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# A molecular framework to identify novel modes of action of endocrine disrupting compounds in shellfish

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# The role of DNA methylation in mediating the effects of estrogens in oysters

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University of Washington

# Outline



17 $\alpha$  ethinylestradiol  
(EE2)



# Outline

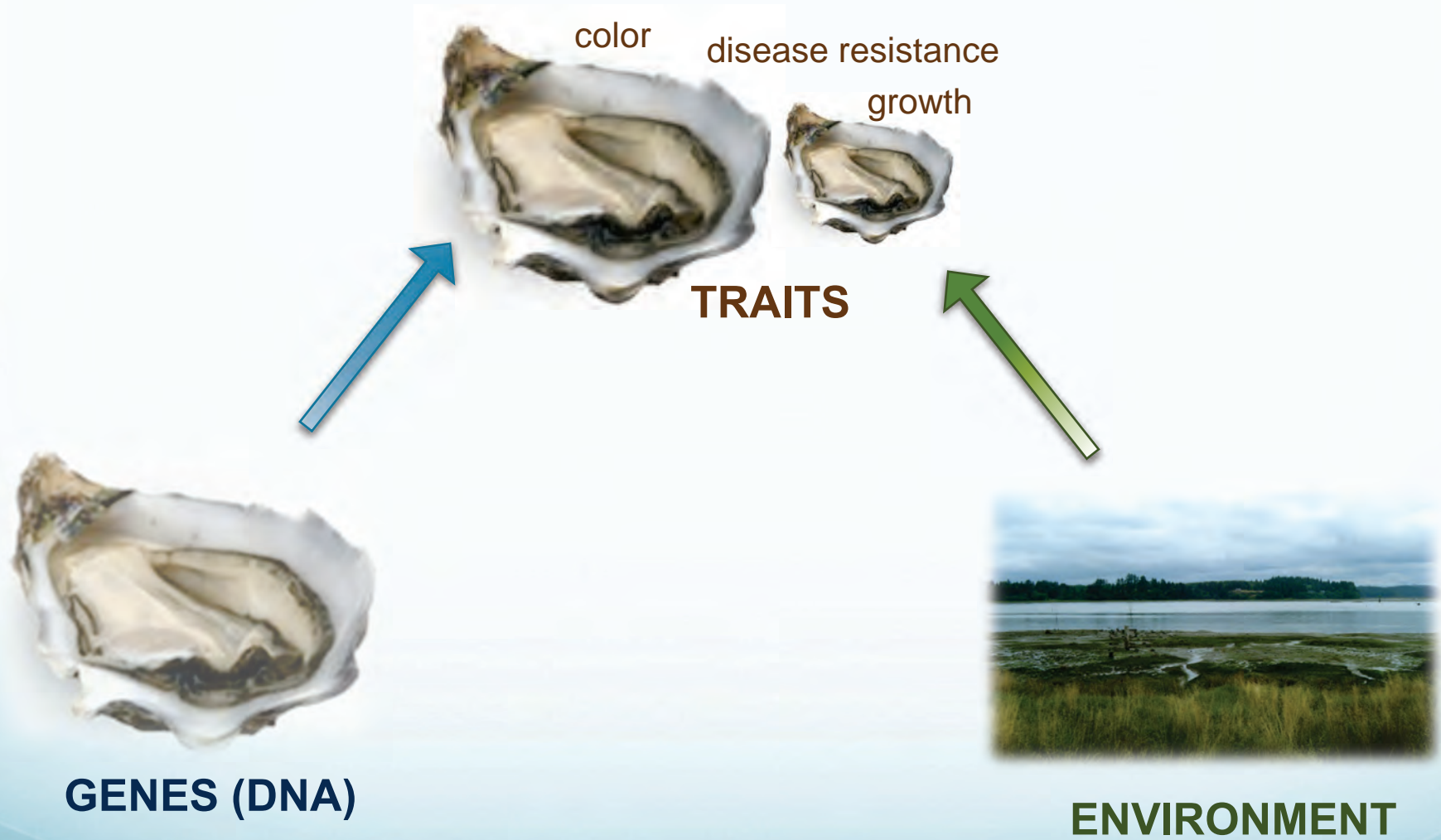
- Background
  - DNA methylation
  - EDCs & bivalves
- Results
- Implications



