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[Article]

Genetic Variation and Structure of Chinook Salmon Life History Types in the Snake River

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Abstract.—We evaluated 25 inland populations of Chinook salmon *Oncorhynchus tshawytscha* in the Snake River with 13 microsatellite loci to test for contemporary genetic differentiation at three scales: between life history types, among regions within life history types, and among populations within regions. The genetic distance and diversity of natural Chinook salmon populations were also contrasted with those of Chinook salmon from several hatcheries. The results provide strong evidence for reproductive isolation among ocean- and stream-type life histories (F_{ST} range, 0.080–0.120). Regional structuring of stream-type Chinook salmon within subbasins was also significant, as all populations were differentiated (F_{ST} range, 0.017–0.045), but populations generally clustered together by region in a neighbor-joining dendrogram. This evidence suggests high levels of philopatry to natal areas in stream-type Chinook salmon, but ocean-type collections were not significantly different from one another (F_{ST} range, 0.001–0.002). Higher levels of genetic diversity in ocean-type (306 total alleles; allelic richness, 16.5) than in stream-type collections (206 total alleles; allelic richness, 12.2) may also reflect variable levels of gene flow within each life history type and colonization history. The genetic similarity of populations within regions suggests gene flow not only from transplanted stocks but also from natural dispersal that provides metapopulation structure. None of the 25 populations in this study offered significant evidence for a genetic bottleneck (M ratio < 0.68) despite apparent demographic bottlenecks in several populations throughout the Snake River drainage in the last century. The combination of dispersal through metapopulation dynamics and transfers of hatchery stocks may be responsible for reducing the genetic bottleneck signal.

Chinook salmon *Oncorhynchus tshawytscha* are a diverse species of Pacific salmon with at least two distinct life history types, ocean and stream (Healey 1991). Ocean-type adult Chinook salmon begin their freshwater migration in early fall, spawning in warmer water near tidewaters or main-stem sections of large rivers. Conversely, stream-type adult Chinook salmon begin migrating in spring, using colder headwater tributaries for spawning. Further, due to early emergence and rapid juvenile growth, ocean-type juveniles migrate to estuaries within 3 months, while stream-type juveniles postpone migration to the sea for 1 year or more. However, each of these life history types includes a wide array of variation throughout their life cycles (e.g., reservoir-type Chinook salmon; Connor et al. 2005). The geographic distribution of ocean-type Chinook salmon is predominantly below 56°N, whereas stream-type salmon are found more commonly north of this latitude (Healey 1991; Brannon et al. 2004). Both of these life history types occur widely throughout the Columbia River basin in the Pacific Northwest of the United States and have overlapping distributions.

Historically, populations of Chinook salmon have been structured through philopatry to natal streams (Quinn and Dittman 1990), geological processes (i.e., the Wisconsin Glaciation; Teel et al. 2000), metapopulation dynamics (Cooper and Mangel 1999), and life history characteristics (Waples et al. 2004). Previous studies have shown that temporal variation within populations is small relative to geographic variation (Utter et al. 1989; Beacham et al. 2003), and populations within drainages tend to be more genetically similar than those in other major watersheds (e.g., Waples et al. 2004). Exceptions to this pattern of geographic structure include strong differentiation of sympatric ocean- and stream-type life histories in the Columbia River (Narum et al. 2004; Waples et al. 2004).

In the last 150 years, populations of Chinook salmon in the interior Columbia River basin have been greatly altered by anthropogenic influences. The impacts from human activities such as habitat destruction and

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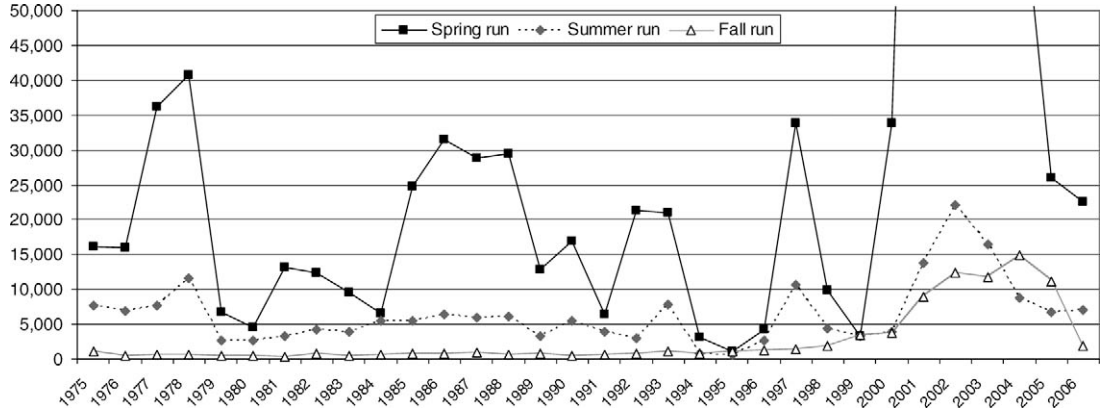

FIGURE 1.—Escapement of Chinook salmon runs over Lower Granite Dam, Idaho, from 1975 to 2006. Data were compiled from www.cbr.washington.edu/dart/adult.html. Escapement of spring-run Chinook salmon in 2001, 2002, 2003, and 2004 was 171,958, 75,025, 70,609, and 70,742, respectively.

overharvest have led to large declines of Chinook salmon (McConnaha et al. 2005) and barriers to the anadromous life history and subsequent mitigation actions may have strongly influenced genetic structure. Hydropower dams built without accommodation for fish passage led to extirpation of Chinook salmon populations in the upper reaches and localized subbasins of the Columbia and Snake rivers (Whitney et al. 2005). Efforts to mitigate for these losses included artificial propagation and outplanting of hatchery-reared Chinook salmon. Planted hatchery stocks have been shown to interbreed with recipient populations and influence their genetic structure and diversity (e.g., Ford et al. 2004).

