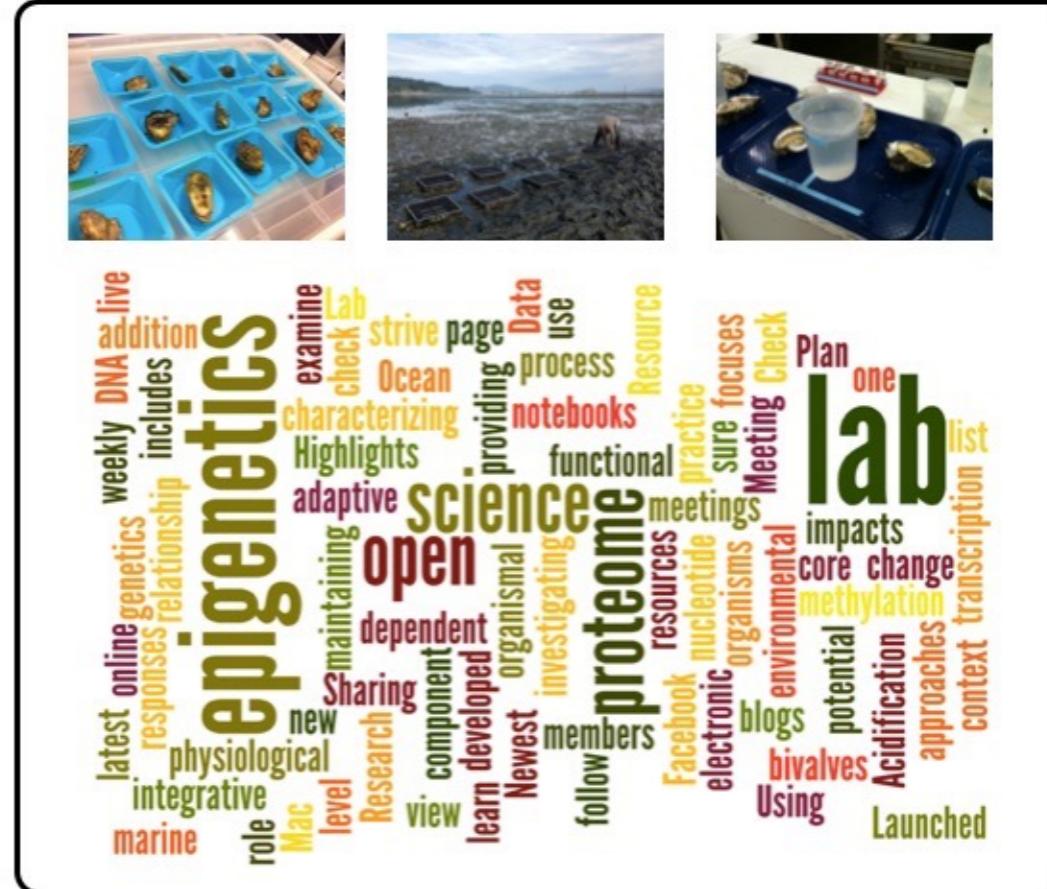


# Does DNA Methylation Facilitate Genome Diversity and Phenotypic Plasticity in Marine Invertebrates?

Steven Roberts  
Associate Professor  
School of Aquatic and Fishery Sciences  
[robertslab.info](http://robertslab.info)  
@sr320



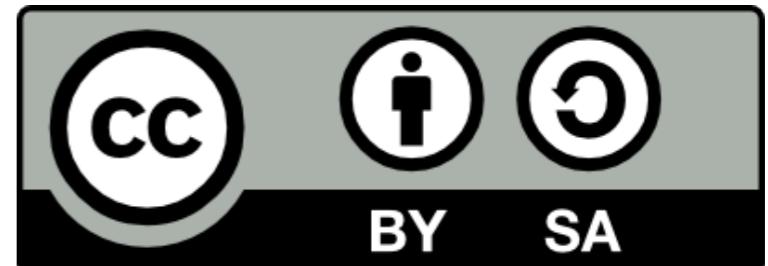
# Open Science

---

- You are free to Share!
- Our lab practices open notebook science



**IP[y]:** IPython  
Interactive Computing



**OpenNotebookScience**

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[onsnetwork.org](http://onsnetwork.org)

- Data, Preprints, Proposals, Lab Meetings, Web Cams, Slidedecks available

These slides plus links @ [robertslab.info](http://robertslab.info)

# Background

disease resistance

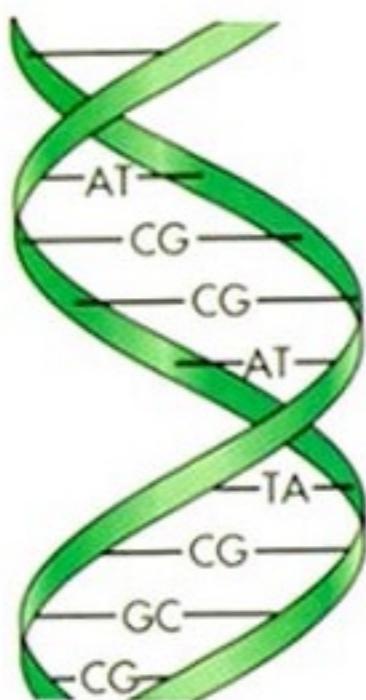


growth

color

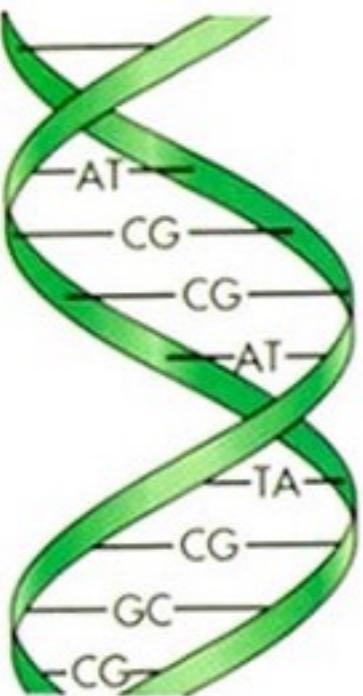
stress  
tolerance

Traits



# Background

Transcriptome



disease resistance



Traits

growth

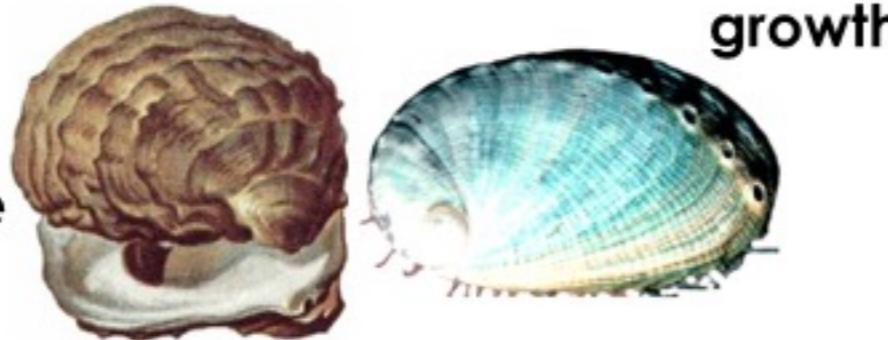
color

stress  
tolerance



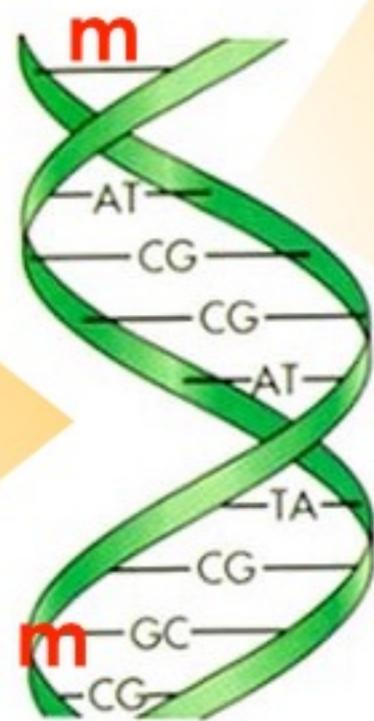
# Background

disease resistance



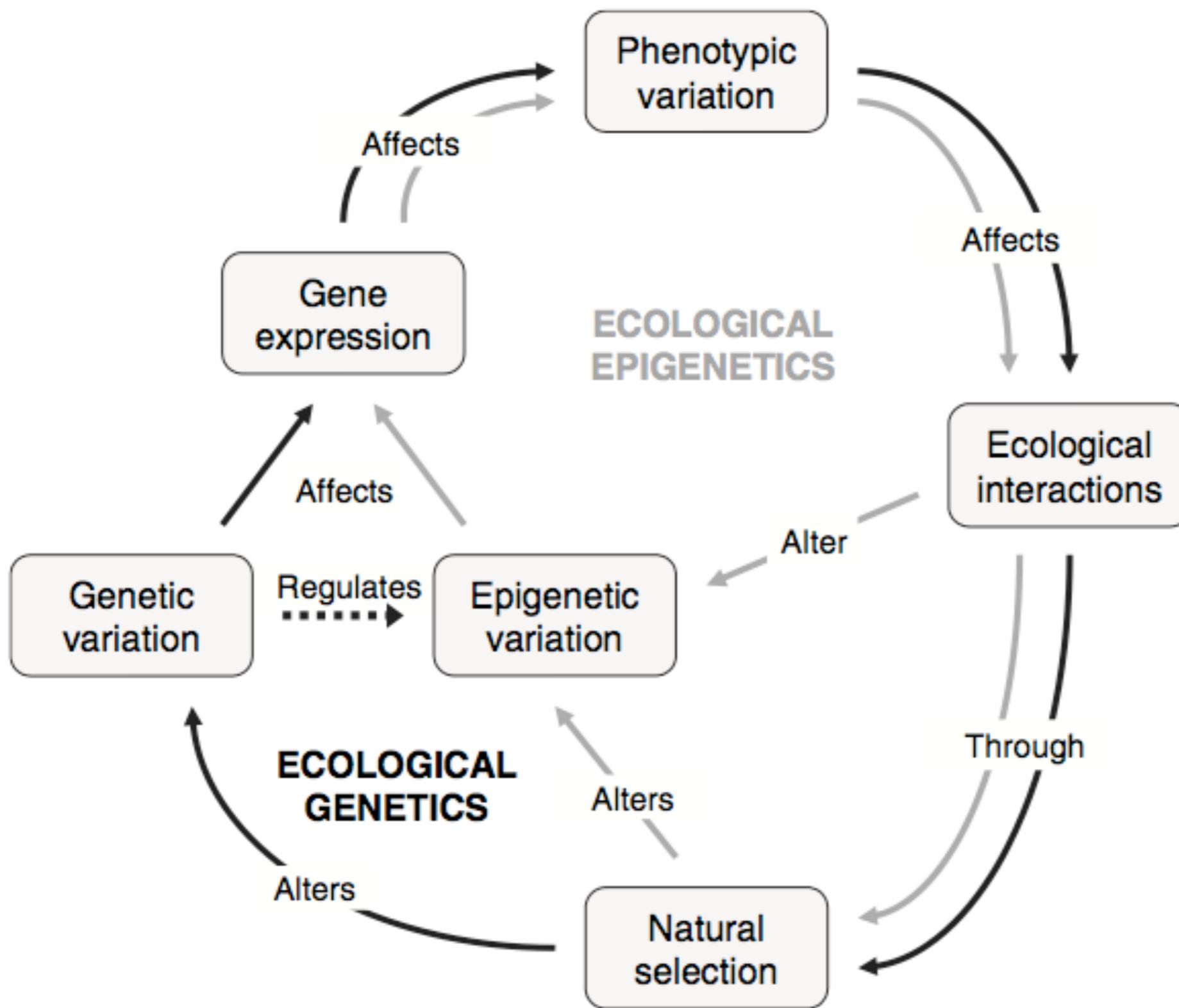
growth

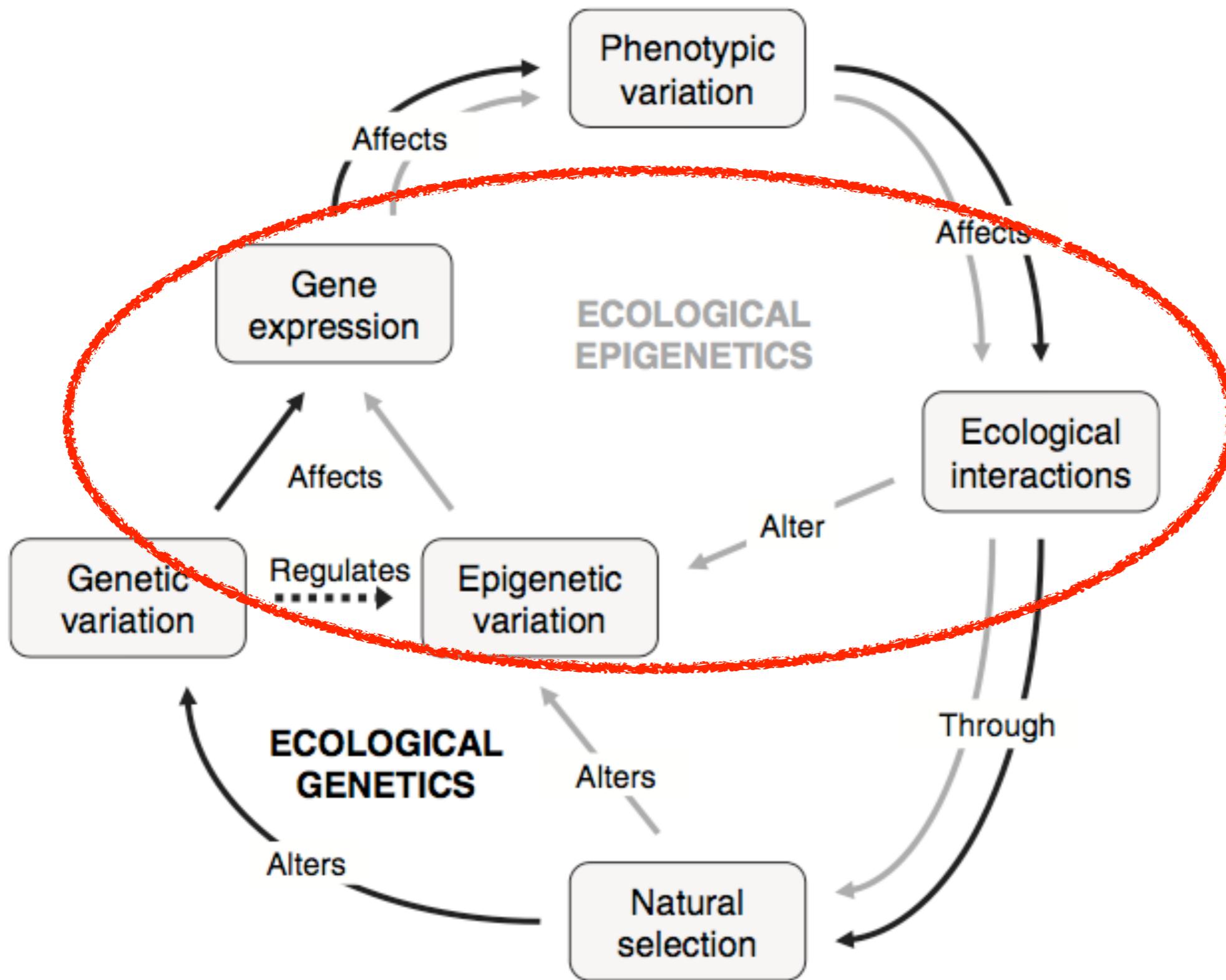
## Traits

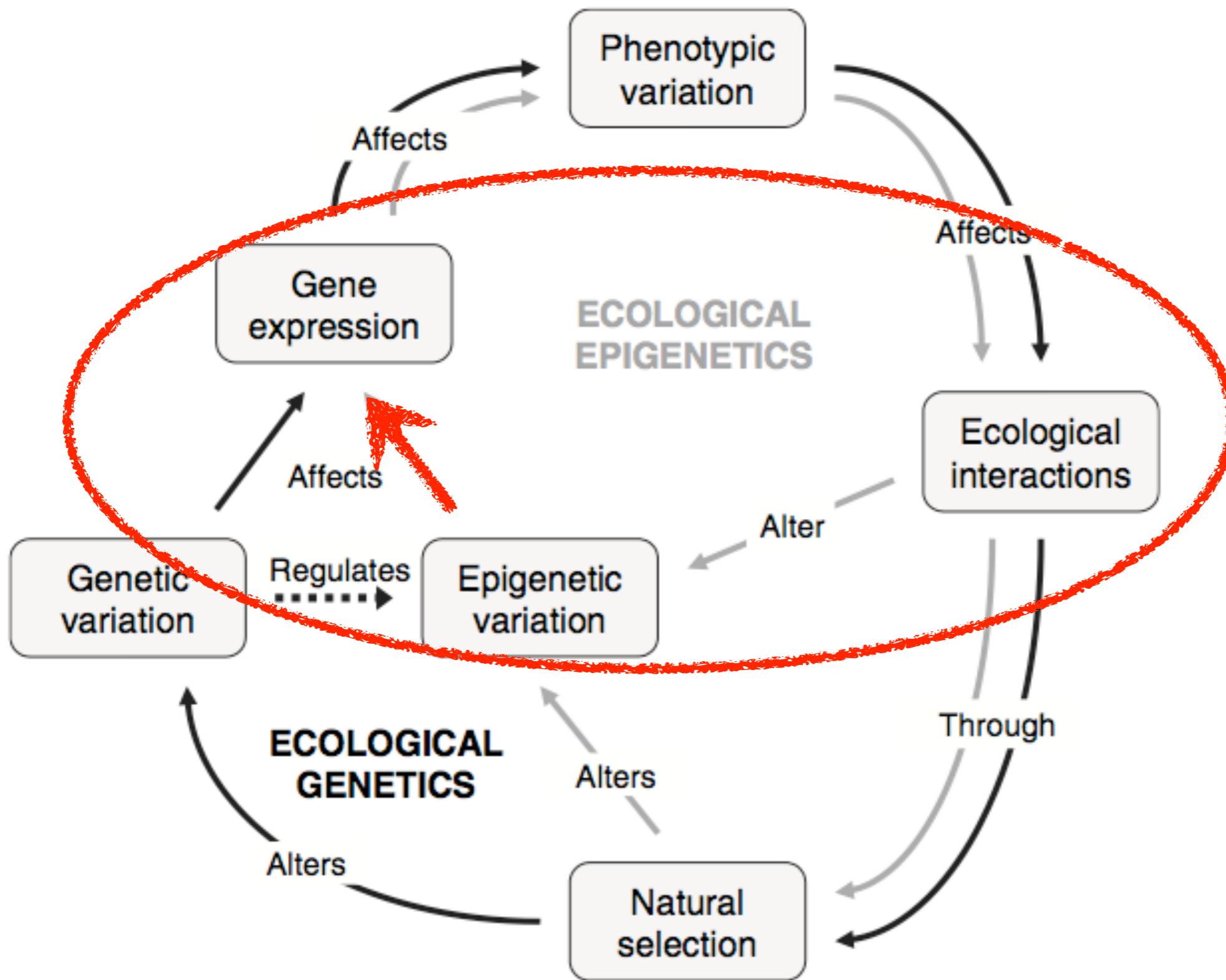


Epigenetics

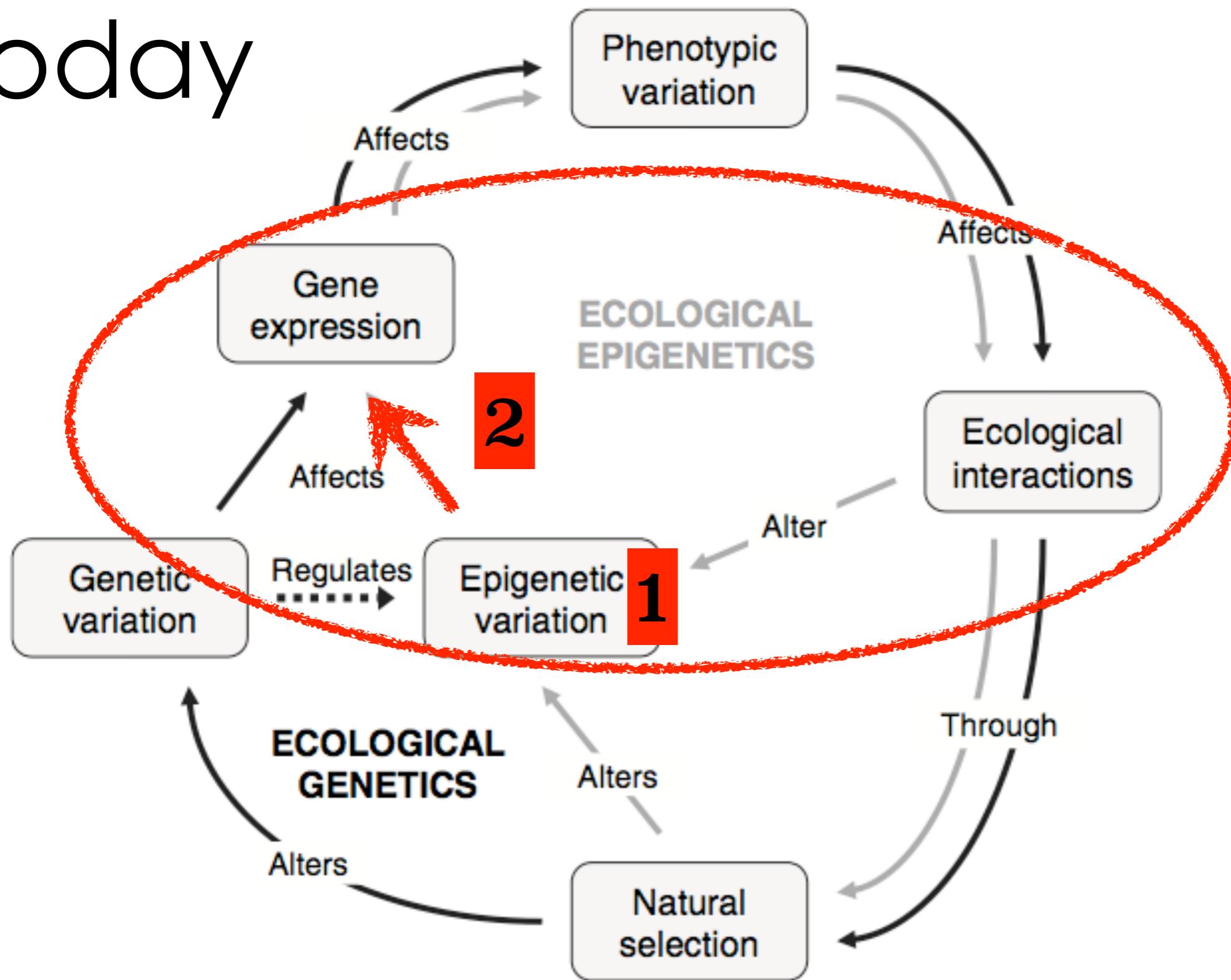








# Today



*Ecology Letters*, (2008) 11: 106–115

doi: 10.1111/j.1461-0248.2007.01130.x

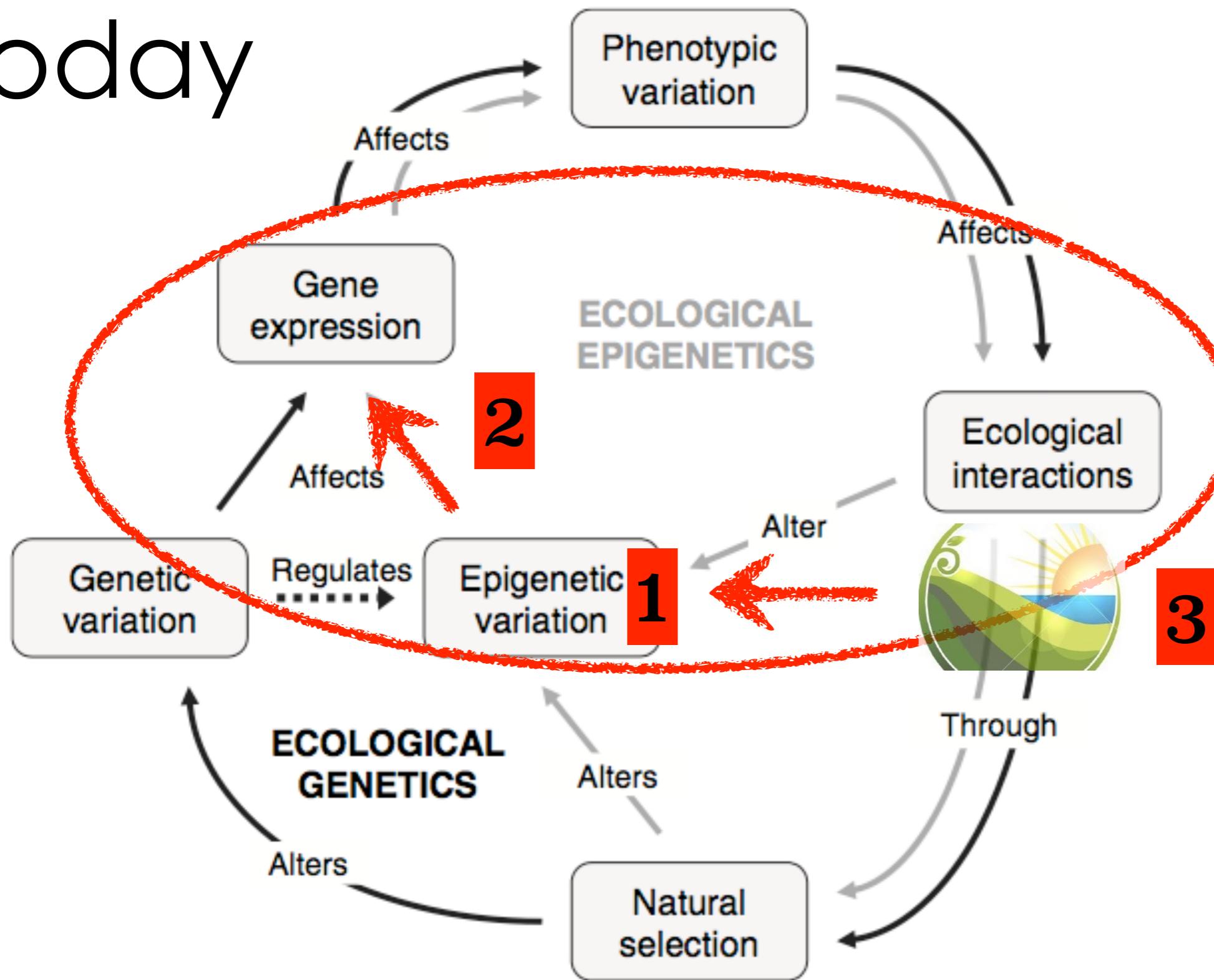
modified from

IDEA AND  
PERSPECTIVE

Epigenetics for ecologists

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

# Today



*Ecology Letters*, (2008) 11: 106–115

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modified from

IDEA AND  
PERSPECTIVE

Epigenetics for ecologists

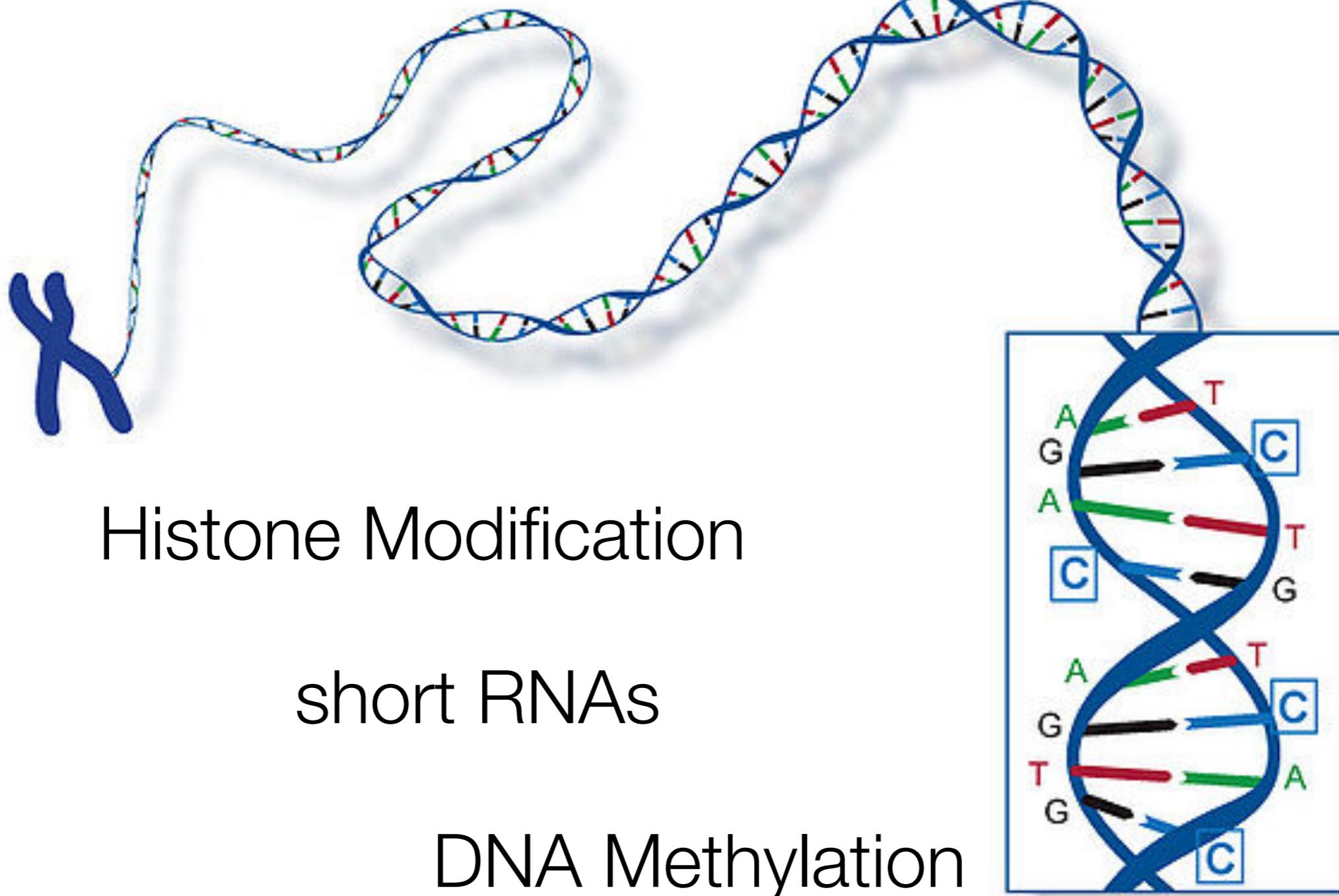
Oliver Bossdorf,<sup>1,\*</sup> Christina L.  
Richards<sup>2</sup> and Massimo Pigliucci<sup>3</sup>



