

NOTES

Variation in the Population Structure of Yukon River Chum and Coho Salmon: Evaluating the Potential Impact of Localized Habitat Degradation

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Abstract.—We used microsatellite and mitochondrial DNA–restriction fragment length polymorphism (mtDNA–RFLP) analyses to test the hypothesis that chum salmon *Oncorhynchus keta* and coho salmon *O. kisutch* in the Yukon River, Alaska, exhibit population structure at differing spatial scales. If the hypothesis is true, then the risk of losing genetic diversity because of habitat degradation from a gold mine near a Yukon River tributary could differ between the two species. For each species, collections were made from two tributaries in both the Innoko and Tanana rivers, which are tributaries to the lower and middle Yukon River. The results revealed a large difference in the degree and spatial distribution of population structure between the two species. For chum salmon, the microsatellite loci (F_{ST} -statistic [F_{ST}] = 0.021) and mtDNA (F_{ST} = -0.008) revealed a low degree of interpopulation genetic diversity on a relatively large geographic scale. This large-scale population structure should minimize, although not eliminate, the risk of genetic diversity loss due to localized habitat degradation. For coho salmon, the microsatellites (F_{ST} = 0.091) and mtDNA (F_{ST} = 0.586) revealed a high degree of interpopulation genetic diversity on a relatively small geographic scale. This small-scale population structure suggests that coho salmon are at a relatively high risk of losing genetic diversity due to localized habitat degradation. Our study underscores the importance of a multispecies approach for evaluating the potential impact of land-use activities on the genetic diversity of Pacific salmon.

Loss of freshwater habitat is a common factor associated with the decline in abundance and diversity of Pacific salmon *Oncorhynchus* spp.

(Reeves et al. 1995). Nevertheless, the impact of localized habitat degradation on genetic diversity may vary among Pacific salmon species. Species composed of populations that occupy large geographic regions and exhibit moderate to high levels of gene flow are less likely to lose genetic diversity as a result of local habitat loss than are species composed of populations occurring on a small geographic scale with low rates of gene flow. Species-specific population structure, therefore, will influence the risk of losing genetic diversity when habitat is impacted by land-use activities such as mining (Frankham et al. 2002).

In this study, we examine the population structure of chum salmon *O. keta* and coho salmon *O. kisutch*, two species of Pacific salmon that co-occur in the Innoko and Tanana rivers, which are tributaries of the lower and middle Yukon River, respectively. The Yukon River is the largest river system within the North American ranges of all five species of Pacific salmon. In contrast to other large North American river systems (e.g., Columbia River, Fraser River), relatively little habitat degradation has occurred in the Yukon River drainage. This study was conducted as part of a comprehensive assessment designed to evaluate the possible environmental and biological impacts of a gold mine in the Innoko River drainage on the lower Yukon River system (Mueller et al. 2000).

Discrete populations of coho salmon have been detected through genetic analysis at both small and large geographic scales in river systems of the Pacific Northwest and Alaska (Small et al. 1998; Beacham et al. 2001; Gharrett et al. 2001; Smith et

