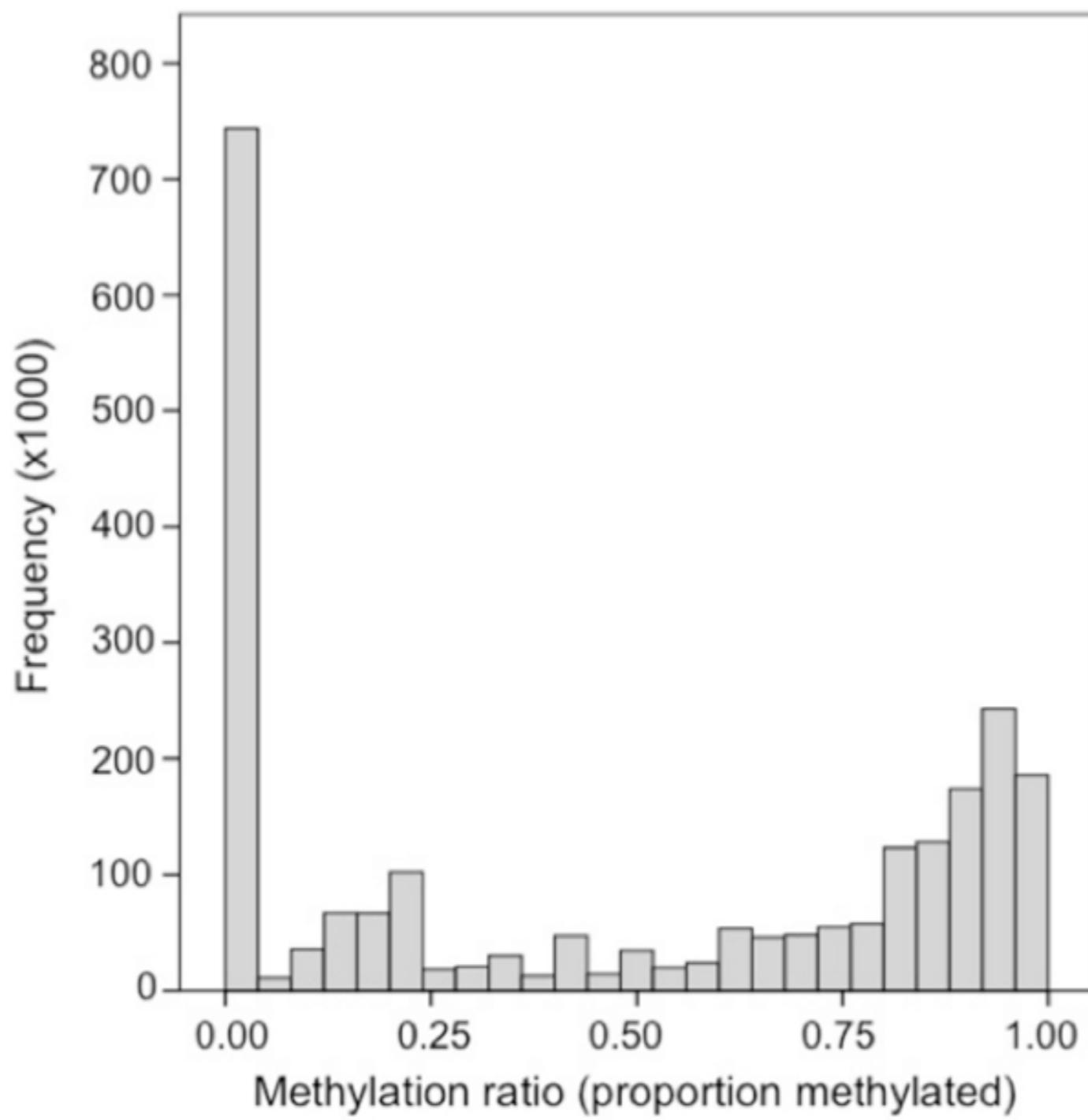
DNA methylation level (0-100%) @ cytosines



*mosaic*

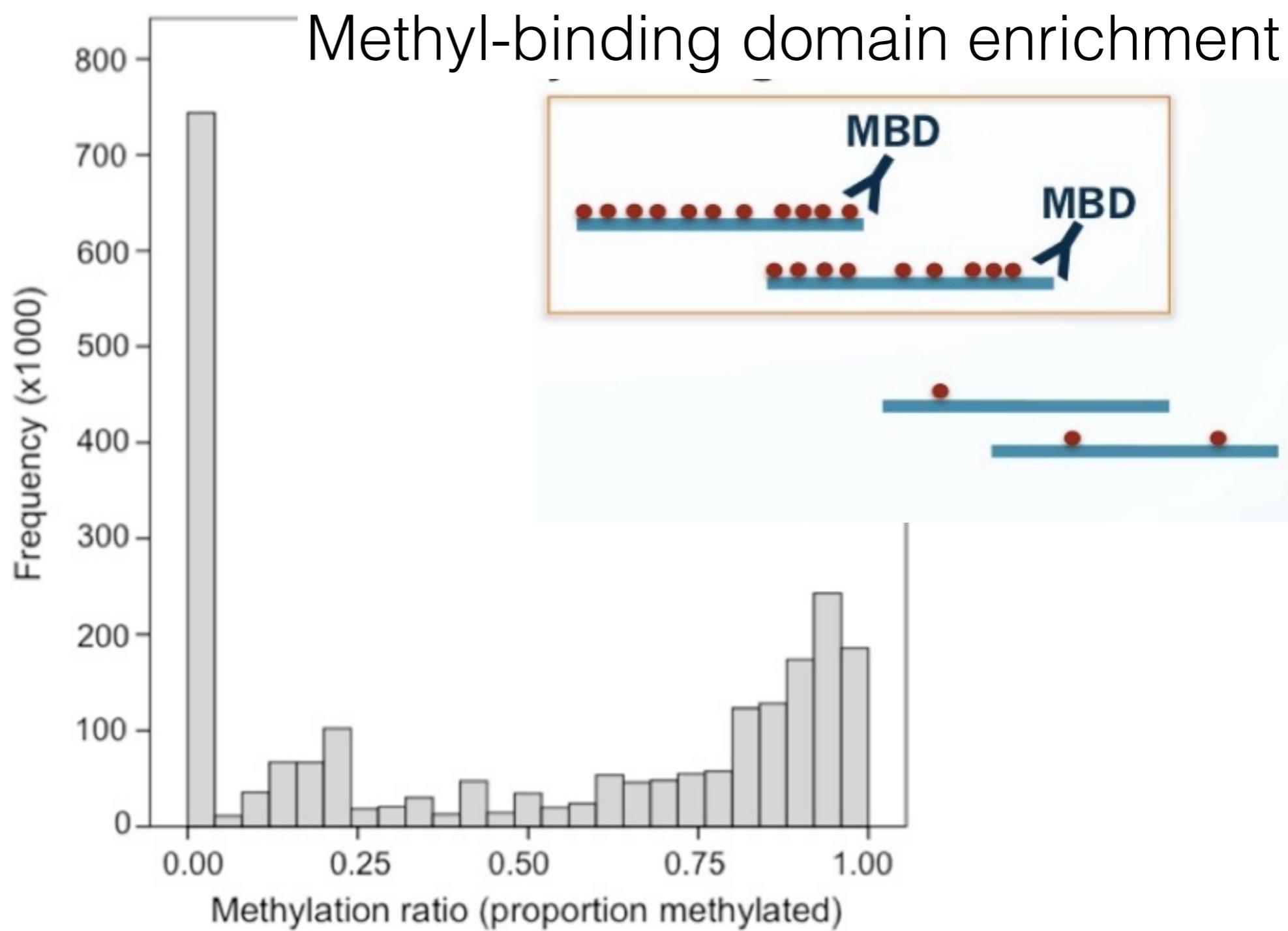
associated with gene bodies

Epigenetic variation **1**



**Figure 1** Frequency distribution of methylation ratios for CpG dinucleotides in oyster gill tissue. A total of 2,625,745 CpG dinucleotides with  $\geq 5 \times$  coverage are represented.

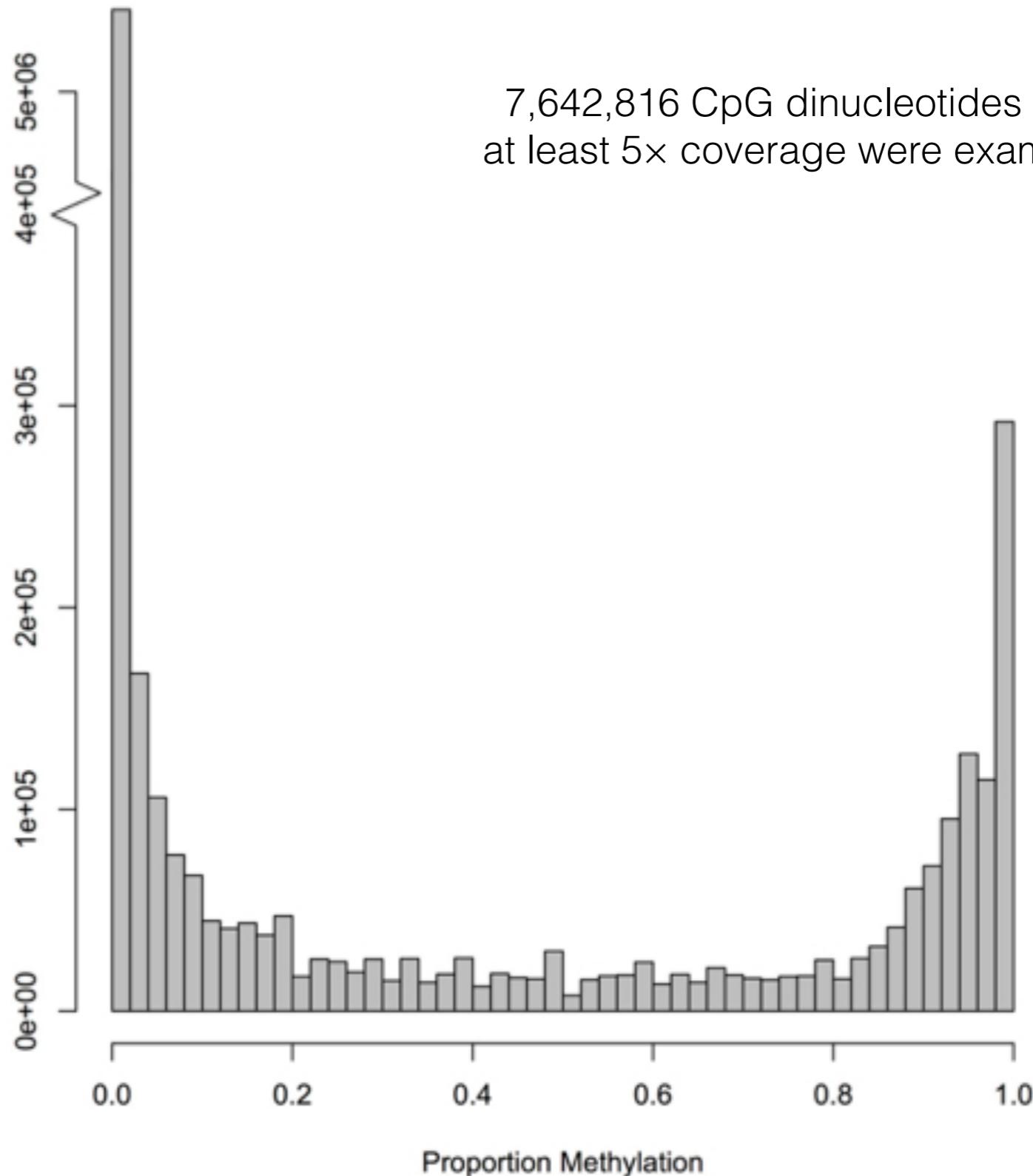
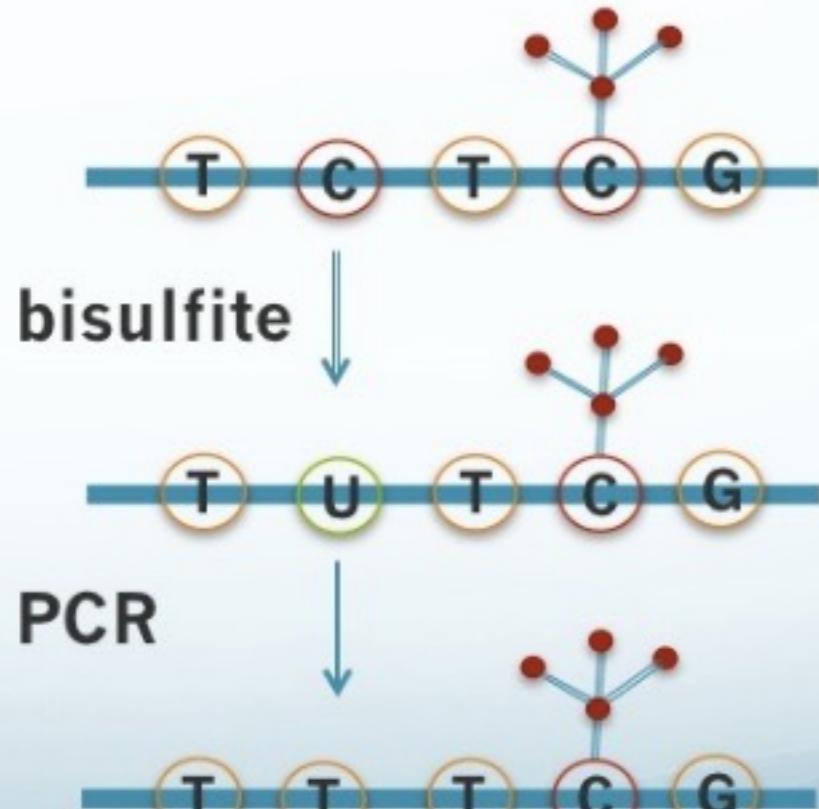
Epigenetic variation **1**



**Figure 1** Frequency distribution of methylation ratios for CpG dinucleotides in oyster gill tissue. A total of 2,625,745 CpG dinucleotides with  $\geq 5 \times$  coverage are represented.

