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Yukon River Coho Salmon Genetics

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Abstract

Here we examine the extent and pattern of genetic diversity in Yukon River coho salmon by assaying eight microsatellite loci. Yukon River coho salmon are geographically structured (G_{ST}=0.103), with a strong genetic disjunction between lower and upper river populations. Upper river populations have much lower levels of genetic diversity in comparison to the lower river populations and to other populations from around Alaska. This deficit is likely the result of a founder or bottleneck effect. Mixed-stock analysis using microsatellite variation assayed here can accurately (95%–99%) and precisely (S.D. 1%–3%) allocate coho salmon in mixtures to regions providing data that can increase the knowledge base and ability to actively manage Yukon River coho salmon. Finer geographic scale management may be possible by increasing baseline sample sizes, improving baseline representation, and, if necessary, assaying additional diverse loci.

Introduction

Implicit in fisheries management is a thorough knowledge of the biology of the species being harvested. Most Pacific salmon (*Oncorhynchus* spp.) are harvested when populations are mixed together, which creates problems for fisheries managers who attempt to achieve sustained yields by balancing harvest and escapement across populations. Knowledge of the population composition in many fisheries is imprecise and not directly measured. If exploitation is significant, then harvest without accounting for genetic consequences can change population parameters through differential harvest within and among populations and result in lost genetic diversity and decreased production (Allendorf et al. 1987). Long term sustained yield, ultimately the goal of fishery management, can only be accomplished through conservation of genetic resources to maintain diversity and a population's adaptive potential in the face of a fluctuating environment (Altukhov and Salmenkova 1987; Nelson and Soule 1987). To bring about effective conservation, the population structure and productivity of the species must be known in order to regulate fisheries to allow optimum escapement of each population. This is a difficult proposition, given the multi-population nature of the fisheries, but one that is possible with genetic mixed-stock analysis (MSA; Pella and Milner 1987).

Coho salmon are distributed in North America from Monterey, California to Point Hope, Alaska and in Asia from North Korea to the Anadyr River in Russia (Sandercock 1991). Like all Pacific salmon, coho salmon are anadromous, philopatric, and spawn semelparously. Fry

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emerge in the spring and spend one to two years in freshwater before migrating to saltwater to mature, with the majority of coho salmon returning to spawn at age three. Most coho salmon spawn in coastal streams after short upstream migrations, but in large rivers they are known to migrate extensively, which has led to two migratory phenotypes, coastal and interior. The coastal type has a larger body and fins while the interior type has a fusiform shape that is more efficient for long distance swimming. Although coho salmon do not have the broadest distribution, they exhibit a wide variety of life histories, which enables them to occupy the most variable spawning habitat of all salmon. Many small streams throughout the range support coho salmon. For example, within Southeast Alaska there are 5,000 known salmon streams, and coho salmon are in 4,000 of them, the most of all the salmon species, whereas Chinook salmon are in only 200 of the streams (Halupka et al. 2003). Colonizing marginal habitats can be risky and precludes many of the populations from attaining large census size, but the ability to adapt to and colonize new habitats, such as found in Glacier Bay, Alaska (Milner et al. 2000), and the shear number of streams that coho salmon occupy appears to be a strategy to offset risk.

Coho salmon spawn throughout the Yukon River drainage, with major known concentrations located in the Tanana River (McBride et al. 1983), but generally little is known about their distribution and abundance. Only the Delta Clearwater River, a tributary of the Tanana River, has an escapement goal, and limited monitoring is conducted on the East Fork of the Andreafsky River and at Pilot Station Sonar. Coho salmon begin entering the Yukon River in late July to early August and continue well into autumn, largely coinciding with fall chum salmon. The similar run timing of coho and fall chum salmon complicates their management. The run size and subsistence demand for fall chum salmon is greater than coho salmon. Consequently, management focus is placed on fall chum salmon, and coho salmon are largely unmanaged and mostly taken as bycatch (Bue 2004), potentially subjecting coho salmon to differential harvest rates. Furthermore, with the recent declines in chum and Chinook salmon resulting in fishing closures and restrictions, demand for coho salmon is increasing (Geiger et al. 1995), and managers are attempting to target coho salmon through selective gear, location, and timing (Bue 2002). As subsistence users fail to meet their needs with chum and Chinook salmon, more and more individuals will take the opportunity to fish coho salmon. The current lack of escapement, run timing, and population structure data for coho salmon in the face of increased pressure is problematic.

Coho salmon exhibit little genetic diversity and population structure at many allozyme loci (Utter et al. 1980; Reisenbichler and Phelps 1987; Wehrhahn and Powell 1987; Bartley et al. 1992), but direct DNA surveys (e.g. microsatellites) reveal that coho salmon are quite diverse and divergent (Small et al. 1998; Beacham et al. 2001; Olsen et al. 2003, 2004). While our understanding of coho salmon genetics is growing, little is known specifically about the genetic structure of Yukon River coho salmon. Recent genetic studies (Gharrett et al. 2001; Smith et al. 2001; Olsen et al. 2003, 2004) have analyzed limited samples of Yukon River coho salmon. Most of these studies (Gharrett et al. 2001; Smith et al. 2001; and Olsen et al. 2003) were focused on large scale phylogeography and population structure for Alaskan and Pacific Northwest coho salmon and only included one or two populations from the Yukon River. The study by Olsen et al. (2004) focused on the impacts of potential habitat degradation from a gold mine on coho salmon by analyzing samples from two tributaries within each