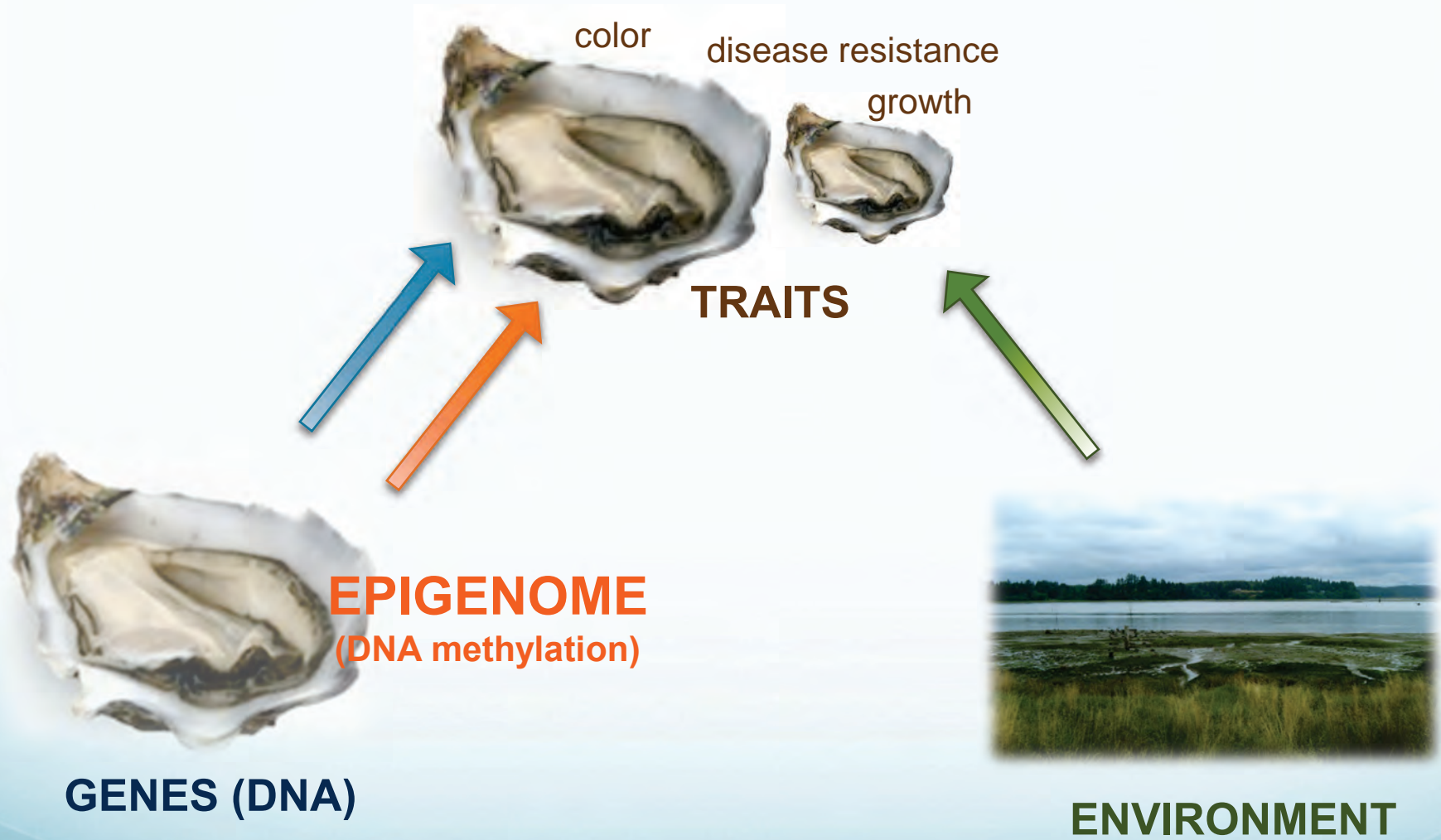
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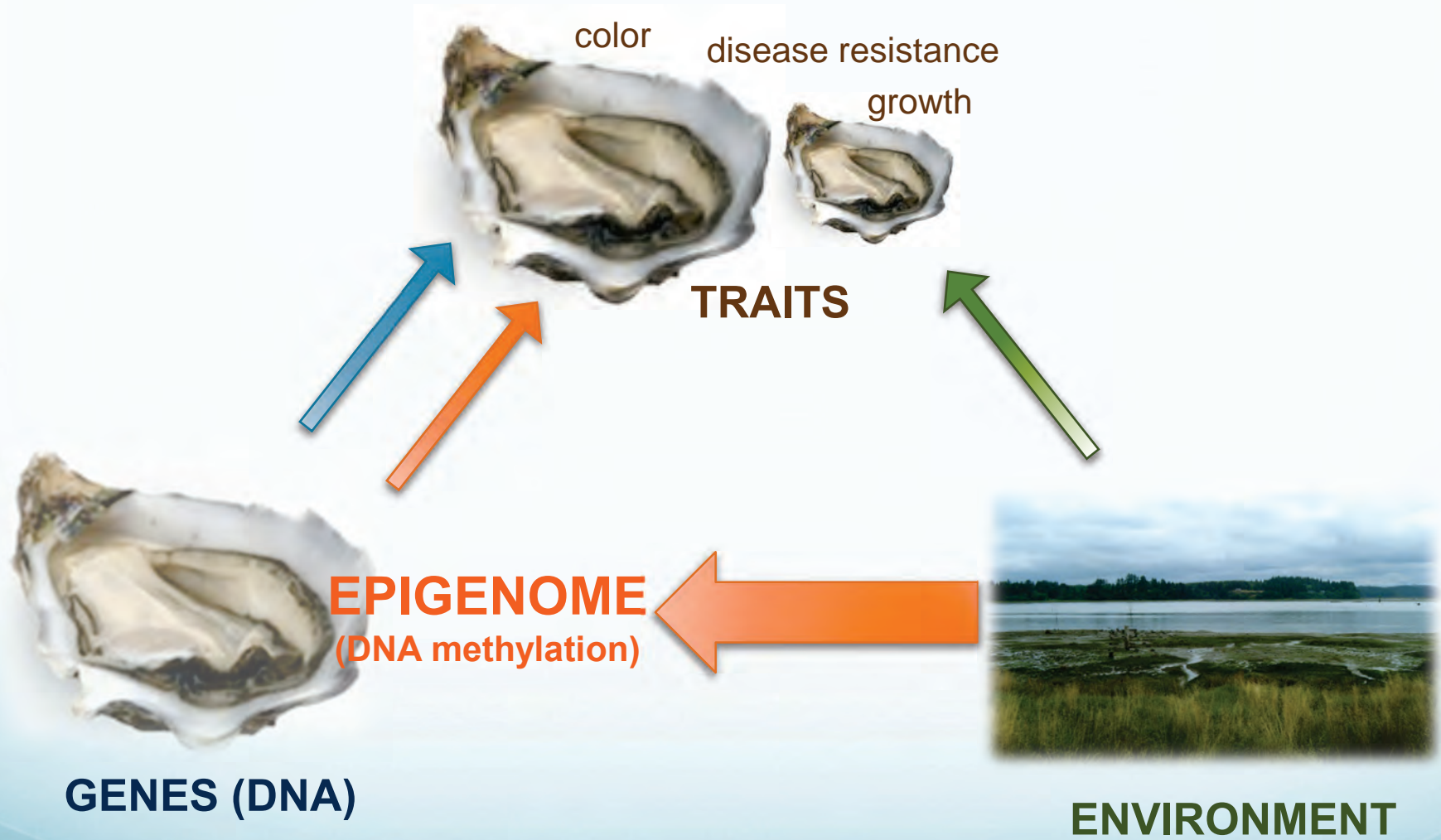
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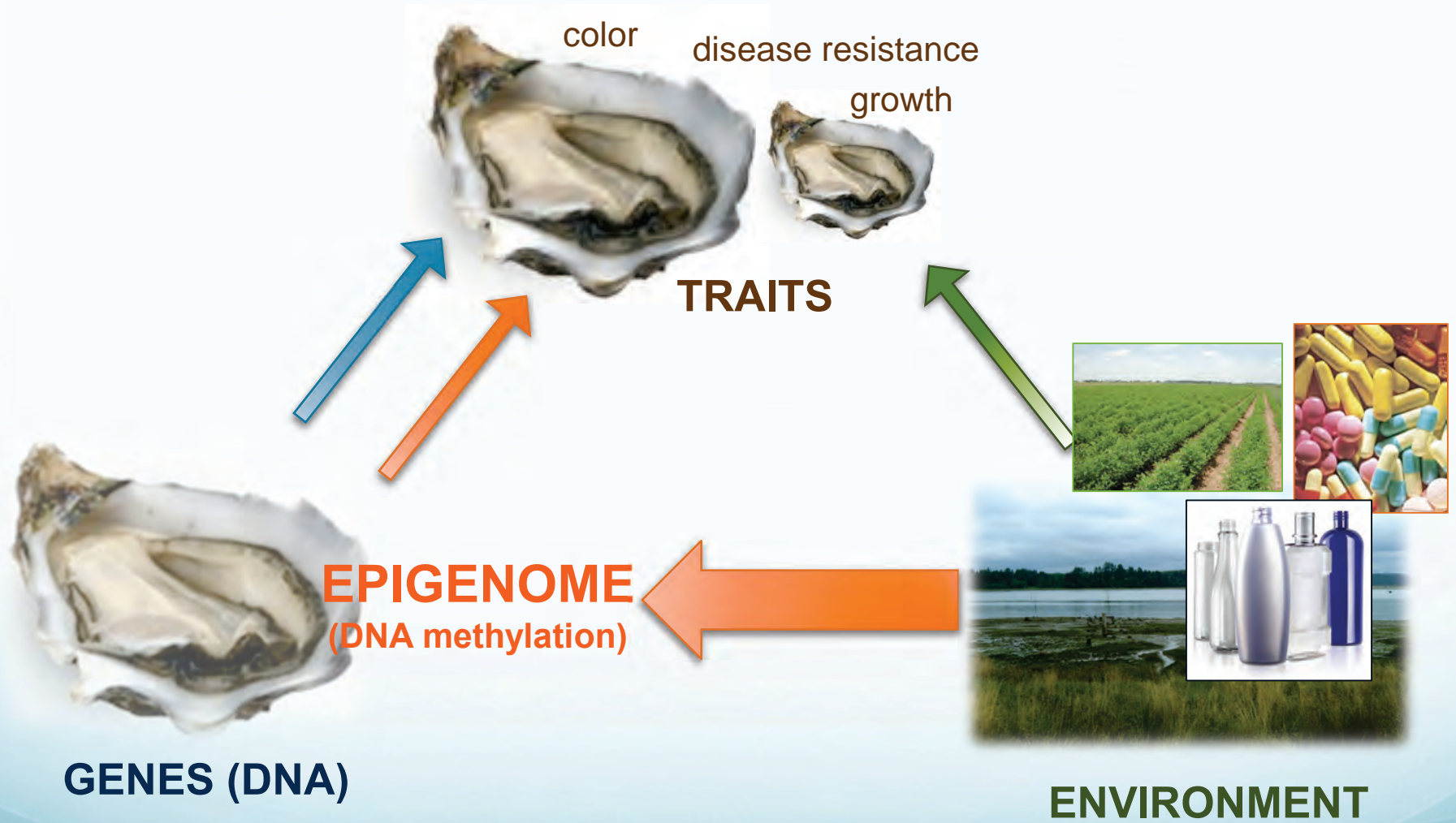
