May 2nd, 8:30 AM - 10:00 AM

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

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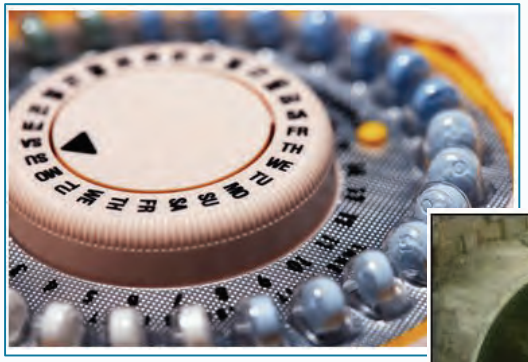
Steven (Steven Beyer) Roberts
University of Washington

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

Mackenzie Gavery & Steven Roberts
School of Aquatic and Fishery Sciences
University of Washington

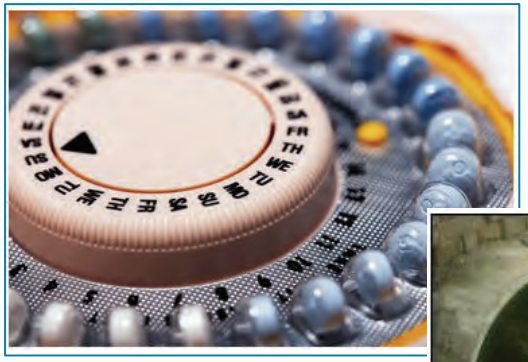
Outline



17α ethinylestradiol (EE2)

Outline

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



17α ethinylestradiol (EE2)

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TRAITS

GENES (DNA)

ENVIRONMENT

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EPIGENOME
(DNA methylation)

