Report on survey of microsatellite variation in northern British Columbia Chinook salmon

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Abstract

Approximately 7,000 Chinook salmon from 49 populations in southern British Columbia were surveyed for variation at 13 microsatellite loci that comprised the suite incorporated in the Genetic Analysis of Pacific Salmon (GAPS) shared database. The multi-locus genotypes of all fish surveyed were provided to Dr. P. Moran of the National Marine Fisheries Service Montlake Laboratory for incorporation into the GAPS database. The number of alleles observed at a locus varied from 11 (Ots9) to 71 (Omm1070), with all loci in Hardy-Weinberg equilibrium (Table 2). The average F_{ST} value was 0.063, with individual locus values ranging from 0.027 (Omm1080) to 0.161 (OtsG474). A regional population structure was observed, with populations clustering into geographic regions.

Introduction

Conservation of Chinook salmon genetic diversity around the Pacific Rim requires an understanding of their origins and the evolutionary processes promoting and maintaining their differentiation, and delineation of phylogenetically and adaptively distinct groups in the distribution. Genetic variation can be employed as a very effective tool to evaluate the population structure of salmonids, is a key component in the elucidation of management units or conservation units in a species, and can be applied to manage fisheries exploiting specific stocks of salmon. For Chinook salmon, variation at allozymes was the initial principal genetic technology employed in evaluation of population structure, ranging from the Yukon River (Beacham et al. 1989), Alaska (Gharrett et al. 1987), Southeast Alaska and northern British Columbia (Guthrie and Wilmot 2004), British Columbia (Teel et al. 2000), to the Pacific northwest (Winans 1989; Utter et al. 1989, 1995; Shaklee et al. 1999). Increased resolution among populations relative to that detected with allozymes became possible with the advent of DNA-level assays. Initial surveys employed variation at mitochondrial DNA (Wilson et al. 1987; Cronin et al. 1993) and minisatellites (Beacham et al. 1996), but these techniques were soon replaced by surveys of microsatellite variation (Banks et al. 2000; Nelson et al. 2001; Beacham et al. 2003). Microsatellites have been recognized as providing the ability to evaluate fine-scale population structure in salmonids (Banks et al. 2000), as well as the capability to investigate population structure on a Pacific Rim basis (Beacham et al. 2006a).

This report outlines the contributions made to a shared microsatellite baseline for Chinook salmon that is commonly known at the Genetic Analysis of Pacific Salmon (GAPS) microsatellite baseline. This baseline is shared by management and assessment agencies from Alaska through California.

Methods and Materials

Collection of DNA samples

Genomic DNA was extracted from either liver, scales, operculum punches or fin clips from Chinook salmon sampled initially using the phenol-chloroform protocol of Miller et al. (1996) and later a chelex resin protocol (Withler et al. 2000). Samples were derived from adults in all areas except for some locations, where due to the difficulty of obtaining adults, juveniles were sampled. Adults could have been sampled and released, freshly killed and sampled, or samples could have been obtained from carcasses on the spawning grounds.

Template DNA was amplified via the polymerase chain reaction (PCR) at a total of 13 microsatellite loci (Table 1) including nine tetranucleotide microsatellite loci, *Oki*100 (K. Miller, unpub. data), *OMM*1080 (Rexroad *et al.* 2001), *Ots*211, *Ots*212, *Ots*213, *Ots*201b, *Ots*208b (Grieg *et al.* 2003), *Ots*G474 (Williamson *et al.* 2002), *Ssa*408 (Cairney *et al.* 2000) and four dinucleotide loci, *Ogo*2, *Ogo*4 (Olsen *et al.* 1998), *Ots*3, *Ots*9 (Banks *et al.* 1999). All multi-locus genotypes were provided to Dr. P. Moran of the National Marine Fisheries Service Montlake Laboratory for incorporation into the Genetic Analysis of Pacific Salmon (GAPS) database for Chinook salmon. These data can be accessed at http://webapps.nwfsc.noaa.gov/gaps.

Data Analysis

All annual samples available for a location were combined to estimate population allele frequencies, as was recommended by Waples (1990). Weir and Cockerham's (1984) F_{ST} estimates for each locus over all populations were calculated with FSTAT version 2.9.3.2 (Goudet 1995). Cavalli-Sforza and Edwards (CSE) (1967) chord distance was

used to estimate genetic distances among all populations. An unrooted neighbor-joining tree based upon CSE was generated using NJPLOT (Perriere and Gouy 1996). Computation of the number of alleles observed per locus was carried out with GDA (Lewis and Zaykin 2001).