Epigenetic variation **1**

– Bisulfite conversion

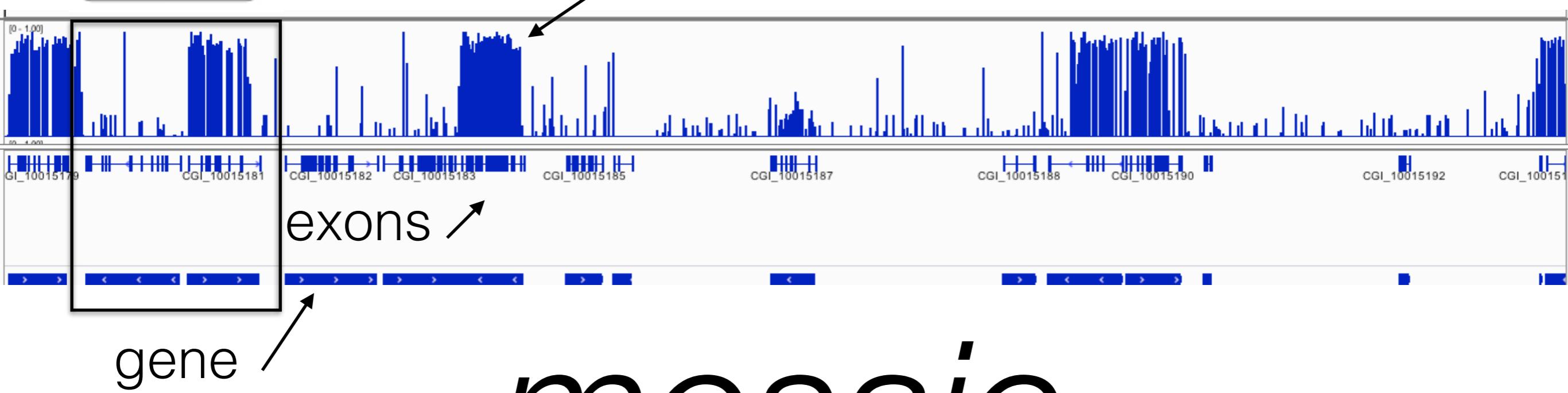


7,642,816 CpG dinucleotides with at least 5x coverage were examined



Epigenetic variation **1**

DNA methylation level (0-100%) @ cytosines

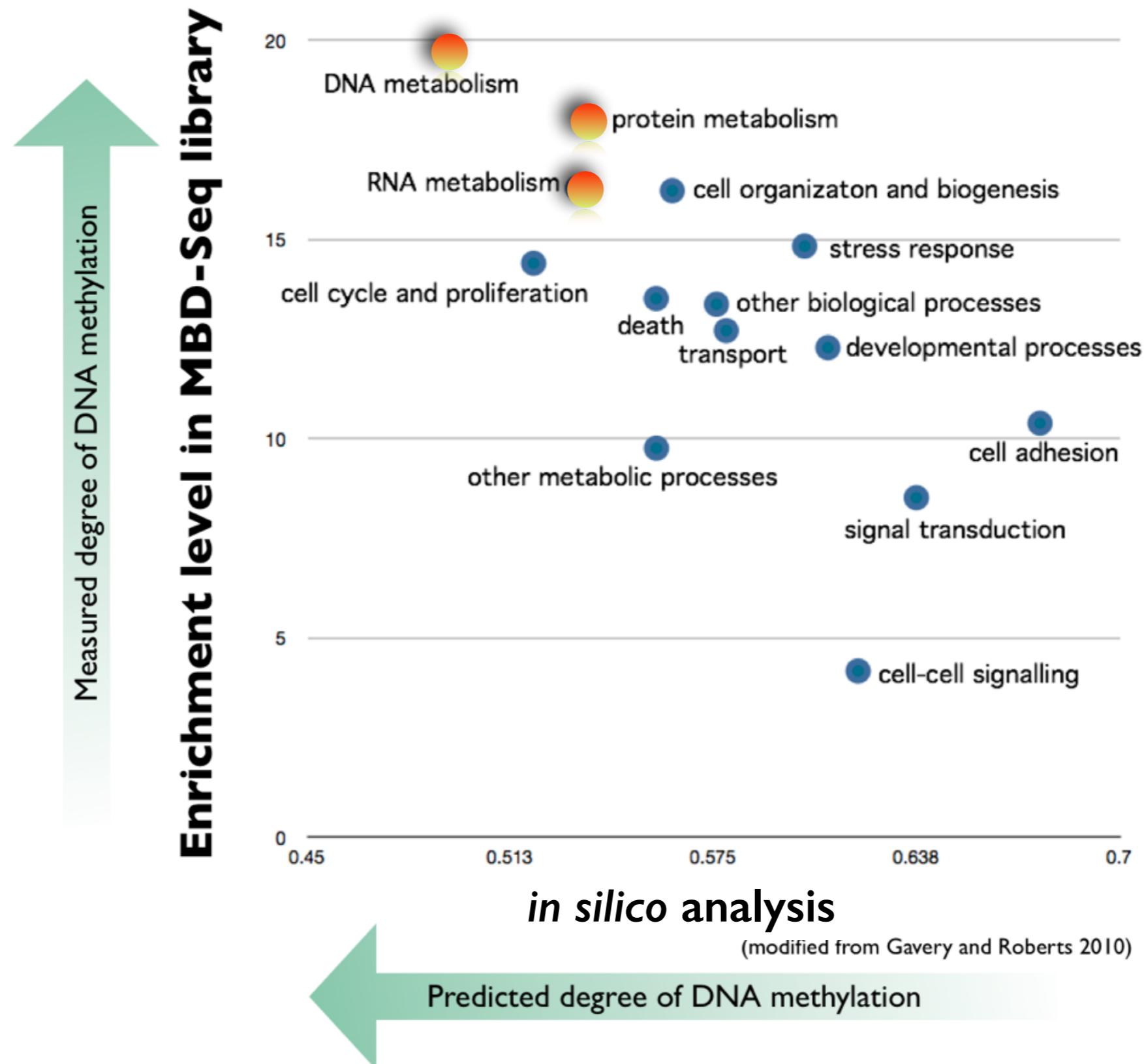


*mosaic*

**Why are only a subset of genes methylated?**

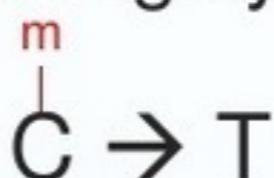
associated with gene bodies

Epigenetic variation **1**



# *in silico* approach

- Principle:
  - Methylated cytosines are highly mutable



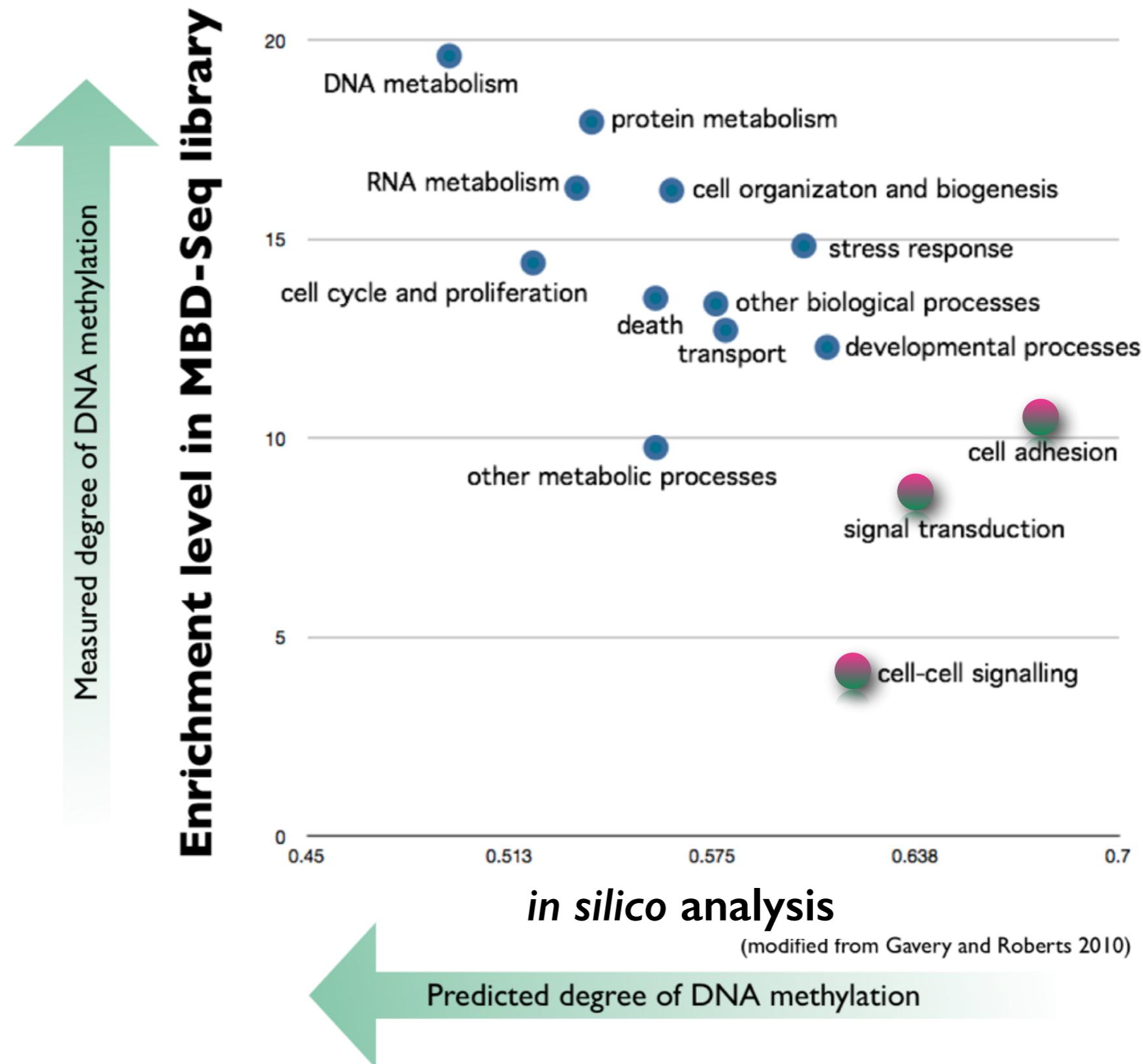
- Methylated regions of DNA are depleted of CpG dinucleotides over evolutionary time (CpG to TpG)

$$\text{CpG O/E} = \frac{\text{CpG observed}}{\text{CpG expected}}$$



high = unmethylated  
*approaching 1*

Epigenetic variation **1**



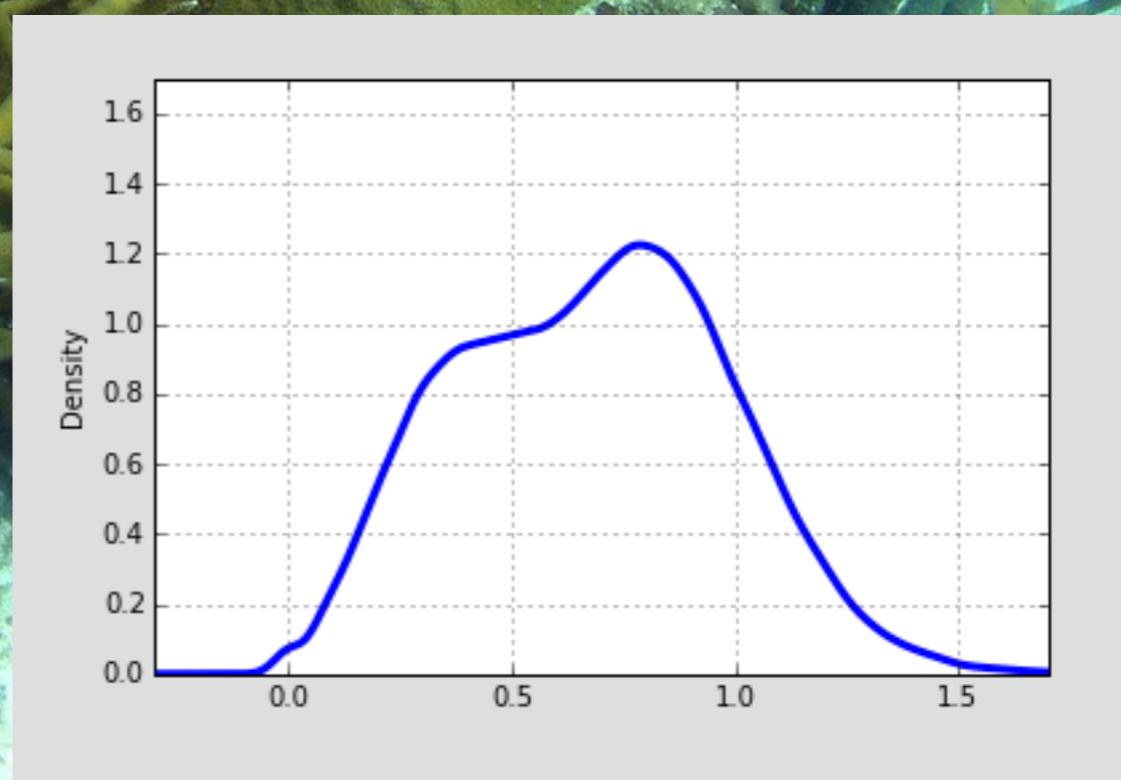
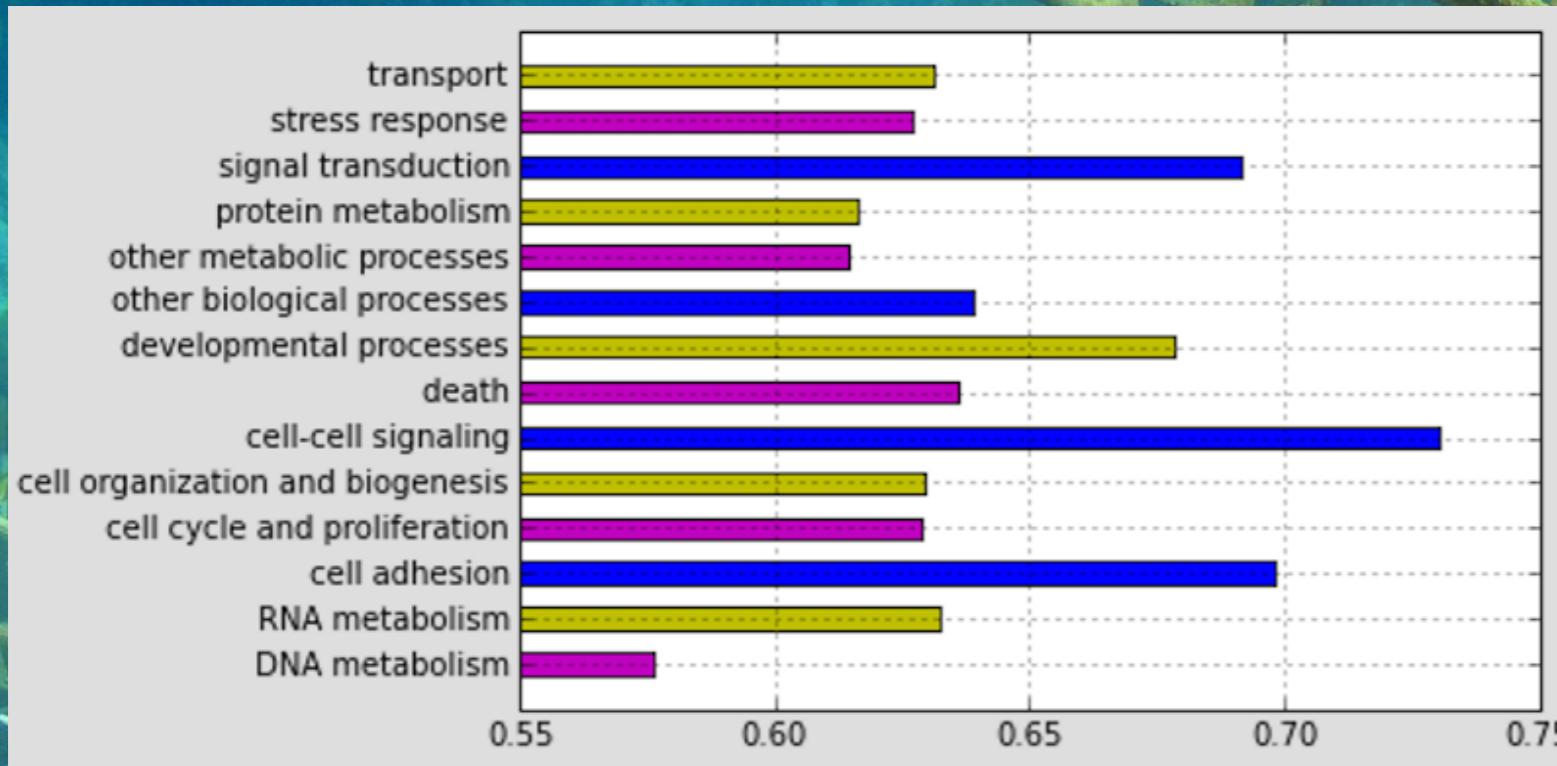
Jay Dimond

*Acropora palmata*

*Acropora cervicornis*

# Jay Dimond

*Acropora palmata*



# Jay Dimond

Special Issue

## **Germline DNA methylation in reef corals: patterns and potential roles in response to environmental change**

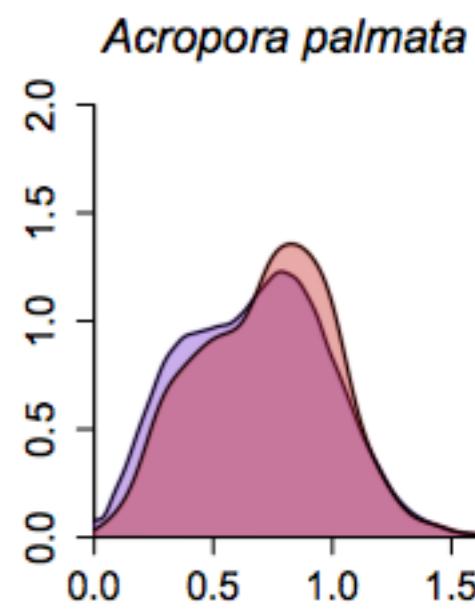
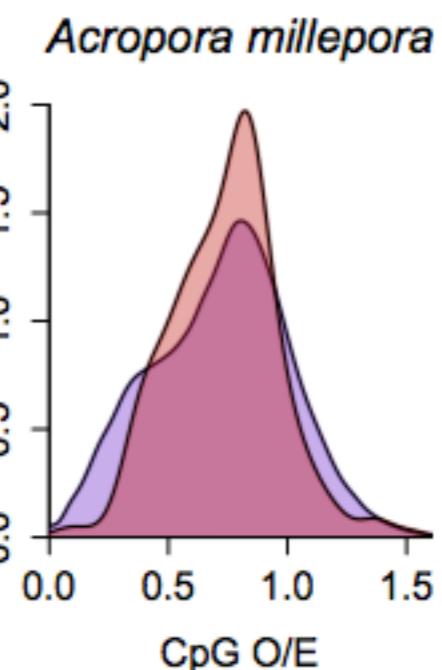
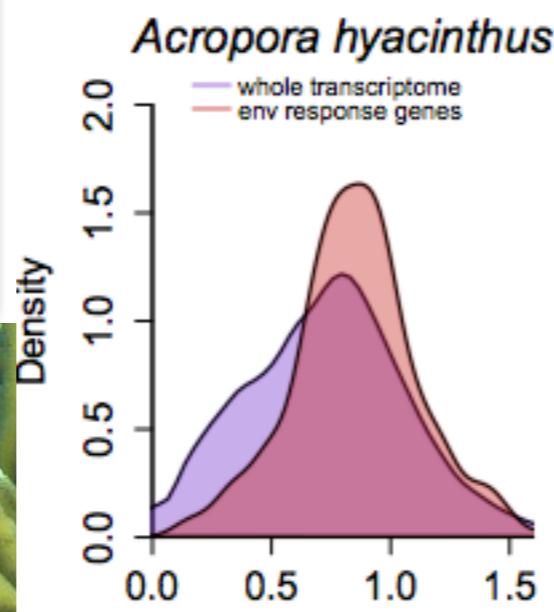
James L. Dimond and Steven B. Roberts\*

DOI: 10.1111/mec.13414

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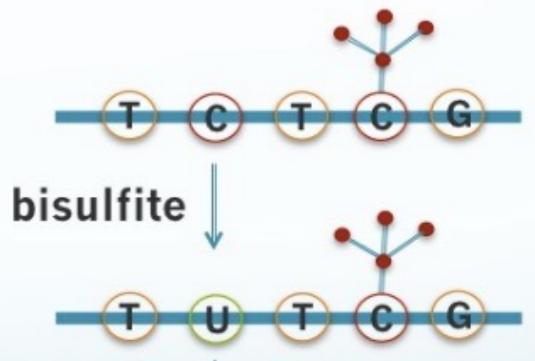


Issue



Epigenetic variation **1**

# Family and Developmental Variation



Sperm &  
Larvae  
(72h & 120h)



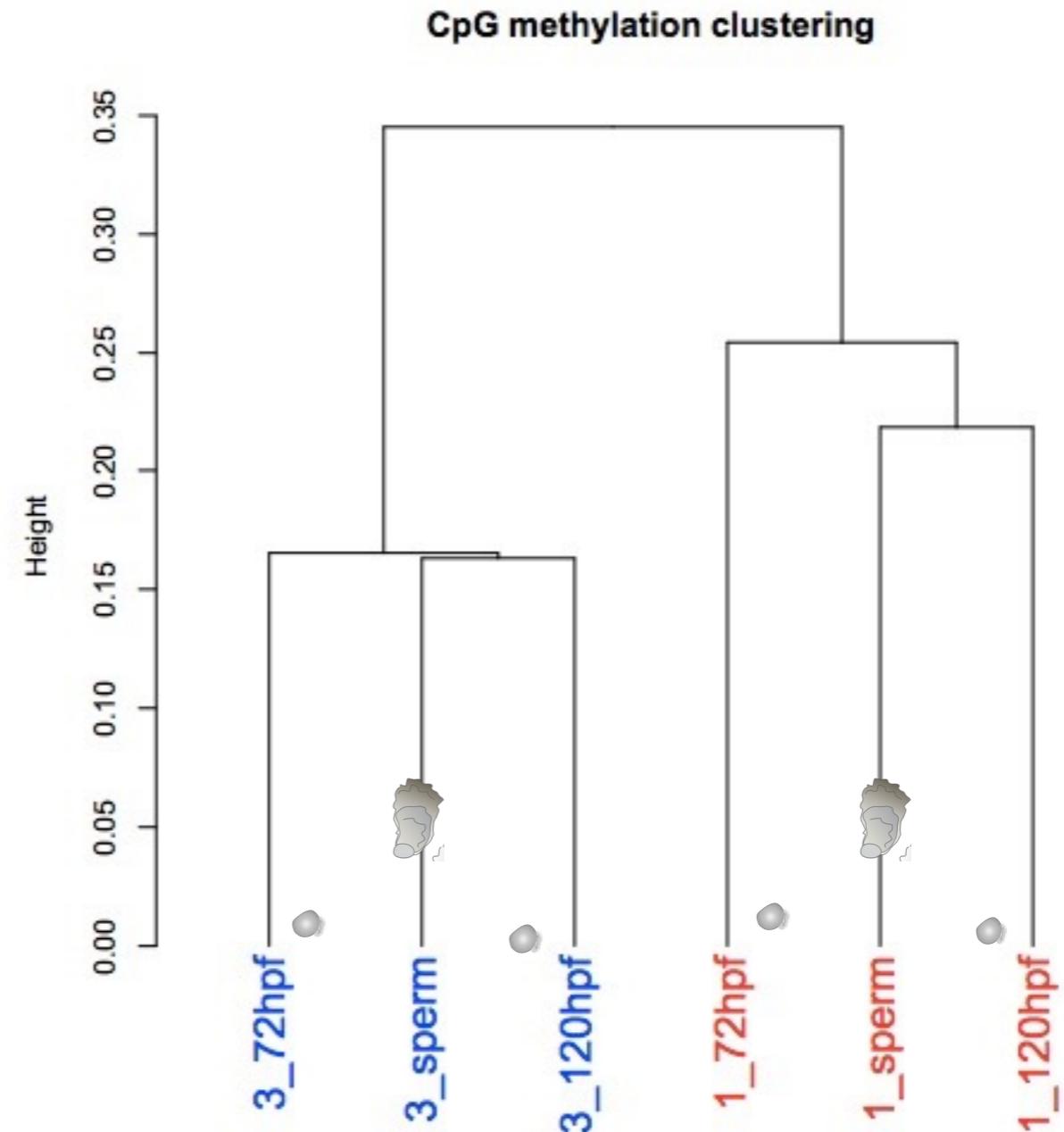
**bioRxiv**  
beta  
THE PREPRINT SERVER FOR BIOLOGY

New Results

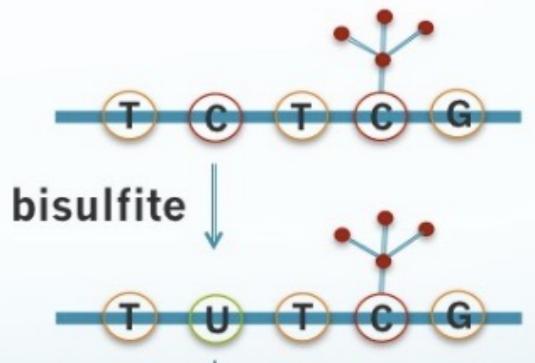
**Indication of family-specific DNA methylation patterns in developing oysters**