In the Snake River, a major tributary to the Columbia River, stocks of Chinook salmon have been decimated and are unstable (Figure 1) owing to four primary factors described as the "four *H*s": habitat, hydropower, hatcheries, and harvest. There are two evolutionarily significant units (ESUs) of Chinook salmon in the Snake River, spring–summer run (stream-type) and fall run (ocean-type), and both are listed as threatened under the Endangered Species Act (ESA; Myers et al. 1998). These life history types of Chinook salmon have overlapping spawning times in the Snake River, but peak spawning time differs considerably (peak for stream-type is summer; peak for ocean-type is fall). In addition to these extant populations, the historical distribution of Chinook salmon included 391 km of the Snake River that is now unavailable due to construction of hydropower dams in Hells Canyon in the 1960s (Whitney et al. 2005). Extirpated populations include the largest historical stock of fall run Chinook salmon in the Marsing region of Idaho (Connor et al. 2005), along with several populations of spring–summer run Chinook salmon. Lewiston Dam was another upstream barrier built in the Clearwater River in 1927 that probably extirpated Chinook salmon in the drainage before removal of the dam in 1973. Relative to other Chinook salmon in the Snake River basin, populations in the Salmon River are generally regarded as the least impacted by the four *H*s (Liss et al. 2005). However, native salmon in the Salmon River drainage have experienced extreme fluctuations in abundance as annual redd counts in some regions suggest demographic bottlenecks (Isaak and Thurow 2006).

To mitigate losses from the hydroelectric system and to boost threatened populations, supplementation of natural populations with hatchery-reared Chinook salmon has been and is currently occurring in the Snake River. In the Clearwater River, stream-type Chinook salmon in the entire drainage have been reestablished with stocks from both within the ESU (>50 million Rapid River Hatchery outplants since 1968) and outside of the ESU (>9 million Carson Hatchery outplants since 1968; Keifer et al. 1992; LSRCP 1998; Myers et al. 1998). Ocean-type Chinook salmon have been reintroduced into the Clearwater River with Lyons Ferry Hatchery stock, with more than 28 million outplanted fish since 1945 (Myers et al. 1998). Natural production of stream-type Chinook salmon in many Snake River drainages has been supplemented by both native and nonnative stocks and a summary of stocking history by drainage follows (Myers et al. 1998). The main-stem Salmon River has received outplants of Rapid River Hatchery and Sawtooth Hatchery stocks (>72 million outplants since 1970), and the upper Salmon River has been planted with Sawtooth Hatchery stock (>15 million since

1974). The South Fork Salmon River has been supplemented, but primarily with fish from local broodstock reared at McCall Hatchery (>12 million since 1976). The Imnaha River has received Rapid River Hatchery outplants (>4 million since 1984). The Grande Ronde River has had a combination of outplants from two stocks, Rapid River Hatchery (>8 million since 1982) and Carson Hatchery (>7 million since 1982). The Tucannon River has received outplants largely from Lyons Ferry Hatchery (>1.5 million since 1962). In general, most of the major drainages in the Snake River, with the exception of the Middle Fork Salmon River, have been planted with hatchery reared fish to improve population stability, increase harvest opportunities, and mitigate for losses due to anthropogenic causes.

We evaluated Snake River populations of ocean- and stream-type Chinook salmon with 13 microsatellite loci to determine the contemporary genetic variation within this species in this heavily impacted river basin. Genetic differentiation was tested at three scales: among life history types, among populations, and temporal variation within populations. Genetic distance and diversity of natural Chinook salmon populations were also contrasted with those of hatchery-reared Chinook salmon. Further, we tested for genetic bottlenecks in all populations to determine if genetic diversity of Snake River Chinook salmon has been reduced due to highly variable escapement. We also evaluated genetic diversity to test for evidence of unique remnant populations of native Chinook salmon in the Clearwater River. Genetic data can often facilitate management decisions (Ryman and Utter 1987) such as determining population units, evaluating impacts from introduced stocks, and estimating reproductive contribution of individuals or stocks.