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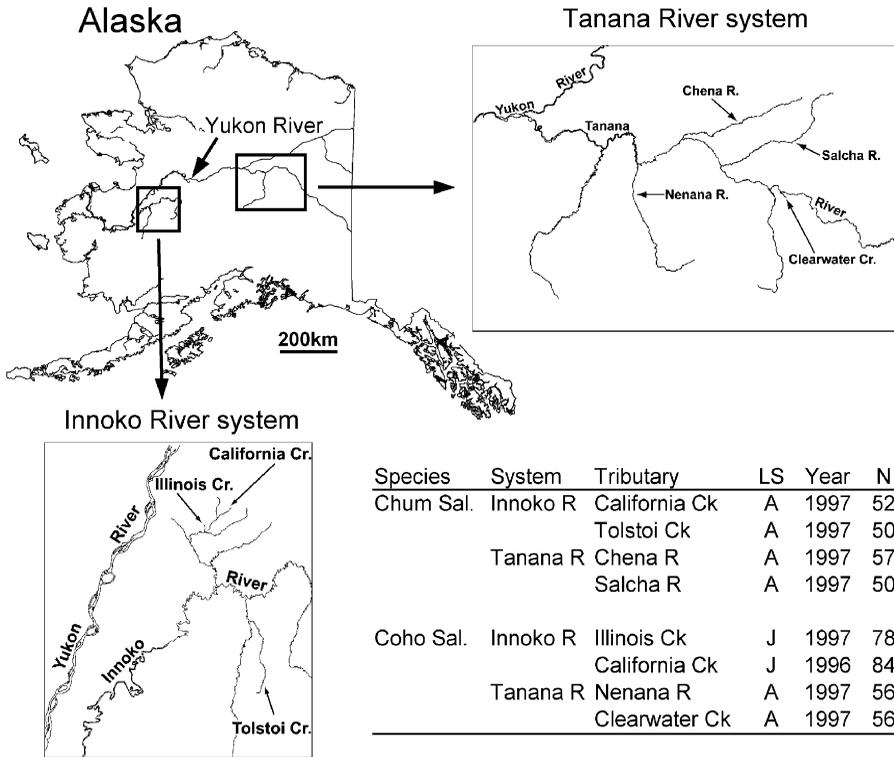


FIGURE 1.—Map of Alaska, showing the Yukon River and the tributaries sampled within the Innoko and Tanana rivers. The table (inset) shows the sample size (*N*), sample year, and life stage (LS) of fish sampled from each tributary for each species. Life stage is denoted as adult (A) or juvenile (J).

al. 2001; Olsen et al., 2003). Chum salmon populations, with some exceptions, generally appear to be organized on a large geographic scale encompassing multiple river systems (Park et al. 1993; Wilmot et al. 1994; Seeb and Crane 1999). Our hypothesis is that chum salmon and coho salmon in the Yukon River exhibit population structure at differing spatial scales. If this hypothesis is true, then the risk of losing genetic diversity because of habitat degradation in a Yukon River tributary could differ between the two species.

Methods

Sample collection and DNA preparation.—Fin tissue samples were collected from coho salmon and summer-run chum salmon in both the Innoko and Tanana rivers (Figure 1). We sampled each species from two tributaries of each river. All chum salmon were sampled as adults, as were coho salmon from the Tanana River. Coho salmon from the Innoko River were sampled as juveniles because adults were difficult to obtain.

Samples of juveniles may be dominated by a small number of families, thus creating possible

bias in estimates of allele frequencies in a population (Allendorf and Phelps 1981). Therefore, we used a sampling protocol that minimized the probability of “family sampling.” Coho salmon were captured in minnow traps that retained mostly 1-year-old juveniles (sibling groups likely disperse after 1 year; Hansen et al. 1997). Also, multiple locations were sampled in each tributary of the Innoko River. In California Creek, we sampled 18 locations over approximately 16 km. In Illinois Creek, a smaller stream, we sampled six locations over approximately 150 m. Hansen et al. (1997) found no evidence of family sampling in brown trout *Salmo trutta* when 1-year-old juveniles were sampled in multiple locations within a stream.

Tissue samples were stored in 100% ethanol until processed. Total genomic DNA was isolated from approximately 25 mg of fin tissue by use of a Puregene DNA isolation kit (Gentra Systems, Minneapolis, Minnesota). Isolated DNA was quantified with a TKO 100 fluorometer (Hoefer, Inc.) and diluted to 50 ng/μL for use in polymerase chain reaction (PCR) amplifications.

Microsatellite assay.—Five microsatellite loci

were screened for variation in chum salmon using the procedures of Scribner et al. (1998). The loci were *One μ 1*, *One μ 10*, *One μ 18* (Scribner et al. 1996), *Ssa14* (McConnell et al. 1995), and *Ssa197* (O'Reilly et al. 1996). Polymerase chain reactions were conducted in 25–30- μ L volumes with 0.25–0.30 units of *Taq* polymerase, ~150 ng of total genomic DNA, 10 mM tris-HCl (pH 8.3), 1.5–4.0 mM MgCl₂, 50 mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton X-100, 200 μ M of each deoxynucleotide triphosphate (dNTP), 0.06–0.40 μ M of each primer, and deionized H₂O. The amplification profile was one cycle of 94°C (2 min), 30–35 cycles of 94°C (1 min), 50–58°C (depending on the locus, 1 min), and 70°C (1 min), and one cycle of 68°C (5 min for *Ssa14*).