Claire E. Olson , Steven B. Roberts

doi: <http://dx.doi.org/10.1101/012831>



# Family and Developmental Variation



## Inheritance



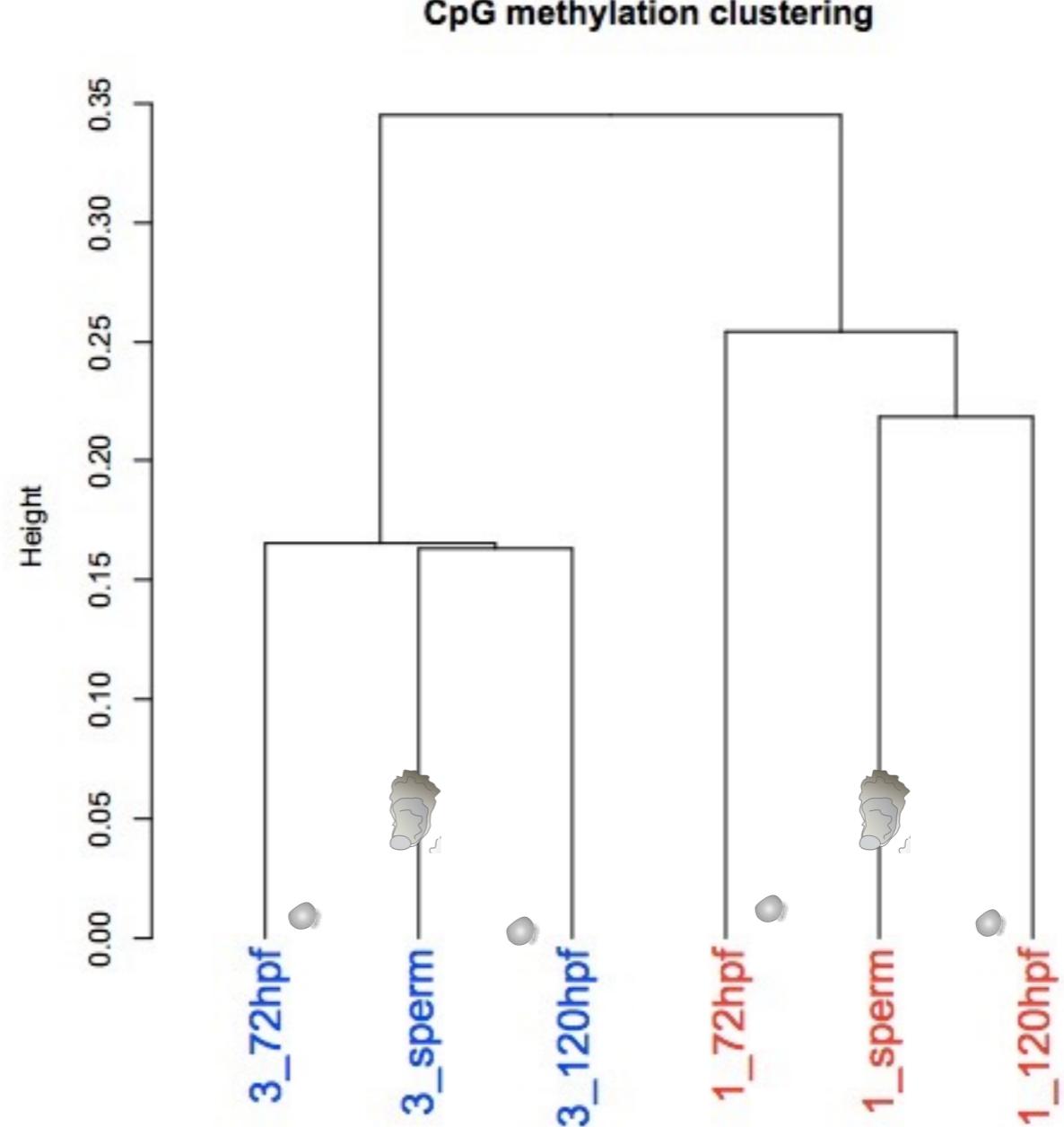
**bioRxiv**  
beta  
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New Results

**Indication of family-specific DNA methylation patterns in developing oysters**

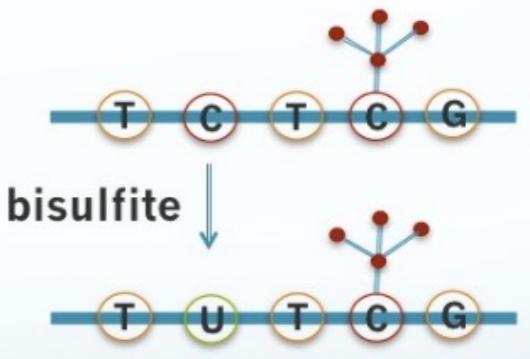
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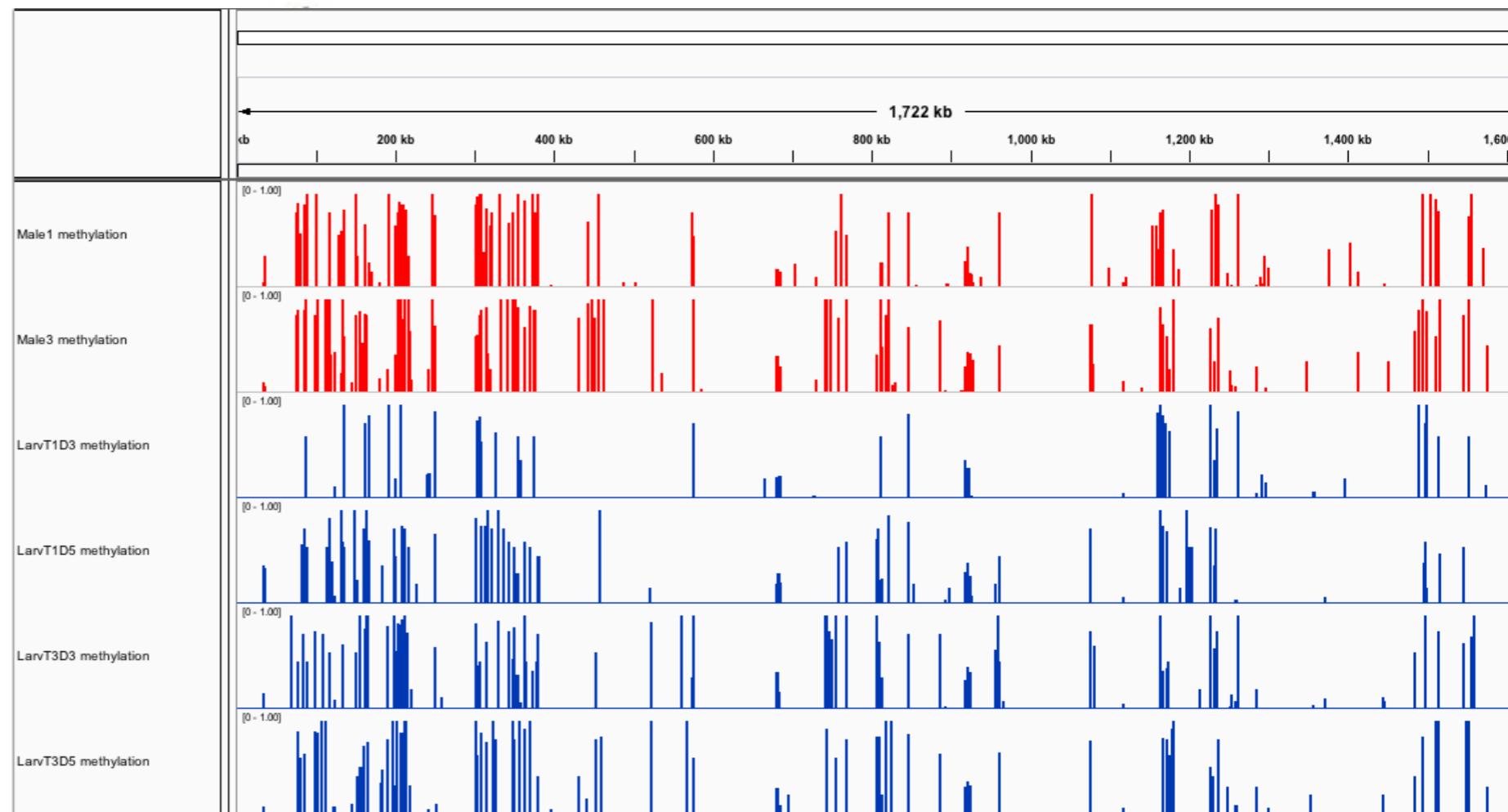


## Epigenetic variation 1

# *Family and Developmental Variation*



# Sperm & Larvae (72h & 120h)



DNA methylation level (0-100%) @ cytosines



bioRxiv

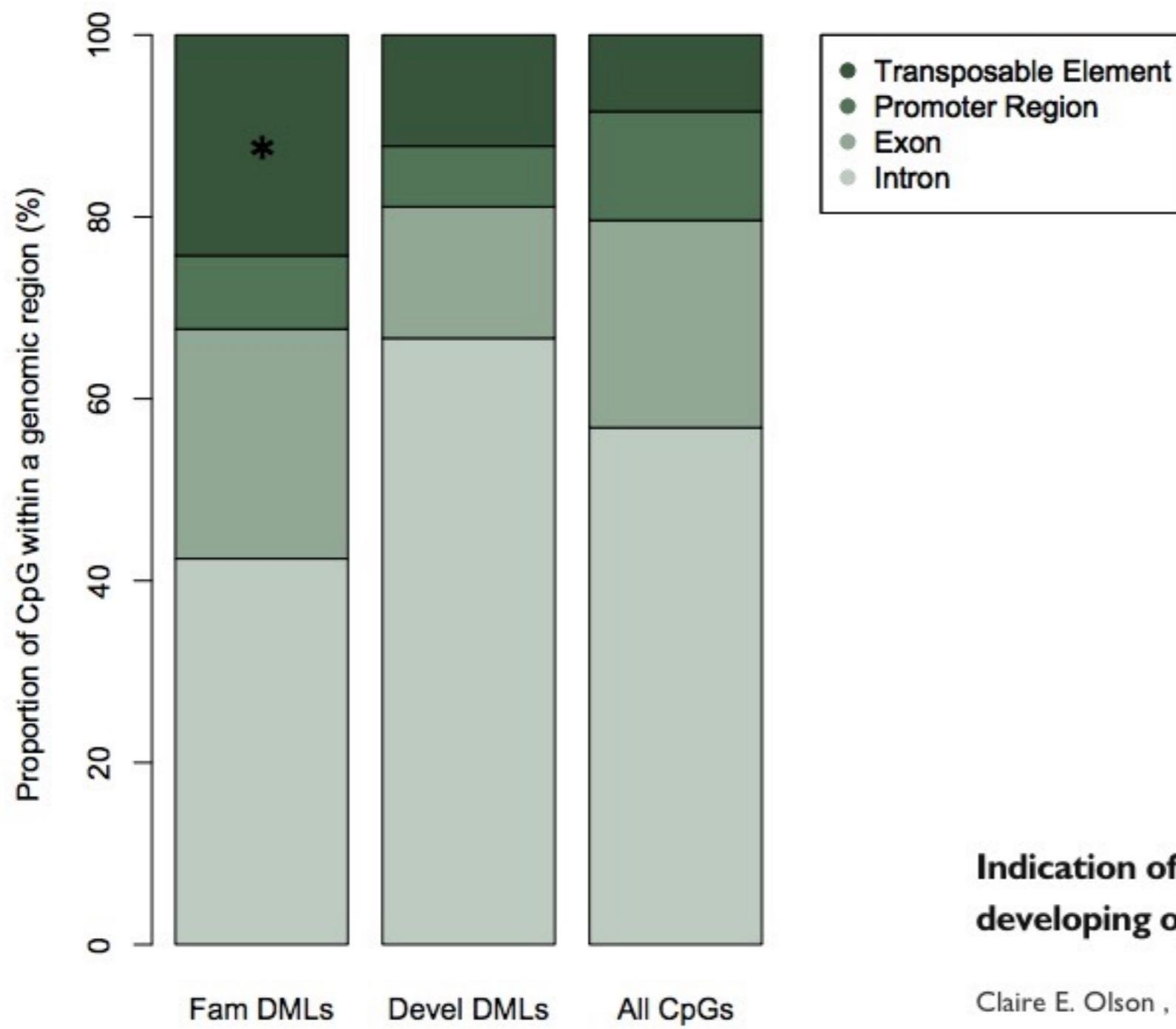
THE PREPRINT SERVER FOR BIOLOGY

New Results

## Indication of family-specific DNA methylation patterns in developing oysters

Claire E. Olson , Steven B. Roberts  
doi: <http://dx.doi.org/10.1101/01283>

# Where are the differences?

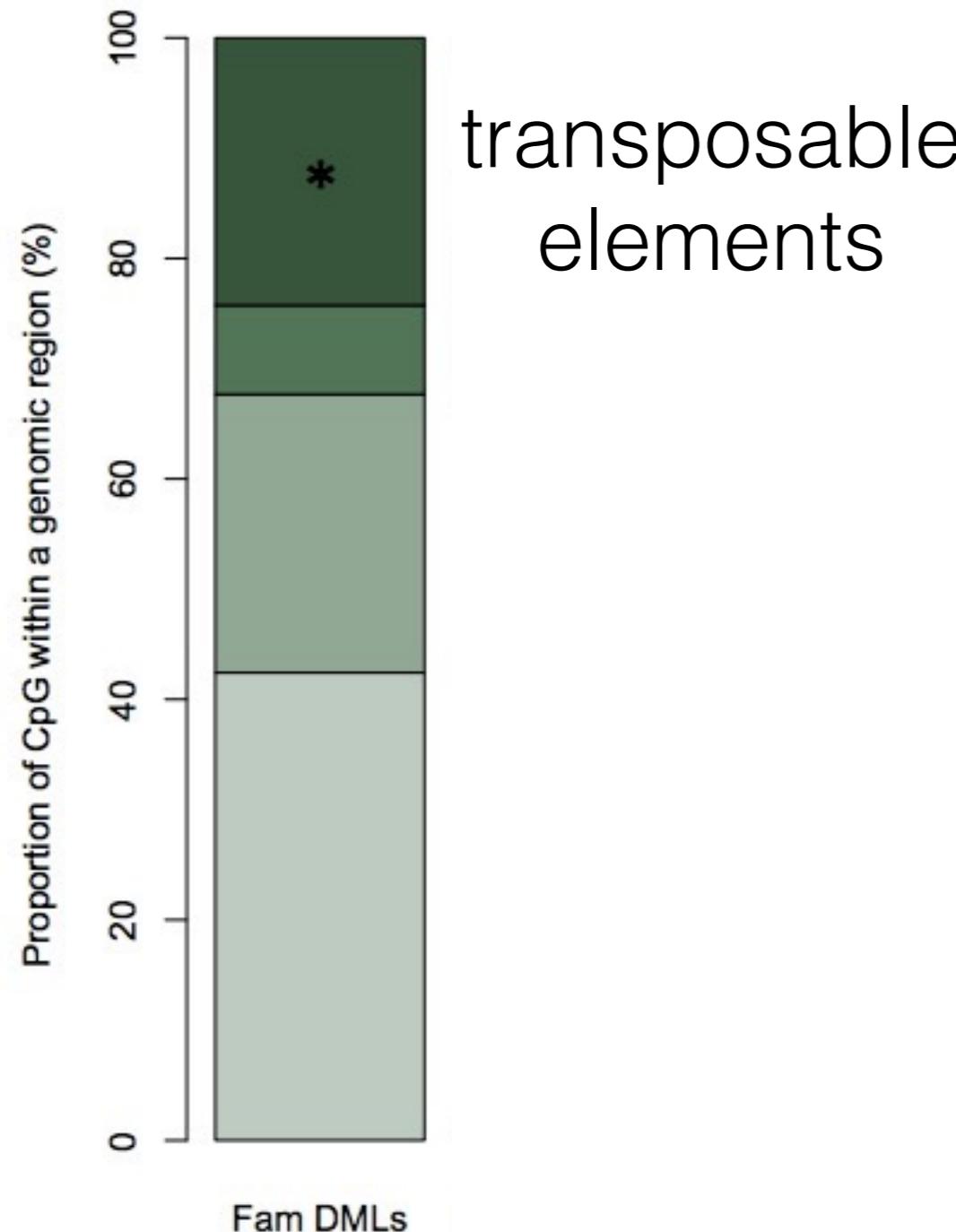


**Indication of family-specific DNA methylation patterns in developing oysters**

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Epigenetic variation **1**



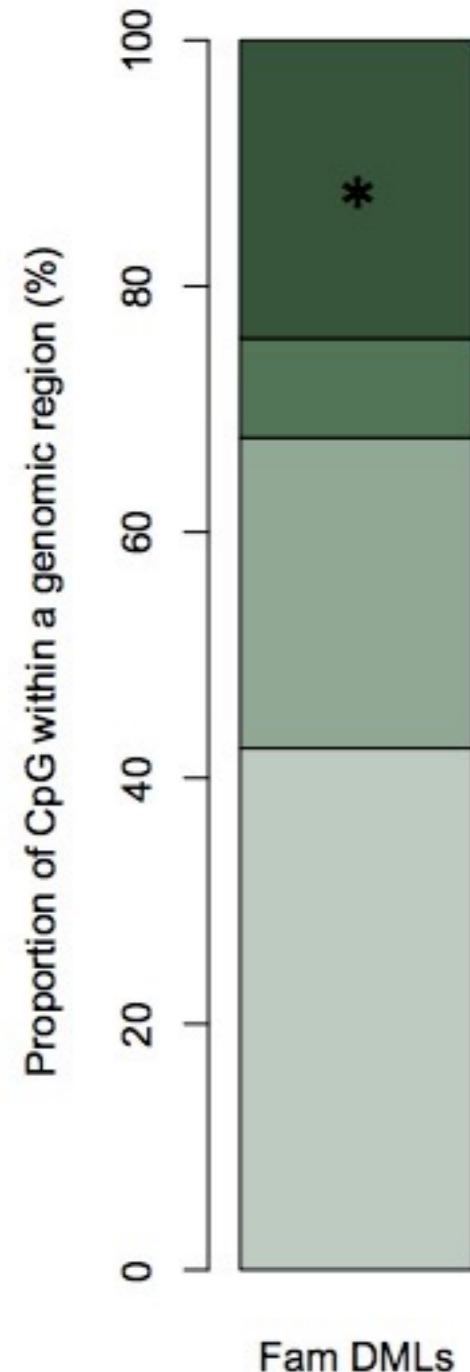
Differentially methylated loci in gene bodies could be lethal or deleterious, as they would alter gene expression.

Another possibility is that differentially methylated loci may provide advantageous phenotypic variation by increasing transposable element mobility.

