Three Genetic Stocks of Upriver Bright Fall Chinook Salmon Detected in the Columbia River Basin, USA

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In order to detect stock structure in the Columbia River basin, we analyzed 694 upriver bright fall chinook salmon samples from seven locations at seven microsatellite loci. Results indicate three main stocks of upriver bright fall chinook salmon in the Columbia basin above Bonneville Dam. These three stocks are Deschutes River, Snake River (natural origin), and Columbia River mainstem (plus Lyons Ferry Hatchery). Samples from 424 unknown origin upriver bright fall chinook salmon passing Bonneville Dam in 1999 were assigned to one of the three genetic stocks detected in this study.

Columbia River Basin upriver bright fall chinook salmon are heavily harvested in the Alaskan, Canadian, coastal, and the Columbia River in-river fisheries. Ocean harvest of Columbia River salmon in mixed stock fisheries has been difficult to quantify due to the large number of wild fish, and incomplete coded wire tagging of hatchery fish. Current genetic methods may prove to be a strong indicator of stock composition in mixed fisheries by utilizing genetic markers inherent in all salmon (Beacham et al. 2003). In order to detect stock structure in the Columbia River basin, we analyzed 694 upriver bright fall chinook samples from seven locations at seven microsatellite loci, and assigned unknown individuals to stocks of origin based on these data.

Sample collections represent the major upriver bright fall chinook salmon producing locations in the Columbia Basin. These sample locations (and year collected) include the Grande Ronde River 1998 (n = 38), Clearwater River 1998 (n = 66), Hanford Reach 1998 (n = 54) & 1999 (n = 81), Lyons Ferry Hatchery 2000 (n = 85), Priest Rapids Hatchery 1998 (n = 36) & 1999 (n = 106), upper Deschutes River 1998 (n = 95) & 1999 (n = 91), and lower Deschutes River 1999 (n = 42). Further, unknown samples of upriver bright fall Chinook passing Bonneville Dam were collected from August 2 to October 28 in 1999 (n = 424).

Fin clips were digested and DNA extracted using standard manufacture's protocols from Qiagen® DNeasy™ in conjunction with a Qiagen® 3000 robot. Genomic DNA was quantified and arrayed into 96 well plates for high throughput genotyping. Polymerase chain reaction (PCR) was used to amplify seven microsatellite loci (Table 1; also see Table 1 for PCR conditions). Forward primers were fluorescently labeled (Applied Biosystems®), and PCR products were genotyped using manufacture's protocols with an Applied Biosystems® model 3100 genetic analyzer.

Table 1. Microsatellite loci and total number of alleles (A) in all samples. Annealing temperatures (°C) for PCR are shown as performed with the AmpliTaq Reagent System (Applied Biosystems) and 25ng genomic DNA in 15ul total volume.

Locus	Reference	Annealing Temp.	Total A
*Ots*G68	Williamson et al. 2002	52	43
*Ots*G78	Williamson et al. 2002	52	46
*Ots*G249	Williamson et al. 2002	52	44
*Ots*311	Williamson et al. 2002	52	52
*Ots*G432	Williamson et al. 2002	52	30
*Ots*4	Banks et al. 1999	58	13
*Ogo*4	Olsen et al. 1998	58	18

To estimate the level of within-population genetic diversity, observed unbiased gene diversity and allelic richness (average alleles per locus corrected for sample size) were calculated for all microsatellite loci (FSTAT; Goudet 1995). Genetic variance was calculated from allele frequencies (F_{ST}; Weir and Cockerham 1984) using

