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July 6, 2016

MEMORANDUM

TO: **Council members**

FROM: **Mark Fritsch, project implementation manager**

SUBJECT: **Briefing on domestication selection and epigenetic mechanisms to reduce fitness loss in hatchery steelhead.**

BACKGROUND:

Presenter: Dr. Penny Swanson (Supervisory Research Physiologist, Program Manager for the Environmental Physiology Program) and Dr. Barry Berejikian (Supervisory Research Fisheries Biologist, Acting Program Manager for the Hatchery Reform Science Program) of NOAA's Northwest Fisheries Science Center.

Summary: Hatchery salmon and steelhead populations suffer fitness loss (lower capacity to reproduce) in the natural environment. Domestication selection (adaptation to the hatchery environment) has been implicated as a primary mechanism causing fitness loss in hatchery populations. The presentation will discuss progress towards reducing domestication selection through practical changes to hatchery rearing environments and evaluate the potential for heritable epigenetic change in hatchery populations. Epigenetics addresses the question of how modification to the structure of DNA influences individual traits. We have identified epigenetic differences between hatchery and natural steelhead from the Methow River Basin. Important remaining questions include: Does this happen in a single generation? How do epigenetic changes affect gene expression and ultimately fitness? What aspects of the hatchery environments influence DNA methylation? To what extent are DNA methylation patterns inherited across generations? The goal is to improve the fitness of hatchery populations spawning in nature, so that existing management frameworks (e.g., Columbia River Hatchery Reform) can better contribute to recovery.

More Info: Please see attached presentation slides

NOAA FISHERIES

Northwest
Fisheries
Science Center

Understanding domestication selection and epigenetic mechanisms to reduce fitness loss in hatchery steelhead

Presenters: Penny Swanson, Barry Berejikian

Chris Tatara
Jeff Hard
Don Van Doornik
Mackenzie Gavery
Krista Nichols
Don Larsen
Mollie Middleton (UW)
Jon Dickey (UW)

July 13, 2016

In collaboration with the USFWS

BPA Project: 1993-056-00 – Research to advance hatchery reform

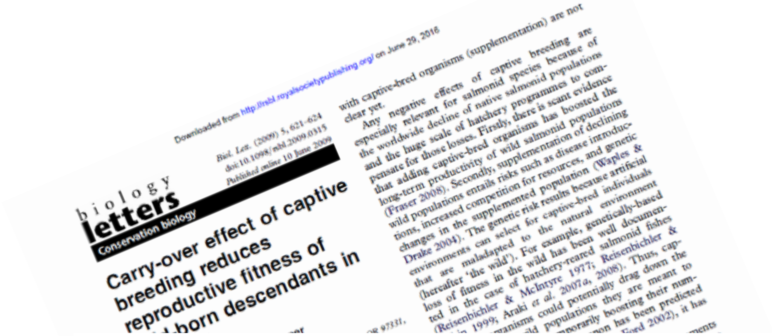
Topics

- Fitness loss in hatchery salmon and steelhead

- Current management approach

- What causes heritable fitness loss?
  - Domestication selection
  - Epigenetic mechanisms

Problem: Fitness loss in steelhead can be heritable



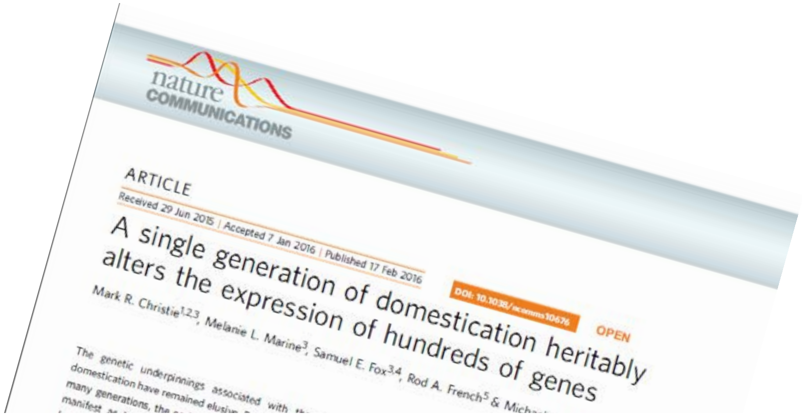
Biol. Lett. (2009) 5, 621–624
doi:10.1098/rsbl.2009.0315
Published online 10 June 2009

biology letters
Conservation biology

Carry-over effect of captive breeding reduces reproductive fitness of wild-born descendants in

with captive-bred organisms (supplementation) are not clear yet.

Any negative effects of captive breeding are especially relevant for salmonid species because of the worldwide decline of native salmonid populations and the huge scale of hatchery programmes to compensate for those losses. Firstly, there is scant evidence that adding captive-bred organisms has boosted the long-term productivity of wild salmonid populations (Fraser 2008). Secondly, supplementation of declining wild populations entails risks such as disease introductions, increased competition for resources, and genetic changes in the supplemented population (Waples & Drake 2004). The genetic risk results because artificial environments can select for captive-bred individuals that are maladapted to the natural environment (hereafter 'the wild'). For example, genetically-based loss of fitness in the wild has been well documented in the case of hatchery-reared salmonid fishes (Reisenbichler & McIntyre 1977; Reisenbichler & Rubin 1999; Araki et al. 2007a, 2008). Thus, captive-bred organisms could potentially drag down the wild populations they are meant to temporarily boosting their numbers


nature COMMUNICATIONS

ARTICLE

Received 29 Jun 2015 | Accepted 7 Jan 2016 | Published 17 Feb 2016

DOI: 10.1038/ncomms10676 OPEN

A single generation of domestication heritably alters the expression of hundreds of genes

Mark R. Christie[1,2,3], Melanie L. Marine[3], Samuel E. Fox[3,4], Rod A. French[5] & Micha

These differences in fitness and gene expression could be due to inherited genetic and/or epigenetic differences


captive-bred and one wild parents). Our

which has negative influence on the size

period.

Keywords: captive breeding; reproductive success; parentage; steelhead trout

1. INTRODUCTION

Many conservation programmes now employ captive breeding to aid endangered and threatened species (Olney et al. 1994; Frankham et al. 2002; Allendorf & Luikart 2007). It is estimated that thousands of species will require captive breeding to prevent their extinction over the next 200 years (Allendorf & Luikart 2007). However, the effects of supplementing wild populations

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2009.0315 or via http://rsbl.royalsocietypublishing.org.

Received 26 April 2009
Accepted 18 May 2009

(figure 1). This

owing to any carry-over effect

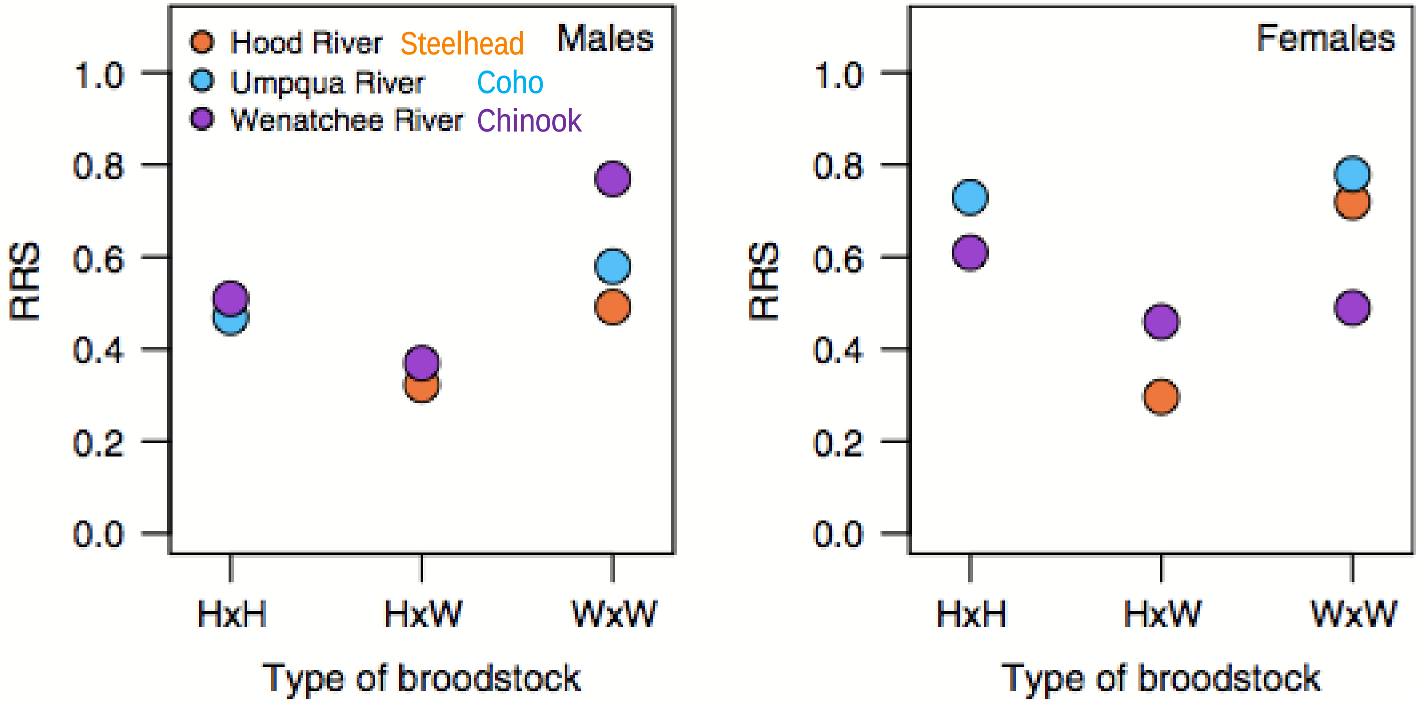
2. MATERIAL AND METHODS

621

NPCC's Fish Propagation Theme

PRIORITY UNCERTAINTY: Because supplementation of salmonid populations is a widely used management strategy its effects on natural populations need to be understood. Important questions remain about how deleterious genetic changes may occur in cultured populations.

Fitness loss in salmon and steelhead



Christie et al. 2014. Evolutionary Applications [Volume 7, Issue 8,]pages 883-896, 9 JUL 2014 DOI: 10.1111/eva.12183

HATCHERY SCIENTIFIC REVIEW GROUP

REPORT TO CONGRESS ON THE
SCIENCE OF HATCHERIES:

An updated persepctive on the role of hatcheries in salmon and steelhead management in the Pacific Northwest

June 2014

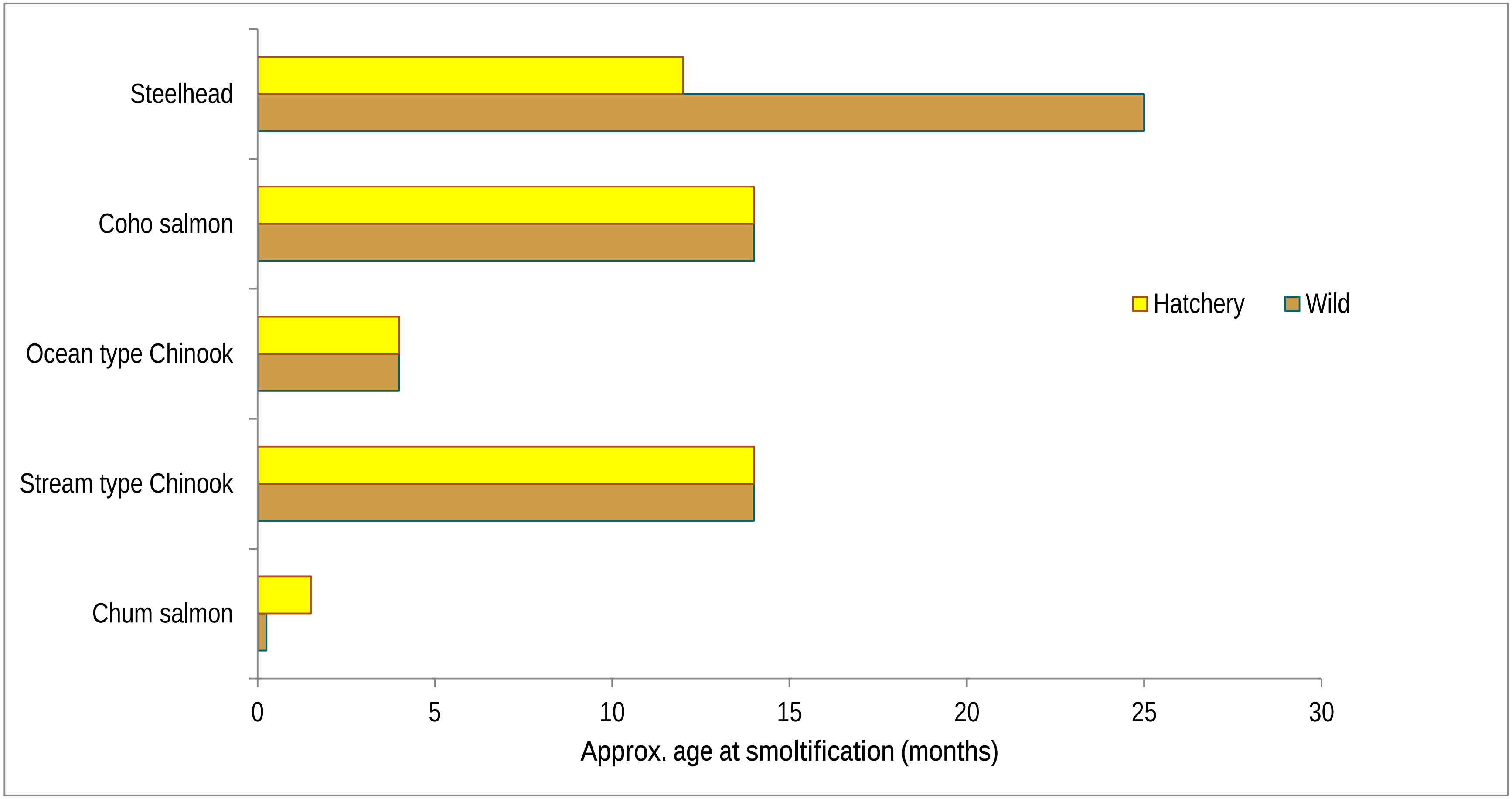
Hatchery Scientific Review Group
Pacific Salmon Hatchery Reform

