ABSTRACT

Investigation of Fine Spatial Scale Population Genetic Structure
in Two Alaskan Salmonids

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Fidelity to natal habitat influences fine spatial scale genetic population structure in salmonids. We analyzed genomic diversity of two species of Alaskan salmonids, coho salmon (*Oncorhynchus kisutch*) and Dolly Varden char (*Salvenius malma*) in Kenai Peninsula of south-central Alaska using Single Nucleotide Polymorphisms (SNPs). We examined SNPs for patterns of molecular diversity and divergence. Sample design compared within- and among-catchment genomic variation at first-order streams. Genomic diversity and divergence were spatially distributed unevenly across sites. Populations in adjacent habitats showed different patterns of genetic migration. Our results suggest first order streams support locally diverged populations in coho salmon but not in Dolly Varden char and that multiple drainages may house metapopulations. This could result from interaction between ecological selection and philopatry. Conservation considering species-specific distribution of genetic diversity may avoid omission of crucial diversity and improve the capacity of populations to adapt to future conditions.

Investigation of Fine Spatial Scale Population Genetic Structure in Two Alaskan Salmonids

by

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This would not have been possible without my wonderful family and friends, who have supported me throughout my life. Especially to Mom, Dad, Colleen, Mike and Moncie, thank you from the bottom of my heart.

DEDICATION

To my family, you make it all worthwhile.

CHAPTER ONE

Introduction

The Giving Tree (Fig. 1.1, Silverstein 1964) is a tragic story of how human growth can destroy the natural world that has nurtured us. Just as the symbolic child grows to adulthood and requires more of the tree, the human demand for natural resources is expanding. In the story, the tree looses crucial functional pieces to the child's use: leaves, branches and trunk. The loss of those functional pieces impairs the trees ability to heal and continue providing for the child. Humanity's use of resources, similarly, can cause declines in biodiversity. Such declines are associated with reductions in the ability of ecosystems to provide resources to human populations. Simultaneous increase in demand and impairment of ecosystems makes the importance of effectiveness in conservation practices paramount.

Figure 1.1: The final illustration of Shel Silverstein's *The Giving Tree*

Maintenance of biodiversity is critical for the future of the global ecosystem (Brooks et al. 2006), and its provision of services to humanity (Sweeney et al. 2004). Essential to this maintenance is understanding how biodiversity is distributed. This task becomes difficult, however, in light of the complex relationships among organisms and environments. For any organism or community, habitat distribution and quality, dispersal and biological interactions all affect diversity at a local scale (Taylor 1991). At larger spatial scales, factors such as climate change, geographic barriers and habitat disturbance can affect regional diversity. These factors affect species differently as a result of different life histories, feeding characteristics, and habitat preferences.

Understanding the different responses of species to changing environments requires specific investigation to inform proper conservation. While some species have maintained robust populations in the face of intensive exploitation (Kennington et al. 2013), other widely distributed species have experienced population collapses (Van Hyning 1968, Hutchings 2000, Hutchings and Reynolds 2004). A clear instance of the devastating effect of human exploitation on natural populations can be found in Pacific salmonids. Though they are distributed across the entire Pacific Rim, and at regional scales have large census sizes, some Pacific salmonids have experienced declining population sizes and local extinctions(Garwood 2012, National Marine Fisheries Service 2016). Such negative reaction to exploitation, and the failure of subsequent conservation efforts, are partly due to the specific biological characteristics of Pacific salmonids. In the

pursuit of more effective conservation, this study attempts to further understanding of the relationship between salmonid biology and the environment.

Pacific salmonids include fishes of the genuses *Oncorhychus* and *Salvenius*, commonly salmon, trout and char. These taxa have diverse, complex life histories (Quinn 2011) that are the result of extensive morphological, behavioral, and physiological adaptations; these adaptations make Pacific salmonids unique among fishes and also ecologically important. Conservationists' efforts over the past century have begun to illuminate how the biological characteristics of some salmonids make them especially vulnerable to exploitation. Despite understanding their vulnerability, humans have continued to exploit Pacific salmonids. In some cases human exploitation has resulted in extinction across large portions of their historical range. For example, both Chinook and coho salmon (O. tschawycha, O. kitsuch) are threatened, endangered or extirpated across their entire historical habitat in the United States.

Quinn (Quinn 2011) describes three crucial themes in salmonid biology that contribute to their vulnerability and ecological importance: Anadromy, Semelparity, and Homing. All salmonids hatch and spawn in freshwater. After hatching, many Salmonids exhibit Anadromy: they migrate to sea to feed and grow, then return to freshwater to spawn. Fishes undergo profound physiological changes to survive the salinity gradient during both directions of this migration. Anadromy varies in extent among species. Even among populations of an Anadramous species, some may be life-long stream residents (effectively non-Anadramous) while others may make wide migrations at sea for many years. Generally, Anadramous salmonids feed at sea from about six months to three years and then return to freshwater to spawn.

Semelparous salonids play a unique part in the transport of resources between ocean and stream ecosystems (Quinn 2011). Some salmonid species die immediately after their first spawning. This is referred to as Semelparity, and represents an important part of the ecological role of salmonids. Mature adult salmonids expend most of their energy reserves in migrating long distances and crossing the salinity gradient. As a result, most die near spawning sites and transport large amounts of organic material from the ocean into streams. This influx enriches stream and terrestrial ecosystems and connects them to the ocean energetically. The upstream transport by salmonids is unusual and runs counter to the removal of resources from streams by the physical transport of water flow. Almost all anadramous salmonids are semelparous.

Many salmonids exhibit Homing in their return to freshwater for spawning (Quinn 2011). Homing in salmonids is the preference for and ability to locate their natal habitat in their return to freshwater. This behavior is remarkably site specific, though its precision and accuracy vary among species. As a result of Homing, salmonids often spawn only with others that hatched at their natal site. Such reproductive isolation makes possible quick adaptation to local conditions at small spatial scales. Further, individual salmonids will experience reduced fitness in nearby, but ecologically different habitats. Homing and local adaptation, therefore, have produced complex distributions of biodiversity in salmonid species. These distributions cannot be captured by studies that make incorrect assumptions about spatial scale. To illustrate this, our study investigates biodiversity at a spatial scale that is informed by the species-specific characteristics of two salmonids.

The scale of the distribution of biodiversity is important to conservation(Brooks et al. 2006, Reiss et al. 2009). In evaluating diversity, conservationists run the risk of obfuscating diversity by sampling with low resolution and, or at too large a spatial scale. This could result in the unintentional agglomeration of distinct populations, or the inverse. Applying the same conservation measures to isolated, differentially adapted populations may do more harm than good. For example, efforts to replenish natural salmonid populations with hatchery fish, which are not adapted to the natural environment, have had negative effects(Waples 1991a, Johnson et al. 2012). By accounting for the biological characteristics of salmonid populations, we have attempted to reveal the distribution of diversity and how it may be conserved.

Identifying units for effective conservation presents a challenge, especially for species with complex relationships to their environment. A number of criteria have been developed to define and preserve these units (Brooks et al. 2006). These approaches emphasize the uniqueness or irreplaceability of those units relative to their vulnerability. A notable approach is the refinement of the U.S. Endangered Species Act in designating and protecting Evolutionarily Significant Units (ESU, Waples 1991). ESUs are reproductively isolated groups within a species that are important to the evolutionary future of that species.