of the Innoko (lower Yukon River) and Tanana Rivers (upper Yukon River). Their findings indicate that coho salmon populations are highly divergent on a small geographic scale and that there is a spatial trend in the levels of genetic diversity, with greater intrapopulation genetic diversity within coho salmon from the Innoko River. Low levels of genetic diversity were also observed within coho salmon from the Tanana River as compared to coho salmon from outside the Yukon River (Gharrett et al. 2001; Olsen et al. 2003), a founder effect or bottleneck in the middle Yukon River populations may be an explanation (Olsen et al. 2004). These results suggest that lower Yukon River coho salmon contain a large component of the overall genetic diversity, and that populations are generally small with little gene flow occurring among them (Olsen et al. 2004), which can exacerbate the harmful effects of an unmanaged harvest. Such a contrast in genetic diversity and apparent fine scale population structure emphasizes the need for a more thorough investigation to better understand the genetic structure of Yukon River coho salmon.

In this study, we assay variation at eight microsatellite loci to: 1) evaluate patterns of genetic diversity within and among 11 putative coho salmon populations distributed throughout the Yukon River drainage; and 2) provide preliminary estimates of the power of genetic data for use in various mixed-stock analyses (MSA) of Yukon River coho salmon.

Methods

Sample collection

Fin clips were collected from 11 putative coho salmon populations within the Yukon River (Table 1, Figure 1). The following collections were performed by seine netting adult coho salmon on their spawning grounds over a one to two week time period: Archuelinguk, Anvik, Rodo, Kantishna, Nenana, Otter, and Delta. The Andreafsky collection of adult coho salmon occurred at the weir located on the east fork over a two week period. Due to a lack of knowledge of spawning ground location or to inclement weather during spawning, juvenile coho salmon were collected at three locations (Clear, Old Crow, and Fishing Branch) by setting minnow traps over a large spatial area (>>100 meters) for a one to two month period as recommended by Hansen et al. (1997).

Genetic analysis

Total genomic DNA was extracted from the tissue (~25mg) using proteinase K with the Dneasy™ DNA isolation kit (Qiagen Inc., Valencia, CA). Concentrations of DNA were quantified by fluorometry and diluted to 50 ng/µl. The following micosatellite loci were assayed for genetic variation: *Oke-2, -3, -4* (Buchholz et al. 2001); *Oki-1, -3, -11* (Smith et al. 1998); *One-3* (Scribner et al. 1996); and *Ots-105* (Nelson 1998). Polymerase Chain Reaction (PCR) DNA amplification was done in 10 µl volumes; general conditions were: 2.5 mM $MgCl_2$, 1X PCR buffer (20 mM Tris-HCL pH 8.0, 50 mM KCL), 250 µM for each dNTP, 0.03µM fluorescently labeled forward primer, 0.37 µM unlabeled forward primer, 0.40 µM unlabeled reverse primer, 0.008 units *Taq* polymerase, and 1 µl of DNA (50ng/µl). PCR was carried out in an MJResearch DNA Engine® thermal cycler. Standard thermal cycling conditions were: initial denaturation cycle of 92°C for 2 minutes, followed by 92°C for 15 seconds, 50–62°C for 15 seconds (range of locus-specific annealing temperatures), and 72°C for 30 seconds, with a final single cycle of 72°C for 10 minutes. The PCR product was mixed in equal parts with load-

Table 1. Population, region, population label, sample collection years, number of fish sampled per year (*N*), and life stage of sampled Yukon River coho salmon.

Population	Region	Label	Year	N	Life stage
Archuelinguk	Lower	1	2005	50	Adult
Andreafsky	Lower	2	1998	93	Adult
Anvik	Lower	3	2002	56	Adult
Rodo	Lower	4	2005	51	Adult
Clear	Lower	5	2004	47	Juvenile
Kantishna	Upper	6	2001	250	Adult
Nenana	Upper	7	1997	100	Adult
Otter	Upper	8	2003, 2004	50, 50	Adult
Delta	Upper	9	1997	100	Adult
Old Crow	Upper	10	1998	100	Juvenile
Fishing Branch	Upper	11	1998, 2000	74, 200	Juvenile