Methods

Sampling and genetic data collection.—A total of 2,959 tissue samples were taken from ocean- and stream-type Chinook salmon in the Snake River basin (Figure 2) to represent 25 natural and hatchery collections (Table A.1 in the appendix). The term "natural" is used hereafter to indicate collections of fish that were reared in the wild (from parents of unknown origin) with intact adipose fins. Three collections contained a mix of natural and hatchery supplementation samples including Catherine Creek (<5% hatchery), Sawtooth Hatchery (50% hatchery), and Pahsimeroi River (50% hatchery). Samples were collected over multiple years for all but seven locations (Pahsimeroi River, Dworshak Hatchery, Red River, Powell Hatchery, South Fork Clearwater River, Tucannon River, and West Fork Yankee Fork). Temporal samples within populations were not significantly different with exact tests and were pooled for statistical analyses with the exception of Big Creek collections. Consequently, two collections for Big Creek (a for 2001, b for 2002 and 2003) were included in all analyses. Fin or opercle tissue was collected from juveniles and adults and stored in nondenatured ethanol (Table A.1).

We extracted DNA from all samples using Qiagen DNeasy protocols in conjunction with a Qiagen 3000 BioRobot. The DNA was then arrayed in 96-well plates for high-throughput genotyping. Template DNA was amplified by means of the polymerase chain reaction (PCR) at nine tetranucleotide microsatellite loci, namely, *Oki100* (K. Miller, Fisheries and Oceans Canada, unpublished data), *OMM1080* (Rexroad et al. 2001), *Ots211*, *Ots212*, *Ots213*, *Ots201b*, *Ots208b* (Greig et al. 2003), *OtsG474* (Williamson et al. 2002), and *Ssa408* (Cairney et al. 2000), and four dinucleotide loci, *Ogo2*, *Ogo4* (Olsen et al. 1998), *Ots3*, and *Ots9* (Banks et al. 1999; Greig and Banks 1999). Fluorescently labeled PCR products were separated with an Applied Biosystems 3730 Genetic Analyzer and genotyped with GeneMapper software.

Statistical analysis.—Deviation from Hardy–Weinberg equilibrium was evaluated at each locus and population using the Markov chain–Monte Carlo algorithm implemented in GENEPOP version 3.3 (Raymond and Rousset 1995). Tests for linkage disequilibrium between all pairs of loci were also performed using simulated exact tests in GENEPOP. Because multiple comparisons were involved, corrections were made against type I error in both tests with the Bonferroni method (Rice 1989).

To estimate the genetic diversity of each collection, the unbiased heterozygosity (H_E), observed heterozygosity (H_O), total number alleles, and allelic richness (average alleles per locus corrected for a sample size of 27) were estimated for all microsatellite loci in FSTAT version 2.9.3.2 (Goudet 2001). Private allelic richness (PAR) was estimated with HP-Rare v.4.1 (Kalinowski 2005). Pairwise Wilcoxon rank-sum tests were completed with "R" (www.r-project.org) to test for differences in H_E, allelic richness, or private allelic richness among collections of life history type (ocean-type and stream-type) and among regions of stream-type Chinook salmon (Tucannon River, Rapid River Hatchery, Lostine River, Imnaha River, South Fork Salmon River, Middle Fork Salmon River, and upper Salmon River).

Tests for reduced population size and recent bottleneck events were conducted with Garza and Williamson's (2001) *M* ratio of allele number to allele size range under a stepwise mutation model. Published