- Broodstock management
- Harvest management
- Assumes genetic fitness loss
- Managing around the problem
- Challenges:
    - Segregation is very difficult to achieve
    - Integration requires robust natural populations

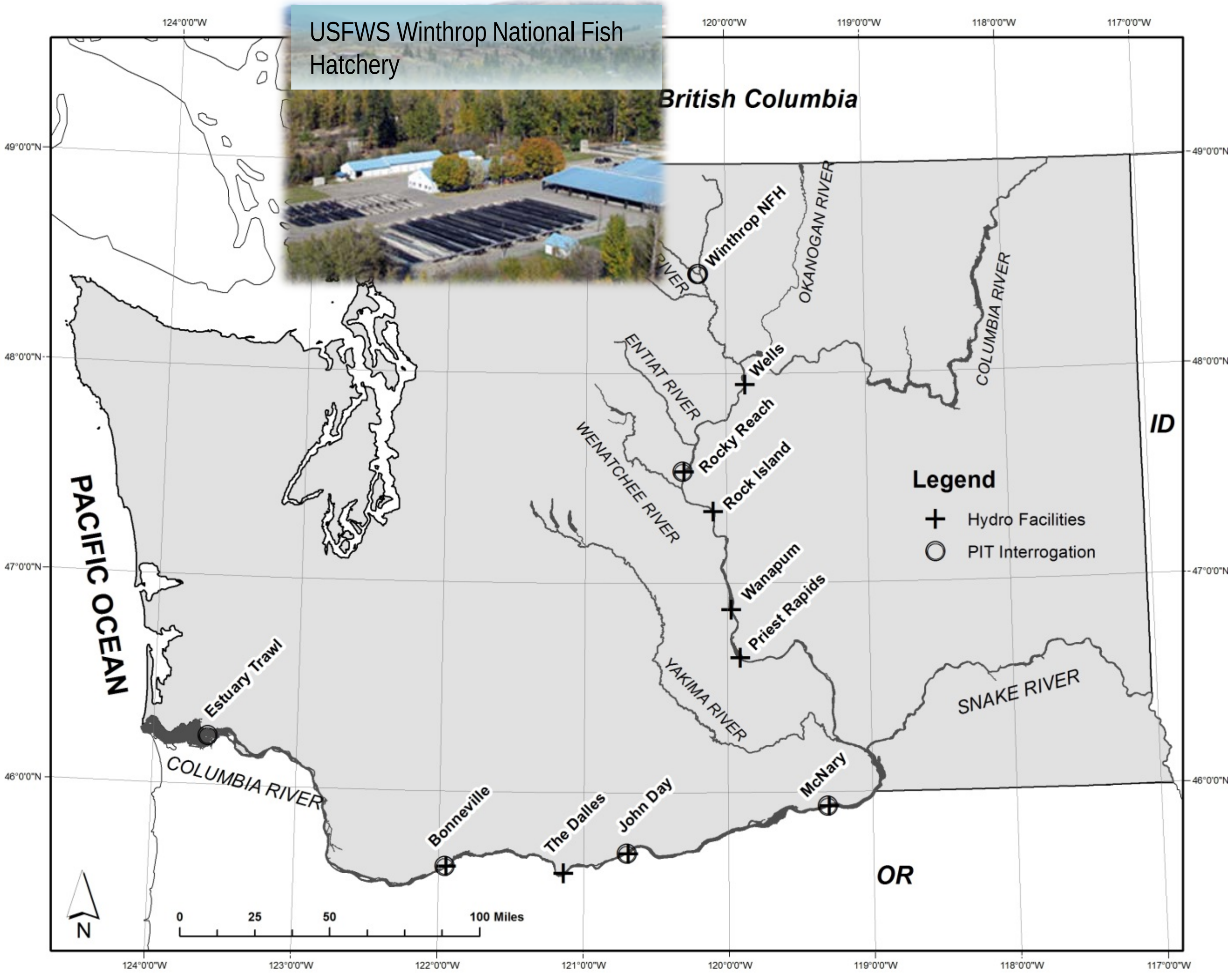
Potential causes of heritable fitness loss

- Deleterious mutations
- Inbreeding depression
- Genetic drift
- Domestication selection: adaptation to the hatchery environment
  - Selection on traits in captivity (or after release) that are different than what would occur in the natural environment
  - Heritability for the trait
  - Growth rate (and correlated traits: aggression, risk-taking, metabolic rate)

Why is growth rate important?



Natural-origin (wild) broodstock program in the Methow River

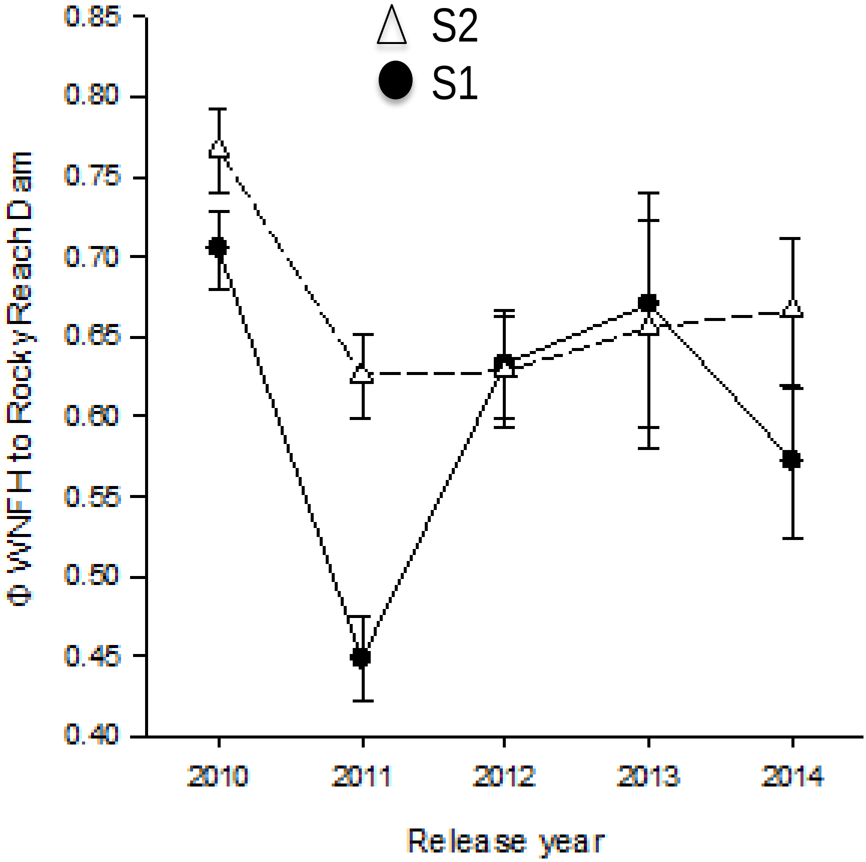


USFWS Winthrop National Fish Hatchery

Tatara et al. in prep

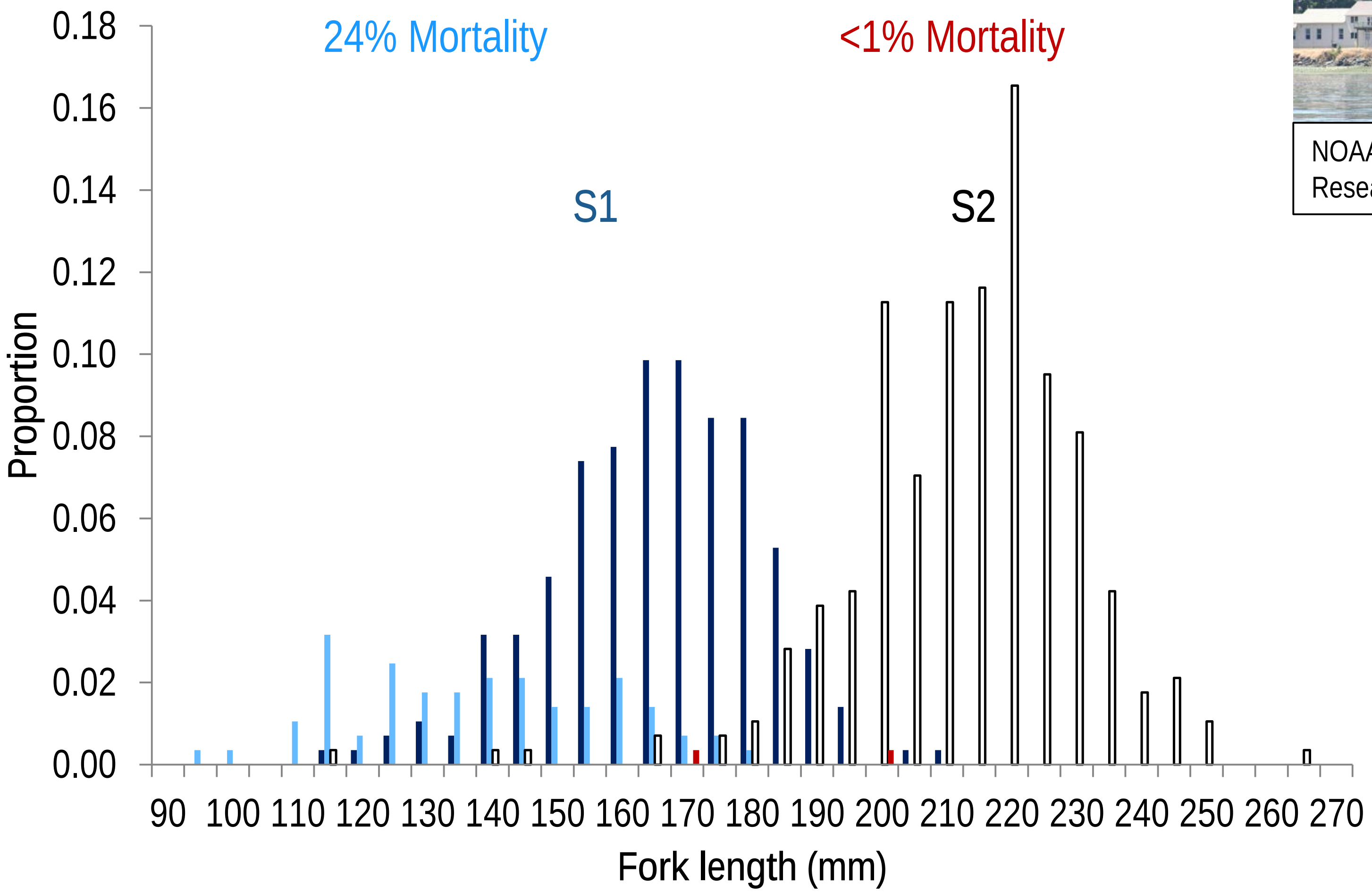
Size affects downstream migration

Release year	Rearing type	Fork Length	Mass (g)
2011	S1	158.3 (35.1)	48.2 (30.0)
	S2	186.6 (25.7)	72.3 (29.2)
2012	S1	172.2 (28.4)	60.6 (25.4)
	S2	185.5 (21.7)	70.0 (22.8)
2013	S1	195.1 (21.6)	82.6 (26.0)
	S2	193.7 (22.0)	83.6 (25.9)
2014	S1	177.2 (23.4)	62.8 (22.3)
	S2	190.6 (18.6)	76.0 (20.5)



Tatara et al. in prep.