# Genome Resources



# Epigenetics



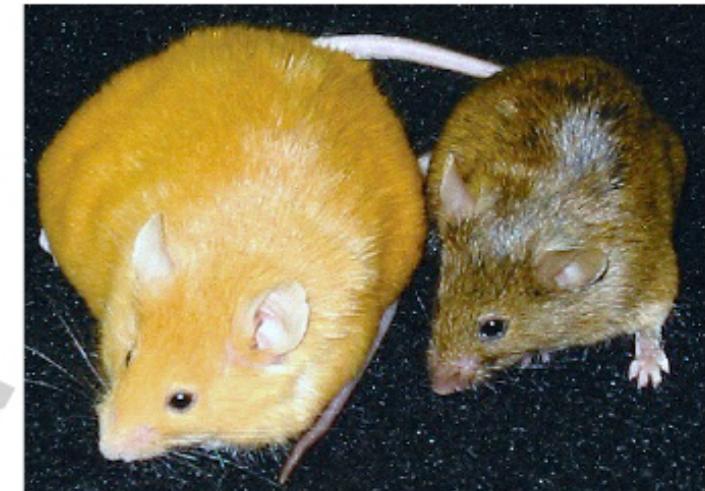
# Epigenetics



Photo credit: Flickr, Creative Commons, he-boden



# Epigenetics



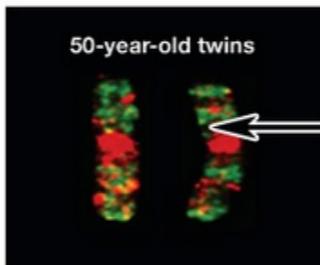
## Nature AND Nurture

### Chromosome 3 Pairs

3-year old twins vs. 50-year-old twins



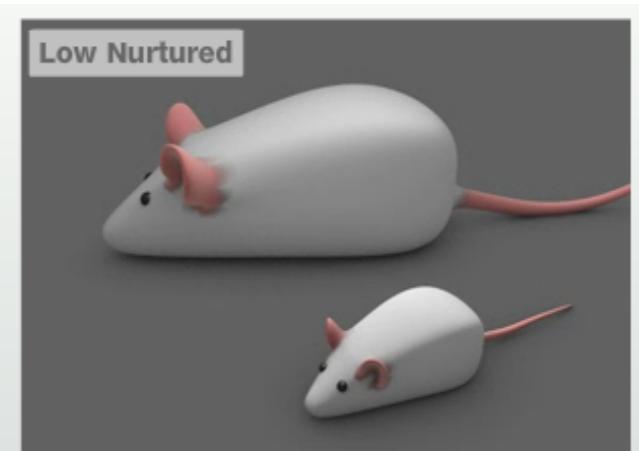
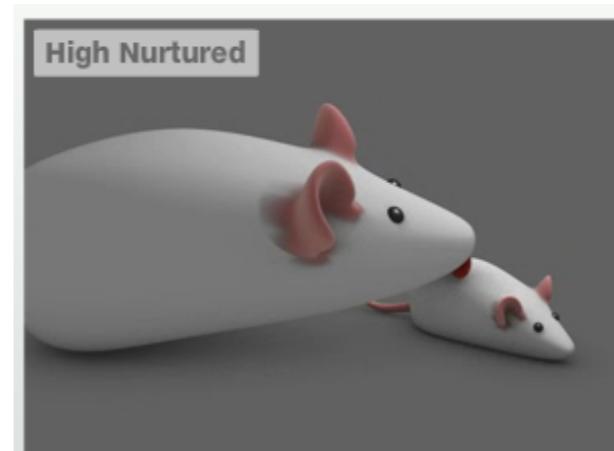
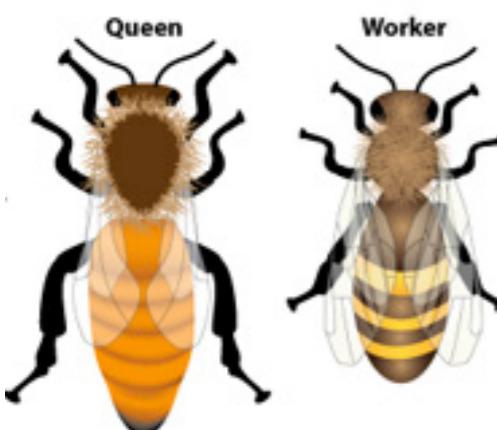
Yellow shows where the twins have epigenetic tags in the same place.



Red and green show where the twins have epigenetic tags in different places.

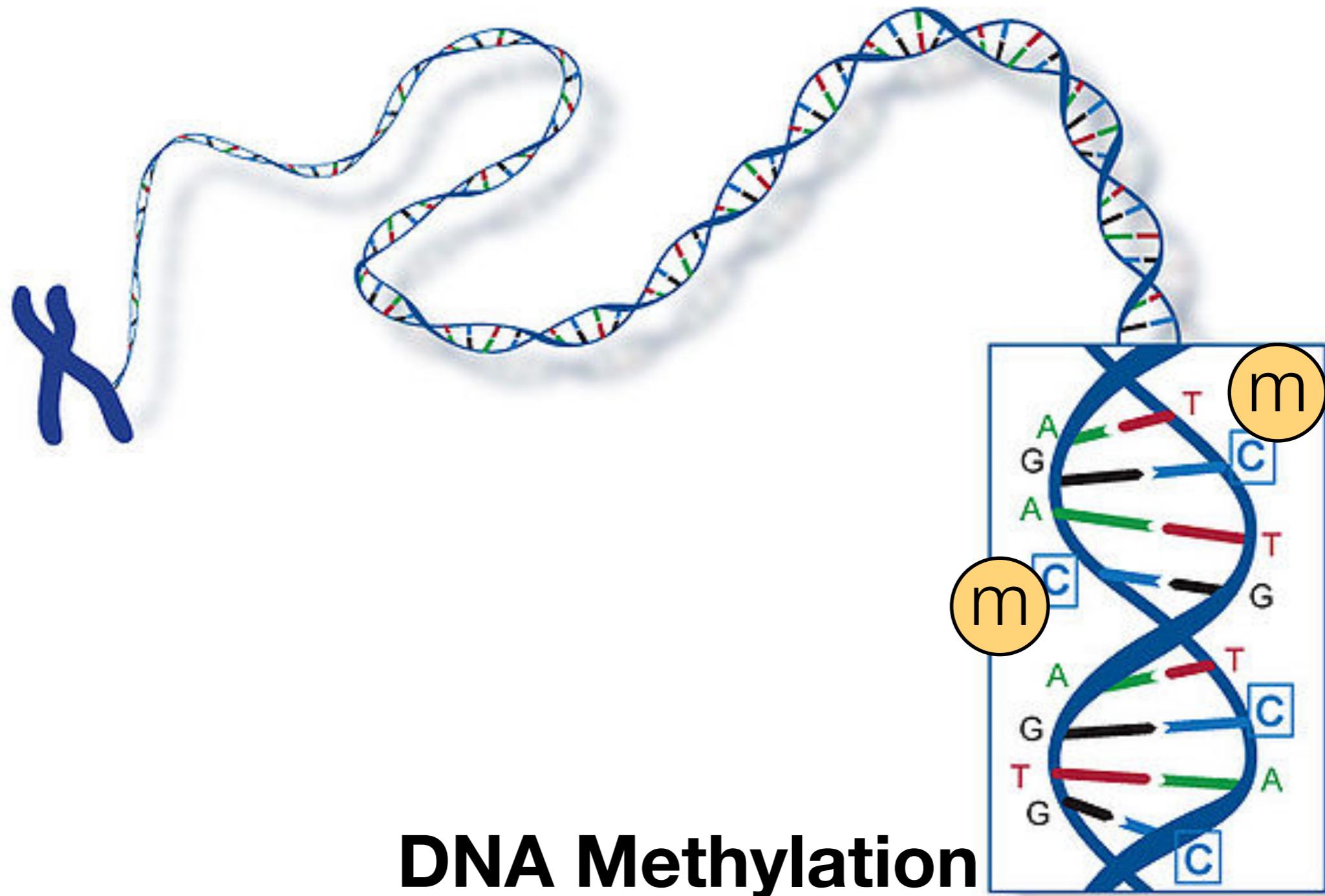


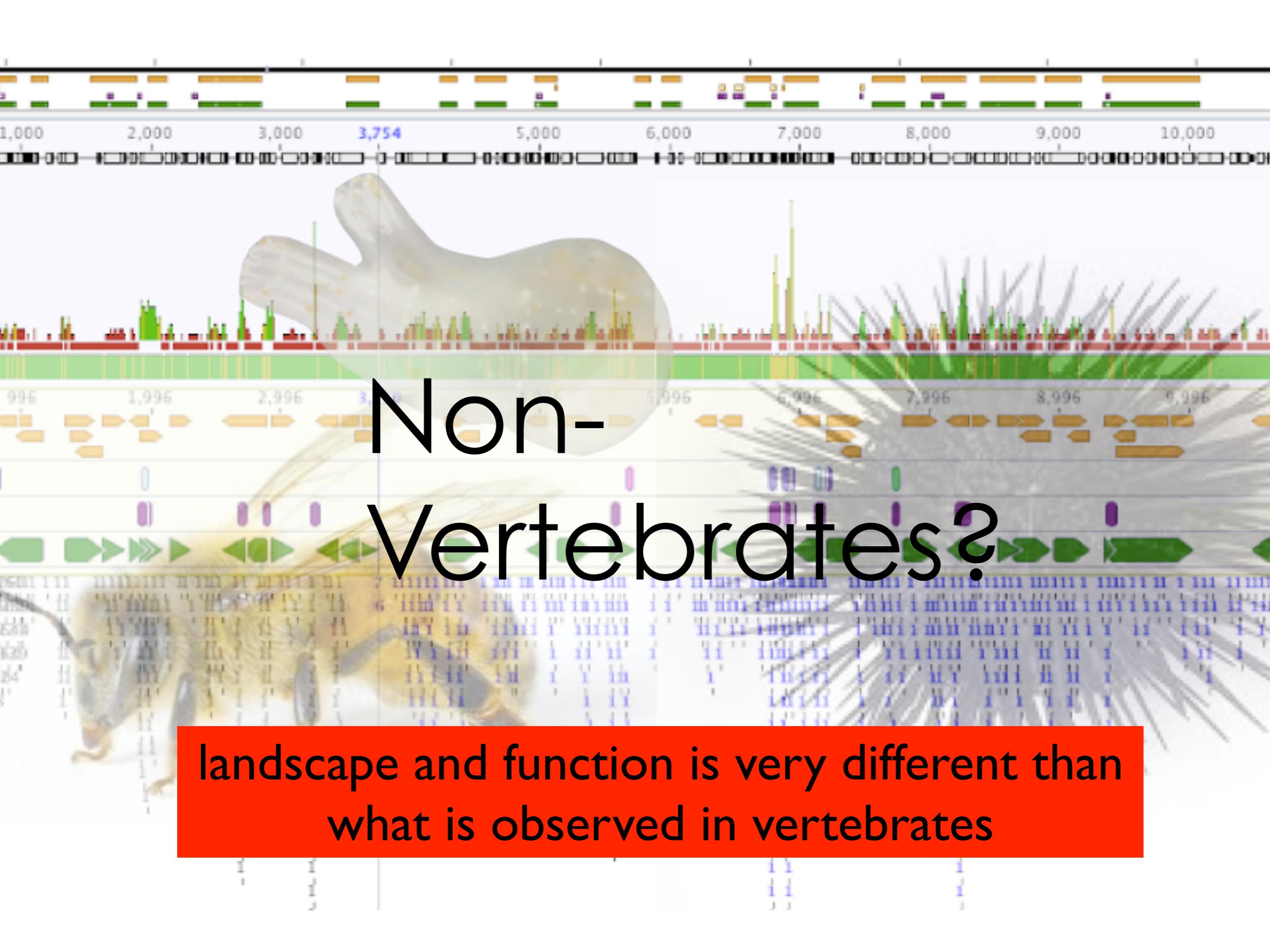
Queen Bee Larvae: Queens are raised in specially constructed cells called "queen cups," which are filled with royal jelly.



These mothers come from a long line of inbred rats, so their genomes are highly similar. But they care for their pups very differently.

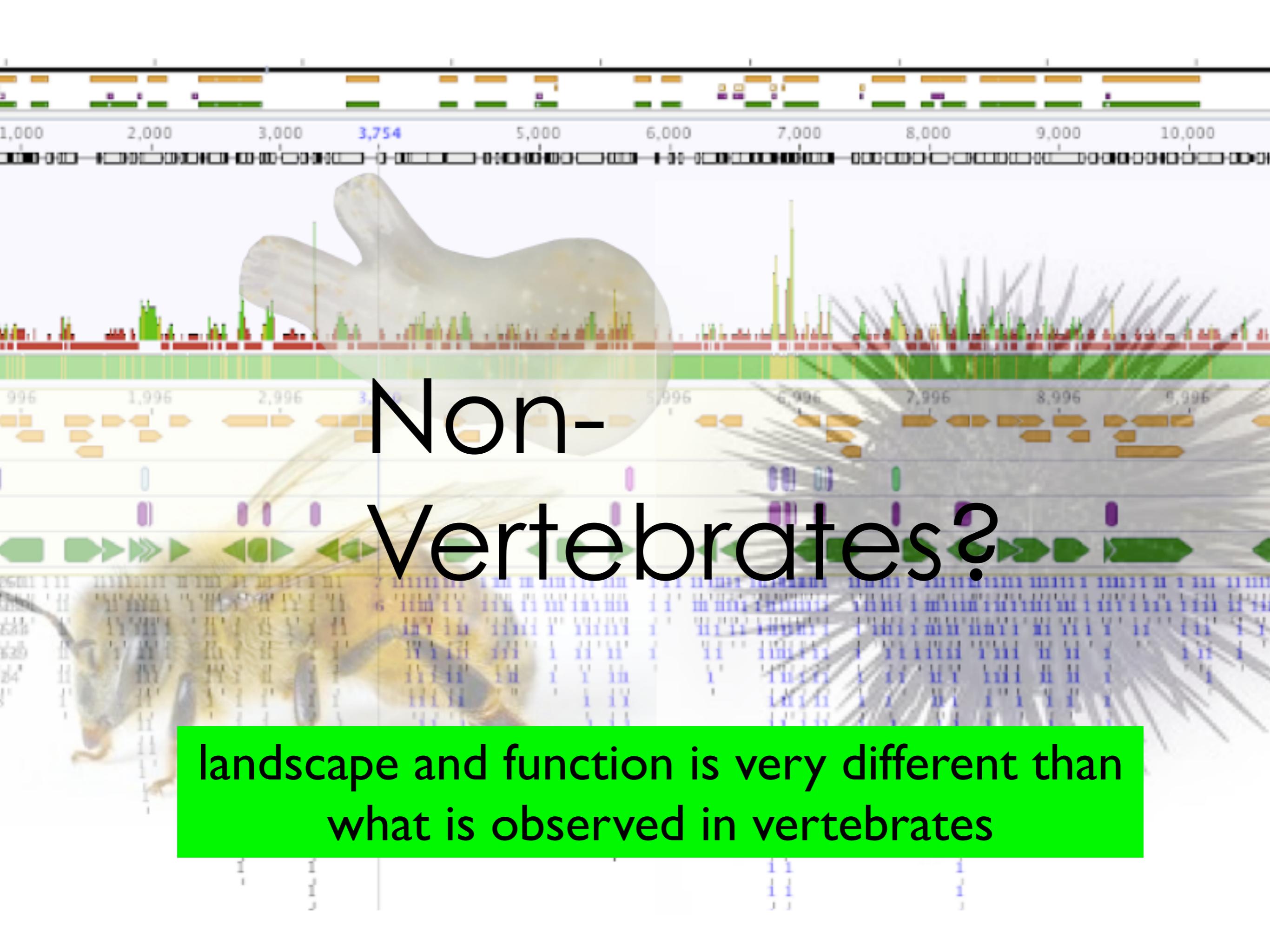
AUDIO





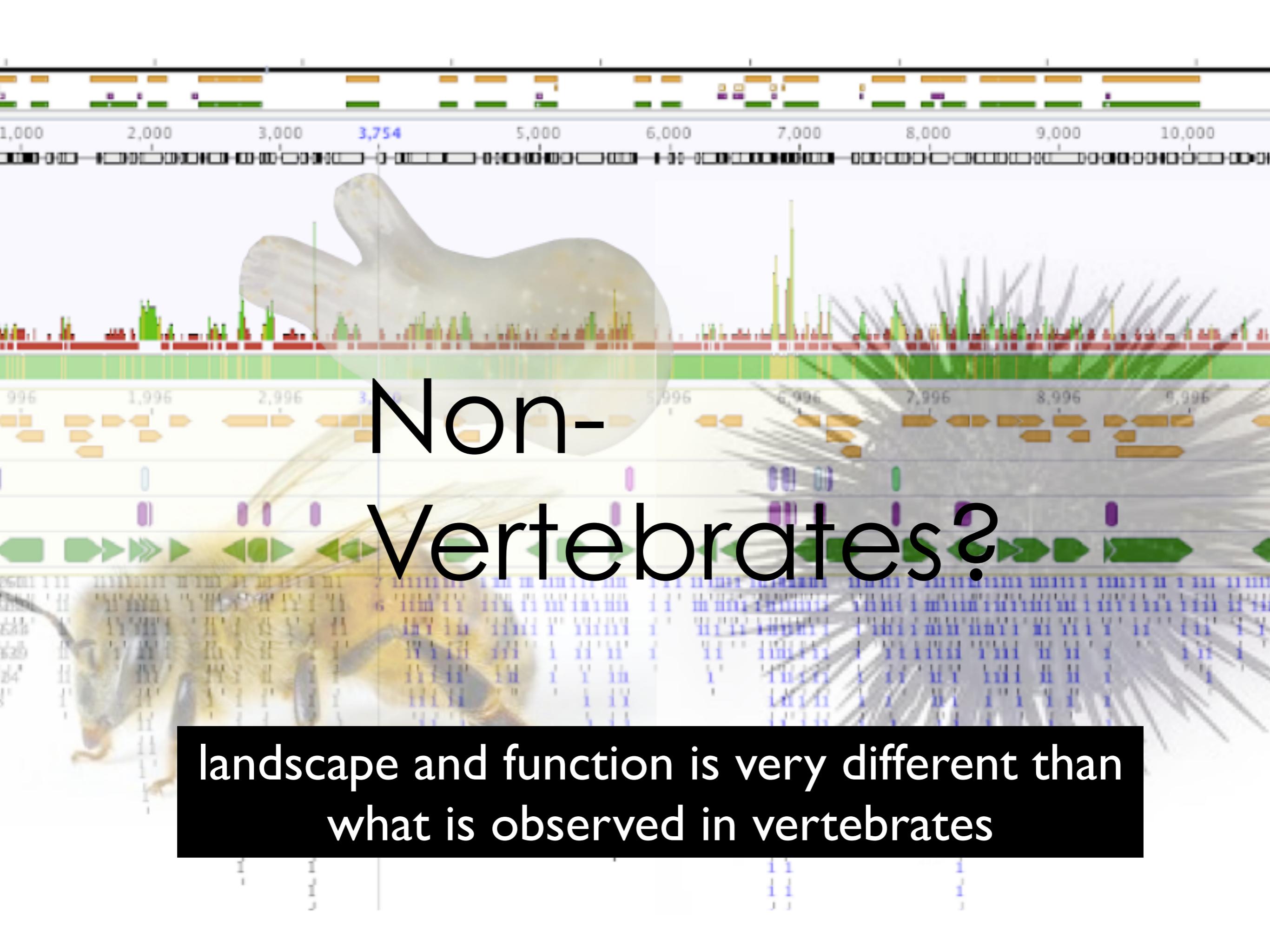
# Non- Vertebrates?

landscape and function is very different than  
what is observed in vertebrates



# Non- Vertebrates?

landscape and function is very different than  
what is observed in vertebrates



# Non- Vertebrates?

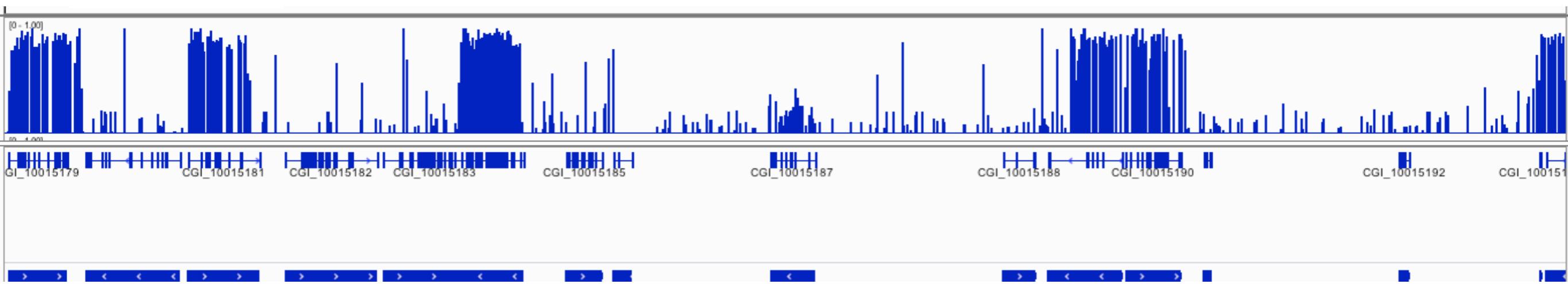
landscape and function is very different than  
what is observed in vertebrates

*Absent in  
several  
model  
organisms*



Oysters?

