INFLUENCE OF LANDSCAPE AND ENVIRONMENT ON SALMONID GENETICS

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Shawn R. Narum, Nathan R. Campbell, Andrew P. Matala, Ben Hecht, Jon Hess

Columbia River Inter-Tribal Fish Commission (CRITFC), Hagerman, ID, 83332

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Executive Summary

a. Fish Population RM&E

This project addresses two objectives related to environmental and landscape features that contribute to population structure, life history diversification, and adaptation of salmonids.

Objective 1) Environment & Landscape Genetics – Evaluate genetic structure of natural populations of salmonids relative to their environment and identify candidate markers associated with traits that are related to adaptation of steelhead and Chinook salmon populations

For Objective 1, work has progressed on sequencing Chinook salmon and steelhead throughout the Columbia River Basin to evaluate adaptive genetic variation related to environmental features.

Objective 2) Controlled Experiments – experiments with controlled environmental variables to validate phenotypic response of fish with given genotypes.

For Objective 2, empirical work was done to further advance our understanding multiple traits related to recovery of salmonids in the Columbia River. Work focused on genomic regions associated with run-timing in Chinook salmon and steelhead, and patterns of gene expression of thermally adapted strains of redband trout under heat stress. Further, progress was also made towards developing projects to investigate the genomic basis for age-at-maturity in Chinook salmon and thermal tolerance in *O. mykiss*.

2. Introduction

Environmental and landscape features can greatly contribute to the population structure, life history diversification, and local adaptation of organisms in aquatic habitats (reviewed in Storfer et al. 2006). Geographic barriers to dispersal include recent events that may have been human induced (e.g., dams) as well as ancient events such as glaciations and formation of mountain chains (e.g., Castric et al. 2001). However, other environmental characteristics such as elevation, temperature, forest cover, and precipitation may influence distribution, adaptation, and gene flow of species (Dionne et al. 2008; Narum et al. 2008). For example, the geographic distributions of species ranges' are often determined by thermal tolerance (Brannon et al. 2004) and may necessitate adaptations for survival in extreme environments (Rodnick et al. 2004).

Screening with many genetic markers provides the opportunity to investigate local adaptation in natural populations and identify candidate genes under selection (Beaumont and Nichols 1996; Beaumont and Balding 2004; Excoffier et al. 2009). This has become a commonly employed approach in ecological and population genetics studies to detect outlier loci that are putatively under selection (e.g., Vasemagi and Primmer 2005; Nosil et al. 2008). Additionally, correlation methods can be highly informative to identify markers in coding and cis-regulatory regions of known functional genes that are associated with specific selective pressures or phenotypes (Lyman and Mackay 1998; Chase et al. 2009; Torgerson et al. 2009). With increasing genomic information available for non-model organisms, single nucleotide polymorphisms (SNPs) have begun to see increased use as genetic markers for population genetic studies (e.g., Morin et al. 2004). These sequence polymorphisms are densely scattered throughout the genome of most organisms, and are commonly observed in both coding and non-coding regions of functional genes making them ideal markers to study adaptive molecular variation (e.g., Akey et al. 2002). In a large suite of unlinked SNPs that are distributed across the genome (e.g., Campbell et al. 2009), it is possible to utilize both functionally neutral and adaptive markers within a single study. This combination of information provides a powerful approach to study questions in ecological genetics since both demographic processes (i.e., gene flow and genetic drift) and local adaptation (i.e., selection) may be inferred.

Molecular techniques such as RNA-seq (Wolf 2013) provide the opportunity to investigate transcriptional response to thermal stress and further identify mechanisms for thermal adaptation. Patterns of gene expression under heat stress are important to determining evolutionary adaptation among conspecific populations that occupy various environments. Multiple genes have been shown to be involved in heat tolerance across many species, including highly conserved heat shock proteins (hsps)

that are upregulated under stressful conditions such as exposure to heat (Morimoto et al. 1992; Sorensen et al. 2003). An adaptive heat shock response has additionally been shown to occur among conspecific populations that occupy variable environments (e.g., Dahlhoff and Rank 2000; Sorensen et al. 2001). However, many genes are known to have a role in regulating the effects of temperature and are likely to be involved in thermal adaptation (Sorensen et al. 2005; Kassahn et al. 2007). Thus, RNA-seq provides the opportunity to investigate differential expression across the transcriptome and identify biological pathways involved in evolutionary response to thermal stress.