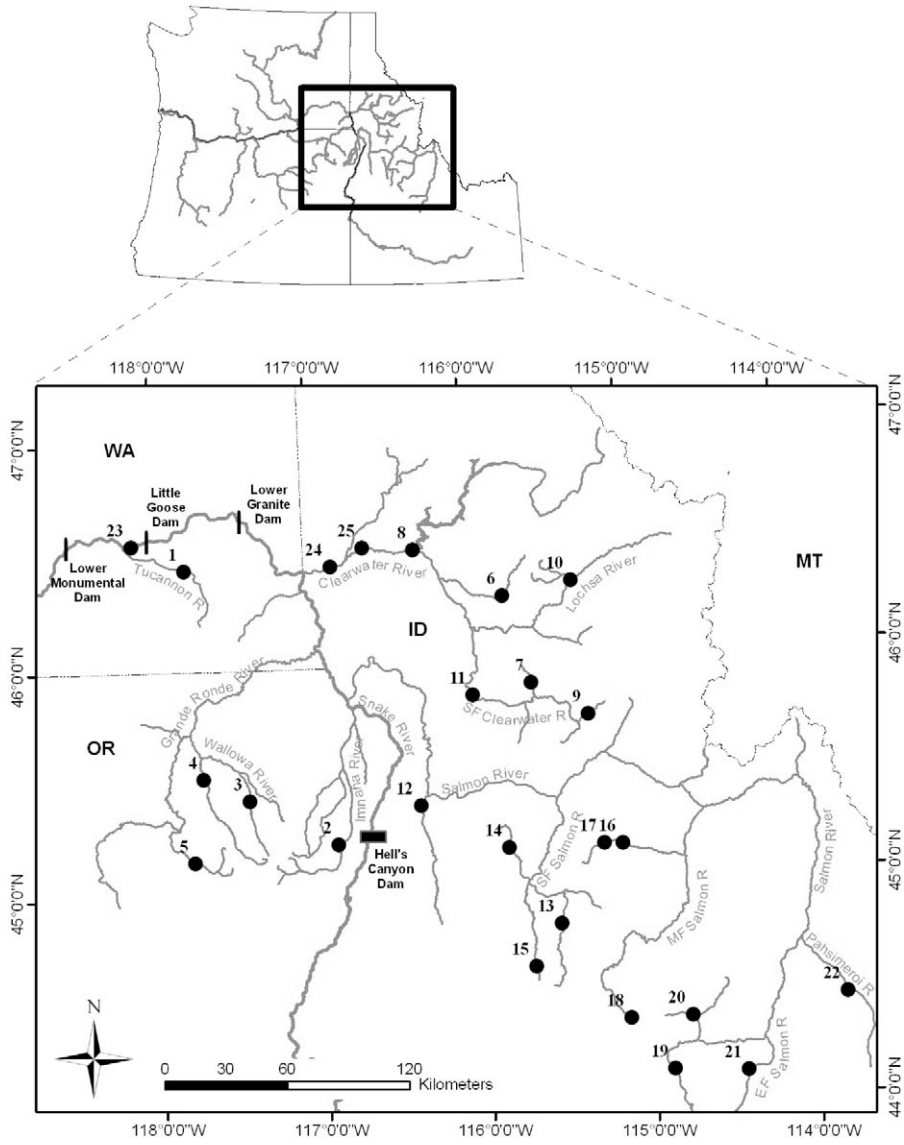

FIGURE 2.—The Snake River drainage with collection sites indicated by black circles. All collections are of stream-type Chinook salmon except for sites 23–25, which are of ocean-type fish. Circles are as follows: (1) Tucannon River (TUC), (2) Imnaha River (IMN), (3) Lostine River (LOST), (4) Minam Creek (MIN), (5) Catherine Creek (CAT), (6) Lolo Creek (LOLO), (7) Newsome Creek (NEWS), (8) Dworshak Hatchery (DWO-H), (9) Red River (RED), (10) Lochsa River Powell Hatchery (POW), (11) South Fork Clearwater River (SFCW), (12) Rapid River Hatchery (RAP-H), (13) Johnson Creek (JOHN), (14) Secesh River (SEC), (15) Johnson Creek Supplementation (JOHN-H), (16) Big Creek (a) (BIGa), (17) Big Creek (b) (BIGb), (18) Marsh Creek (MAR), (19) Sawtooth Hatchery weir (SAW), (20) West Fork Yankee Fork (WFYF), (21) East Fork Salmon River (EFSR), (22) Pahsimeroi River (PAH), (23) Lyons Ferry Hatchery ocean-type (LFH-H), (24) Clearwater River ocean-type (CLW), and (25) Nez Perce Tribal Hatchery ocean-type (NPTH-H).

literature suggests an M ratio less than 0.68 is evidence to indicate a probable bottleneck and reduced effective population size (Garza and Williamson 2001; Shrimpton and Heath 2003).

Pairwise genetic variance (temporal and geographic) was estimated from allele frequencies (F_{ST}; Weir and Cockerham 1984) in GENEPOP. Exact tests were performed in GENEPOP to determine significance of pairwise genetic variance. Significance levels were adjusted for multiple tests with a modified version of

TABLE 1.—Genetic diversity statistics and *M* ratios for 25 populations of Chinook salmon in the Snake River. Statistics are as follows: H_E = unbiased heterozygosity, H_O = observed heterozygosity, A = total alleles, AR = allelic richness, and PAR = private allelic richness. Population abbreviations are given in Figure 2.

Population	*n*	H_E	H_O	*A*	AR	PAR	*M* ratio
TUC	161	0.791	0.803	210	11.6	0.09	0.75
IMN	137	0.783	0.793	229	12.7	0.07	0.78
LOST	101	0.754	0.763	178	11.0	0.12	0.68
MIN	138	0.790	0.788	239	13.5	0.07	0.77
CAT	124	0.775	0.776	217	12.7	0.10	0.76
LOLO	109	0.787	0.767	232	13.6	0.05	0.76
NEWS	109	0.765	0.760	205	12.0	0.03	0.78
DWO-H	92	0.793	0.792	222	13.5	0.05	0.75
RED	86	0.795	0.795	215	13.0	0.04	0.79
POW	138	0.788	0.789	220	13.1	0.02	0.75
SFCW	187	0.785	0.782	228	12.8	0.03	0.76
RAP-H	141	0.762	0.767	195	11.3	0.06	0.77
JOHN	143	0.776	0.775	209	11.9	0.06	0.78
SEC	137	0.773	0.763	215	12.1	0.04	0.81
JOHN-H	105	0.779	0.776	205	12.2	0.07	0.77
BIGa	69	0.754	0.764	188	11.7	0.00	0.79
BIGb	69	0.760	0.782	178	11.3	0.02	0.75
MAR	46	0.782	0.777	173	12.1	0.23	0.71
SAW	181	0.790	0.793	228	13.0	0.02	0.81
WFYF	59	0.758	0.779	161	10.3	0.08	0.73
EFSR	141	0.769	0.757	210	12.0	0.02	0.80
PAH	105	0.780	0.790	189	11.5	0.06	0.77
LFH-H	137	0.870	0.845	314	16.4	0.61	0.87
CLW	110	0.856	0.858	296	16.8	0.74	0.85
NPTH-H	134	0.866	0.866	308	16.3	0.61	0.84
Average	2,959[a]	0.777	0.779	207	12.2	0.06	0.76

[a] Total.

the False Discovery Rate program referred to as the B-Y FDR (Benjamini and Yekutieli 2001; Narum 2006).

