**Introduction**

Genetic data for assessment have relied largely on putatively neutral markers such as microsatellites. However, thorough representation of the genome is particularly critical for exploited organisms with relatively high gene flow to distinguish subtle patterns of differentiation that may be associated with local adaptation. Next-generation sequencing has provided new tools to identify large numbers of informative single-nucleotide polymorphisms (SNPs) across the genome, which includes surveys of both neutral and adaptive loci used to determine levels of genetic diversity and genetic differentiation. Sequencing of restriction-site-associated DNA (RAD) tags (Miller et al. 2007) simultaneously facilitates both marker discovery and genotyping-by-sequencing (Narum et al. 2013).

Eulachon are anadromous smelts found in the North Pacific Ocean ranging from northern California to the southeastern Bering Sea along the Alaska coast (Gustafson et al. 2012) Females spawn during the spring, upstream of the mouth of large river systems, and fertilized eggs adhere to the substrate, where they hatch in about 20–40 d depending on water temperature (Hay & McCarter 2000). Once hatched, the larvae are immediately flushed to sea where they appear to be dispersed by estuarine and ocean currents (Barraclough 1964; Hay & McCarter 2000). The objective of the analysis is to determine population differentiation in eulachon using putatively adaptive SNP panels obtained from Dryad.

**Methods**

Tissue samples of eulachon from 494 individuals were obtained from 12 different rivers in Alaska, British Columbia and Washington from 2001 to 2012. Tissue collections used in the analysis came from the US Fish and Wildlife Service (USFWS), Washington Department of Fish and Wildlife (WDFW), and the Department of Fisheries and Oceans (DFO) laboratory, Pacific Biological Station, Nanaimo, British Columbia. DNA extraction were performed with Qiagen DNeasy kits (Qiagen, Valencia, CA, USA).

Libraries for RAD-seq were prepared using methods previously described by Miller et al. (2012) with slight modifications. DNA in the samples was quantified in 96-well assay plates using the Quant-iT dsDNA pico-green assay kit (Life Technologies, Grand Island, NY, USA) and a PerkinElmer Victor 5 plate reader. 250 ng of each sample was used in 100-lL restriction enzyme digests (SbfI, New England Biolabs, Ipswich, MA, USA). Each sample then was tagged with sticky end ligation with one of 96 uniquely barcoded adapters (P1 adapter) to the SbfI cut sites using T4 DNA ligase.

The samples were mixed together into libraries of 96 individuals and each pooled library was sheared to an average size of 500 bp with a Bioruptor UCD-300 sonicator (Diagenode, Denville, NJ, USA). library was concentrated to a volume of 100 lL using Qiagen MinElute columns. Size selection was performed on each library with Agencourt AMPure XP beads. Prior to sequencing, each library was quantified by qPCR using standard Illumina PCR primers and Power Sybr qPCR master mix (Life Technologies) on an ABI 7900HT instrument (Life Technologies). The libraries then were sequenced by single-end 100 base reads using an Illumina HiSeq1500 sequencer (Illumina Inc., San Diego, CA, USA).

SNP discovery and genotyping were performed using a bioinformatics pipeline provided and detailed in Miller et al. (2012). SNPs were identified using the first 500 K sequencing reads from a subset of 12 individuals that were selected to represent genetic variation from each collection site.

Detection of putative adaptive SNP loci

An FST outlier approach was used to determine a set of candidate SNPs that had significantly higher FST values than expected under a neutral model of selection. A set of 4104 SNPs from the 12 collections was analyzed with LOSITAN (Antao et al. 2008), a panel of 193 SNPs was found with a high FST, greater than the probability of 0.975 as putatively under divergent selection.

**Results**

The panel of 193 SNPs putatively under divergent selection was used in the analysis. The mean diversity measures between populations for the putative adaptive SNPs displayed a high mean number of alleles which indicates genetic diversity in the northern GOA populations(TMRO1, KENON ) , trend of increasing values from the southern Fraser-Columbia region (Fraser, CR12, COW02 ) populations and SE Alaska-B.C(SS08, BELCO3, KC02, KEM, KLK02, SKEL10, STIK06) population was intermediate. Mean AR ranged from 3.23 to 3.42 in the population. The test for differentiation showed an overall FST of 0.05. and pairwise FST genetic differentiation between all population comparisons was highly significant (P < 0.05).

DAPC analysis showed that the optimal number of clusters of individual genotypes was three using the package adegenet. The second principal axis differentiated the northern GOA populations from SE Alaska-BC and Columbia-Fraser populations. the first axis expressed the variability between SE Alaska- BC and Columbia-Fraser

The Neighbour-joining tree results also indicate that there is a three-population with respect to the clustering of each sample group; southern Columbia-Fraser group (Cowlitz, Columbia, and Fraser rivers), a seven-population British Columbia (BC) – SE Alaska group (Stikine, Nass, Skeena, Klinaklini, Kingcome, Kemano and Bella Coola rivers) and a two-population northern Gulf of Alaska (GOA) group (Twenty Mile and Kenai rivers).

**Discussion and conclusion**

RAD sequencing data is used to discover putatively adaptive SNP variation in eulachon population. The putatively adaptive SNP panel demonstrated considerably higher levels of differentiation in the population level, higher mean and overall FST values. the adaptive panel showed more variation between regional groups and less variation within populations and this can be attributed to differences in selective pressures among recently colonized environments creating the patterns of diversity observed among the putatively adaptive SNPs indicated in (Candy et al., 2015).The genetic diversity in the three regions may be related to a demographic expansion from a refuge where leading- edge colonizers following glacial retreat would have both lower allelic richness and expected heterozygosity (Hewitt 1996).

In summary, patterns of regional stock structure for eulachon seem to be conserved across adaptive variation. Population-specific differentiation of eulachon is low within each region however regional groups were well differentiated. (Candy et al., 2015) also indicated that, this is possibly due to the life history of eulachon, or possibly due to the lingering historical effects of demographic radiation from glacial refugia.

**References**

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