Conservation Genetics (2006) 7:65–78
DOI 10.1007/s10592-005-7967-2

Genetic structure of chum salmon (*Oncorhynchus keta*) populations in the lower Columbia River: are chum salmon in Cascade tributaries remnant populations?

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Received 17 January 2005; accepted 26 May 2005

Key words: chum salmon, microsatellites, population structure, endangered salmonids, *Oncorhynchus keta*, Columbia River

Abstract

The lower Columbia River drainage once supported a run of over a million chum salmon. By the late 1950s, the run had decreased to often a few hundred fish. With the exception of Grays River near the coast and an aggregation of chum salmon spawning in creeks and the main stem near Bonneville Dam in the Columbia Gorge, most populations were thought to be extinct. However, chum salmon consistently return in low numbers to tributaries originating in the Cascade Range: the Cowlitz, Lewis, and Washougal rivers. To assess whether Cascade spawners were strays or remnants of former populations, chum salmon from the Coastal, Cascade and Gorge ecoregional zones were characterized at 17 microsatellite loci. Significant heterogeneity in genotype distributions was detected between zones and collections formed regional groups in a neighbor-joining tree. Cascade collections had higher allelic richness and private alleles, and the Cowlitz River supported genetically divergent fall and summer runs, the only summer chum salmon run extant in the Columbia River drainage. We propose that chum salmon in the Cascade zone are remnants of original populations. We attribute the divergence between zonal groups to diverse ecological conditions in each zone, which promoted regional genetic adaptation, and to genetic drift experienced in small populations.

Introduction

Chum salmon (*Oncorhynchus keta*), once a run of over a million in the lower Columbia River in Washington State (WA) (NOAA 2004), were nearly extirpated in the 20thcentury. Declines resulted from overfishing coupled with spawning habitat destruction in tributaries and rearing habitat destruction in the Columbia River estuary (Sherwood *et al.* 1990; Johnson *et al.* 1997). Historically, chum salmon were present in 16 tributaries (Myers *et al.* 2002) and throughout the main stem of the Columbia River below Celilo Falls (river kilometer (rkm) 320, Johnson *et al.* 1997). Runs were in decline by the 1930s and by the late 1950s, sometimes fewer than 100 fish returned and chum salmon had disappeared from most tributaries (Fulton 1970). Limited hatchery supplementation was implemented with regional (56%) and out of region (44%) broodstock in tributaries near the mouth of the Columbia River in Oregon (OR) in 1929 and in WA in 1958 (Johnson *et al.* 1997). Supplementation with non-native fish likely had little genetic effect upon remaining native chum salmon since juveniles were planted in tributaries where native runs were extinct (Johnson *et al.* 1997) and the modest efforts (mean less than 500,000 fry per year) failed to restore populations. Further, genetic analyses (Phelps *et al.* 1994) indicated that lower Columbia River chum salmon

remain genetically distinct from other chum salmon in Washington State that had been planted as hatchery fish. Despite supplementation efforts and a moratorium on commercial exploitation implemented in the late 1950s, the chum salmon run remains around 2500 fish, roughly 3% of historical abundance (WDFW 2000). Throughout the decrease, two populations survived, one in Grays River at the coast and the other centered in the mainstem and tributaries near Bonneville Dam in the Columbia Gorge (WDFW 2000, see Figure 1). However, consistent low returns (up to 30 fish) have been documented in tributaries in other, ecologically different regions of the lower Columbia drainage that formerly supported viable chum salmon populations. Thus, other populations may persist at low numbers (Keller 2001; J. Hymer Washington Department of Fish and Wildlife (WDFW) unpublished data) and harbor important genetic diversity.

Chum salmon have the widest distribution of Pacific salmonids, ranging north to Arctic shores and south to Korea and California (Johnson *et al.* 1997). Although large, averaging 3.6 to 6.8 kg, chum salmon rarely surmount rapids and generally spawn in coastal areas and lower reaches of rivers (Salo 1991). Redds (salmon nests) are constructed in gravel in main stems and side channels, preferably where groundwater percolates through the gravel (Salo 1991). Eggs hatch after 3–4 months and alevins remain in the gravel 3–5 weeks. Emerging juveniles migrate directly to salt water, spending about a month in estuaries before heading to the ocean (Salo 1991). Marine residence varies and spawners in the Columbia River return mainly after 3 years (Johnson *et al.* 1997), with 4 and 5 year returns also common (Keller 2001). Run timing follows a cline from summer in the north to fall in the south, although summer and fall runs often occur in the same stream (Johnson *et al.* 1997).

Population declines in chum salmon prompted a status review, which divided chum salmon in WA into four evolutionarily significant units (ESU's): Puget Sound/Strait of Georgia, Hood Canal summer-run, Pacific coast and Columbia River (Johnson *et al.* 1997). The Columbia River ESU was listed as threatened in 1999 (NMFS 1999) and recovery criteria were outlined by the Willamette/Lower Columbia Technical Recovery Team (WLC-TRT) (Myers *et al.* 2002; McElhany *et al.* 2003). The WLC-TRT identified distinct ecological-hydrological regions (zones) occupied by the Columbia River ESU based upon Environmental Protection Act ecozones: the Coastal zone with low elevation tributaries dependent upon seasonal rainfall; the Western Cascade zone with large tributaries originating in the Cascade mountains and fed by snowmelt; and the Columbia Gorge zone with small tributaries fed by ground and rainwater, some traversing hot, dry habitat. Tributaries within these zones provide