**Indication of family-specific DNA methylation patterns in developing oysters**

Claire E. Olson , Steven B. Roberts

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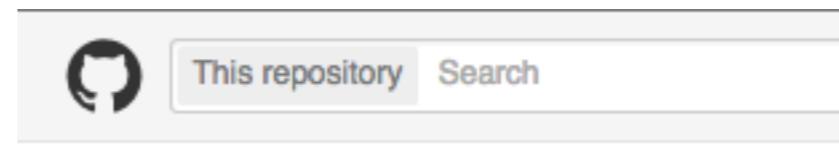
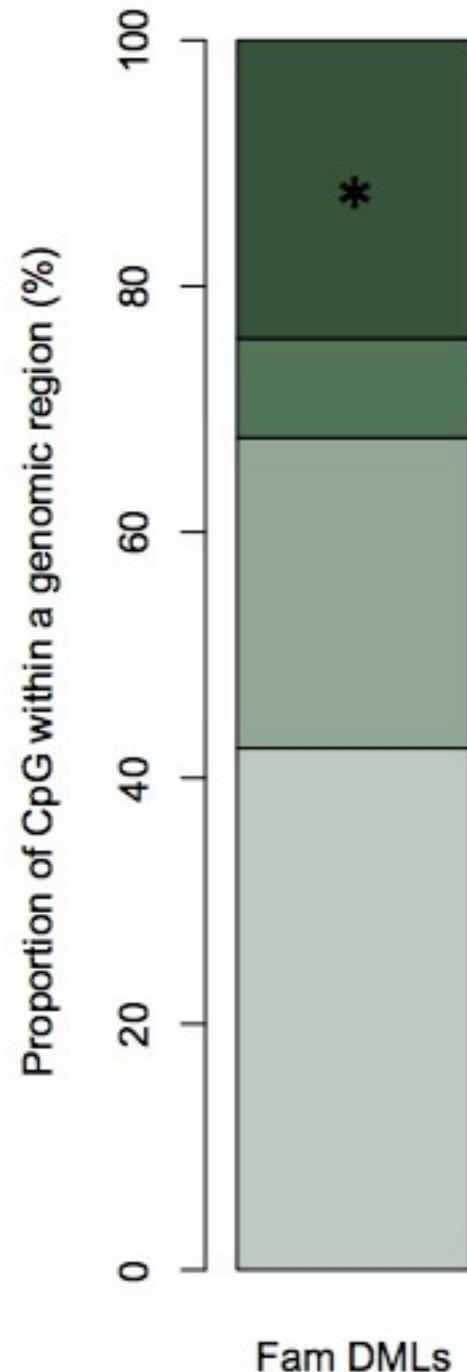


***Assumes some form  
of random process***  
occurring during  
gametogenesis?

**Indication of family-specific DNA methylation patterns in developing oysters**

Claire E. Olson , Steven B. Roberts  
doi: <http://dx.doi.org/10.1101/012831>

Epigenetic variation **1**



[che625 / olson-ms-nb](#)



**IPython Notebook for downloading and analyzing data  
the manuscript: "Indication of family-specific DNA  
methylation patterns in developing oysters"**

bioRxiv preprint - <http://dx.doi.org/10.1101/012831>

To execute the IPython Notebook in its entirety you will need:

- IPython - [install instructions](#)
- BSMAP - [install instructions](#)
- bedtools - [install instructions](#)
- R - [install instructions](#)
- rpy2 (interface to R from Python) - [install instructions](#)

- Sparsely (~16 %), mosaic methylated genome
- Gene body methylation correlated with function
- DNA methylation patterns are inherited
- DMRs are predominant in transposable elements

Gene  
expression



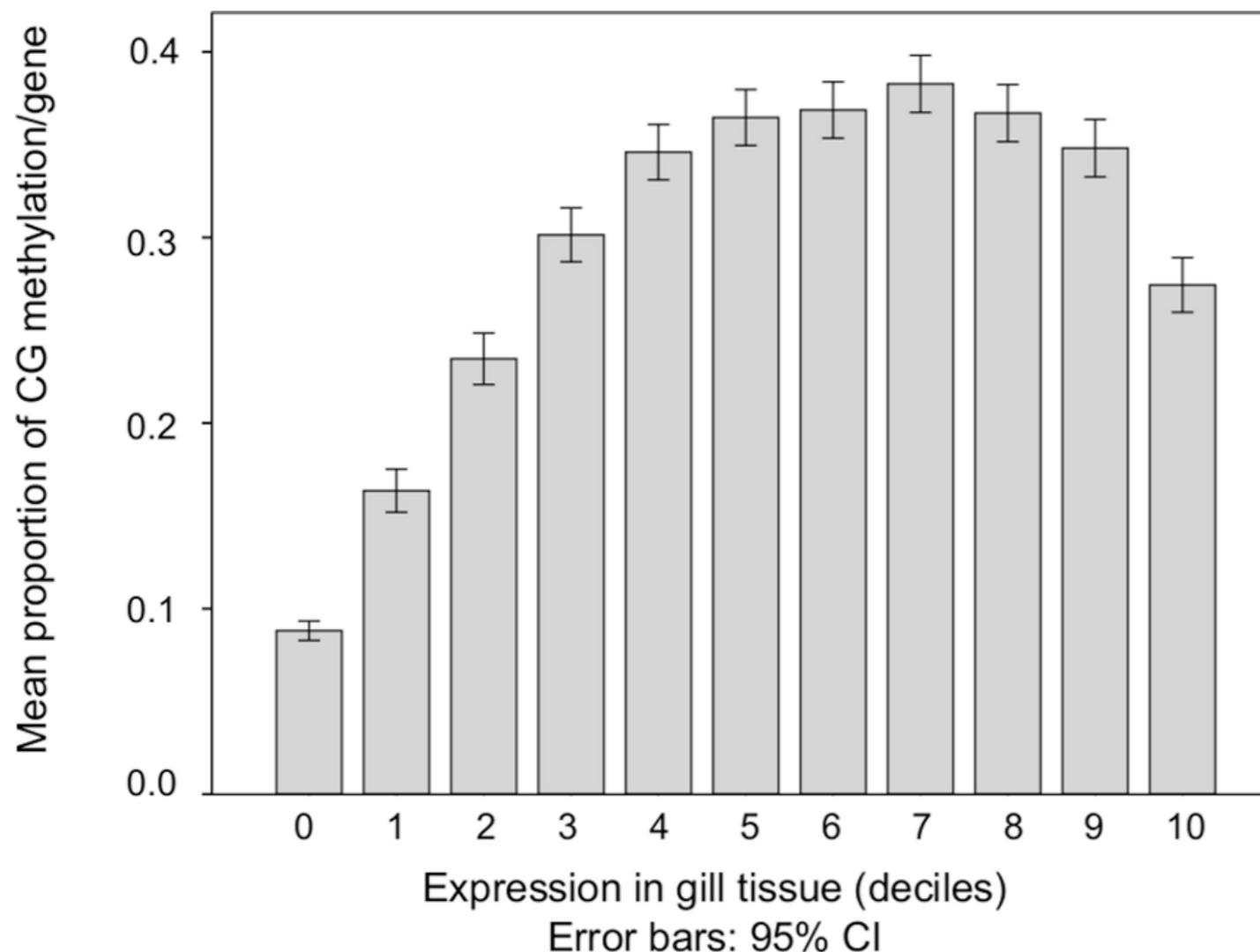
Epigenetic  
variation

# Function?

Gene expression

2

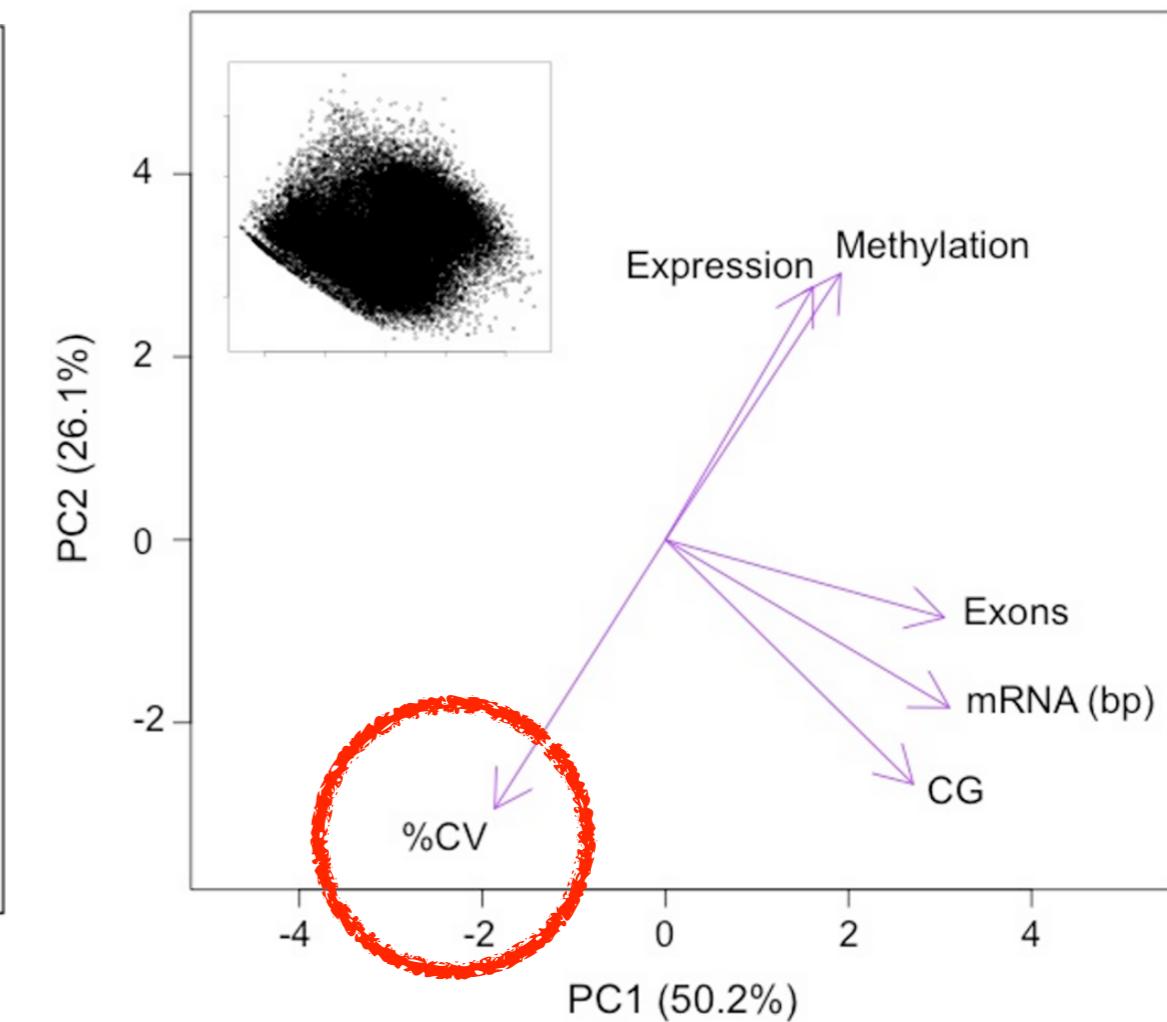
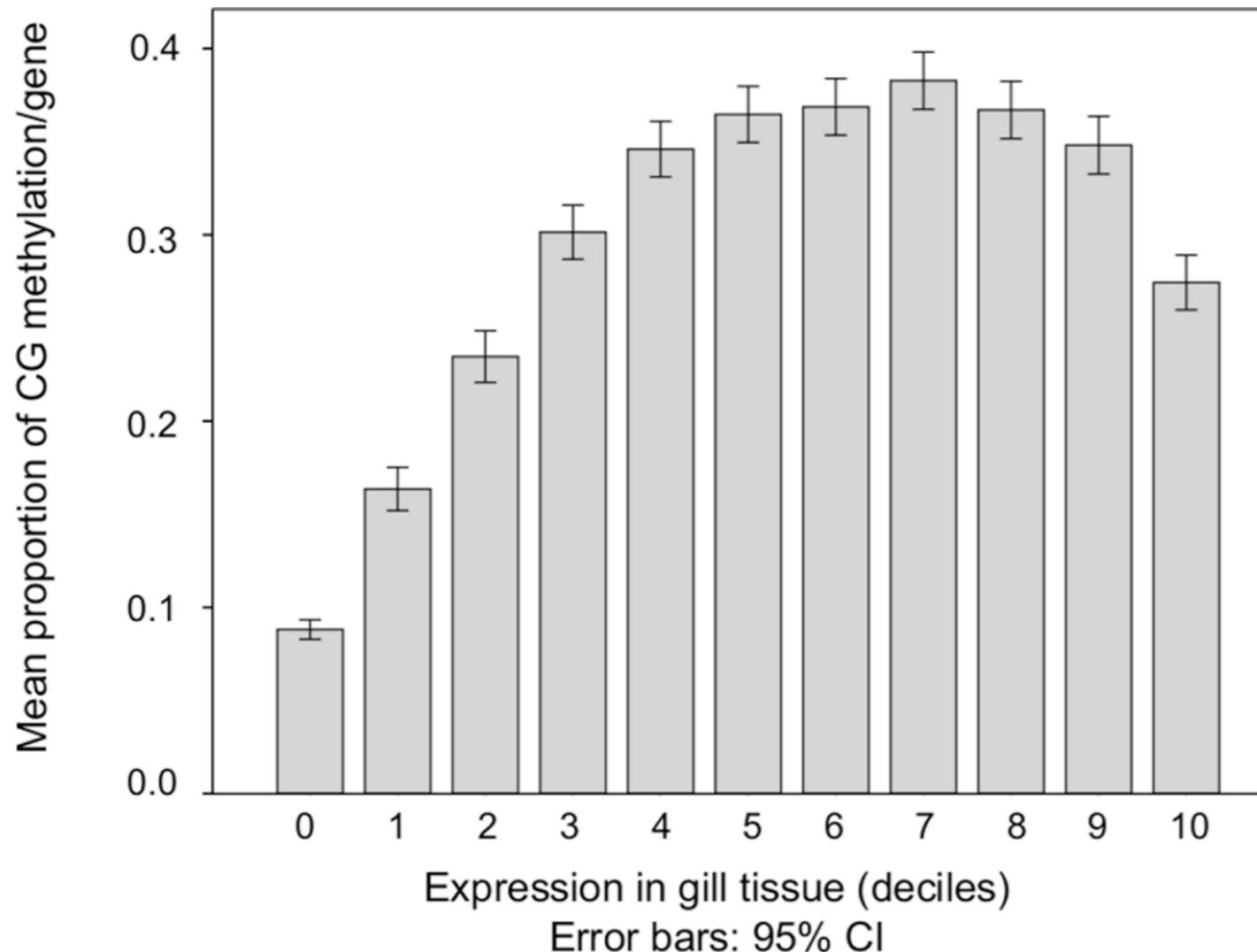
Epigenetic variation



Gene expression

2

Epigenetic variation



## Predominant intragenic methylation is associated with gene expression characteristics in a bivalve mollusc

Mackenzie R. Gavery and Steven B. Roberts

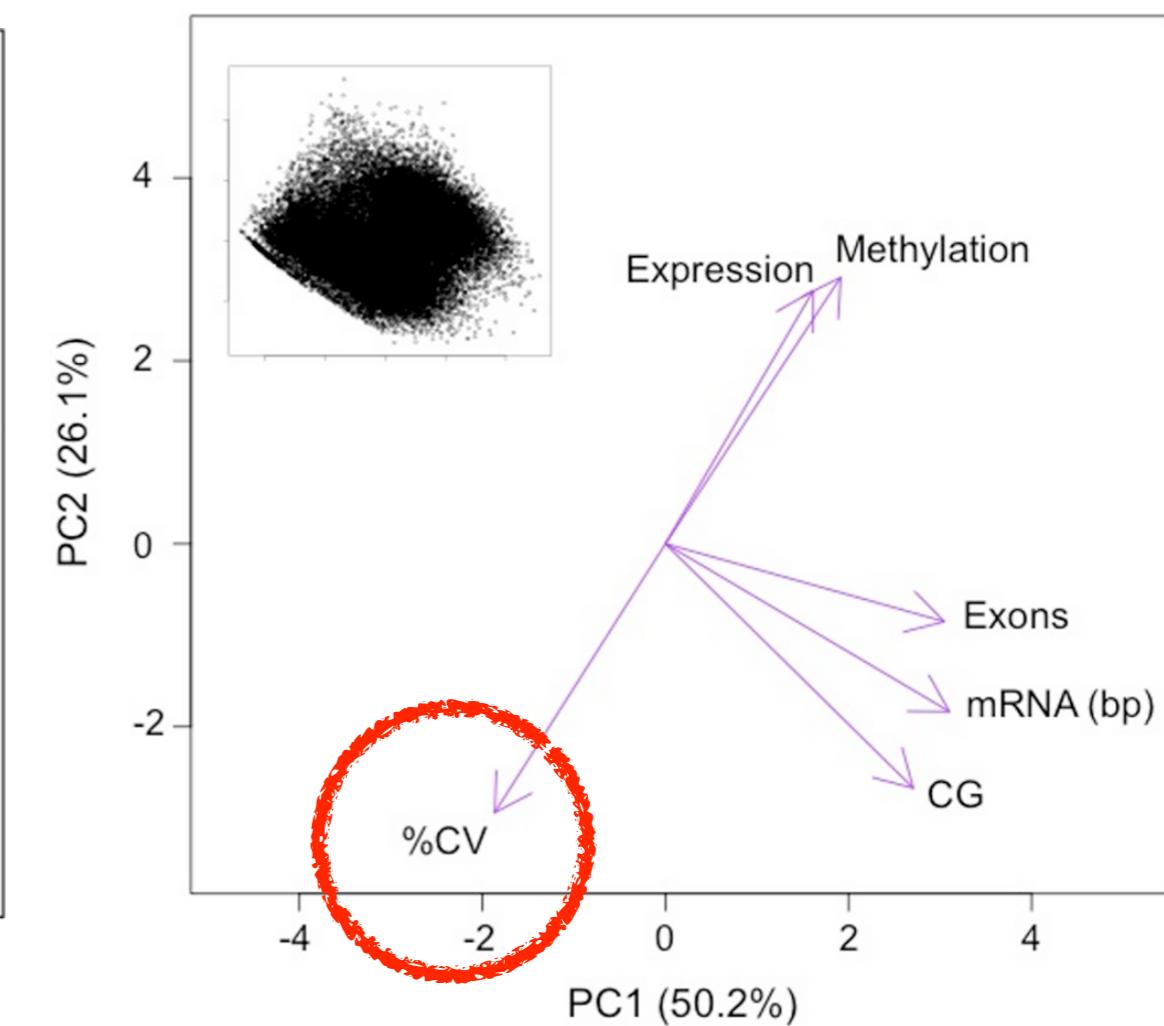
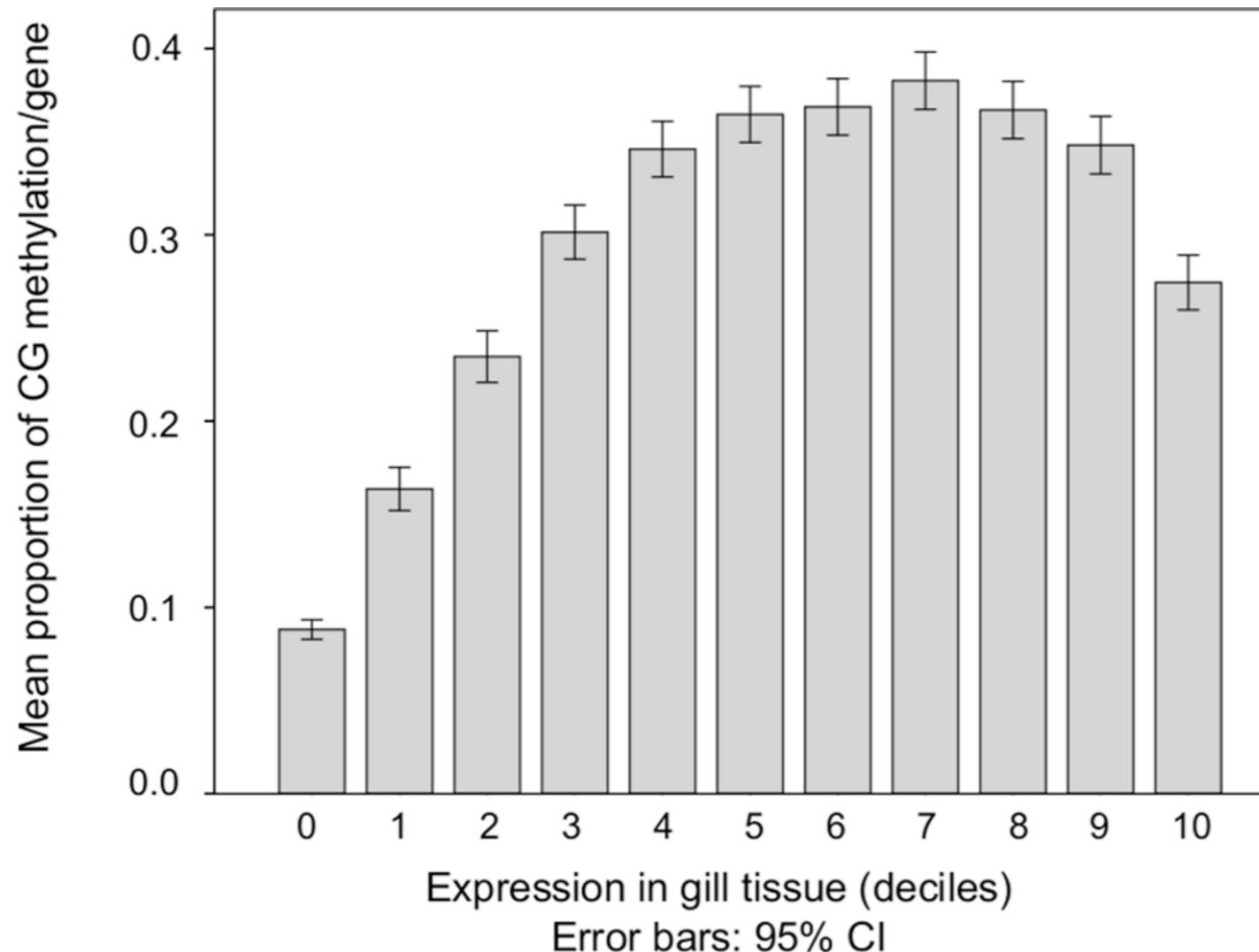
School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA, USA

Gene expression

2

Epigenetic variation

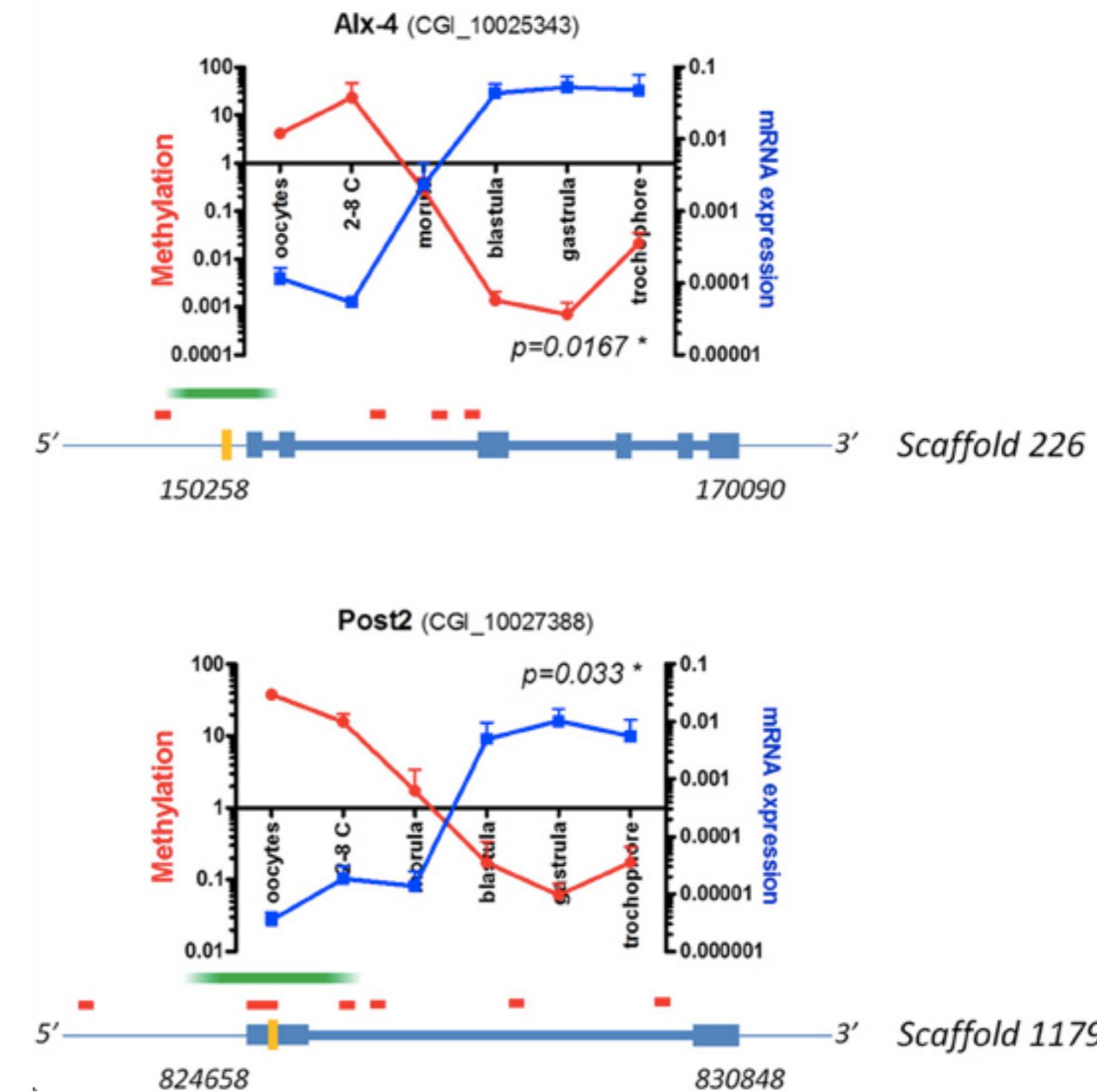
Theory: **Does not** influence expression level  
but rather alternative splicing.