Biologists conceived of ESUs in order to address the complex characteristics of Pacific salmonids, and these groups have served as a framework for studies of biodiversity in those species (Bucklin et al. 2007). However, some of these investigations may have been under-resolved as a result of the spatial-scale implied by the current geographical definitions of ESUs (NOAA Fisheries 2016), which can include hundreds of

miles of coast and many catchment basins (see below). Aareas of similar size to existing ESUs include salmonid populations experiencing reproductive isolation and fitness differentials (Quinn 2005). Biologists might expect such an outcome, as rivers are fundamentally dynamic, multi-scale systems (Larsen et al. 2015).

Within salmonid ESUs, a natural delineation of river habitat can be found in catchment basins. For the purposes of many salmonid studies, including this one, a catchment basin is the area from which rainfall flows to a single point downstream at an outlet to the ocean. This outlet is the first point of recognition that an anadramous fish must find to return to its natal habitat. Past evaluations of salmonid diversity have considered the fish sampled in a catchment to be a united population(Bucklin et al. 2007). However, important isolation and local adaptation have been observed on the sub-catchment scale in salmonids and other fishes (Bond et al. 2014, Waters and Burridge 2016). It is not clear that catchments basins provide the best scale for studying salmonid diversity. In this study we investigate the scale of habitat housing united populations by considering species or population-specific biological characteristics and habitat utilization.

Spawning is a crucial part of salmonid habitat use (Quinn 2011). All salmonids spawn in the gravel beds lining freshwater habitat. Some salmonids prefer certain parts of the river habitat. Pink and Sockeye salmon (*O. gorbuscha* and *O. nerka*), for example, generally spawn in the lower reaches of rivers, near the ocean. On the other hand, coho salmon (*Oncorhynchus kisutch*) and Dolly Varden char (*Salvenius malma*) both prefer to spawn further upstream, often in first order streams. It is possible that the scale of

population isolation and local adaptation corresponds to the scale of spawning habitat, and is, therefore, different among salmonid species.

First order streams are the smallest and most common river-system component, and are furthest from the end or confluence of the stream. First order streams, especially headwater streams (HWS) are uniquely and heterogeneously affected by their terrestrial surroundings because of their small size (King et al. 2012, Mazza and Olson 2015). This environmental heterogeneity may drive fine-scale local adaptation in fish using first order streams as critical spawning habitat (Taylor 1991). While larger river components have been studied for conservation or restored, first order streams often remain unprotected because they are so numerous and often found on private land.

In the following study, we have examined spatial scale of habitat that contains populations of coho salmon and Dolly Varden char. We have also examined the possibility that first order streams contain important diversity in those species. We took tissue samples from salmonids at several sites within on catchment basin (within-catchment) and samples from a single site at several adjacent catchment basins (among-catchment). Within-catchment sites were distributed along the longitudinal continuum of South Anchor River (Vannote et al. 1980) at upper-river, mid-river and lower-river sites.

We evaluated an important component of biodiversity, standing genetic variation. Genetic variation may allow a species to persist in quickly changing environments (Barrett and Schluter 2008). Standing genetic variation can be thought of as the diversity of genetic information available to a population. Some of this information may be useful for adaptation, and affect the fitness of populations. Some variation may not affect fitness, and provide no adaptive substrate. Standing genetic variation can make a

population irreplaceable if it confers the flexibility for future adaptation (Taylor et al. 2011) and it is exclusive to that population. Further, the exclusivity of variation can be evaluated by measuring the genetic divergence between populations. Genetic divergence can be thought of as the absence of migration, and can be measured in terms of the departure from complete population genetic mixture and the resulting hybrid individuals and loci.

We employed a genome scan technique, Restriction site Associated Digest Sequencing (RADseq), to sample the genomes of all individuals collected. We then searched these genome scans for Single Nucleotide Polymorphisms (SNPs) and analyzed SNPs for diversity within populations and divergence among populations. Our sampling design and analysis allowed us to compare the magnitude of among-catchment difference between populations to the within-catchment differences.

If important differences exist among populations at the sub-catchment scale, conservation informed by these differences will be more effective. Sub-catchment diversity may require multiple conservation strategies at ostensibly similar sites within a catchment. Such targeted conservation may improve salmonid population health and productivity, thus maintaining an ecologically and commercially important fish. Generally, we hope this work may illuminate how the relationship between complex organisms and their environment can make conservation more effective in a changing global ecosystem

CHAPTER TWO

Investigation of Fine Spatial Scale Population Genetic Structure in Two Alaskan Salmonids

Abstract

Pacific salmonids provide a useful model system to study how species use of habitat drives genetic diversity in complex environments. Diverging traits like philopatric homing and life-history among salmonid species potentially interact with the environment to maintain different fine spatial scale genetic population structures. To assess this, we analyzed genomic diversity of two species of Alaskan salmonids, coho salmon (*Oncorhynchus kisutch*) and Dolly Varden char (*Salvenius malma*), that are co-distributed in the lower Kenai Peninsula of south-central Alaska using Single Nucleotide Polymorphisms (SNPs) identified in 2bRAD libraries. We examined SNPs for patterns of molecular diversity and divergence in F_{ST}. We designed the sample to compare within- and among-catchment genomic variation and to examine the importance of first order streams to these two species. Population differentiation was higher than expected if reproductive isolation were driven by distance at this scale in some populations of coho salmon but not Dolly Varden. AMOVA suggests discrete populations may be catchments do not represent discrete populations. Genomic diversity and divergence were spatially distributed unevenly across sites. Populations in adjacent habitats showed different patterns of genomic admixture, possibly and indicating inter-drainage metapopulation. Our results suggest first order streams support locally diverged

populations in coho salmon but not in Dolly Varden char. These patterns may reflect an interaction between ecological selection and philopatry. Conservation strategies should consider factor driving species-specific distribution of genetic diversity. This may avoid omission of crucial diversity and improve the capacity of populations to adapt to future conditions.

Introduction

Biodiversity not distributed uniformly because it is driven by different factors at multiple spatial and temporal scales (Harding et al. 1998, Smale 2010, Taylor et al. 2015, Larsen et al. 2015). An important component of biodiversity is within-species genetic variation. For some taxa, distribution (Legalle et al. 2005, King et al. 2012), dispersal(Row et al. 2010, Gil et al. 2016) and gene flow (Razgour et al. 2014) have different patterns across spatial scales. Understanding the sensitivity of within-species genetic variation to scale could be crucial to human efforts to protect habitat for these species.

Species-specific biological characteristics interact with the environment to define the distribution and use of important habitat. These are commonly scale-dependent interactions. For example, recent studies of fine spatial scale population genetics revealed that previously undescribed sub-species types may be genetically isolated due to habitat preference (Waters and Burridge 2016) or life-history divergence (Kazyak et al. 2016). Information about habitat use and fidelity for a species can refine our understanding of the mechanisms of maintaining biotic diversity. The utilization of first order streams by

anadromous salmonids is potentially an ideal model system for understanding habitat preference and diversity-promoting processes.