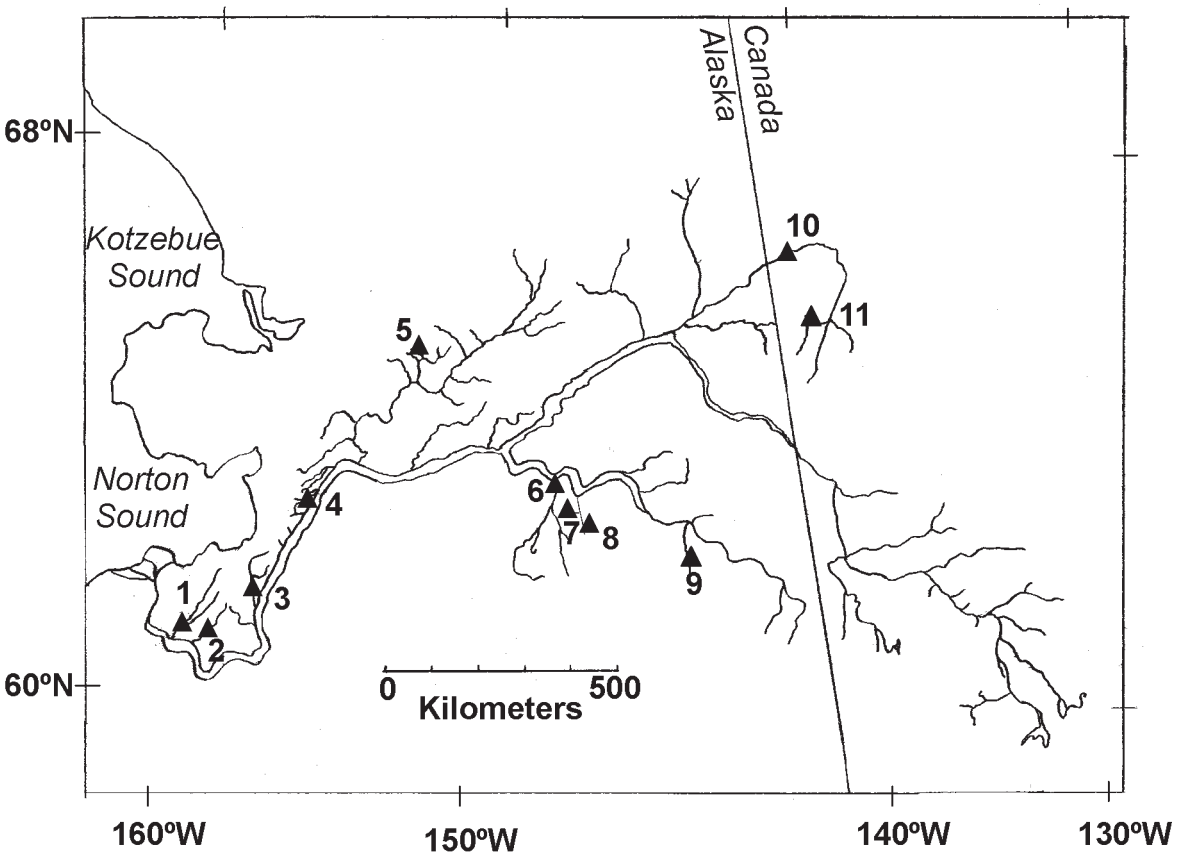

Figure 1. Sampling locations: 1) Archuelinguk, 2) Andreafsky, 3) Anvik, 4) Rodo, 5) Clear, 6) Kantishna, 7) Nenana, 8) Otter, 9) Delta, 10) Old Crow, 11) Fishing Branch.

ing dye (98% formamide, 10mM EDTA, 2 mg/ml Orange G), placed on a 92°C heating block for two minutes, and then one µl of this mixture was electrophoresed and visualized on a denaturing 6% polyacrylamide gel (19:1 acrylamide:bisacrylamide) using a Li-Cor IR2® DNA scanner. The sizes of bands were estimated and scored by the computer program Saga GT ver 3.0 (Li-Cor, Lincoln, NE). Li-Cor size standards (50bp–350bp) and allele ladders were run every sixteen lanes to ensure consistency of allele scores. All scores were verified by visual inspection. Alleles were scored by two independent researchers, with any discrepancies being resolved by re-running the samples in question and repeating the double scoring process until scores matched.

Data analysis

Intrapopulation genetic diversity—Hardy-Weinberg and gametic phase equilibrium were analyzed for each population using the program BIOSYS-2 (Swofford and Selander 1981) and LINKDIS (Black and Krafsur 1985), respectively. Juvenile samples were analyzed for relatedness using the Queller and Goodnight (1989) method implemented in the program IDENTIX (Belkhir et al. 2002). These tests were done to determine if the samples represent randomly mating, Mendelian populations. Significant tests of disequilibrium ($P<0.05$) were compared to binomial expectations to determine if chance alone explained the results (Apostal et al. 1996). In addition, BIOSYS-2 calculated the percentage of polymorphic loci at the 95% criterion and expected and observed heterozygosity for the populations. The program FSTAT 2.9.3 (Goudet 1995) was used to calculate estimates of allelic richness for loci and populations and expected and observed heterozygosity for loci. The program POPGENE 1.32 (Yeh and Boyle 1997) was used to estimate the effective number of alleles. The diversity values for populations from the lower and upper regions of the Yukon River were tested for statistical differences by a one-tailed Mann-Whitney test.

Interpopulation genetic diversity—Using PHYLIP 3.57 (Felsenstein 1993), population pairwise chord (CSE) distances (Cavalli-Sforza and Edwards 1967) were calculated from allele frequencies wherefrom a neighbor-joining (Saitou and Nei 1987) dendrogram was constructed by MEGA 2.1 (Kumar et al. 2001) to evaluate the spatial pattern of the genetic variation. To determine if the data conform to the isolation by distance model (IBD) (Wright 1943), population pairwise matrices of genetic distance (F_{ST}), estimated by the program GENEPOP 3.4 (Raymond and Rousset 1995), and geographical distance (river miles) were analyzed by standard linear regression and lowess smoothing. The Mantel test (Mantel 1967), performed in GENEPOP 3.4 with 10,000 randomizations, determined if the two matrices were significantly correlated.

The data were analyzed hierarchically, based on the population structure depicted in the neighbor-joining dendrogram and on geographic units currently used in fisheries management, by means of log-likelihood ratio, gene diversity, and mixed-stock analyses.

Homogeneity among populations, among populations within regions, and between regions was examined by log-likelihood ratio tests of allelic frequencies (Sokal and Rohlf 1995). Pooling of neighboring alleles occurred when expected overall values were less than three (Sokal and Rohlf 1995). An approximate *F*-statistic was used to contrast heterogeneity in the hierarchy (Smouse and Ward 1978). In cases where the same hypothesis was tested multiple

times, the alpha was corrected to maintain a type I error rate of 0.05 (Cooper 1968). Additional population pairwise tests of allelic frequency homogeneity were conducted using a Markov chain Monte carlo exact procedure in GENEPOP 3.4.

