9.2 What is epigenetics and why could it be relevant to recovery and management of protected resources?

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Problem

• Hatchery programs may reduce the “fitness” of salmon and steelhead spawning in the nature

• Plausible mechanisms:
  • Hatchery-induced selection that causes rapid genetic change
  • Environmentally-induced changes in juveniles that alter:
    • Adult phenotype and reduce breeding success
    • Non-genetic factors in germ cells that affect offspring survival
  • Both
Genetic x Environment ➔ Phenotype

Genome (DNA Sequence)

Expression (mRNA, protein)

Physiology/Behavior

Biology/Disease

Genome, primary determinant of an organism’s phenotype
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Genetic x Environment $\Rightarrow$ Phenotype

- Genome (DNA Sequence)
- Epigenome
- Expression (mRNA, protein)
- Physiology/Behavior
- Biology/Disease

Environment
Heritable changes that affect phenotype

Genome (DNA Sequence) → Epigenome → Expression (mRNA, protein) → Physiology/Behavior → Biology/Disease

Environment

Heritable through cell lineage
Heritable across generations through germ cells (eggs/sperm)

DNA mutations, rare
DNA Methylation

• Most well understood epigenetic mechanism is DNA methylation

- Methylation of CpG occurs in most plants and animals
- Typically* associated with gene silencing if in promoter region of gene
- Methylation in gene bodies associated with enhanced transcription, formation of splice variants
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
Implications for Evolution

• Genome changes slowly, through random mutation and natural selection on the phenotype. Takes many generations for genetic trait to be common in a population.

• Epigenome changes rapidly in response to signals from the environment and can happen in many individuals at once. Some MAY be passed on to future generations. Through epigenetic inheritance, experiences of parents may pass to future generations.

• Epigenome is flexible, allows organism to continually adjust gene expression according to environment without changing DNA code.
Why could epigenetics be relevant to understanding loss of fitness of hatchery fish, or rapid adaptation and evolution?
Hatchery rearing environment differs from nature and is manipulated to meet production goals

- Embryo incubation temperature alters seasonal timing of emergence & ponding of fry
- Energy content of diet higher than natural diet
- Seasonal pattern of juvenile growth different than in nature
- Rearing density higher (can affect stress)
- Lack of predators
Potential factors that could induce heritable changes in the epigenome

- Photoperiod
- Temperature
- Olfactory cues
- Reproductive cells that will generate F1s
- Hormones in the yolk
- Water chemistry (pH, organics, toxins, O2)
- Nutrients (yolk and exogenous food)
Questions

Are there discernable epigenetic and/or genetic differences between hatchery and natural origin steelhead that *could* be passed to subsequent generations?

If so, does it differ between hatchery rearing protocols (S1 vs S2 smolt)?
Experimental Design:
Compare genetic and epigenetic variation in natural and hatchery origin Methow River steelhead

- Returning Methow River steelhead adults collected by angling
- Collect fin clip, blood, gametes
- **Genetic analysis**: Genome wide SNP analysis, 15,000 SNPs, RAD sequencing
- **Epigenetic analysis**: Genome wide DNA methylation analysis in sperm, RRBS
Experimental Design:
Compare genetic and epigenetic variation in natural and hatchery origin Methow River steelhead

- Returning Methow River steelhead adults collected by angling
- Collect fin clip, blood, gametes

No discrimination between natural & hatchery origin fish

- Epigenetic analysis: Genome wide DNA methylation analysis in sperm, RRBS
Preliminary Results: Epigenetics

- 74 differentially methylated CpG Regions (DMRs) in sperm DNA
  - 45 hypermethylated in hatchery fish
  - 29 hypomethylated in hatchery fish

- Almost half of the DMRs are located within 5kb of genes (potential gene regulatory regions), one third within gene bodies

- Differences could be due to early rearing environment, but cannot rule out age.