To infer the degree of relatedness between sample collections, pairwise genetic distances (Cavalli-Sforza and Edwards 1967) were calculated between all populations using GENDIST in PHYLIP version 3.5 (Felsenstein 1993). Genetic chord distances were then used to construct a neighbor joining tree of sample populations with NEIGHBOR (PHYLIP version 3.5). Bootstrap replicates of 1,000 iterations were attained using SEQBOOT and a consensus tree was made with CONSENSE in PHYLIP version 3.5.

Results

Of 325 tests for deviations from Hardy–Weinberg equilibrium, only three were significant (Bonferroni-corrected $\alpha = 0.05/325$ tests $= 0.0002$). The three significant tests were observed for Big Creek (collection b) at *Oki100* (heterozygote excess), West Fork Yankee Fork at *OMM1080* (heterozygote deficit), and Lyons Ferry Hatchery at *Ots212* (heterozygote deficit), but no populations or loci consistently deviated from Hardy–Weinberg equilibrium. No tests for linkage disequilibrium were significant.

Estimates of unbiased H_E averaged 0.777, with a range of 0.754 (Big Creek [collection a]) to 0.870 (Nez Perce Tribal Hatchery), and H_O estimates were similar (Table 1). Populations with ocean-type life history had more total alleles than any of the stream-type populations (averages, 306.0 and 206.6, respectively). The dissimilarity in the number of observed alleles among life history types were not attributed to differences in sample size as allelic richness was also much higher for the ocean-type (average, 16.5) than for the stream-type (average, 12.2; Table 1). Regions of stream-type Chinook salmon with consistently low measures of genetic diversity included Lostine River, Tucannon River, South Fork Salmon River, and Middle Fork Salmon River relative to regions with higher diversity such as the Clearwater River and Grande Ronde River. Private allelic richness in stream-type salmon was lowest in populations in the Clearwater River (average, 0.040) and highest in the Lostine River (0.124). However, the single largest observation of private allelic richness in an individual stream-type population was Marsh Creek (0.23) and highest private allelic richness in an individual ocean-type population was Clearwater River (0.74). Wilcoxon rank-sum tests of H_E, allelic richness, and private allelic richness were significant among ocean- and stream-type life history types (all three P-values < 0.001). However, none of the measures of genetic diversity were significant in pairwise Wilcoxon rank-sum tests among regions of stream-type populations (all P-values > 0.10), partly

TABLE 2.—Average pairwise F_{ST} values of Chinook salmon populations within (along the diagonal [bold italics]) and between regions (below the diagonal). Values for individual populations and their significance levels are given in the text. All populations are stream-type except where noted. Abbreviations are as follows: TUC = Tucannon River, IMN = Imnaha River, LOST = Lostine River, RAP = Rapid River Hatchery–Clearwater River, SFSR = South Fork Salmon River, MFSR = Middle Fork Salmon River, and UPSR= upper Salmon River; "na" refers to regions with only one population, in which no within-region comparison was possible.

Population	TUC	IMN	LOST	RAP	SFSR	MFSR	UPSR	Ocean-type
TUC	na							
IMN	0.033	na						
LOST	0.040	0.033	na					
RAP	0.030	0.017	0.033	***0.006***				
SFSR	0.036	0.020	0.041	0.021	***0.010***			
MFSR	0.043	0.022	0.045	0.033	0.027	***0.020***		
UPSR	0.039	0.021	0.038	0.021	0.023	0.031	***0.017***	
Ocean-type	0.083	0.101	0.113	0.095	0.098	0.110	0.103	***0.001***

owing to the limited number of populations in some regions.

No population in this study had an *M* ratio less than 0.68 (significant bottleneck), but Lostine River (0.68), Marsh Creek (0.71), and West Fork Yankee Fork (0.73) all had low *M* ratios (Table 1). The highest value for stream-type Chinook salmon was observed in the Secesh River and Sawtooth Hatchery populations (0.81). The three ocean-type collections had relatively high *M* ratios ranging from 0.84 (Nez Perce Tribal Hatchery) to 0.87 (Lyons Ferry Hatchery).

All but 3 of 300 pairwise exact tests among collections were statistically significant (B-Y FDR modified critical value for 300 tests = 0.005), indicating genetic differentiation of populations. The only three pairwise tests that were not significant were among the ocean-type collections (Lyons Ferry Hatchery and Clearwater River, $P = 0.093$; Lyons Ferry Hatchery and Nez Perce Tribal Hatchery, $P = 0.038$; and Clearwater River and Nez Perce Tribal Hatchery, $P = 0.217$). Pairwise F_{ST} values were highest among ocean- and stream-type populations (range, 0.080–0.120). When pairwise F_{ST} values of stream-type populations were averaged within and between regions (Table 2), differentiation within any region was always less than pairwise comparisons between that and any other region. However, average F_{ST} values within the Middle Fork Salmon River (0.020) and upper Salmon River (0.017) regions were relatively high compared with other regions. Among regions of stream-type Chinook salmon, the highest average differentiation was observed between the Middle Fork Salmon and Lostine rivers (0.045) and lowest between Imnaha River and Rapid River Hatchery (0.017).