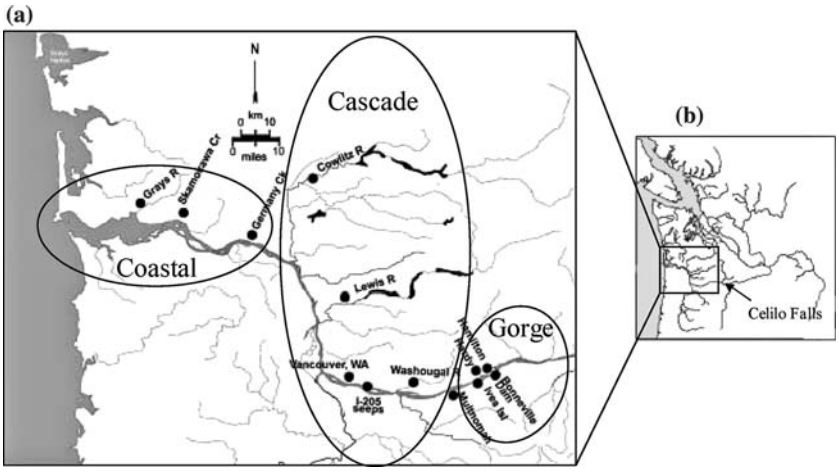

Figure 1. Detailed map (a) (from Jim Shaklee, WDFW) indicates collection sites in lower Columbia River with filled circle over each collection tributary and filled circle showing Vancouver, WA. Ecological zones are labeled and delineated approximately. Map (b). indicates major drainages in Washington State with a box around collection area for lower Columbia River chum salmon (map from Shelly Snyder and Darrell Pruit at WDFW).

diverse chum salmon habitat, which can be expected to promote regional adaptation and genetic diversity (Taylor 1991). However, although all chum salmon populations in Cascade tributaries were suspected to be extinct, some spawners return annually to the Cowlitz, Lewis and Washougal rivers. WDFW and cooperating regional enhancement groups have initiated efforts to repopulate streams that historically supported chum salmon. Successful recovery depends on understanding genetic diversity in the system, and determining if spawners in Cascade tributaries are strays or original populations.

Previous allozyme analysis by WDFW confirmed that extant chum salmon groups in the lower Columbia River were genetically distinct: the Grays River in the Coast zone, and the Bonneville Dam region in the Columbia Gorge zone (Myers *et al*. 2002). As in other salmonids, population genetic structure in chum salmon is a result of natal homing, which reduces gene flow among populations spawning in different tributaries, colonization history, adaptation to local environments and random genetic drift at neutral loci (McConnell *et al*. 1997; Small *et al*. 1998; Spidle *et al*. 2001). We surveyed genetic variation at neutral loci in chum salmon samples from tributaries in the Coast, Cascade and Gorge ecological zones. We examined partitioning of genetic variation among collections and among zones to assess population structure and show that regional genetic structure was partitioned among ecological zones. We used multi-locus genotypes to explore ancestry at individual and population levels to examine hypotheses that fish returning to Cascade tributaries were strays from extant groups or remnants of original populations. We present data suggesting that endemic populations have survived undetected in Cascade tributaries and we document a degree of genetic diversity that is remarkable given the history of extirpation and low run numbers in the lower Columbia River.

Materials and methods

Samples

Adult chum salmon tissue samples (fin clips or scales) were collected from 12 sites within the lower Columbia River between 1992 and 2002 (Table 1, Figure 1). Several collections included multiple collection years (see Table 1). Since year classes overlap in chum salmon, subsequent collection years include members of the same year classes and are generally genetically indistinguishable. Earlier analysis (Small *et al*. unpublished data) indicated genetic difference between Grays River samples collected after the first hatchery supplementation return in 2001 (a hatchery program was started in the Grays River in 1998) and prior samples. Thus, samples prior to 2001 were combined (1992, 1997, 2000) and the 2001 sample was analyzed separately (see Table 1). Collection sites in the Coastal ecological zone (Coastal) included Grays River (river kilometer (rkm) 35) and Skamokawa (rkm 60) and Germany creeks (rkm 90), (Figure 1). Cascade ecological zone collections (Cascade) included the Cowlitz (rkm 109) and Lewis (rkm 140) rivers (Figure 1) (see below for Washougal River). Cowlitz River chum have a bimodal run, peaking in September and November, and may thus include summer and fall runs. We explored this possibility by running analyses with fish grouped and again with fish separated by run. We designated fish collected before October 15 as summer-run and after October 15 as fall-run, following the cut-off time designated for run timing in Hood Canal rivers in WA which support summer and fall runs (Johnson *et al*. 1997). The Washougal River (rkm 195, Figure 1) was different from other Cascade tributaries, being a modest-sized drainage fed by groundwater and rainfall (Todd Hillson, WDFW, personal communication). Since earlier genetic analyses indicated it was part of the Gorge group (Small *et al*. unpublished data) we included Washougal River with Gorge zone collections. Other Columbia Gorge collections (Gorge) included Hamilton (rkm 228) and Hardy creeks (rkm 229), Ives Island (rkm 227), the trap at Bonneville Dam (rkm 235), Multnomah Creek (rkm 218 on the Oregon side), and I–205 seeps in the main stem near Vancouver, WA (rkm 180).

DNA processing

Genomic DNA was extracted from tissues using a chelex resin protocol (Small *et al*. 1998). Microsatellite alleles at 17 loci were PCR-amplified using fluorescently labeled primers (see Table 2 for detailed PCR information). PCR's were conducted on a MJResearch PTC-200 thermocycler in 96 well plates

Table 1. Table of lower Columbia River chum salmon collection statistics

Collection years	Collections	Region	N	link	Hexp	Hobs	HWE *P*	Allelic rich	Priv	M	var M
1992,1997,2000	GraysC	Coastal	231	5	0.823	0.798	< **0.0001**	10.30	0.52	0.174	0.044
2001	Grays01	Coastal	100	7	0.822	0.816	0.0382	10.38	0.73	0.178	0.044
2000 – 2002	Skamokawa	Coastal	53	1	0.826	0.832	0.3631	10.29	0.51	0.170	0.037
2003	Germany	Coastal	33		0.835	0.804	0.1054	11.58	0.77	0.206	0.073
2000 – 2003	*Cowlitz C*	*Cascade*	*64*	*20*	*0.859*	*0.819*	< ***0.0001***	*12.77*	*1.07*	*0.239*	*0.125*
2000 – 2003	Cowlitz F	Cascade	27		0.823	0.836	0.2507	12.33	1.26	0.224	0.092
2000 – 2003	Cowlitz S	Cascade	37	26	0.864	0.804	< **0.0001**	12.60	2.14	0.237	0.125
2000 – 2003	Lewis	Cascade	62	4	0.845	0.815	< **0.0001**	12.02	1.11	0.225	0.108
2000	Washougal	Gorge	35		0.820	0.811	0.1258	10.70	0.56	0.184	0.051
2002,2003	Vancouver	Gorge	88	2	0.815	0.802	0.0181	11.20	0.90	0.203	0.081
2002	Multnomah	Gorge	97	2	0.814	0.796	0.0191	10.30	0.54	0.175	0.046
1992,1996,1997,2002	Hamilton	Gorge	190	4	0.816	0.787	< **0.0001**	10.52	0.55	0.184	0.056
2000 – 2002	Ives Island	Gorge	146	7	0.806	0.735	< **0.0001**	10.17	0.58	0.176	0.047
1996,1997,2002	Hardy	Gorge	134	3	0.813	0.794	< **0.0001**	10.18	0.50	0.179	0.052
2000 – 2002	Bonneville	Gorge	46	1	0.824	0.778	0.0081	10.52	0.64	0.186	0.059
			1374								
		Coastal						13.76	1.54		
		Cascade						16.52	3.52		
		Gorge						13.64	1.52		