Results and Discussion

During the study, approximately 7,000 fish from 49 populations in southern British Columbia were surveyed at 13 microsatellite loci that comprised the Genetic Analysis of Pacific Salmon suite (Table1). The number of alleles observed at a locus varied from 11 (Ots9) to 71 (Omm1070), with all loci in Hardy-Weinberg equilibrium (Table 2). The average F_{ST} value was 0.063, with individual locus values ranging from 0.027 (Omm1080) to 0.161 (OtsG474).

Analysis of genetic relatedness among populations indicated that there was a regional population structure. Populations clustered into the following regions: west coast Vancouver Island, east coast Vancouver Island, southern British Columbia mainland, upper Fraser River, middle Fraser River, lower Fraser River, South Thompson River, North Thompson River, lower Thompson River, and Boundary Bay (Figure 2).

Funding was originally provided to survey variation in approximately 4,800 individual Chinook salmon. However, during the course of the survey, the Molecular Genetics Laboratory was able to upgrade the automated DNA sequencers in the laboratory, reducing the cost of analysis of individual fish. These cost savings were incorporated in the survey of Chinook salmon microsatellite variation, with approximately 7,000 individuals surveyed instead of the proposed 4,800 individuals.

The multi-locus genotypes of all individuals surveyed in the study have been provided to Dr. P. Moran (NMFS, Seattle) for incorporation into the GAPS database. A

number of publications are anticipated from the analysis of these data, and some are currently under scientific review.

This project also included an upgrade of the infrastructure in the Molecular Genetics Laboratory. A report was previously provided to the Pacific Salmon Commission outlining the purchase of a 3730 model DNA sequencer, as well as robotics for DNA extraction. Additional funding was later secured for increasing the number of thermal cyclers available in the laboratory, as well as some additional robotics. These upgrades to the laboratory infrastructure were accomplished as envisioned, with the equipment used in applications of interest to the Pacific Salmon Commission.

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Table 1. Summary of population surveyed, region, and number of fish analyzed for variation at 13 microsatellite loci in the study. Regions are Nass (Nass River), NOMN (Northern BC mainland), QCI (Queen Charlotte Islands), Skeena Bulkley (Bulkey River), Skeena Lower (Lower Skeena River), Skeena Mid (Middle Skeena River), Skeena Upper (upper Skeena River), Stikine (Stikine River), Taku (Taku River), Alsek (Alsek River).

Population	Region	Number
Cranberry	NASS	211
Damdochax	NASS	15
Ishkheenickh	NASS	216
Kincolith	NASS	143
Kiteen	NASS	54
Kwinageese	NASS	71
Meziadin	NASS	152
Owegee__	NASS	110
Teigen___	NASS	30
Tseax___	NASS	196
Chuckwalla	NOMN	64
Dean@Main	NOMN	19
Dean_River	NOMN	219
Docee__	NOMN	42
Kateen	NOMN	132
Kilbella	NOMN	68
Kildala_	NOMN	181
Kitlope	NOMN	194

Takia_River	NOMN	47
U._Dean	NOMN	194
Yakoun__	QCI	137
Bulkley	Skeena Bulkley	226
Morice__	Skeena Bulkley	150
Ecstall_	Skeena Lower	39
L._Kalum	Skeena Lower	157
L._Kalum@AC	Skeena Lower	112
Thomas_Creek	Skeena Lower	21
Kispiox_	Skeena Mid	104
Kitwanga	Skeena Mid	183
Skeena@Terrace	Skeena Mid	37
Sweetin_River	Skeena Mid	54
Bear____	Skeena Upper	109
Slamgeesh	Skeena Upper	48
Sustut__	Skeena Upper	184
Christina	Stikine	222
Craig_River	Stikine	107
Johnny_Tashoot	Stikine	26
Little_Tahltan	Stikine	130
Shakes_Creek	Stikine	137
Verrett	Stikine	422
Dudidontu_R	Taku	239
Kowatua	Taku	164
Little_Tatsam.	Taku	403

Little_Trapper	Taku	78
Nahlin__	Taku	216
Nakina	Taku	291
Blanchard	Alsek	372
Klukshu_	Alsek	72
Takhanne	Alsek	183

Table 2. Number of alleles, expected heterozygosity (H_E), observed heterozygosity (H_O), and genetic differentiation (F_{ST}) for 13 microsatellites examined in 49 collections of Chinook salmon.