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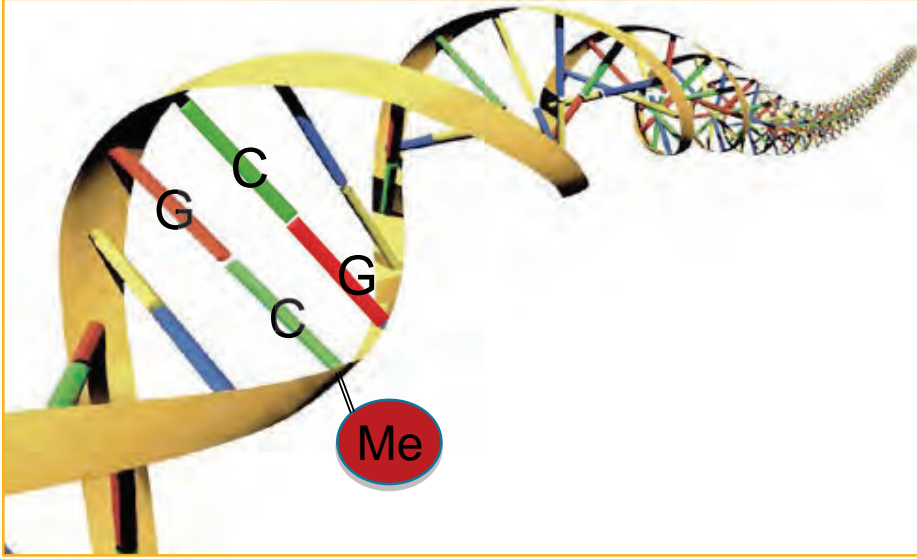
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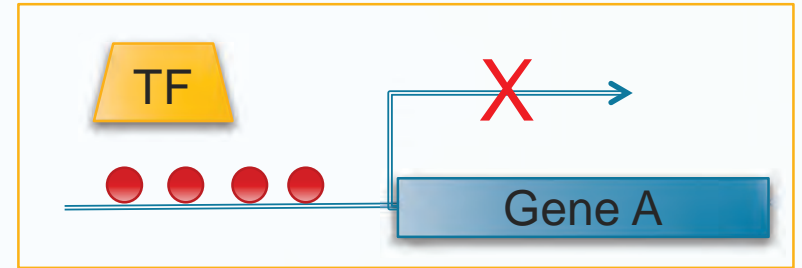
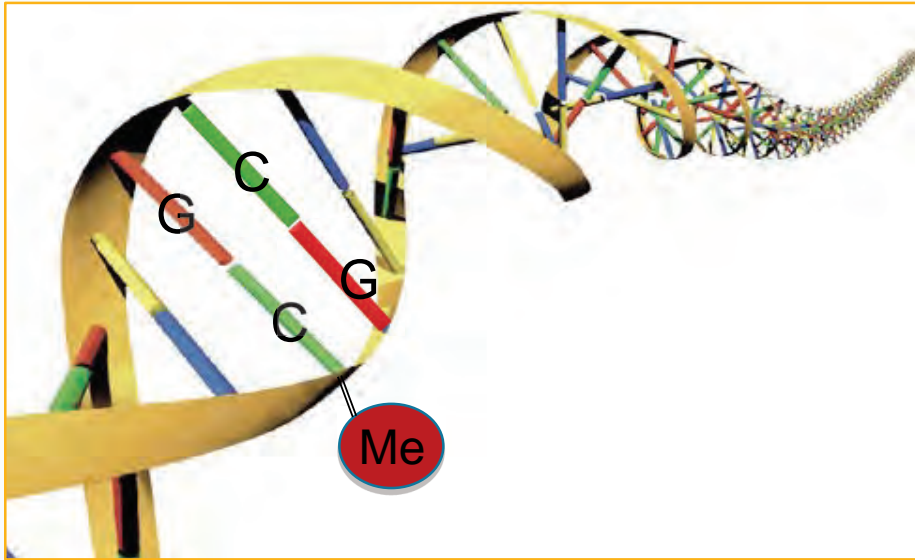