Three microsatellite loci were screened for variation in coho salmon by use of the procedures of Olsen et al. (in press). The loci were *One μ 3* (Scribner et al. 1996), *Okil* (Smith et al. 1998), and *Ots1* (Banks et al. 1999). The PCR amplifications were performed in 25- μ L volumes containing 0.125 units of *Taq* polymerase, ~100 ng of total genomic DNA, 10 mM of tris-HCl (pH 8.3), 1.5 mM of MgCl₂, 50 mM of KCl, 0.01% pectin, 200 μ M of each dNTP, 0.40 μ M of HEX-labeled forward primer, 0.40 μ M of reverse primer, and deionized H₂O. The amplification profile was one cycle of 95°C (3 min), 30 cycles of 95°C (1 min), 56–58°C (depending on the locus, 1 min), and 70°C (1 min), and one cycle of 70°C (5 min).

Mitochondrial DNA assay.—Regions of the mitochondrial DNA (mtDNA) genome were examined for polymorphism by use of restriction enzymes. The mtDNA region and enzyme combinations for chum salmon were NADH dehydrogenase-5/6 (*ND5/6*)—*Bst*N I and *Ase* I, and for coho salmon they were *ND5/6*—*Dde* I and cytochrome *b*—*Bfa* I, *Bsa*J I, and *Bst*N I. For both chum salmon and coho salmon, the *ND5/6* region was amplified by PCR with primers designed from rainbow trout *O. mykiss* (GenBank access number L29771: forward 5'-GCTCATCCATTGGTCTTAGGAACC-3', reverse 5'-ATAACAACGGTGGTTTTTCAAGTCAT-3'). For coho salmon only, the cytochrome-*b* region was also amplified by PCR with primers from Bickham et al. (1995). PCR amplifications were performed in a 25- μ L volume containing 0.125 units of *Taq* polymerase, ~150 ng total genomic DNA, 10 mM tris-HCl (pH 9.5), 1.5 mM MgCl₂, 50 mM KCl, 200 μ M each dNTP, 0.20 μ M of each primer, and deionized H₂O. The amplification profile for *ND5/6* was one cycle of 95°C (3 min), 32 cycles of 95°C (50 s), 54°C (50 s), and 70°C (2.5 min), and one cycle of 70°C (5

min). The amplification profile for cytochrome *b* was one cycle of 95°C (3 min), 32 cycles of 95°C (45 s), 50°C (50 s), and 70°C (2.5 min), and one cycle of 70°C (5 min). Restriction digests consisted of 5 units of a restriction enzyme, 5 μ L of amplified PCR product, 1.5 μ L of each enzyme's 10 \times buffer, and deionized H₂O added to a final volume of 15 μ L. The restriction digests were screened for variation by use of procedures similar to those of Burger et al. (1997). A composite haplotype was generated for each individual by inferring the presence or absence of restriction sites for all restriction enzymes and mtDNA fragments (Lansman et al. 1981).

Analysis of juvenile samples.—Juvenile chinook salmon (*O. tshawytscha*) are sometimes mistaken for coho salmon when field identification methods are used. Because both species occur in the Innoko River, we used the mtDNA fragment patterns generated by the assay described above to verify the species identity of each juvenile sample. Individuals that exhibited a chinook salmon composite haplotype were removed from the sample prior to data analysis. These species-specific haplotypes were determined a priori by genotyping adult samples of chinook salmon and coho salmon (S. J. Miller, U.S. Fish and Wildlife Service, unpublished data). Based on this analysis, 90 juvenile samples were removed from the data set: 87 samples from California Creek and 3 samples from Illinois Creek.