Ecological zone regional location is indicated in ''Region'' column. The combined summer and fall Cowlitz River collection is named ''CowlitzC'' and italicized. Other statistics include: the number of genotypic linkage disequilibria (''link''), gene diversity (Hexp), observed heterozygosity (Hobs), Hardy Weinberg equilibrium (HWE) with significant *P*-values (HWE *P*) in bold type, allelic richness (rich), private allelic richness (Priv), M values and their variance over all loci.

Table 2. Information for multiplexes and loci: number of alleles in this study, size range (in base pairs), observed and expected heterozygosity (Hobs and Hexp), repeat unit size (in base pairs), and *P*-value for deviation from Hardy Weinberg equilibrium (HWE)

Multiplex	anneal T	Locus	Primer conc [μM]	Dye	#alleles	Range	Hobs	Hexp	repeat	HWE *P*	Source
OkeA	50	*Ots*-G311	0.4	6fam	55	240–485	0.873	0.957	4	< **0.0001**	Williamson *et al.* (2002)
		Oke-3	0.4	hex	16	315–445	0.613	0.621	13	0.0056	Bucholz *et al.* (2001)
		Omy-1011	0.1	ned	17	196–257	0.868	0.869	4	0.0536	Paul Bentzen, personal communication
OkeB	50	*One*-102	0.5	6fam	24	212–323	0.910	0.904	4	0.2387	Olsen *et al.* (2000)
		One-114	0.4	hex	31	198–297	0.916	0.923	4	0.0088	Olsen *et al.* (2000)
		Ots-3M	0.1	ned	16	128–167	0.716	0.720	2	0.0199	Banks *et al.* (1999)
OkeC	50	*Ots*-1	0.15	6fam	24	152–239	0.749	0.811	2	< **0.0001**	Banks *et al.* (1999)
		Omm-1137	0.06	hex	27	98–162	0.549	0.814	2	< **0.0001**	Rexroad III *et al.* (2001)
		One-101	0.07	ned	38	119–275	0.840	0.893	4	< **0.0001**	Olsen *et al.* (2000)
OkeE	53	*One*-106	0.1	6fam	37	168–320	0.897	0.936	4	< **0.0001**	Olsen *et al.* (2000)
		Ssa-419	0.05	hex	14	258–310	0.801	0.818	4	0.5317	Cainey *et al.* (2000)
		One-18	0.04	ned	8	163–188	0.650	0.673	2	0.2059	Scribner *et al.* (1996)
OkeF	53	*One*-111	0.2	6fam	64	170–356	0.858	0.875	2	0.013	Olsen *et al.* (2000)
		Oki-1	0.1	hex	13	188–240	0.820	0.841	4	0.1695	Smith *et al.* (1998)
		Ots-2M	0.12	ned	5	143–157	0.464	0.458	2	0.4458	Banks *et al.* (1999)
OkeG	45	*One*-108	0.1	6fam	48	160–386	0.906	0.932	4	< **0.0001**	Olsen *et al.* (2000)
		Ots-103	0.28	hex	38	91–241	0.921	0.926	4	< **0.0001**	Beacham *et al.* (1998)

Values out of equilibrium after Bonferroni corrections are in bold. Primer sequence source citation is in ''Source'' column.

in 5 μl volumes employing 1 μl template with final concentrations of 1.5 mM $MgCl_2$ and 1× Promega PCR buffer. After initial three minute denature at 92 °C, 33 cycles consisting of 92 °C for 15s annealing (temp in Table 2) for 30s extension at 72 °C for 60s were followed by a 30min extension at 72 °C. Samples were run on an ABI 3730 automated sequencer and alleles were sized (to base pairs, bp) and binned using an internal lane size standard (GS500Liz from Applied Biosystems) and Genemapper software (Applied Biosystems).

Within collection site data analysis

Statistical tests were conducted on loci and samples from each collection site to assess conformation to Hardy Weinberg expectations (Hardy Weinberg equilibrium, HWE), and genotypic heterogeneity using GENEPOP version 3.3 (Raymond and Rousset 1995); FSTAT 2.9.3 (Goudet 2001) and MSA (Dieringer and Schlötterer 2003). The Cowlitz River collection was analyzed with run groups combined and then separated into putative fall and summer groups. Loci and collections were tested for deficits of heterozygotes and homozygotes (HWE) across all loci and across all collections using GENEPOP with 100 batches and 2000 iterations. Loci were tested for large allele dropout and null alleles using MICRO-CHECKER Version 2.2.1 (Van Oosterhout *et al*. 2004). F_{IS} values were calculated for each locus in each collection and over all loci using FSTAT. Linkage disequilibrium was assessed using GENEPOP with 200 batches and 3000 iterations. Allelic richness and private allelic richness were calculated for individual collections and for regional groups using rarefaction as implemented in HP-RARE v1.4 (Kalinowski 2005). FSTAT was employed to calculate observed heterozygosity and Nei's (1987) gene diversity. Collections were examined for recent bottlenecks by calculating Garza and Williamson's ''M'' (Garza and Williamson 2001).