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ENVIRONMENT

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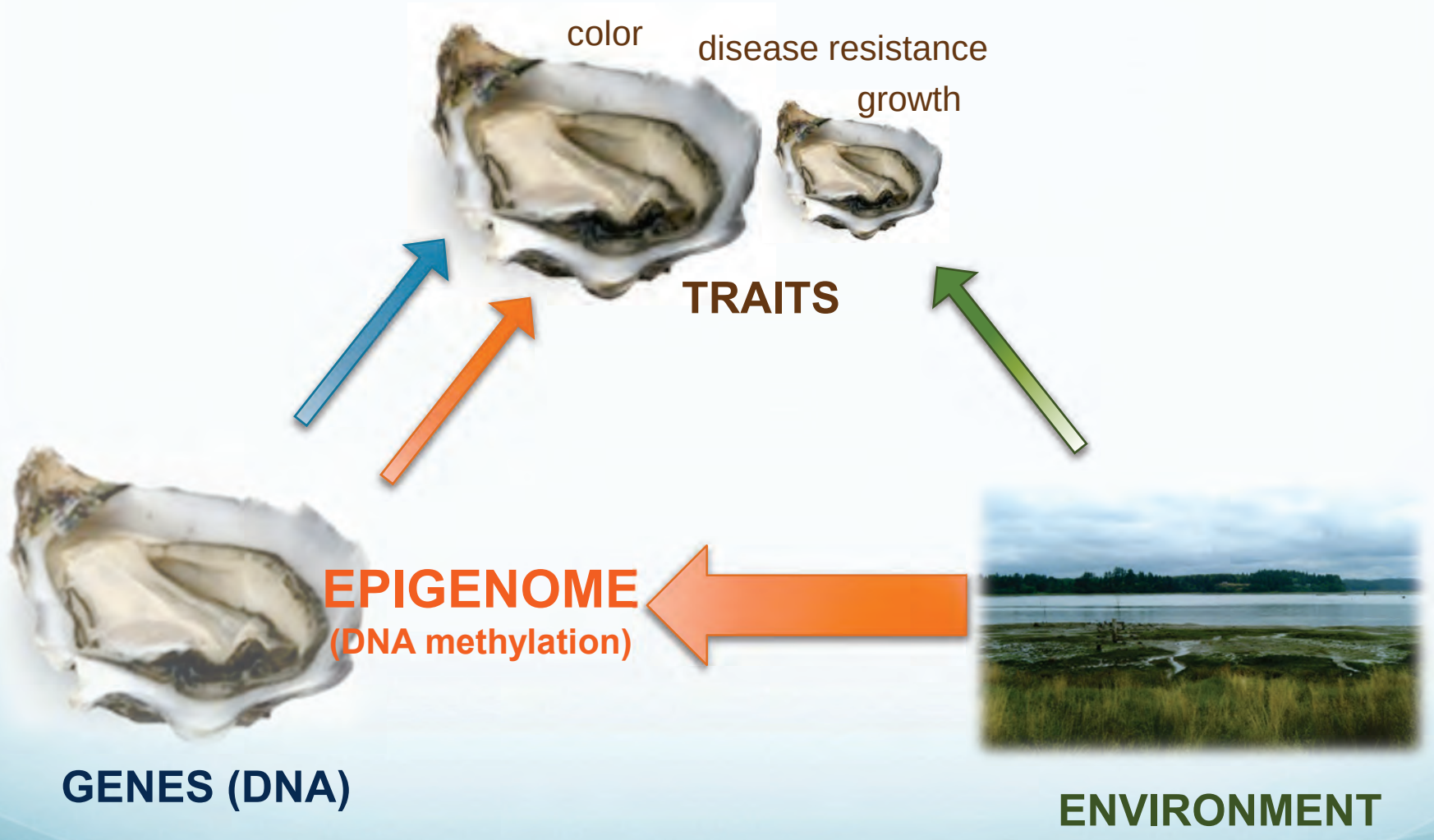
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