Body size and seawater survival

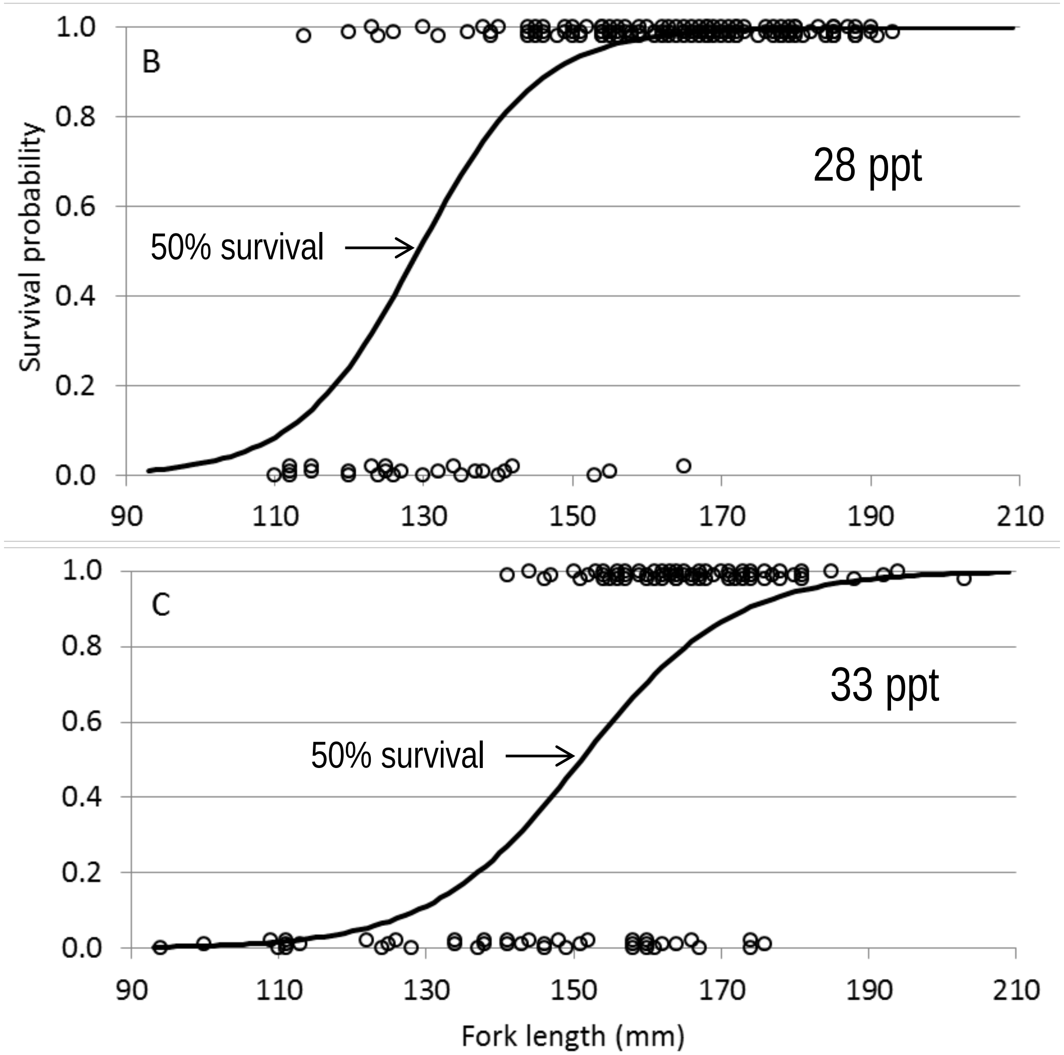


NOAA, NWFSC, Manchester Research Station

NOAA FISHERIES

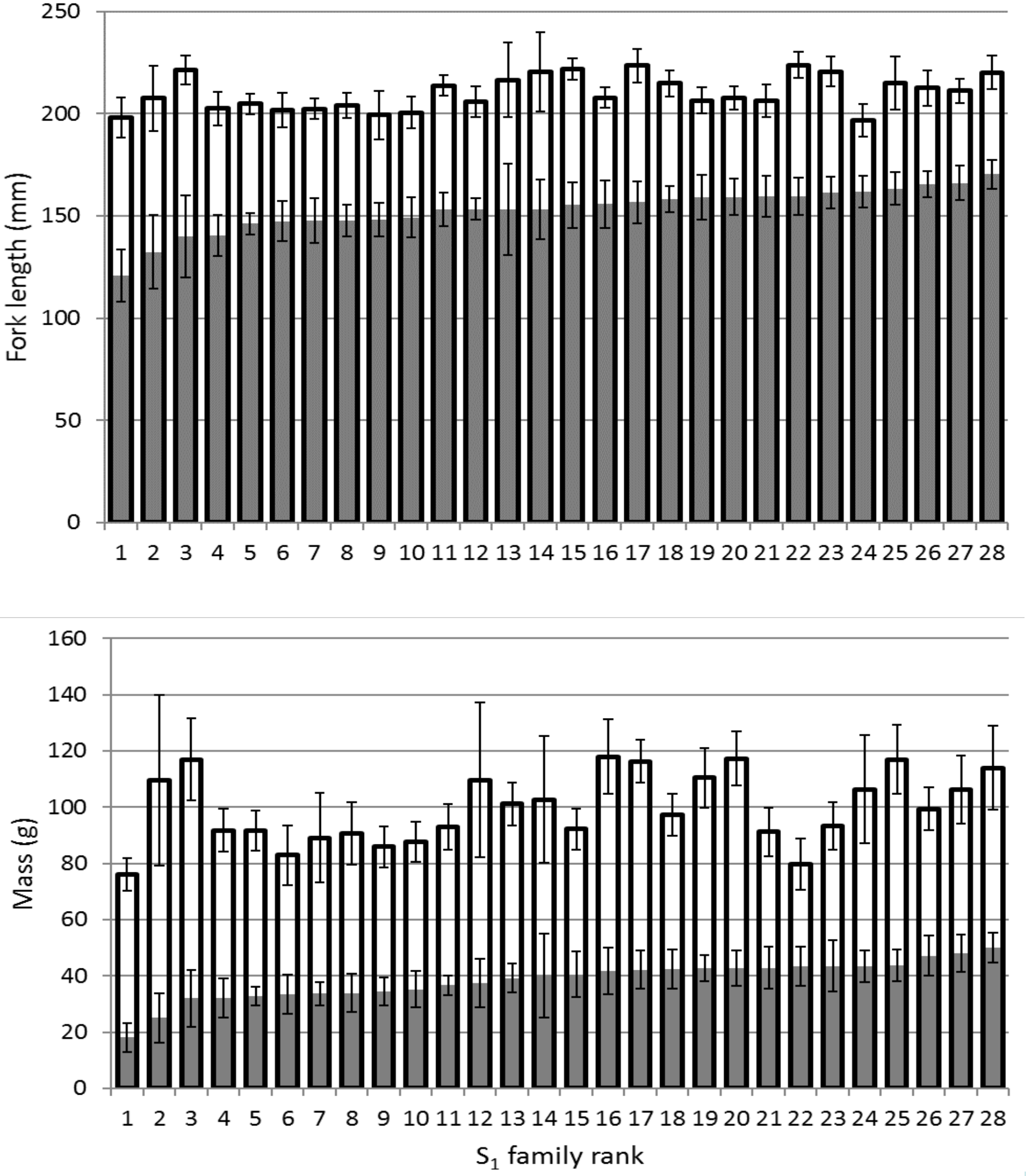
Berejikian et al. accepted. Can. J. Fish. Aquat. Sci

Size-selective mortality in S1 steelhead smolts



Berejikian et al. accepted. Can. J. Fish. Aquat. Sci

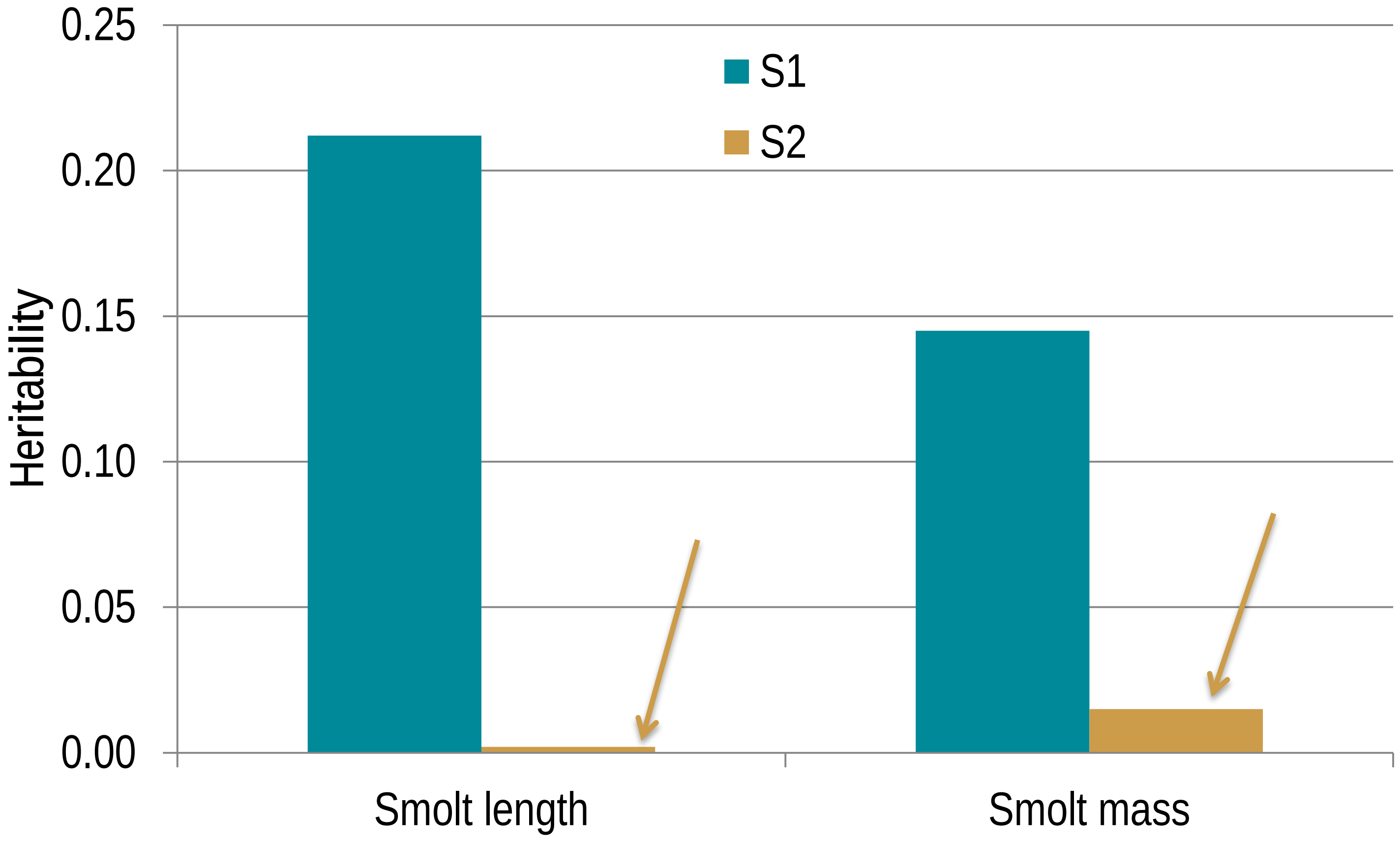
Body size varies among families



Berejikian et al. accepted. Can. J. Fish. Aquat. Sci.

Heritability of body size at release

Within a population, how much of the variability is due to inherited differences (range 0 to 1)?