Between collection site data analysis

ARLEQUIN 2.000 (Schneider *et al*. 2000) was used to perform analyses of molecular variance (AMOVA) tests with collections divided into regional groups. The AMOVA was based upon a distance matrix with 100,000 permutations. The program WHICHRUN 4.2 (Banks and Eichert 2000) was used for jackknife assignment tests. Individual fish were given the most likely assignment to a collection based upon the genotype of the fish and the allele frequencies in collections. STRUCTURE 2.1 (Pritchard *et al*. 2000) was used to estimate the proportion of ancestry shared among regions and the ancestry of individuals. Relationships among collections were examined with pairwise tests: collections were tested for heterogeneity in genotypic distributions at each locus and across all loci using GENEPOP with 300 batches and 3000 iterations. Pairwise F_{ST} values and their significance were evaluated using FSTAT with 100,000 permutations. FSTAT was also employed to test for differences between zones in allelic richness, F_{IS} and F_{ST}. Test results were adjusted for multiple comparisons using Bonferroni corrections.

Genetic relationships among collections were further explored with a cluster analysis and presented in a dendrogram. Population allele frequencies were generated from genotypic data using CONVERT 1.3 (Glaubitz 2004). Pairwise chord distances (Cavalli-Sforza and Edwards, 1967) among collections were calculated from allele frequencies using GENDIST in PHYLIP 3.5 c (Felsenstein 1993). We employ chord distance since the distance is based upon drift rather than a mutation model. Genetic drift is often more important in small and isolated population and microsatellite DNA often follows complex mutational patterns. Chord distances were used to construct a neighbor-joining tree using PHYLIP. To test the repeatability of NJ tree branching, 10,000 bootstrap replicates of the allele frequency file were generated using SEQBOOT. Tree topologies were created for replicates using NEIGHBOR, a consensus tree was generated using CONSENSE and plotted using TREEVIEW 1.6.6 (Page 2001).

Results

Several collections deviated from Hardy Weinberg equilibrium (HWE) for homozygote excess when calculated over all loci (Table 1). In individual tests, after Bonferroni corrections (adjusted *P* value 0.00021 for 238 tests) five collections, excluding CowlitzC, were out of HWE at the *Ots*-G311 locus (Table 3). Most collections were out of HWE at *Omm*-1137 (Table 3), which was removed from subsequent analyses when MICRO-CHECKER

Table 3. Loci information for collections

Locus	GraysC	Grays01	Skam	Germ	CowC	CowF	CowS	Lewis	Wash	Van	Mult	Ham	Ives	Hardy	BDam
Oke-3	0.041	0.003	−0.121	0.000	0.133	−0.173	0.164	0.030	−0.220	−0.032	0.020	0.005	0.183	−0.036	0.028
Omy-10	0.060	0.018	−0.038	0.012	0.016	−0.104	0.071	0.029	−0.074	0.009	−0.152	0.064	0.029	0.042	−0.010
Ots-G311	**0.106**	0.063	0.037	−0.002	0.157	0.010	0.295	−0.015	0.121	0.045	0.114	**0.136**	**0.244**	**0.113**	0.094
One-102	0.024	−0.008	−0.118	0.004	−0.037	−0.057	−0.013	0.046	−0.062	0.003	0.028	0.035	0.029	0.024	0.056
One-114	0.017	−0.028	0.007	−0.051	0.042	−0.007	0.073	0.035	−0.066	−0.043	−0.026	0.026	**0.099**	0.064	−0.026
Ots-3M	−0.018	0.005	−0.036	0.019	0.041	−0.125	0.081	−0.078	−0.039	−0.031	−0.005	−0.024	0.112	0.036	0.081
Omm-1137	**0.323**	**0.405**	**0.285**	**0.292**	**0.363**	0.290	**0.420**	**0.382**	**0.420**	**0.223**	**0.237**	**0.296**	**0.388**	**0.286**	**0.476**
One-101	0.086	0.000	−0.041	0.119	0.012	−0.056	0.026	0.055	0.114	0.003	**0.152**	**0.105**	**0.182**	0.096	0.035
Ots-1	0.091	0.049	0.034	0.146	0.099	0.025	0.139	0.154	0.073	0.004	0.031	0.120	0.095	0.006	0.124
One-106	0.007	−0.014	0.027	−0.046	0.061	0.009	0.096	0.097	0.085	−0.011	0.106	0.000	**0.216**	0.018	0.079
One-18	0.005	−0.002	−0.023	0.139	0.026	0.082	−0.006	0.022	0.054	0.121	−0.054	−0.005	0.034	−0.010	0.155
Ssa-419	−0.039	0.027	−0.006	0.033	0.054	−0.066	0.105	0.215	−0.029	−0.012	0.041	−0.016	−0.010	0.001	0.066
Oki-1	0.006	−0.012	0.018	0.019	0.075	0.233	−0.046	0.011	0.155	0.005	−0.028	0.002	0.020	−0.043	0.190
One-111	0.032	0.017	−0.018	0.069	0.073	−0.001	0.102	−0.007	0.061	0.012	0.025	0.019	0.012	−0.004	−0.022
Ots-2M	−0.028	0.042	0.052	0.233	−0.103	−0.289	−0.073	−0.121	−0.252	0.173	0.106	−0.003	0.023	−0.027	0.198
One-108	0.019	−0.02	0.027	0.019	0.030	0.042	0.019	0.041	0.023	0.040	0.014	0.039	0.058	0.045	0.013
Ots-103	0.035	−0.018	0.034	0.025	0.022	0.012	0.013	−0.046	−0.021	0.038	−0.011	0.026	0.036	−0.032	−0.050
Overall F_{IS}	0.031	0.007	−0.008	0.037	0.047	−0.017	0.069	0.036	0.010	0.015	0.022	0.037	0.088	0.023	0.056
P-value	< **0.0001**	0.2478	0.7092	0.0154	**0.0003**	0.7957	< **0.0001**	**0.0028**	0.291	0.0737	0.0537	< **0.0001**	< **0.0001**	0.0098	**0.0001**

Under each collection is the F_{IS} value at each locus (values significant after correction in bold) and the *P*-value for the F_{IS} value over all loci for each collection (calculated without *Omm-1137*). The following abbreviations were employed: GraysC = Grays River combined years; Skam = Skamokawa Creek; Germ = Germany Creek; Cow = Cowlitz River, C = combined (combined values italicized), F = fall, S = summer; Wash = Washougal River; Van = Vancouver; Mult = Multnomah Creek; Ham = Hamilton Creek; Ives = Ives Island; BDam = Bonneville Dam.

indicated a null allele. Deviations from HWE were detected in 28 other tests (Table 3, CowlitzC excluded) and five remained significant after corrections. When tested over all collections, 7/17 loci deviated from HWE (adjusted P value 0.0029 for 17 tests, Table 2). Since most deviations at these loci were insignificant within collections, the loci remained in the analysis. When calculated over all loci, several collections had significant, positive F_{IS} values (bottom row, Table 3), suggesting recent admixture or differences between collection years in multi-year collections. Cowlitz summer and fall runs had different F_{IS} values at each locus and overall (Table 3).