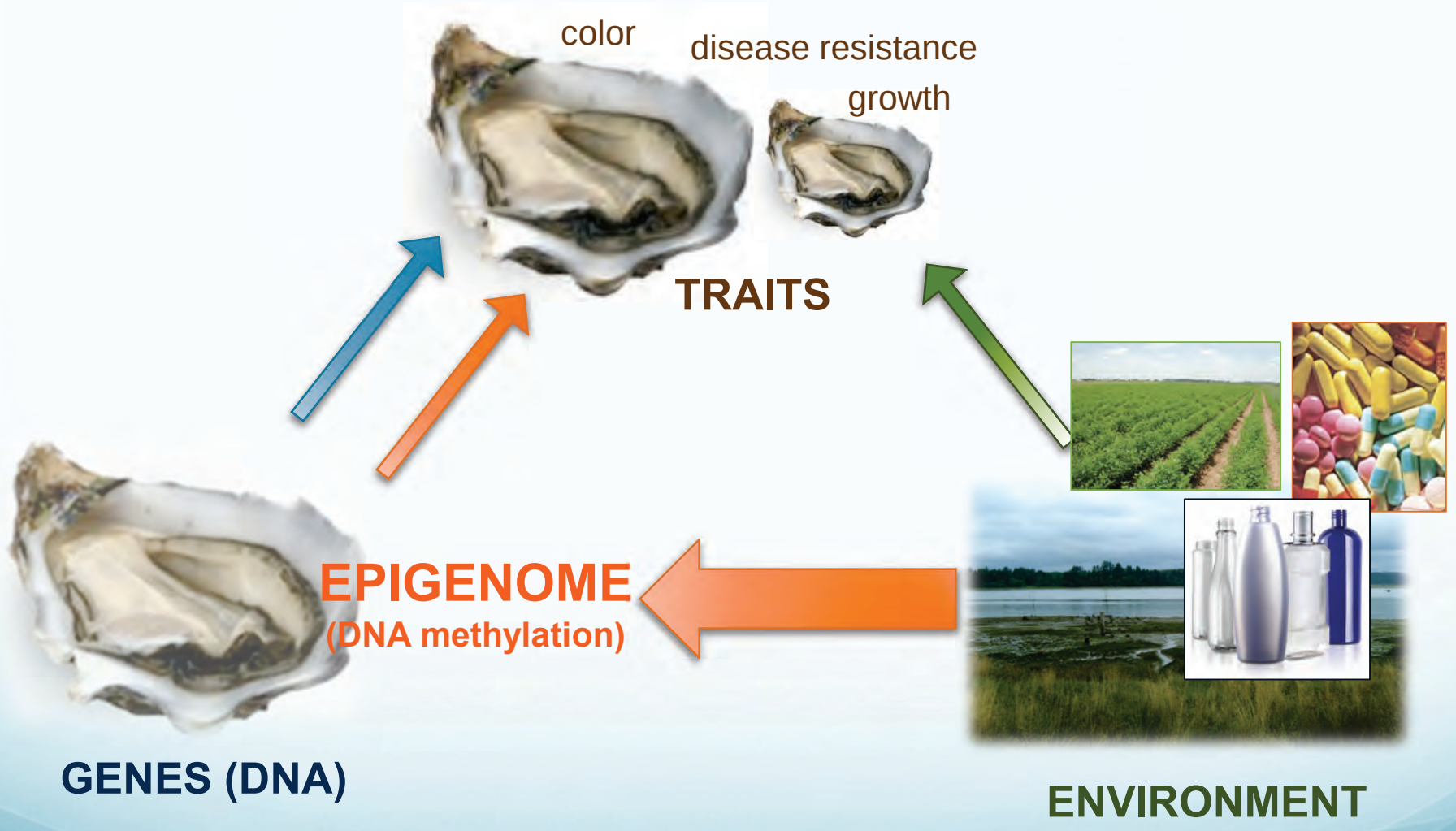
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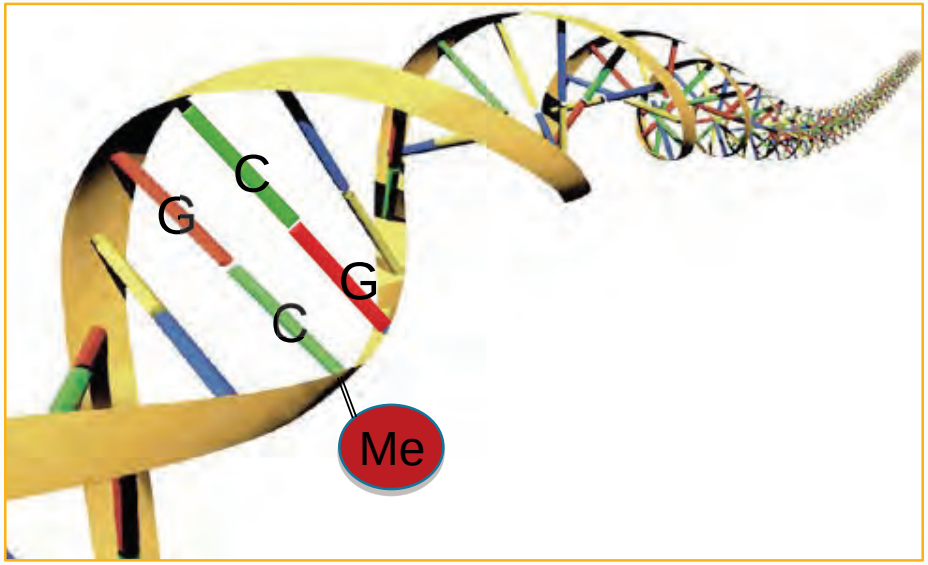
C
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