Fishes in the family Salmonidae (salmonids) are commercially important (Utter 2004) and are naturally vulnerable to perturbation. Salmonids exhibit high levels of genetic population structure due to distance, ecology and behavior (Taylor 1991, Waples 1991a, Hendry et al. 2004, Waples et al. 2008). Some salmonid species exhibit high levels of reproductive isolation among populations that are geographically co-occurring (Bond et al. 2014). Specifically, salmonids return to their natal habitat for spawning (Homing) and undergo local adaptation(Hendry et al. 2004). These biological characteristics isolate salmonid populations and make them vulnerable to human activities. Fishing, urbanization, hydropower installations and interactions with hatchery reared fish affect these populations (Van Hyning 1968, Waples 1991a, Hard et al. 2008) through population size reduction, destruction of habitat, interruption of migratory routes and dilution of locally adapted lineages. Anthropogenic effects on salmonids vary among species (Hard et al. 2008) and across the range of some species.

Targeted conservation of salmonid species in the United States has included the development of Evolutionary Significant Units (ESUs): populations that are reproductively isolated, and important for future evolution (Waples 1991b). Seven ESUs are defined for coho salmon (*Oncorhynchus kisutch*), four of which are currently endangered or threatened (Weitkamp et al. 1995, Starks 2014). Though they are monitored, the codistributed salmonid Dolly Varden (*Salvenius* malma) is not threatened in its North American range and has no defined ESUs. Coho salmon and Dolly Varden

have experienced similar pressures from human activities and have similar ecological requirements.

Coho salmon ESUs encompass large geographic regions. Stock enhancements of ESUs have been based on genetic information at large scales including many catchments (NOAA Fisheries 2016). Efforts to conserve and restore ESUs have experienced reduced returns that have been attributed to environmental variability (NMFS 2015). There may also be lowered genetic diversity in those populations (Hauser et al. 2002). Understanding how factors such as habitat usage, life-history traits and local adaptation drive genetic diversity in different species could improve conservation efficiency.

First order streams in the Pacific Northwest of North America serve as crucial spawning habitat for many salmonid species including coho salmon and Dolly Varden (Quinn 2011). Spatially fine-scale river components such as first order streams are biologically diverse and environmentally variable ecosystems (King et al. 2012, Göthe et al. 2014). Headwaters are also highly connected to the total river (Meyer et al. 2007). Downstream flow transports nutrients and organic matter out of first order streams. Organisms like salmonids disperse to and from of first order streams to feed and spawn. Headwaters have unique combinations of riparian cover, geomorphology, and human land-use that may vary widely over small distances (King et al. 2012, Clark et al. 2014). The combination of habitat variation across first order streams and salmonid fidelity to natal first order streams for spawning may reinforce disruptive selection and maintain complex genetic population structure within a single watershed. Though the potential ecological importance of first order streams has been clearly acknowledged (Lowe and

Likens 2005, Mazza and Olson 2015), within-drainage diversity of first order streams is not considered and many first order streams are not protected (King et al. 2012).

Past studies of impacted salmonid populations have not accounted for the role of first order streams in maintaining salmonid genetic diversity (Bucklin et al. 2007, Johnson and Banks 2008). Some studies have conflated catchment with population by designating the salmonids within a catchment as a discrete population. Salmonids in a catchment may not form a population that is either coherent (Kazyak et al. 2016) or isolated (Keefer and Caudill 2014, Jensen et al. 2015). Rather, some salmonid species first order streams may be cores of metapopulation diversity spanning adjacent watersheds (Huntsman 2014). Salmonids exhibiting such a pattern cannot be genetically characterized without regard for the scale and distribution of important habitat, especially first order streams.

In this study we used population genomic methods to investigate the spatial scale and distribution of genetic diversity in two co-distributed salmonid species: coho salmon (*Oncorhynchus kisutch*) and Dolly Varden char (*Salvenius malma*).
We selected sites on the lower Kenai Peninsula of south-central Alaska (Fig 2.1) where coho salmon and Dolly Varden char were expected to occur (King et al. 2012) and sampled individuals of each species for genetic analysis. We examined genetic diversity and divergence of populations from thousands of Single Nucleotide Polymorphisms (SNPs). Spatially, sites were distributed along the longitudinal continuum within the South Anchor River drainage, and at first order streams of three adjacent drainages to allow for analysis of multi-scale partitioning of genetic variance.

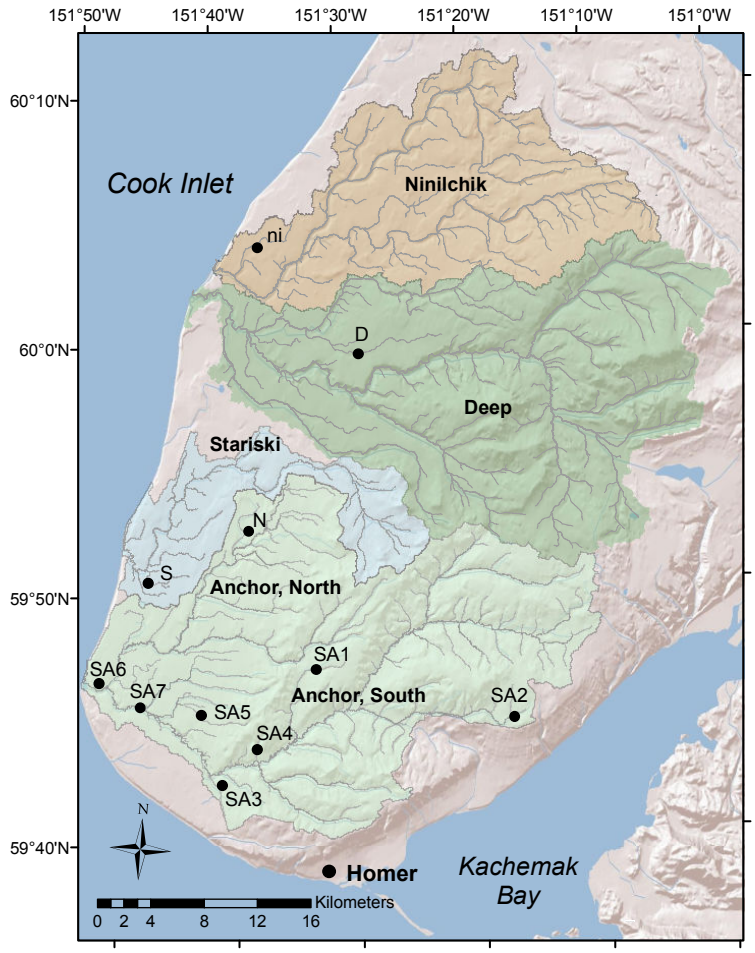

Figure 2.1: Map of sample sites for coho salmon and Dolly Varden char in the lower Kenai Peninsula of southcentral Alaska. Sites are identified by Catchment ID.

Methods

Sampling Design

Fin clips were taken from coho salmon and Dolly Varden char collected from 23 August to 20 September 2013. Individuals were selected on the basis of size and associated juvenile markings (parr marks) to support the assumption that the sample location was nearby their natal habitat. Dolly Varden from the lower river site in South Anchor River were adult. Coho salmon were sampled in Starisky Creek, North Anchor

River and South Anchor River drainages. Dolly Varden char were collected in Starisky Creek, North Anchor River, Ninilchik River, Deep Creek and South Anchor River drainages. Samples were taken from one location in each drainage, except the South Anchor River drainage.