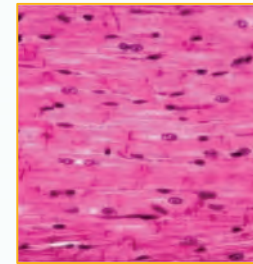
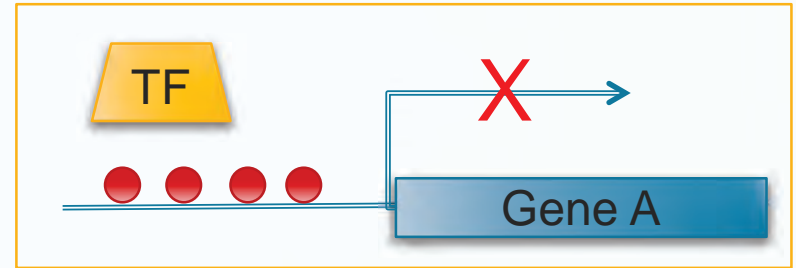
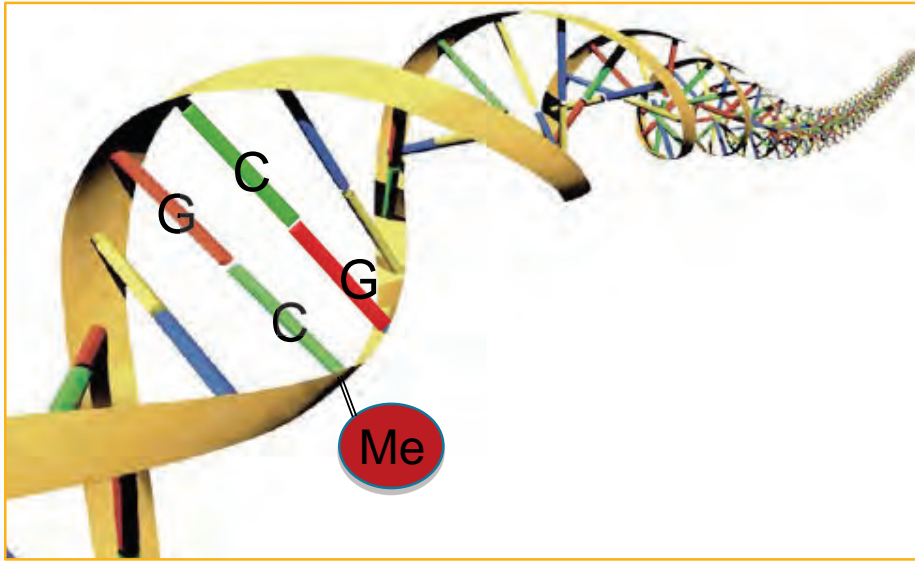
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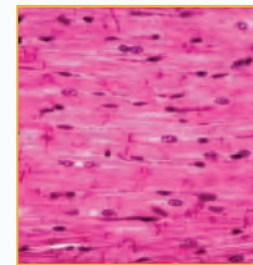
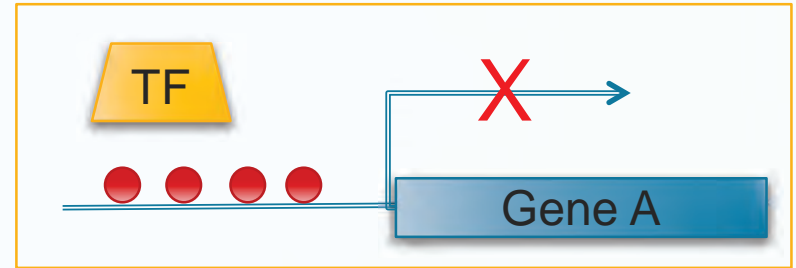
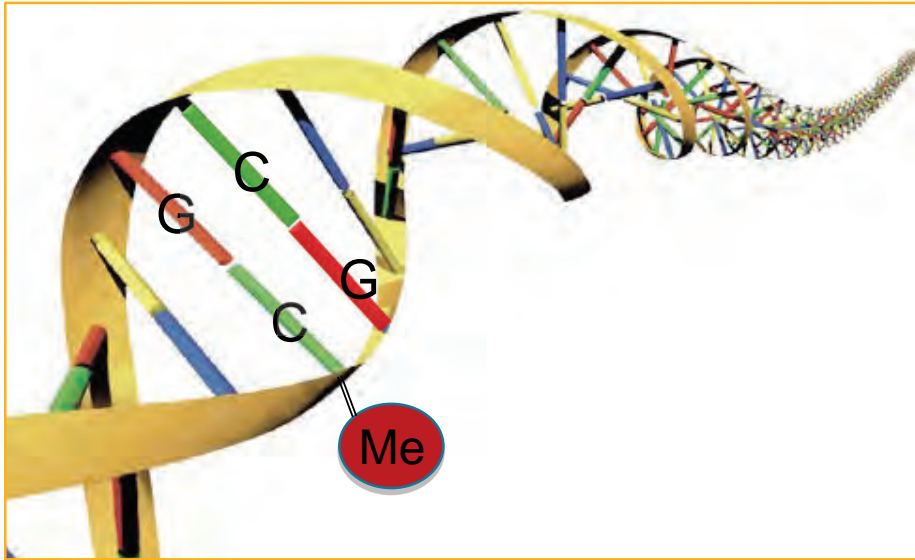