The relative magnitude of genetic variation resulting from population heterogeneity was assigned to the different hierarchical levels through G_{ST}-statistics (Chakraborty and Leimar 1987; Nei and Chesser 1983). The hierarchical log-likelihood ratio tests determined whether the G_{ST}-statistics were significantly different from zero (Chakraborty and Leimar 1987). In terms of a hierarchical island model at equilibrium, G_{ST}-statistics were used to indirectly estimate gene flow (N_em) (Zhivotovsky et al. 1994).

Mixed-stock analysis simulations—The ability of the baseline to correctly apportion mixtures was tested with the program SPAM 3.7 (Debevec et al. 2000), using a Bayesian method (Rananala and Mountain 1997) to estimate allele frequencies. Parametric bootstrap resampling was used to simulate 1000 artificial mixtures (N=400) for each population based on that population's allele frequencies and assumptions of Hardy-Weinberg and gametic phase equilibrium. These 100% mixtures were subjected to conditional maximum likelihood MSA, with parametric bootstrapping of the baseline, to estimate mean allocation and precision. Regional 100% simulations were also performed with equal contribution from populations within the region. For perfect baseline performance, the mean contribution estimate for each population or region would approximate 100%; estimates of approximately 90% were considered robust for mixture analysis (Seeb and Crane 1999). Recently, Alaska members of the Joint Technical Committee, which advises the Yukon River Panel in implementing the Pacific Salmon Treaty, established lower Yukon River and Tanana River geographic management units. Thus, simulations were performed to reflect the current management strategy, which required separating the upper region populations into Tanana and Porcupine River components.

Comparison with coho salmon from around Alaska—Data from the present study were combined with data collected at the same loci for coho salmon from around Alaska (Olsen et al. 2003). Using the previously mentioned programs, estimates of heterozygosity, percent polymorphic loci, allelic richness, and effective number of alleles were calculated for the populations and averaged for populations representing eight geographic regions. A neighbor-joining dendrogram was constructed from CSE distances.

Results

Intrapopulation genetic diversity

Hardy Weinburg and gametic phase disequilibrium (P<0.05) were observed in 4 of 88 and 18 of 308 tests, respectively. These numbers were consistent with a type I error rate of 5% and with binomial expectations. Therefore, the loci and populations were judged to be in equilibrium. The mean estimates of pairwise relatedness among juvenile samples from the Clear, Old Crow and Fishing Branch populations were -0.049, -0.003, and -0.020, respectively. These values were not significant (P>0.05, 1000 permutations) and indicated that the samples were not related.

Allele numbers for the loci ranged from two to eight, whereas allelic richness and expected heterozygosity ranged from 1.4 to 5.1 and 0.023 to 0.678, respectively; only *Oke4* was not

Table 2. Across populations for each locus: number of alleles, allelic richness (A_R), effective number of alleles (A_E), expected heterozygosity (H_E), observed heterozygosity (H_O), and G_{ST}.

Locus	Alleles	A_R	A_E	H_E	H_O	G_{ST}
Oke2	3	1.9	1.2	0.120	0.137	0.151
Oke3	8	4.4	2.1	0.476	0.459	0.066
Oke4	2	1.4	1.0	0.023	0.024	0.021
Oki1	8	5.1	3.2	0.678	0.674	0.082
Oki3	3	2.1	1.6	0.356	0.306	0.269
Oki11	3	2.0	1.2	0.169	0.149	0.048
One3	6	4.1	2.6	0.600	0.607	0.036
Ots105	2	1.9	1.2	0.117	0.121	0.098

Table 3. Across loci for each population: mean sample size (*N*), percentage polymorphic loci at the 95% criterion (*%P*), allelic richness (A_R), effective number of alleles (A_E), expected heterozygosity (H_E), and observed heterozygosity (H_O).

Population	*N*	*%P*	A_R	A_E	H_E	H_O
Archuelinguk	42	87.5	3.1	2.0	0.402	0.396
Andreafsky	90	87.5	3.0	2.1	0.411	0.407
Anvik	53	87.5	3.1	2.0	0.427	0.407
Rodo	50	87.5	3.1	2.0	0.398	0.387
Clear	39	87.5	2.9	2.0	0.405	0.413
Kantishna	243	50.0	2.3	1.5	0.245	0.237
Nenana	85	62.5	2.6	1.7	0.282	0.258
Otter	97	62.5	2.5	1.6	0.270	0.258
Delta	95	62.5	2.3	1.5	0.243	0.245
Old Crow	96	50.0	2.4	1.4	0.209	0.197
Fishing Branch	254	50.0	2.2	1.4	0.201	0.199

polymorphic at the 95% criterion (Table 2). Diversity values varied among the populations (Table 3). Lower Yukon River populations had significantly higher levels of diversity (U=30, P<0.01).