In genotypic disequilibrium tests over all collections, 27/136 tests (adjusted alpha = 0.05/136 = 0.00037) indicated associations between loci. Since different locus pairs were linked in different collections and the same loci transmit independently in other chum populations (Small *et al*. unpublished data), disequilibria likely indicated some inbreeding or a Wahlund effect rather than physical linkage. Strong disequilibria in one collection will generate a positive linkage signal when tested over all collections (Small *et al*. unpublished data). In individual tests, Cowlitz summer had the most linkage (Table 1), suggesting inbreeding from a small population or a Wahlund effect if the October 15 division resulted in summer and fall chum combined in the collection. CowlitzC had 20 linked loci (Table 1), five pairs of which were also linked in the Cowlitz summer collection. The high linkage in CowlitzC was likely a Wahlund effect from combining summer and fall run chum salmon in a single collection.

Numbers of alleles per locus ranged from 5 to 64 and average heterozygosity per locus was 0.785 (Table 1). Collections differed greatly in allelic richness and numbers of private alleles (Table 1). Cowlitz summer had the highest allelic richness and two to five times as many private alleles as any other collection (Table 1). Cascade zone collections also had significantly higher richness ($P < 0.001$) and twice as many private alleles than Coastal or Gorge zone collections (Table 1).

M values (0.174 – 0.225) indicated that all populations had been through bottlenecks (Table 1). Garza and Williamson (2001) suggest that M values < 0.68 imply that the population has experienced a recent bottleneck.

In the AMOVA test, 99% of the variance was within collections, as expected with highly polymorphic microsatellites. No variance was partitioned among collections within zones (–0.3%, NS). Significant variance (1.25%, $F_{ST} = 0.013$, $P < 0.001$) was partitioned among zones, with significantly higher genetic structure within the Cascade zone (Coast $F_{ST} = 0.004$, Cascade $F_{ST} = 0.033$, Gorge $F_{ST} = 0.003$,$P = 0.002$ for comparison among zones, from FSTAT).

The WHICHRUN program uses a jackknife procedure where each fish in turn was removed from the dataset, allele frequencies of all collections were calculated and the fish assigned to the most likely collection based on its genotype. Fish may be incorrectly assigned to small collections since in small collections their allele frequencies are overrepresented – microsatellites are highly polymorphic and large sample sizes are required to adequately represent a population's allelic diversity. Thus, fish from most other collections may have been incorrectly assigned to Germany Creek, Cowlitz fall and Washougal River collections (Table 4). Although Cowlitz summer was also a small collection, few fish from other collections were assigned to it (Table 4), underscoring its genetic distinction. Within individual collections, assignments to collection of origin are termed correct assignments, others are termed misassignments although fish may have originated elsewhere. Most misassignments were to another collection in the same zone (Table 4). Lewis River had the lowest correct assignments in the Cascade group (Table 4). Since the Lewis River collection included small samples from the east and north forks collected over four years, the population may be characterized poorly by the collection. However, misassignments may indicate that Cowlitz fish stray up to the Lewis River and fish from the Gorge zone stray en route to the Gorge region. Misassigned Cowlitz summers were mostly assigned as Cowlitz fall, but misassigned Cowlitz falls were assigned to other fall collections (Table 6): Cowlitz summer was most closely related to Cowlitz falls but Cowlitz fall was genetically more similar to other fall-run chum. When the Cowlitz River fall and summer chum were grouped (CowC in Table 6), correct assignments decreased and incorrect assignments to Germany Creek increased (Table 6). Cowlitz River chum salmon were

characterized better genetically when divided by run timing.

With a large number of collections and much disequilibrium, STRUCTURE (Pritchard *et al.* 2000) was mainly useful for analyzing membership and ancestry in collections at the zone level (Table 5). Since previous analyses indicated three genetic groups, we present data for $K = 3$. However, we tested the data with $K = 2$ to $K = 14$ to explore the possibility of less or more than three genetically distinct groups (results not shown). With K higher than 3, most collections split between two or more groups except Cowlitz summer, which retained high membership in a single cluster shared with other Cascade collections with K up to 10. With $K = 4$, a Cowlitz summer-dominated cluster emerged (91% membership) and Cowlitz fall and Lewis collections had 74% and 62% membership in the cluster, respectively. With $K = 3$, most individuals and collections had at least 68% inferred ancestry in their zone cluster (individual data not shown). However, Germany Creek and Washougal River had 14/33 and 8/35 individuals respectively with at least 75% ancestry in the Cascade cluster and Lewis River had 6/63 individuals with more than 70% ancestry in the Gorge cluster and two individuals with more than 70% ancestry in the Coastal cluster. Since STRUCTURE attempts to minimize linkage disequilibrium when partitioning individuals among clusters, the high disequilibrium in the Cowlitz collection caused the program to simply divide individuals evenly among clusters when analyzing only the Cowlitz River collections.

In pairwise genotypic tests, zone groupings were further supported by high homogeneity within regional clusters (Table 6, upper and lower matrix, single pairwise and overall loci tests corrected for 90 simultaneous tests; adjusted alpha, 0.05/90 = 0.00055). In several instances in single pairwise tests, although no single test was significant after Bonferroni corrections, the test was significant over all loci (Table 6, e.g. comparison between Skamania and 01Grays). In these cases,

Table 4. Summary of assignment test results. Individual fish were given the most likely assignment to a collection based upon the genotype of the fish and the allele frequencies in collections