Microsatellite data analysis.—Estimates of allele frequency, number of alleles (*A*), and observed and expected heterozygosity (*H_O*, *H_E*) per locus and population were calculated with FSTAT version 2.9.3 (Goudet 2001). A randomization test of the statistic *f* was used to test conformity to Hardy-Weinberg equilibrium (HWE) for each locus and population combination (Goudet 2001). A *G*-test of genotypic frequency homogeneity was used to test for genetic differentiation among all population pairs. When simultaneous tests were performed, the threshold for statistical significance ($\alpha = 0.05$) was corrected for *k* simultaneous tests (α/k) with the sequential Bonferroni method (Rice 1989). For each species, a hierarchical analysis of molecular variation for diploid data (AMOVA; Michalakis and Excoffier 1996) was used to evaluate the degree of population structure within and between the Innoko and Tanana rivers. The AMOVA was performed with ARLEQUIN version 2.0 (Schneider et al. 2000) to estimate the relative measure of population structure, or *F*-statistic (*F_{ST}*). For each analysis, the estimate of *F_{ST}* was partitioned into a between-tributary, within-river

component (F_{SR}) and a between-river component (F_{RT}) of spatial population structure. Permutation tests ($N = 5,000$) were used to test whether the estimates of F_{ST} , F_{SR} , and F_{RT} were significantly greater than zero.

Mitochondrial DNA data analysis.—Estimates of haplotype diversity (h) and composite haplotype frequency (S) were computed with ARLEQUIN version 2.0 (Schneider et al. 2000). A probability test of haplotype frequency homogeneity was used to test for genetic differentiation among all population pairs. The degree of population structure was evaluated by use of hierarchical AMOVA, as described above.

Results and Discussion

Intrapopulation Genetic Diversity

The mean H_E over all microsatellite loci for each population ranged from 0.45 to 0.55 for chum salmon and from 0.38 to 0.53 for coho salmon (Table 1). The mean A over all microsatellite loci ranged from 3.8 to 4.4 for chum salmon and from 3.3 to 4.3 for coho salmon (Table 1). The randomization tests of conformity to HWE for each locus and population showed no evidence of a deficit or excess of heterozygote individuals ($P > 0.05$).

For chum salmon, the *ND5/6* region of mtDNA exhibited a lower degree of polymorphism than the microsatellites. For coho salmon, polymorphism in the *ND5/6* and cytochrome-*b* regions of mtDNA was similar to the level of polymorphism in the microsatellites for the Innoko River tributary samples, but not for the Tanana River tributary samples. Haplotype diversity (h) ranged from 0.09 to 0.22 for chum salmon and from 0.00 to 0.66 for coho salmon (Table 1). The number of composite haplotypes (S) ranged from 2.0 to 4.0 in chum salmon and from 1.0 to 6.0 in coho salmon (Table 1).

The estimates of intrapopulation genetic diversity (mean H_E ; h) exhibited a spatial trend in coho salmon but not in chum salmon. For coho salmon, these values indicate that intrapopulation genetic diversity is greater in the Innoko River (lower Yukon River) than in the Tanana River (middle Yukon River). This general trend was apparent from the microsatellite data, but it was most obvious from the mtDNA data. Although extensive surveys of gene diversity have not been conducted on Yukon River coho salmon, two studies reported low microsatellite diversity (Olsen et al. 2003) and mtDNA diversity (Gharrett et al. 2001) for coho salmon populations from the Tanana River relative to other Alaskan coho salmon populations. Inter-

estingly, the difference in haplotype diversity between the Innoko River and Tanana River populations observed in this study is similar in magnitude to that detected between the Tanana River population and other Alaskan coho salmon populations from the Bering Sea and Gulf of Alaska (Gharrett et al. 2001). For chum salmon, the estimates of mean H_E and h indicate that intrapopulation genetic diversity does not vary between the Innoko and Tanana rivers. Similar results were reported by Wilmot et al. (1994) in an extensive population survey of allozyme diversity in Yukon River chum salmon.