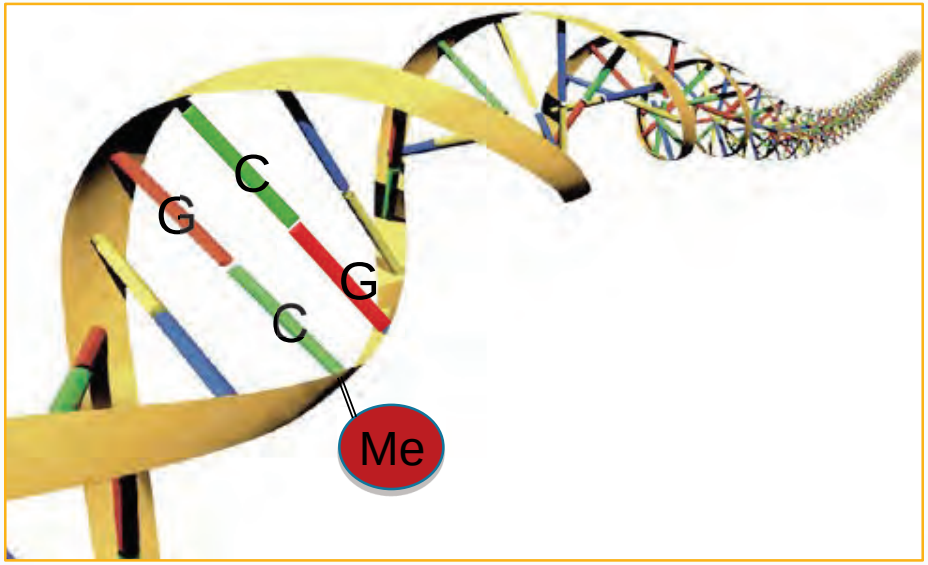
G
C
Me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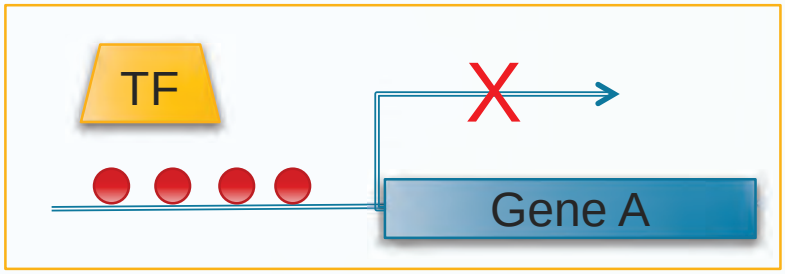
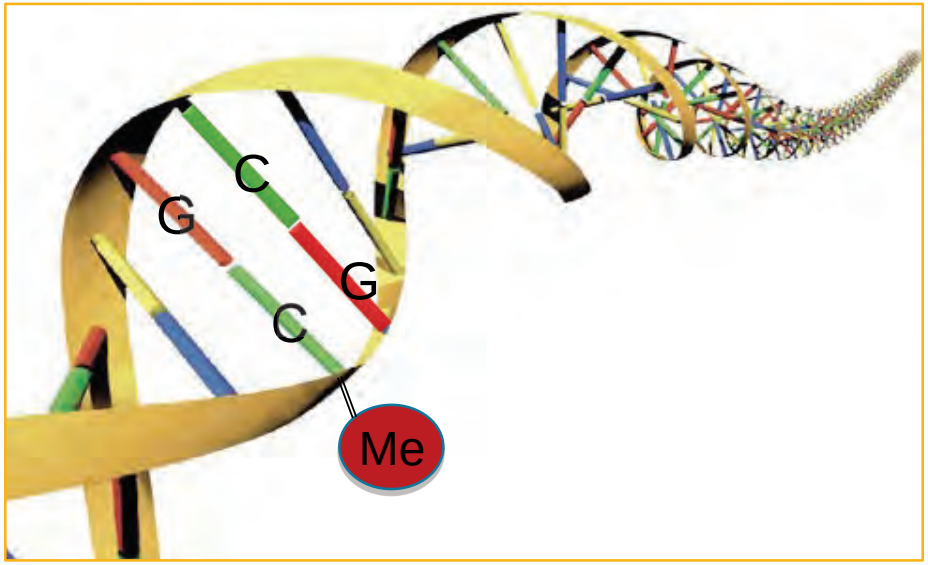