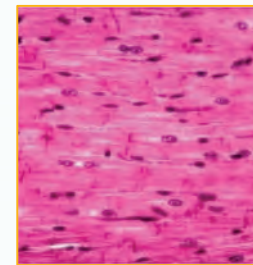
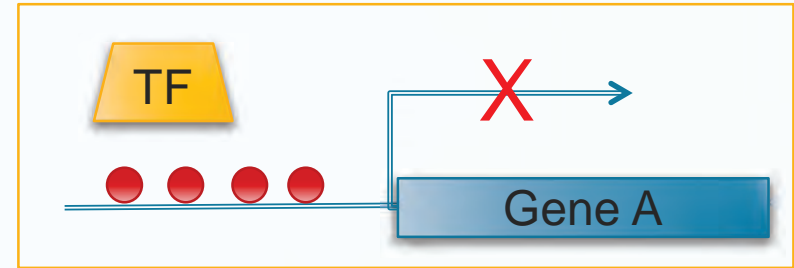
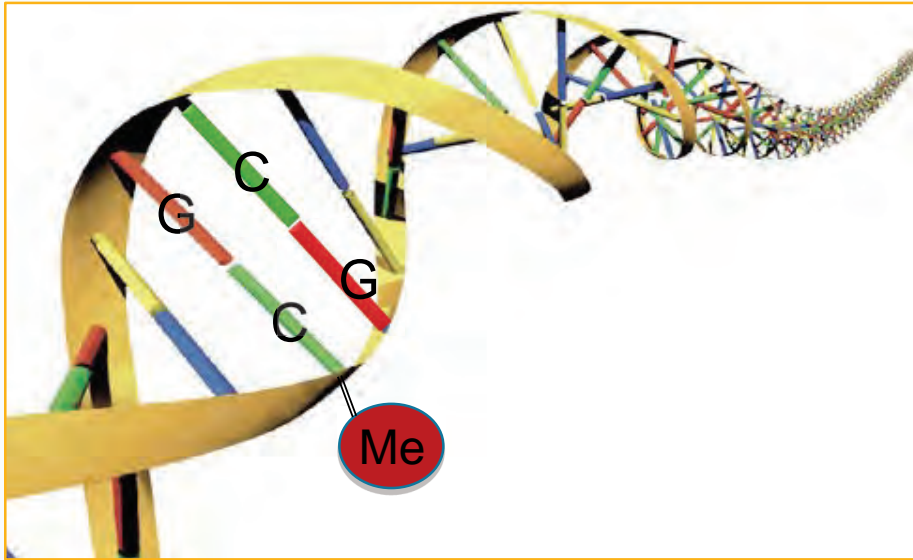