A neighbor-joining dendrogram of Cavalli-Sforza and Edwards (1967) genetic distance supports regional population structure, but it also includes a large cluster of populations related to the Rapid River Hatchery collection (Figure 3). Populations in the Salmon River appeared to cluster tightly by drainage (Middle Fork Salmon River, South Fork Salmon River, and upper Salmon River), as did all three collections of ocean-type life history. While stream-type Chinook salmon populations in the Clearwater River cluster together, they show a strong relationship to the Rapid River Hatchery collection. Additionally, two genetically similar populations from the Grande Ronde River (Catherine Creek and Minam Creek) appear related to collections from the Clearwater River and Rapid River Hatchery. The Lostine River population did not group with others from the Grande Ronde and formed a separate branch. Likewise, the Imnaha River and Tucannon River collections each formed distinct branches, although the Tucannon River clustered with ocean-type populations.

Discussion

Patterns of genetic structure were apparent in this study, with large variation among life history types, intermediate differences among regions, and slight variation within regions. Genetic diversity and distance were highly significant between sympatric ocean- and stream-type Chinook salmon in the Snake River, suggesting nearly complete reproductive isolation among these life history types. Collections of ocean-type fish (Lyons Ferry Hatchery, Clearwater River, and Nez Perce Tribal Hatchery) had similar measures of genetic diversity and were not significantly differentiated from one another. On the other hand, populations of stream-type salmon were all significantly differentiated by allele frequencies, and genetic diversity appeared to be under greater influence from genetic drift (random loss of alleles) than observed in the ocean-type collections. These results from the Snake River are consistent with general patterns observed in the Columbia River basin, as ocean-type Chinook

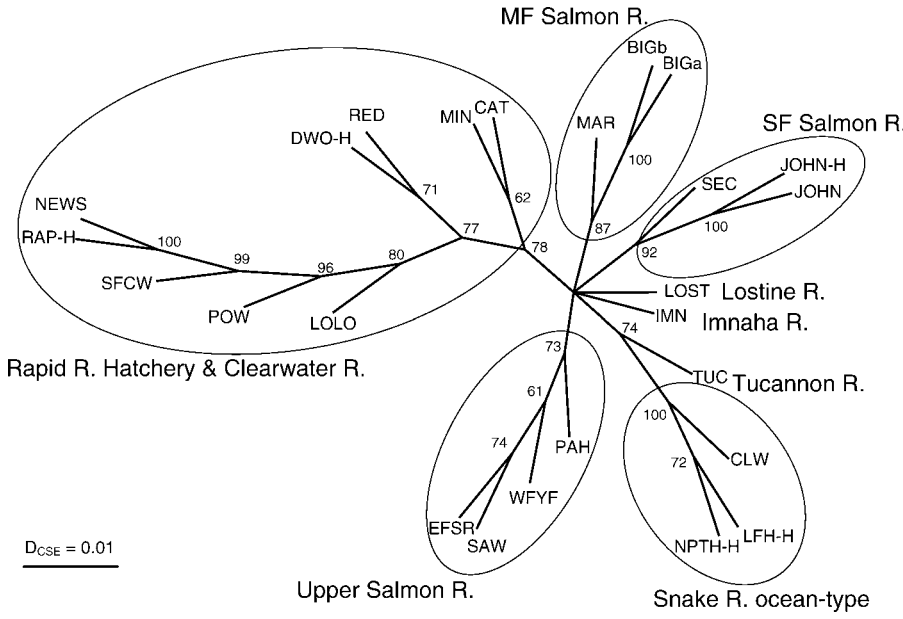

FIGURE 3.—Neighbor-joining dendrogram of 25 Chinook salmon collections from the Snake River based on Cavalli-Sforza and Edwards (1967) chord distance. Population abbreviations are defined in Figure 2. The numbers at the branches indicate the percentage bootstrap support for clades from 1,000 iterations. The scale of the chord distance is shown at the lower left.

salmon have higher genetic diversity and gene flow than stream-type salmon (Winans 1989). The F_{ST} values between populations of ocean- and stream-type Chinook salmon were not exceptionally large (range, 0.080–0.120) except when the average heterozygosity of the loci was taken into consideration. The 13 microsatellite loci in this study were highly variable (total of 386 alleles) with high average heterozygosities (range, 0.754–0.870), thus limiting the maximum value of F_{ST} (Hedrick 2005). Once observed F_{ST} values were translated to G'_{ST} as described by Hedrick (2005, equation [5b]), genetic distances between ocean- and stream-type salmon were very large (range of 0.690–0.880).

The results of this study also indicate regional structuring of stream-type Chinook salmon within subbasins and suggest high levels of philopatry to natal areas. However, populations within regions appear to experience gene flow from natural dispersal that provides metapopulation structure. Genetic similarity of naturally produced populations and collections of Chinook salmon reared at hatcheries suggests that some populations have been influenced by stock transfers. This influence is clearly evident in the Clearwater River where Chinook salmon were re-established with stocks from Rapid River Hatchery and Lyons Ferry Hatchery. All of the stream-type collections from the Clearwater River cluster tightly with those of Rapid River Hatchery, and the collections of natural ocean types from the Clearwater River (three temporal replicates combined) were not significantly different from either Lyons Ferry Hatchery or Nez Perce Tribal Hatchery.