TF
X
Gene A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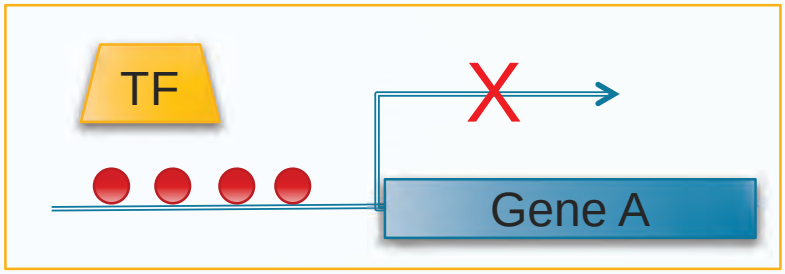
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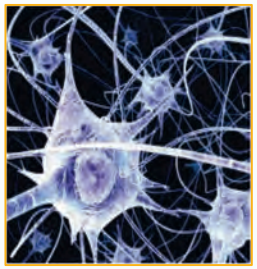

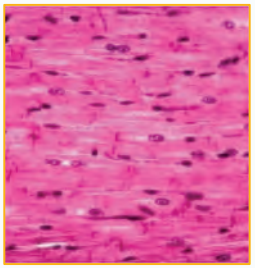

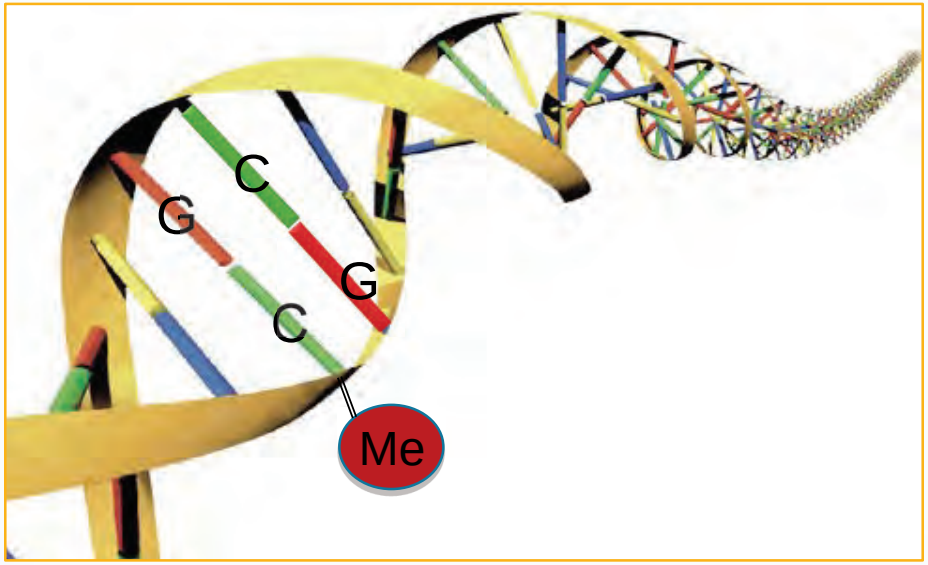
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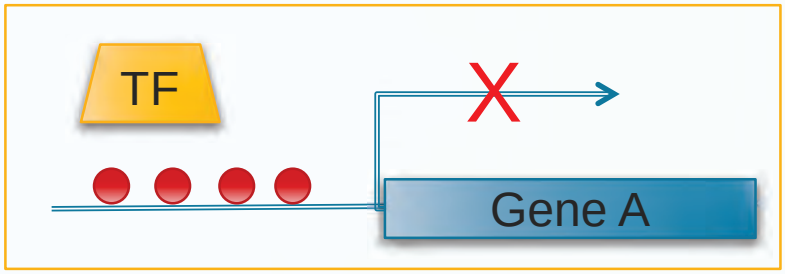
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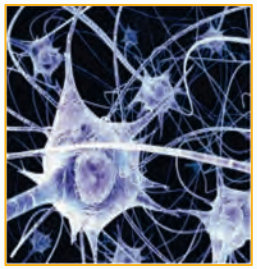
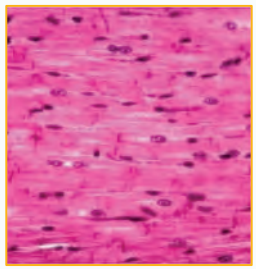
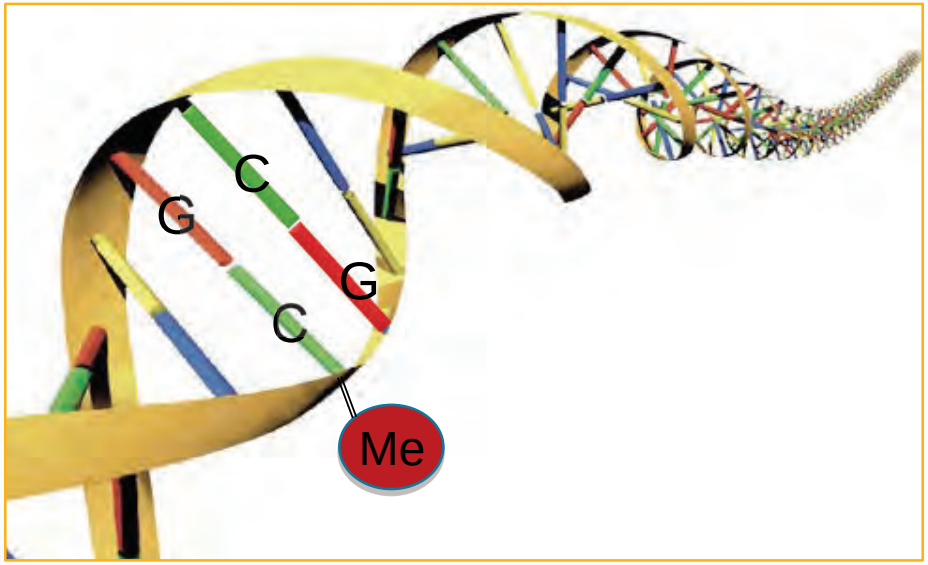
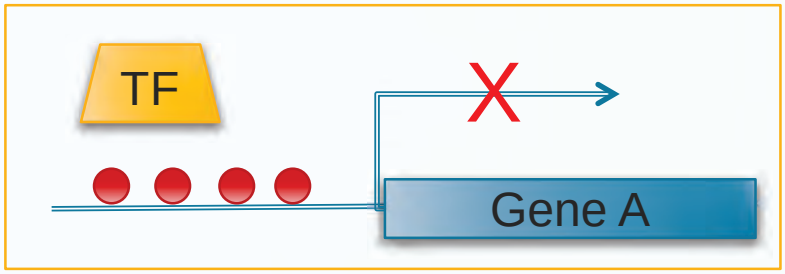
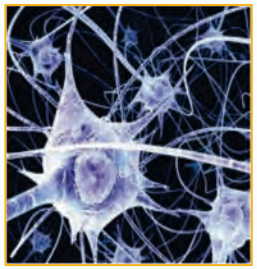


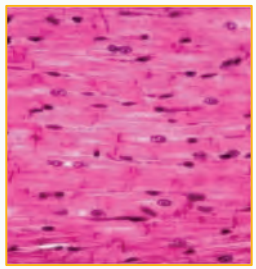



9.5
10.5
11.5
12.5
13.5
14.5
15.5
16.5
17.5
18.5
Neonate
Gestational Ages
in Days










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)

17α ethinylestradiol (EE2)

- ↑ rate of oocyte development (Andrew 2010)

Nonylphenol

- offspring of exposed larvae had ↑ 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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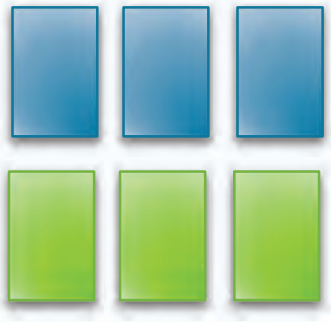
Estrogen Experiment



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

Control: 150 oysters (n=50/tank)