Gene expression



Epigenetic variation



PERSPECTIVE ARTICLE

Front. Physiol., 06 April 2014 | doi: 10.3389/fphys.2014.00129

## Epigenetic features in the oyster *Crassostrea gigas* suggestive of functionally relevant promoter DNA methylation in invertebrates

Guillaume Rivière<sup>1,2\*</sup>



In species that experience a diverse range of environmental conditions, processes have evolved to increase the number of potential phenotypes in a population in order to improve the chances for an individual's survival.



Gene expression

Epigenetic variation  
2



*in silico analysis*

(modified from Gavery and Roberts 2010)

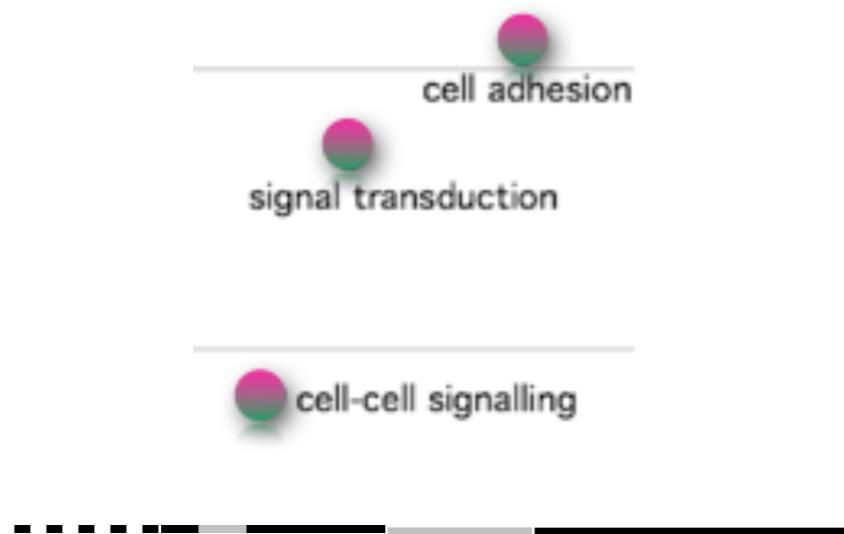
Predicted degree of DNA methylation

Roberts and Gavery 2012

Gene expression

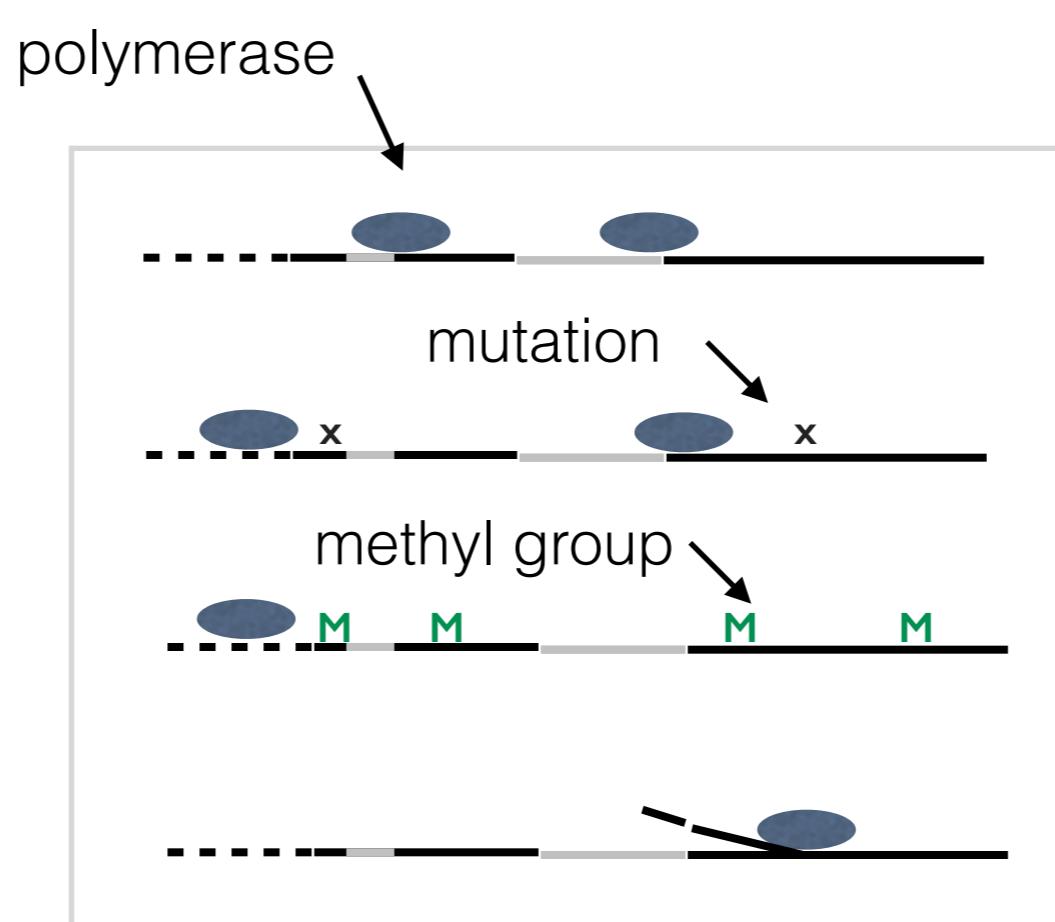
2

Epigenetic variation



## sparse methylation

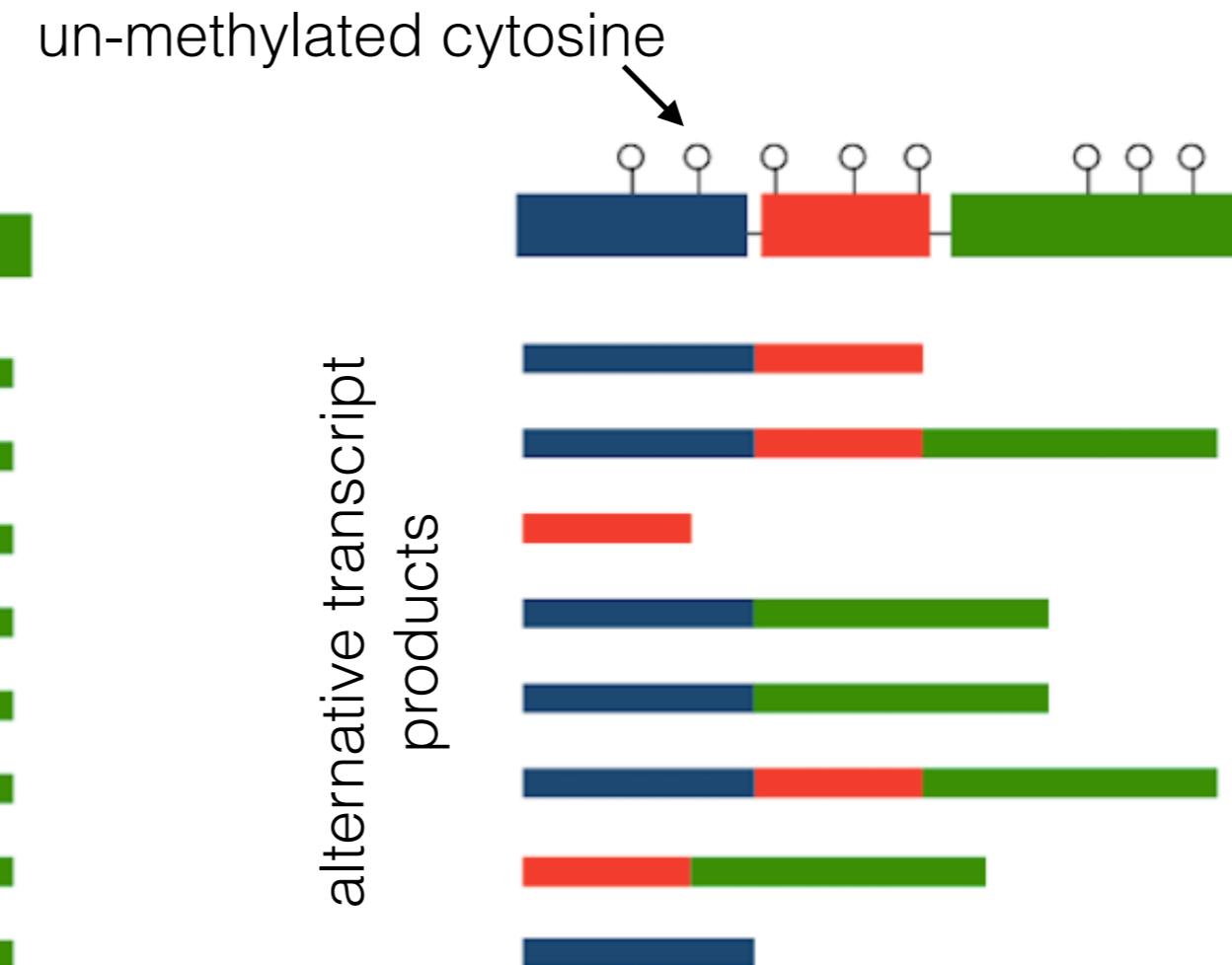
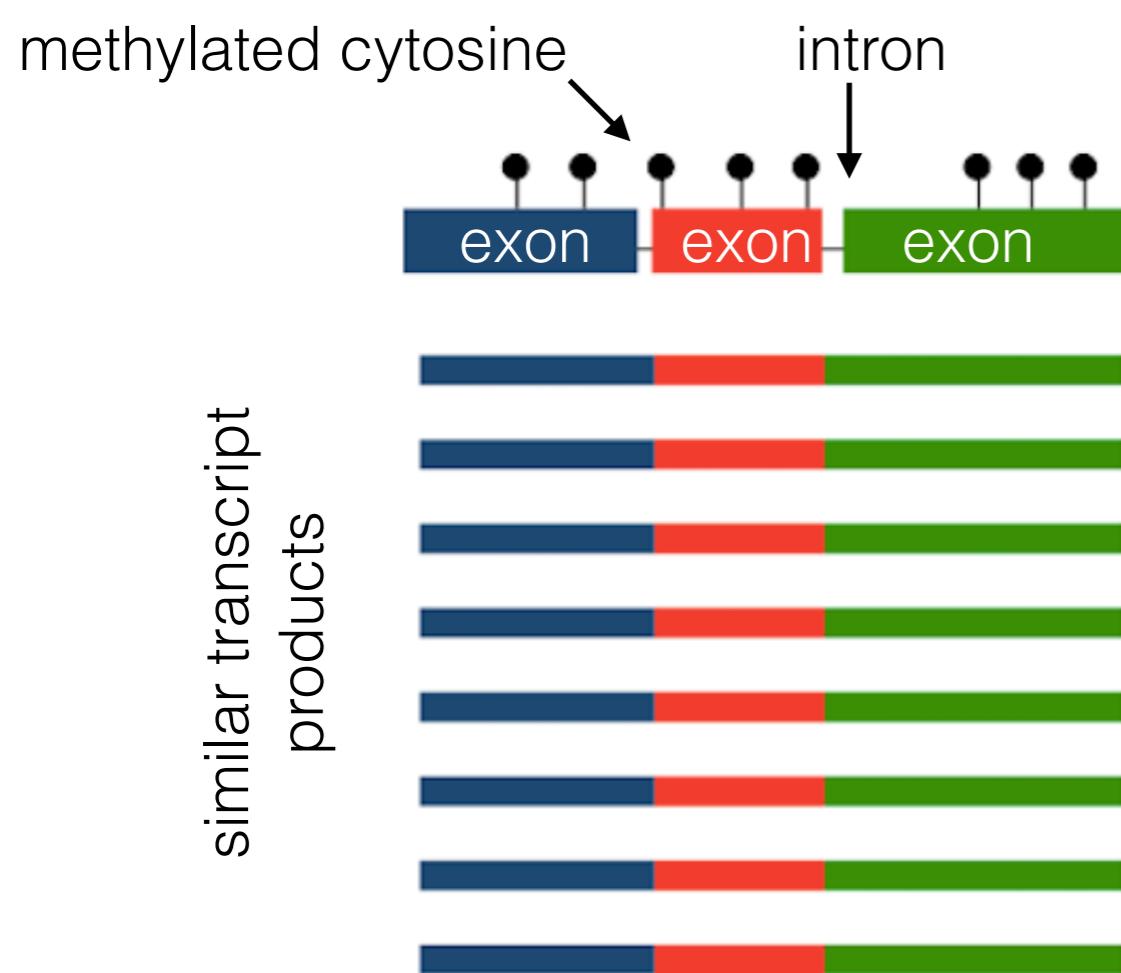
tissue / temporal specific and inducible genes



# Stochastic Variation

**A context dependent role for DNA methylation in bivalves**

Mackenzie R. Gavery and Steven B. Roberts  
Advance Access publication date 7 January 2014



housekeeping

response to  
change

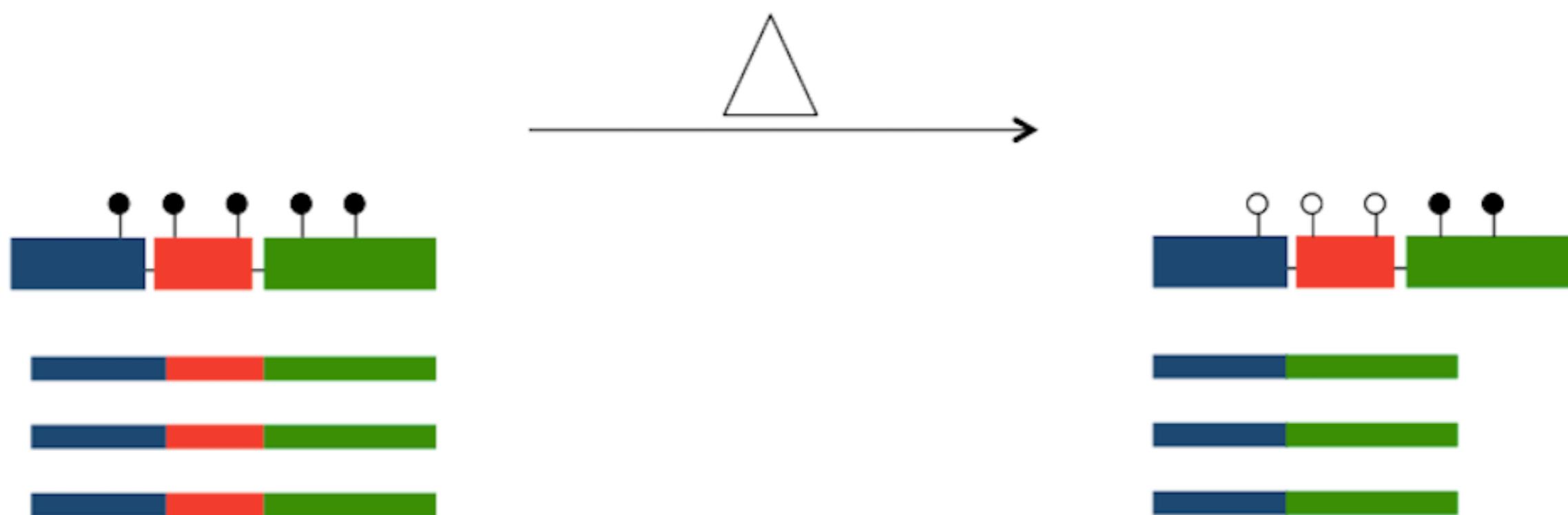


# Targeted Regulation

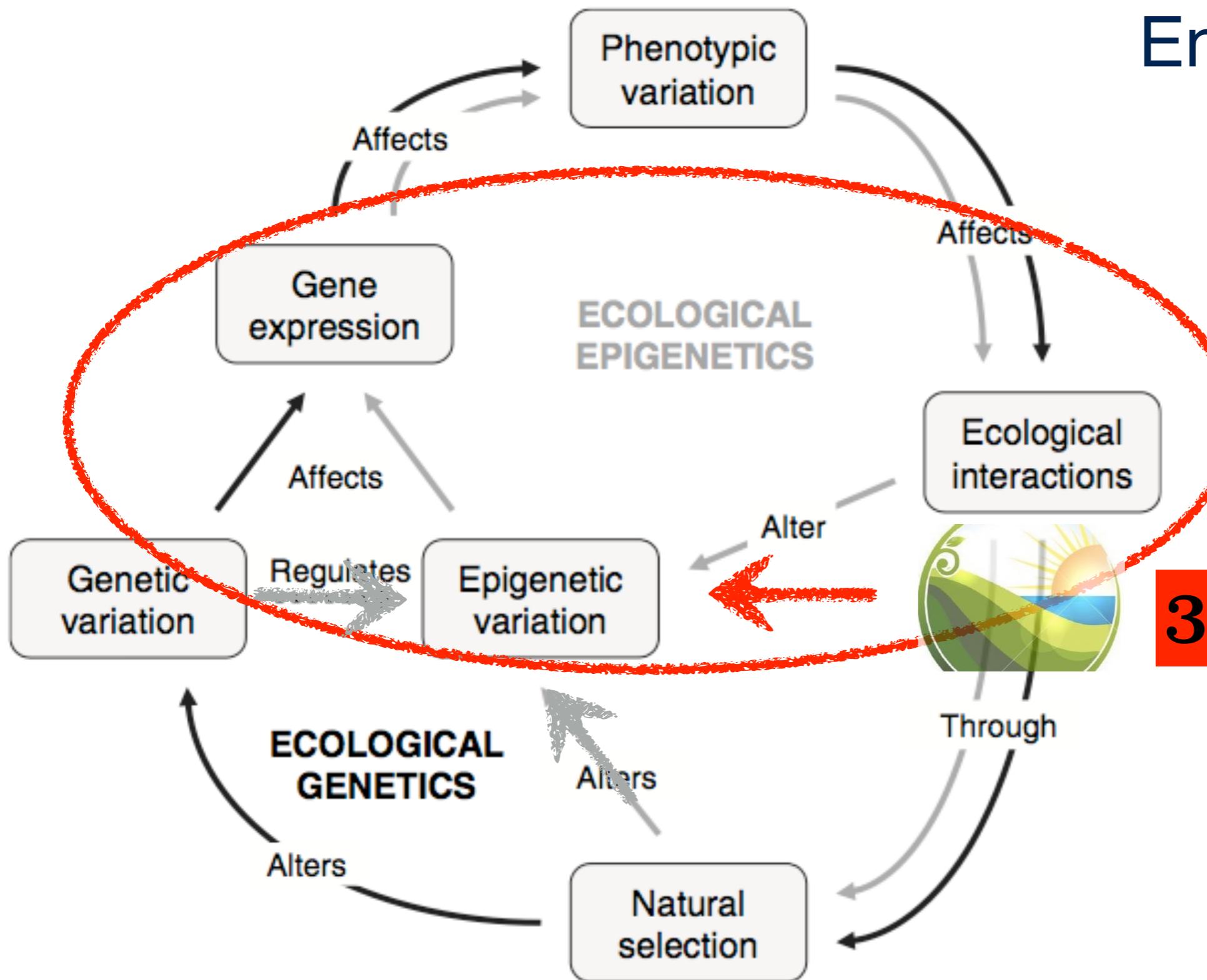
## A context dependent role for DNA methylation in bivalves