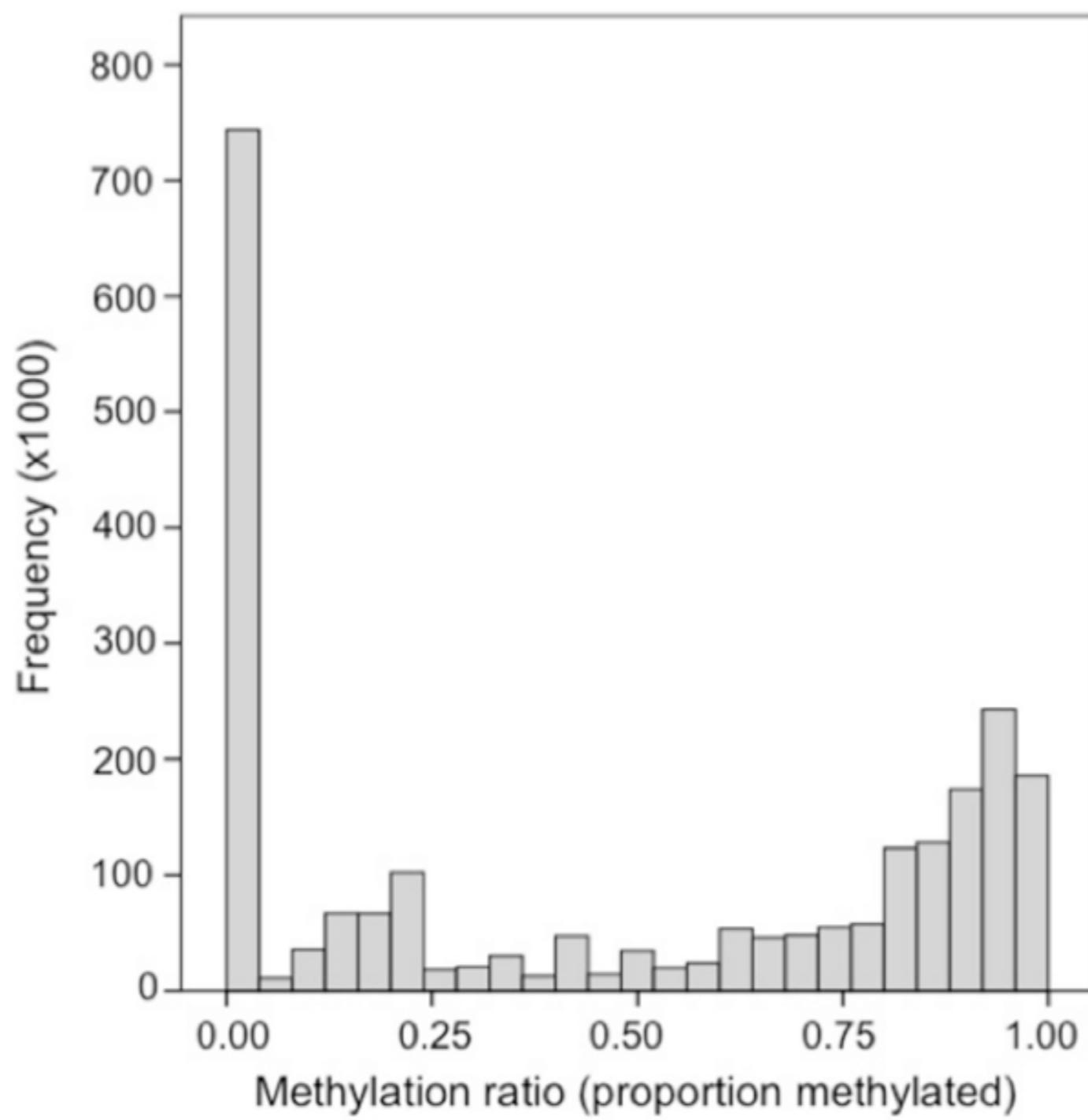
Epigenetic variation **1**



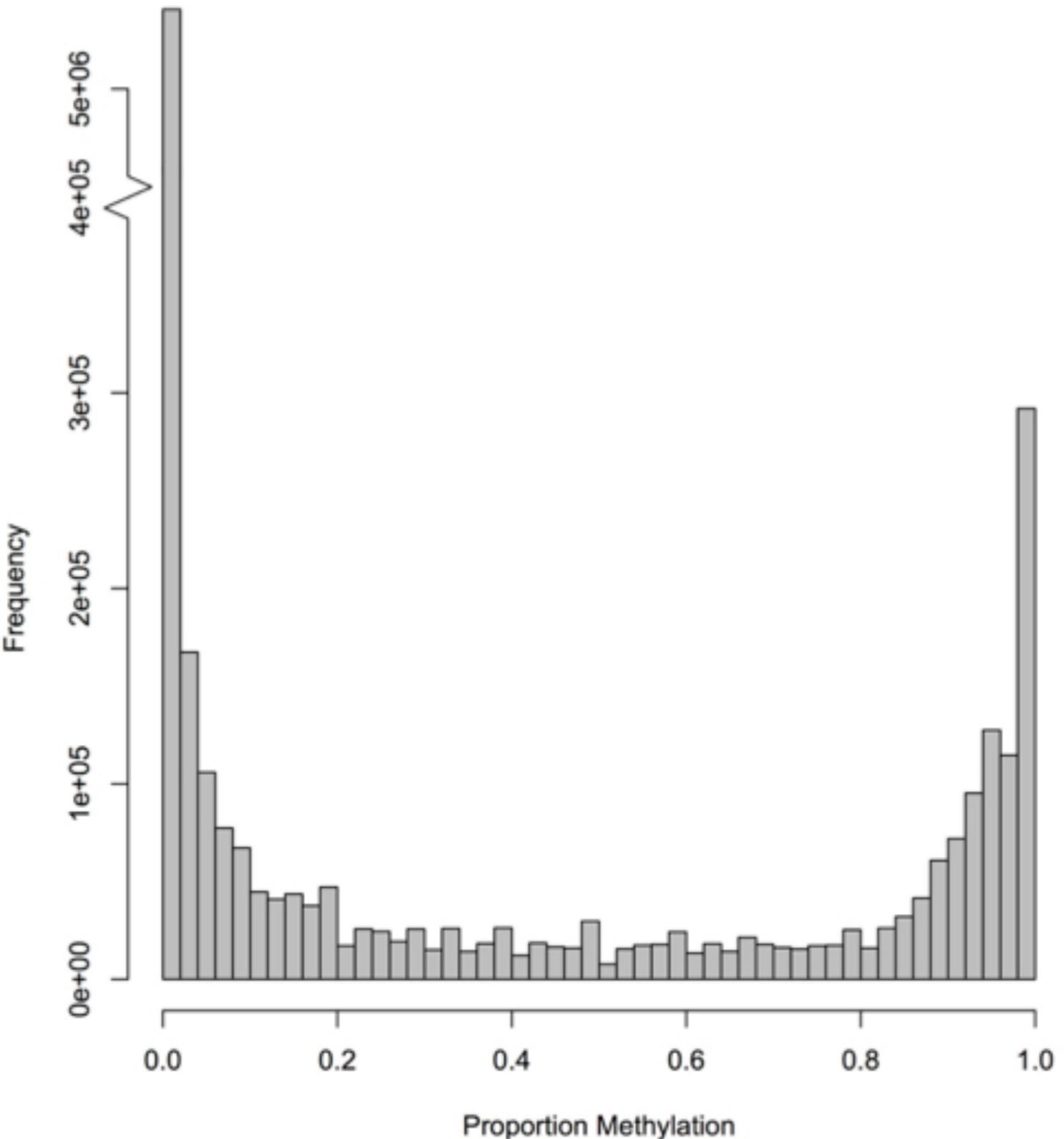
*mosaic*

associated with gene bodies

Epigenetic variation **1**

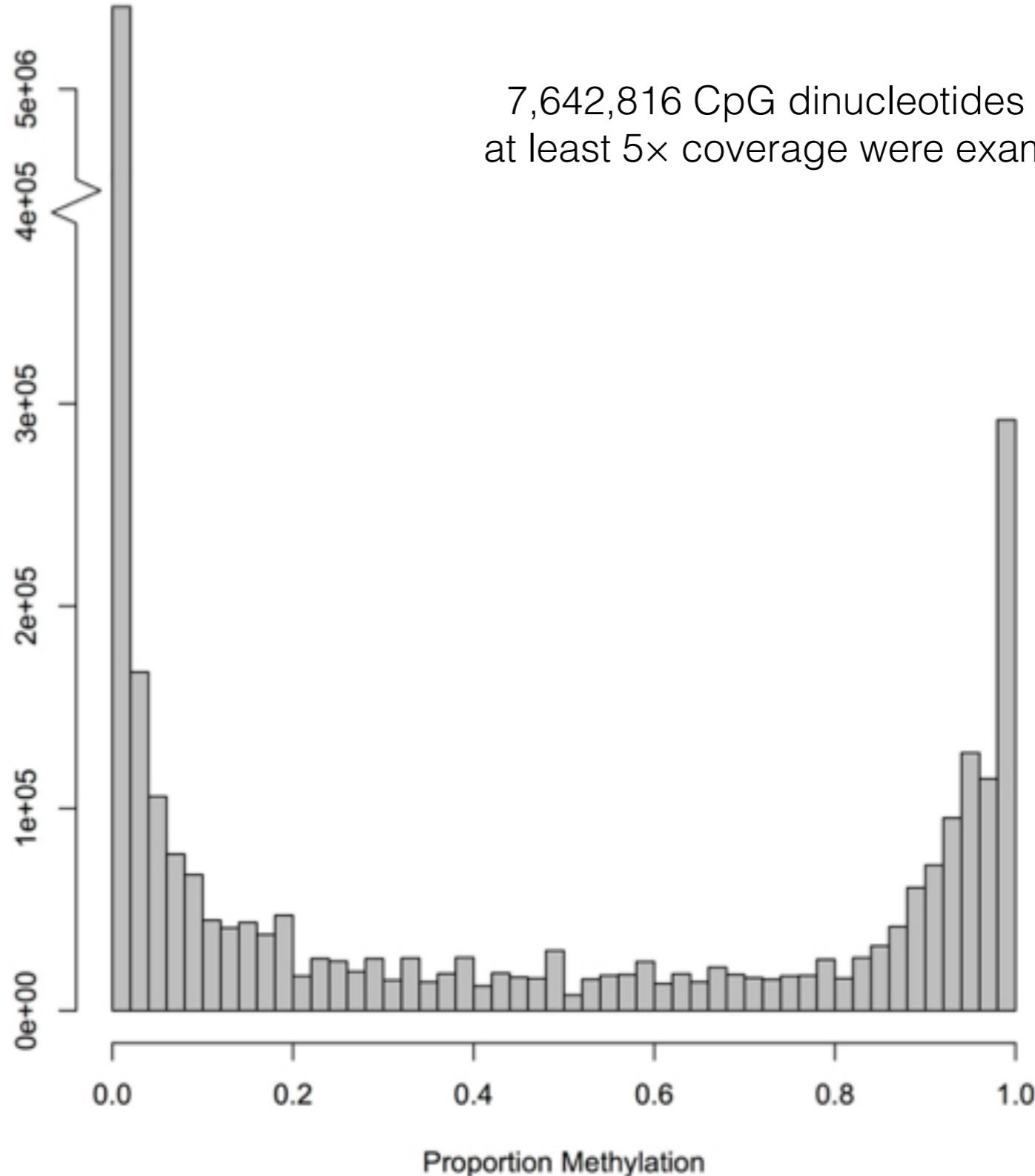
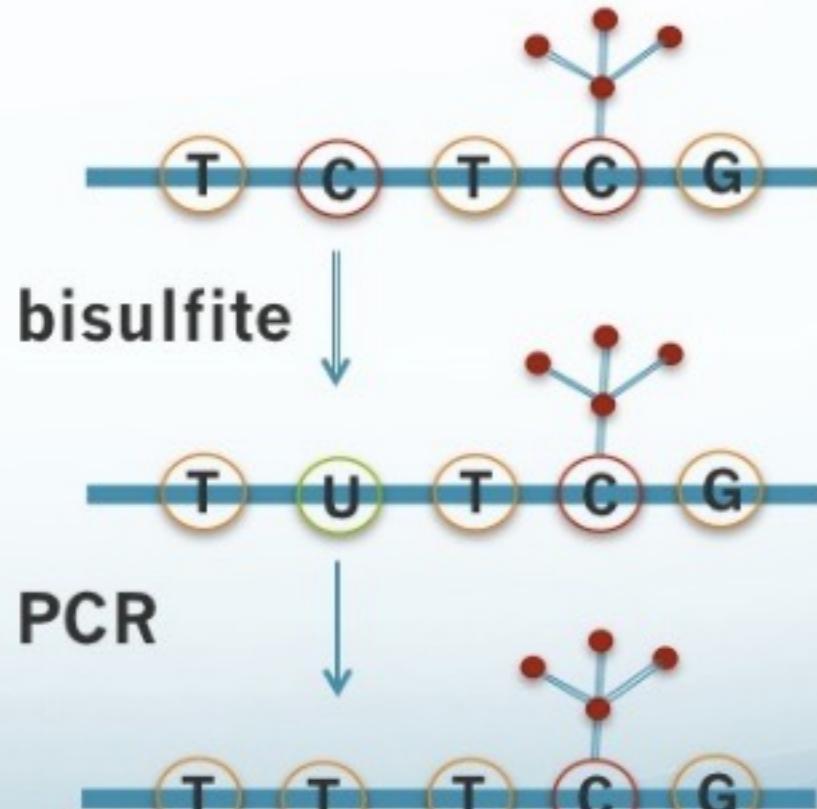


**Figure 1** Frequency distribution of methylation ratios for CpG dinucleotides in oyster gill tissue. A total of 2,625,745 CpG dinucleotides with  $\geq 5 \times$  coverage are represented.



Epigenetic variation **1**

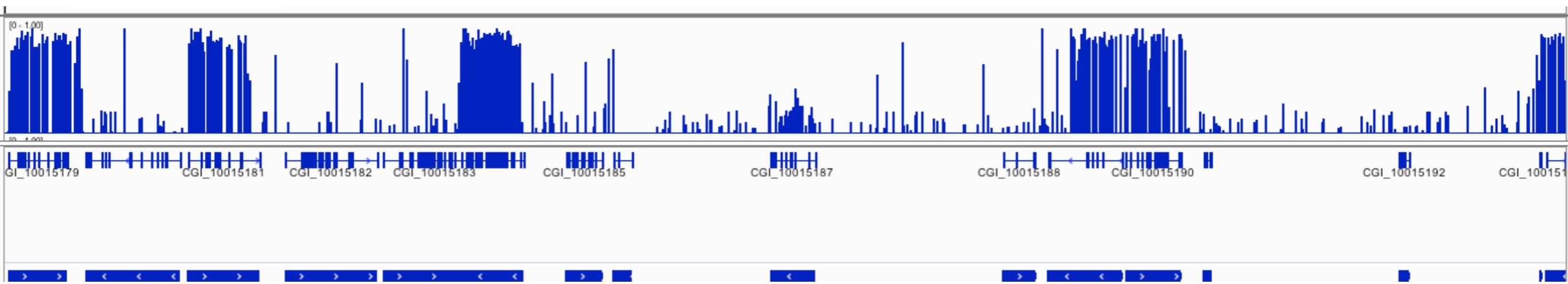
– Bisulfite conversion



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



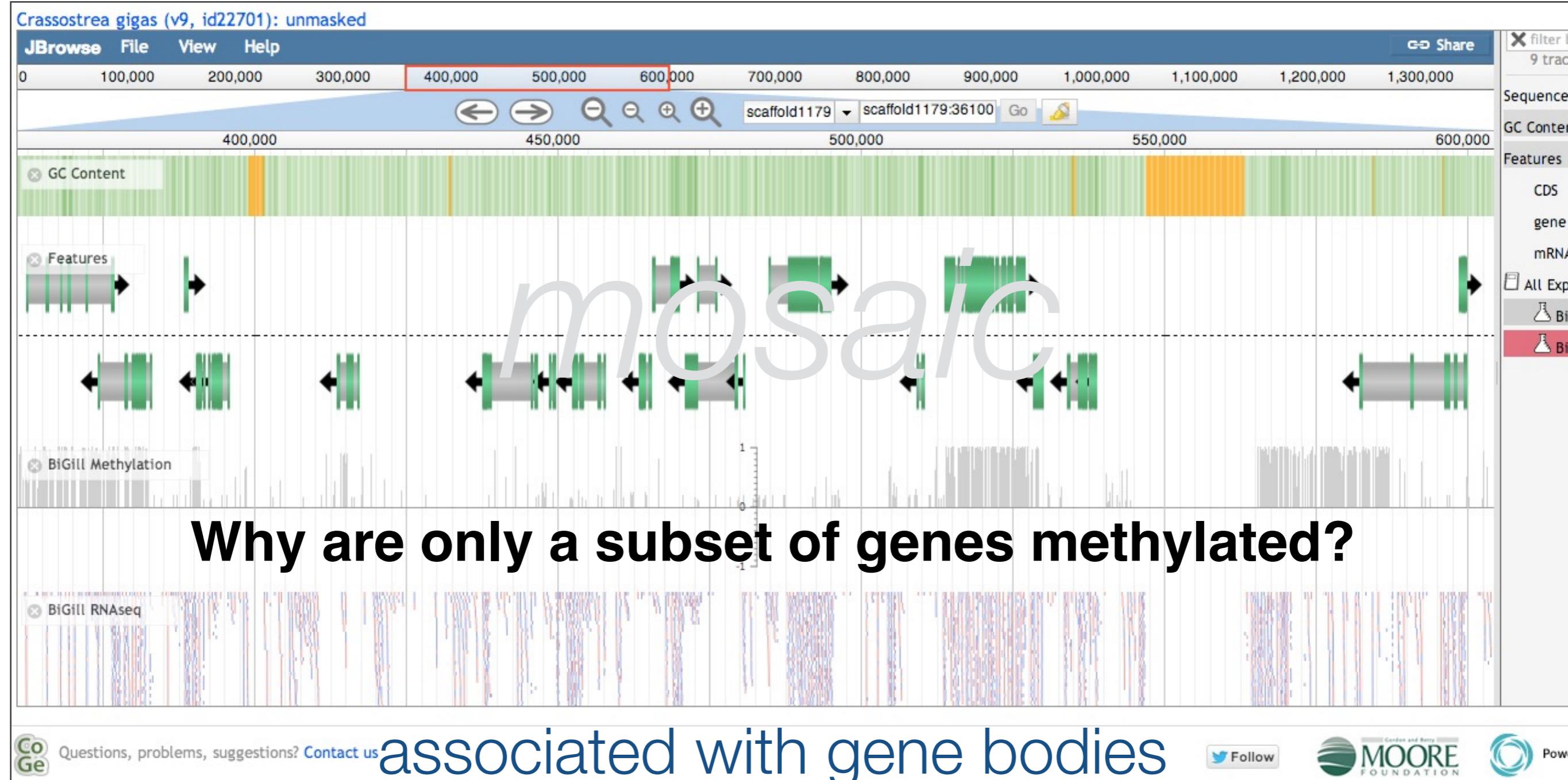
Epigenetic variation **1**



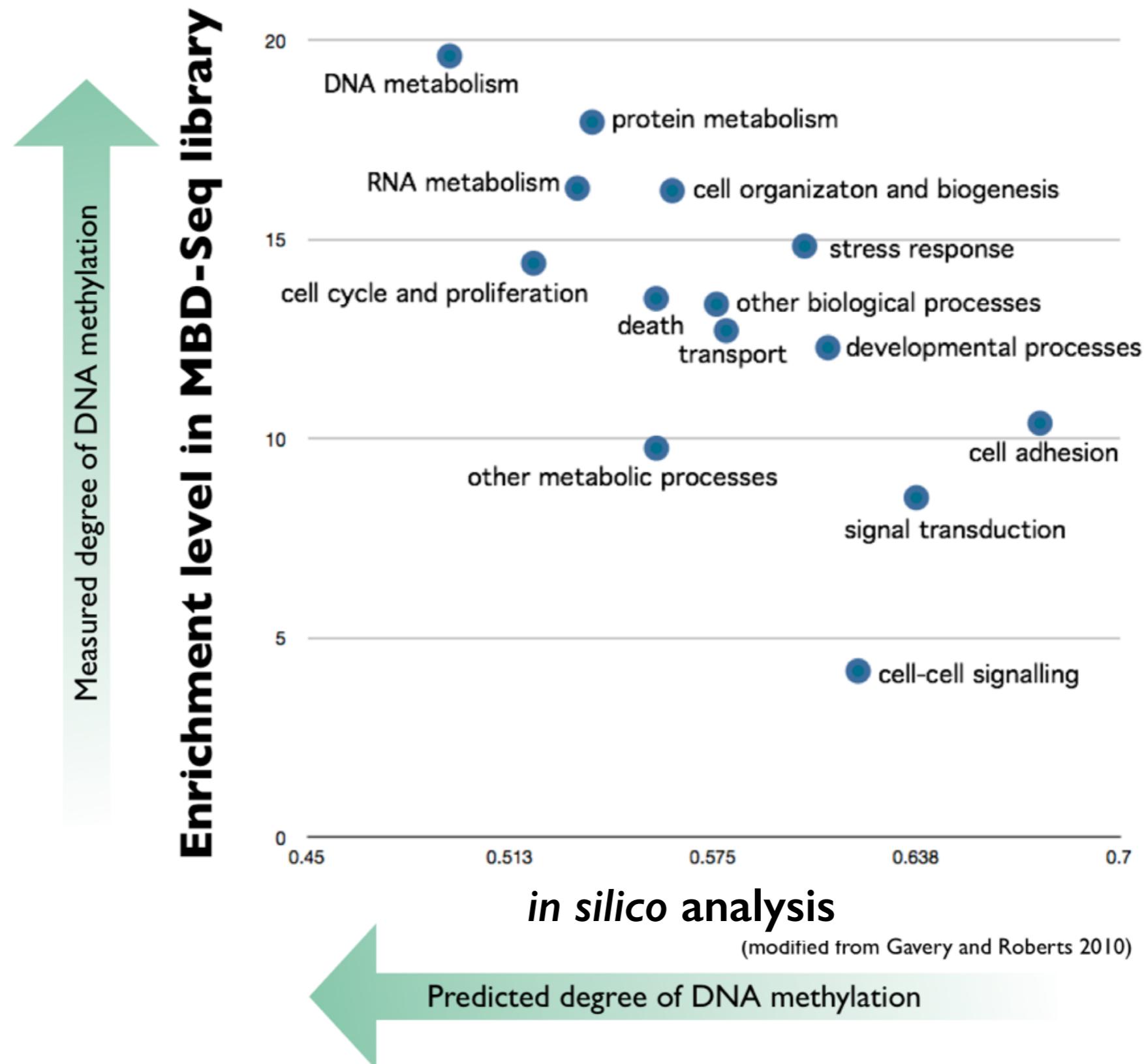
*mosaic*

associated with gene bodies

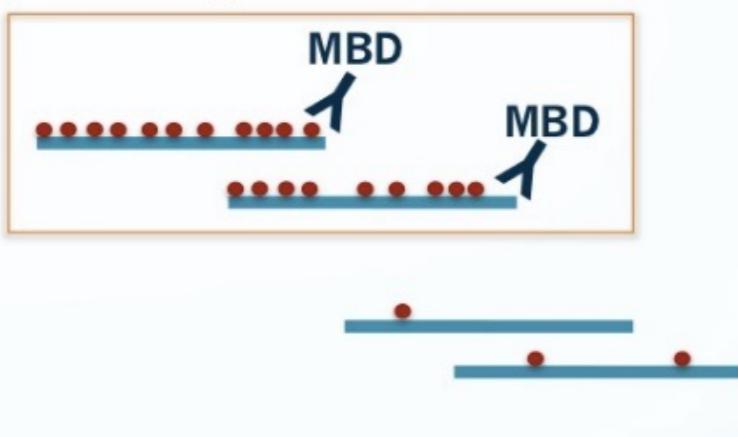




Epigenetic variation **1**

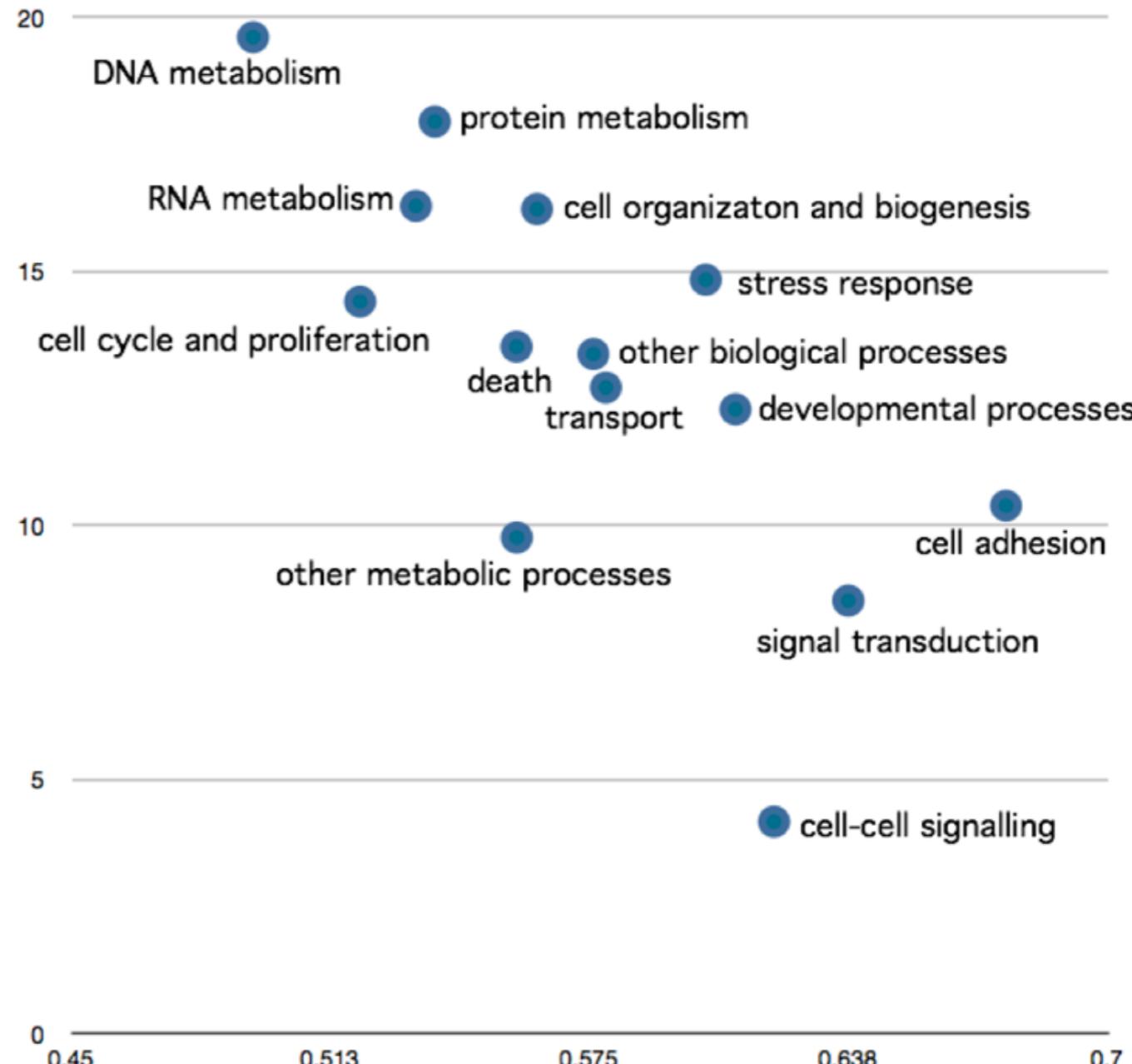


## Epigenetic variation 1



Measured degree of DNA methylation

## Enrichment level in MBD-Seq library



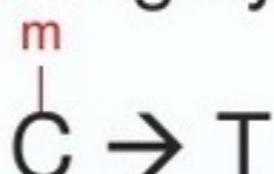
## *in silico* analysis

(modified from Gavery and Roberts 2010)