South Anchor samples were taken at five locations, representing first-order stream sites along the watershed from headwaters to the lower river basin, as well as a mainstem channel location for each species. RAD libraries were sequenced from 108 Coho salmon and 180 Dolly Varden char to identify SNP's (see below). For this, an average of 17 individuals were analyzed per site and species (15 coho salmon and 18 Dolly Varden char). Locations and coordinates are presented in Figure 2.1 and Table 2.1 respectively. These sites spanned four adjacent drainages on the lower Kenai Peninsula (King et al. 2012) to permit the potential partitioning of genetic variation to the within- and between-drainage scale.

Sample Processing

Genomic DNA was extracted from each individual tissue sample using DNeasy Blood and Tissue Kit (Qiagen). DNA concentrations were estimated using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies) and a microplate reader (instrument name, model etc.) in comparison to a geometric series of known concentrations of λ-phage DNA. Samples that did not yield >25 ng/μL were concentrated using a vacuum centrifuge.

Restriction site-associated DNA (RAD) libraries were prepared using the 2b-RAD method (Wang et al. 2012) as developed for multiplexed Illumina sequencing (Matz and

Aglyamova 2014). This technique uses a type 2b restriction enzyme (BcgI) to produce fragments of uniform size, distributed throughout the genome. Reduced genome representation allows for identification of a large number of SNP markers at high confidence without incurring high sequencing costs. Prepared libraries were sequenced using next-generation sequencing (NGS) at the Genomic Sequencing and Analysis Facility at the University of Texas at Austin.

Bioinformatics

Sequence data was initially processed using custom PERL scripts. Reads from an individual spread across separate lanes were concatenated. These sorted data were trimmed of all sequences resulting from restriction digestion, barcoding and amplification. The resulting genotypic data were subjected to basic filtering for quality using the FASTX-Toolkit utility FASTQ Quality Filter. Minimum confidence in any base was set at 99.9% (Phred=30, (Cock et al. 2010). We discarded bases below this threshold and reads with less than 93% of bases above the threshold.

Within species, reads were clustered using cd-hit-est (Fu et al. 2012) with settings optimized for High Performance Computing and 2b-RAD reads. Genotypes were called and filtered using non-parametric variant recalibration based on quality metric validation in experimental replicates. Variants were thinned to one base per read, and filtered for missing data and unlikely heterozygote excess by locus. These protocols were implemented in custom PERL scripts which are available upon request.

We removed all loci that were out of Hardy-Weinberg Equilibrium within any population using VCFtools (Danecek et al. 2011). Finally, data was formatted for various population genetic analysis programs using PGDspider2 (Lischer and Excoffier 2012).

Analysis of Genetic Data

For each species we estimated diversity within populations. Observed Heterozygosity (H_O), Wright's fixation index (F_{IS}) , and nucleotide diversity (π) were calculated using Arlequin 3.5 (Excoffier and Lischer 2010). Divergence among populations was estimated by three methods to resolve the processes giving rise to the observed population pairwise genetic distance. F_{st}, Jost's D and G''_{ST} were calculated in Genodive (Meirmans and Van Tienderen 2004). D is not affected by population size, and may better reflect divergence in allele frequencies between populations. G''_{ST} is an unbiased estimator of F_{st} and is affected by differences in demographic processes in the history of populations. F_{st} was also estimated using Arleqin 3.5 for comparison to other studies (Excoffier and Lischer 2010). Finally, we used a bias-corrected, single sample method based on linkage disequilibrium to estimate effective population size using NeEstimator2 (N_e, Waples and Do 2008 implemented in Do et al. 2014)

We performed Analysis of Molecular Variance (AMOVA, (Excoffier et al. 1992) using the R module *poppr* (Kamvar et al. 2014) to confirm population identity and evaluate whether drainage was a significant partition of genetic variation. We estimated significance of the AMOVA was using a Monte Carlo test with 1,000 permutations using the R module *ade4* (Thioulouse et al. 1997). To examine geographic patterns of F_{ST} variation more broadly, Isolation by Distance was examined. Isolation by distance was

tested using a Mantel test implemented in Genodive. The test examined the relationship between stream distance, estimated in Google Earth, and F_{ST}. We used Genodive to estimate the statistical significance of the result in 10,000 permutations.

After removing F_{ST} outliers (see below), ADMIXTURE was used to further evaluate among population divergence by estimating whole genome patterns of relatedness. ADMIXTURE estimates the most likely number (k) of diverged ancestral populations contributing to contemporary genomic diversity. ADMIXTURE then gives the fraction of each individual genome (q) contributed by those k ancestor populations (Alexander et al. 2009). To identify the impact of population subdivision in coho salmon on ADMIXTURE analysis, we hierarchically removed populations from the ADMIXTURE analysis based on several isolation hypotheses (by drainage, distance and extreme values of F_{ST}).

F_{ST} outliers, SNP loci with F_{ST} values that are likely produced by non-neutral processes, were identified in a model based approach implemented by BayeScan (Foll and Gaggiotti 2008) which measures F_{ST} between each subpopulation and a hypothetical shared gene pool. This F_{ST} coefficient is then decomposed into a locus- specific component (α) and population specific components (β) using logicstic regression(Beaumont and Balding 2004). Values of α that are significantly different than zero indicate selection at a locus. For each locus, BayeScan estimates the posterior probability of an island model with or without selection using a reversible-jump MCMC algorithm. Likelihood of the model including selection as opposed to the neutral model for a given locus was evaluated using Jeffrey's scale for Bayes Factor(Robert et al. 2009). Knowledge of the genes near these outlier SNPs may provide insight into the possible

selective forces elevating the F_{ST} values of these loci. Therefore, the RAD fragments containing these outliers were submitted to NCBI's nucleotide BLAST to find associated genes. These loci were removed from further analysis as they likely do not reflect the neutral evolutionary history of the populations and may introduce bias (Allendorf et al. 2010).

Results

Initial processing produced approximately 25 billion accurately sequenced bases spanning one billion filtered reads. We identified 33,000 putative polymorphic loci in the coho salmon and 43,000 in the Dolly Varden char. Non-parametric quality score recalibration and filtering confirmed 6657 in our coho salmon and 3271 SNPs in our Dolly Varden char samples.

Diversity Within Populations

Analysis of genetic diversity (Fig. 2.2) indicated that all populations had low to moderate genetic diversity. Observed heterozygosity (H_O), nucleotide diversity (π), and Wright's fixation index (F_{IS}) were averaged across all loci for each species. In coho salmon H_O was 0.176(0.053) (mean (standard deviation)) and Dolly Varden char was 0.219(0.067). In coho populations π was 0.150(0.033) and in Dolly Varden char it was 0.172(0.018). Diversity measures (H_O, π) were lowest at North Fork Anchor River and highest at Starisky Creek in coho salmon. In Dolly Varden char, H_O and π were also lowest at the North Anchor site but highest at Ninilchik River,
where they were more than two standard deviations above the mean. The smaller variance and larger median of H_O and π in Dolly Varden char indicates similarity

between populations than coho salmon, and more diversity than coho salmon. The fixation index (F_{IS}) was negative for both species and averaged -0.101(0.073) in coho salmon and -0.103(0.109) in Dolly Varden char. However, none of the observed differences were statistically significant. Molecular diversities within populations were fairly similar and downstream analyses were not likely to be biased by differences within populations.