Summary

- Body size at the time of release from hatcheries influences
  - ➢ Downstream migration rate
  - ➢ Survival in seawater challenge

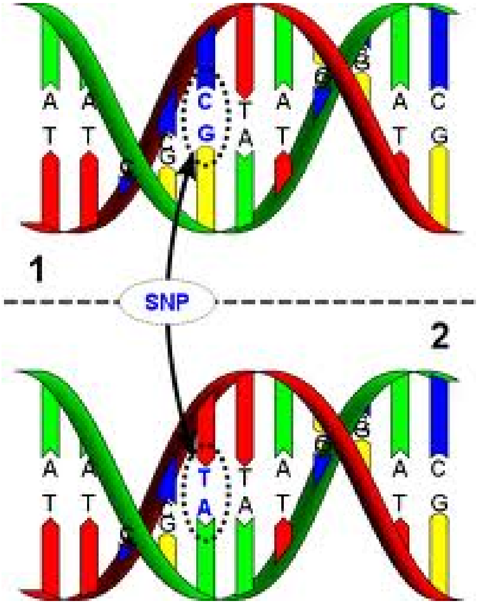
- In S1 rearing regime
  - ➢ Size-selective mortality
  - ➢ Response to selection is possible

- In S2 rearing regime
  - ➢ No size-selective mortality
  - ➢ No possible response to selection (low heritability)

- **Practical changes to hatchery practices show promise for reducing domestication selection**

Next steps: Genetic basis of metabolism, growth and survival in natural and hatchery environments

Credit: Florian Graner



Potential involvement of epigenetic mechanisms in fitness loss of hatchery fish

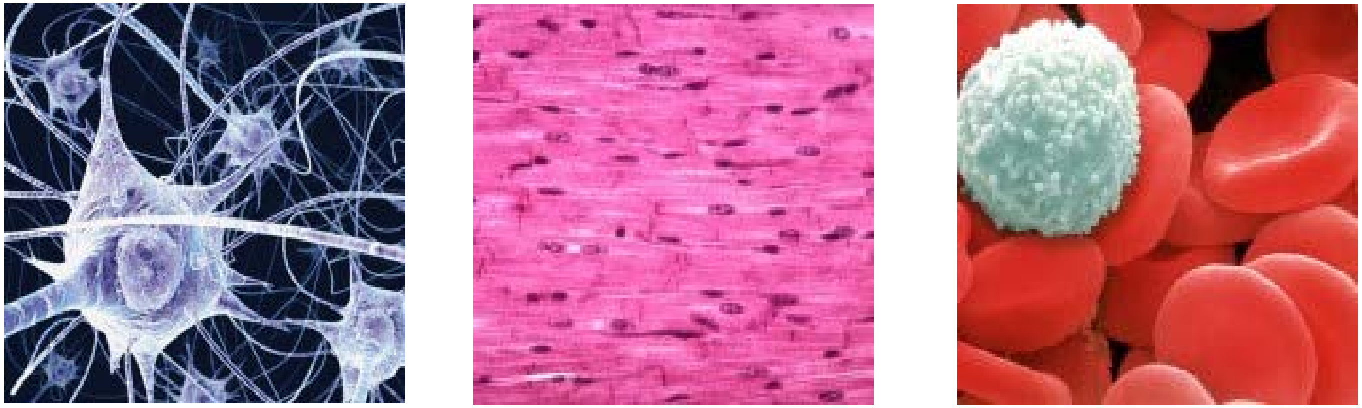
- What can cause heritable fitness loss?
  - Domestication selection
  - Epigenetic mechanisms

How is epigenetics different than genetics?

Hardware vs Software

- Genome (DNA sequence) = Hardware
- Epigenome (structure around DNA) = Software
- Each cell type has the same hardware, but different software
- Programming of software happens during development
- Environmental factors can modify software
    - Can modify function of cell (normal or pathological)
    - Can change normal cell into cancer cell
    - If it happens when egg or sperm are formed, has potential to pass onto offspring

If all cells in an organism have the same DNA, how are only the right genes expressed in a given cell type?

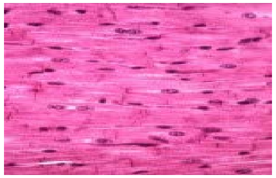


Genotype = DNA sequence (hardware)

Phenotype = characteristics of an individual, trait

Epigenetic Inheritance

Mitotic inheritance (as cells divide): passed through cell lineage, epigenetic marks differ by cell type



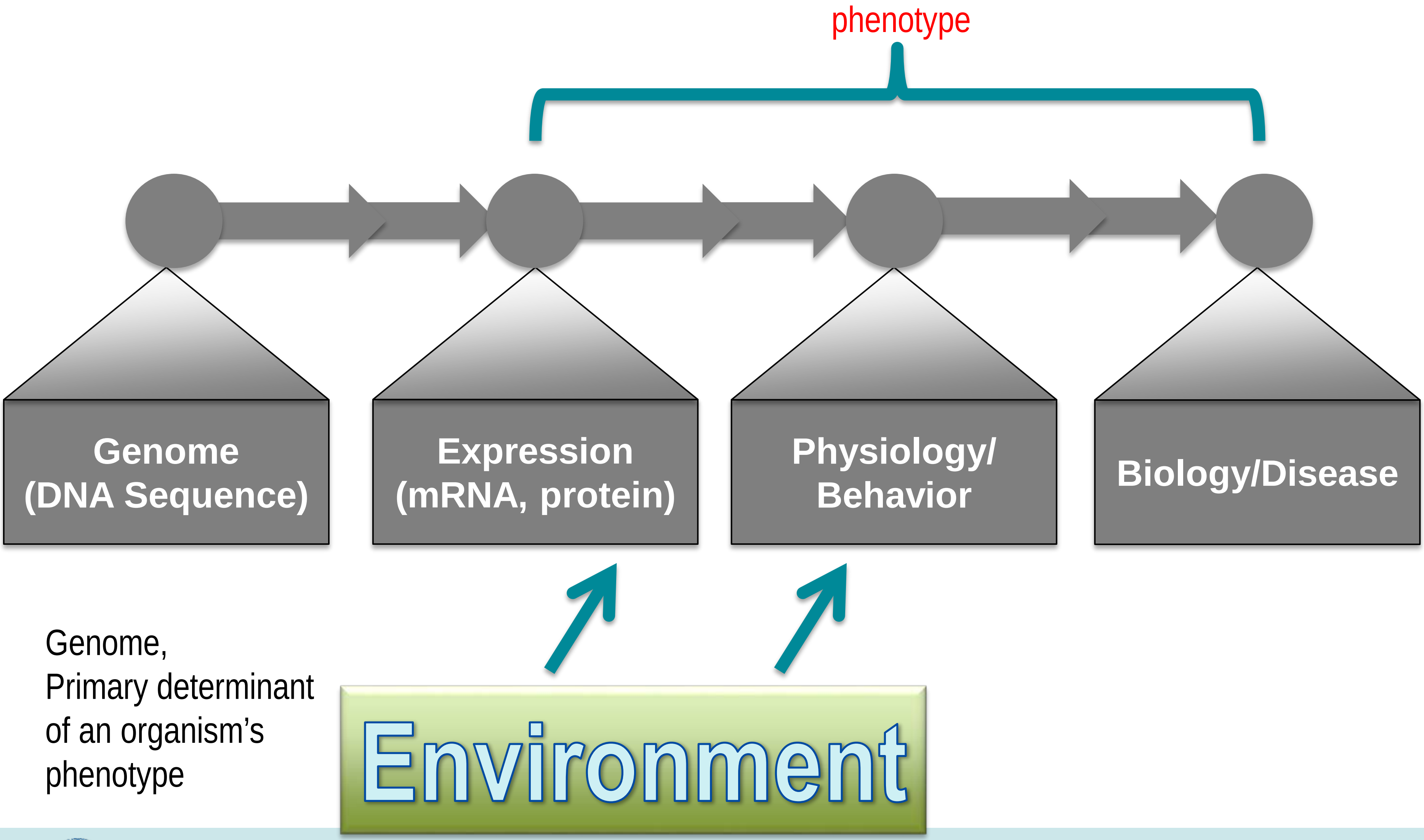
Transgenerational inheritance (from parent to offspring): passed through egg or sperm

- Occurs at critical stages of germ cell development
- phenotype can be expressed at any point in life cycle

How is the epigenome different than genome?

- Dynamic
- Responsive to environment
- May or may not be heritable across generations
- Provides a mechanism for rapid adaptation to environmental change

Genetic + Environment → Phenotype



phenotype

Genome (DNA Sequence)

Expression (mRNA, protein)

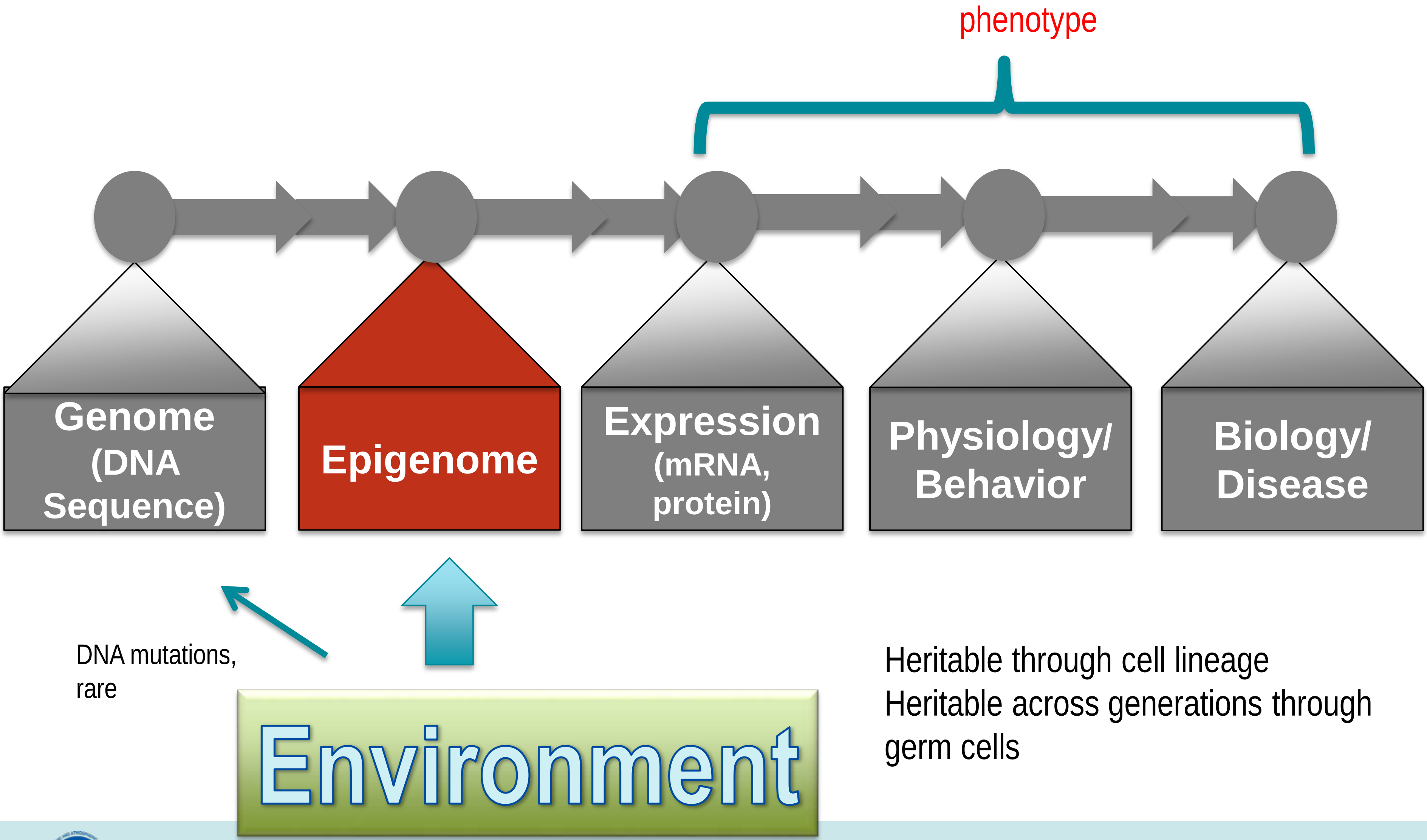
Physiology/ Behavior

Biology/Disease

Environment

Genome,
Primary determinant
of an organism's
phenotype

Heritable changes that affect phenotype



phenotype

Genome (DNA Sequence)