Predicted degree of DNA methylation

# *in silico* approach

- Principle:
  - Methylated cytosines are highly mutable

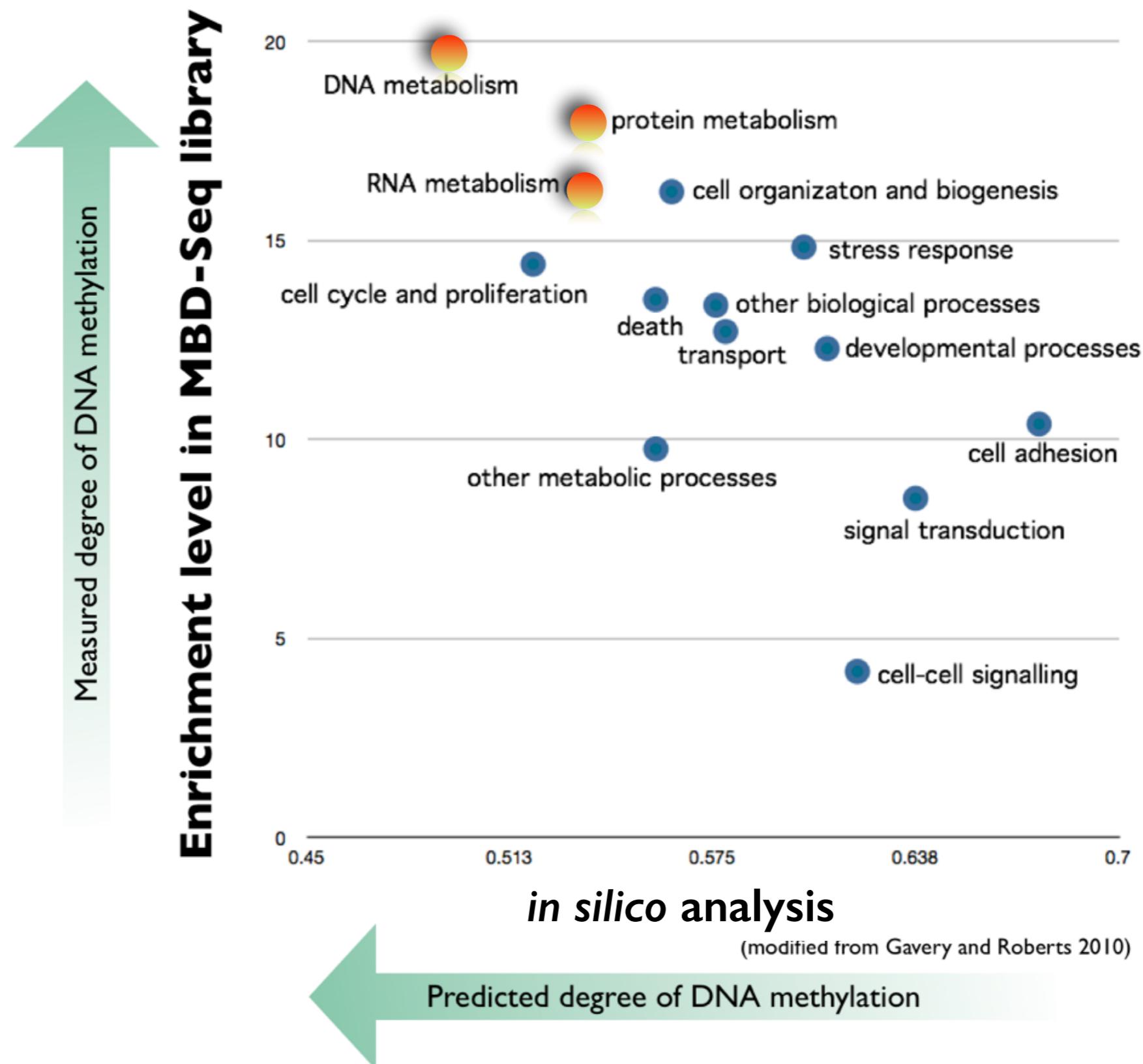


- Methylated regions of DNA are depleted of CpG dinucleotides over evolutionary time (CpG to TpG)

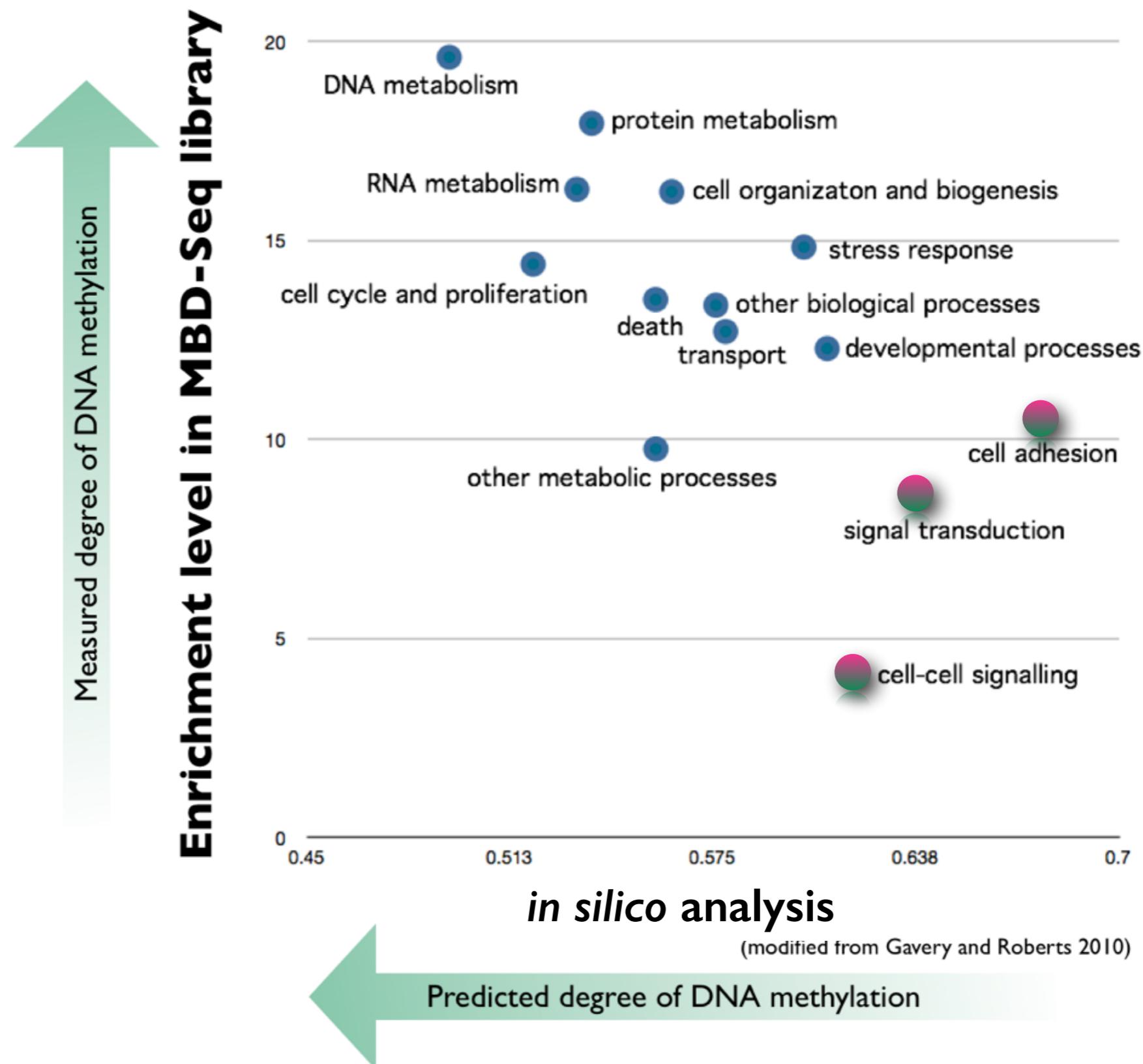
$$\text{CpG O/E} = \frac{\text{CpG observed}}{\text{CpG expected}}$$


high = unmethylated

Epigenetic variation **1**



Epigenetic variation **1**



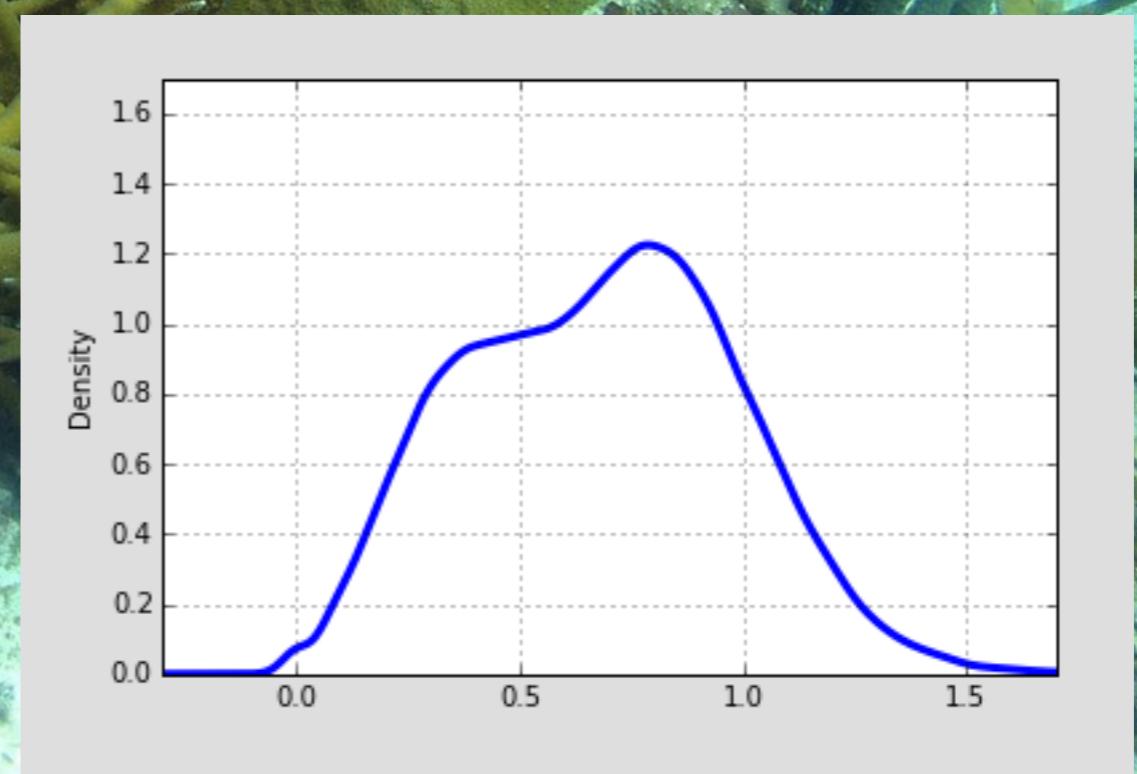
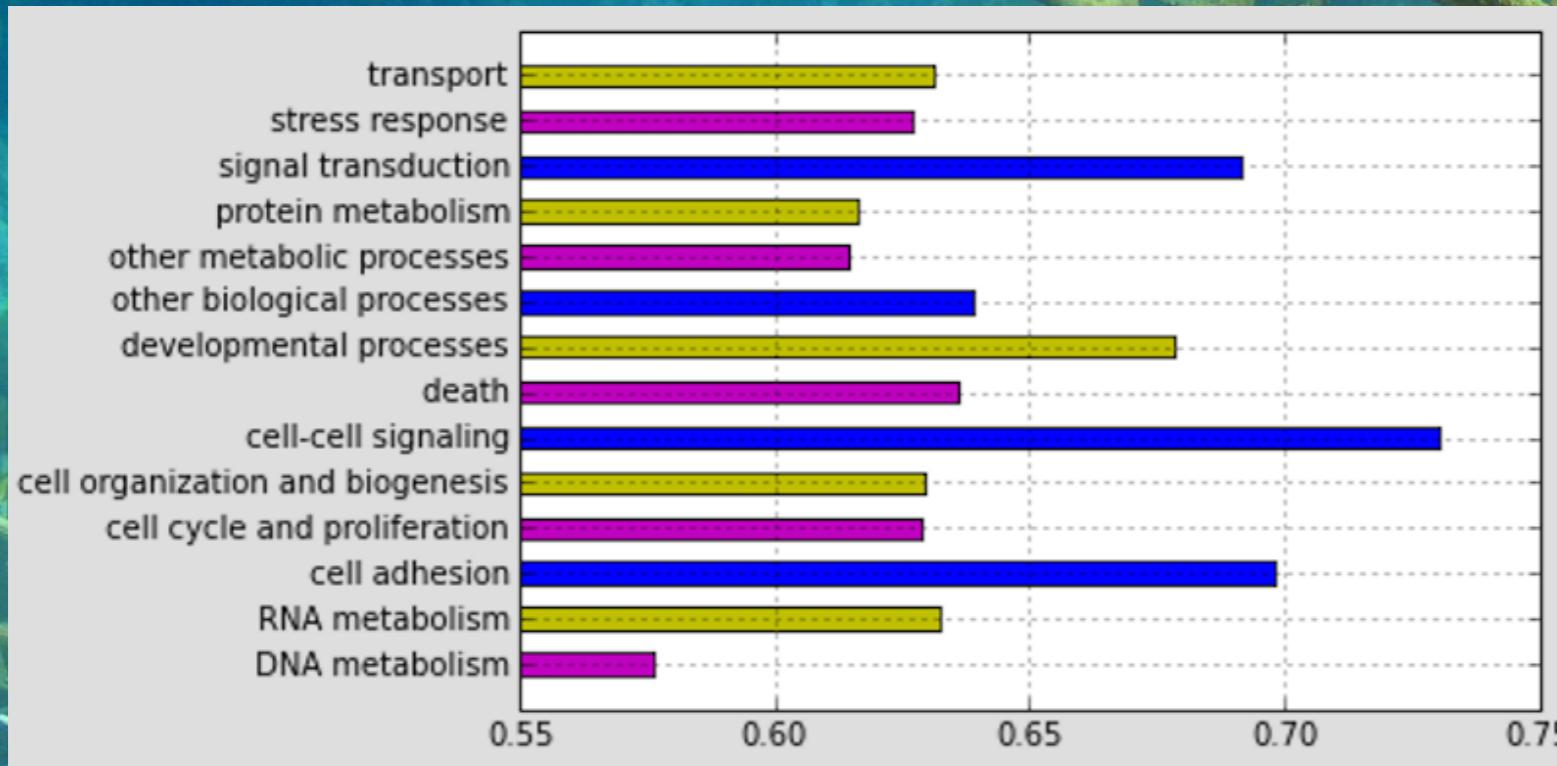
Jay Dimond

*Acropora palmata*

*Acropora cervicornis*

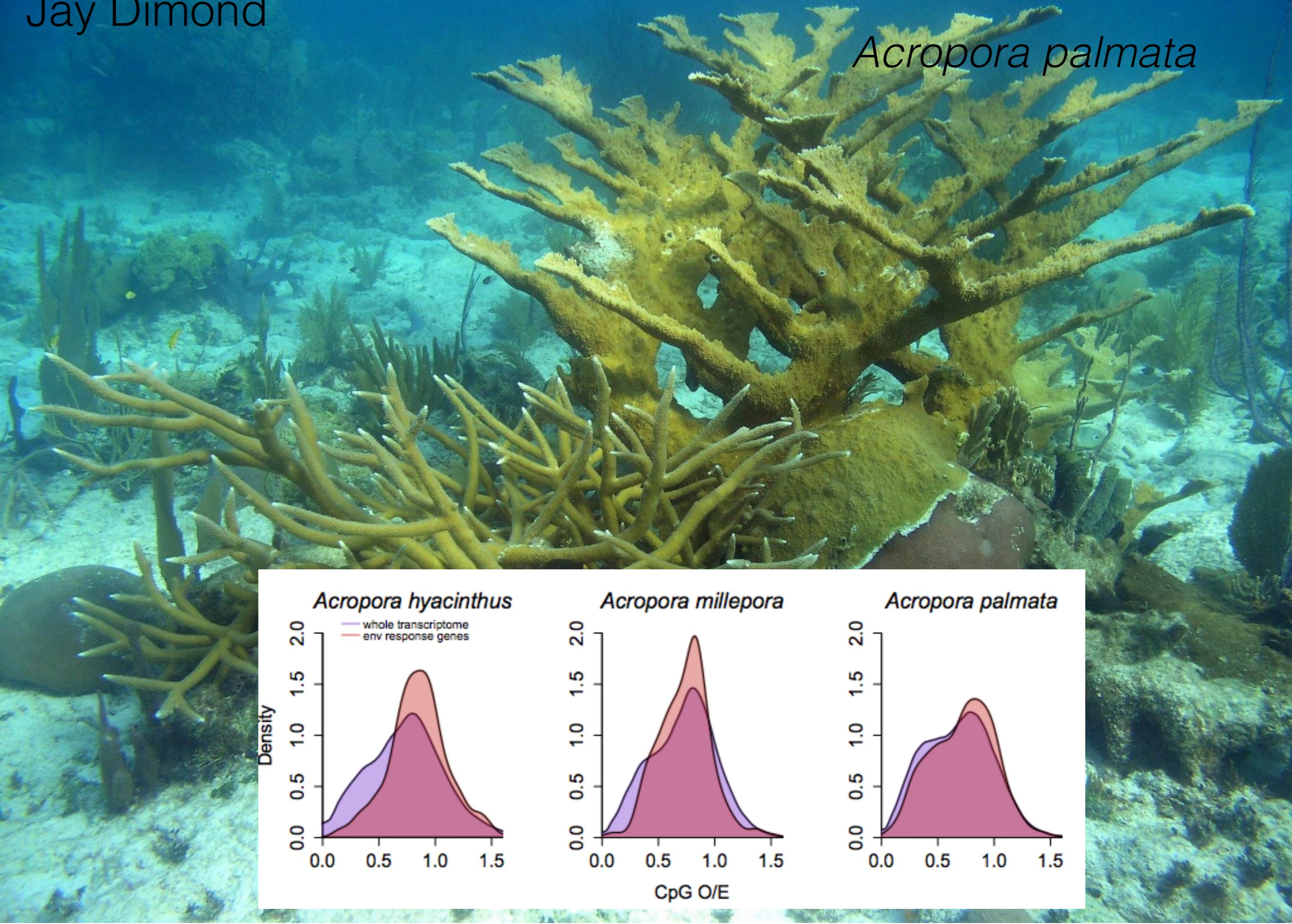
# Jay Dimond

*Acropora palmata*



Jay Dimond

*Acropora palmata*

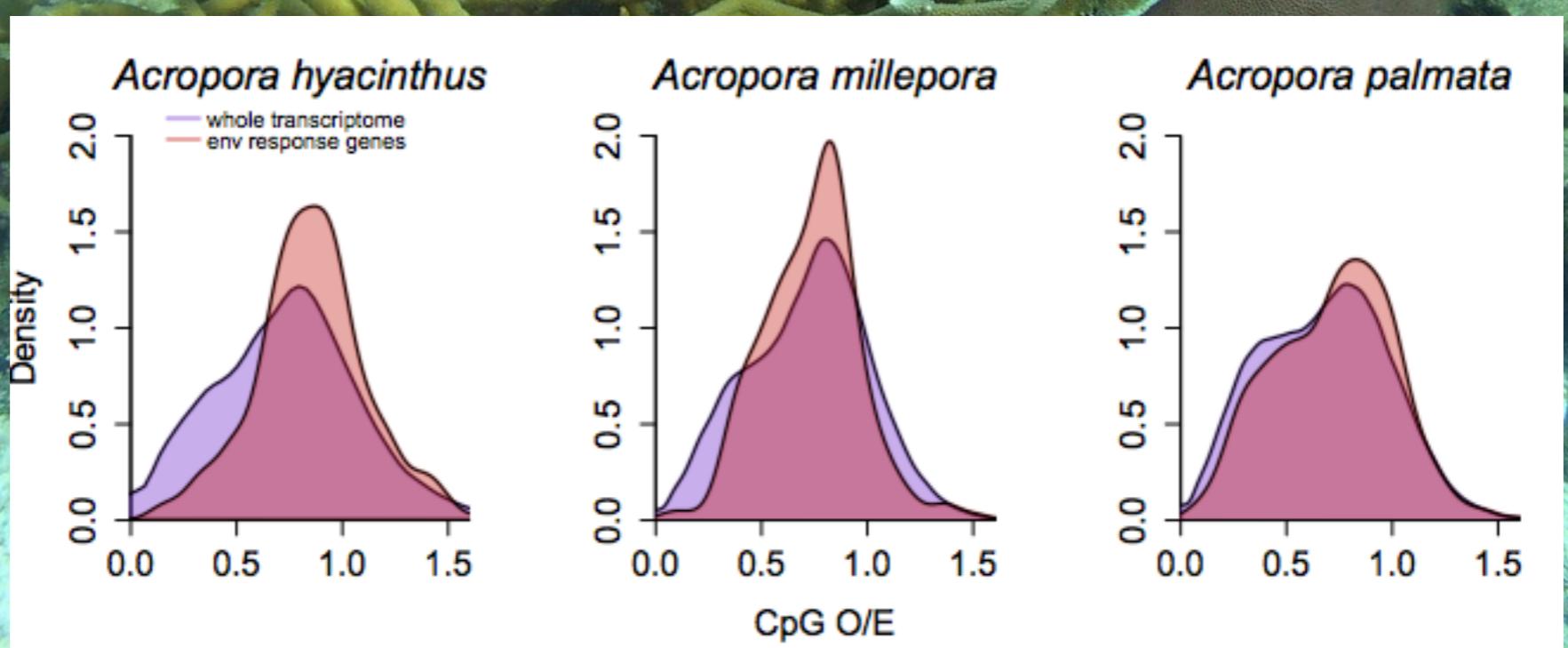


# Jay Dimond

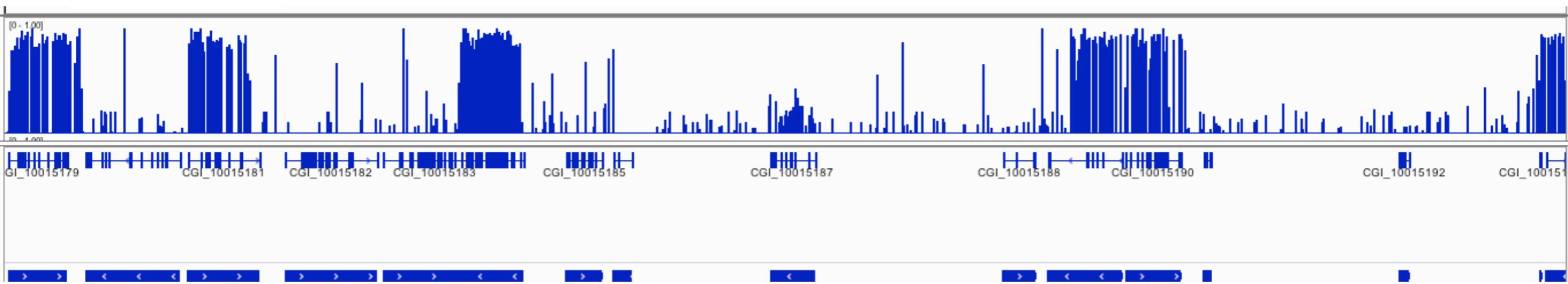


## Germline DNA methylation in five coral transcriptomes

# *Acropora palmata*



Epigenetic variation **1**

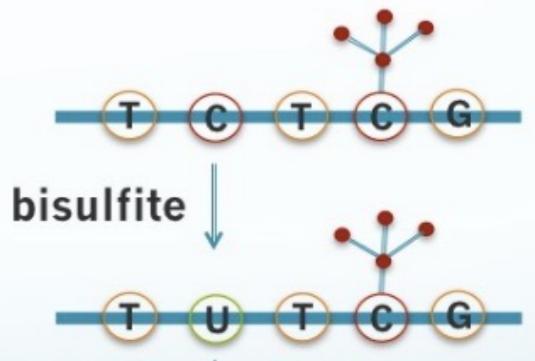


# *mosaic*

associated with gene bodies  
based on gene function

Epigenetic variation **1**

# Family and Developmental Variation



Sperm &  
Larvae  
(72h & 120h)



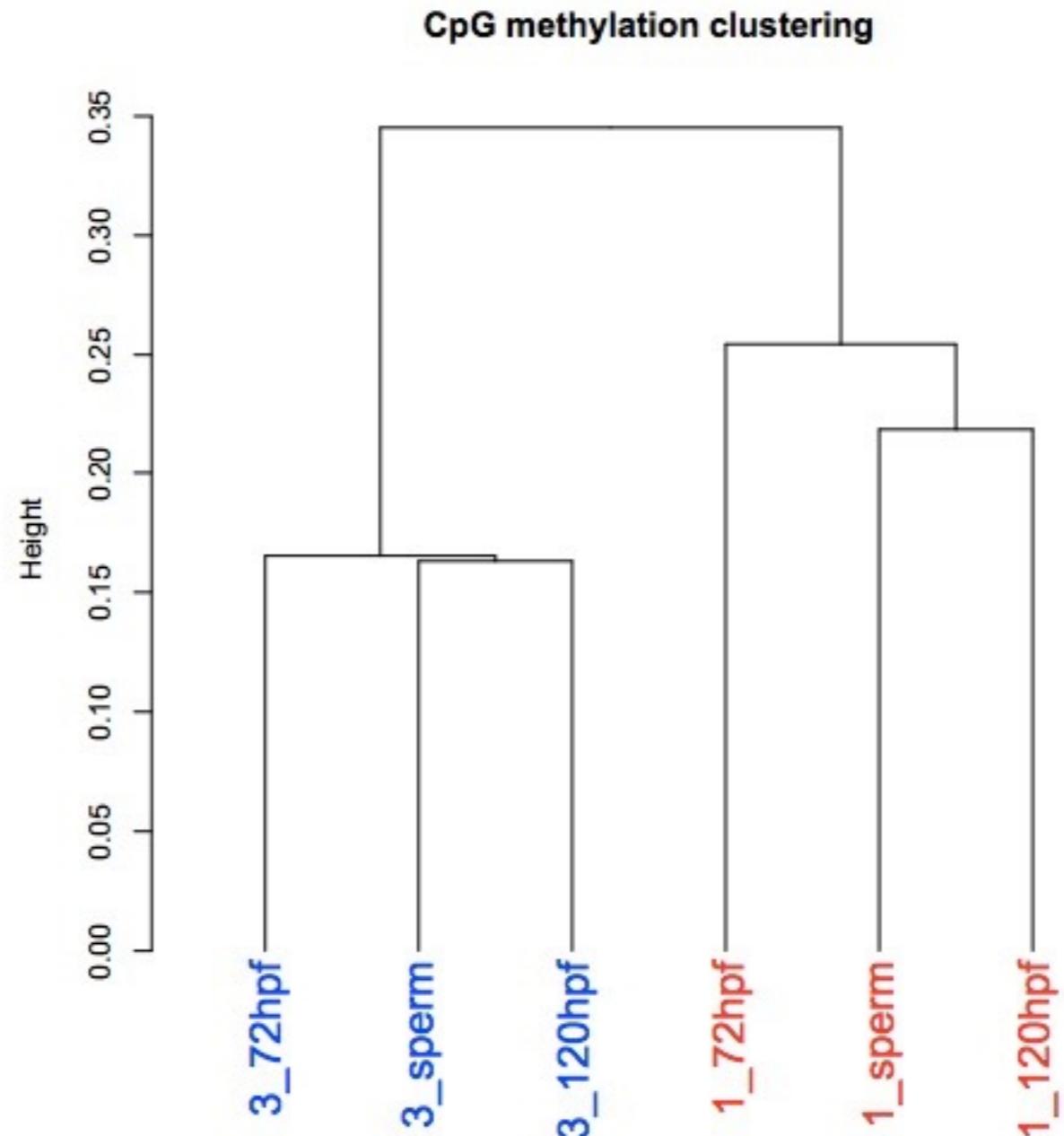
**bioRxiv**  
beta  
THE PREPRINT SERVER FOR BIOLOGY