Population effective size (N_e) estimates and variability in N_e were smaller in coho salmon than in Dolly Varden char (Table 2.1; coho salmon: 34.3(17.24); Dolly Varden char 1535(4289.78)). All populations were lower than published risk levels (see discussion)

with the exception of both species at North Anchor (coho salmon N_e=Infinite; Dolly Varden char N_e=12,972) and Dolly Varden char at Ninilchik River (N_e=Infinite). Populations with infinite N_e were omitted from mean and standard deviation calculations above. The value of N_e was an order of magnitude lower at SA1 (6.7) than any other coho salmon population. The smallest N_e estimated for Doly Varden was (39.1) at Deep Creek. Standard deviation in N_e was different between species ($F_{5,8}$: $p<<0.01$) but not the mean.

Divergence Among Populations and Loci Under Selection

Pairwise F_{st} averaged across all loci was calculated using two different methods in Arlequin 3.5 and Genodive. The pairwise F_{st} average given by Genodive was 0.054(0.029) in coho salmon, and 0.031(0.037) in Dolly Varden char (p=0.009). Population pairwise F_{ST} estimates from Genodive and Arlequin are presented in Table

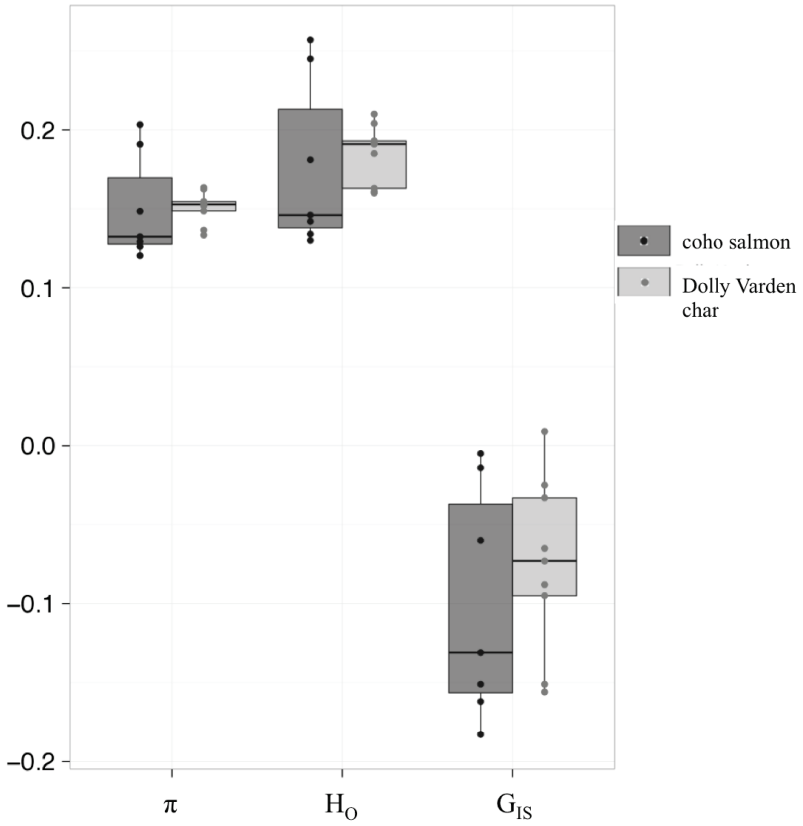

Figure 2.2: Molecular Diversity (H_o, π) and Inbreeding (G_{IS}) in coho salmon and Dolly Varden char (DV) from the lower Kenai Peninsula of south-central Alaska. Indices are estimated from 6657 loci in coho salmon and 3271 loci in Dolly Varden char. Estimation of fixation in Dolly Varden char populations using Gis, an analog of Sewall Wright's classic Fixation Index (F_{IS}). Observed Heterozygosity (Ho), and Inbreeding Coefficient (G_{IS}) were estimated using Genodive. Nucleotide Diversity (PI) was measured using Arleqin 3.5.

2.2. Coho salmon in the South Anchor River site SA1 was the most highly diverged (0.086(0.014)) followed by Starisky Creek (0.066(0.033)) based on F_{ST} estimation.

Populations at sites in the middle reaches of South Branch Anchor River sites (SA2-SA4) were genetically similar (average and standard deviation of F_{ST}: 0.025(0.008); Table 2.2), and were diverged from the headwater site (SA1) (0.077(0.004)) and the lower river site (SA6) (0.045(0.006)). Also, while SA3 is much more geographically distant from the remaining South Anchor river sites, it was

Table 2.1. Population information coho salmon and Dolly Varden char (DV) : Sampling site names and abbreviations, locations, sample sizes (n), number of polymorphic SNP Loci and population effective size (N_e). Sites with only one species have one value n and N_e indicated by "--". Infinite N_e indicates no genetic drift due to finite number of parents.

Symbol	Basin	Catchment ID	Reach ID	Latitude (decimal degrees)	Longitude (decimal degrees)	n (coho/DV)	Loci (coho/DV)	N_e (coho)	N_e (DV)
SA1	Anchor River	ANC-1203	HWS2_1203M	59.779675	-151.5551056	16 / 20	3437 / 2610	6.7	67.9
SA2	Anchor River	ANC-V08	Peri_8	59.7405453	-151.3027552	15 / 19	4301 / 2556	53.8	84.7
SA3	Anchor River	ANC-V03	Val_V03	59.7095941	-151.6987938	17 / 19	4100 / 2278	34.7	37.2
SA4	Anchor River	ANC-V05	Val_V05	59.7510472	-151.7131527	16 / 18	3914 / 2424	50.2	295.2
SA5	Anchor River	ANC-V24	Val_V24	59.7245924	-151.5285954	-- / 19	-- / 2401	--	63.9
SA6	Anchor River	ANC-GW03	NAD83	59.7711751	-151.8435987	18 / --	5635 / --	24.8	--
SA7	Anchor River	ANC-DV	NA	59.756693	-151.783863	-- / 19	-- / 2643	--	150.7
S	Stariski Creek	STAR-171	HWS2_171M	59.8406447	-151.7829479	17 / 20	5423 / 2648	35.6	100.4
N	Anchor River, north branch	ANC-44	HWS2_44L	59.8613647	-151.6583606	9 / 19	3252 / 2532	Infinite	12972
ni	Ninilchik River	NINI-545	HWS2_545L	60.049777	-151.6320347	-- / 7	-- / 1272	--	Infinite
D	Deep Creek	DEEP-V12	Val_V12	59.9900454	-151.4919892	-- / 20	-- / 2512	--	39.1

less strongly diverged from them (0.043(0.025)) than the intervening headwater site SA1 (0.08(0.009)).

Interestingly, the lower river site was not diverged from the sample from the Starisky Creek (S) watershed (F_{ST}=0.009). The estimated G''_{ST} values were similar F_{ST} estimates. Jost's D was smaller between populations that showed stronger signals in F_{ST} (results not presented). Specifically, Jost's D to SA1 were 1.5 standard deviations below the mean D, and G''_{ST} to SA1 were 11 standard deviations above the mean G''_{ST}.