Interpopulation Genetic Diversity

The chum salmon and coho salmon exhibited much different levels of population structure, as revealed by the tests of interpopulation genetic diversity (Tables 2, 3). Both analyses suggest that the geographic scale of chum salmon population structure is large within the Yukon River. The evidence of spatial genetic diversity is limited, and comes from the microsatellite data but not the mtDNA data. The estimate of overall population structure from the microsatellite loci ($F_{ST} = 0.021$), while significant, is low and is due entirely to genetic differences between the two rivers (Innoko and Tanana) and not to genetic differences within rivers (Tables 2, 3). These results also correspond to the findings of Wilmot et al. (1994) and Seeb and Crane (1999), who found significant but weak evidence of population structure in Yukon River chum salmon by use of allozymes. Wilmot et al. (1994) found that most of the genetic variation ($G_{ST} = 0.0049$) among Yukon River chum salmon populations was attributable to run timing (summer versus fall). In the present study, only summer-run chum salmon were examined. Nevertheless, the results of our study and that of Wilmot et al. (1994) suggest that chum salmon population structure in the Yukon River occurs on a large geographic scale.

In contrast, the microsatellite and mtDNA data revealed a significant and high degree of population structure in coho salmon (Tables 2, 3). The values of F_{ST} , F_{SR} , and F_{RT} suggest that population structure for coho salmon in the Yukon River occurs on a much smaller geographic scale than for chum salmon. The hierarchical AMOVA indicates the degree of coho salmon population structure between tributaries within the two rivers (F_{SR}) is greater than the degree of chum salmon population structure between the rivers (F_{RT}). Gharrett et al. (2001) and Olsen et al. (2003) described similar

TABLE 1.—Genetic diversity in four populations of chum salmon and four populations of coho salmon from tributaries of the Innoko and Tanana rivers in the Yukon River system, Alaska (California Creek [Cal], Tolstoi Creek [Tol], Chena River [Che], Salcha River [Sal], Illinois Creek [Ill], Nenana River [Nen], and Clearwater Creek [Cle]). Sample size (*N*), number of alleles (*A*), number of composite haplotypes (*S*), expected and observed heterozygosity (H_E , H_O), and haplotype diversity (*h*) are shown.

Species and locus	Innoko River			Tanana River			
	Cal	Tol	Ill	Che	Sal	Nen	Cle
Chum salmon							
<i>Oneμ1</i>	<i>N</i> (<i>A</i>)	51 (2)	50 (2)	50 (3)	47 (3)		
	H_O	0.26	0.36	0.48	0.32		
	H_E	0.28	0.30	0.48	0.39		
<i>Oneμ18</i>	<i>N</i> (<i>A</i>)	52 (4)	50 (4)	52 (4)	49 (4)		
	H_O	0.65	0.62	0.50	0.49		
	H_E	0.63	0.64	0.50	0.52		
<i>Oneμ10</i>	<i>N</i> (<i>A</i>)	51 (5)	50 (4)	52 (5)	48 (5)		
	H_O	0.53	0.70	0.79	0.73		
	H_E	0.70	0.72	0.73	0.70		
<i>Ssa14</i>	<i>N</i> (<i>A</i>)	52 (7)	50 (6)	51 (5)	46 (6)		
	H_O	0.60	0.64	0.41	0.54		
	H_E	0.60	0.71	0.51	0.50		
<i>Ssa197</i>	<i>N</i> (<i>A</i>)	52 (4)	50 (3)	52 (3)	49 (3)		
	H_O	0.31	0.38	0.19	0.18		
	H_E	0.31	0.36	0.18	0.17		
Mean	<i>A</i>	4.4	3.8	4.0	4.2		
	H_O	0.47	0.54	0.47	0.45		
	H_E	0.50	0.55	0.48	0.45		
mtDNA	<i>N</i> (<i>S</i>)	50 (2)	50 (4)	43 (2)	42 (4)		
	<i>h</i>	0.18	0.19	0.09	0.22		
Coho salmon							
<i>Oneμ3</i>	<i>N</i> (<i>A</i>)	68 (4)		34 (4)		56 (4)	56 (4)
	H_O	0.78		0.68		0.52	0.63
	H_E	0.71		0.68		0.54	0.52
<i>Oki1</i>	<i>N</i> (<i>A</i>)	75 (6)		42 (5)		55 (4)	56 (5)
	H_O	0.67		0.64		0.64	0.59
	H_E	0.73		0.64		0.68	0.63
<i>Ots1</i>	<i>N</i> (<i>A</i>)	78 (3)		41 (2)		56 (2)	56 (1)
	H_O	0.17		0.07		0.02	0.00
	H_E	0.16		0.07		0.02	0.00
Mean	<i>A</i>	4.3		3.7		3.3	3.3
	H_O	0.54		0.46		0.39	0.40
	H_E	0.53		0.46		0.41	0.38
mtDNA	<i>N</i> (<i>S</i>)	84 (4)		78 (6)		51 (2)	52 (1)
	<i>h</i>	0.45		0.66		0.08	0.00

