Patterns of Genetic Diversity in Alaskan Coho Salmon

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Genetic baselines can be used to reveal population structure, perform mixed-stock analysis (MSA), infer the population origin of individuals and characterize patterns of ocean migration in North American and Asian salmon. Extensive genetic baselines have been developed for chinook (*Oncorhynchus tshawytscha*), chum (*O. keta*), and sockeye salmon (*O. nerka*) using allozyme loci. Unfortunately, allozymes show little variation in coho salmon (*O. kisutch*), particularly in Alaska. In this study, we used nine microsatellite loci to genotype 32 putative coho salmon populations ($N = 2{,}581$) from seven regions of Alaska. The objectives were to: 1) estimate the degree and spatial distribution of neutral genetic diversity in Alaskan coho salmon; 2) evaluate the potential for regional-level mixed-stocked analysis and individual assignment.

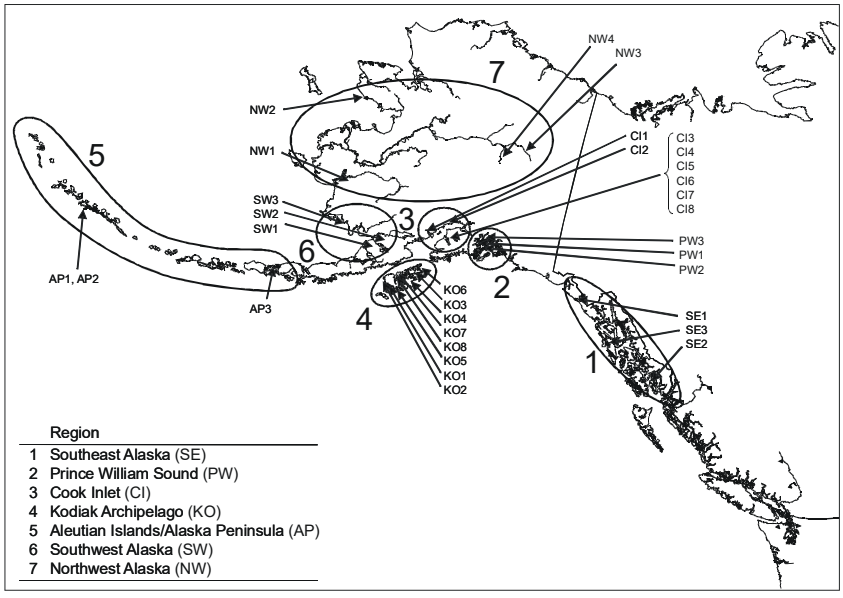
Fin tissue samples for microsatellite analysis were obtained from 32 collections of coho salmon representing seven major regions of Alaska (Fig. 1): Northwest Alaska ($N = 4$); Southwest Alaska ($N = 3$); Aleutian Islands/Alaska peninsula ($N = 3$); Kodiak Archipelago ($N = 8$); Cook Inlet ($N = 8$); Prince William Sound ($N = 3$); Southeast Alaska ($N = 3$). Nine microsatellite loci were used to estimate genetic variation in each population (*Oke2*, *Oke3*, and *Oke4*, Buchholz et al. 2001; *Oki1*, *Oki3*, and *Oki11*, Smith et al. 1998; *One3*, Scribner et al. 1996; *Ots3.1*, Banks et al. 1999; *Ots105*, Nelson and Beacham 1999). Two of these loci (*Oki1*, *Ots3.1*,) have also been used for MSA of coho salmon in British Columbia (Beacham et al. 2001). Total genomic DNA was isolated from approximately 50–100 mg of fin tissue for use in PCR. A description of the microsatellite loci, PCR chemistry, gel electrophoresis and microsatellite allele scoring is given in Olsen et al. (2003).

Three analyses were performed. First, an analysis of molecular variation for diploid data (AMOVA, Michalakis and Excoffier 1996) was used to test for genetic structure within and among the seven regions. AMOVA was performed using ARLEQUIN version 2.0 (Schneider et al. 2000) and the degree of population divergence, F_{ST}, was estimated according to Weir and Cockerham (1984). The estimate of F_{ST} was partitioned into within-region (F_{SR}) and between-region (F_{RT}) genetic variation. Randomization tests were used to test if the estimates of F_{ST}, F_{SR}, and F_{RT} were significantly greater than zero.

Second, MSA simulations were performed using the direct maximum likelihood method implemented in the program SPAM 3.7 (Debevec et al. 2000). Parametric bootstrap resampling of both the baseline and mixture was carried out 1,000 times to derive the mean allocation estimate and to evaluate precision. Artificially simulated mixtures ($N = 400$) representing 100% of each individual population were subjected to MSA as a test of baseline performance. Mean allocations to individual populations were then summed for geographically defined regions. The allele frequencies for the baseline samples were estimated using the Bayesian modeling approach of Rannala and Mountain (1997).

Finally, the program GENECLASS version 1.0.02 (Cornuet et al. 1999) was used to conduct a (re)assignment test by the partial Bayesian method with leave-one-out classification.

Fig. 1. Sample location and region for 32 coho salmon populations from Alaska.