Thus, genome scans with large numbers of SNP markers (e.g., RAD sequencing) and gene expression (e.g., RNA-seq) approaches may be effective tools for identifying the genetic architecture underlying specific traits such as thermal tolerance, run-timing, disease resistance, age-at-maturity, etc... Once these underlying genomic regions are identified, they can be broadly screened throughout the Columbia River Basin to facilitate management for long term conservation and recovery of salmonids.

a. Fish Population RM&E

F&W Program Strategy: Assess the status and trend of diversity of natural and hatchery origin fish populations.

F&W Program Management Question: What are the status and trend of diversity of natural and hatchery origin fish populations?

Uncertainty Research

Identify and compare adaptive genetic variation relative to neutral variation in salmonid stocks in the Columbia River.

Project Map:

http://www.cbfish.org/Project.mvc/Map/2009-005-00

Contract Map(s):

http://www.cbfish.org/Contract.mvc/Map/61839

http://www.cbfish.org/Contract.mvc/Map/65575

3. Methods: Protocols, Study Designs, and Study Area

Method Title: RAD sequencing v1.0

Method Link: http://www.monitoringmethods.org/Method/Details/4144

Method Summary:

RAD sequencing is a technique for tagging DNA at restriction enzyme cut sites with adapters used in massively parallel sequencing. This method allows thousands of SNPs to be discovered and genotyped in several individuals. Through the use of sample specific DNA barcodes included in the adapters, information for specific samples can be separated in silico following sequencing. This method effectively reduces sequence complexity by targeting only sequence surrounding restriction enzyme cut sites making alignments among sequencing reads far less computationally intense. The sequence alignments among samples can then be analyzed for both identification and genotyping of SNPs (Single Nucleotide Polymorphisms). This method was first described by Baird et al. (2008).

Method Title: Obtain gene expression data via RNAseq v1.0

Method Link: http://www.monitoringmethods.org/Method/Details/607

Method Summary:

Compare gene expression between fish of different genetic backgrounds but raised in the same environment. Molecular techniques such as RNAseq provide the opportunity to investigate transcriptional response and further identify mechanisms for thermal adaptation. Patterns of gene expression are important to determining evolutionary adaptation among conspecific populations that occupy various environments.

4. Results

a. Fish Population RM&E

Objective 1)

Results from broad scale analyses identified three primary environmental factors associated with local adaptation in Chinook Salmon (Hecht et al. 2015). We used a multivariate method, redundancy analysis (RDA), to identify polygenic correlations between 19,703 SNP loci and a suite of environmental variables in 46 collections of Chinook salmon (1,956 total individuals) distributed throughout much of its North American range. Models in RDA were conducted on both range-wide and regional

scales by hierarchical partitioning of the populations into three distinct genetic lineages. Our results indicate that between 5.8 – 21.8% of genomic variation can be accounted for by environmental features, and 566 putatively adaptive loci were identified as targets of environmental adaptation. The most influential drivers of adaptive divergence included precipitation in the driest quarter of the year (Range-wide and North Coastal Lineage, ANOVA p=0.002 and 0.01, respectively), precipitation in the wettest quarter of the year (Interior Columbia River Stream-Type Lineage, ANOVA p=0.03), variation in mean diurnal range in temperature (South Coastal Lineage, ANOVA p=0.005), and migration distance (Range-wide, ANOVA p=0.001). Our results indicate that environmental features are strong drivers of adaptive genomic divergence in this species, and provide a foundation to investigate how Chinook salmon might respond to global environmental change.