Mackenzie R. Gavery and Steven B. Roberts

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# Environmental Influence



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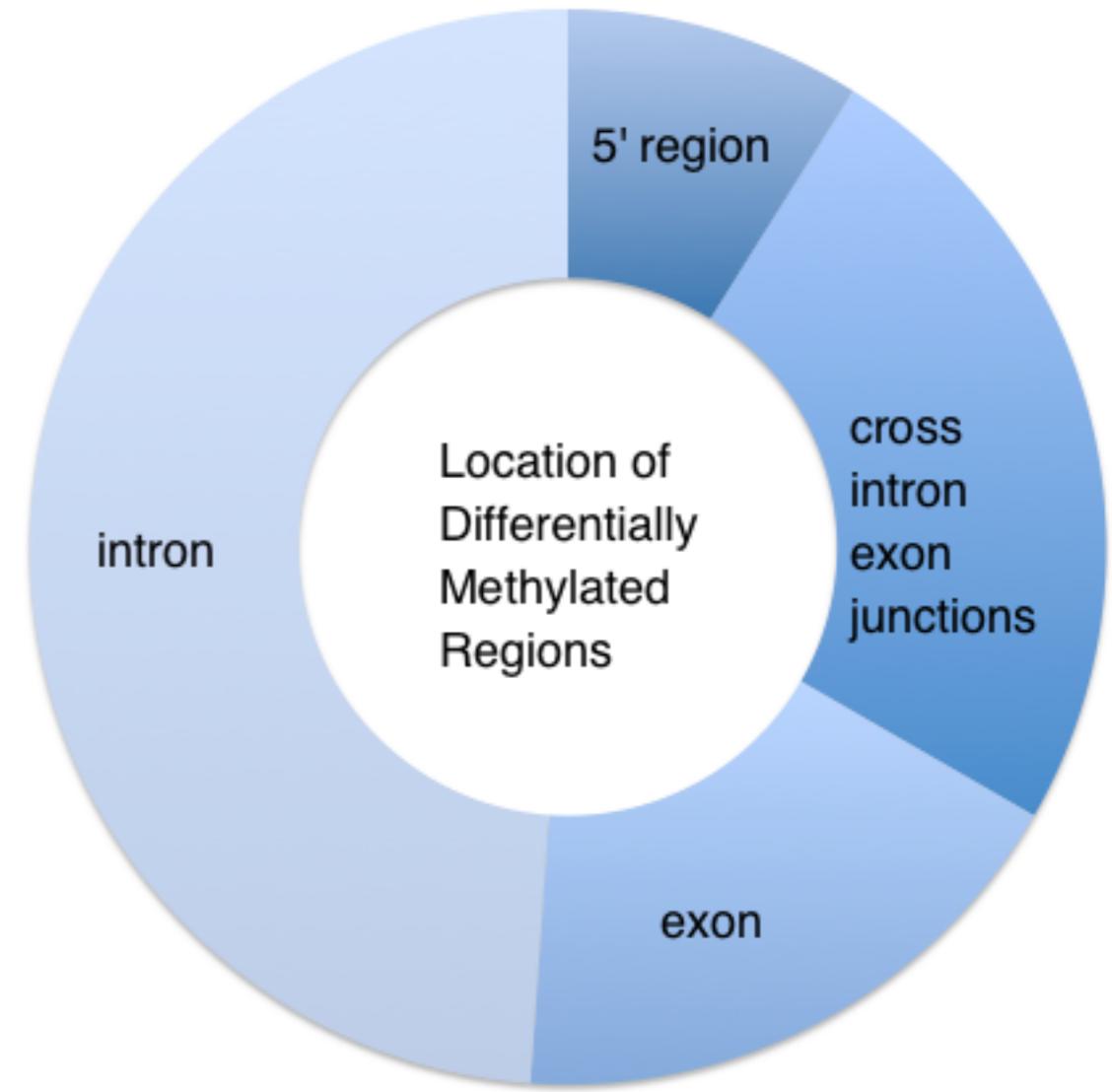
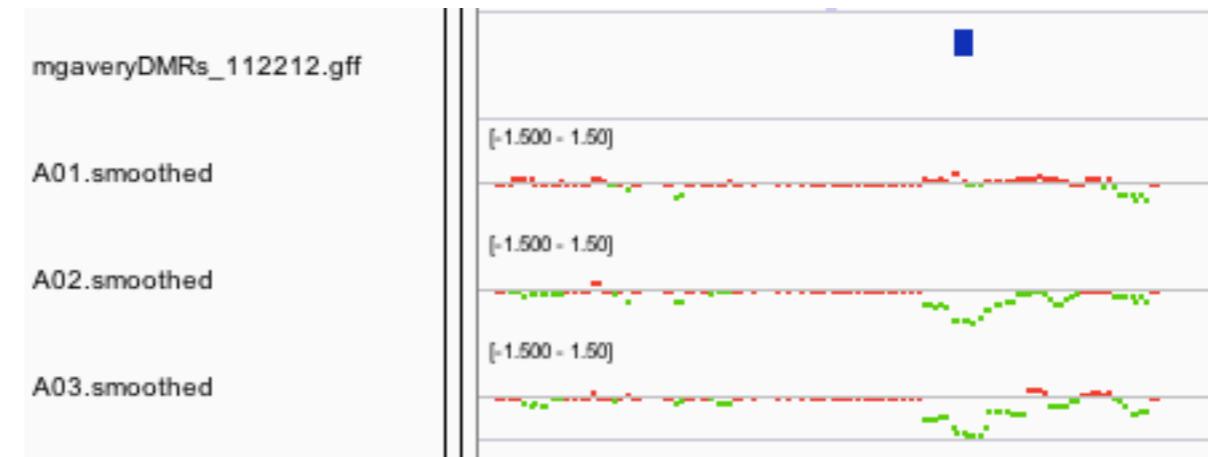
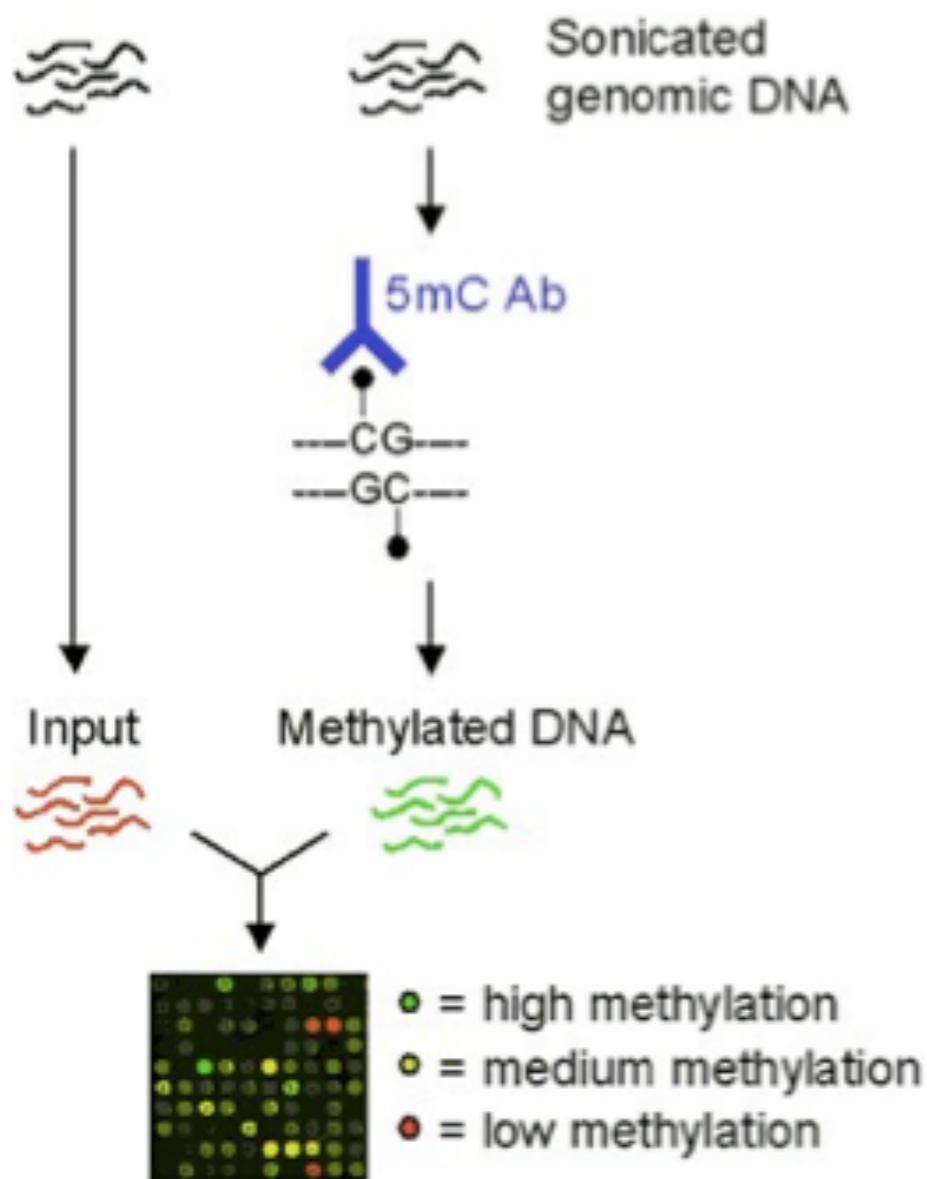
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IDEA AND  
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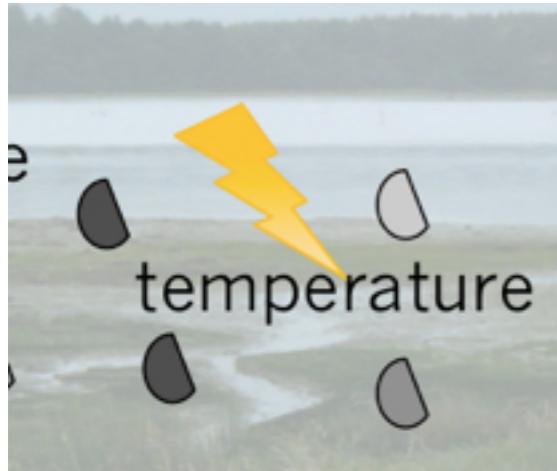
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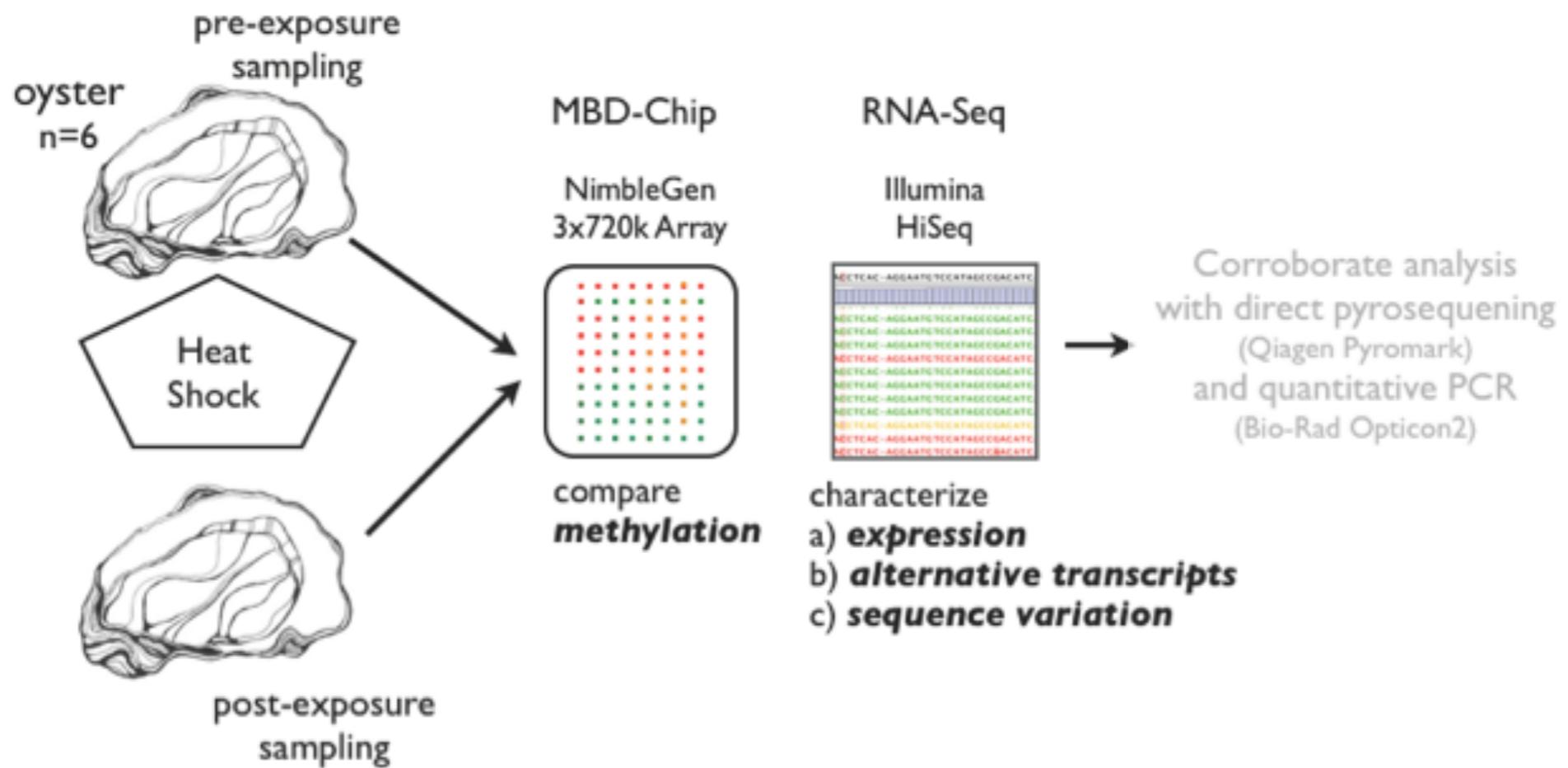
# Environmental impact (Estrogens)



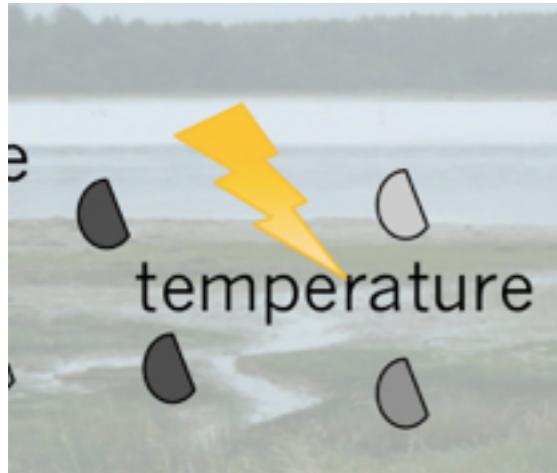
# Environment and gene expression



*stochastic or targeted?*



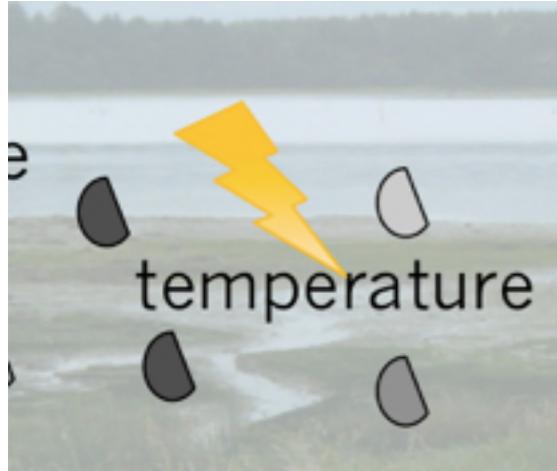
# Environment and gene expression



*stochastic or targeted?*

Oyster	Hypo-methylated	Hyper-methylated
2	7224	2803
4	6560	3587
6	7645	4044

# Environment and gene expression

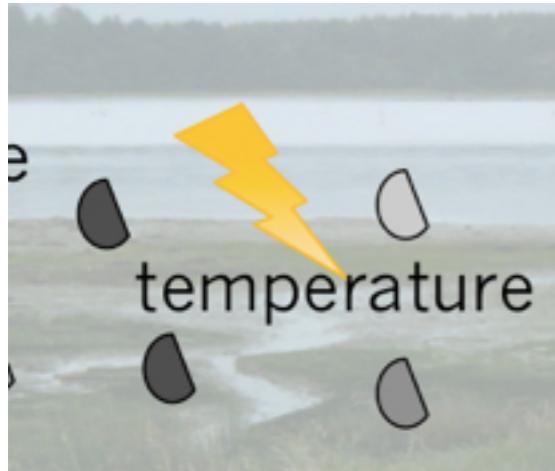


*stochastic or targeted?*

Oyster	Hypo-methylated	Hyper-methylated
2	7224	2803
4	6560	3587
6	7645	4044

No obvious association  
with genome feature  
including *differentially*  
*expressed*  
*genes*