Interpopulation genetic diversity

A major subdivision between lower and upper Yukon River coho salmon was revealed from neighbor-joining analysis (Figure 2). Mean CSE and F_{ST} distances within subdivisions were 0.02 and 0.03 whereas estimates between subdivisions were 0.08 and 0.16, respectively. Further structure within these major subdivisions was suggested by the branching of the Clear, Old Crow, and Fishing Branch populations from others in their respective groups. The ge-

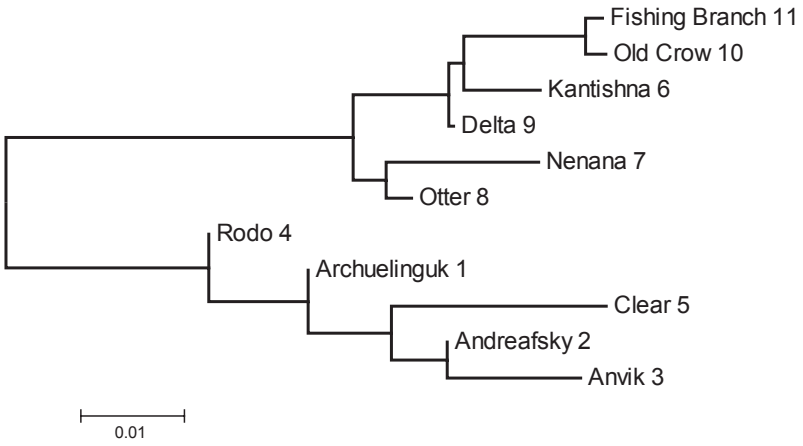

Figure 2. Neighbor-joining dendrogram of CSE distances among 11 Yukon River coho salmon populations.

netic variation followed an IBD model (P<0.0001, Figure 3). Geographic distance explained 41.3% of the genetic variation among populations.

The populations exhibited genetic heterogeneity (P<0.00001) within and between regions as measured by differences in allelic frequencies (Table 4). Due to low cell counts, *Oke4* was dropped from the analysis. The between regions component accounted for approximately 19 times more heterogeneity than within regions ($F_{12,108}$=18.6, P<0.00001). The upper region exhibited approximately four times the heterogeneity ($F_{60,48}$=3.9, P<0.00001) than the lower region. Populations within the lower and upper regions were heterogeneous at two and six individual loci, respectively. Of the 55 pairwise tests of allelic frequency homogeneity, 49 were significant (Table 5).

Individuals varying within populations accounted for 89.7% of the gene diversity while variation among populations accounted for 10.3% (Table 6). Most of the diversity among populations resulted from regional divergence (8.1%), with the balance (2.2%) due to divergence within regions. Log-likelihood ratio analysis rejected the null hypothesis (P<0.00001) that these gene diversity estimates equaled zero. The effective number of migrants per generation (N_em) was 2.2 overall, 0.7 between regions, and 11.2 within regions.

Mixed-stock analysis simulations

The individual population MSA simulation accuracies ranged from 59%–95%, only allocations to Kantishna, and Fishing Branch were ≥90% accurate although Clear, Nenana, and Otter were close, ranging from 86%–89% (Table 7). Misallocation was generally to geographically proximate populations. Improvement in MSA accuracy and precision occurred when estimates for individual populations were summed to region, and when simulations were performed on regional aggregates (Table 8).

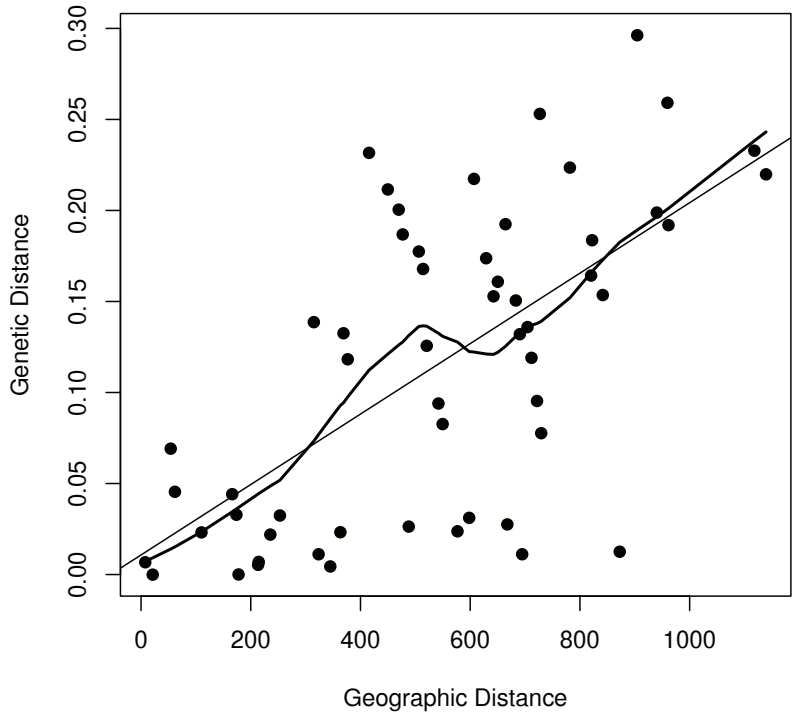

Figure 3. Scatter plot of genetic distance (F_{ST}) on geographic distance (miles). Linear (y=0.0002x + 0.0107, r^2=0.4131, P<0.0001) and lowess trend lines are displayed.

Comparison with coho salmon from around Alaska

A range of diversity values was observed among the regions (Table 9). Expected heterozygosity and percent polymorphic loci ranged from 0.242–0.419 and 56.3%–97.5%. Allelic richness and effective number of alleles ranged from 2.4–3.6 and 1.5–2.3. The upper Yukon River populations were the least diverse at all measures. The neighbor-joining dendrogram revealed that the upper Yukon River populations were quite distinct while the lower Yukon River populations were similar to those of Western Alaska (Figure 4).