Other populations of stream-type Chinook salmon also appear to have been influenced by hatchery-reared fish. The collection from Tucannon River represents a stream-type population that clusters closely with ocean-type populations, possibly due to hybridization in hatchery crosses of broodstock or natural spawning events. Two populations in the Grande Ronde River, Catherine and Minam creeks, clustered with Rapid River Hatchery population rather than by region with the Lostine River population (Figure 3). Straying and planting of hatchery-reared Chinook salmon into the Grande Ronde River subbasin may have resulted in the lack of regional structure observed in the Catherine Creek and Minam Creek collections. These tributaries have experienced supplementation with a stock founded from the one at Rapid River Hatchery (Keifer et al. 1992; Crateau 1997; Myers et al. 1998) with very little divergence over time (Waples et al. 1993). The stocks propagated in the upper and South Fork portions of the Salmon River also appear to have experienced gene flow from Rapid River Hatchery since F_{ST} values are low. Since straying is more common in transplanted salmon (Quinn 1993), even populations that were not

directly planted with artificially propagated fish may have been influenced by supplementation in other drainages. While hatchery influence can negatively affect natural populations (e.g., Ryman et al. 1994; Waples 1994), some level of introgression from dispersal may be beneficial by increasing genetic diversity of localized populations.

In the Snake River, escapement of Chinook salmon has been highly variable over the last 30 years (Figure 1), with a high potential for population bottlenecks. Interestingly, however, significant evidence for genetic bottlenecks was not observed in any collection, although those in the Lostine River, Marsh Creek, and West Fork Yankee Fork all had relatively low *M* ratios (0.68, 0.71, and 0.73, respectively). Given that demographic bottlenecks have occurred in some Snake River populations (e.g., Middle Fork Salmon River; Isaak et al. 2003), metapopulation dynamics may be responsible for maintaining diversity in fluctuating Chinook salmon populations. Dispersal of naturally spawned fish within drainages likely reduces the genetic effects of demographic bottlenecks while still maintaining regional population structure (e.g., Neville et al. 2007). Straying of supplemented Chinook salmon into nontarget areas would also provide "recovery" from genetic bottlenecks, but could also reduce distinct population structure and potentially important adaptive traits. The significant temporal genetic variation in Big Creek is probably due to wildly fluctuating census size (Isaak et al. 2003). Interestingly, the Lostine River population provided the only suggestion of a genetic bottleneck (*M* ratio = 0.68) and did not cluster with nearby Grande Ronde River collections or any others in the Snake River. It is possible that Chinook salmon in the Lostine River experience limited gene flow with other populations and represent a bottlenecked population.

Populations of both ocean- and stream-type Chinook salmon in the Clearwater River have recovered after removal of Lewiston Dam and reintroduction efforts (Narum et al., in press); however, there was no significant evidence for unique remnant genetic variation in this subbasin. Natural ocean-type Chinook salmon were not significantly differentiated from those from hatcheries, and private allelic richness was only slightly higher in natural populations (PAR = 0.74) than in the hatchery stocks (PAR = 0.61; Table 1). All collections of stream-type Chinook salmon in the Clearwater River had significantly different allele frequencies from one another indicating distinct populations with reduced gene flow, but the measures of private allelic richness in these populations were the smallest of any observed in this study. Before the removal of Lewiston Dam in 1973, the number of Chinook salmon at the dam never reached zero but was greatly reduced from predam levels (J. White, Idaho Department of Fish and Game, personal communication). While there may have been remnant individuals that contributed to re-established populations, any unique rare alleles probably would have been lost through genetic drift and the current population reflects the transplanted stock with no significant signal of unique genetic variation from native populations.

Evidence for reproductive isolation among ocean and stream life history types is strong ($P < 0.00001$) and indicates that these two types have been geographically isolated since at least the last glaciation event (Teel et al. 2000; Waples et al. 2004). Thus, the relatively large levels of observed differentiation among sympatric ocean- and stream-type Chinook salmon in the Snake River are most probably due to isolation followed by secondary contact rather than adaptation and evolution of a single lineage (e.g., Brannon et al. 2004). Also, the significantly higher genetic diversity in ocean-type than in stream-type populations suggests larger founding populations for ocean-type Chinook salmon, episodic colonization events of ocean-type fish, hatchery influence on diversity of populations, demographic bottlenecks and genetic drift for stream-type salmon, or some combination of these factors.

The significant regional structure observed in this study may also serve as a resource for identification of fish of unknown origin based on genotypic data. The genetic relationships of populations (Figure 3) provide a genetic baseline for determining stock proportions in mixed stock analyses (Shaklee et al. 1999; Beacham et al. 2006). Simulations of proportional stock assignment with these 25 populations were quite high with an average accuracy of 89.9% to populations (S. R. Narum, unpublished). Mixed stock analysis has a large number of potential applications, including estimation of adult escapement to subbasins and the population composition of mixed stock commercial and recreational harvests. Thus, population structure as revealed by genetic markers not only provides information regarding population diversity and interbreeding but also provides a tool with which to manage fisheries.