Epigenome

Expression (mRNA, protein)

Physiology/ Behavior

Biology/ Disease

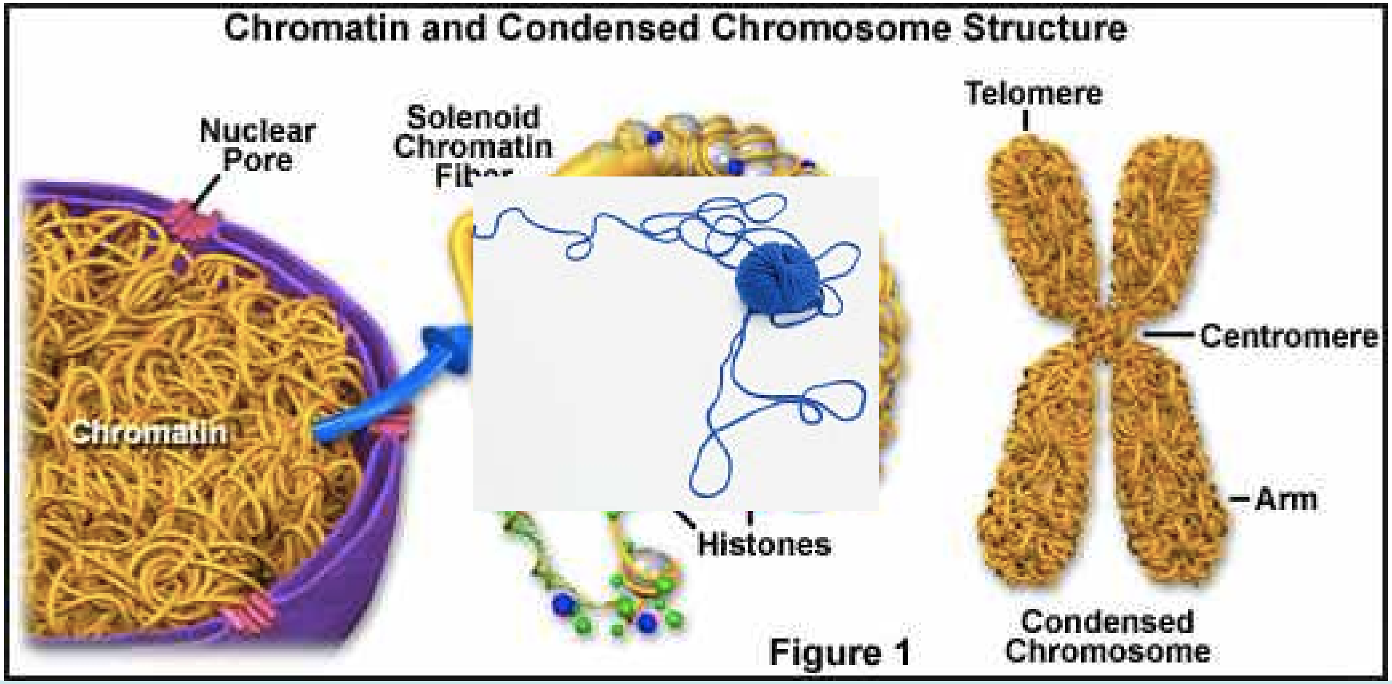
DNA mutations, rare

Environment

Heritable through cell lineage
Heritable across generations through germ cells

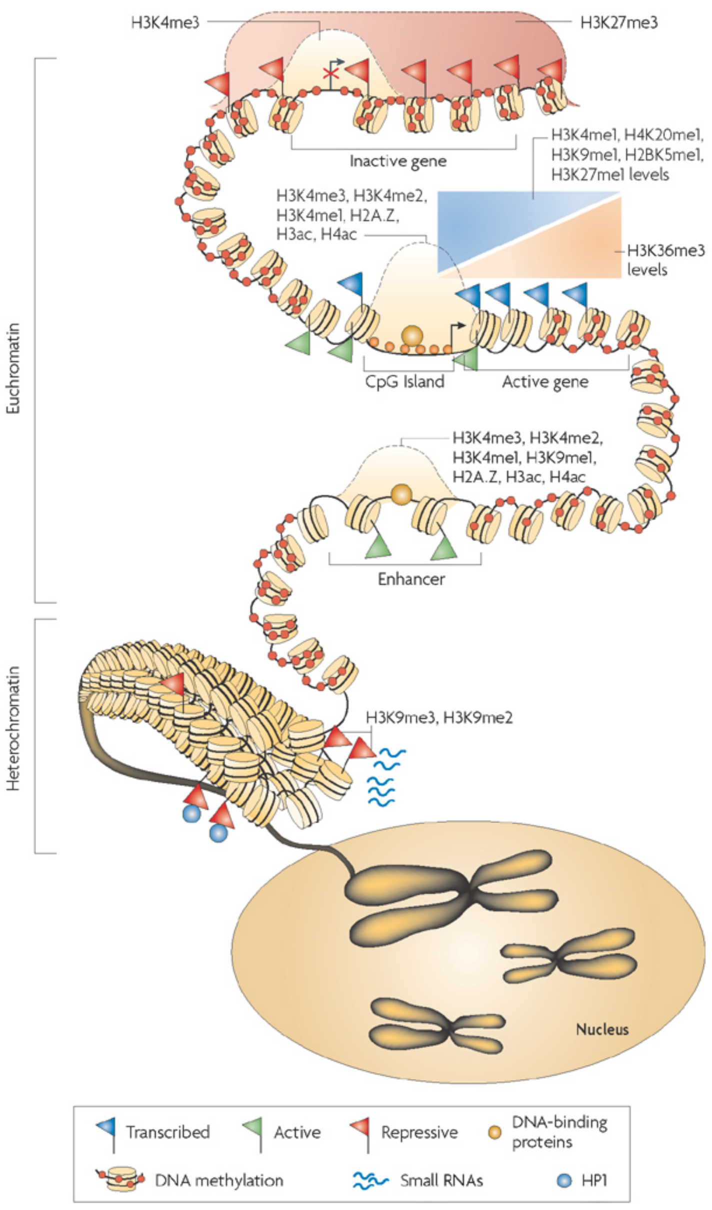
Most of the genome is inaccessible

- Nearly 6 ft of DNA is packed into the nucleus of every cell
- DNA structure must be opened up for genes to be expressed



Epigenetic Mechanisms of Gene Regulation

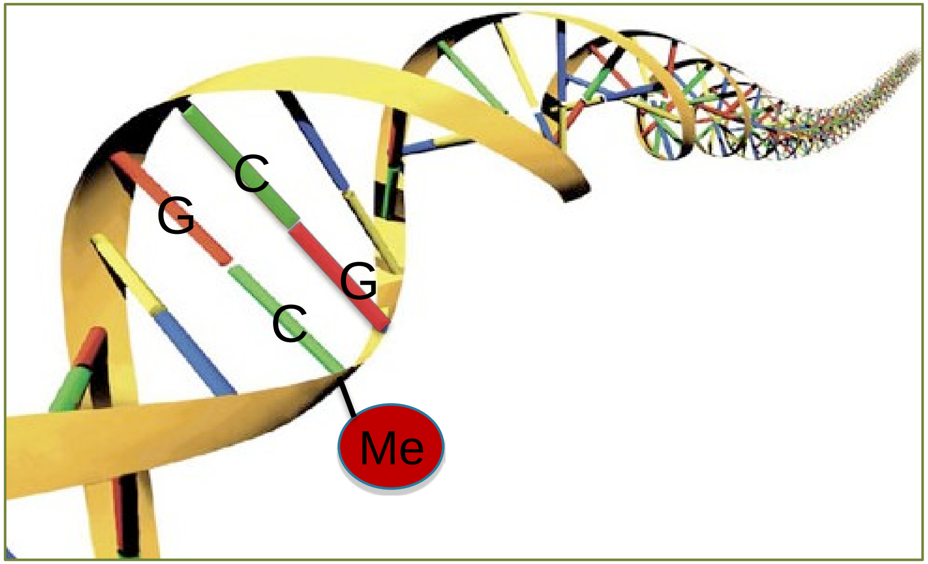
- **DNA methylation**
- Histone modifications
- Chromatin Structure
- DNA organization into domains (loops)
- Nuclear compartmentalization (e.g. nuclear matrix)
- Noncoding functional RNAs