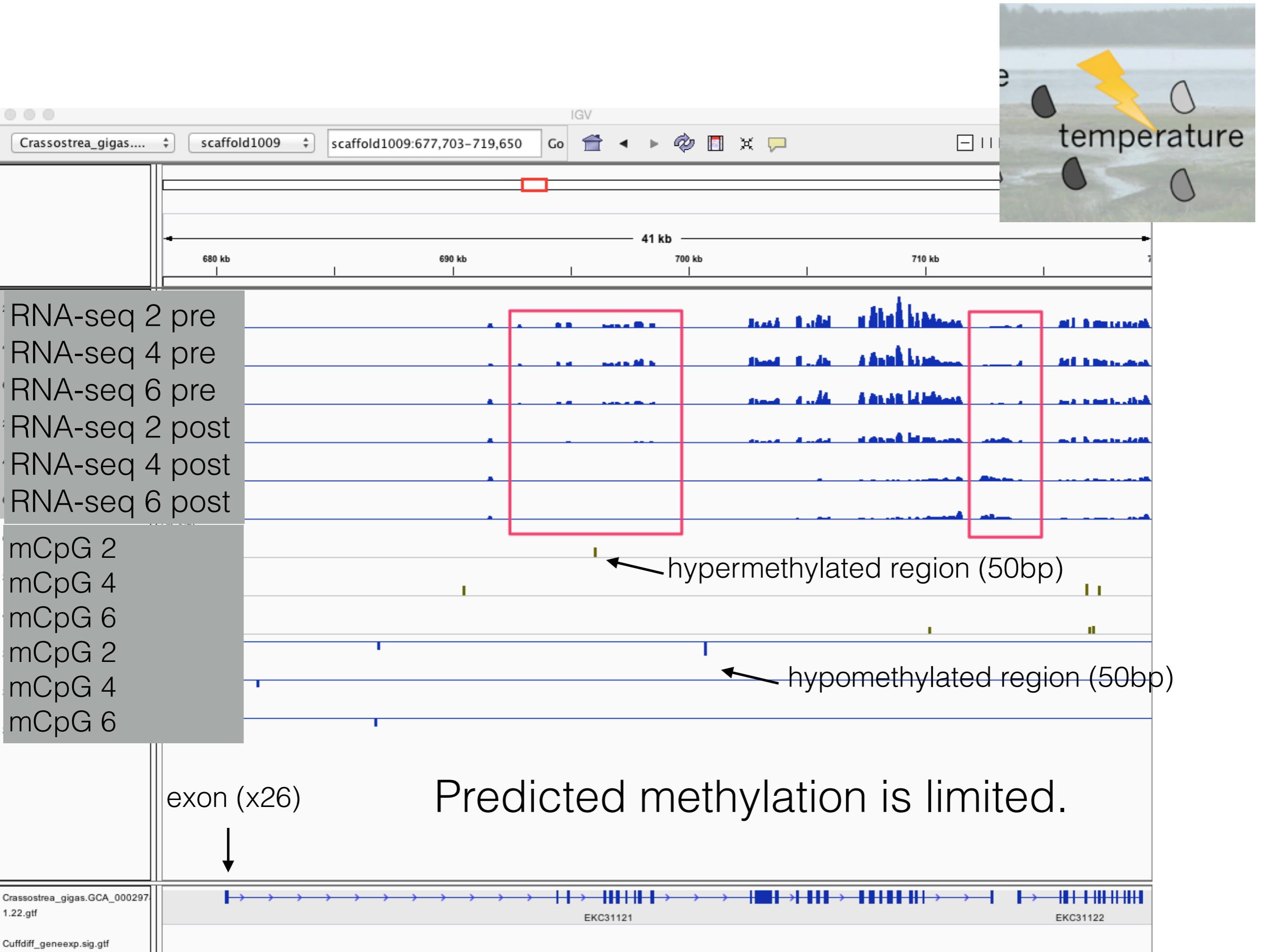
# Environment and gene expression



*stochastic or targeted  
or ..?*

Oyster	Hypo-methylated	Hyper-methylated
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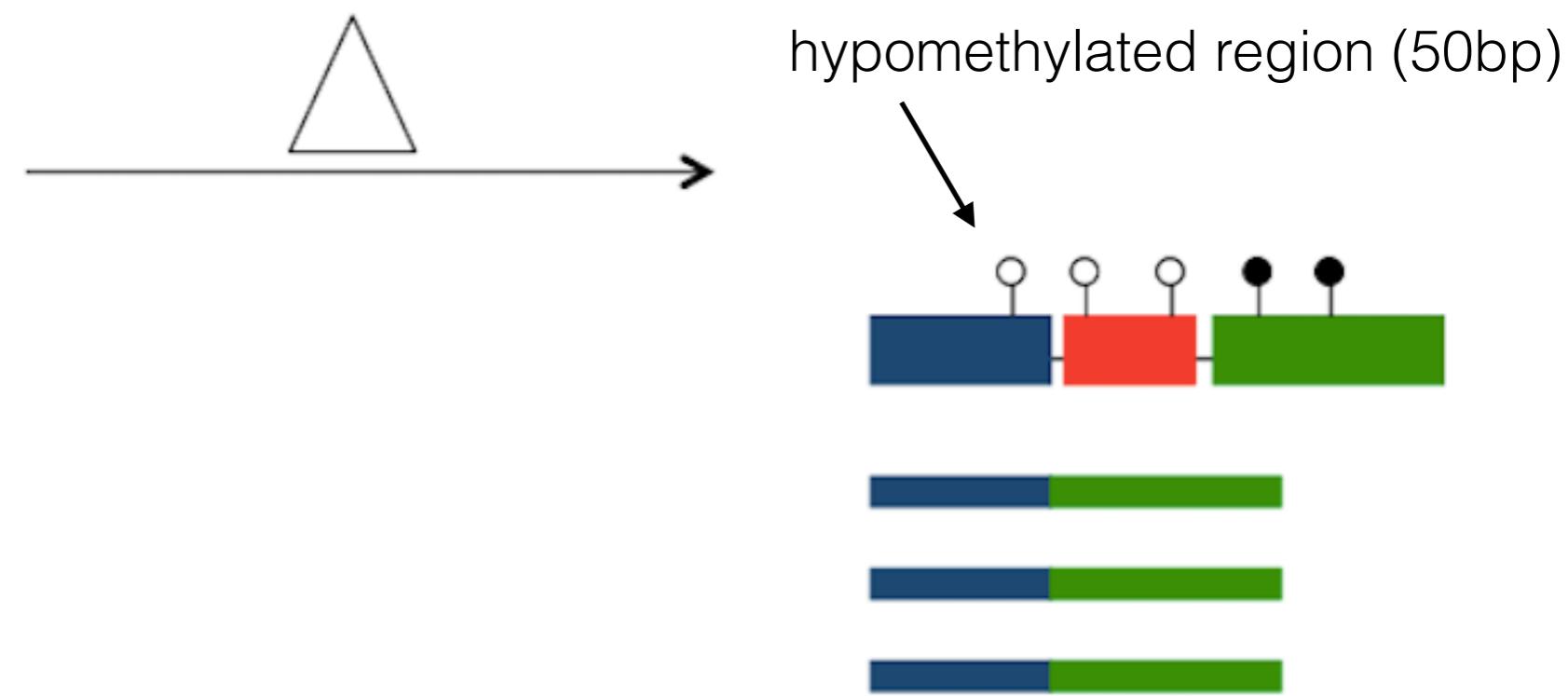
Changes in methylation (either direction) are more prevalent in introns, repeats, and transposable elements.



Gene  
expression

2

Epigenetic  
variation



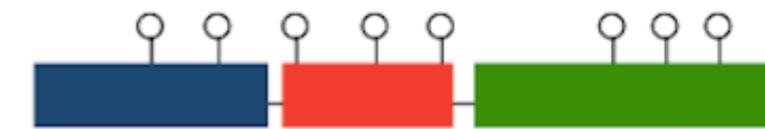
**not** in this experiment

not even consistent methylation changes at loci level

Gene  
expression

2

Epigenetic  
variation



more questions....  
interesting but what is controlling?

# Take Home

*Oyster genome has a fantastic degree of diversity contributing to phenotypic plasticity & adaptation potential.*

# Take Home

*Oyster genome has a fantastic degree of diversity contributing to phenotypic plasticity & adaptation potential.*

- large gene families
- very high mutation rate (snp/50bp)
- numerous exons per gene (potential for alternatives)
- genome full of repeats region
- high number of transposable elements
- lack of methylation of transposable suggest mobility
- family variation of methylation
- limited methylation environmental response genes  
is associated with spurious transcription
- inheritance of epigenetic marks as mechanism of improved adaptation

A photograph of a dense tropical forest. In the foreground, a large tree trunk with dark, textured bark dominates the left side. To its right, several thick branches hang down, covered in bright green moss. The background is filled with more trees and foliage, creating a sense of depth and complexity.

## Next Steps

Transposable  
Elements

Consider other  
epigenetic process

# Next Steps

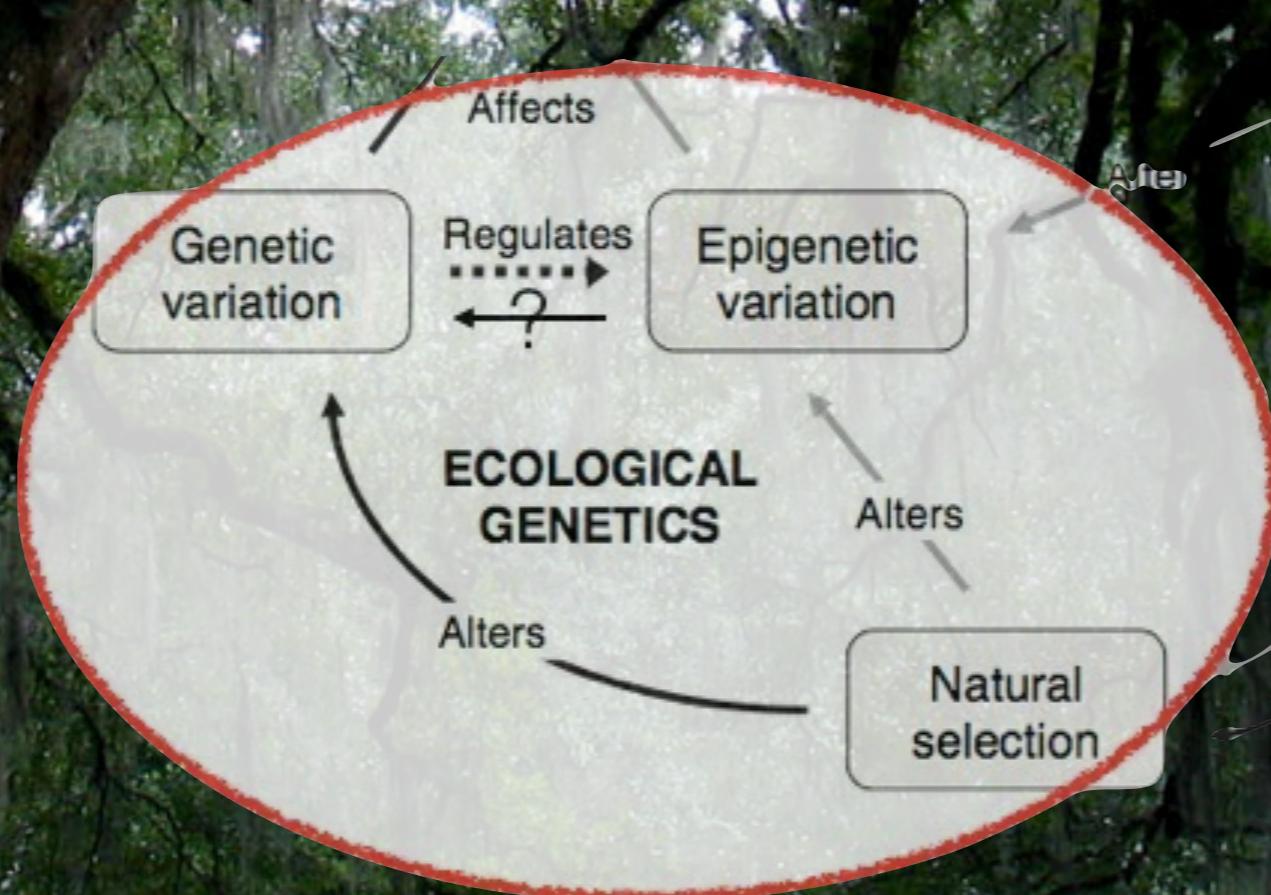
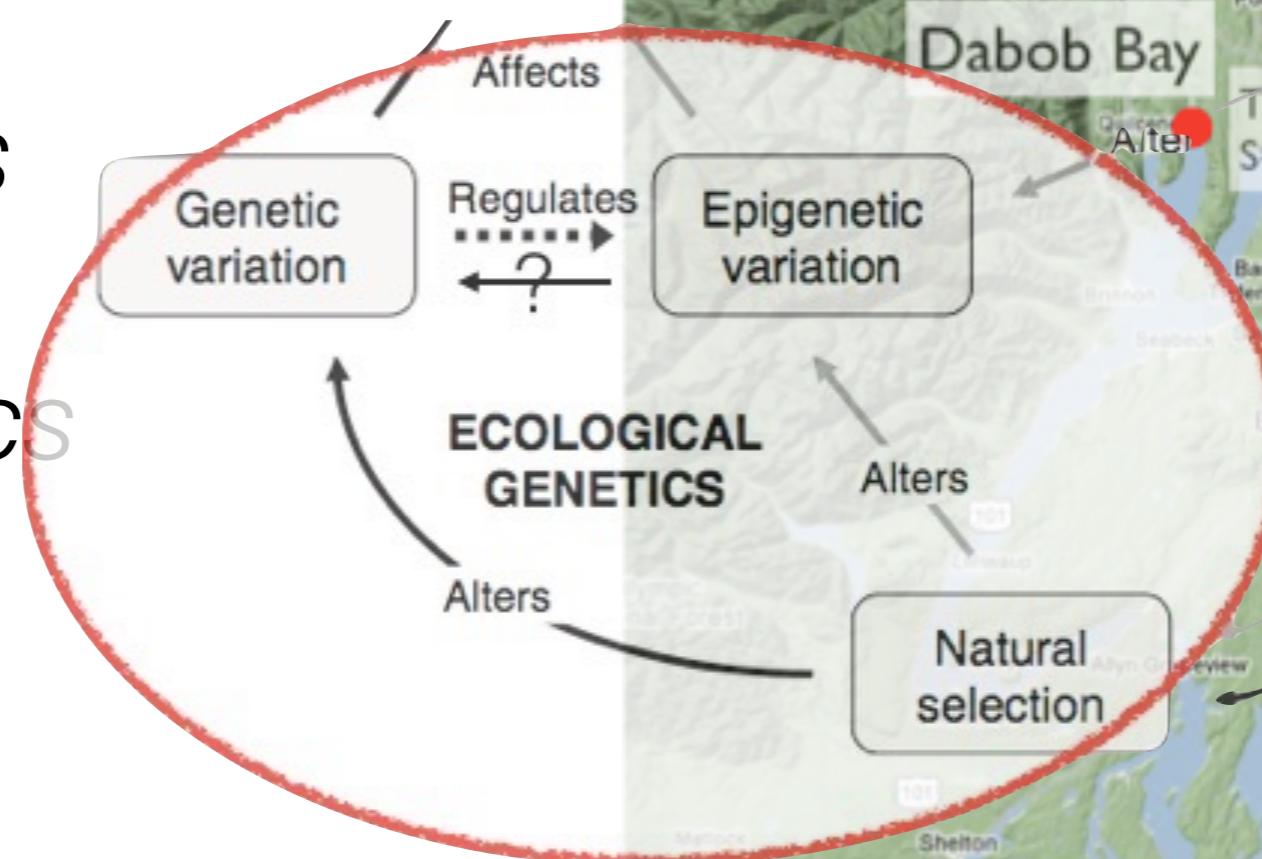


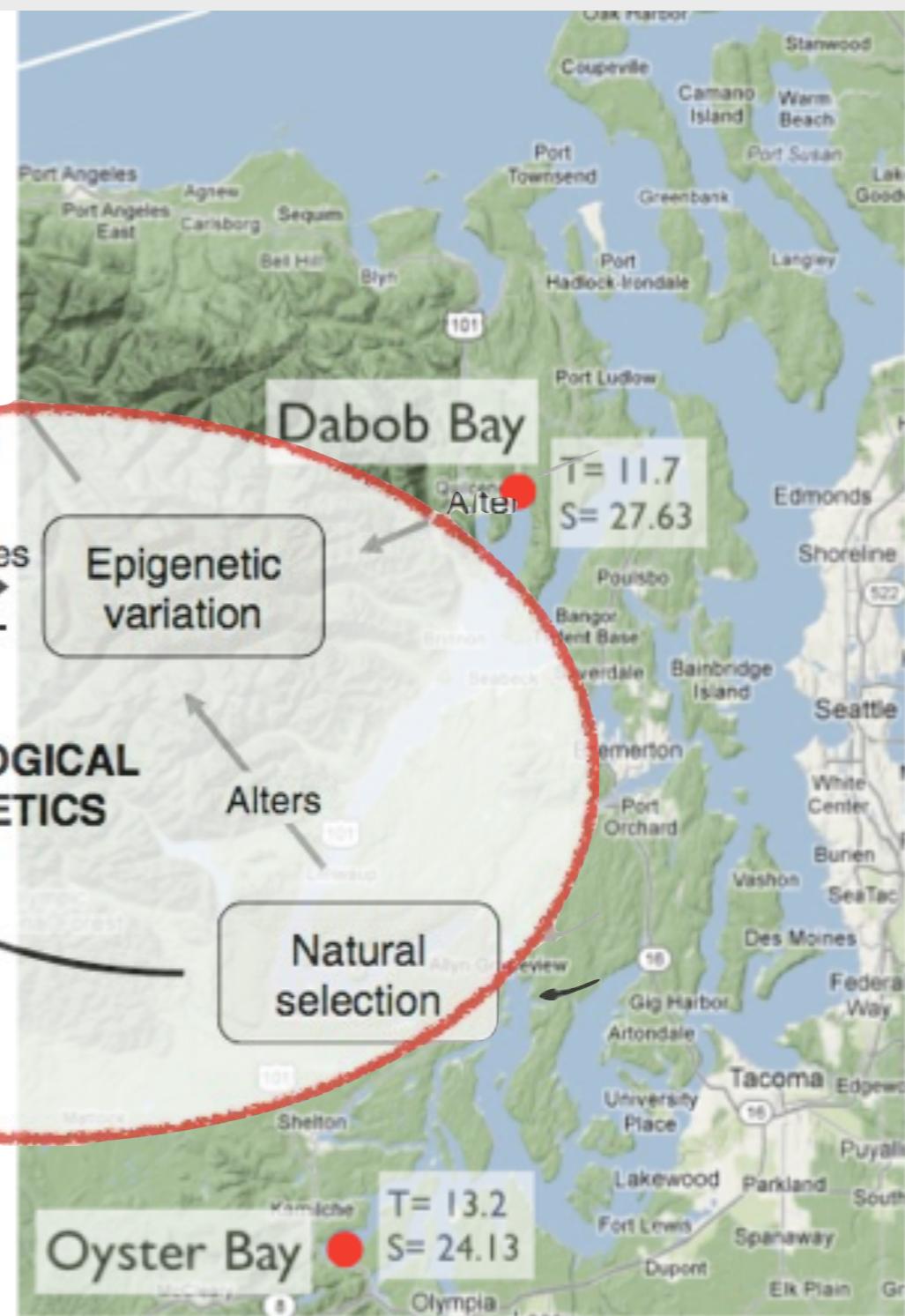
Photo credit: Flickr, Creative Commons, csesums