New Results

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

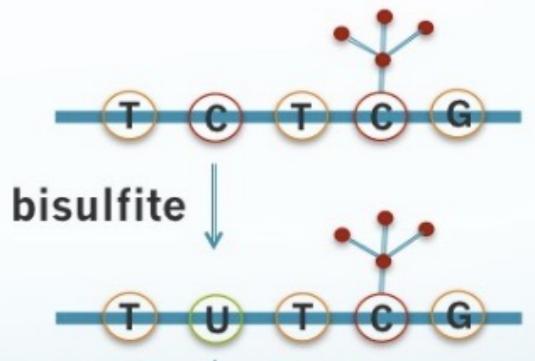
Claire E. Olson , Steven B. Roberts

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



Epigenetic variation **1**

# Family and Developmental Variation



Sperm &  
Larvae  
(72h & 120h)



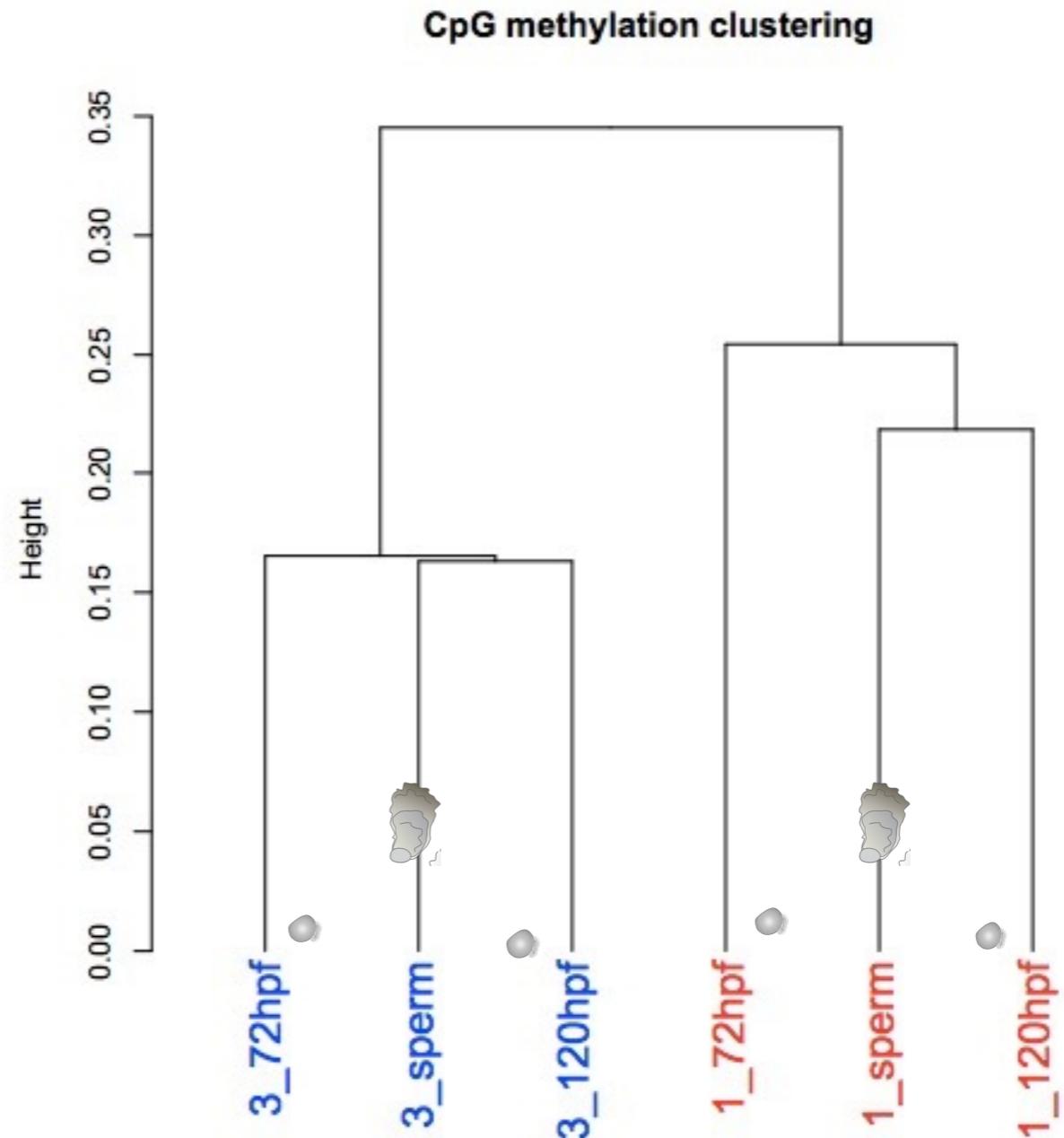
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New Results

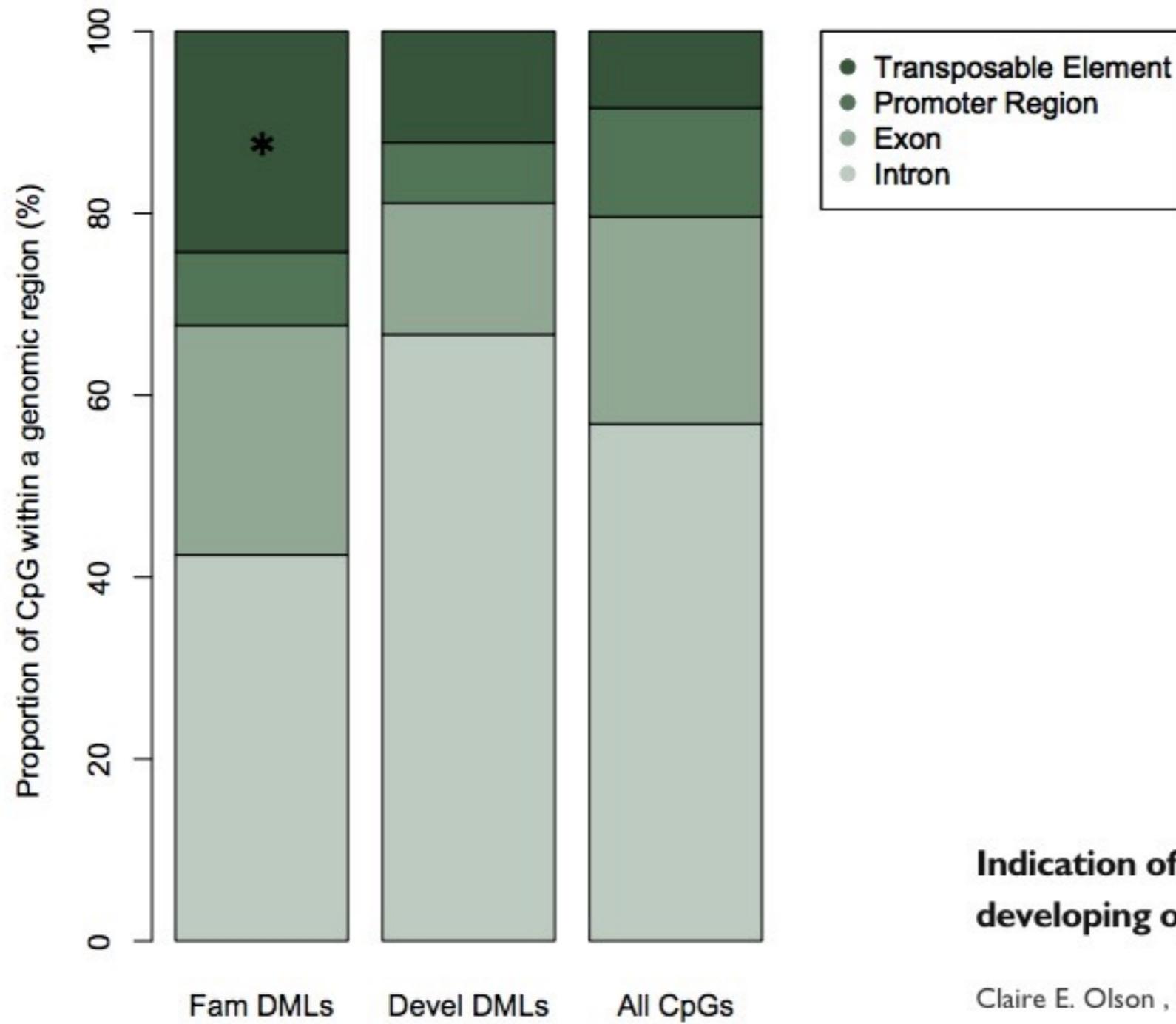
**Indication of family-specific DNA methylation patterns in developing oysters**

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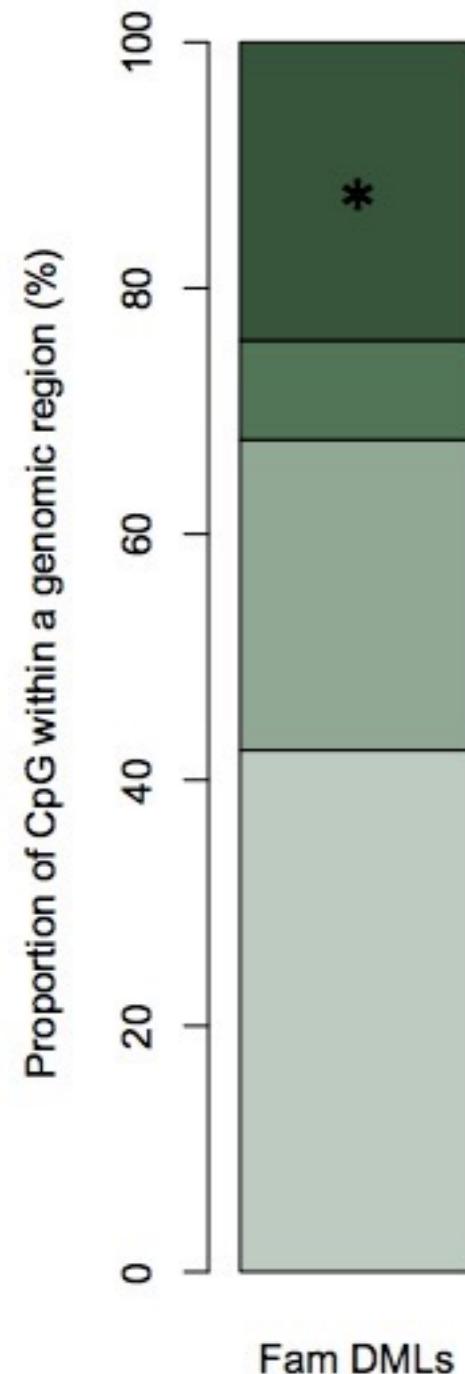
Epigenetic variation **1**



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

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Epigenetic variation **1**



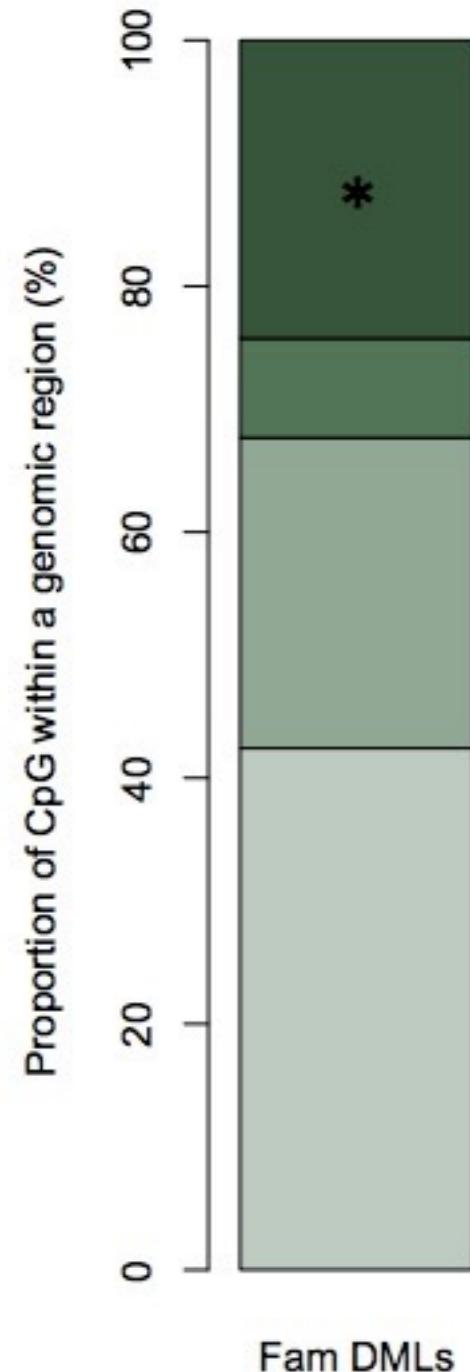
Differentially methylated loci in gene bodies could be lethal or deleterious, as they would alter gene expression.

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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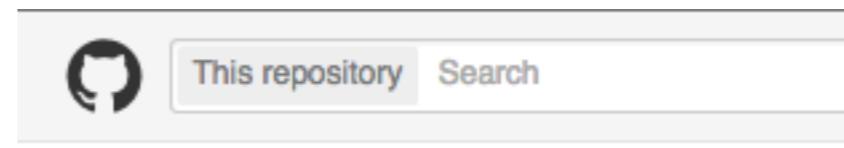
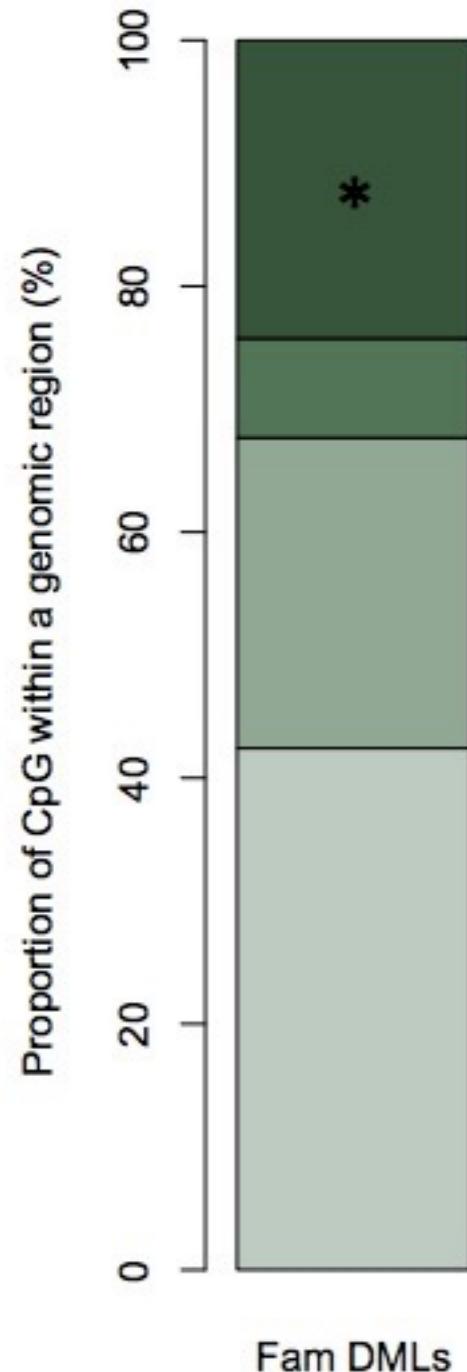


***Assumes some form  
of random process***  
occurring during  
gametogenesis?

**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 %) methylated genome
- Limited variation between cell type
- Gene body methylation correlated with function
- Evidence indicates DMRs are predominant in transposable elements

Gene  
expression



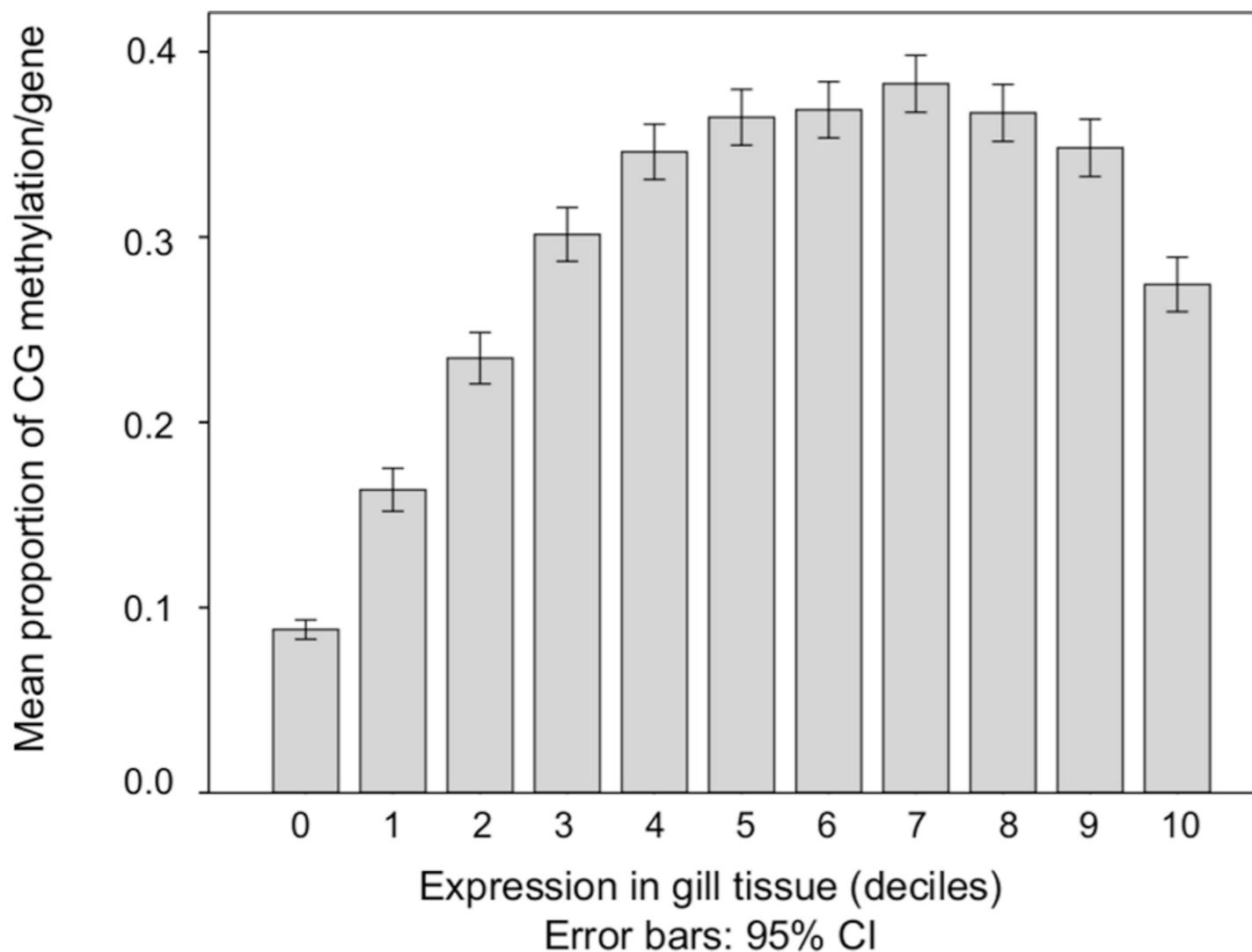
Epigenetic  
variation

# Function?

Gene expression

2

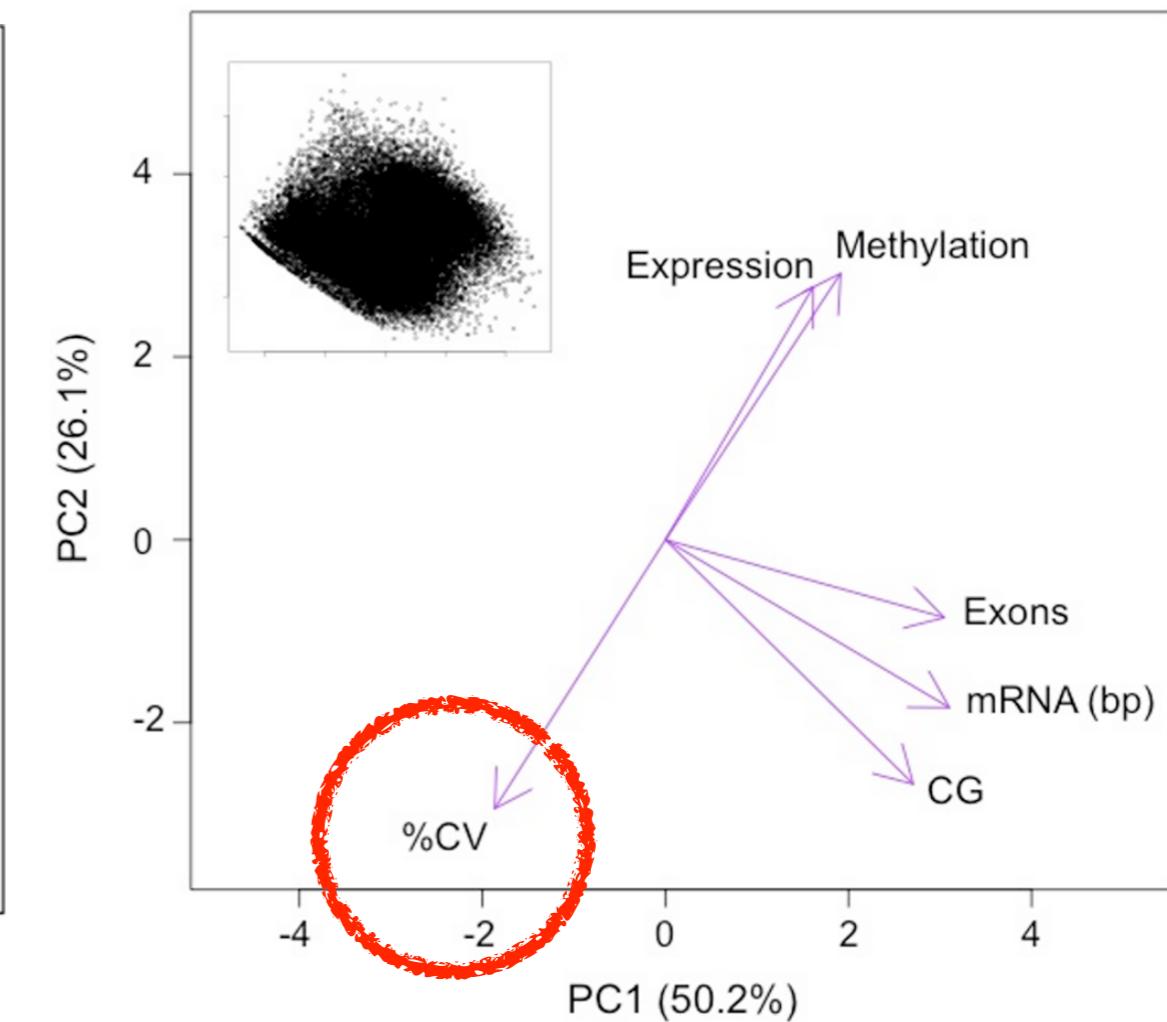
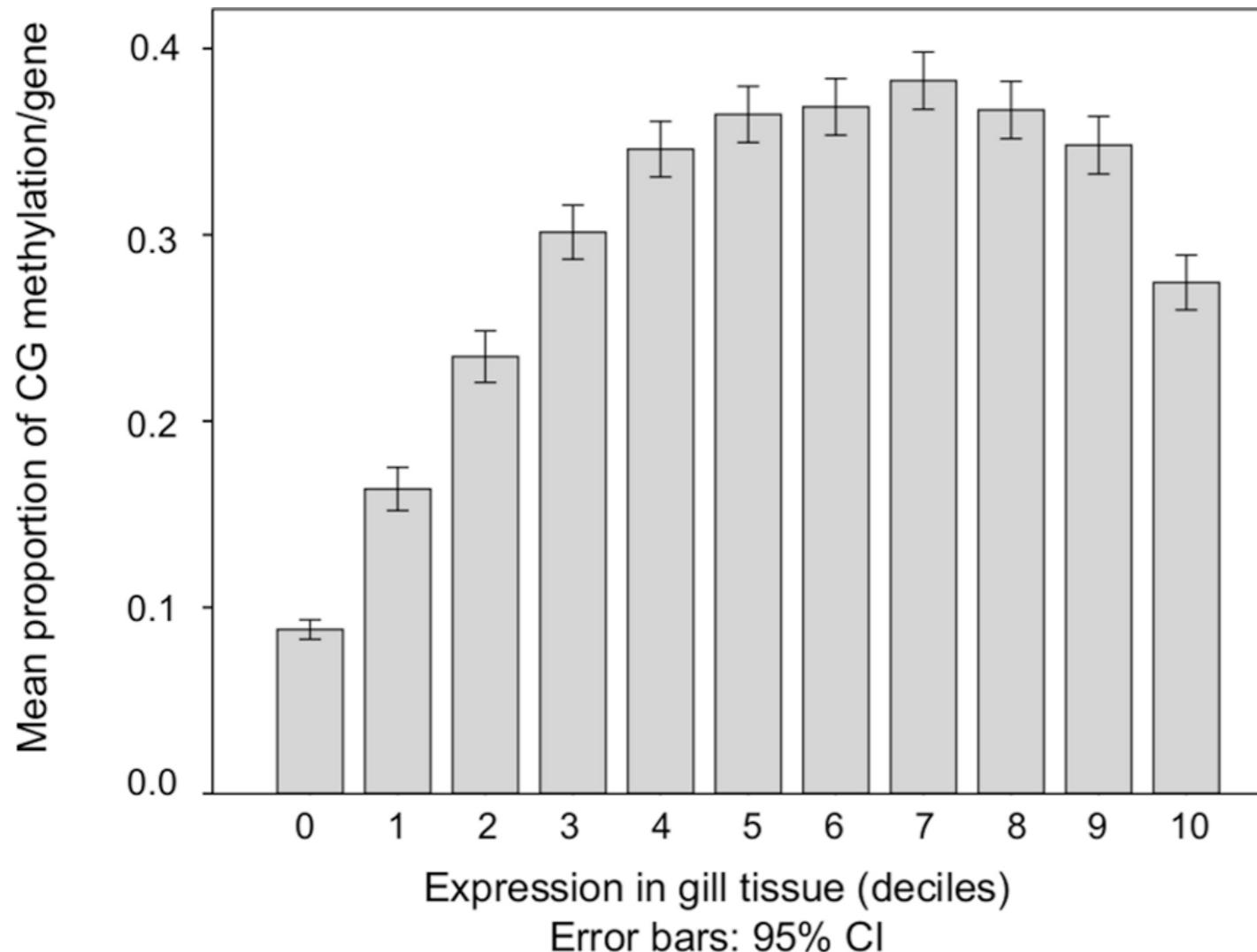
Epigenetic variation