degrees of small-scale population structure, and concluded that Alaskan coho salmon populations are generally small and discrete, and therefore should be managed at a fine geographic scale to conserve genetic diversity. The results of this study support a similar conclusion for Yukon River coho salmon.

The unusually large estimate of F_{ST} from the coho salmon mtDNA analysis (0.586) is atypical of most genetic studies of Pacific salmon, especially at this spatial scale. Nevertheless, this result is not unexpected given the data from Gharrett et al. (2001). We used their haplotype frequency data to estimate F_{ST} for the western Alaska coho salmon populations they studied: the Clearwater River, the Eek River, and the Kanektok River. The Clearwater

River population is from the middle Yukon River and the other two populations are from the lower Kuskokwim River area, the next major river system south of the Yukon River. The F_{ST} estimate (0.406) we calculated from their data is similar to the value we report for the four Yukon River populations. We believe this large genetic signal may reflect a founder event or bottleneck in the middle Yukon River populations, and such a result underscores the need for a more extensive survey of coho salmon in this region.

The large difference between mtDNA and microsatellite estimates of F_{ST} and F_{SR} in coho salmon is likely due to the unique attributes of the two marker types. The low genetic diversity in the mtDNA (as evidenced by low *h*) may persist fol-

TABLE 2.—Results of genotypic frequency (below diagonal) and haplotypic frequency (above diagonal) homogeneity tests for population pairs of chum salmon and coho salmon from the Innoko and Tanana rivers in the Yukon River system, Alaska. See Table 1 for abbreviations of tributaries. The critical value ($\alpha = 0.05$) was adjusted for six simultaneous tests (Bonferroni adjusted $\alpha = 0.0083$); significant tests are indicated by asterisks; NS = not significant.

Tribu- tary	Chum salmon				Tributary	Coho salmon			
	Innoko		Tanana			Innoko		Tanana	
	Cal	Tol	Che	Sal		Cal	Ill	Nen	Cle
Cal		NS	NS	NS	Cal		*	*	*
Tol	NS		NS	NS	Ill	NS		*	*
Che	*	NS		NS	Nen	*	*		NS
Sal	*	NS	NS		Cle	*	*	*	

lowing a bottleneck in the middle Yukon River populations because the effective population size for mtDNA is one-fourth that of nuclear genes (Birky et al. 1983). Furthermore, microsatellites often have a higher mutation rate than mtDNA, and therefore may exhibit higher levels of intra-population genetic variation and consequently lower values of F_{ST} (Hedrick 1999).

Interestingly, Smith et al. (2001) found very little mtDNA variation in five populations of Alaskan coho salmon, including samples from the middle Yukon River, Kuskokwim River, and Gulf of Alaska. With the exception of the middle Yukon River, their results differ from ours and from those of Gharrett et al. (2001). We believe there are two likely explanations for these contradictory results. First, Smith et al. (2001) examined only one mtDNA region (D-loop), whereas we examined two regions (*ND5/6* and cytochrome *b*) and Gharrett et al. (2001) examined the entire mtDNA genome. Second, independent analysis of the different mtDNA regions (“genes”) may provide contradictory results, presumably because the forces influencing genetic variation (e.g., mutation) in these genes differ (Churikov et al. 2001). Thus, we infer from the three studies that the mtDNA D-loop is probably not a good candidate for examination of small- to moderate-scale population structure in Alaskan coho salmon, whereas it is informative for large-scale biogeographic analyses, such as that described by Smith et al. (2001).