Table 2.2. Estimates of population differentiation (pairwise F_{ST}) among six populations of coho salmon in the lower Kenai Peninsula. Above the diagonal are F_{ST} estimated by Genodive 2. Below the diagonal are F_{ST} estimated by Arlequin 3.5. Site abbreviations as indicated in Table 2.1. Empty cells indicate estimates that were not significant ($\alpha = 0.05$).

F_{ST}	*SA1*	SA2	SA3	SA4	SA6	S	NA
SA1		0.082	0.074	0.074	0.091	0.112	0.082
SA2	0.064		0.03	0.03	0.038	0.063	0.033
SA3	0.070	0.013		0.016	0.05	0.075	
SA4	0.068	0.009	0.012		0.048	0.072	0.019
SA6	0.096	0.025	0.054	0.038			0.043
S	0.110	0.041	0.073	0.054	0.001		0.067
NA	0.076	0.019	0.015	0.016	0.052	0.068	

Estimates of divergence in Dolly Varden char (Table 2.3) suggest that the South Anchor headwater (SA1) and mid-river sites (SA2-SA5) were not qualitatively different (among mid-river F_{ST} 0.012(0.002); SA1 to mid-river 0.011(0.001)), as was the case with coho salmon. The South Anchor lower river site (SA7) was also less diverged than higher river sites within the South Anchor drainage (0.007(0.003)). There was a nominal increase in F_{ST} when considering Deep Creek (0.023(0.003)), but the values from the

other two adjacent drainage samples were not different from the South Anchor drainage (S: 0.01(0.003); NA:0.01(0.004)).

The results of nested AMOVA with populations nested within drainages found that there was significant genetic variation among populations within drainages in either species (p=0.001), but not among drainages (coho salmon: p= 0.637, Dolly Varden char: p=0.671). Mantel Test results indicate that neither genetic differentiation in coho salmon (p=0.162) nor in Dolly Varden char (p=0.132) was significantly associated with stream distance in these samples.

Table 2.3. Estimates of population differentiation (pairwise F_{ST}) among ten populations of Dolly Varden char in the lower Kenai Peninsula. Above the diagonal are F_{ST} estimated by Genodive 2. Site abbreviations as indicated in Table 2.1. Empty cells indicate estimates that were not significant ($\alpha = 0.05$).

F_{ST}	*SA1*	SA2	SA3	SA4	SA5	SA7	S	NA	N	D
SA1		0.01	0.012	0.01	0.013	0.007	0.009	0.01	0.097	0.019
SA2			0.014	0.01	0.009		0.009	0.009	0.083	0.021
SA3				0.013	0.015	0.012		0.014	0.09	0.025
SA4	0.002	0.007			0.013		0.01	0.009	0.119	0.025
SA5						0.009	0.014	0.013	0.099	0.025
SA7	0.002	0.004					0.008		0.112	0.021
S		0.002		0.005				0.011	0.097	0.02
NA	0.002	0.008		0.009	0.004		0.009		0.122	0.022
N										0.104
D	0.007	0.012		0.016			0.013	0.015		

ADMIXTURE analysis suggested that three ancestral lineages contributed to the contemporary genomic diversity in coho salmon (Fig. 2.3) Interpretation of ADMIXTURE results suggested a lineage dominating the gene pool at SA1 that is found only in small proportions of genomes at all other sites (genomic ancestry fraction: $q<2^{-4}$). Another second lineage was predominant ($q>2^{-1}$) in South Anchor River sites except for

SA1. The third lineage in coho salmon contributed heavily to genomes ($q>2^{-1}$ in at least half of individuals) at the South Anchor river-margin site (SA6) and the at the site in Starisky Creek (S). At SA6 and S sites ADMIXTURE estimated genomes had a gradient of admixture with nearly equal overall contributions of two major lineages. Most other drainages had fewer mixed genomes with smaller genomic contributions minor lineages. The most likely ADMIXTURE model had only one lineage contributing to Dolly Varden char genetic diversity in these samples.

Ancestry fractions (q-values) estimated by ADMIXTURE in sub-groups of our sample indicated the importance of structure due to separation by drainage, geographic distance and genetic distance (Fig. 2.3). Neither the exclusion of sites outside of South Anchor River, nor the exclusion of the most geographically distant South Anchor (SA2) site affected the model solution (k=3). However, the exclusion of the South Anchor headwater site (SA1) caused ADMIXTURE to find only two contributing ancestral populations (k=2). This was the case regardless of inclusion of individuals from adjacent drainages.

We used Bayescan (Foll and Gaggiotti 2008) to evaluate the likelihood that selection s acting on each locus across all populations. The false discovery rate was set to a stringent level (FDR: $q < 0.01$). In coho salmon 72 loci were highly likely to be influenced by selection. In Dolly Varden char only one such loci was found. These loci were submitted to NCBI nucleotide BLAST to gain insight on their putative identity and function. Two RAD fragments aligned with one and three reference sequences, respectively. All of these alignments had 100% identity and a Bit Score of 58.4 on 36 bases. One alignment (Bit Score = 58.4) of note was in a genomic region containing

salmonid MHC II genes (*Salmo salar* MHC class II antigen chains alpha and beta) which BayeScan analysis suggested was under strong diversifying selection ($\alpha = 1.65$). The same region contained also contained *S. salar* genes for neurogranin, TIP41-like protein, leucine rich repeat containing 35-like protein, and alpha-tectorin-like protein(Harstad et al. 2008). A second RAD fragment aligned with *S. salar* E3 ubiquitin-protein ligase Hakai mRNA (Leong et al. 2010).