	GrayC	Grays01	Skam	Germ	CowC	CowF	CowS	Lewis	Van	Wash	Mult	Ham	Ives	Hardy	BDam	Total
Coastal																
GraysC	**83**	34	60	24		7	1	1	1	7	6	2	0	0	5	231
Grays01	24	**38**	22	6		2	1	0	1	4	0	0	1	0	1	100
Skamokawa	14	8	**17**	6		3	0	1	0	3	0	0	1	0	0	53
Germany	2	2	4	**10**		9	1	1	0	2	0	1	0	0	1	33
Cascade																
CowC	1	0	2	17	**21**			11	0	11	0	0	0	0	1	64
Cascade																
CowF	1	0	0	4		**13**	1	4	0	4	0	0	0	0	0	27
CowS	0	0	1	3		11	**17**	2	0	2	1	0	0	0	0	37
Lewis	0	0	1	11		20	2	**10**	2	7	2	1	2	2	2	62
Gorge																
Vancouver	1	1	0	4		4	0	0	**20**	19	14	4	5	4	12	88
Washougal	0	0	2	2		5	0	1	6	**5**	5	0	3	2	4	35
Multnomah	0	1	3	3		5	0	1	7	12	**29**	8	8	10	10	97
Hamilton	2	0	8	6		20	1	1	15	24	15	**38**	18	21	21	190
Ives	0	0	1	3		4	0	5	7	18	20	17	**39**	16	16	146
Hardy	2	1	2	4		6	0	0	8	15	19	17	13	**30**	17	134
Bdam	0	0	2	0		4	0	2	4	6	8	3	1	7	**9**	46
Sample size	231	100	53	33	64	27	37	62	88	35	97	190	146	134	46	
correct	83	38	17	10	21	13	17	10	20	5	29	38	39	30	9	
% correct	35.93	38.00	32.08	30.30	32.81	48.15	45.95	16.13	22.73	14.29	29.90	20.00	26.71	22.39	19.57	

Assignments back to collection of origin are in bold type, assignments to other collections are in normal type with total number of fish at end of the row (Total). Assignment test was conducted with Cowlitz River collections separated (all results shown) and with Cowlitz River collections combined (only results for CowC shown, in italics). Abbreviations follow Table 3.

Table 5. Bayesian ancestry analyses at population (Pop) and zone (Grouped) levels for chum salmon from Lower Columbia River

Pop	1	2	3	N
Coastal				
GraysC	**0.816**	0.122	0.062	231
Grays01	**0.851**	0.112	0.036	100
Skamokawa	**0.832**	0.11	0.058	53
Germany	0.32	**0.555**	0.126	33
Cascade				
CowF	0.08	**0.867**	0.052	27
CowS	0.022	**0.956**	0.022	37
Lewis	0.096	**0.725**	0.178	62
Gorge				
Vancouver	0.098	0.248	**0.654**	88
Washougal	0.101	0.362	**0.537**	35
Multnomah	0.105	0.188	**0.706**	97
Hamilton	0.102	0.244	**0.654**	190
Ives	0.079	0.235	**0.686**	147
Hardy	0.093	0.19	**0.717**	134
BDam	0.078	0.197	**0.725**	46
			Ln =	−82537
Grouped				
Coastal	**0.785**	0.155	0.061	417
var	0.074	0.057	0.013	
Cascade	0.07	**0.824**	0.106	126
var	0.021	0.067	0.045	
Gorge	0.094	0.222	**0.684**	737
var	0.020	0.071	0.093	
			Ln =	−82565

Numbers in the row indicate the portion of the collection assigned to each cluster, with highest values in bold type. The Ln data value is the probability of the number of clusters. Name abbreviations follow Table 3.

there were at least four loci with significant differences in distributions among populations before corrections (P value between 0.00055 and 0.05). Tests involving Grays01 and other Coastal collections were heterogeneous (Table 6, upper and lower matrices). The Grays River hatchery program was implemented in 1998 and in 2001, three-year-old fish returned in large numbers (Steve Schroder, WDFW, personal communication), altering the genetic profile. Comparisons involving Germany Creek were similarly heterogeneous in tests with Coastal and Cascade fall collections (Table 6). Cowlitz summer was significantly different from all other collections, but shared the most genotypic distributions with Cowlitz fall. Genotypic overlap between Cascade collections and Washougal River and Germany Creek suggest some gene flow among adjacent tributaries or may be artifacts caused by small collection sizes. Pairwise F_{ST} values (Table 6) were mostly concordant with genotypic tests except for Germany Creek and Lewis River, which were undifferentiated from some Gorge collections.

The NJ tree indicated strong grouping by zone (Figure 2). The tree shows three major clusters with high bootstrap values for Coast, Cascade and Gorge branches. Branch lengths in the Cascade cluster indicated greater genetic distances among Cascade collections. Although Germany Creek joined the Coast branch some distance from the Grays River and Skamokawa Creek collections, strong bootstrap support indicated definitive membership in the Coastal group. A tree with Cowlitz River run groups combined yielded bootstrap values of 88% for Coast, 97% for Cascade and 100% for Gorge branches (not shown).

Discussion

Origin of the Cascade zone chum salmon

In the Cascade zone, consistent low returns have been observed in the Cowlitz and Lewis rivers (Keller 2001; Myers *et al.* 2002; NOAA 2004). These tributaries formerly supported large chum salmon populations that likely contained important components of genetic diversity within the lower Columbia River. If chum salmon in the Cascade tributaries are remnants of former populations, this genetic structure should be preserved and fostered in rehabilitation efforts. If the tributaries were recolonized by strays from the Coastal and Gorge regions, supplementation for the Cascade region could utilize the larger populations at the Coast and in the Gorge. However, our data suggested that the Cascade group is genetically distinct. The distinction could arise from extreme genetic drift following recolonization, or may be a signal from remnant original structure. We discuss two hypotheses: that tributaries were recolonized within the past 50 years by strays from the Coast and Gorge zones, or that collections represent original populations.