Discussion

Extent and pattern of genetic diversity

In the Yukon River, coho salmon exhibit a high degree of geographically based genetic structure (G_{ST}=0.103), more than has been observed for either chum (Scribner et al. 1998; Crane et al 2001; Flannery 2004) or Chinook salmon (Smith et al. 2005; Templin et al. 2005). This level of divergence is similar to the estimate for coho salmon across Alaska (Olsen et al 2003) although the patterns of population structure differ. Generally, the genetic variation of coho salmon in Alaska has weak geographic associations, likely resulting from small, locally adapted populations, with little gene flow (Gharrett et al. 2001; Olsen et al. 2003). This is not the case for Yukon River coho salmon, which show strong regional structuring. However, these results are not surprising given the dimensional nature of the Yukon River. One-dimensional habitats, such as a river, wherein populations are linearly distributed and gene flow

Table 4. Hierarchical tests of homogeneity using log-likelihood ratio analysis (Sokal and Rohlf 1995) of allele frequencies from seven microsatellite loci (one locus dropped because of expected counts below three) among populations, among populations within regions, and between regions. Total df and *G*-test are degrees of freedom and log-likelihood ratio summed overall loci for the respective hierarchical levels.

Source of variation	Total df	Total *G*-test
Between Regions within Total	12	1536.33*
Among Populations within Regions	108	744.92*
Lower	48	127.51*
Upper	60	617.41*
Total	120	2281.25*

**P*<0.00001

Table 5. Population pairwise tests of allelic frequency homogeneity. S=significant test (*P*<0.05); NS=not significant.

Population	Pairwise Significance									
Archuelinguk (1)										
Andreafsky (2)	NS									
Anvik (3)	S	NS								
Rodo (4)	NS	NS	NS							
Clear (5)	S	S	S	S						
Kantishna (6)	S	S	S	S	S					
Nenana (7)	S	S	S	S	S	S				
Otter (8)	S	S	S	S	S	S	S			
Delta (9)	S	S	S	S	S	S	S	S		
Old Crow (10)	S	S	S	S	S	S	S	S	S	
Fishing Branch (11)	S	S	S	S	S	S	S	S	S	NS

follows a stepping-stone model, are more likely to have a geographic basis for the genetic variation (Slatkin 1993; Olsen et al. 2003). Indeed, similar results have been observed for coho salmon populations within the Kenai River (Olsen et al. 2003).

Although Yukon River coho salmon exhibit regional genetic relationships, the subdivision between lower and upper river populations represents an especially strong genetic disjunction (mean pairwise F_{ST}=0.160). Compared to upper river populations, those in the lower river maintain more genetic diversity, are more genetically similar to one another and to Western

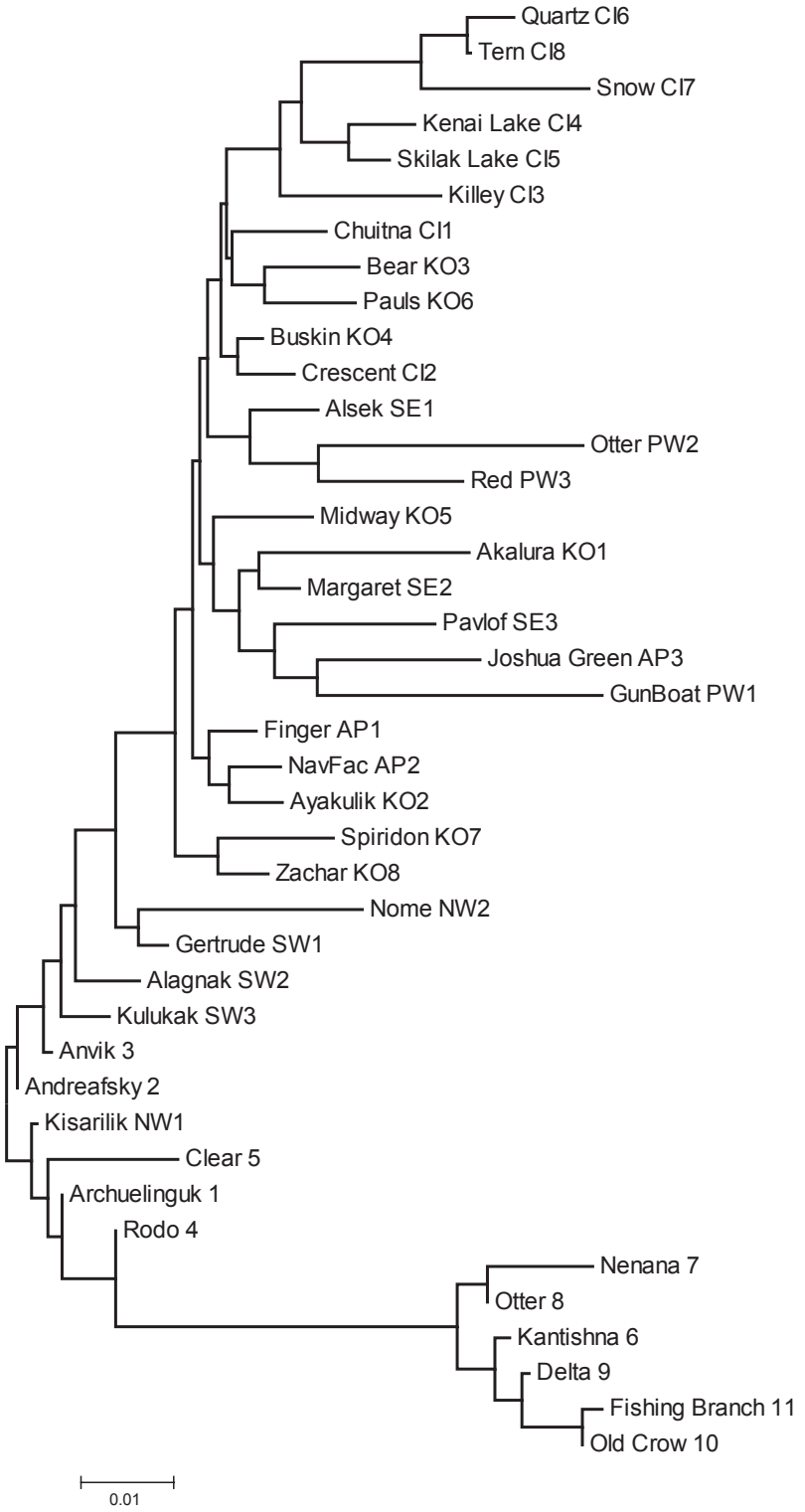

Figure 4. Neighbor-joining dendrogram of CSE distances among 41 Alaskan coho salmon populations.