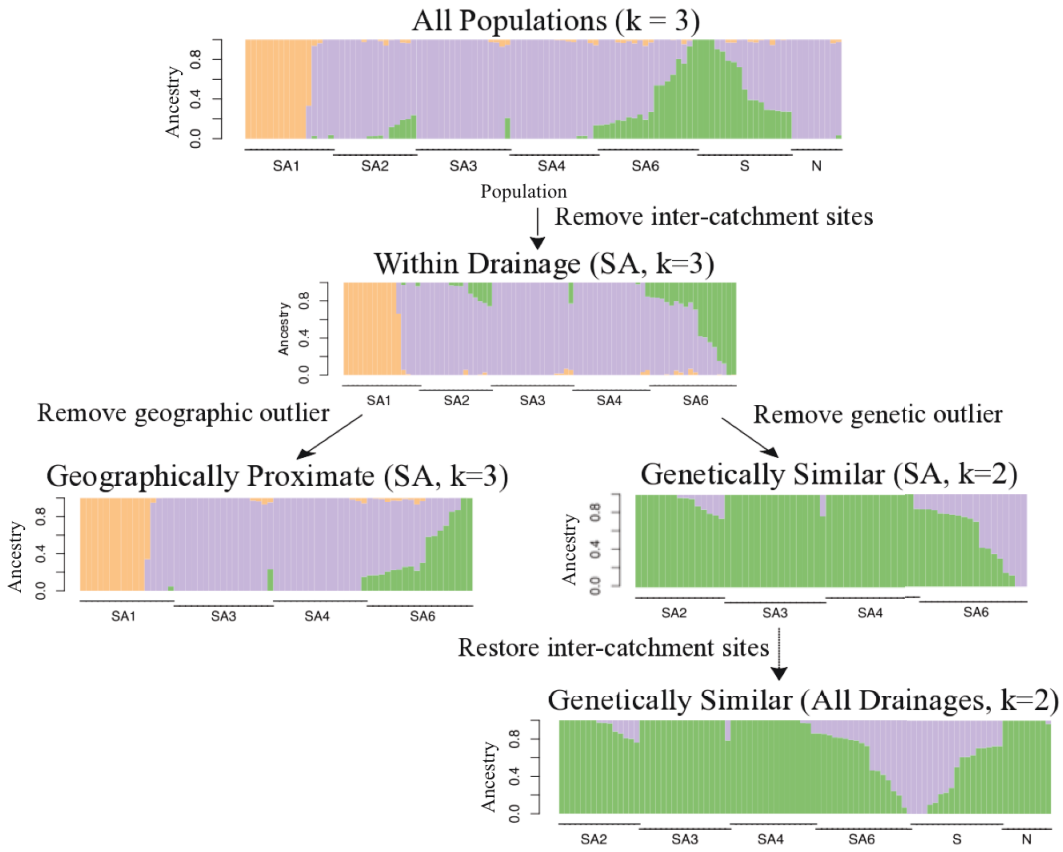

Figure 2.3: Genome contributions (Q-estimates) from each of the most likely number of ancestral populations (k) as estimated by ADMIXTURE for each subset of populations. Stacked bars represent the genome of a sampled individual, partitioned into k groups of loci from maximally diverged ancestral populations. Neither drainage differences nor geographic distance impact the model as strongly as the upper river site SA1, which contains a highly diverged lineage (orange) that is nearly exclusive to that site.

Discussion

Population genomic analyses of organisms that have complex relationships with their environment may not detect important patterns if they are not informed by species-specific hypotheses. Our results suggest that two related species of Pacific salmonids in the same first-order streams have similar levels of within population diversity, but very different genetic population structure. The patterns observed in coho salmon indicate multiple non-neutral processes occurring both within and between watersheds. Dolly Varden char occur in panmixis across the same streams.

There were similar levels of genetic diversity within populations of both species with the exception of one sample. The sample of Dolly Varden char at Ninilchik River likely showed a different pattern due to missing data at many loci. Filling these loci with randomly generated data drawn from the empirical distribution of either the total sample or the population, changed the molecular diversity of this population to near median levels of each metric we examined. Assuming this adjustment renders the data more realistic, the genetic diversity of this whole sample falls within recently published ranges for heterozygosity of SNPs in globally distributed coho salmon populations (Starks et al. 2016). Multiple estimates per drainage of within population molecular diversity in SNP loci may not be directly informative for conservation. On the other hand, the utility of these metrics varies by genetic data type, and important processes may manifest as differences in diversity in other locations and study designs.

All values of F_{IS} were negative in our sample for both species, probably indicating the effect of small population size and a sampling artifact. Negative F_{IS} can result from differences in allele frequencies that occur randomly between breeding males and

females in small populations (Pudovkin et al. 1996). The results of N_e estimation confirm that populations at our sample sites are very small, especially in coho salmon. Negative F_{IS} may also result if the local breeding population geographically extends beyond our sample area (Neel et al. 2013). N_e results are generally larger in Dolly Varden char, supporting the possibility that this artifact affected F_{IS} for that species. Such artifacts may be unavoidable in salmonids at small spatial scales due to their semi-continuous distribution, and potential for existing in metapopulations. Nonetheless, AMOVA supports the hypothesis that the sample sites represent discrete units of genetic variation. Non-equilibrium processes like balancing selection or heterozygote advantage may have also contributed to negative F_{IS} in these populations. Locally adapted lineages present in the Kenai Peninsula drainages that form high-fitness hybrids could sustain high levels of heterozygosity. However, theoretical evidence suggests that outbreeding depression is more likely in locally adapted salmonids (Emlen 1991). Selection is unlikely to have affected all populations of both species in the same way.

The low estimates of N_e suggest that these sub-populations are at risk for extirpation due to stochastic effects or land use changes. Nearly all sites had N_e estimates below the threshold of concern for extinction risk according to published risk thresholds (Waples 1990, Allendorf et al. 1997). Many populations are below the threshold for high extinction risk (N_e<50). It has been proposed that even in the absence of other threats, a population with such a low N_e has 20% chance of extinction over the next 20 years (*in sensu* population viability analysis). However, this estimate is conservative, as it does not account for recent impacts or demographic trends (Allendorf et al. 1997). Further information may be difficult to obtain as many sites are first-order streams on private

land. In the event of local extirpation it is possible that other local sub-populations could recolonize the habitat. However, crucial genetic variation shaped by local adaptation could still be lost if adjacent populations are genetically diverged.

Coho salmon populations in our sample were highly diverged given the geographic extent of our sampling area. For comparison, a meta-analysis has shown that the species most closely related to coho salmon((Crête-Lafrenière et al. 2012, Zhivotovsky 2015)) have F_{ST} less than 0.1 between populations at 500km (*O. tshavytscha*: $F_{ST} < 0.1$, (Hendry et al. 2004)). Divergence of coho salmon sample from SA1 was equivalent to 86% of the 500km F_{ST} benchmark. However, SA1 is only 27.52(18.97) kilometers separated from other sites. SA1 was more diverged within its watershed than other South Anchor sites. Further, SA1 shows higher levels of divergence than any inter-drainage comparison excluding SA1. This pattern is likely due to demographic processes and not mutation, as G''_{ST} is elevated but not Jost's D (Meirmans and Hedrick 2011). Low N_e at this site (6.7) further supports the influence of demography. Unlike coho salmon at SA1, relatively low divergence was observed between samples at the most geographically isolated sites (SA6, SA2, and Starisky Creek). Qualitatively, there seemed to be no pattern of increased divergence between drainages in coho salmon. Divergence was generally an order of magnitude lower in Dolly Varden char. In Dolly Varden char elevated divergence between drainages was evident, though not significant.

Spatial analyses (AMOVA, Mantel Test) found that neither drainage nor distance were significant isolating factors at this scale for these species. This may be due to the predominant action of other processes in coho salmon such as demography or selection.

The low divergence signal in Dolly Varden char at this scale may have been overwhelmed by stochasticity or noise resulting from incomplete sampling. IBD is thought to be an important process for Pacific salmonids at most spatial scales (Hendry et al. 2004, Petrou et al. 2014). A more evenly spaced and, or higher density spatial sample would likely reveal isolation by distance (IBD) in these drainages. However, IBD is not likely a comprehensive model for Pacific salmonids at this scale (Ackerman et al. 2013, Bond et al. 2014, Harris et al. 2015, Waters and Burridge 2016). Reanalysis by removal of the highly diverged Anchor River SA1 site caused a distinct increase in the fit of the Isolation by Distance (IBD) model to this sample. The spike in divergence at SA1 may be evidence that other important processes, such as local adaptation, affect coho salmon within this drainage. Samples that do not emphasize first order streams may not detect such local phenomena. In contrast to the pattern in coho salmon, Dolly Varden char at this scale shows very little divergence, and may effectively be a single breeding population.