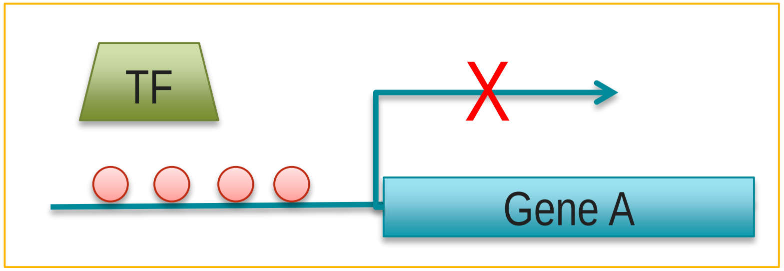


Nature Reviews | Genetics

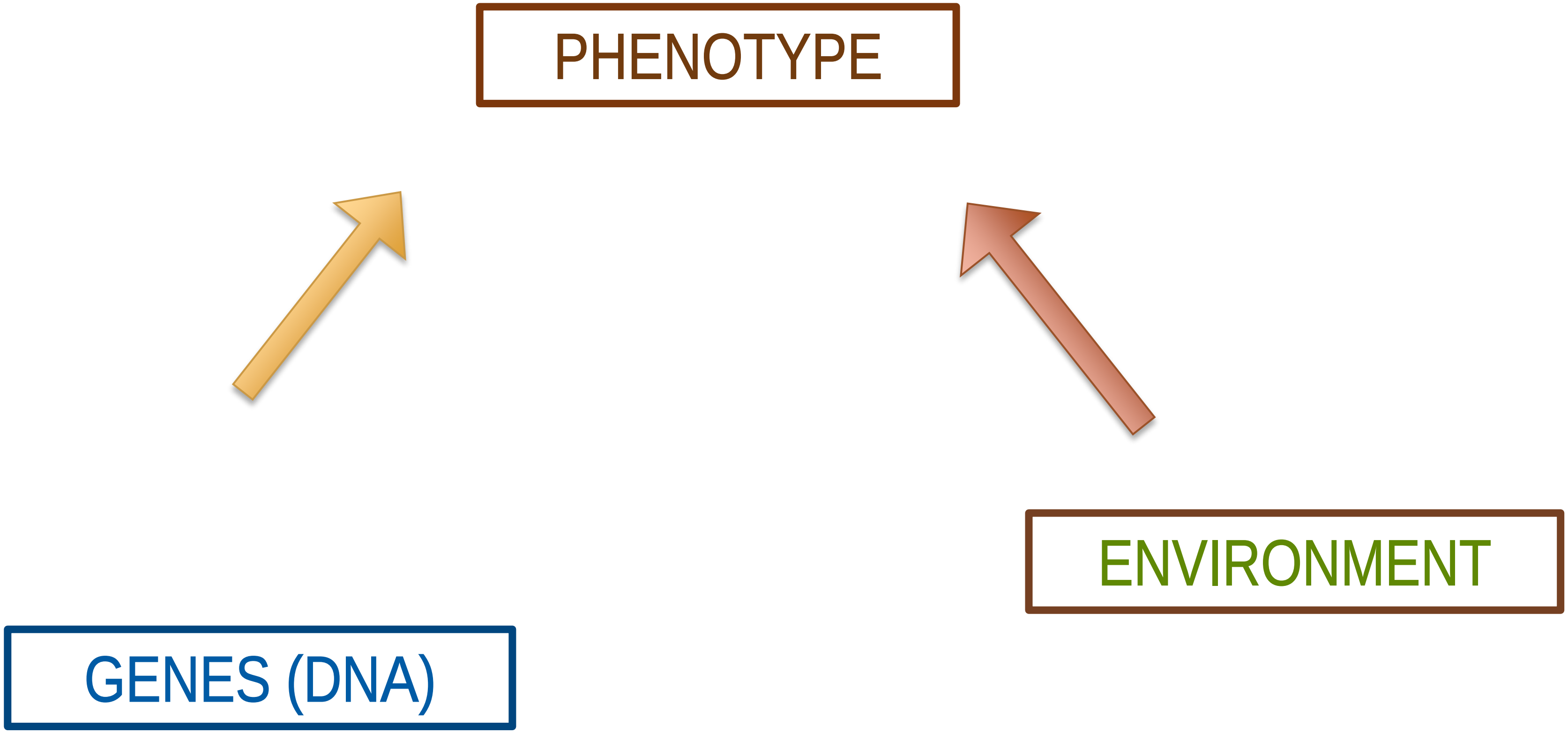
DNA Methylation

- Most well understood epigenetic mechanism is DNA methylation





DNA methylation & environment

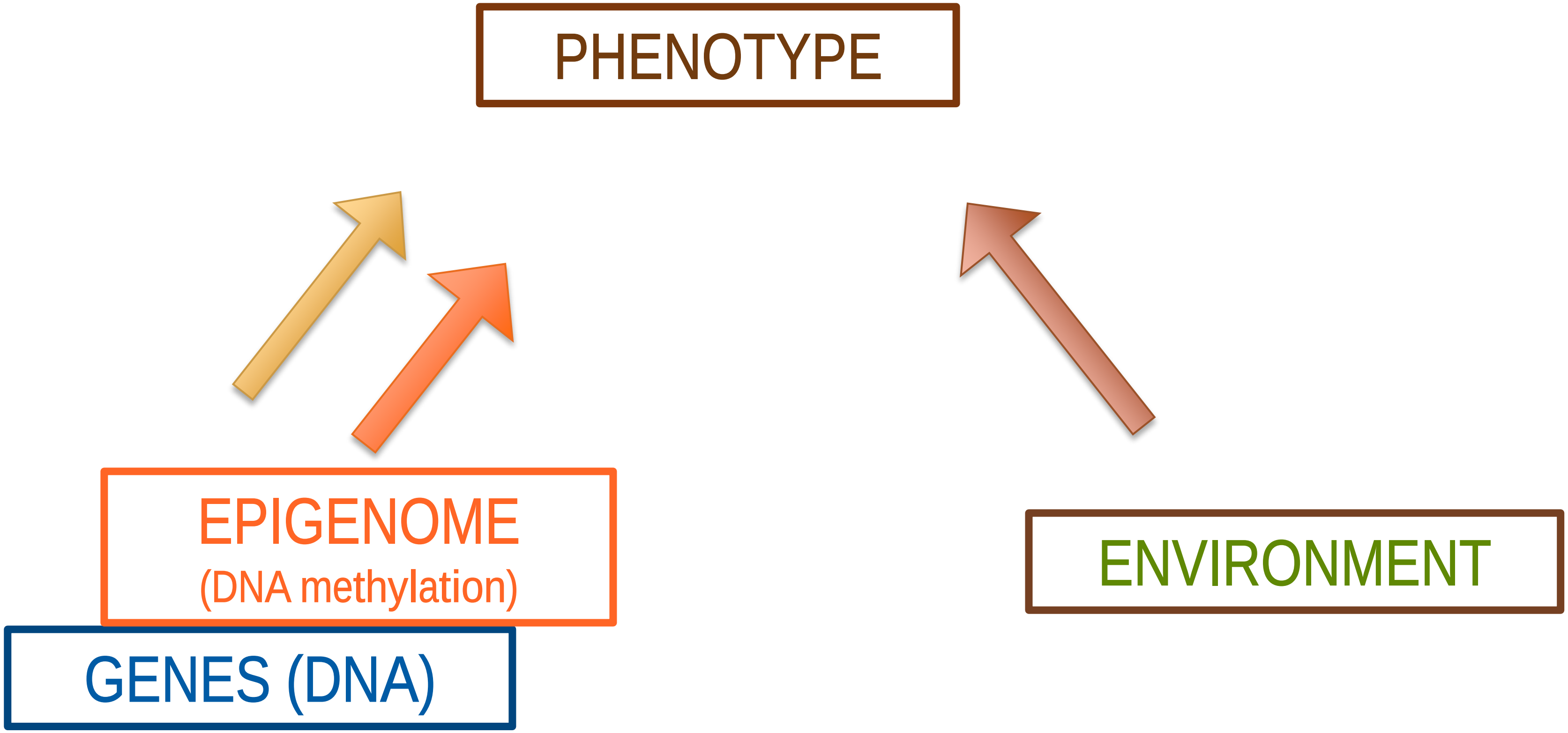


PHENOTYPE

ENVIRONMENT

GENES (DNA)

DNA methylation & environment



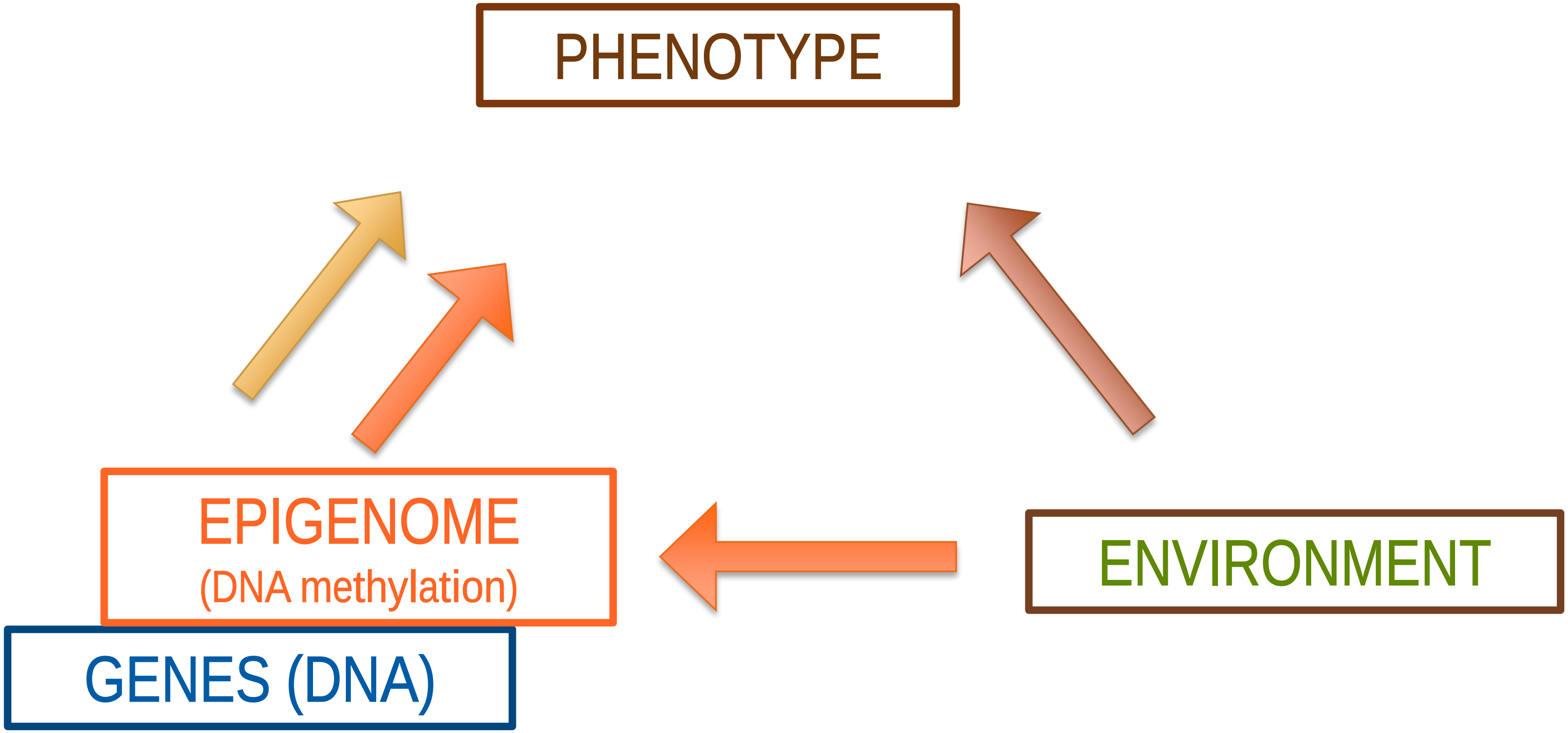
PHENOTYPE

EPIGENOME
(DNA methylation)

GENES (DNA)

ENVIRONMENT

DNA methylation & environment



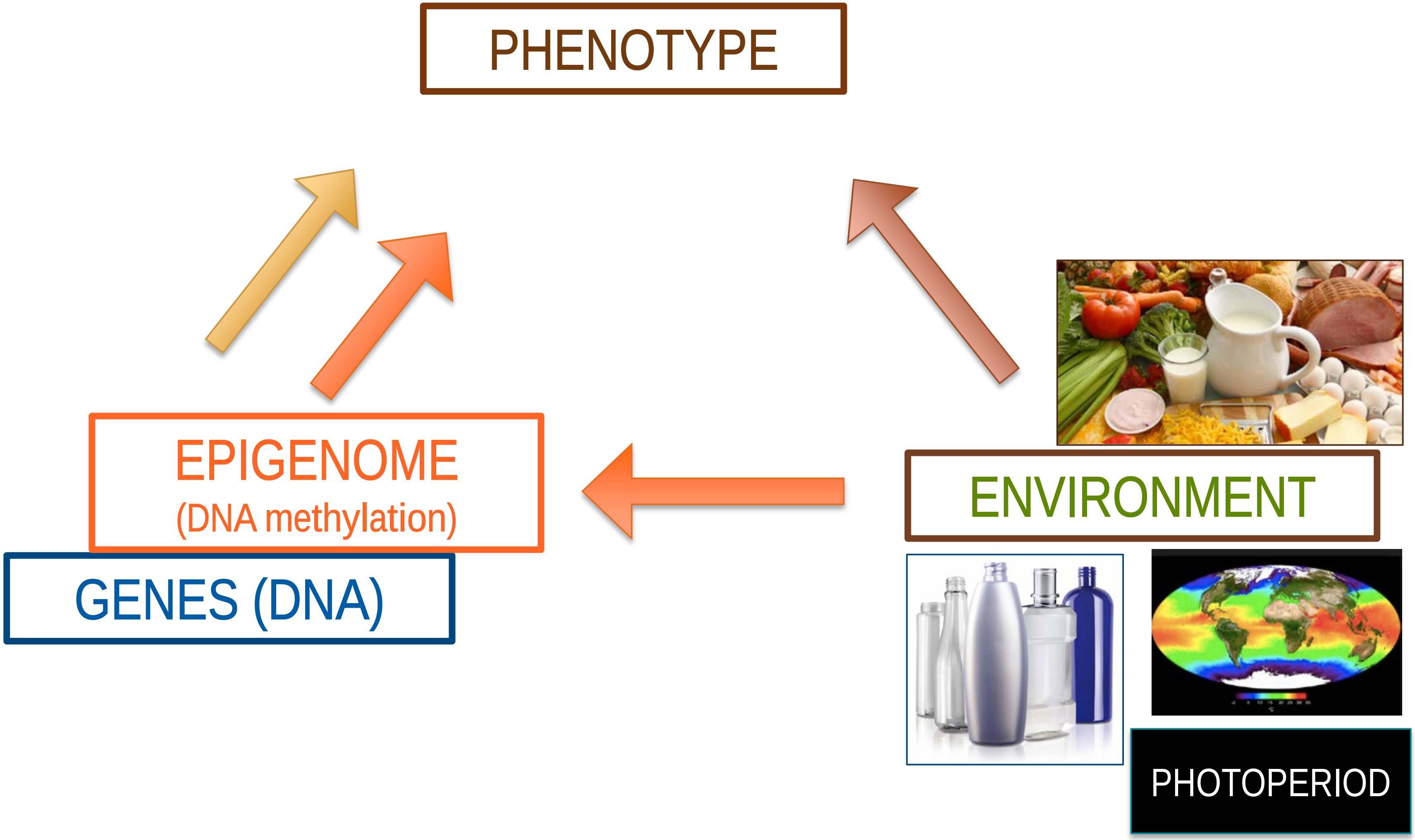
PHENOTYPE

EPIGENOME
(DNA methylation)

GENES (DNA)