As Gorge region fish pass Cascade tributaries en route to natal spawning grounds, they would be likely candidates for exploring underutilized spawning areas. We saw a few Cascade fish with

Table 6. Table of pairwise comparisons among lower Columbia River chum salmon collections

	GraysC	Grays01	Skam	Germ	CowF	CowS	Lewis	Wash	Van	Mult	Ham	Ives	Hardy	BDam
Coastal														
GraysC		2	0	2*	7*	13*	12*	8*	12*	10*	13*	14*	14*	12*
Grays01	**0.0026**		0*	1*	7*	10*	12*	7*	10*	12*	13*	12*	14*	11*
Skam	0.0021	0.0037		0	3*	9*	5*	3*	8*	6*	11*	11*	10*	3*
Germ	**0.0074**	**0.0096**	**0.0079**		0	2*	0*	1	2*	4*	3*	2*	4*	0*
Cascade														
CowF	**0.0223**	**0.0279**	**0.0266**	0.0086		1*	0	0*	4*	3*	3*	2*	4*	2*
CowS	**0.0283**	**0.0300**	**0.0284**	**0.0215**	**0.0284**		2*	4*	9*	7*	9*	10*	10*	9*
Lewis	**0.0141**	**0.0175**	**0.0156**	**0.0046**	0.0033	**0.0172**		0*	4*	4*	7*	4*	8*	1*
Gorge														
Wash	**0.0127**	**0.0130**	**0.0154**	0.0094	0.0147	**0.0269**	0.0077		0	0	0	0	1*	0
Van	**0.0158**	**0.0150**	**0.0158**	**0.0133**	**0.0252**	**0.0286**	**0.0139**	0.0030		1	0*	3*	1*	1
Mult	**0.0151**	**0.0152**	**0.0169**	0.0115	**0.0273**	**0.0303**	0.0144	0.0013	0.0031		1*	2*	1*	0
Ham	**0.0136**	**0.0142**	**0.0149**	**0.0123**	**0.0195**	**0.0261**	**0.0123**	0.0025	0.0026	0.0028		0*	0*	0
Ives	**0.0149**	**0.0151**	**0.0161**	0.0127	**0.0269**	**0.0312**	0.0149	0.0046	0.0046	0.0013	0.0018		1*	0
Hardy	**0.0146**	**0.0151**	**0.0144**	**0.0136**	**0.0254**	**0.0292**	**0.0132**	0.0038	0.0031	0.0021	0.0014	**0.0028**		0
BDam	**0.0139**	**0.0143**	**0.0143**	**0.0107**	**0.0188**	**0.0261**	**0.0105**	−0.0006	0.0018	0.0001	−0.0002	0.0018	0.001	

Lower matrix shows pairwise F_{ST} values, with values significant after Bonferroni corrections in bold type. Upper matrix shows the number of loci with significant different genotypic distributions after corrections (adjusted alpha 0.05/90 = 0.00055). Asterisks indicate significant comparisons when summed over all loci after Bonferroni corrections.

high ancestry to Coast and Gorge zones, so it is possible that these were strays or alternatively their genotypes may reflect shared common ancestry. If populations had been recolonized within the past 50 years, a strong bottleneck signal might persist with low values for diversity, allelic richness, and heterozygosity and no private alleles (Kinnison *et al*. 2002; Ramstad *et al*. 2004). However, if both zones contributed founders, diversity and heterozygosity might be enhanced. Yet, since zones differ ecologically, strays from other zones may have suffered low colonizing success (Spidle 2001) and private alleles would be absent. Given the genetic drift associated with founder effects (Ramstad *et al*. 2004) in both of these cases we would also expect no association among Cascade collections. However, we saw strong genetic association among Cascade collections and little genotypic overlap and low individual ancestry to other zones. Cascade collections also had higher heterozygosity, allelic diversity, and private alleles than collections in other zones, decreasing the likelihood that they were recently recolonized. Further, colonists from extant groups were unlikely to have founded the genetically unique Cowlitz River population with summer run timing. In salmonids, run timing is genetically controlled (Hansen and Johnson 1991; Smoker *et al*. 1998; Quinn *et al*. 2000). Run timings naturally diverged by a month over 100 years in Chinook introduced in New Zealand (Quinn *et al*. 2000). However, Coastal and Gorge populations

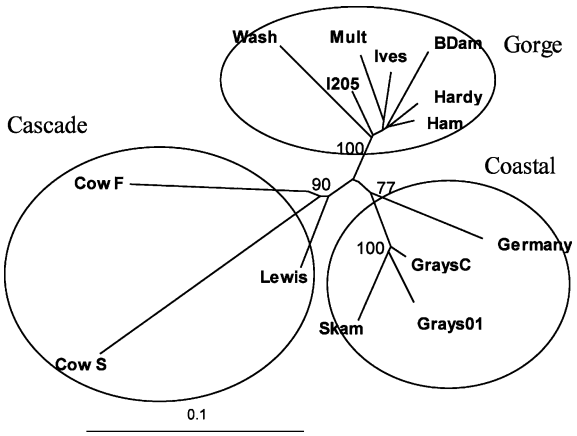

Figure 2. Consensus neighbor-joining tree of Cavalli-Sforza and Edwards distances among lower Columbia chum salmon collections. Numbers at the nodes indicate the percentage of 10,000 trees in which collections grouped together. Zone branches are circled and labeled. The following collection name abbreviations were employed: GraysC = Grays River combined, Grays01 = Grays River 2001; Skam = Skamokawa Creek; CowF = Cowlitz River fall; CowS = Cowlitz River summer; Ives = Ives Island; BDam = Bonneville Dam; Wash = Washougal River; Mult = Multnomah Creek; Ham = Hamilton Creek.

return starting in October and lack early returning individuals that could have founded a run starting in July, and a three-month run divergence is unlikely within the past 50 years.

Another possibility is that native Cascade populations persisted unobserved, at low numbers for several generations. Native populations of Atlantic salmon, thought to be extinct have been discovered persisting in low abundance (Nielsen *et al*. 1997, 1999, 2001). Although Cascade chum salmon populations were considered extinct, spawners were found occasionally when biologists surveyed for other salmonid species (Johnson *et al*. 1997) and low numbers consistently appeared at the Cowlitz salmon hatchery (Dan Rawdings, WDFW, unpublished data). Since biologists were not surveying potential chum salmon spawning areas and chum spawners are difficult to detect due to deep water in mainstems and high rainfall and turbidity during spawning times, we suspect that chum salmon abundance was underestimated in the Cascade region. Run timing diversity within the Cowlitz River suggests that the large drainage contains different environments, which fostered ecological and genetic diversity. Since chum salmon have overlapping year classes and a propensity to stray (Johnson *et al*. 1997), low population numbers and diversity would be increased by mating among brood years (Waples 1990) and reinforced by movement within the drainage and possibly strays from other regions. Although competitive exclusion may have limited the success of strays adapted to other zones (Heard 1991; Spidle *et al*. 2001), strays may have contributed to the higher allelic richness in Cascade collections. If numbers remained low after initial population declines, a bottleneck signal would be evident: genetic drift would be pronounced, collections would display inbreeding but a regional association might persist. Most collections show signs of continued small effective population size with excess homozygosity and low M values. In comparison to less perturbed collections of South Puget Sound, WA summer chum salmon, gene diversity is lower in Columbia River chum salmon (Small *et al*., unpublished data). Results suggest high genetic drift, since Cascade collections were more distant from each other than were Coastal or Gorge collections, but original populations may have been more distant genetically. We saw a within zone association in allelic richness, genetic ancestry, and in the NJ tree, but genotypic comparisons to collections from other zones were confounded by small sample sizes. However, given the private alleles, life history divergence within the Cowlitz River and within-zone association, we suspect that Cascade tributaries contain remnants of original populations. We offer that ecological differences between zones fostered and maintained regional (zonal) genetic divergence.