The ADMIXTURE analysis of Dolly Varden char samples supports a single unified ancestral lineage at all sites. On the other hand, our interpretation of this analysis was that coho salmon genomes represent three diverged lineages. Genomes were relatively homogeneous within most coho salmon populations, with one ancestral lineage dominating all genomes. Two sites (SA6, S) exhibited a gradient of ancestry composition. These sites are in different drainages and both are in close proximity to the ocean. Frequent interbreeding among inter-drainage populations is clearly possible in coho salmon. One lineage involved in this interbreeding is the primary constituent of genomes in most other South Anchor River populations. This pattern may be the result

of salmonid homing behavior to the South Anchor River. The other among-drainage mixed lineage does not dominate any individual genomes outside SA6 and S. The distribution of this lineage indicates fidelity to near-ocean habitat, in addition to a lack of barriers to reproduction with members of diverged population. If there is no habitat where this lineage is dominant, it may represent an introgression with hatchery reared fish (Waples 1991a). Alternatively, we may have not sampled the location containing the 'pure' population of this lineage.

The sample at SA1 contained several individuals with high genome fractions from a lineage that was barely present at any other site. This indicates that a unique subpopulation at SA1 has diverged from the other sites in the South Anchor River. This could be the result of differences in local ecological conditions such as stream velocity, dissolved oxygen content, substrate composition, canopy and macroinvertebrate community (Taylor 1991, King et al. 2012, Ackerman et al. 2013) or life history changes (Gomez-Uchida et al. 2011). We have attempted to further support these hypotheses by examining the effect of genetic population structure on our ADMIXTURE results. Removal of SA1 (and retention of inter-drainage and geographically distant sites) caused the model to find one less contributing ancestral lineage (k=2). This further supports the presence of a diverged lineage at SA1.

For coho salmon, F_{ST} and ADMIXTURE results together suggest the presence of distinct but connected lineages in a single watershed, and a high level of genetic migration between watersheds. The South Anchor river and adjacent drainages seem to house an inter-drainage metapopulation. Past studies of Pacific salmonids have implicitly assumed that the drainage is the primary unit of importance for studying structured

populations. Studies have therefore examined diversity and isolation at the inter-drainage scale. Yet watersheds may be a spurious unit for studying diversity in some species. Similar habitat can be distributed among drainages and salmonid species are known to move among drainages and may stray from natal habitat for spawning (Hendry et al. 2004, Jensen et al. 2015). We have provided evidence for the importance of both specific river components and multi-scale interactions (within and among drainage) to determining genetic population structure in Pacific salmonids.

Habitat differences may affect genetic population structure thru habitat preference and local adaptation. Preference for sub-drainage habitat type has been implicated in the fine-scale divergence of ecotypes in New Zealand *Galaxias* fish (Bond et al. 2014, Waters and Burridge 2016). The distribution of spawning habitat affects the spatial auto-correlation of genotypes in *Oncorhynchus* species and other taxa (Neville et al. 2006, Row et al. 2010). Additionally, associations between fine-scale differences in habitat type and genetic differentiation indicate that neighboring populations may be isolated by adaptation in both Pacific char and salmon (Ackerman et al. 2013, Bond et al. 2014). The direct affects of habitat may interact with philopatry to influence Pacific salmonid genetic populations structure.

A number of studies have examined straying rates, and factors influencing homing and straying (Keefer and Caudill 2014). Straying varies greatly among studies of the same species. Some of these differences are likely influenced by the spatial scale considered. Some processes affecting straying may only be relevant at certain scales. For example, the effects of mate choice and site-selection on straying and homing may not affect patterns observed above the reach scale (Quinn 1995). Changes in

geomorphology, climate and land use that affect natal habitat may interact differently at different scales alter straying in salmonids. Specifically, alterations of migration corridors, stream levels, stream detritus and local spawner density have been shown to affect breeding site selection and juvenile imprinting (Keefer and Caudill 2014, Clark et al. 2014). These alterations may vary among adjacent first order streams, but observed levels may remain constant at the drainage scale.

Environmental and community diversity may occur on a fine scale in many dimensions, especially across highly variable first order streams (Gomi 2002, King et al. 2012, Göthe et al. 2014). First order streams differ in flow regimes, substrate, water chemistry, and community composition and density. These site variations may drive local adaptation and maintain sub-drainage diversity in immunity, physiological tolerance, and life-history characteristics (Taylor 1991, Dionne et al. 2009, Harris et al. 2015). If adjacent populations are sufficiently diverged, these local populations may represent irreplaceable genetic diversity (Waples 1991b). Our results suggest that some salmonid populations diverge at very fine geographic scales, but mix at larger scales.

The diversity in coho salmon may represent colonization by diverged lineages following the Pleistocene Glacial Maximum. However, (Smith et al. 2001) found that this area may be inhabited by a single lineage. While their results were inconclusive, Smith suggested that local adaptation could maintain genetic variation and limit migration. Our BayeScan results gave decisive evidence suggest that selection is influencing the pattern of genetic divergence among our sample of coho salmon at 72 loci but only one among Dolly Varden char (FDR < 0.01,(Berger and Pericchi 1996, Foll and Gaggiotti 2008)). This suggests that selective pressures experienced by coho salmon are

species specific, that Dolly Varden char are not subject to the selective pressures acting on the loci we sampled, or that Dolly Varden char lack the genetic variability to respond to selective pressure.

BLAST revealed several high scoring alignments in coho salmon sequences under putative selection, and none in Dolly Varden char. The alignment of a putatively selected coho salmon RAD-tag with a genomic region containing MHC II in *Salmo salar* may indicate differential immunity among our populations. Standing genetic variation of MHC in salmonid has been associated with variable pathogen resistance and selection in wild populations (Dionne et al. 2009, de Eyto et al. 2011, Miller et al. 2014). Also, other important genes including neurogranin, TIP41-like protein, leucine rich repeat containing 35-like protein, and alpha-tectorin-like protein genes, and E3 ubiquitin-protein ligase Hakai, which are not yet completely understood in *Salmo* or *Onchorhynchus*. Further study may be conducted to ascertain whether they play roles in responding to changes in chemical or biological stream environment, or in evolution of alternative life-histories and phenologies such as run-timing in Pacific salmonids.

Basic models of isolation such as IBD are not adequate for salmonids on the lower Kenai Peninsula. Grouping species for analysis can result in the misapplication of a basic model. While Dolly Varden char appear panmictic on these sites, coho salmon exhibit clear genetic population structure. Additionally, without considering species-specific biological characteristics and habitat utilization, even extensive sampling may not capture information that could be crucial to conservation efforts. In this study, our focus on the importance of first order streams to coho salmon as spawning habitat allowed us to capture localized genetic diversity.

Approaching the genetic study of populations with species-specific information may reveal the underlying processes that maintain robust wild populations. While some species have maintained a large N_e and historical distributions in the face of rampant overexploitation (Kennington et al. 2013), other widely distributed species have experienced population declines or collapses (Van Hyning 1968, Hutchings 2000, Hutchings and Reynolds 2004, Miller et al. 2014). Understanding how landscape characteristics interact with species-specific biology to influence genetic diversity should improve conservation efficacy (Smit et al. 2010, Row et al. 2010). In the lower Kenai Peninsula, individual sub-drainage scale river components such as first order streams, maintain the genetic diversity in wild populations. Headwaters themselves are highly diverse, and may present diverse selective pressures that maintain within-drainage variation and genetic population structure. This variation could be crucial to the capacity of a species to weather stochastic perturbations, and respond to a changing climate. Therefore, identifying and understanding headwater stream habitat on both public and private lands may be crucial for conservation of endangered riverine species

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