ENVIRONMENT

DNA methylation & environment



PHENOTYPE

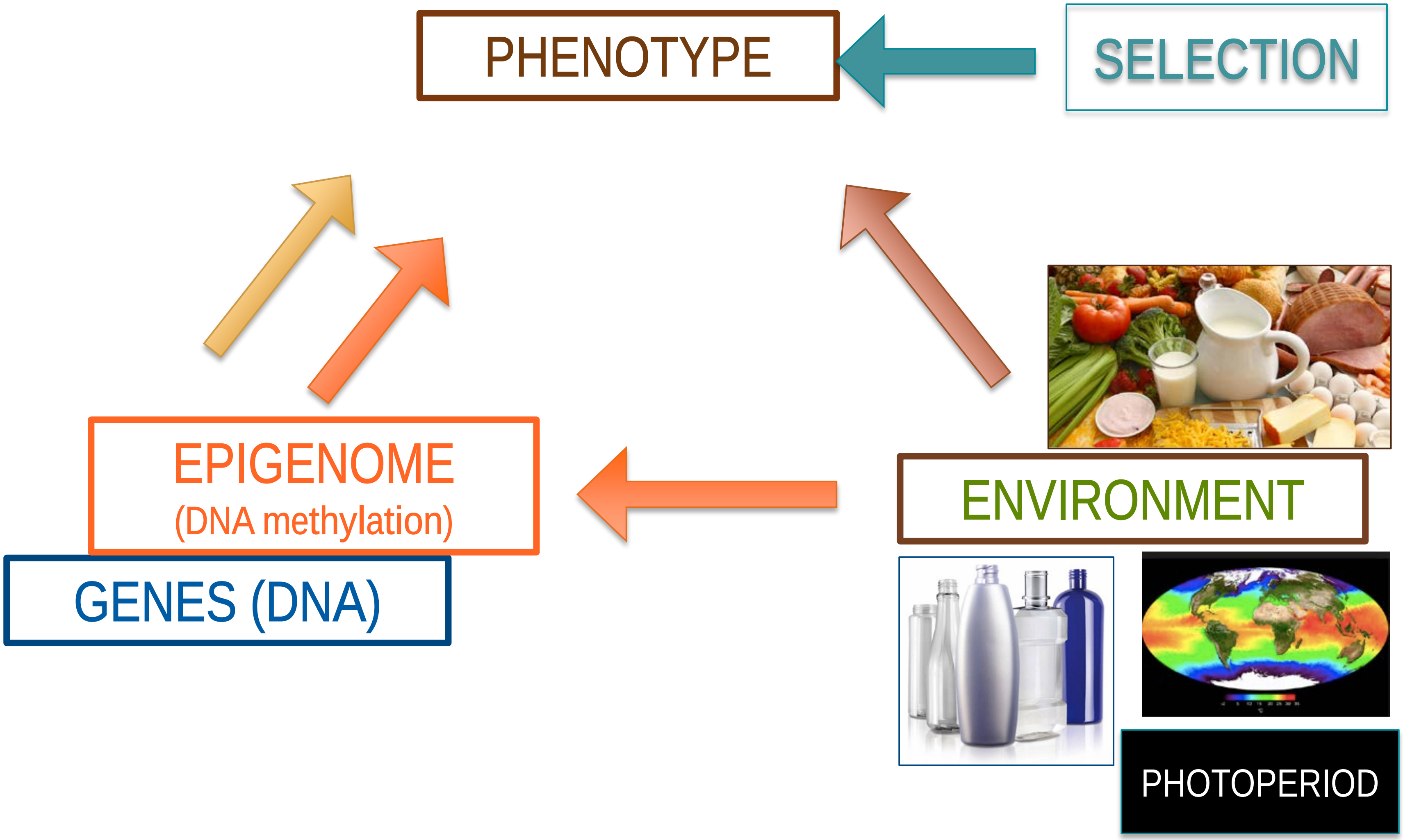
EPIGENOME
(DNA methylation)

GENES (DNA)

ENVIRONMENT

PHOTOPERIOD

DNA methylation & environment



PHENOTYPE

SELECTION

EPIGENOME
(DNA methylation)

GENES (DNA)

ENVIRONMENT

PHOTOPERIOD

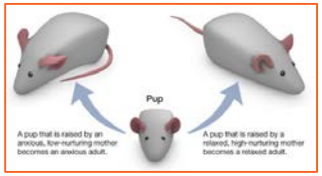
Examples of change in DNA methylation associated with phenotypic change

- Temperature- sex change
- Aging- twins
- Domestication-stress response
- Behavior- maternal care, anxiety
- Nutrition/Toxins-coat color, obesity


PLoS Genet 7: e1002447


PNAS (2005)102:10604-10609

BMC Genomics (2012) 13: 59



http://learn.genetics.utah.edu/content/epigenetics/rats/

Nature Neuroscience (2004) 7:847–854


Environ. Health Perspect (2006)114:567–572

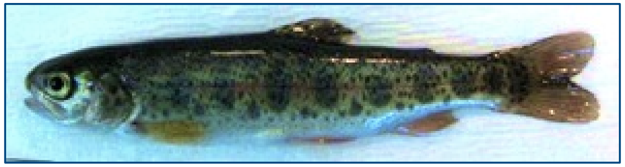
How is epigenetics relevant to understanding (rapid) fitness loss of hatchery fish?

Hatchery vs. natural environment

Photoperiod

Temperature

Stress



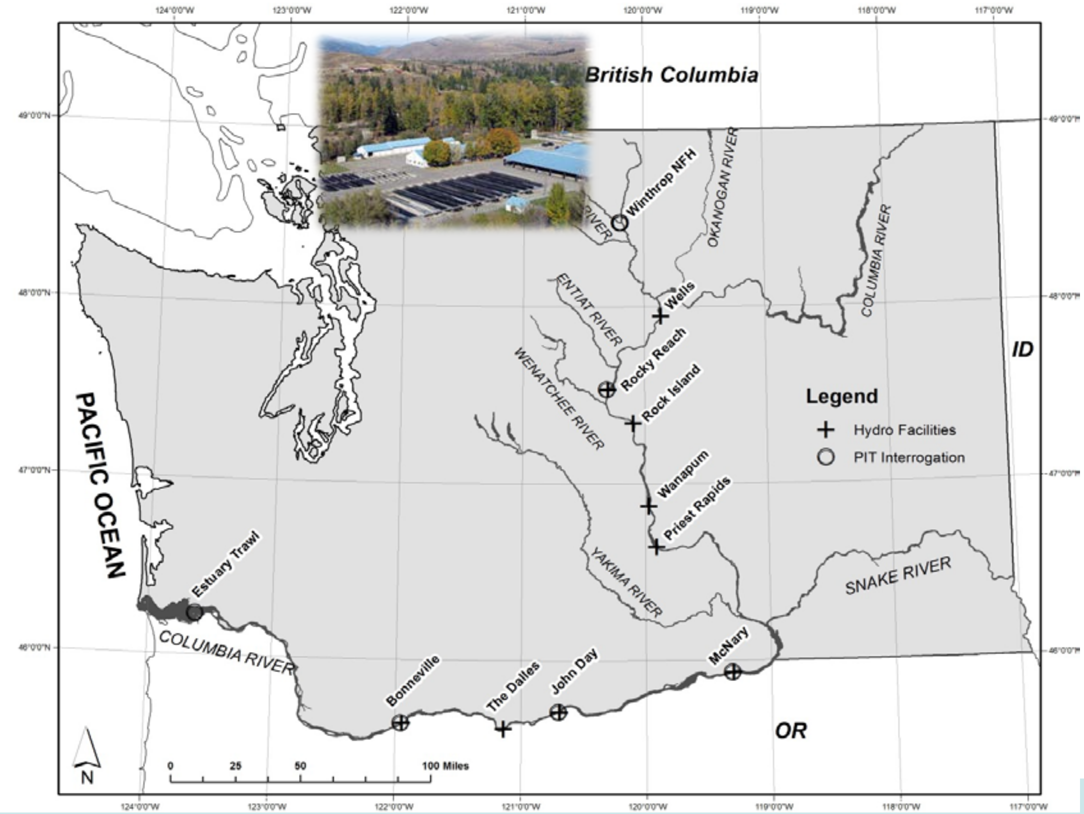
Water chemistry (pH, organics, toxins, O2)

Olfactory cues

Nutrients (yolk and exogenous food)

Question

Are there discernible genetic and/or epigenetic differences between hatchery and natural origin steelhead in Methow River?



Approach

- Returning adult steelhead were collected in 2014
- Identified as hatchery or natural origin
  - Hatchery = fin clipped, tagged
  - Natural = unmarked
- Collected sperm and red blood cells

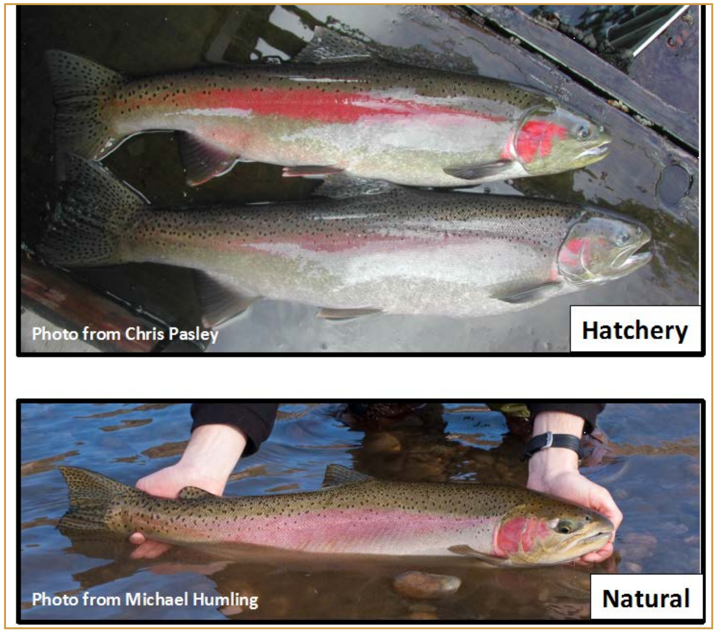


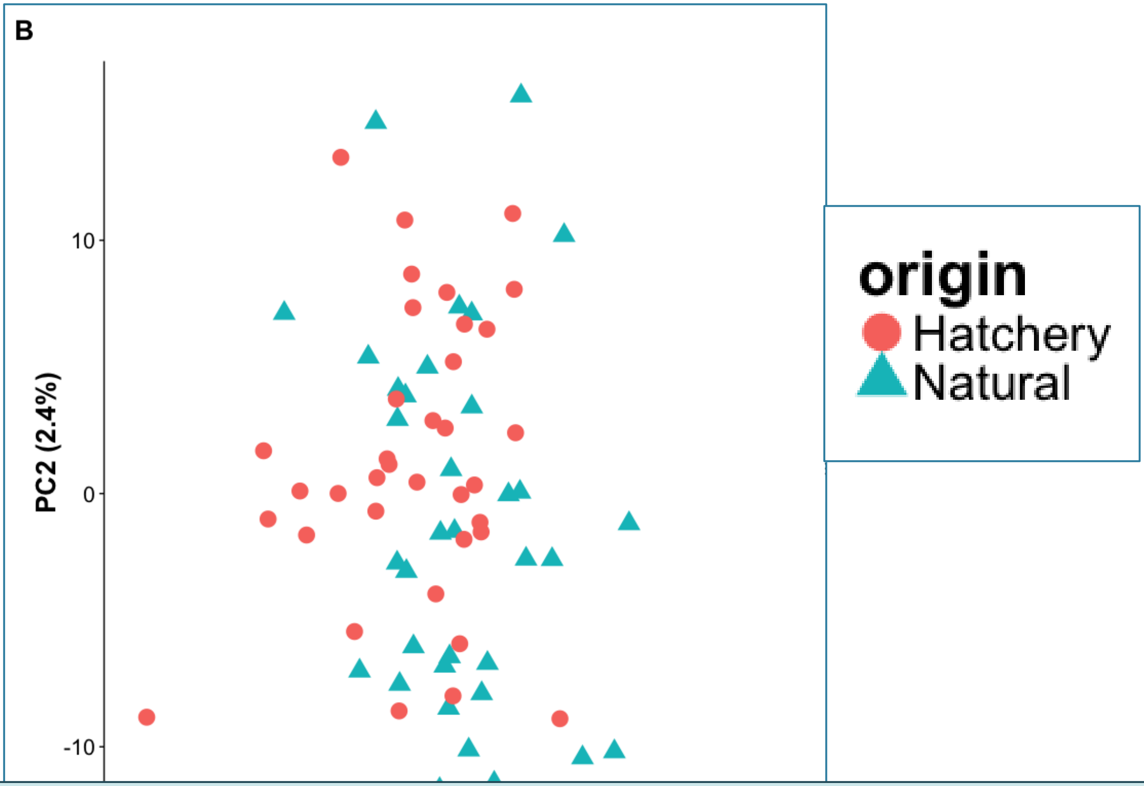
Photo from Chris Pasley

Photo from Michael Humling

Approach

- Genetic analysis: Restriction site associated DNA sequencing (RAD-Seq)
  - 72 fish (n=36 hatchery, n=36 natural-origin)
  - ~ 3 million reads per individual
  - Aligned to *O. mykiss* scaffolds (Berthelot et al., 2014)
  - Very stringent filtering: 936 Single Nucleotide Polymorphisms (SNPs)