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A chi-square test was used to determine if the number of successful classifications was significantly different than expected when individuals were randomly assigned to region.

The AMOVA results indicated that the degree of differentiation (F_{ST} = 0.093) among populations of Alaskan coho salmon was significantly greater than zero ($P < 0.001$) and was as large or larger than that reported for other Pacific salmon species in Alaska (Table 1). AMOVA also showed that Alaskan coho salmon exhibit significant inter- and intra-regional population structure. Estimates of among-region (F_{RT} = 0.036) and within-region (F_{SR} = 0.057) genetic differentiation were significantly greater than zero ($P < 0.001$). F_{SR} was greater than F_{RT}, indicating that intra-regional variation is a large component of the overall genetic population structure in Alaskan coho salmon. The relatively high degree of intra-regional genetic variation suggests that population structure in Alaskan coho salmon is organized on a relatively small geographic scale and these populations should be managed to conserve this fine-scale genetic diversity.

The potential for accurate regional-level MSA is good. The mixture estimates ranged from 87.2% (SE) to 95.9% (PW) for simulated mixtures containing only fish from a single region (100% simulations, Table 2). The 95% confidence intervals were above 80% for all but the SE region (79.4%). The results suggest MSA could be a useful tool to estimate stock proportions in a regionally heterogeneous sample of coho salmon.

The potential for accurate regional-level individual assignment varies by region but is generally low. Of the 2,581 coho, 1,396 (54.1%) were correctly assigned to their true region. The percent of correct assignments ranged from 34.5% (AP) to 80.6% (PW) and was greater than 50% in four of the seven regions (Table 3). The percentage of individuals correctly assigned to region varied greatly among regions. The number of individual assignments was always greatest for the region of origin and was significantly different than expected when individuals were randomly assigned to region ($P < 0.001$, Table 3). Although the percentage of individuals (re)assigned to their true region is significantly larger than expected based on random assignment, a large percentage (mean = 45.9%) were assigned to the wrong region. Moreover, the number of miss-classified individuals assigned to the seven regions was not consistent with spatial distributions. For example, 20 individuals from Cook Inlet are misclassified to the adjacent Prince William Sound but 35 individuals are misclassified to the more distant Southwest Alaska region.

In summary, the microsatellite loci used in this study have high potential for accurate regional-level MSA of Alaskan coho salmon, but relatively low potential for accurate regional-level individual assignment. This is most likely because MSA makes better use of the genetic data by computing a single likelihood for all the mixed-sample genotypes and is therefore a more powerful method (Millar 1990). The accuracy of both methods will likely be improved by adding loci and increasing the sample size of the baseline populations (Cornuet et al. 1999). Simulation results by Cornuet et al. (1999) suggest 20 or more loci may be required to achieve near 100% assignment accuracy given the relatively low inter-regional genetic differentiation (F_{RT} = 0.036) in Alaskan coho salmon.

Table 1. Hierarchical gene diversity analysis of 32 coho salmon population samples from Alaska. An asterisk (*) denotes $P < 0.001$ the value is not greater than zero; RT = among regions; SR = within regions.

Grouping Strategy	Source of variation	σ^2	Percent of total	F_{ST}	F_{RT}	F_{SR}
Seven regions[a]	Total	1.711	100.00			
	Within populations	1.555	90.88			
	Between populations	0.156	9.12	0.093*		
	Between regions	0.062	3.64		0.036*	
	Between populations within regions	0.094	5.48			0.057*

Table 2. Results of mixed-stock analysis simulations for 100% contribution by region. Mean% is the average percent contribution over 1000 simulated mixtures. LCI and UCI are the bounds for the lower and upper 95% confidence intervals. Region abbreviations are as indicated in Fig. 1.

Region	Mean%	LCI	UCI
NW	95.5	91.2	98.7
SW	90.3	82.6	96.0
AP	92.9	86.9	97.4
KO	90.0	81.6	96.0
CI	91.4	85.5	96.8
PW	95.9	92.8	98.5
SE	87.2	79.4	94.5

Table 3. Assignment test results for 2,581 coho salmon from seven regions of Alaska. The number of correct assignments (bold print), sample size (*n*), percent correct assignments (%CA), percent expected assignments by chance (%EA), number of expected assignments by chance (EA), and the chi-square test *P*-value are shown for each region. Region abbreviations are as indicated in Fig. 1.

Region	NW	SW	AP	KO	CI	PW	SE	*N*	%CA	%EA	EA	*P*-value
NW	**209**	33	4	28	23	2	3	302	69.2	12.5	37.8	<0.001
SW	46	**101**	12	44	57	7	13	280	36.1	9.4	26.3	<0.001
AP	14	19	**131**	92	75	17	32	380	34.5	9.4	35.6	<0.001
KO	17	29	33	**340**	90	33	45	587	57.9	25.0	146.8	<0.001
CI	21	35	42	98	**346**	20	21	583	59.3	25.0	145.8	<0.001
PW	1	2	4	15	16	**191**	8	237	80.6	9.4	22.2	<0.001
SE	11	7	18	43	39	16	**78**	212	36.8	9.4	19.9	<0.001

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