Table 6. Hierarchical gene diversity analysis of Yukon River coho salmon using eight microsatellite loci. H_T is the total gene diversity; H_S is the gene diversity within populations; D_{SR} is the gene diversity among populations within regions; D_{RT} is the gene diversity among regions; H_S/H_T is the relative gene diversity due to variation within populations; G_{ST} is the relative gene diversity due to variation among populations; G_{RT} is the relative gene diversity due to variation among regions; G_{SR} is the relative gene diversity due to variation among populations within regions; N_em is the number of effective migrants per generation.

Source	Gene diversity	G_{ST}-statistics	N_em
Average within populations	H_S=0.317	H_S/H_T=0.897	
Average among populations within regions	D_{SR}=0.008	G_{SR}=0.022*	11.2
Average between regions	D_{RT}=0.029	G_{RT}=0.081*	0.7
Total gene diversity	H_T=0.354	G_{ST}=0.103*	2.2

*P<0.00001 inferred from hierarchical tests of homogeneity.

Alaskan populations, and have comparable levels of diversity to those from other Alaskan regions. While previous studies have observed limited genetic diversity for Yukon River coho salmon, the populations analyzed were limited to the upper river (Gharrett et al. 2001; Olsen et al. 2003), a region where populations likely represent a separate lineage exhibiting relatively low diversity within populations and high divergence among populations.

Similar findings of genetic as well as morphometric (i.e. coastal vs. interior) disjunction and spatial differences in genetic divergence and diversity exist for Fraser River coho salmon (Taylor and McPhail 1985; Small et al. 1998; Beacham et al. 2001). Fraser River coho salmon have been extensively sampled, and no hybrid zone has been found, with the point of disjuncture at Hell's Gate. This has led to the conclusion that based on the geologic record and similar disjunctions for sockeye and Chinook salmon that different colonizing sources and local adaptation, which prevents or severely limits gene flow between regions, are responsible for this divide (Small et al. 1998). Parallel conclusions are not yet possible for Yukon River coho salmon as further sampling in the middle section between the Rodo and Tanana Rivers is required to rule out a contact zone or identify a point of disjuncture. Until such work is completed, two possible colonization scenarios exist. The geologic record provides the possibility of an alternative colonizing source as it has been postulated that the upper and middle Yukon River once drained to the south into the Gulf of Alaska before being rerouted by glacial damming (Lindsey and McPhail 1986). Also, differences exist between lower and upper Yukon River chum and Chinook salmon (Wilmot et al 1994; Templin et al. 2005), although not of the same magnitude as for coho salmon. Alternatively, recent divergence from a single colonizing source due to founding/bottleneck effects, which Olsen et al. (2004) suggest, may also be an explanation. Regardless, upper Yukon River coho salmon occupy habitat at the extremes of both geographic distribution and freshwater migratory distance and have a level of genetic distinction similar to upper Fraser River coho salmon, which Small et al. (1998) deem an evolutionary significant unit.

Table 7. Mixed-stock analysis accuracy for 100% individual population simulations. Accuracy listed above the standard deviation.

	Mean allocation by population										
Population	Archuelinguk	Andreafsky	Anvik	Rodo	Clear	Kantishna	Nenana	Otter	Delta	Old Crow	Fishing Br
Archuelinguk	**0.59**	0.22	0.03	0.11	0.03	0.00	0.00	0.00	0.00	0.00	0.00
	0.10	0.11	0.03	0.08	0.03	0.00	0.01	0.01	0.00	0.00	0.00
Andreafsky	0.08	**0.75**	0.09	0.05	0.01	0.00	0.00	0.00	0.00	0.00	0.00
	0.07	**0.10**	0.07	0.05	0.02	0.00	0.00	0.01	0.00	0.00	0.00
Anvik	0.02	0.19	**0.71**	0.05	0.02	0.00	0.00	0.00	0.00	0.00	0.00
	0.03	0.10	**0.10**	0.05	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Rodo	0.09	0.10	0.04	**0.73**	0.01	0.00	0.00	0.00	0.01	0.00	0.00
	0.07	0.07	0.04	**0.08**	0.02	0.00	0.00	0.01	0.01	0.01	0.01
Clear	0.03	0.03	0.03	0.04	**0.86**	0.00	0.00	0.00	0.00	0.00	0.00
	0.04	0.03	0.03	0.04	**0.06**	0.00	0.00	0.00	0.00	0.00	0.00
Kantishna	0.00	0.00	0.00	0.00	0.00	**0.95**	0.00	0.01	0.02	0.01	0.01
	0.00	0.00	0.00	0.00	0.00	**0.03**	0.00	0.01	0.02	0.02	0.02
Nenana	0.00	0.00	0.00	0.00	0.00	0.00	**0.86**	0.10	0.02	0.01	0.00
	0.00	0.00	0.00	0.00	0.00	0.01	**0.06**	0.06	0.03	0.01	0.01
Otter	0.00	0.00	0.00	0.00	0.00	0.03	0.02	**0.89**	0.04	0.00	0.01
	0.01	0.01	0.00	0.00	0.00	0.04	0.02	**0.05**	0.04	0.01	0.01
Delta	0.00	0.00	0.00	0.00	0.00	0.12	0.02	0.07	**0.73**	0.03	0.03
	0.00	0.00	0.00	0.01	0.00	0.07	0.02	0.05	**0.09**	0.04	0.04
Old Crow	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.04	**0.68**	0.26
	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.01	0.04	**0.12**	0.12
Fishing Br	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.07	**0.90**
	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.02	0.08	**0.08**