Results: RAD-Seq

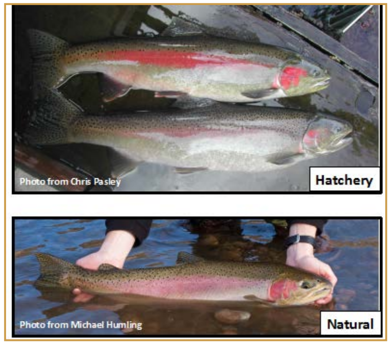


Hatchery and natural-origin Methow River Steelhead cannot be genetically distinguished at the population level

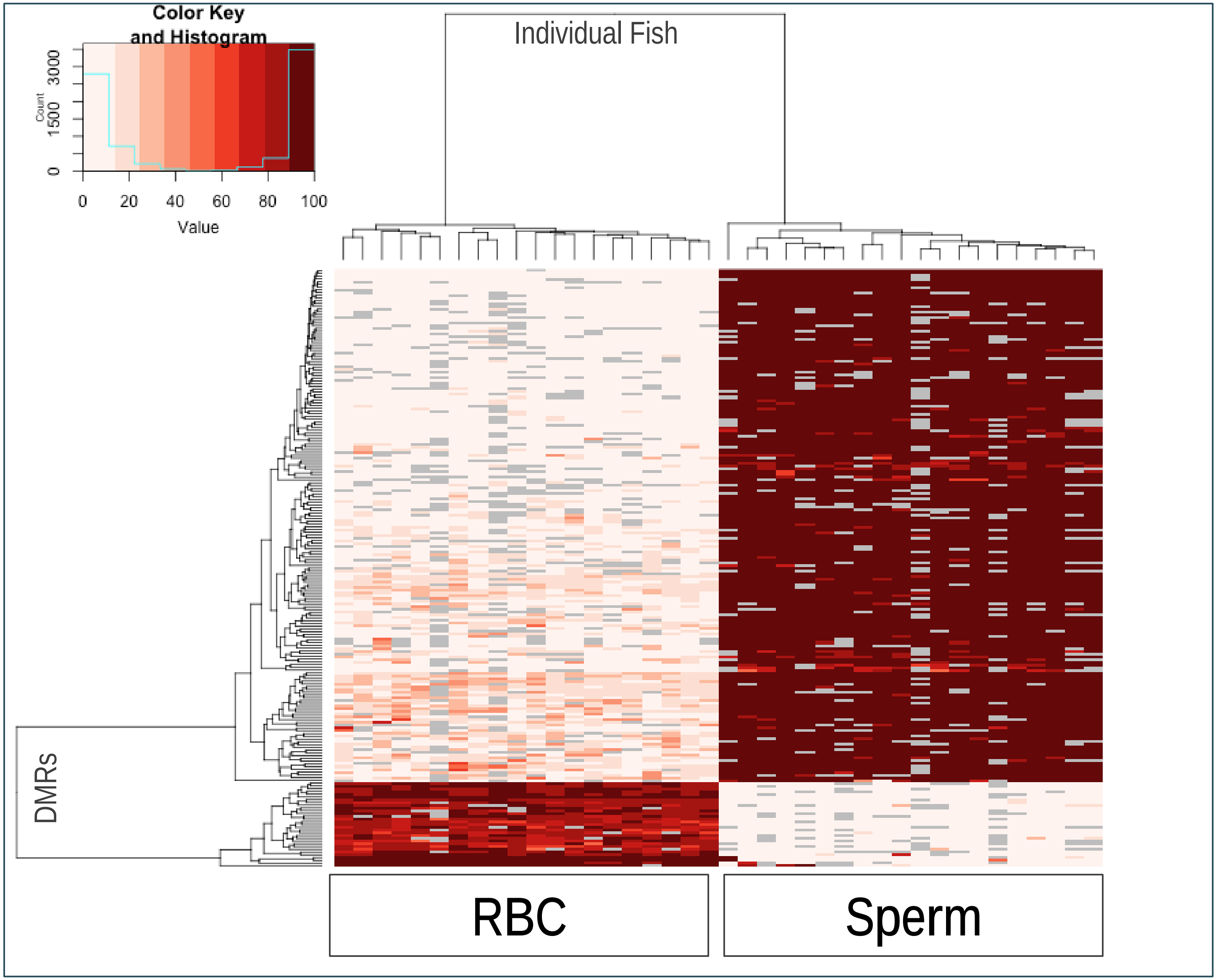
Gavery et al. In prep

Approach

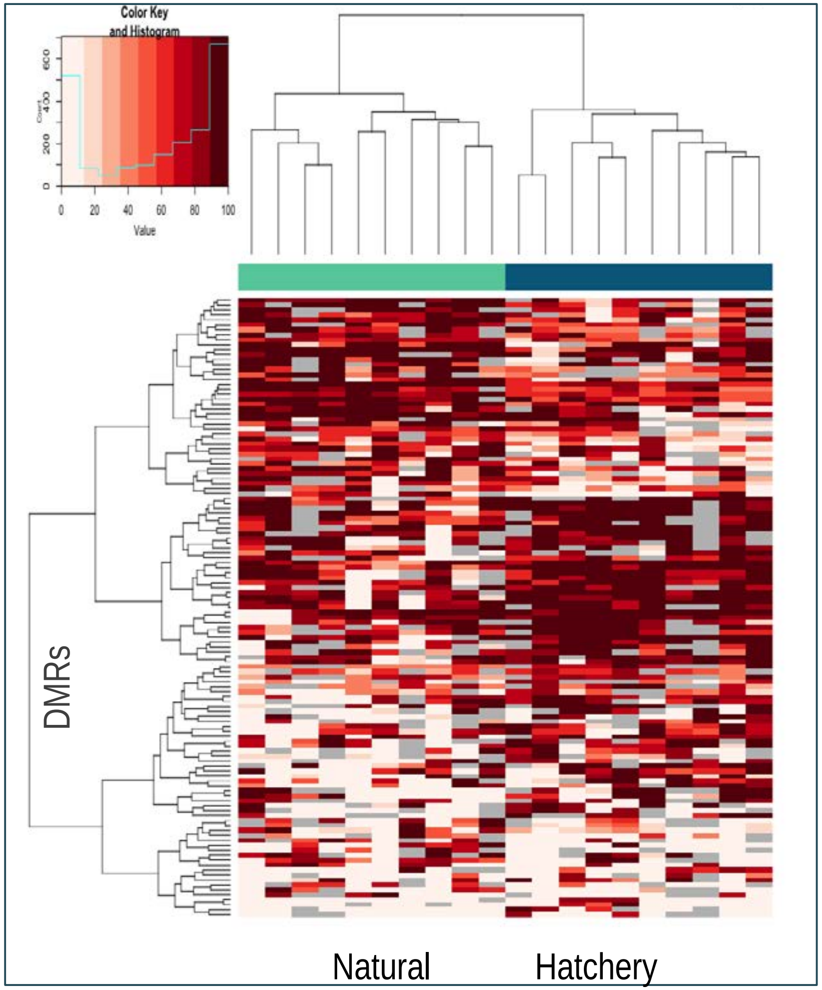
- DNA methylation analysis
- Two different approaches
  - ➢Red blood cells (RBC)
  - ➢Sperm



Cell-type specific methylation



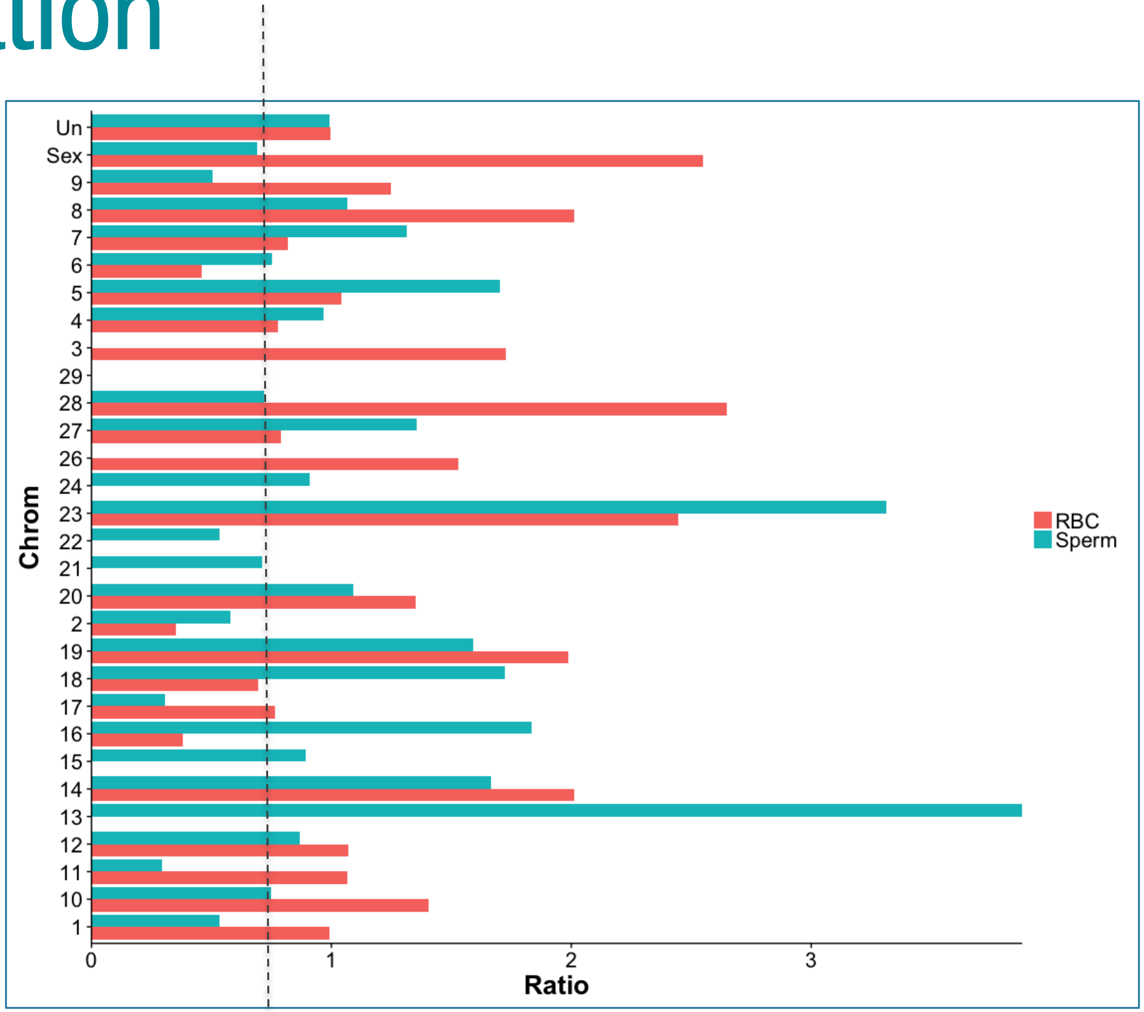
Fish origin specific methylation



Sperm

Genomic location of origin-specific methylation

Chromosome Number



Ratio of Observed to Expected

Gavery et al. In prep

Summary

There are differences in DNA methylation between natural and hatchery origin steelhead in both cell types

Individual variation in DNA methylation high suggesting some probably inherited from parents

Still need to know

- Does this happen in a single generation?*
- How do these DNA methylation changes affect gene expression and ultimately fitness?
- What aspects of the hatchery environments influence DNA methylation?
- To what extent are DNA methylation patterns transgenerationally inherited?

Critical to establish role in selection and fitness loss

Effects of rearing environment on epigenome

Hypothesis: early rearing environment alters epigenetic programming of steelhead germ line

20 families split across two rearing environments

Hatchery Tanks

Quasi-natural Stream

Goal: Develop methods to improve hatcheries for the long-term health of natural salmon and steelhead population

- Minimize heritable fitness loss in hatchery populations

- Reduce negative impacts to natural populations

- ….so that existing management frameworks (e.g.,HSRG) can be more effective and better contribute to recovery.