- Currently analyzing additional samples- comparing two hatchery rearing methods (yearling vs 2-year old smolt) allows us to test for age and rearing environment effects

Unpublished data from Gavery et al.
What about transgenerational effects?

Establishing epigenetic differences in sperm DNA from natural and hatchery origin fish is compelling, but does not prove these are heritable.
Challenges of Proving Transgenerational Epigenetic Inheritance

- Must rule out possibility of genetic change
- Must show that the epigenetic effect can pass through several generations to rule out possibility of direct exposure

Environmental exposure

$F_0$ 

Reproductive cells that will generate F1s
New Study- Transgenerational Epigenetic Inheritance
Hypothesis- Early Rearing Environment Alters Epigenetic Programming of Steelhead Germ Line

20 families split across two rearing environments

Hatchery

Artificial Stream

Manchester Research Station facilities
Phenotype of Steelhead from Hatchery vs Artificial Stream Environment: 8 months of rearing

Next steps: 1-2 more years of rearing needed to obtain maturing males for sperm DNA methylation analysis. Test for rearing environment effects. Compare paternal and offspring methylation patterns.
Summary

• Assembled a strong interdisciplinary team to investigate role of epigenetics in phenotypic plasticity, adaptation and evolution in O. mykiss
  • Expertise in molecular and quantitative genetics, functional genomics, fish physiology, behavioral ecology, and bioinformatics
  • Facilities for lab studies, hatchery rearing, computer cluster for bioinformatics
  • Partnerships with USFWS, UW, WSU, and Institut National de la Recherche Agronomique (INRA)

• Established methodology for analyzing DNA methylation and bioinformatic pipelines to analyze data

• Found significant differences in methylation of sperm DNA due to hatchery rearing or age. Suggest potential for heritable epigenetic effects of early rearing environment.

• Studies initiated to test for transgenerational epigenetic inheritance
What are the challenges?

• Bioinformatics challenging because of genome duplications in salmonines

• Data analyses in other Pacific salmon species without genome sequence difficult

• Linking specific differences in epigenetic programming to functional differences, phenotypes

• Costs of the analysis

• Long-term funding for transgenerational studies in species with 2-4 year generation times
Where could this research go?

- Reforming hatchery rearing practices (diet, feeding regimes, early rearing protocols) to reduce impacts
- Development of epigenetic signatures of wild and hatchery fish of same stock that cannot be discriminated with genetic analysis
- Understanding scope of adaptation to extreme changes in environment
- Numerous applications to habitat restoration and toxicological research
- If epigenetic changes occur, can they readjust in several generations in the wild? Epigenome is more dynamic than genome.
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• Mike Skinner, Washington State University
Extra slides
Epigenetic Mechanisms: Link Between Nature and Nurture

Environmental Factors

- Environmental toxins
- Diet
- Stress
- Pathogens
- Temperature
- Photoperiod
- Olfactory cues

DNA methylation

Histone modifications

Chromatin remodeling

Signaling molecules, noncoding RNAs

Gene Expression

Change in phenotype

Morphology

Physiology/stress response

Disease susceptibility

Behavior

Olfactory imprinting

Modified from, Tammon et al. (2013) Molecular Aspects of Medicine 34:753 - 764
Results: Genetics

Genetic Analysis of Natural and Hatchery Origin Methow River Steelhead: Genome Wide Assessment of SNPs

Preliminary results:

- 15,000 SNPs analyzed
- No obvious discrimination between hatchery and natural fish
- No association with brood year/age, rearing environment, or sex

Unpublished data from Gavery et al.
Example of gene with DMR:
HTRA1, regulates insulin like growth factor (IGF) availability, cleaves IGF binding proteins

Cluster of 7 CG hyper-methylated in hatchery fish

High-temperature requirement A serine peptidase 1
Chromosomal locations of differentially methylated regions of sperm DNA in natural vs hatchery origin (S1) Methow River steelhead

Data generated in collaboration with Prof. Mike Skinner, WSU