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

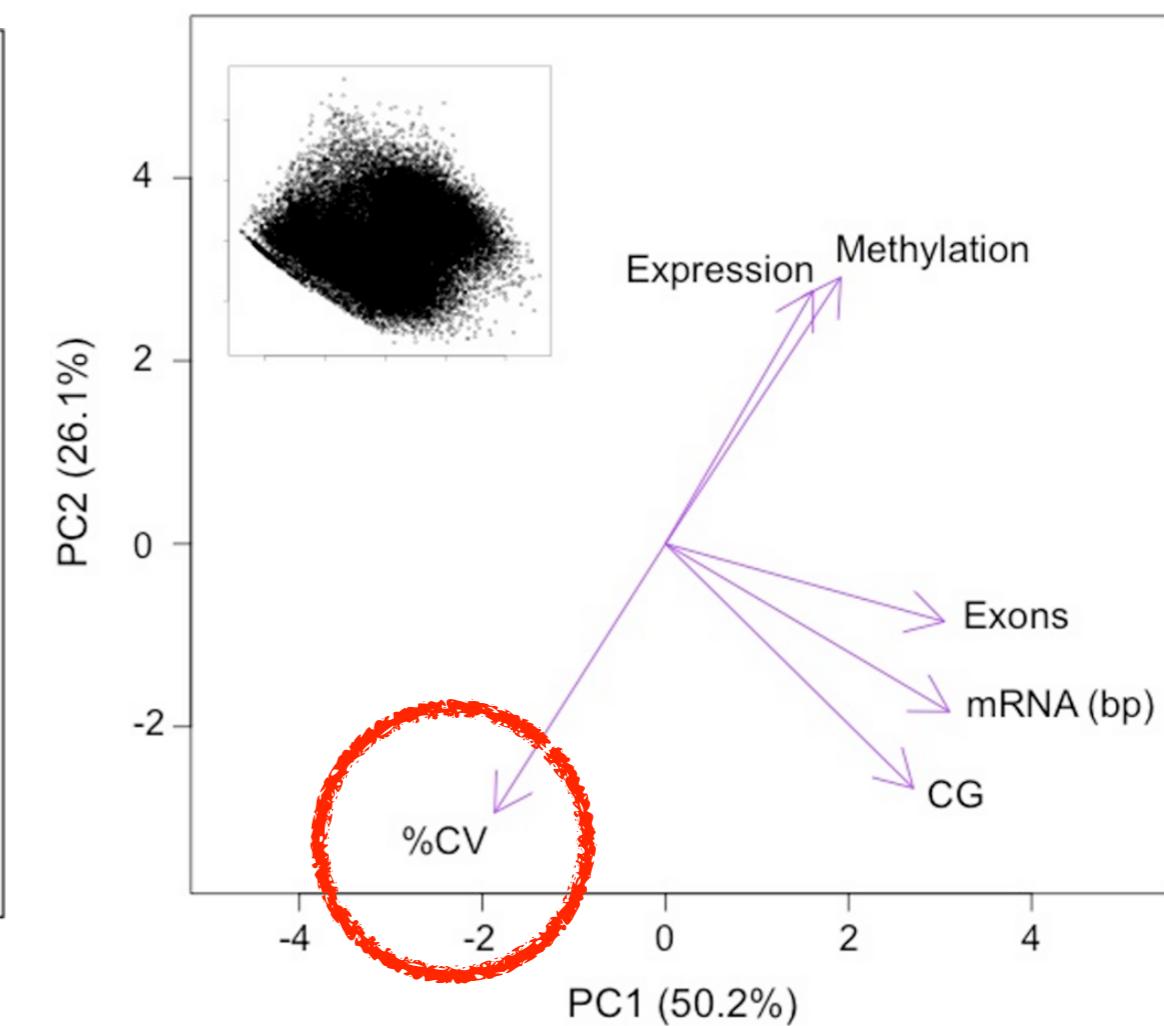
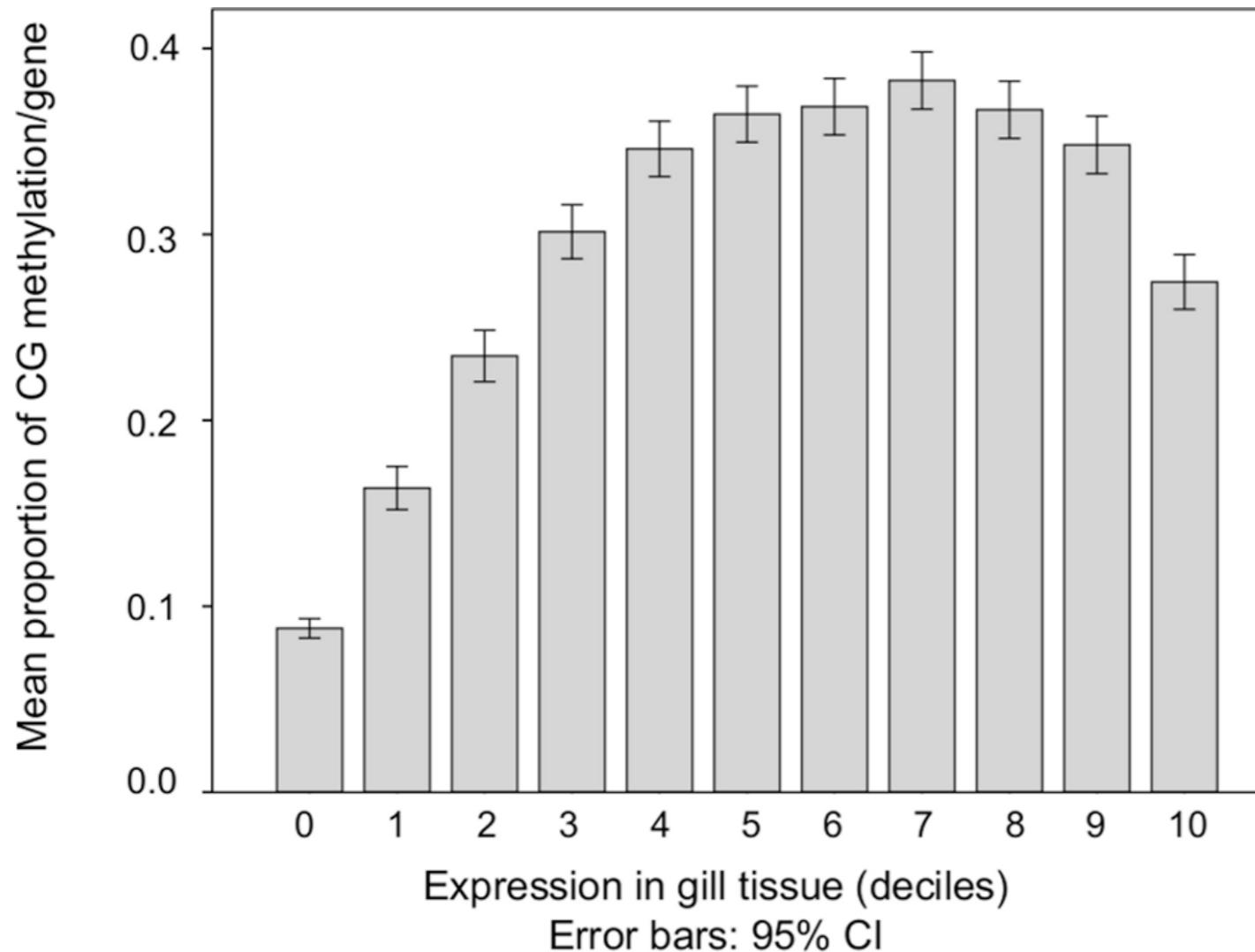
School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA, USA

Gene expression

2

Epigenetic variation

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



What?

## A context dependent role for DNA methylation in bivalves

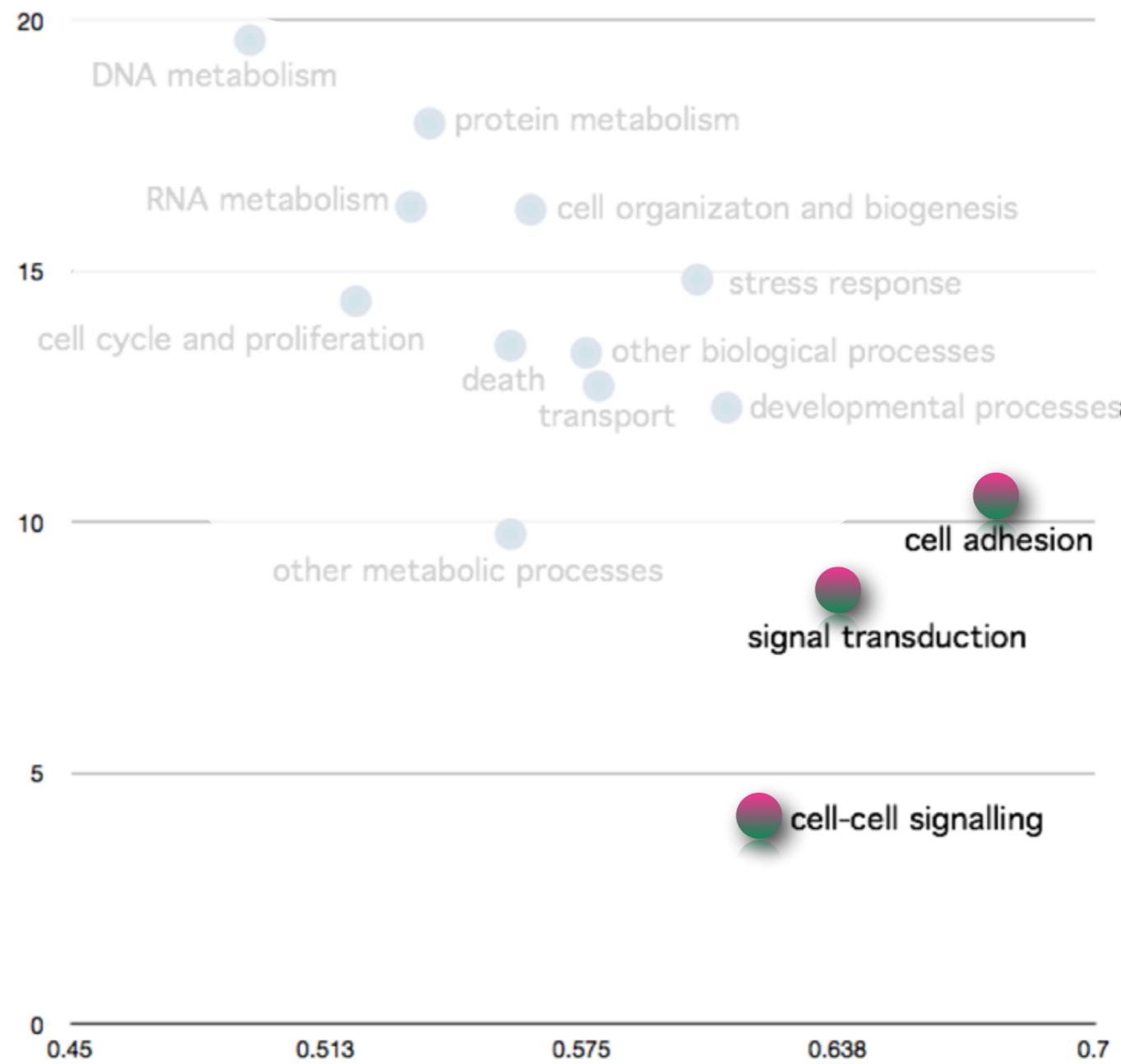
Mackenzie R. Gavery and Steven B. Roberts

Advance Access publication date 7 January 2014

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



Roberts and Gavery 2012

Gene expression

2

Epigenetic variation

cell adhesion

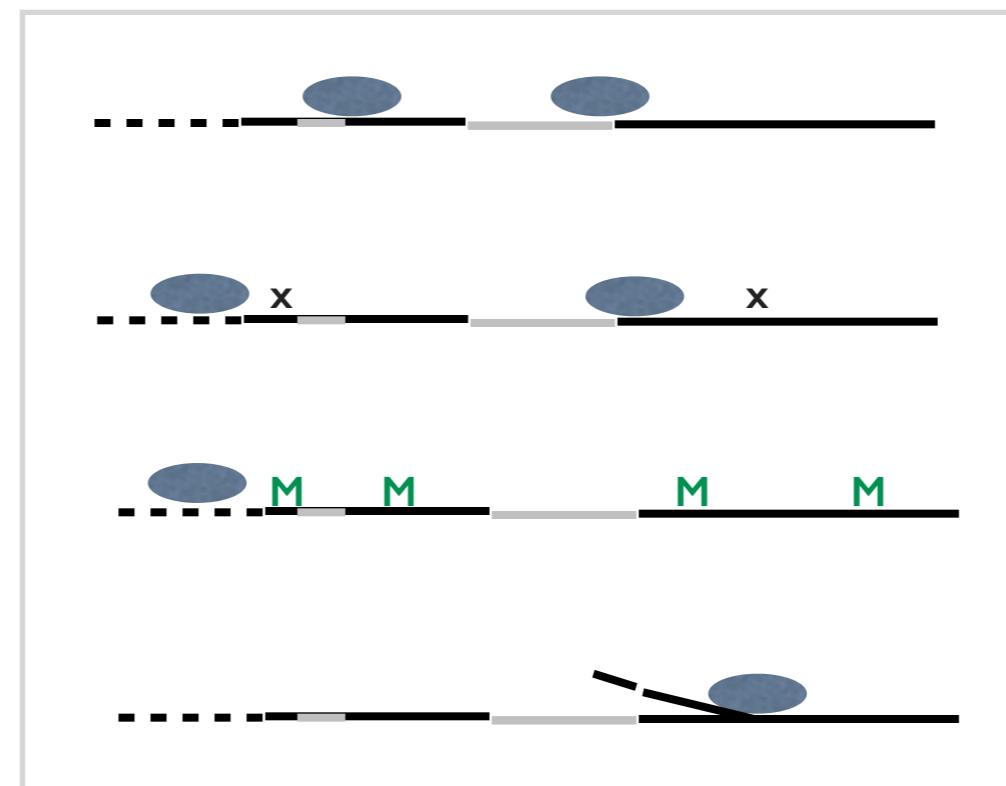
signal transduction

cell-cell signalling

**sparse methylation**

*tissue / temporal specific and inducible genes*

## Transcriptional opportunities



alternative start sites

sequence mutation  
change AA, premature stop codon

conventional transcription  
*transient methylation*

alternate transcript  
exon skipping

Gene expression



Epigenetic variation

DNA metabolism

protein metabolism

RNA metabolism



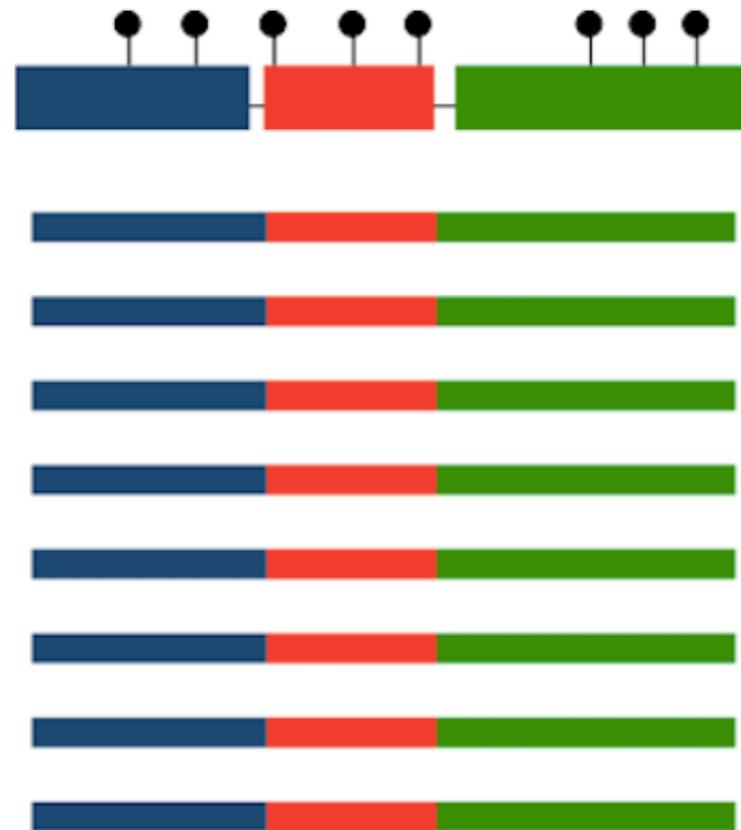
germline methylation

*ubiquitously expressed,  
critical genes*

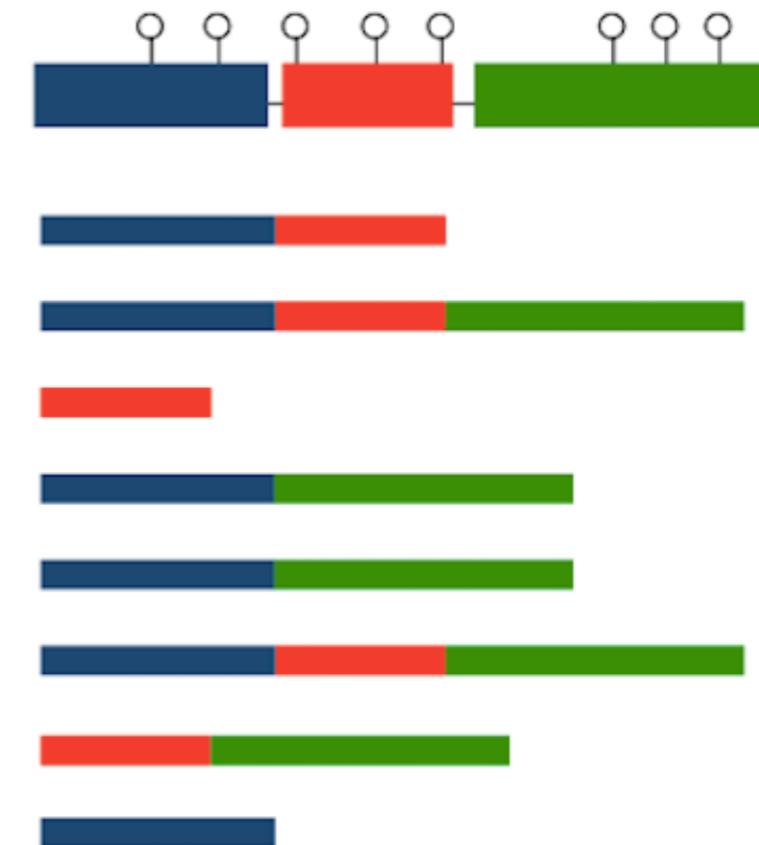
## Transcriptional opportunities



conventional transcription  
5' UTR promotor



housekeeping



response to  
change

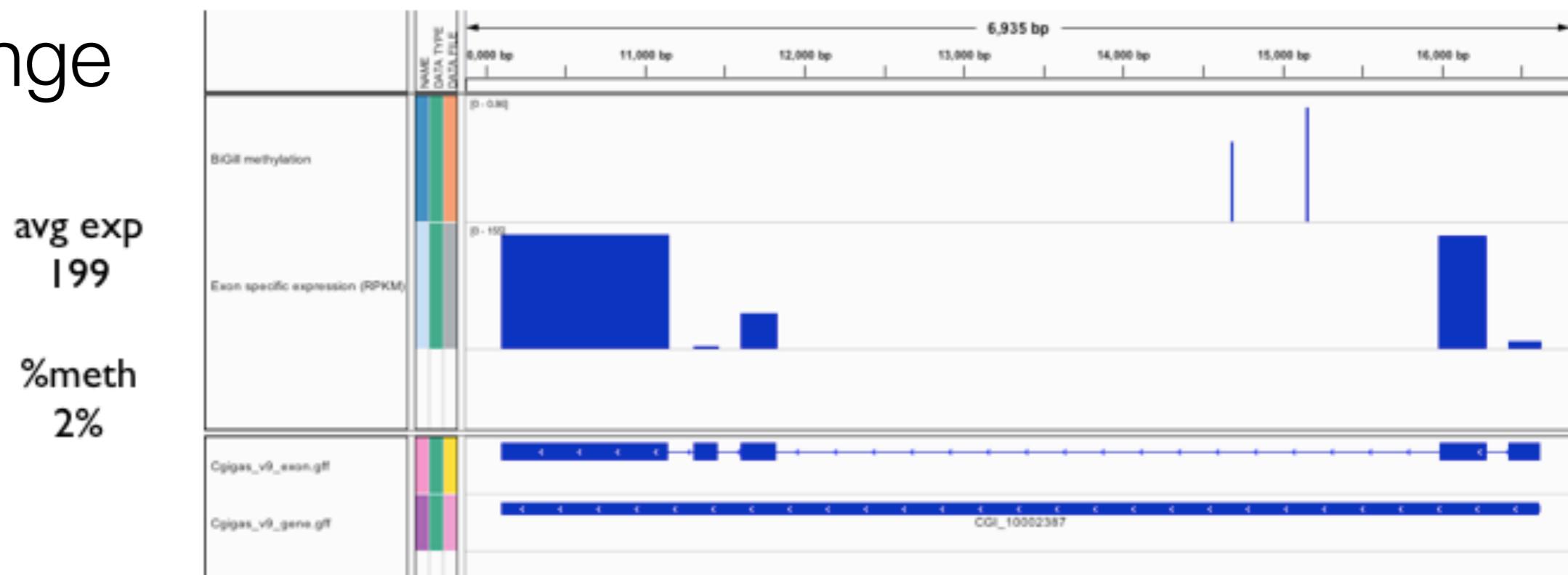
# A context dependent role for DNA methylation in bivalves

Mackenzie R. Gavery and Steven B. Roberts

Advance Access publication date 7 January 2014

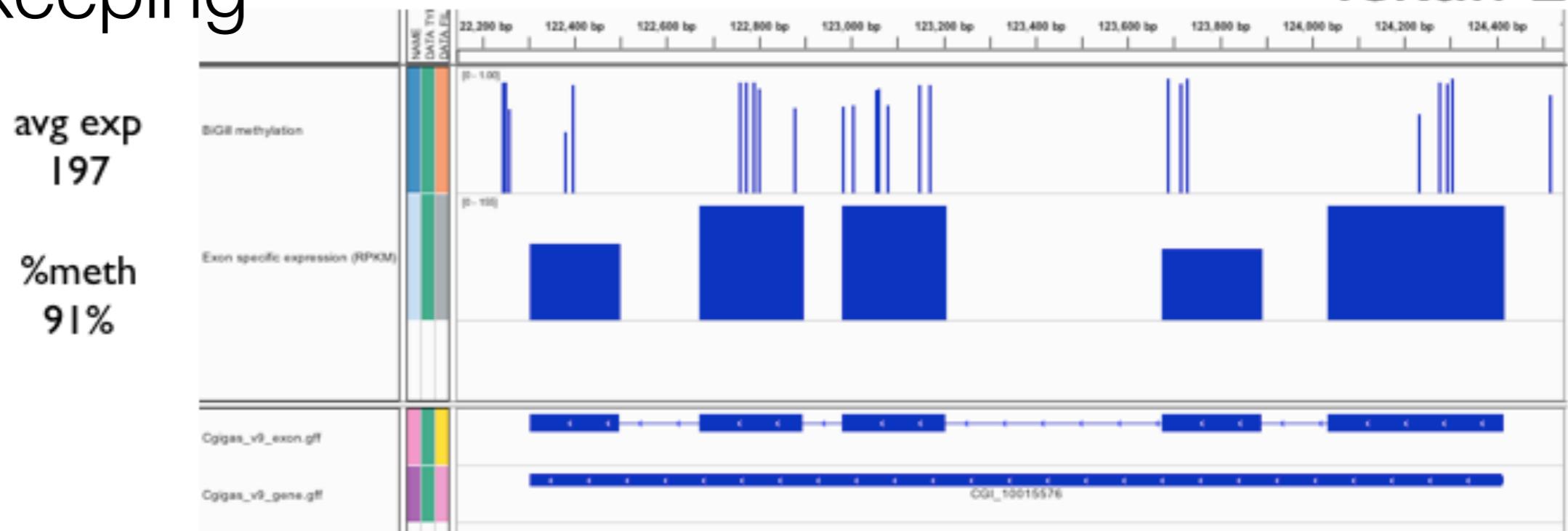
response to  
change

# Heat shock 70 kDa protein 12A



housekeeping

# Tektin-2



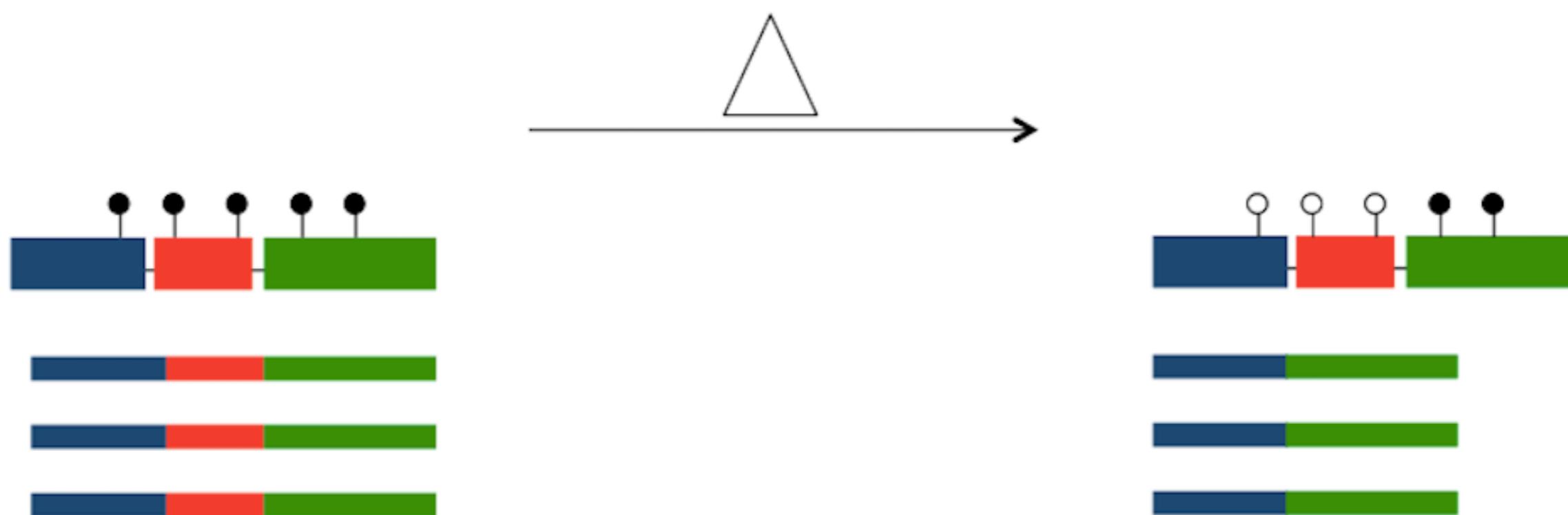


# Targeted Regulation

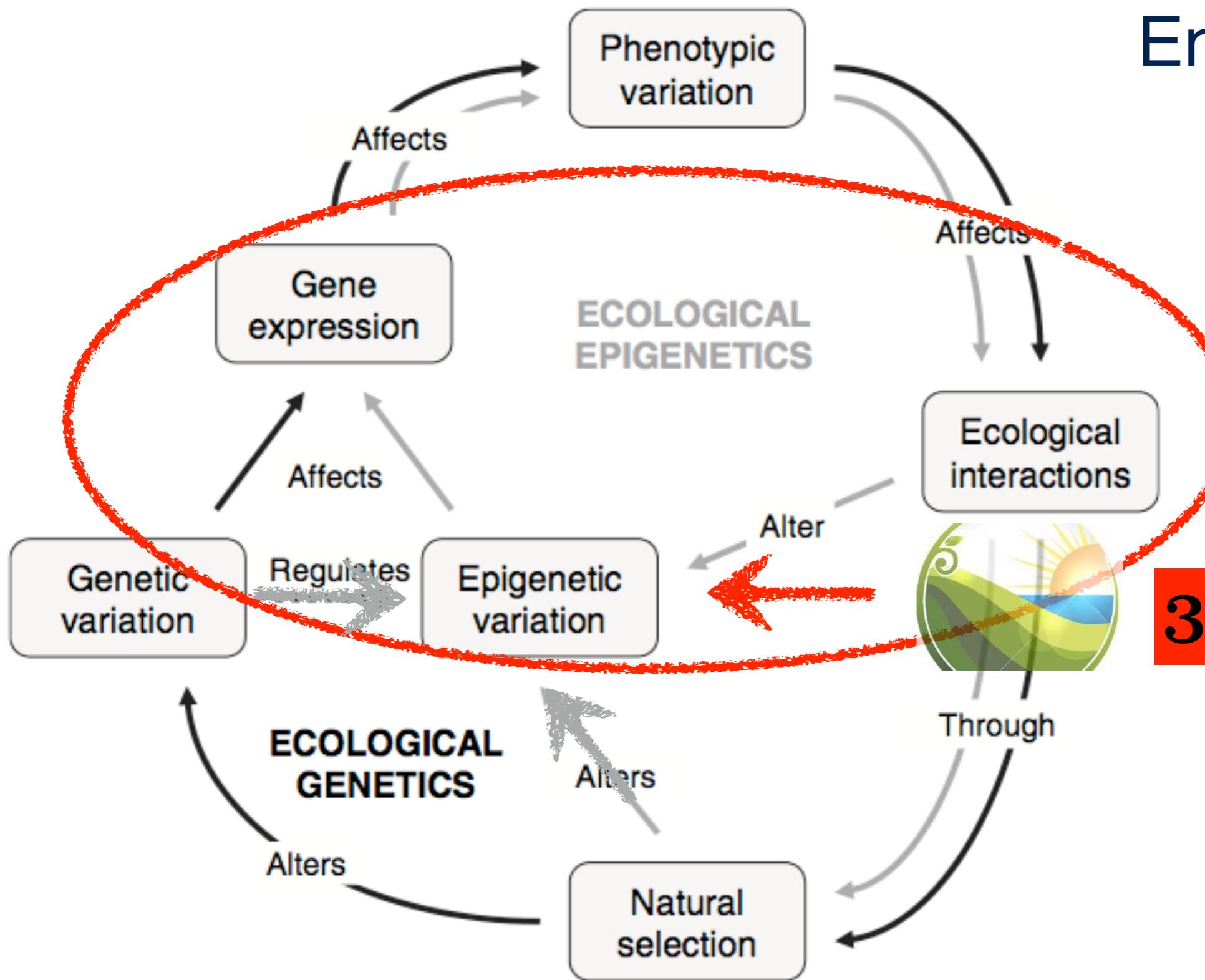
## A context dependent role for DNA methylation in bivalves