Objective 2)

The ability to adapt to increased water temperatures will be of paramount importance for many fish species as the climate continues to warm and water resources become limited. Because increased water temperatures will reduce the dissolved oxygen available for fish, we hypothesized that adaptation to low oxygen environments would involve improved respiration through oxidative phosphorylation (OXPHOS). To test this hypothesis, we subjected individuals from two ecologically divergent populations of inland (redband) rainbow trout (*Oncorhynchus mykiss gairdneri*) with historically different temperature regimes (desert and montane) and their F1 progeny to diel cycles of temperature stress and then examined gene expression data for 82 nuclear- and mitochondrial-encoded OXPHOS subunits that participate in respiration. Of the 82 transcripts, 7 showed ≥ 2 fold difference in expression levels in gill tissue from desert fish under heat stress while the montane fish had none and the F1 only had one differentially expressed gene. A structural analysis of the proteins encoded by those genes suggests the response could coordinate the formation of supercomplexes and oligomers. Supercomplexes may increase the efficiency of respiration because complexes I, III, and IV are brought into close proximity and oligomerization of complex V alters the macro-structure of mitochondria to improve respiration. Significant differences in gene expression patterns in response to heat stress in a common environment indicate the response was not due to plasticity but had a genetic basis. These results are published in Garvin et al. (2015).

5. Synthesis of Findings: Discussion/Conclusions

a. Fish Population RM&E

Objective 1)

Our recent results indicate that environmental features are strong drivers of adaptive genomic divergence in this species, and provide a foundation to investigate how Chinook salmon might respond to global environmental change (Hecht et al. 2015). Broad geographic patterns of neutral and non-neutral variation demonstrated here can be used to accommodate priorities for regional management and inform long-term conservation of Chinook salmon.

Objective 2)
Results from gene-expression profiles (Narum et al. 2015; Garvin et al. 2015) suggest an adaptive response of *Oncorhynchus mykiss gairdneri* to survive under increasing temperatures, and this is valuable information for fisheries and conservation efforts. These patterns could be used to monitor the health of populations of fish that are exposed to higher temperature regimes as the climate continues to warm. Theoretically, populations that display these gene expression patterns may show some protection against the increased water temperatures and those that don't may be at risk.

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Appendix A: Detailed Results

Figure 1. Results from Hecht et al. (2015) in Chinook Salmon that demonstrate the significant environmental factors associated with local adaptation (Fig. 1a) and the candidate SNP markers under selection (Fig. 1b).

Fig. 1a Triplots of global range-wide partial redundancy analysis (RDA) results for 46 Chinook Salmon populations throughout the North American range (Hecht et al. 2015). Population scores for canonical RDA axis 1 and 2 are represented by the three letter abbreviation for each population, colored to represent the lineage assignment of that population. SNP variance is indicated by the position of grey "+" symbols radiating from the plot centroid, each representing a different locus. Environmental factors are depicted as black vectors (arrows), where the length of the vector is a representation of the magnitude of the contribution of that environmental variable in explaining SNP variance. The angle between environmental variable vectors is a representation of the correlation between those variables. Vectors and points are plotted with symmetrical scaling (scale = 3) to preserve the relationship between scores, without focusing on a single score. (Hecht et al. 2015)