Source: Randy Jirtle



# Reproduction in oysters

- Pacific oysters are sequential hermaphrodites
- Sex determination has a genetic component, but influenced by environmental factors



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## Estradiol

- induces sex reversal (Mori 1969)

## 17 $\alpha$ ethinylestradiol (EE2)

- $\uparrow$  rate of oocyte development (Andrew 2010)

## Nonylphenol

- offspring of exposed larvae had  $\uparrow$  intersex (Nice et al. 2003)



# Hypotheses

- EE2 exposure will result in phenotypes such as skewed sex ratios and increased rate of gonad development
- DNA methylation patterns will be altered in oysters exposed to EE2



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- DNA methylation patterns will be altered in oysters in upon exposure to EE2

# Estrogen Experiment



500 ng/L EE2: 150 oysters (n=50/tank)



Control: 150 oysters (n=50/tank)

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Day 0



Day 7



Day 60

- Samples: histology, gonad tissue

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Day 0



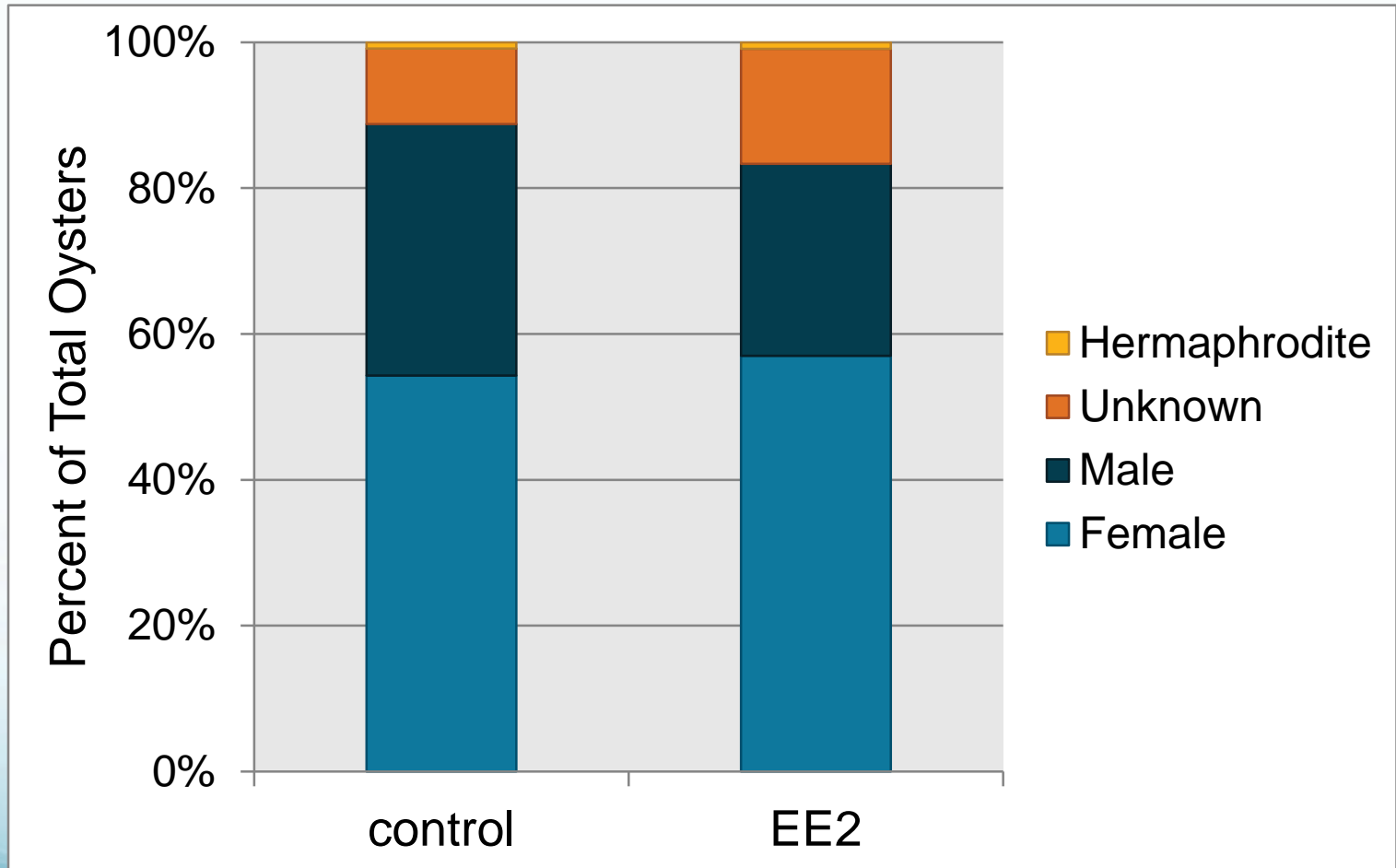
Day 7



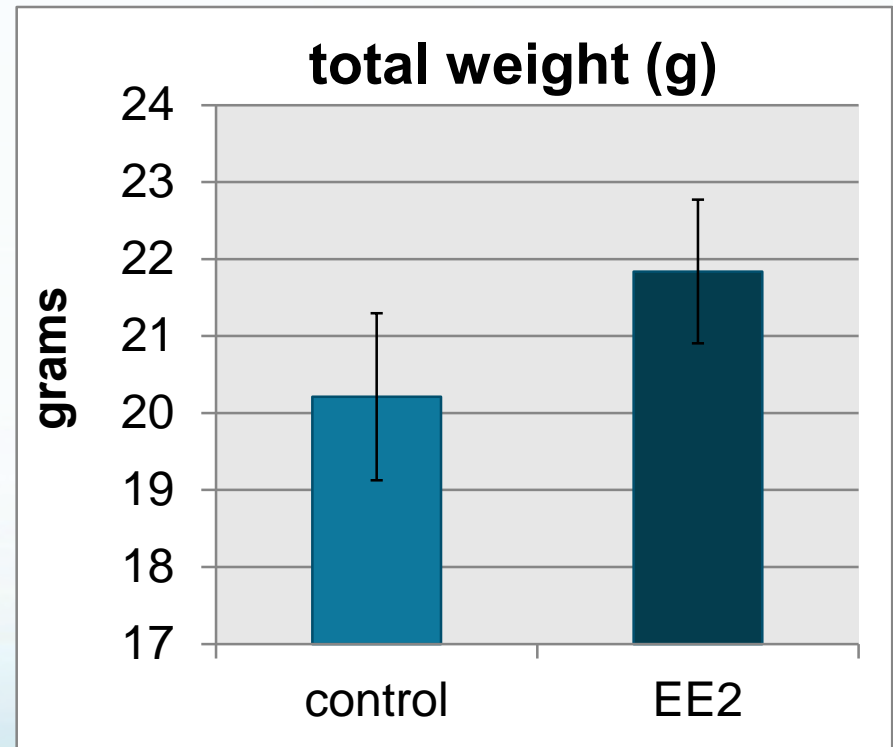
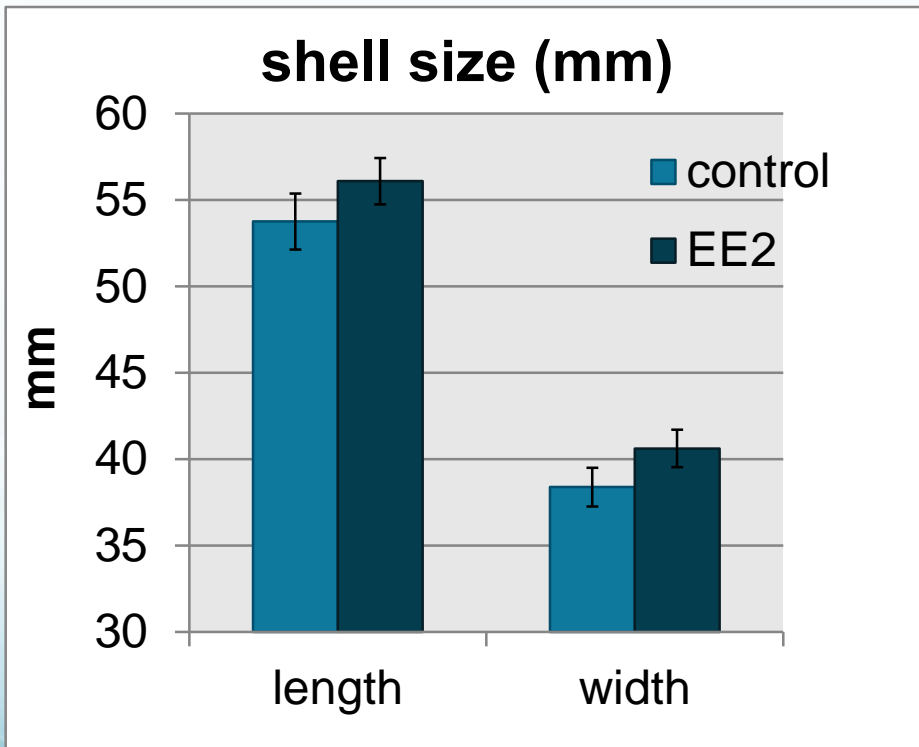
Day 60

- Samples: histology, gonad tissue

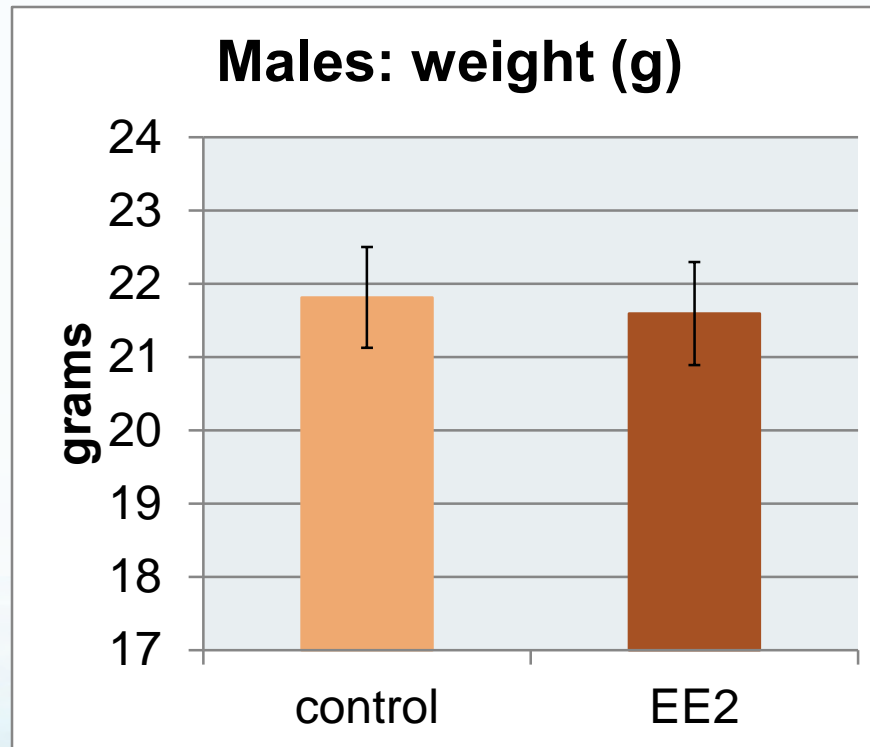
# Results: Day 60 sex determination



# Results: Day 60 size of females



# Results: Day 60 size of males



# Results: DNA methylation



EE2 (500ng/L) 150 oysters (n=50/tank)



control 150 oysters (n=50/tank)