Locus	Number of alleles	H_E	H_O	F_{ST}
*Ogo*2	22	0.66	0.67	0.101
*Ogo*4	19	0.75	0.75	0.065
*Oki*100	43	0.94	0.94	0.021
*Omm*1080	57	0.94	0.94	0.024
*Ots*201b	35	0.90	0.91	0.044
*Ots*208b	50	0.94	0.93	0.025
*Ots*211	40	0.92	0.92	0.032
*Ots*212	30	0.80	0.80	0.048
*Ots*213	42	0.93	0.93	0.033
*Ots*3M	13	0.69	0.69	0.104
*Ots*9	12	0.50	0.50	0.045
*Ots*G474	12	0.32	0.32	0.088
*Ssa*408	29	0.84	0.82	0.090
Overall				0.052

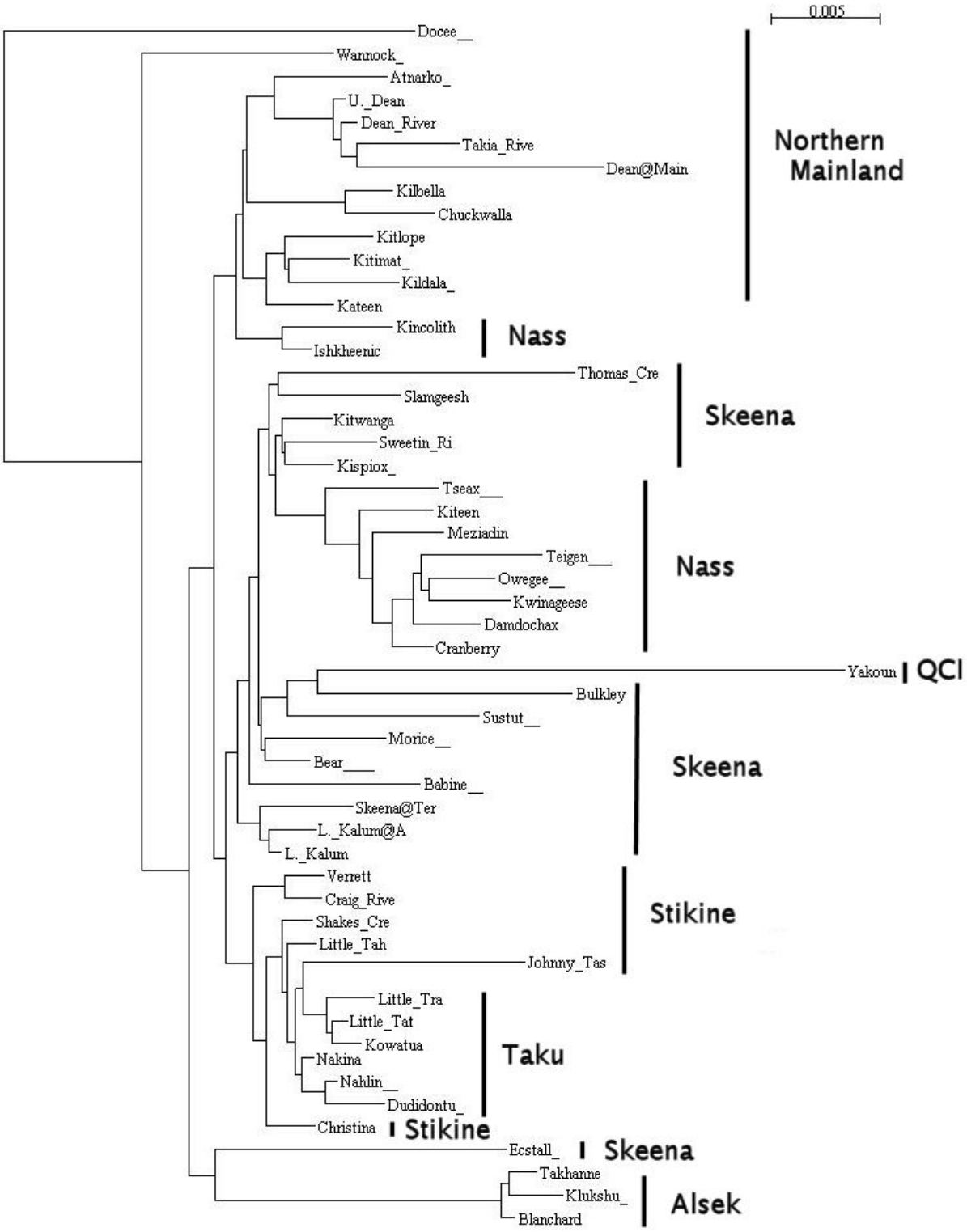

Figure 1. Neighbour-joining dendrogram of Cavalli-Sforza and Edwards (1967) chord distance for southern British Columbia populations of Chinook salmon surveyed at 13 microsatellite loci.