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# Environmental Influence



*Ecology Letters*, (2008) 11: 106–115

doi: 10.1111/j.1461-0248.2007.01130.x

modified from

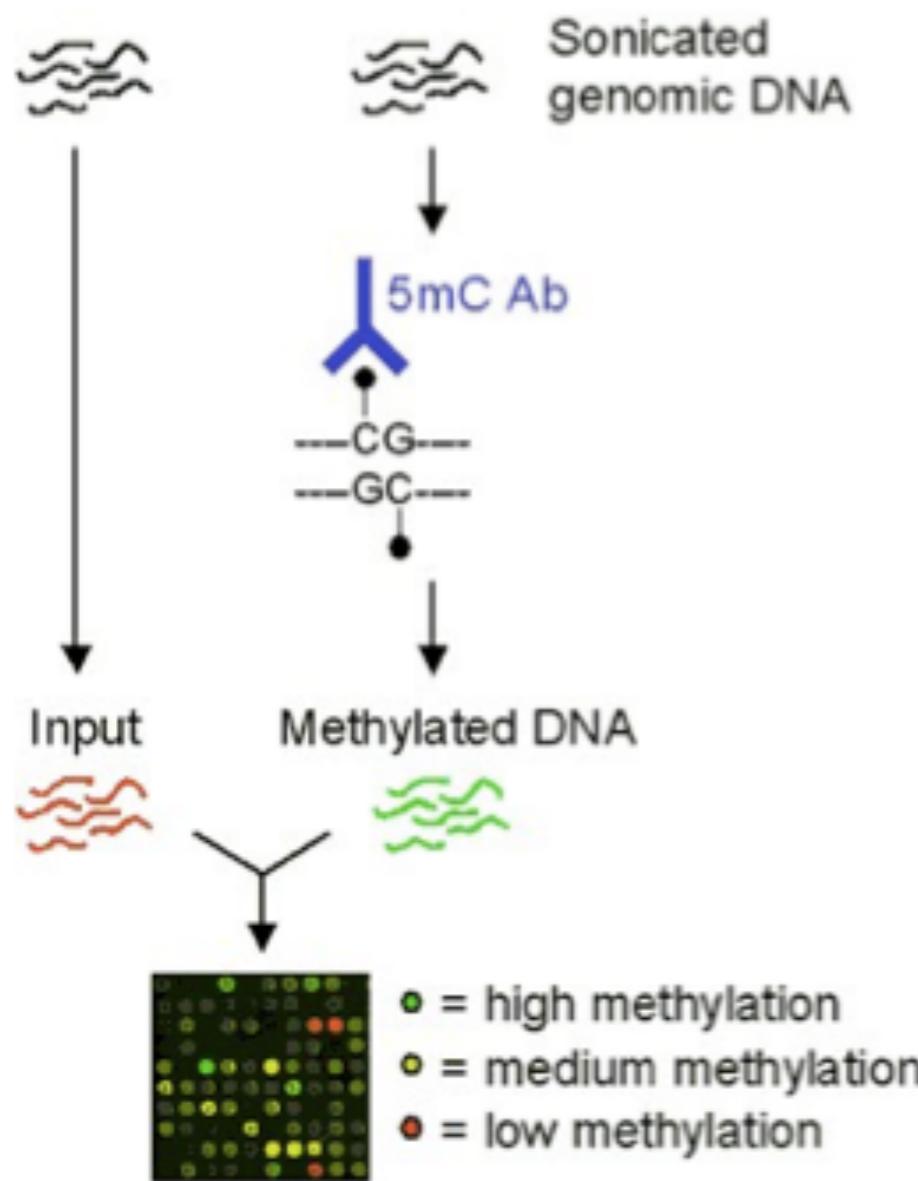
IDEA AND  
PERSPECTIVE

Epigenetics for ecologists

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

# Very new data

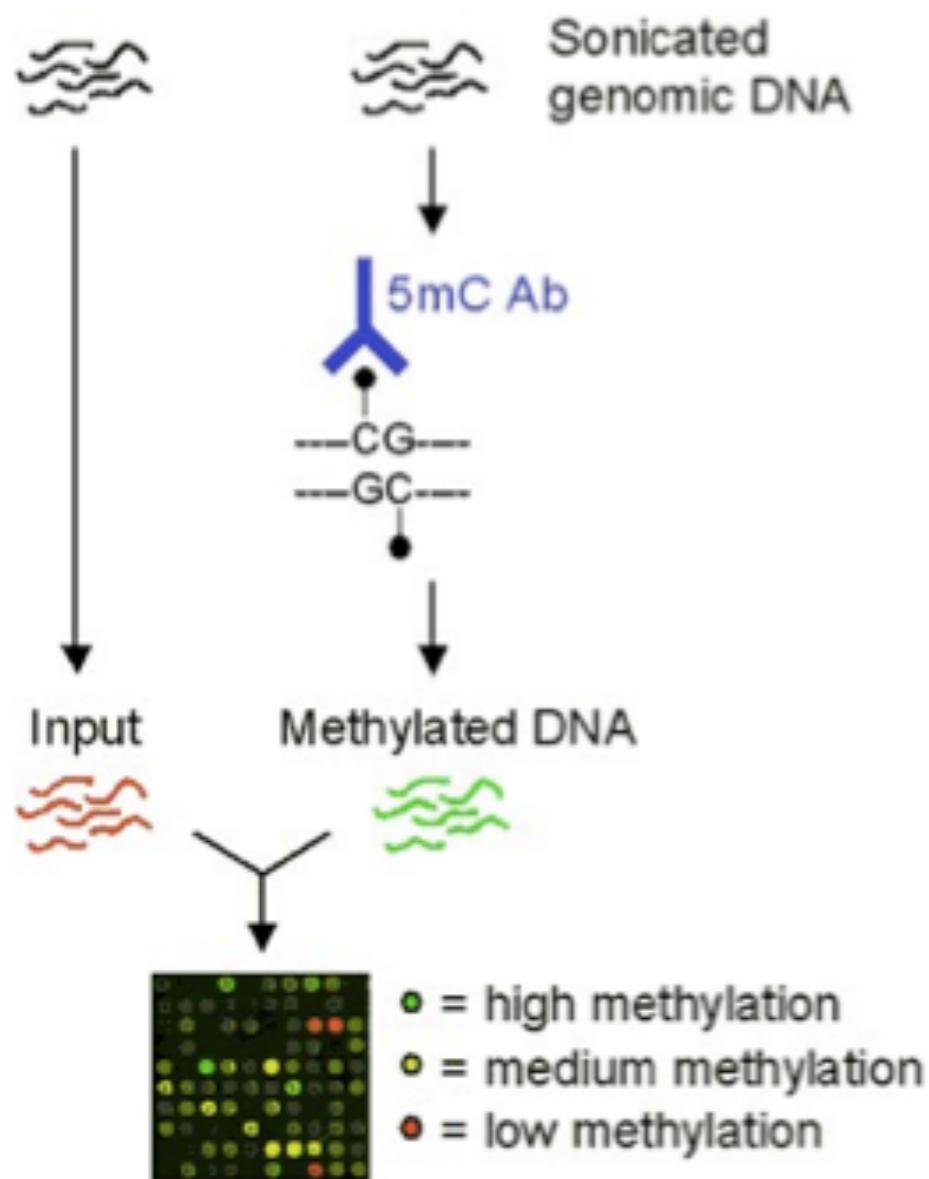
## Environmental impact (Estrogens)



- 96 regions that were hypermethylated in EE2
  - 90 are in genes
  - 52 of these cross exon/intron boundary another
  - 32 are in introns
  - 6 are just in exons
- 287 regions that were hypomethylated in EE2
  - 256 are in genes
  - 138 cross exon/intron boundary
  - 114 are in introns only and
  - 4 are just in exons

# Very new data

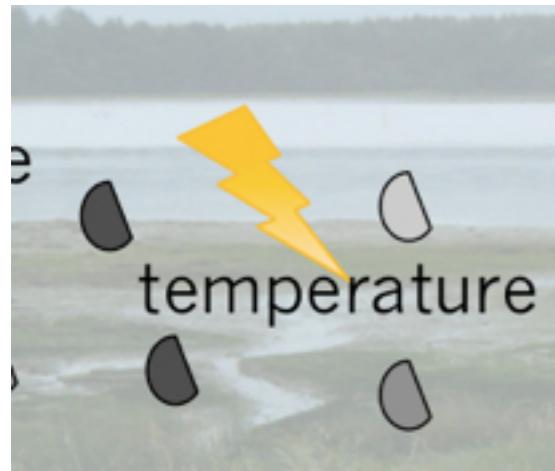
## Environmental impact (Estrogens)



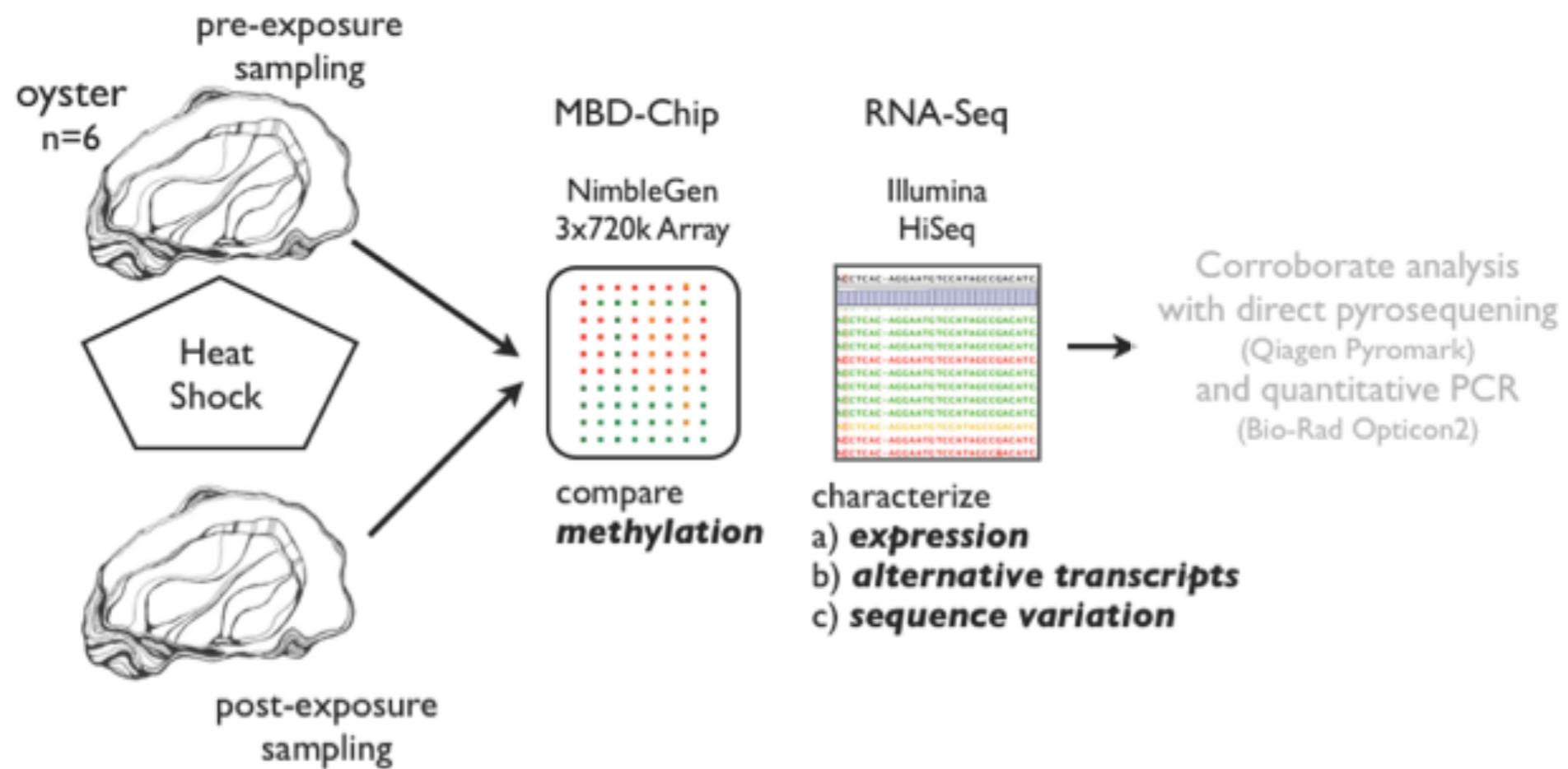
Exposure results in significant hypomethylation

# Very new data

## Environment and gene expression

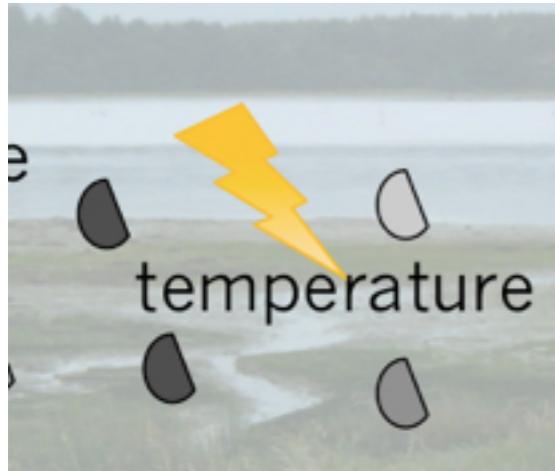


*stochastic or targeted?*



# Very new data

## Environment and gene expression

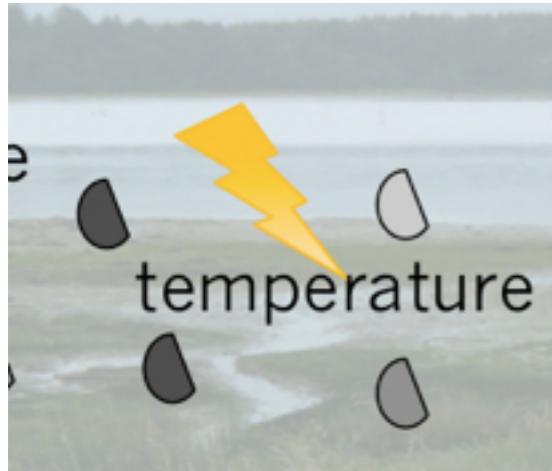


*stochastic or targeted?*

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

# Very new data

## Environment and gene expression



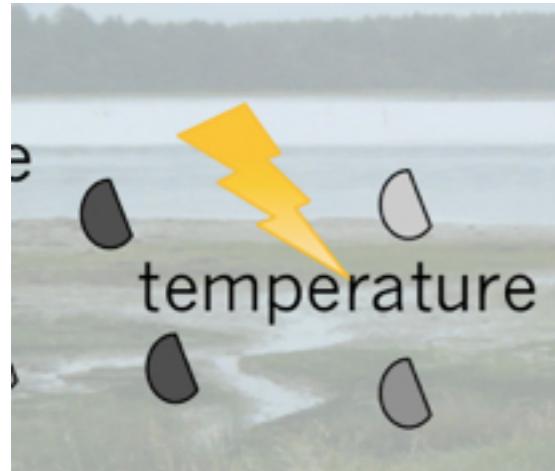
*stochastic or targeted?*

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

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

# Very new data

## Environment and gene expression

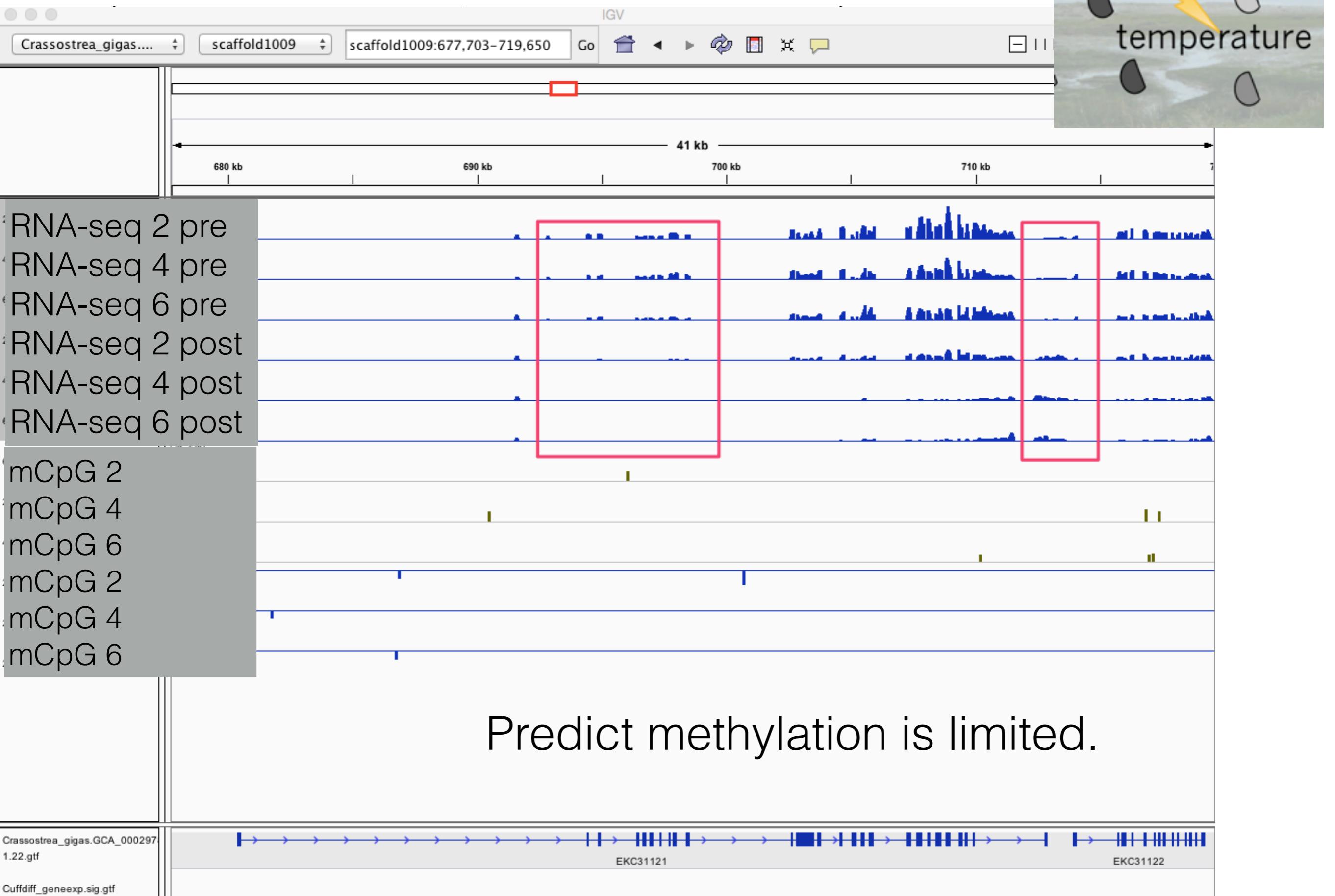


*stochastic or targeted  
or ..?*

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

Changes in methylation (either direction) are more prevalent in introns, repeats, and transposable elements.

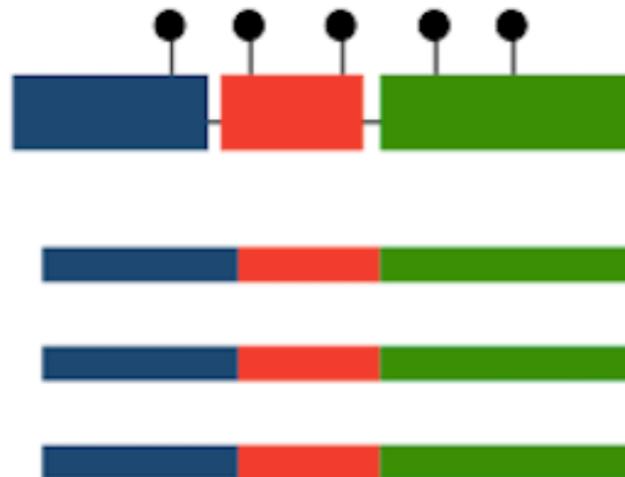
# Very new data



Gene  
expression

2

Epigenetic  
variation



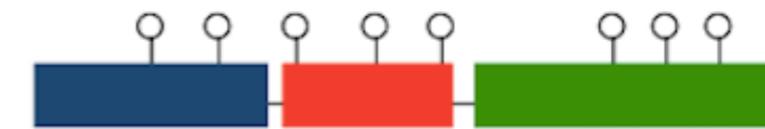
not in this experiment

not even consistent methylation changes at loci level

Gene  
expression

2

Epigenetic  
variation

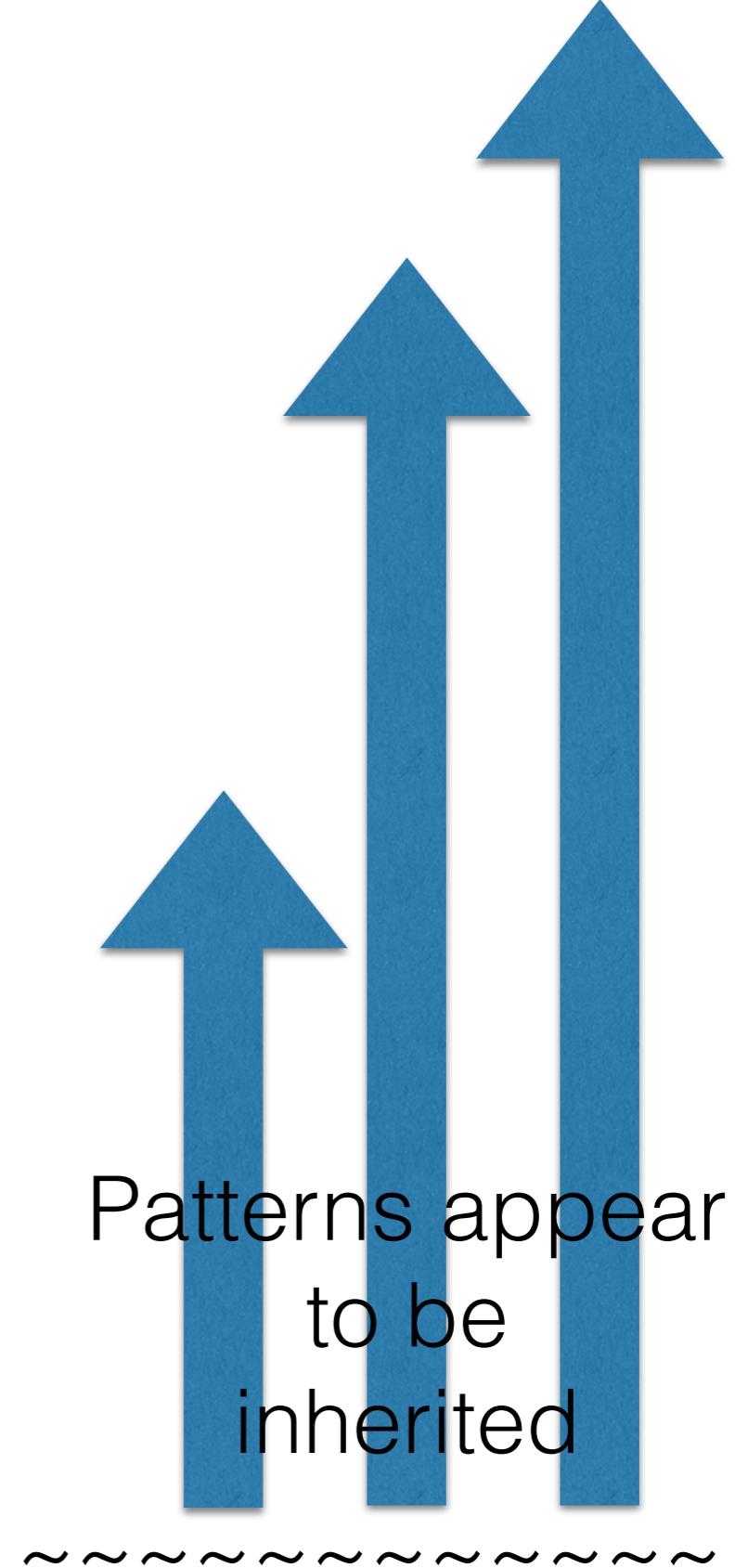


more questions....  
interesting but what is controlling?