Day 0



Day 7



Day 60



# Results: DNA methylation



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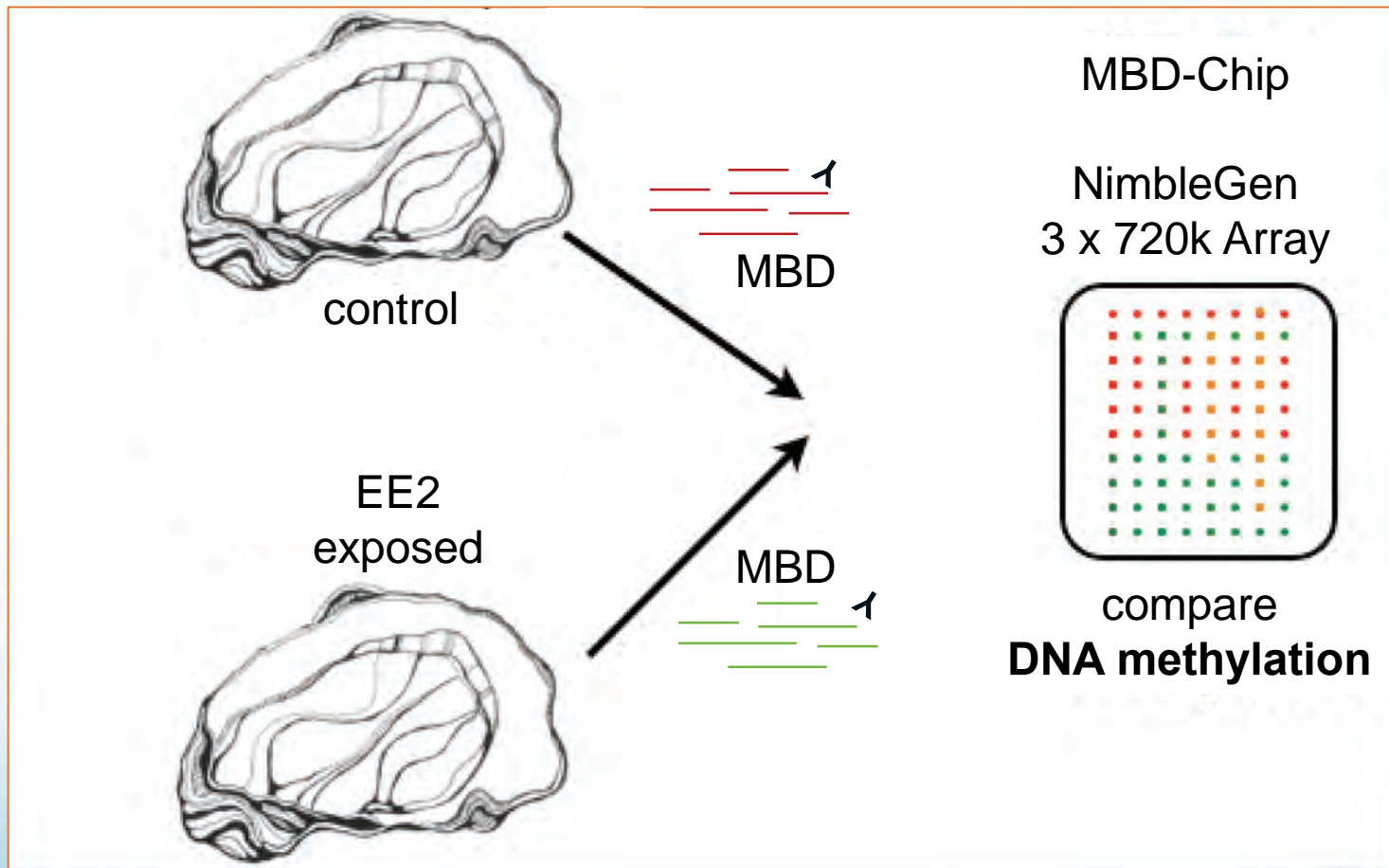


Day 7



Day 60

# Results: DNA methylation



# Results: DNA methylation

- Results:
  - 45 differentially methylated regions (DMR)
  - DMRs were located in 38 different genes

## Protein names

5-hydroxytryptamine receptor 1B  
ATP-binding cassette sub-family G member 1  
Angiotensin-converting enzyme  
Neuronal acetylcholine receptor subunit alpha-6  
Anaphase-promoting complex subunit 1  
Arrestin domain-containing protein 3  
Calmodulin  
Corticotropin-releasing factor receptor 2  
Carnosine synthase 1  
E3 ubiquitin-protein ligase DTX3L  
Dynein gamma chain, flagellar outer arm  
Elongator complex protein 2  
Ryncolin-1  
Glutamine synthetase  
Glutaredoxin 3  
Granulins  
Translation factor Guf1, mitochondrial  
Apoptosis inhibitor IAP  
Interferon-induced protein 44  
Kelch-like protein 24  
Liprin-beta-1  
Low-density lipoprotein receptor-related protein 6  
Unconventional myosin-Vb  
NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial  
Nose resistant to fluoxetine protein 6  
Peptidase M20 domain-containing protein 2  
60 kDa SS-A/Ro ribonucleoprotein  
Solute carrier family 28 member 3  
Solute carrier family 45 member 3  
Protein transport protein Sec16A  
Small integral membrane protein 14  
Src kinase-associated phosphoprotein 2-B  
DNA topoisomerase 1  
tRNA pseudouridine synthase A, mitochondrial  
Vasorin  
Vacuolar protein sorting-associated protein 13C  
WASH complex subunit 7

Protein names  
 5-hydroxytryptamine receptor 1B  
 ATP-binding cassette sub-family G member 1  
 Angiotensin-converting enzyme  
 Neuronal acetylcholine receptor subunit alpha-6

Gene Ontology (GO Slim)	Count
transport	10
cell organization and biogenesis	8
other metabolic processes	7
signal transduction	6
protein metabolism	5
RNA metabolism	5
developmental processes	4
cell cycle and proliferation	3
death	2
stress response	2
cell-cell signaling	1
DNA metabolism	1

Small integral membrane protein 14  
 Src kinase-associated phosphoprotein 2-B  
 DNA topoisomerase 1  
 tRNA pseudouridine synthase A, mitochondrial  
 Vasorin  
 Vacuolar protein sorting-associated protein 13C  
 WASH complex subunit 7

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 Vacuolar protein sorting-associated protein 13C  
 WASH complex subunit 7

- ATP-binding cassette protein
- Serotonin receptor
- Low density lipoprotein receptor
- Granulin

# Summary

- EE2 treatment did not affect sex ratios, but exposed females were larger than controls
- DMRs were identified within 1 week of EE2 exposure
- Genes with DMRs are functionally diverse (e.g. growth, immune, reproduction)



# Implications

- DNA methylation may play a role in mediating responses to EDCs in bivalves
- Epigenetic marks may provide early indicators of EDC exposure in aquatic species



# Acknowledgements

- Roberts Lab:  
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