Broad-scale population structure

The Columbia River is a large drainage traversing diverse ecological zones. The Coastal, Cascade and Gorge zones have distinctive hydrological and ecological qualities that would promote adaptive variation among fish utilizing the tributaries (Taylor 1991) and serve as strong cues supporting regional fidelity. Water temperature and availability, velocity, turbidity as well as geology and topography establish zone and tributary character. In this study, chum salmon spawning in the distinct freshwater habitats within the three ecological zones were genetically similar and differentiated from chum salmon spawning in different zones, suggesting reproductive isolation among zones. Associations within ecological zones would develop as chum salmon moved among adjacent tributaries where adaptive variation fostered their survival. Chum salmon have been described as more flexible in their homing than other salmonids since they spawn in lower reaches of rivers and in the main stem if tributaries lack adequate water (Johnson *et al*. 1997). We hypothesize that genetic associations within zones in the Columbia River developed from shared common ancestry within zones and genetic differences among zones resulted from ecological differences. Similar genetic distinction within a smaller drainage suffering a history of near extirpation was found among Atlantic salmon in tributaries of the Penobscot River (Spidle *et al*. 2001) and was attributed to ecological diversity in the drainage.

Germany Creek had an ambiguous genetic profile. It is possible that the sample size was too small to adequately characterize the population genetically. However, since the mouth of the Cowlitz River is only 20 km distant, Cowlitz River fall chum salmon (or other upriver fish passing by) could stray into Germany Creek, linking the gene pools and contributing to Germany Creek's

intermediate genetic status. The higher allelic richness in Germany Creek in comparison to other Coast collections, genotypic overlap and ancestry shared with other zones would also suggest that strays contributed to diversity in the population. The Washougal River collection was also small, but the sample unambiguously joined Gorge collections in the NJ tree. While geographically located within the Cascade region, the Washougal drainage is a small, lower-elevation watershed on south-facing mountains, which retain no snow pack. Similar to Gorge tributaries, flow is derived from rainfall and groundwater and the river mouth is close to Gorge tributaries. Since the drainage is ecologically more similar to the Gorge zone and Washougal River is a member of the Gorge genetic group, it should be included in the Gorge stratum (combined life history and ecological zone group described in McElhany *et al.* 2003).

Chum salmon were recently discovered spawning in main stem seeps near the I-205 bridge at Vancouver, WA (Myers *et al.* 2002). We examined their relationship to other spawner groups to assess whether they represent recent opportunistic colonization or show evidence of longer establishment. The lack of differentiation from Washougal River and Multnomah creek, the closest tributaries, suggest colonization from these groups. Allelic richness and heterozygosity were higher than other Gorge collections, suggesting input from diverse populations. However, Vancouver genotypes were in Hardy-Weinberg equilibrium, so enough time has elapsed, if colonization was recent, for the population to stabilize.

With exception of the Cowlitz River, chum salmon in the lower Columbia River have fall run timings. The Cowlitz River, the largest drainage in the lower Columbia River in WA, appears to support both fall and summer runs of chum salmon. Chum salmon arrive at the Cowlitz Hatchery starting in July (Dan Rawding, WDFW, unpublished data). Although Cowlitz summer chum salmon were members of the Cascade zone genetic group, they were genetically unique, which suggested temporal isolation from Cowlitz fall chum salmon. Summer and fall runs of chum salmon also occur in the same rivers in Hood Canal and Puget Sound tributaries, (Johnson *et al.* 1997). While Hood Canal summer chum salmon form an ESU separate from Hood Canal fall chum salmon, Puget Sound summer and fall chum salmon form an ESU together (Phelps *et al.* 1994; Johnson *et al.* 1997). Similar to Puget Sound chum salmon, Cowlitz summer chum salmon are most closely related to Cowlitz fall chum salmon. Ecotypic divergence within this large watershed may have been fostered by early availability of water as well as spatial and habitat diversity. This summer run represents an important surviving component of genetic diversity in chum salmon within the Cascade zone and within the lower Columbia River.

Conservation and restoration implications

Our data suggest that remnants of native populations have survived at low numbers in Cascade tributaries, presenting the opportunity for native genetic diversity to be protected in restoration efforts. Although Cascade populations appear to receive some strays, genetic diversity is partitioned among ecological zones, indicating regional adaptation. Thus, hatchery supplementation would be more effective if implemented using broodstock from within the same ecological zone (Nielsen *et al.* 1999, 2001). However, populations remained depressed despite a moratorium on fishing for over 50 years, indicating that spawning and rearing habitat must also be restored to re-establish stable populations. Chum salmon spawn in the lower reaches of tributaries where logging, agriculture and development is concentrated (Johnson *et al.* 1997), and juveniles rear in the Columbia River estuary, 40% of which has been altered for industrial purposes (Sherwood *et al.* 1990).

Acknowledgements

Cherrill Bowman, WDFW, provided technical support. Samples were collected by staff from Washington Department of Fish and Wildlife, Oregon Department of Fish and Wildlife, US Fish and Wildlife Service and Pacific Salmon Marine Fisheries Commission. Work was supported by Washington State General Funds and Bonneville Power Administration. Ann Blakley, Craig Busack, Dan Harmon, Denise Hawkins, Todd Hillson, Joe Hymer, Anne Marshall, Dan Rawding,

Steve Schroder, Jim Shak, Jack Tipping, Ken Warheit (WDFW), two anonymous reviewers and editor Daniel Ruzzante provided helpful comments on the project and manuscript.

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