A potential criticism of our study is that too few microsatellite loci were used to detect genetic differentiation and estimate the degree of population structure. It is true that more loci would add statistical power and precision, and increase the probability of detecting significant population structure, especially among weakly differentiated populations like chum salmon. Additional DNA is not available, however, and the remoteness of the sampling locations renders collections of new samples

cost prohibitive. Nevertheless, we believe this sample of microsatellite loci is sufficient to reveal accurate levels of relative population structure in the two species we studied. This assertion is supported by the fact that, for both chum salmon and coho salmon, the estimates of F_{ST} were similar among loci and did not appear to be dominated by a single locus. For chum salmon, the F_{ST} estimates for *One μ 1*, *One μ 10*, *One μ 18*, *Ssa14*, and *Ssa197* were 0.041, 0.001, 0.021, 0.024, and 0.037, respectively. For coho salmon, the F_{ST} estimates for *One μ 3*, *Okil*, and *Ots1* were 0.109, 0.081, and 0.043, respectively.

Implications for Conservation

Our study supports the hypothesis that chum salmon and coho salmon in the Yukon River exhibit population structure at differing spatial scales. Spatial genetic diversity in Yukon River chum salmon appears to be distributed over a relatively large geographic area. This large-scale population structure should minimize, although not eliminate, the risk of loss of genetic diversity in the event that some chum salmon spawning aggregations vanish due to localized habitat degradation. It must be emphasized, however, that this study examined only neutral genetic markers (genes not under selection). It is possible that small-scale population structure may exist in genes controlling traits of adaptive importance. Also, regardless of whether or not genetic diversity is lost, the loss of chum salmon from a tributary would likely reduce the overall abundance of Yukon River chum salmon. Such a loss could have many other impacts (e.g., ecological, cultural, or economic).

In contrast, coho salmon populations in the lower and middle Yukon River are highly differentiated, and the lower-river populations (such as in the Innoko River) harbor a large component of the overall genetic diversity observed at these loci. Because coho salmon populations are relatively

TABLE 3.—Hierarchical gene diversity analysis (microsatellites [microsat] and mtDNA) of four coho salmon populations and four chum salmon populations sampled from the Yukon River system. An asterisk denotes a probability less than 0.05 that the value is not greater than zero. The relative measure of population structure, or F -statistic (F_{ST}), is partitioned into a between-river component (F_{RT}) and a between-tributary, within-river component (F_{SR}).

Species and DNA type	Source of variation	σ^2	% of total	F_{ST}	F_{RT}	F_{SR}
Chum salmon						
Microsat	Total	1.264	100.00			
	Within populations	1.238	97.94			
	Between populations	0.026	2.06	0.021*		
	Between rivers (Innoko, Tanana)	0.026	2.06		0.021*	
	Between tributaries within rivers	0.000	0.00			0.00
mtDNA	Total	0.086	100.00			
	Within populations	0.086	100.00			
	Between populations	0.000	0.00	-0.008		
	Between rivers (Innoko, Tanana)	0.000	0.00		-0.005	
	Between tributaries within rivers	0.000	0.00			-0.002
Coho salmon						
Microsat	Total	0.746	100.00			
	Within populations	0.678	90.88			
	Between populations	0.068	9.12	0.091*		
	Between rivers (Innoko, Tanana)	0.045	6.04		0.061*	
	Between tributaries within rivers	0.023	3.08			0.032*
mtDNA	Total	0.426	100.00			
	Within populations	0.176	41.30			
	Between populations	0.250	58.70	0.586*		
	Between rivers (Innoko, Tanana)	0.240	56.30		0.564*	
	Between tributaries within rivers	0.010	2.40			0.052*

small and discrete, the likelihood of localized habitat degradation affecting an entire population is greater with coho salmon than with chum salmon. In addition, the AMOVA results suggest that gene flow between lower- and middle-river populations is extremely low and that these populations, if extirpated, are unlikely to be rapidly recolonized by other coho salmon populations.

Specifically, our study suggests that localized habitat degradation due to land-use activities in the Yukon River, such as mining in the Innoko River drainage, could impact the genetic diversity of coho salmon to a greater degree than chum salmon. In general, our results demonstrate that species-specific conservation plans may be required to maintain genetic diversity of salmon species occupying the same geographical area. Thus, a multispecies approach is recommended for evaluating the potential impact of land-use activities on genetic diversity of Pacific salmon.

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