Very new data  
Heritability  
Plasticity  
Local Adaptation

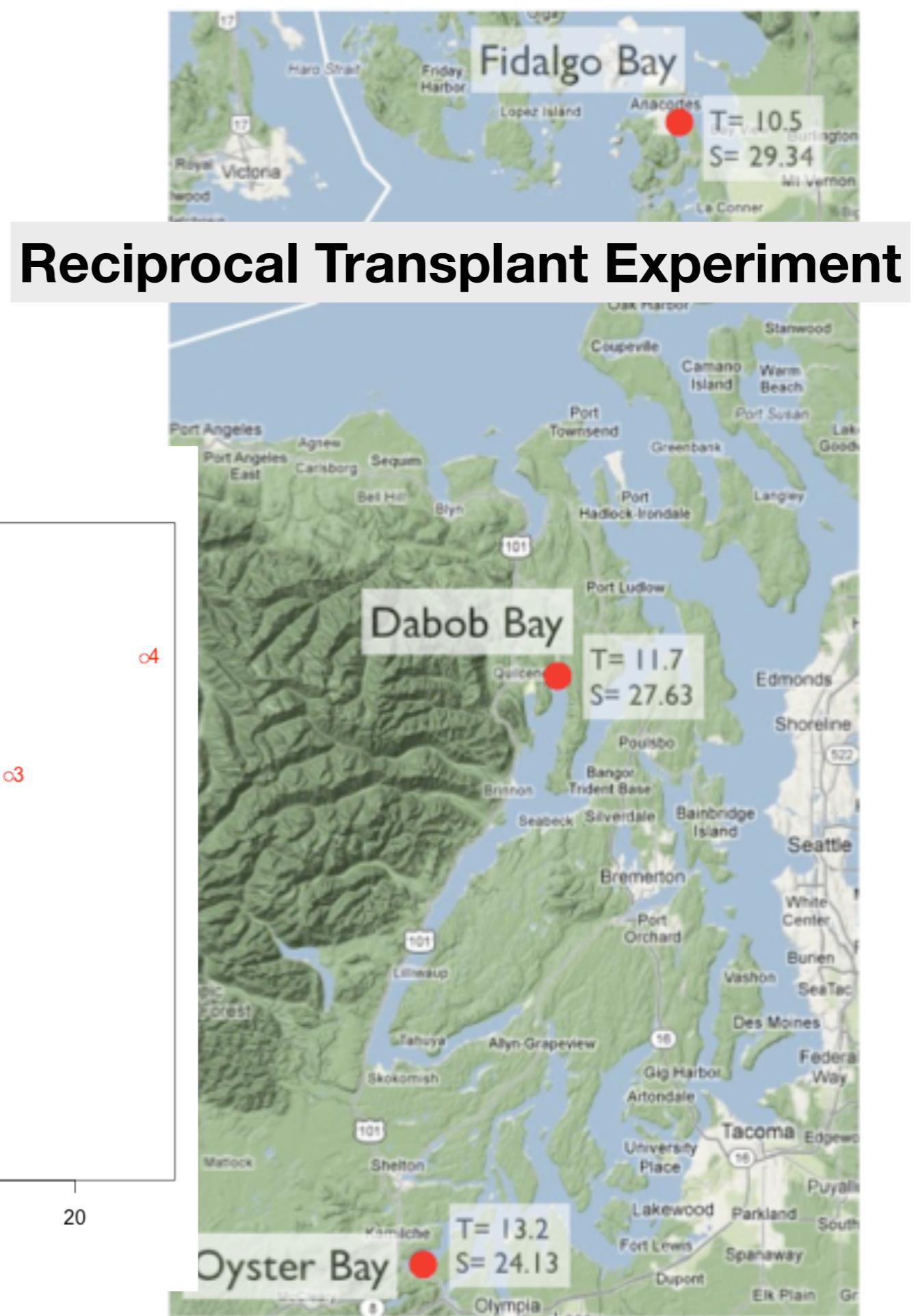
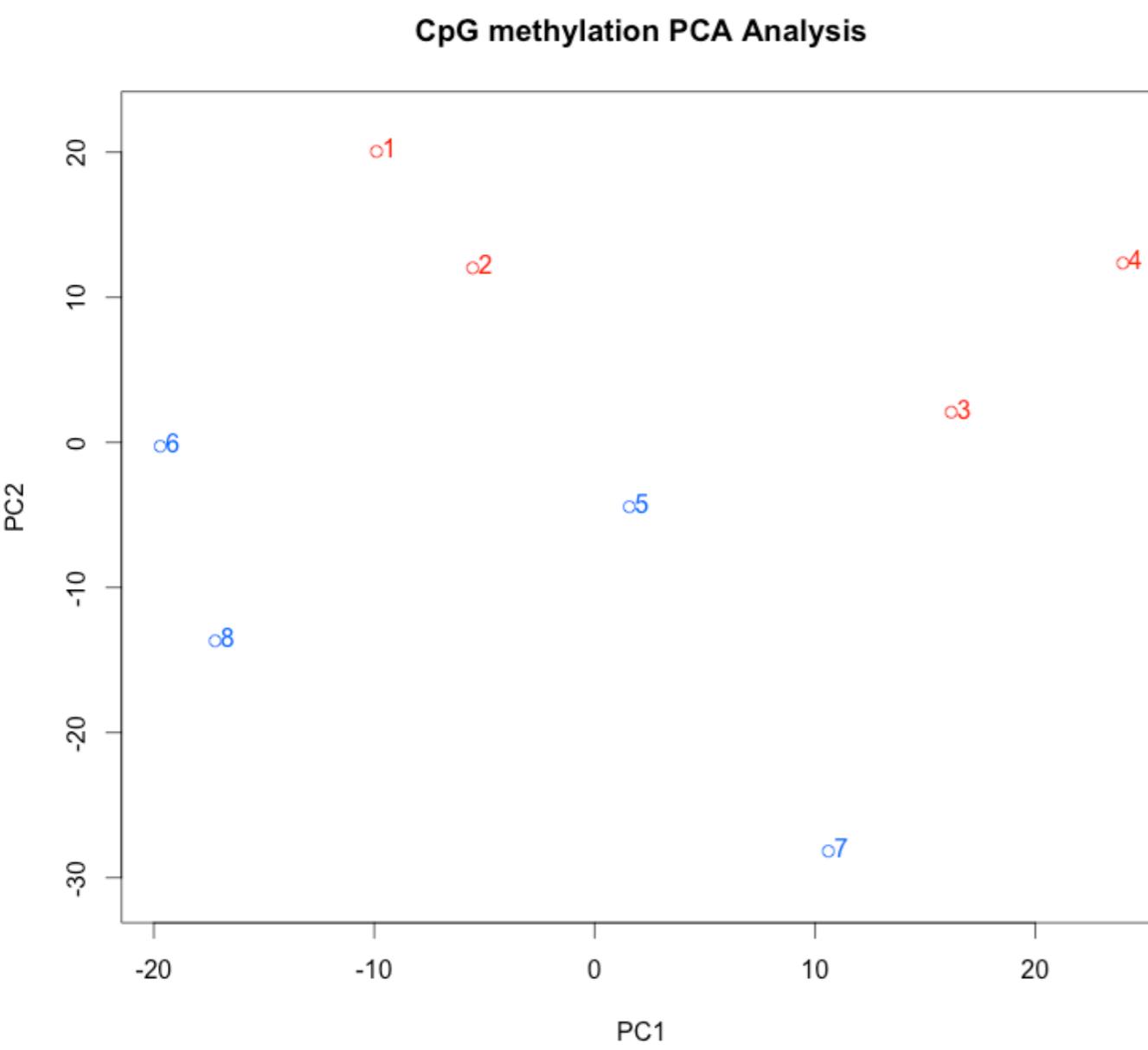
Genetics  
versus  
*Epigenetics*



## Reciprocal Transplant Experiment



Very new data  
Heritability  
Plasticity  
Local Adaptation

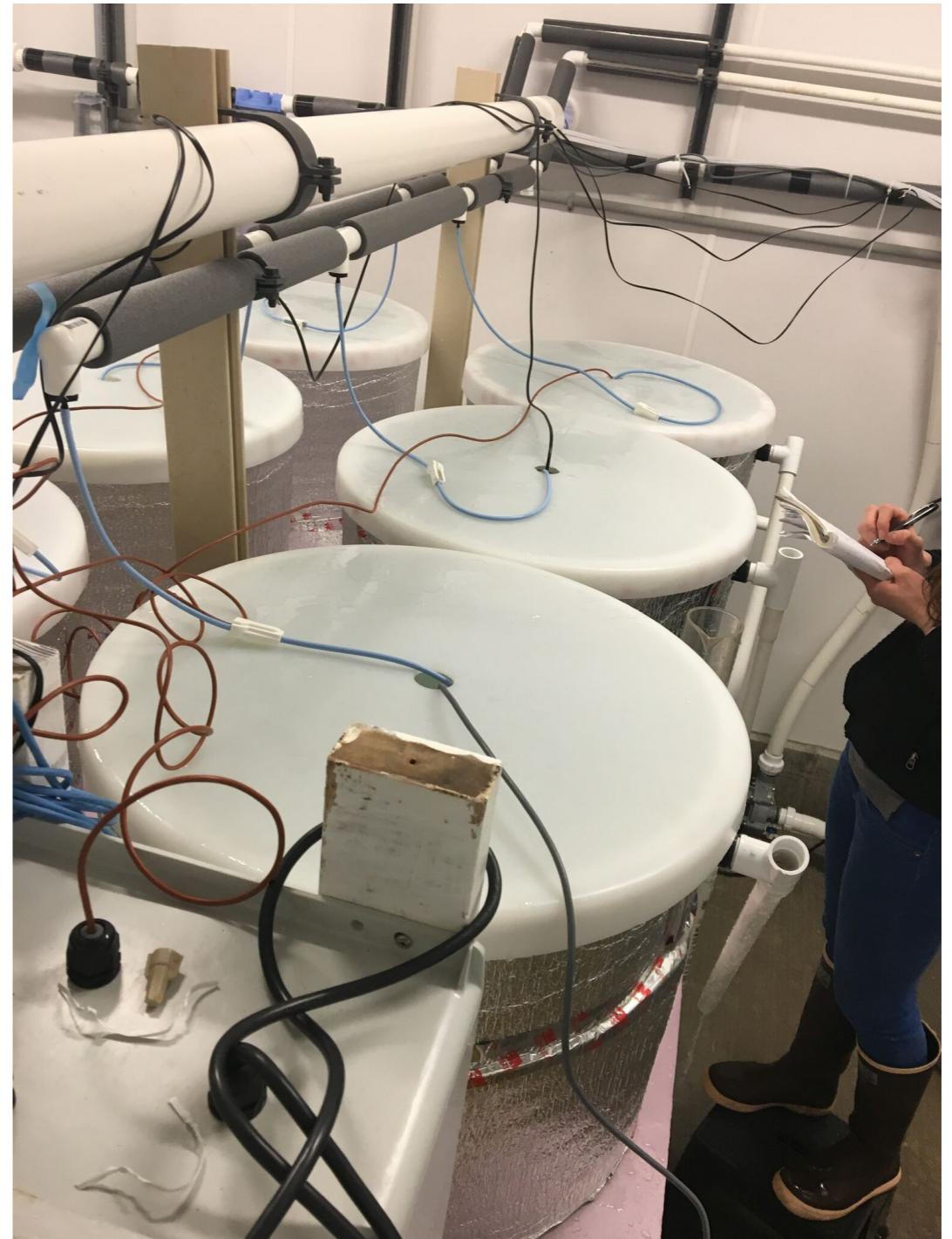


# Very new ~~data~~

## Selection

## Ocean Acidification

*Panopea generosa*



# Very new data

## Selection Ocean Acidification

Day 10

Control: Random Mortality

~42% ACGCTGATCGT

~38% ACGCTAATCGT

Day 1

Proportion of sequences in pooled  
larvae sample with given allele (G vs A)

~42% ACGCTGATCGT

~38% ACGCTAATCGT

Day 10

High  $p\text{CO}_2$ : Non-random  
Mortality

~80% ACGCTGATCGT

~20% ACGCTAATCGT

Day 1

Evidence of allele beneficial (G) to survival  
under High  $p\text{CO}_2$  conditions

High  $p\text{CO}_2$   
Ambient temperature

High  $p\text{CO}_2$   
High temperature

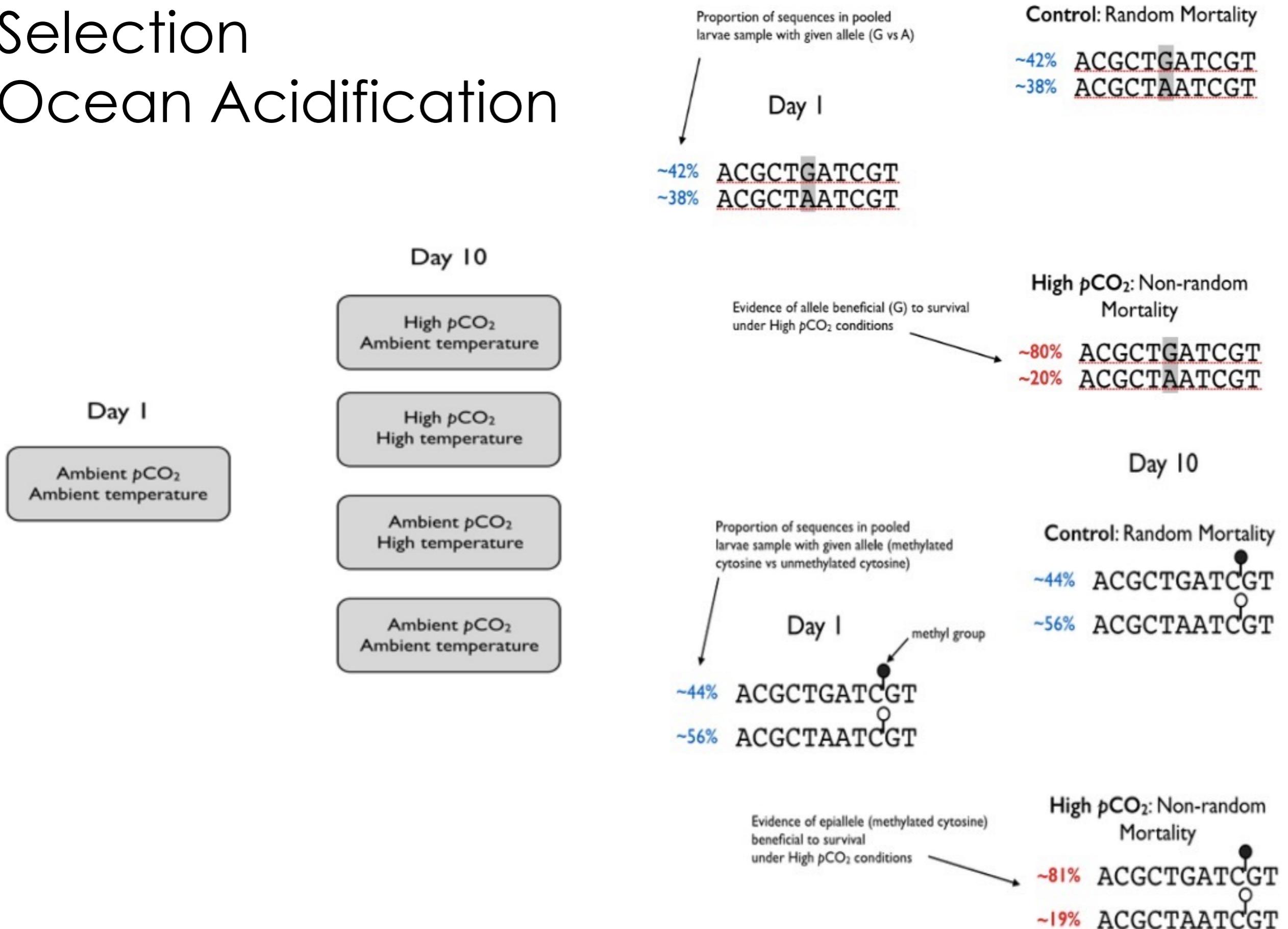
Ambient  $p\text{CO}_2$   
High temperature

Ambient  $p\text{CO}_2$   
Ambient temperature

Ambient  $p\text{CO}_2$   
Ambient temperature

# Very new data

## Selection Ocean Acidification



# Open Science

about science

web-native scholarship

# Sharing



Photo credit: Flickr, Creative Commons, speechless

# Open Data

# Open Methods

# Approach



Data Lake

# Raw Data

1) As sequencing facility provides data, files are downloaded to our local NAS (owl), in the root `nightingales` directory.  
<http://owl.fish.washington.edu/nightingales/>

2) The Nightingales Google Spreadsheet is updated.

3) Update the Nightingales Google Fusion Table with new information from the Nightingales Google Spreadsheet. This is accomplished by:

- deleting all rows in the Nightingales Google Fusion Table (Edit > Delete all rows)
- Importing data from the Nightingales Google Spreadsheet (File > Import more rows...)

4) Once metadata is included in the Google Fusion Table [Nightingales](#), respective data files are moved to subdirectory labelled with first letter of genus followed by species ie `c_gigas`. Check url in Nightingales Fusion table to ensure it is accurate.

**Nightingales**

Imported at Wed Jun 25 07:25:38 PDT 2014 from Nightin  
Add Attribution - Edited on December 15, 2014

File Edit Tools Help Rows 1 ▾

Filter ▾ No filters applied. Sorted by SeqDat

◀ ▶ 1-100 of 153 ▶

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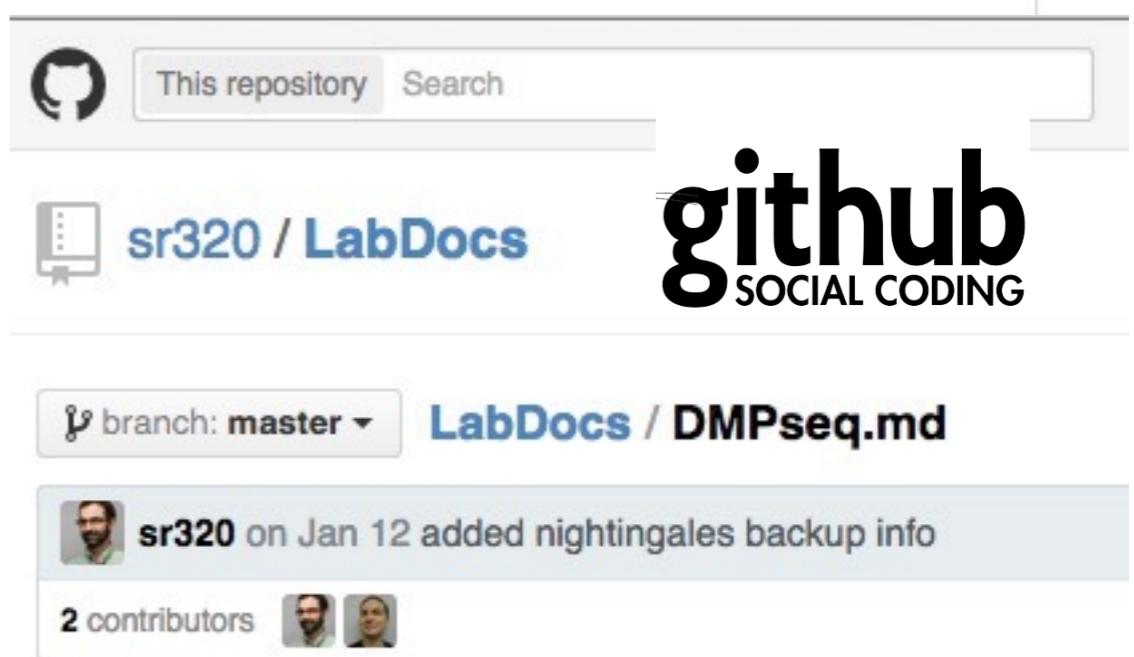
This repository Search

sr320 / LabDocs

branch: master LabDocs / DMPseq.md

sr320 on Jan 12 added nightingales backup info

2 contributors



# Open Methods

# Open Methods

reproducibility

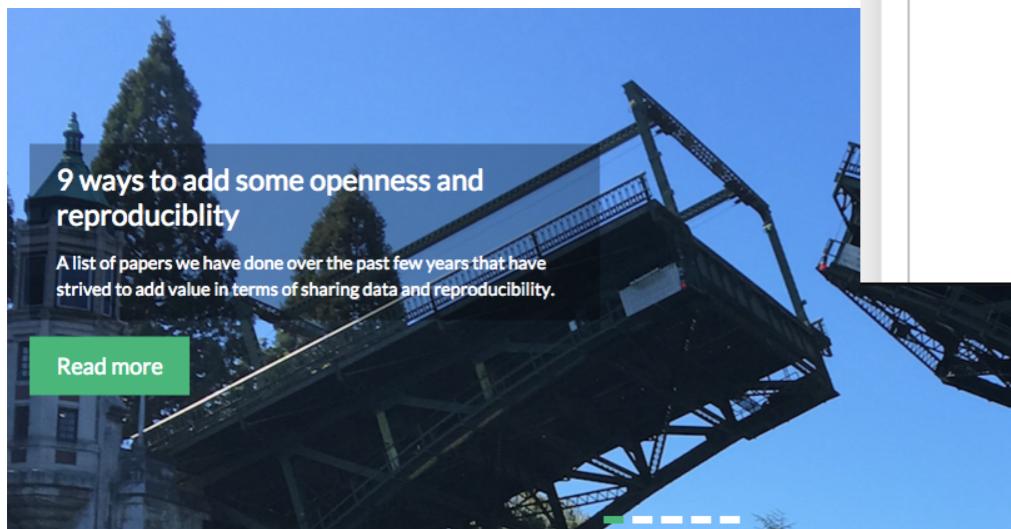
# Data Acquisition and Analysis

---

Open  
Notebook  
Science

# Data Acquisition and Analysis

# Open Notebook Science



jupyter Welcome3 (autosaved)

File Edit View Insert Cell Kernel Help Python 3

Cell Toolbar: None

## Welcome

Welcome to Pineapple, the next generation scientific notebook.

### Run Python code

```
In [1]: 2 ** 64
```

```
Out[1]: 18446744073709551616
```

### You can make plots right in the notebook

```
In [2]: %matplotlib inline
import matplotlib.pyplot as plt
import numpy as np
x = np.linspace(0, 10)
plt.plot(x, np.sin(x));
```

jldimond / Coral-CpG

Unwatch 2

Repository associated with article "Germline DNA methylation in reef corals: patterns and potential roles in response to environmental change"

22 commits 1 branch 0 releases 2 contributors

Branch: master +

Update READMEs

jldimond authored 5 days ago latest commit d94c7b5aec

analyses Update READMEs 5 days ago

data typo 7 days ago

ipynb Update READMEs 5 days ago

README.md typo 7 days ago

README.md

Analyses and data associated with manuscript:  
*Germline DNA methylation in six species of reef corals: patterns and potential roles in response to environmental change*



# Acknowledgements

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Dan Halperin



Hollie Putnam  
Laura Spencer

slides, data & more @  
[github.com/sr320/talk-univ-perp-2016](https://github.com/sr320/talk-univ-perp-2016)