# Considerations

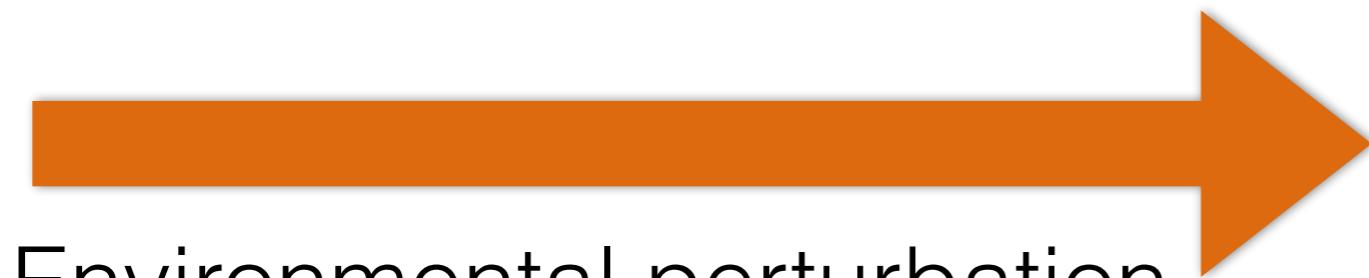


Germline methylation encoded with a pattern  
product of evolutionary forces

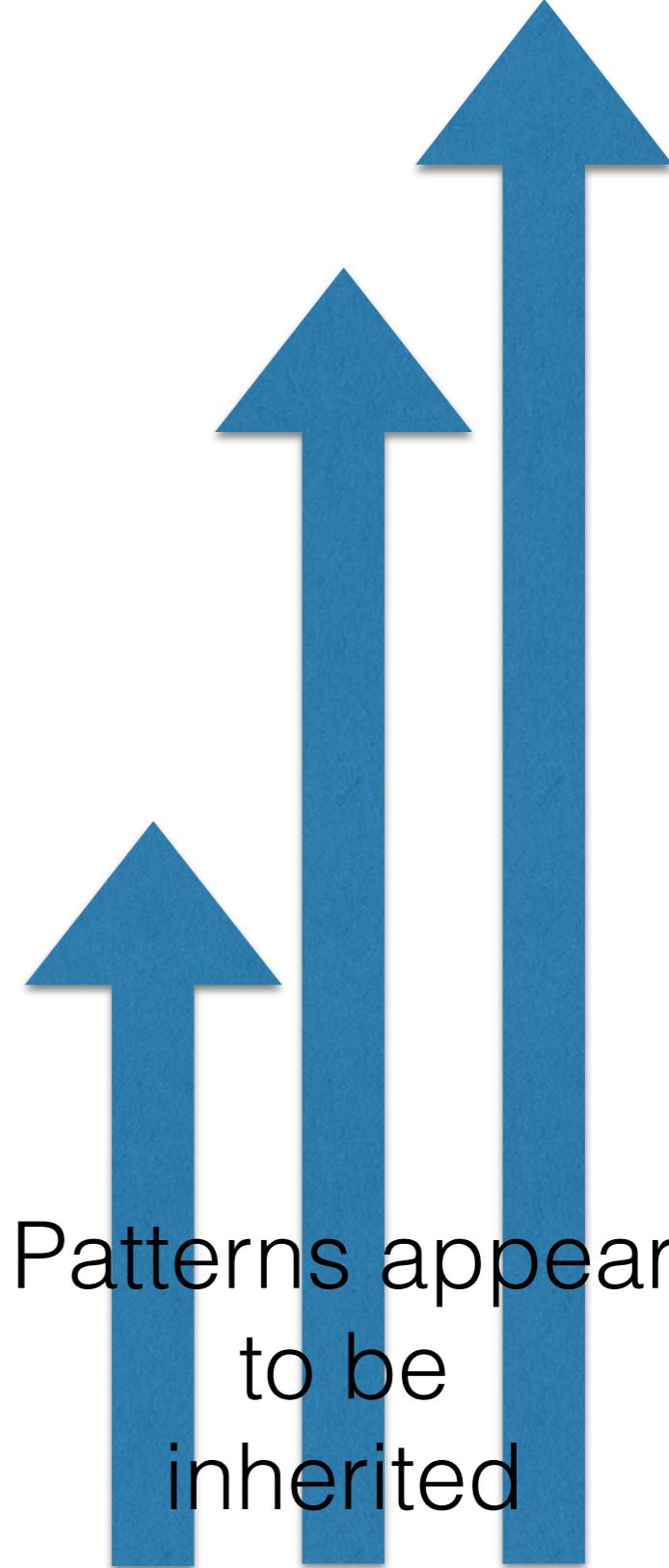


Patterns appear  
to be  
inherited

# Considerations



Environmental perturbation  
impacts DNA methylation  
(predominantly demethylation)

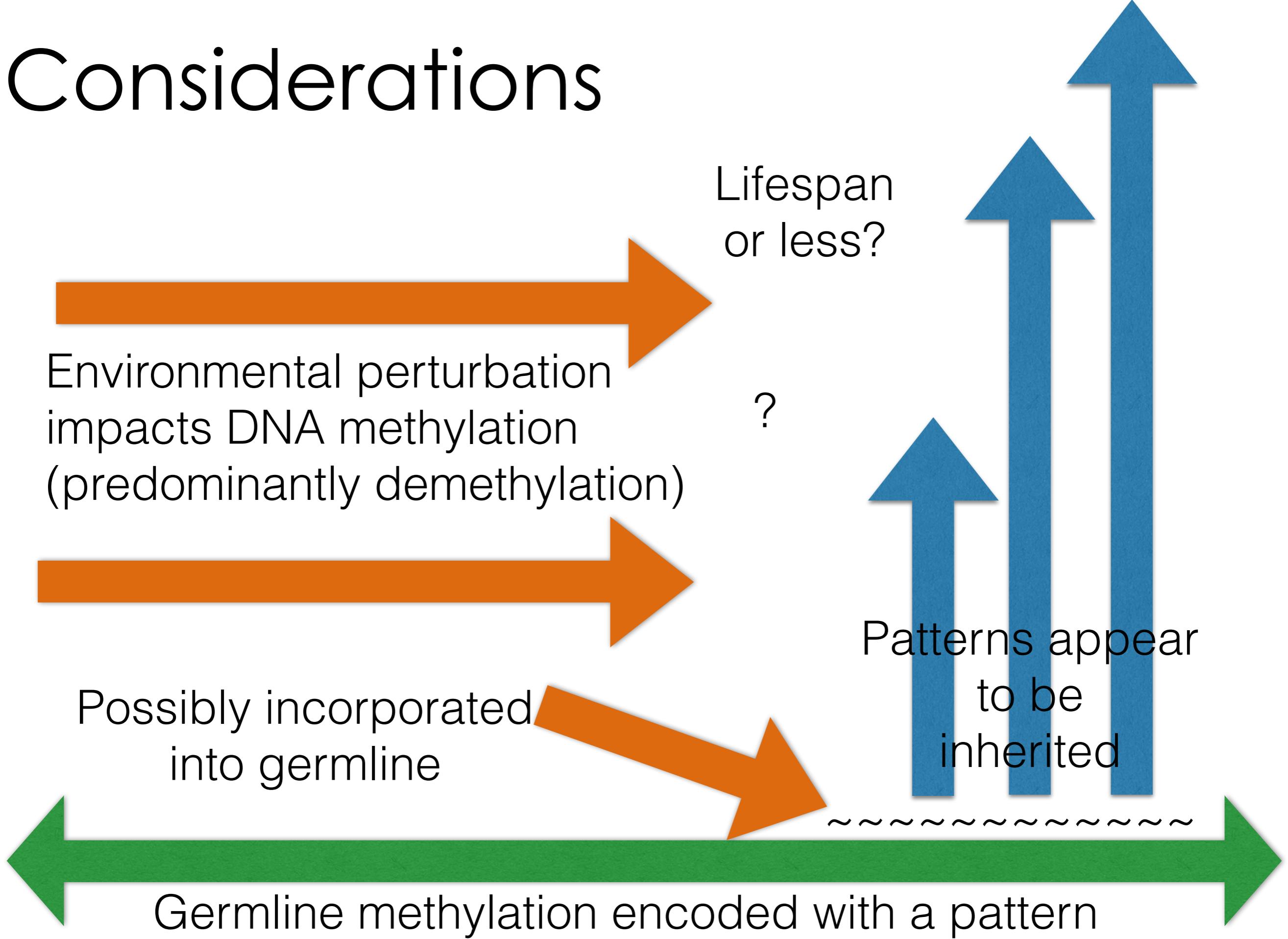


Patterns appear  
to be  
inherited



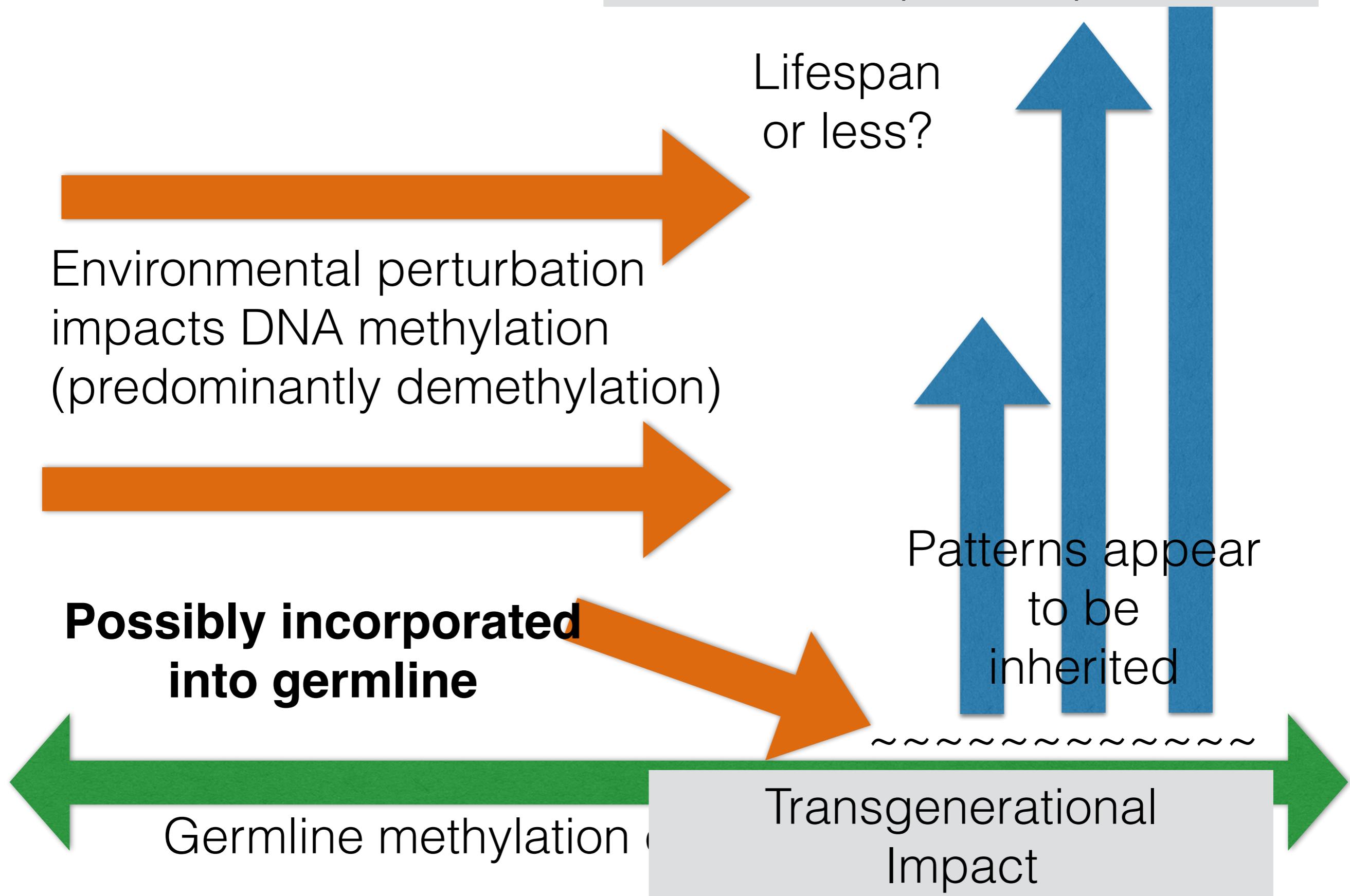
Germline methylation encoded with a pattern  
product of evolutionary forces

# Considerations



# Consideration

Could this provide a “memory”  
for subsequent exposure?



## Next Steps

Dig into the  
heat-shock data

Transposable  
Elements

Consider other  
epigenetic process

# Next Steps

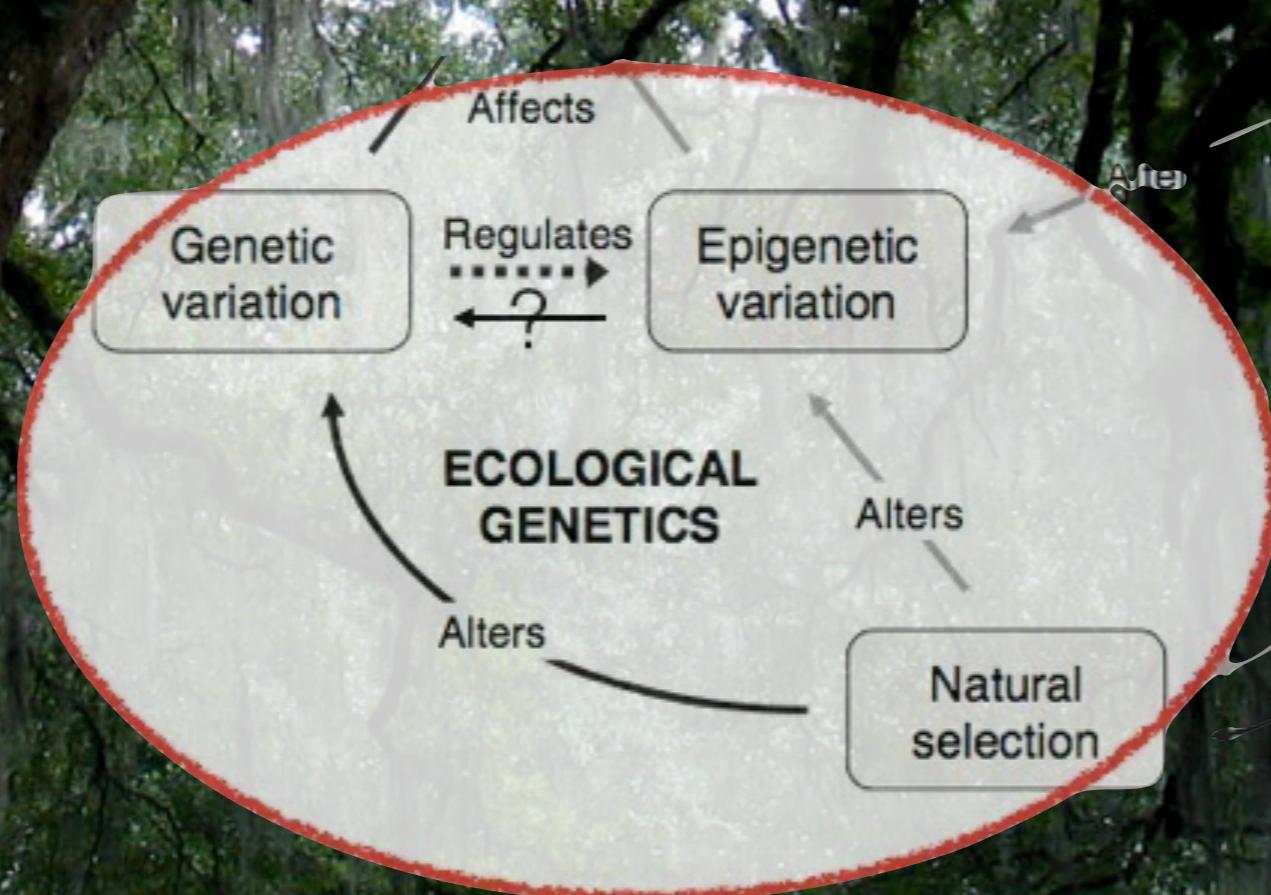


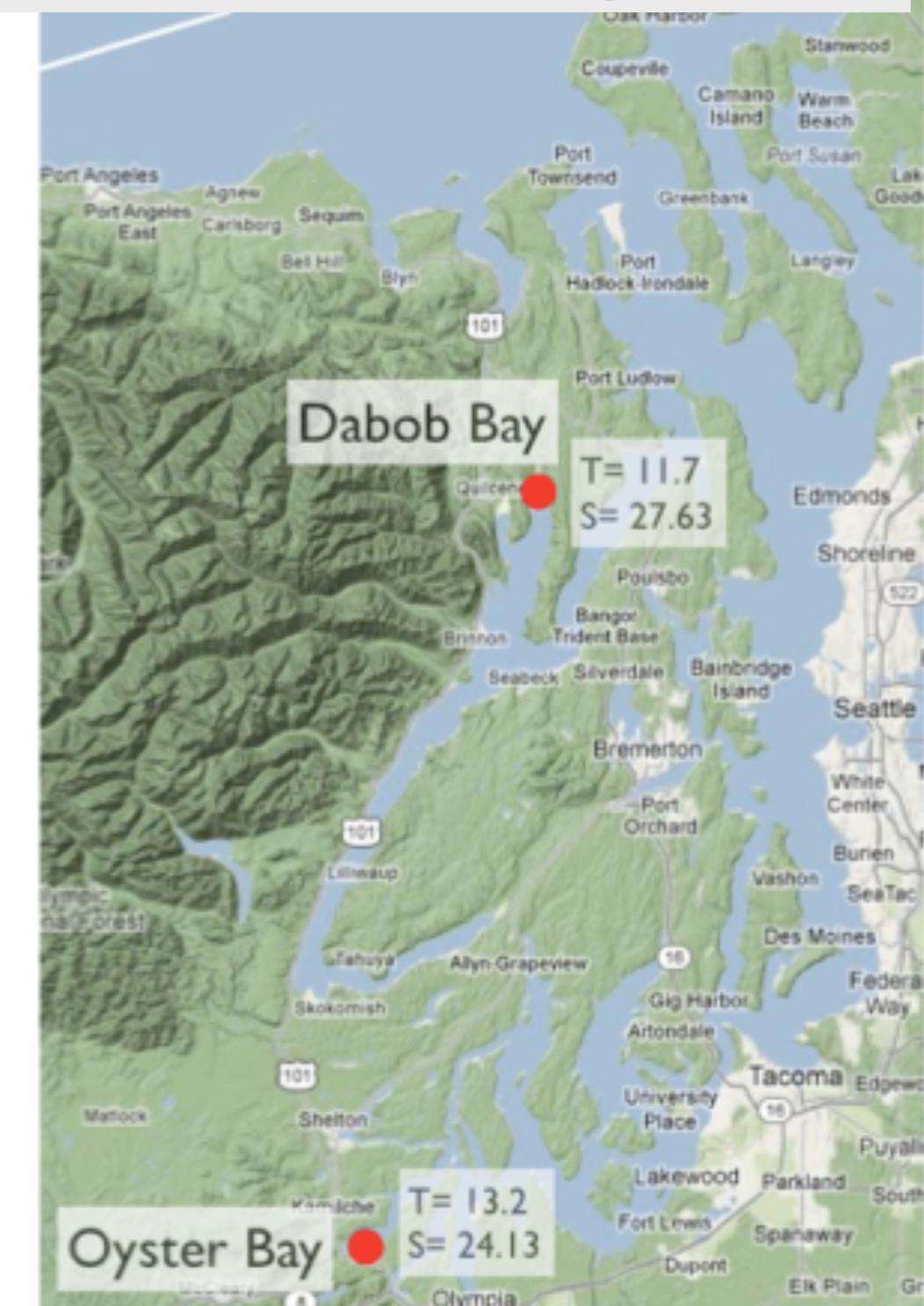
Photo credit: Flickr, Creative Commons, csessums

~~Very new data~~  
Heritability  
Plasticity  
Local Adaptation

*Genetics  
versus  
Epigenetics*

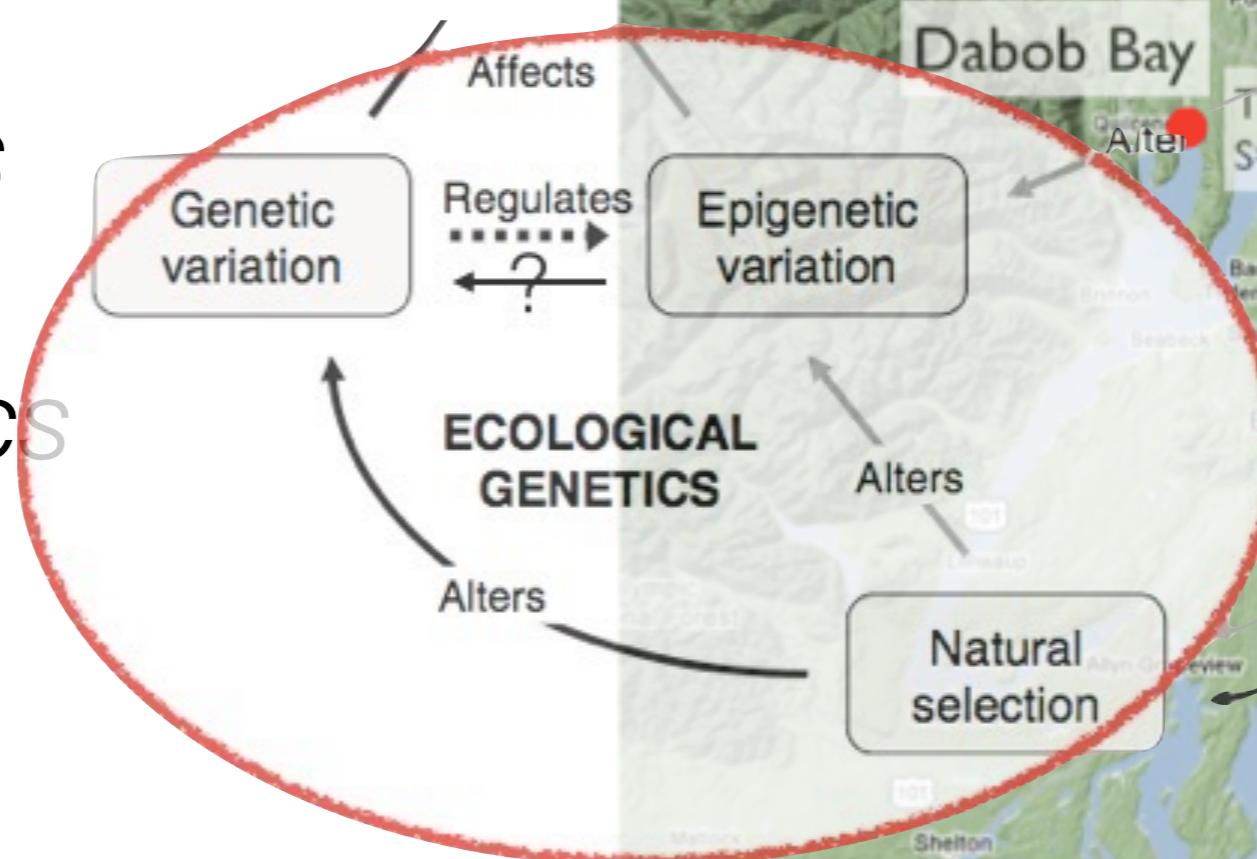


## Common Garden Experiment

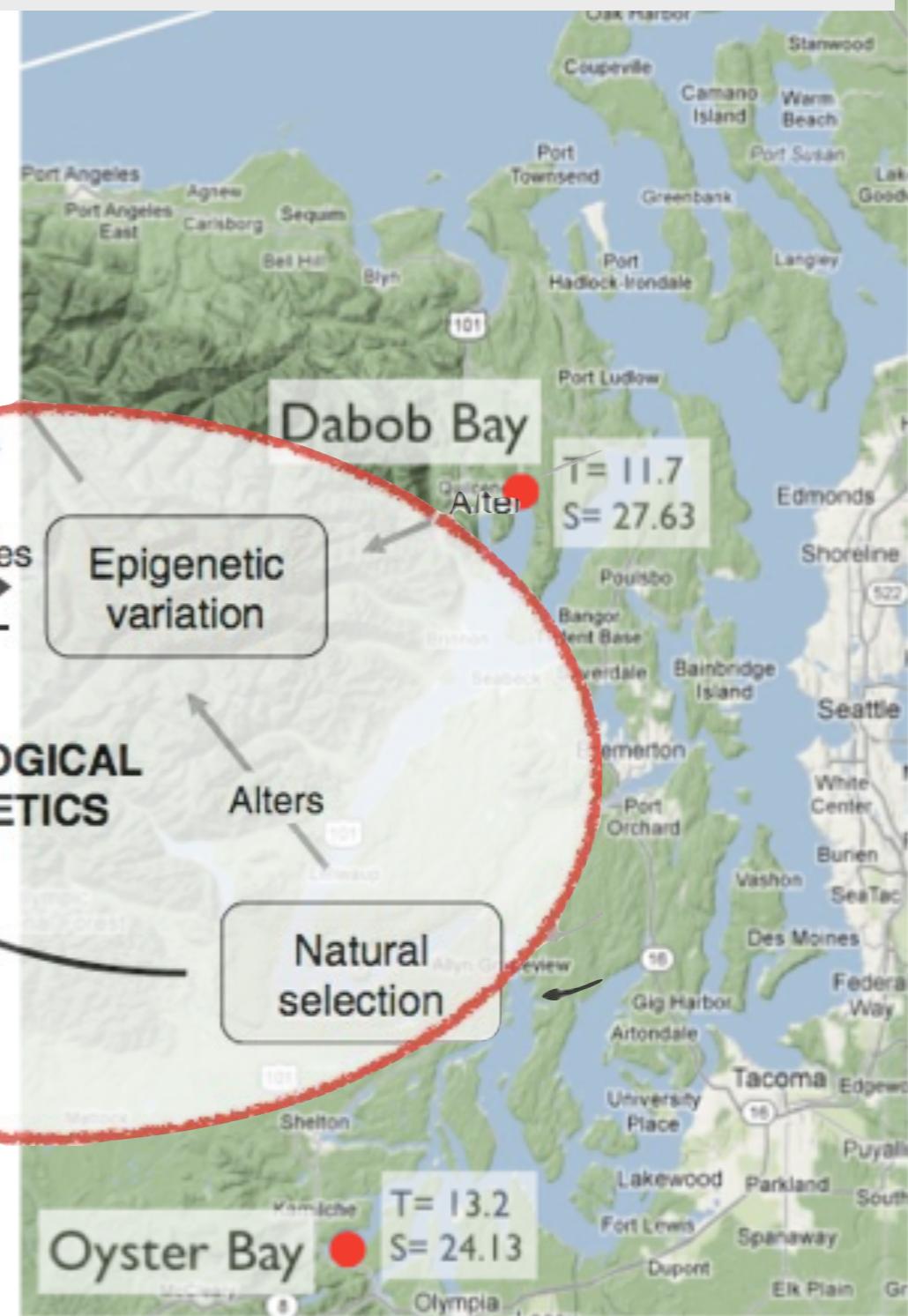


~~Very new data~~  
Heritability  
Plasticity  
Local Adaptation

Genetics  
*versus*  
Epigenetics



## Common Garden Experiment



# Acknowledgements

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slides, data & more @ [robertslab.info](http://robertslab.info)