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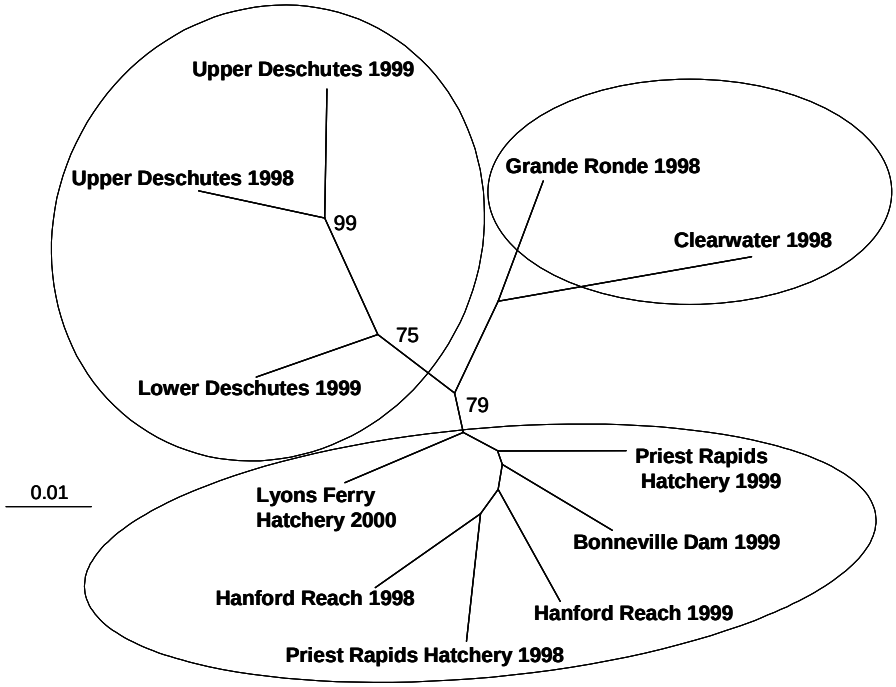
GENEPOP v. 3.3 (Raymond and Rousset 1995) to estimate pairwise genetic divergence among collections from the Columbia River Basin. Exact-significance testing methods were used to evaluate conformance to Hardy-Weinberg, linkage equilibria, and differences in allele frequency distributions (temporally and geographically). Unbiased estimators of exact significance probabilities were obtained using the Markov-Chain algorithm described in Guo and Thompson (1993), as implemented in GENEPOP v. 3.3 (Raymond and Rousset 1995), using 500,000 steps. Corrections were made against Type I error in exact tests using the sequential Bonferroni method (Rice 1989). Assignment tests were performed using the Bayesian method in GeneClass (Cornuet et al. 1999) to assign unknown individuals to populations of origin. In order to infer the degree of relatedness between sample collections, pairwise genetic distances (Cavalli-Sforza and Edwards 1967) were calculated between all populations and were used to construct a neighbor joining (NJ) tree with 1000 iterations (PHYLIP v. 3.5; Felsenstein 1993).

A high number of alleles were detected in all samples with an average of 36 alleles per locus (average allelic richness = 21.6; Table 2). Gene diversity was also high with an average of 0.897 (Table 2). Results from the NJ tree indicate three main stocks of fall chinook salmon in the Columbia basin (Fig. 1). These three stocks are Deschutes River, Snake River (natural origin), and Columbia River mainstem (plus Lyons Ferry Hatchery). Sample collections from hatchery (Priest Rapids Hatchery, Lions Ferry Hatchery) and wild (Hanford Reach) stocks were not significantly different and displayed little genetic differentiation (F_{ST} = 0.005). Temporal collections within locations also displayed little genetic differentiation and none were significantly different. Samples from 424 unknown origin upriver bright fall chinook passing Bonneville Dam in 1999 were assigned to one of the three genetic stocks detected in this study. The majority of unknown samples assigned to the Columbia River mainstem stock (68%), 20% assigned to the Deschutes River, and 12% assigned to the Snake River.

Table 2. Diversity statistics (Allelic Richness = AR; and Gene Diversity) for three populations of upriver bright fall chinook salmon.

Population	n	AR	Gene Diversity
Snake River	104	21.6	0.913
Columbia River	362	22.8	0.888
Deschutes	228	20.4	0.891

Fig. 1. Neighbor Joining Tree constructed with chord distances (Cavalli-Sforza and Edwards 1967) from 1000 iterations. Bootstrap values above 50% are shown at branch nodes. Scale chord distance of 0.01 is shown.



The populations of fall chinook salmon in this study are some of the healthiest stocks in the Columbia River Basin, but intermingled among them is the threatened Snake River fall chinook stock. Genetic analyses may be useful in determining the presence of Snake River stock in unknown catches of fall chinook salmon in mixed stock fisheries in the North Pacific Ocean (Shaklee et al. 1999). Results from this study indicate that data from additional microsatellite loci are necessary to obtain more precise stock structure (specifically for Lyons Ferry Hatchery as per Marshall et al. 2000) and increased assignment fidelity. Seven genetics laboratories in North America (including our lab in Hagerman, Idaho) have begun efforts to standardize microsatellite loci for generating a North American coastwide chinook salmon database. Upon completion of standardized loci between the seven labs, we plan to further analyze this data set with these loci to provide standardized baseline data of Columbia Basin upriver bright fall chinook salmon stocks.

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