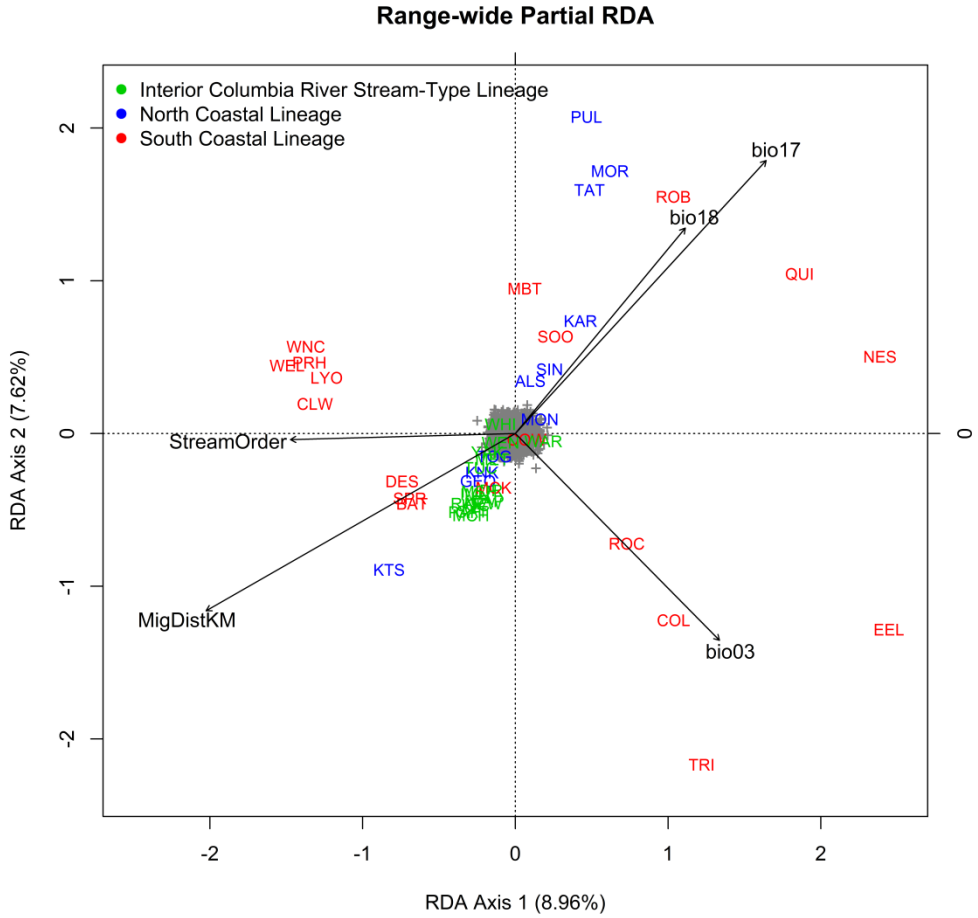

Fig. 1b. Genome-wide Manhattan plots of squared SNP scores (correlations) to the first multivariate climatic redundancy analysis (RDA) axis (RDA1) for global range-wide analyses for 46 Chinook Salmon populations throughout the North American range (Hecht et al. 2015). Genetic map position and chromosome assignment are listed on x-axis with bin for chromosome 35 representing all unmapped loci with arbitrary genetic position assigned. The red line represents the 0.5% outlier threshold, with all loci above the line considered putatively adaptive. (Hecht et al. 2015)