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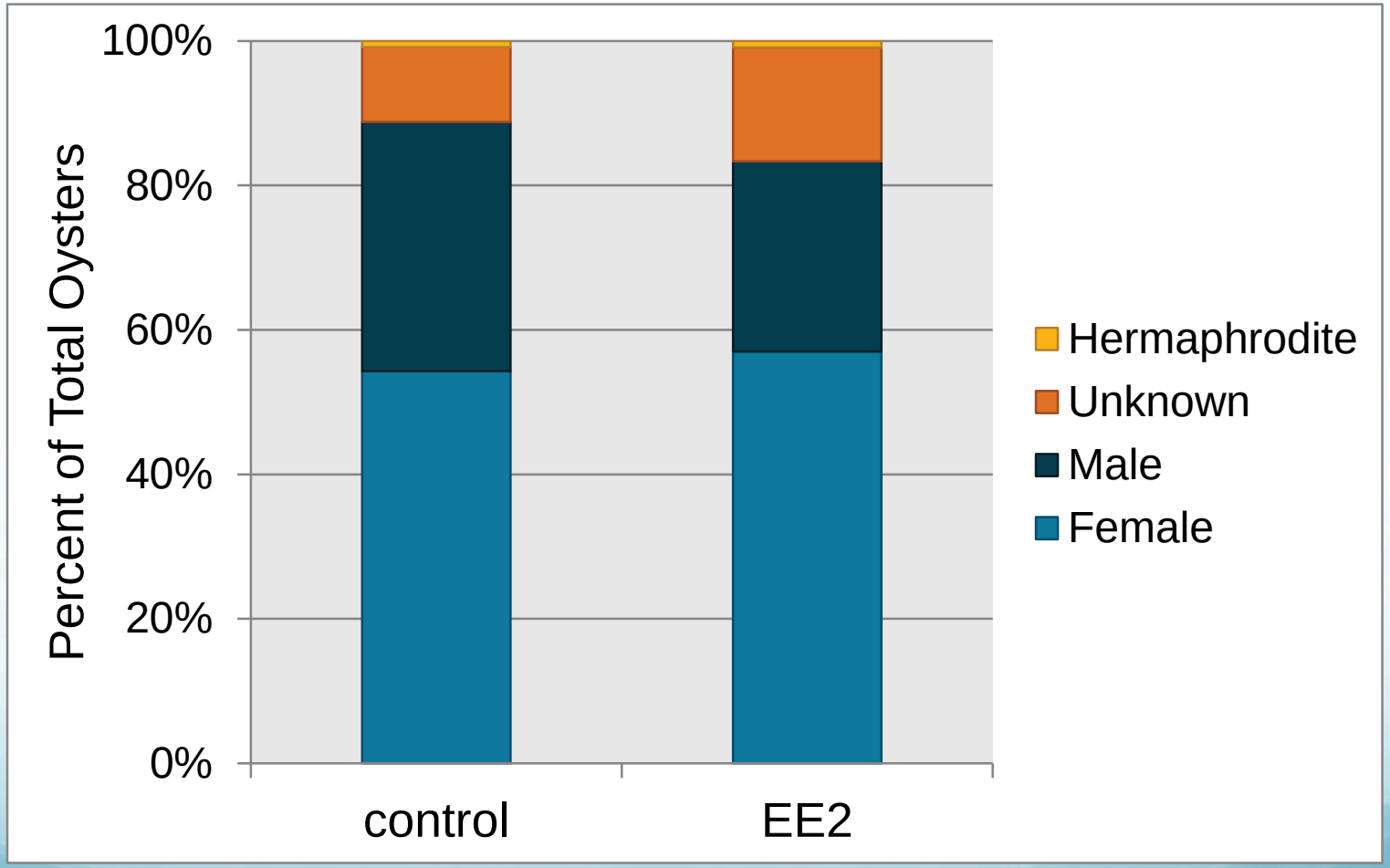