Conclusions

Complicated patterns of genetic variation were apparent in the Chinook salmon of the Snake River drainage. Life history types were highly distinct even in sympatry. Within life history type, the three collections of ocean-type Chinook salmon were not differentiated in contrast to all 22 collections of stream-type salmon. Regional genetic structure within stream-type life history was observed through clustering of nearby populations, although evidence for hatchery

influence was widespread in nearly all collections. Despite evidence for gene flow of hatchery stocks into natural populations, regional population structure is apparent and may be used for mixed stock analysis.

A combination of natural and artificial production may be necessary to assist the recovery of Chinook salmon in the Snake River, and the approach should vary depending on life history type. Highly philopatric stream-type populations will diverge owing to genetic drift and natural selection, and thus supplementation efforts of stream-type populations should focus on using locally adapted broodstock with a minor level of input from nearby populations. Conversely, ocean-type Chinook salmon appear to benefit from dispersal and gene flow that maintains higher genetic diversity, and supplementation programs of ocean-type populations should give high priority to genetic diversity (e.g., broodstock that reflect high gene flow among natural populations) and place emphasis on regional rather than local stock integrity. Several studies have demonstrated that supplementation can be successful with native broodstock over multiple generations (Hedrick et al. 2000; Olsen et al. 2000; Wang and Ryman 2001; Duchesne and Bernatchez 2002). However, in most situations it is preferable to renew broodstock (Duchesne and Bernatchez 2002) and avoid captive breeding programs to reduce adaptations arising from artificial culture (Gilligan and Frankham 2003) such as domestication selection (Ford 2002).

Given the threatened status of Snake River Chinook salmon under the ESA, further efforts are needed in fisheries management to assist the recovery of this species. Novel research tools such as those offered by this study need to be further applied to increase our understanding of natural populations and how they are affected by human activities such as habitat alteration. Additional research also needs to be devoted to determining the human induced and environmental factors that limit productivity and survival. Ultimately, management efforts aimed at maximizing life history and genetic diversity are necessary to allow the adaptation of Chinook salmon populations to the changing environment.

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Appendix: Snake River Chinook Salmon Samples

TABLE A.1.—Summary of sample collections of Chinook salmon from the Snake River basin. Origin was considered "natural" if the adipose fin was intact, "hatchery" if the adipose fin had been clipped, or "mixed" if the collection contained both natural and hatchery fish; n = sample size.

Population	Drainage	Abbreviation	n	Year(s) collected	Adult or juvenile	Origin	Life history type
Tucannon River	Tucannon River	TUC	161	2003	Adult	Natural	Stream
Imnaha River	Imnaha River	IMN	137	1998, 2002, 2003	Juvenile	Natural	Stream
Lostine River	Grande Ronde River	LOST	101	2001, 2002	Adult	Natural	Stream
Minam Creek	Grande Ronde River	MIN	138	1994, 2002, 2003	Juvenile	Natural	Stream
Catherine Creek	Grande Ronde River	CAT	124	2002, 2003	Adult	Mixed	Stream
Lolo Creek	Clearwater River	LOLO	109	2001, 2002	Both	Natural	Stream
Newsome Creek	Clearwater River	NEWS	109	2001, 2002	Both	Natural	Stream
Dworshak Hatchery	Clearwater River	DWO-H	92	2005	Adult	Hatchery	Stream
Red River	Clearwater River	RED	86	2005	Adult	Natural	Stream
Powell Trap (Lochsa River)	Clearwater River	POW	138	2005	Adult	Natural	Stream
South Fork Clearwater River	Clearwater River	SFCW	187	2005	Adult	Natural	Stream
Rapid River Hatchery	Salmon River	RAP-H	141	1997, 1999, 2002	Juvenile	Hatchery	Stream
Johnson Creek	South Fork Salmon River	JOHN	143	2002, 2003	Adult	Natural	Stream
Secesh River	South Fork Salmon River	SEC	137	2001, 2002, 2003	Juvenile	Natural	Stream
Johnson Creek Suppl.	South Fork Salmon River	JOHN-H	105	2002, 2003, 2004	Juvenile	Hatchery	Stream
Big Creek (a)[a]	Middle Fork Salmon River	BIGa	69	2001	Adult	Natural	Stream
Big Creek (b)[a]	Middle Fork Salmon River	BIGb	69	2002, 2003	Adult	Natural	Stream
Marsh Creek	Middle Fork Salmon River	MAR	46	2003, 2004	Adult	Natural	Stream
Sawtooth Hatchery	Upper Salmon River	SAW	181	2002, 2003	Adult	Mixed	Stream
West Fork Yankee Fork	Upper Salmon River	WFYF	59	2005	Adult	Natural	Stream
East Fork Salmon River	Upper Salmon River	EFSR	141	2004, 2005	Adult	Natural	Stream
Pahsimeroi River	Upper Salmon River	PAH	105	2002	Adult	Mixed	Stream
Lyons Ferry Hatchery	Snake River	LFH-H	137	2002, 2003	Adult	Hatchery	Ocean
Clearwater River	Snake River	CLW	110	2000, 2001, 2002	Adult	Natural	Ocean
Nez Perce Tribal Hatchery	Snake River	NPTH-H	134	2003, 2004	Adult	Hatchery	Ocean

[a] Two temporal collections of samples from Big Creek (a and b) are listed separately since exact tests of allele frequencies were statistically significant.