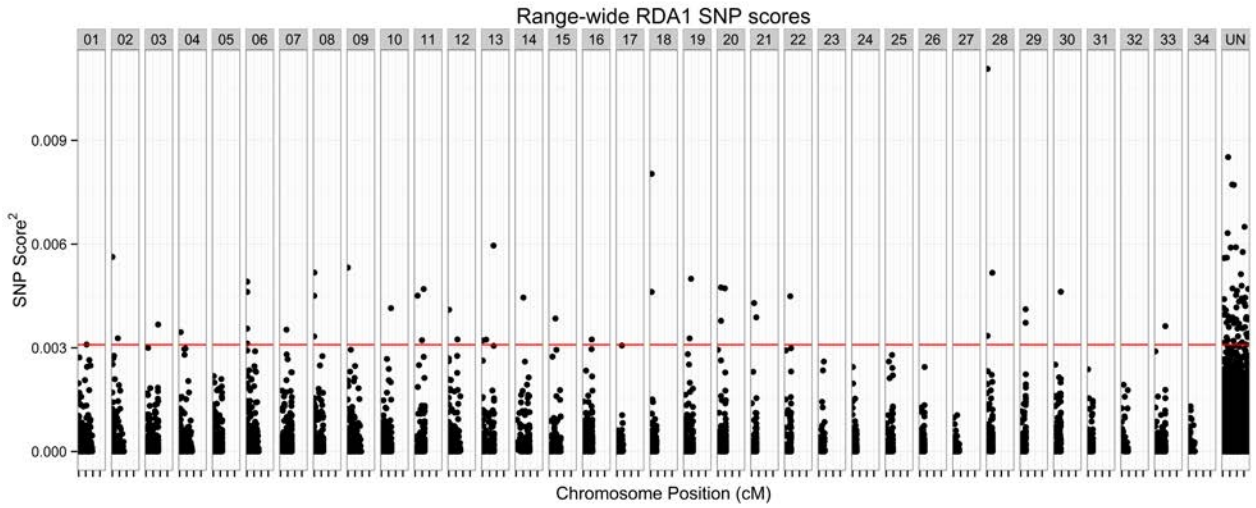

Figure 2: Summary of significantly expressed genes among ecologically divergent strains of redband trout from desert (LJ = Little Jacks Cr.) and montane (K = Keithly Cr.) environments, and their F1 cross (Garvin et al. 2015). Numbers indicate fold difference between the two treatments. Green is upregulated, red is downregulated, yellow is not significant. A singlet asterisk indicates $p < 0.01$ and a double asterisk $p < 0.001$. (Garvin et al. 2015)

Gene	Genome	Complex	LJ	K	F1
MTND1	Mitochondrial	I	2.0 *	1.0	1.0
MTND5	Mitochondrial	I	3.8 *	1.0	1.0
NDUFA07	Nuclear	I	1.0	1.0	-1.3 *
NDUFA10	Nuclear	I	1.0	1.0	-1.3 **
NDUFB02	Nuclear	I	1.0	1.0	-1.1 **
NDUFB08	Nuclear	I	1.4 **	-1.4 *	1.4 **
NDUFS01	Nuclear	I	1.0	-1.4 **	1.0
NDUFS02	Nuclear	I	1.3 *	-1.3 **	1.0
NDUFS03	Nuclear	I	1.2 **	1.0	1.0
NDUFS06	Nuclear	I	1.5 **	1.0	1.0
NDUFV01	Nuclear	I	1.3 **	1.0	1.0
NDUFV02	Nuclear	I	1.3 *	1.0	1.0
QCR02	Nuclear	III	1.4 **	1.2 **	1.2 **
QCR06A	Nuclear	III	2.6 **	2.7 **	2.7 **
QCR07	Nuclear	III	1.3 **	1.0	1.0
UCRI	Nuclear	III	1.0	-1.3 *	1.0
CYC1	Nuclear	III	1.2 *	1.0	1.1 **
CYCS	Nuclear	III	2.4 **	1.0	1.4 *
MTCO1	Mitochondrial	IV	-1.3 **	1.0	1.0
COX6B2	Nuclear	IV	4.3 **	1.0	1.0
MTATP8	Mitochondrial	V	2.1 **	1.0	1.0
MTATP6	Mitochondrial	V	2.5 **	1.0	1.0
ATP5E	Nuclear	V	1.8 **	1.0	1.0
ATP5G3	Nuclear	V	1.0	1.1 **	-1.3 **
ATP5I	Nuclear	V	1.6 **	1.0	1.0
ATP5L	Nuclear	V	1.0	1.0	-1.3 **

Appendix B: List of Metrics and Indicators

Category	Subcategory	Subcategory Focus 1	Subcategory Focus 2	Specific Metric Title
Fish	Composition: Fish Species Assemblage	Fish Life Stage: Juvenile - Alevin	Fish Origin: Natural	Fish stock analysis based on genetics
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		Understand genetic relationship of steelhead
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Natural		Steelhead diveristy and variation based on genetics
Fish	Presence/Absence: Fish	Fish Life Stage: Juvenile - Stream Type		Hatchery/out of basin wild steelhead presence based on genetics
Fish	Stray Rate	Fish Origin: Both		out of basin stray spawning or introgression rate
Fish	Tissue Sample: Fish			Fish tissue samples for genetics
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Composition: Fish Species Assemblage	Fish Life Stage: Juvenile - Alevin	Fish Origin: Natural	Fish stock analysis based on genetics
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		Understand genetic relationship of steelhead

Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Natural		Steelhead diveristy and variation based on genetics
Fish	Presence/Absence: Fish	Fish Life Stage: Juvenile - Stream Type		Hatchery/out of basin wild steelhead presence based on genetics
Fish	Stray Rate	Fish Origin: Both		out of basin stray spawning or introgression rate
Fish	Tissue Sample: Fish			Fish tissue samples for genetics
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		