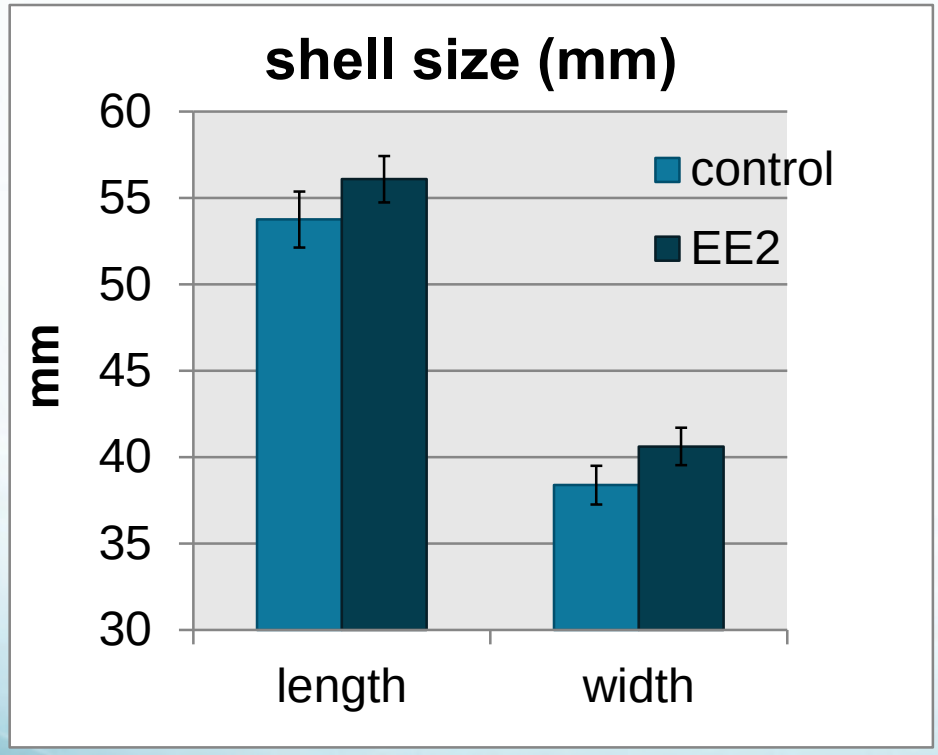
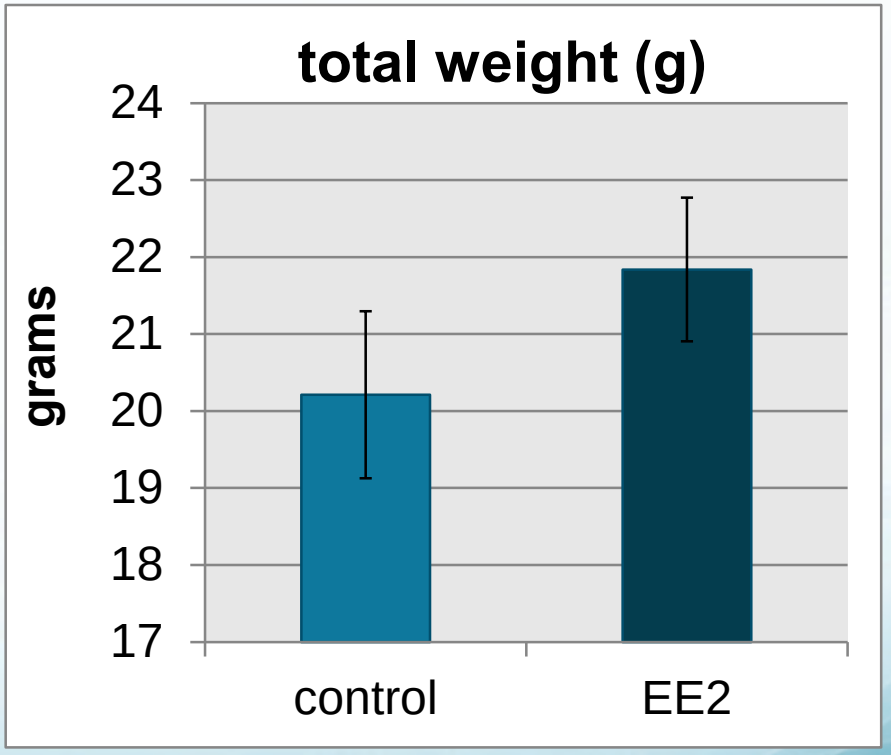
Results: Day 60 sex determination