Table 8. Mixed-stock analysis accuracy for 100% individual population simulations summed to regions and for 100% regional aggregate simulations. Accuracy listed above the standard deviation.

		Mean Allocation by Region		
Region	**Population**	**Lower**	**Tanana**	**Porcupine**
Lower		**0.99**	0.00	0.00
		0.01	0.01	0.00
	Archuelinguk	**0.99**	0.01	0.00
		0.01	0.01	0.01
	Andreafsky	**0.99**	0.01	0.00
		0.01	0.01	0.00
	Anvik	**1.00**	0.00	0.00
		0.00	0.00	0.00
	Rodo	**0.98**	0.01	0.01
		0.01	0.01	0.01
	Clear	**1.00**	0.00	0.00
		0.01	0.00	0.00
Tanana		0.00	**0.97**	0.02
		0.01	**0.02**	0.02
	Kantishna	0.00	**0.98**	0.02
		0.00	**0.02**	0.02
	Nenana	0.01	**0.99**	0.01
		0.01	**0.01**	0.01
	Otter	0.01	**0.98**	0.01
		0.01	**0.02**	0.01
	Delta	0.00	**0.93**	0.06
		0.01	**0.05**	0.05
Porcupine		0.00	0.04	**0.95**
		0.00	0.03	**0.03**
	Old Crow	0.00	0.06	**0.93**
		0.01	0.04	**0.04**
	Fishing Br	0.00	0.03	**0.97**
		0.00	0.03	**0.03**

Fishery management

Two coho salmon lineages occur within the Yukon River and should be conserved in order to preserve the species evolutionary ability. Within these lineages are demographically independent stocks that should be treated as such when it comes to managing harvests in order to maintain production and the viability of the larger lineage. Additional structure, not accounted for by the present management units, exists within both the lower and upper lineages. In

Table 9. Percentage polymorphic loci at the 95% criterion (%P), allelic richness (A_R), effective number of alleles (A_E), expected heterozygosity (H_E), and observed heterozygosity (H_O) for eight Alaska regions.

Region	*%P*	A_R	A_E	H_E	H_O
Western Alaska	97.5	3.0	2.1	0.411	0.412
Aleutians/Alaska Pen.	79.2	3.4	2.1	0.382	0.382
Kodiak	84.4	3.4	2.1	0.390	0.380
Cook Inlet	79.7	3.3	2.2	0.388	0.396
Prince William Sound	75.0	2.8	2.0	0.363	0.366
Southeast Alaska	87.5	3.6	2.3	0.419	0.414
Lower Yukon River	87.5	3.0	2.1	0.408	0.402
Upper Yukon River	56.3	2.4	1.5	0.242	0.232

lower river lineage, two management units are apparent from the significant pairwise tests of allelic divergence: mainstem tributaries and Koyukuk River. Much more structure occurs in the upper river lineage, with all pairwise tests significant except between Old Crow and Fishing Branch of the Porcupine River. This knowledge, as well as the general indication that coho salmon populations are small and genetically discrete (Olsen et al. 2003, 2004), suggests that finer scale management may be necessary.

Simulation results are encouraging should finer scale management be desired. Simulation accuracies for the individual populations within the Koyukuk and Tanana Rivers range from 73%–95%. While only the Kantishna population exceeds the 90% accuracy threshold (Seeb and Crane 1999), most are close, which is quite good considering the low numbers of alleles and individuals per population in the analyses. However, a better assessment of the management units and ability to apportion mixtures would be facilitated by increasing baseline sample sizes and improving baseline representation if additional stocks exist, which is not clear due to data scarcity. These are definite priorities and requirements for finer scale management. If these measures are not satisfactory then assaying more diverse loci to increase resolution and power of MSA is another option.

Regarding the present management units, genetic divergence among these regions is sufficient with the current microsatellite baseline to allow accurate and precise estimates of stock compositions from fishery harvests (Seeb and Crane 1999). Moreover, the concordance between geography and genetic variation assures that accurate apportionment estimates to regional groups is possible with the present baseline because the fraction of the mixture from unsampled populations will allocate to neighboring populations (Beacham et al. 2003). These stock composition estimates can provide information on run-timing, migratory patterns, and, when combined with Pilot Station sonar enumeration data, stock return sizes, vastly increasing our knowledge of and, hence, our ability to manage Yukon River coho salmon.

Conclusions

1) Yukon River coho salmon exhibit a high degree of population structure.

2) Upper Yukon River coho salmon have lower levels of genetic diversity.

3) Accurate (>90%) apportionment to regions is possible with the current microsatellite baseline.

Recommendations

1) Increase sample sizes for those populations currently below 200 individuals.

2) Improve baseline representation if other stocks are identified.

3) Assay additional microsatellite loci that have greater than 20 alleles, if necessary, after completing 1 and 2.

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