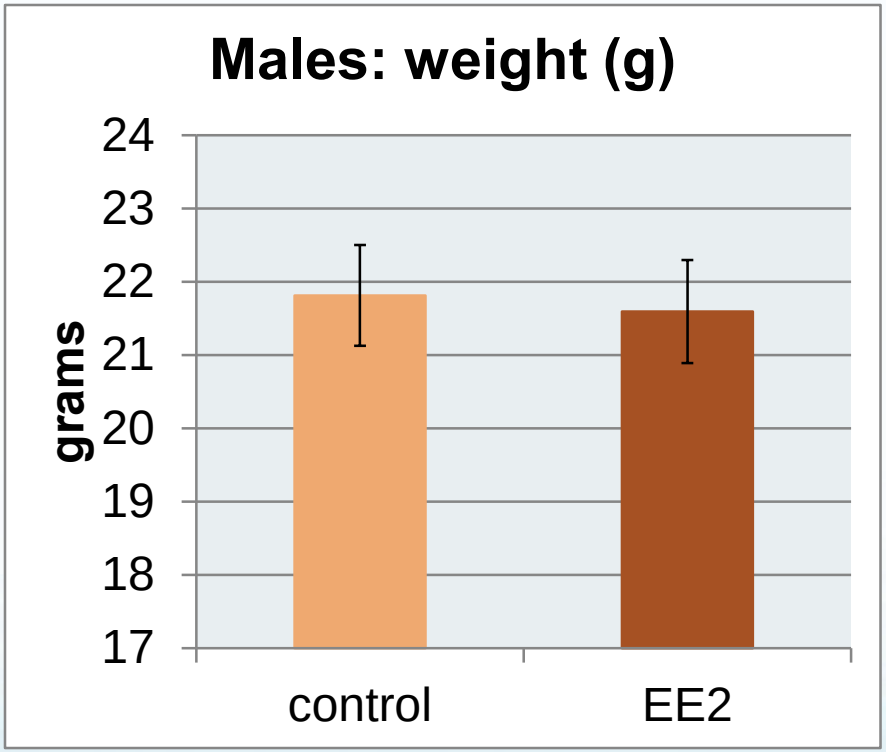


Results: Day 60 size of females

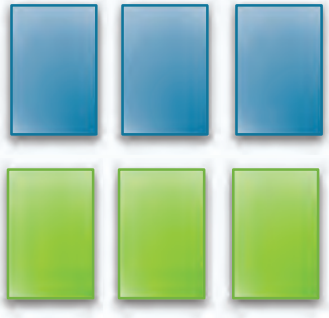




Results: Day 60 size of males



Results: DNA methylation



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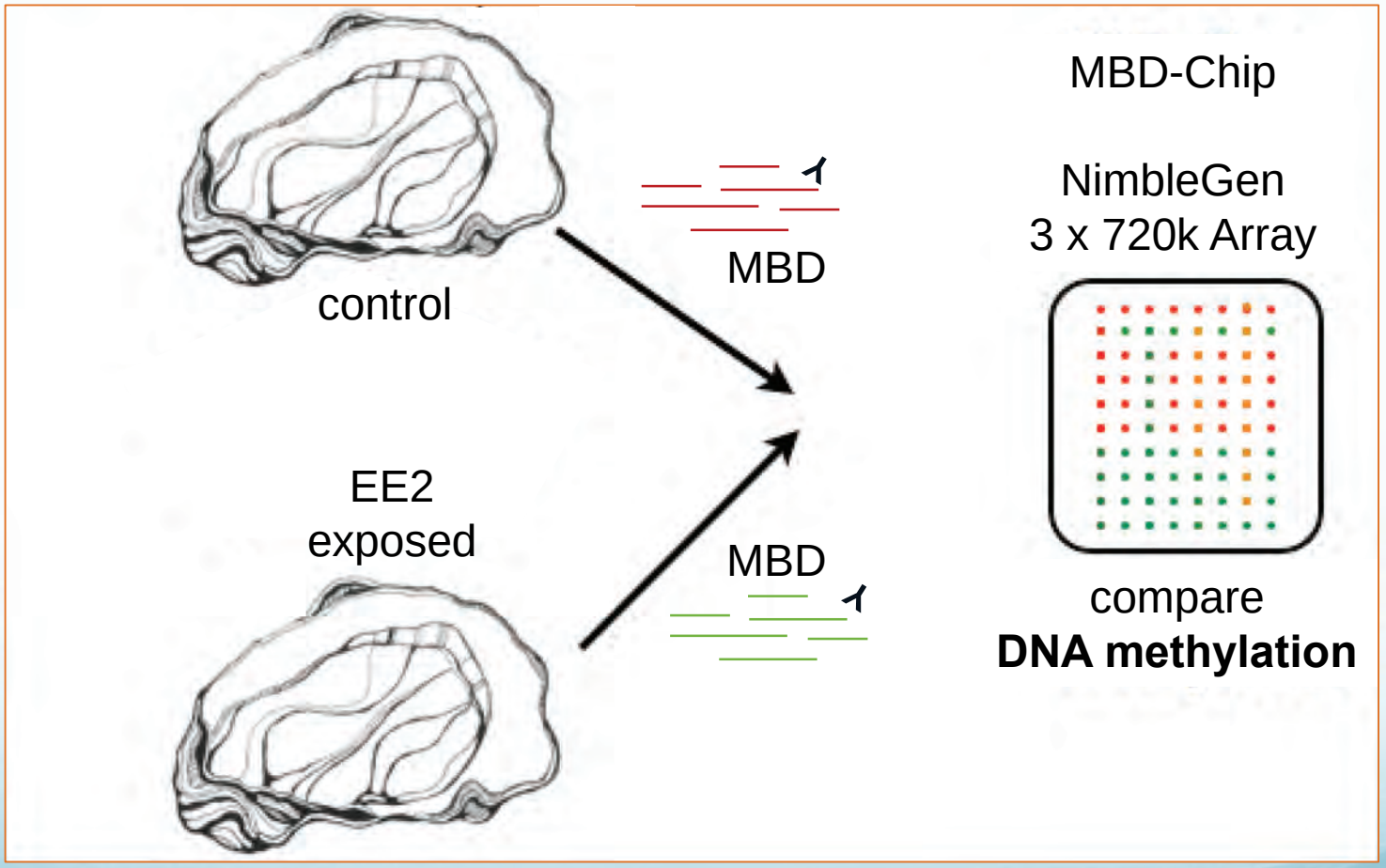

Results: DNA methylation

- 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

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
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- 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:
  Steven Roberts
  Samuel White
  Emma Timmins-Schiffman
  Claire Ellis
  Brent Vadopalas
  Jake Heare

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  Molly Jackson

- Irv Shultz (Battelle PNNL)