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[Note]

Localized Genetic Structure Persists in Wild Populations of Chinook Salmon in the John Day River Despite Gene Flow from Outside Sources

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Abstract.—Samples of Chinook salmon *Oncorhynchus tshawytscha* collected from four spawning areas in the John Day River, Oregon ($n = 330$), were genotyped with 13 microsatellite loci to test for bottlenecks and temporal stability within sites as well as genetic differentiation among sites, and to estimate gene flow from outside populations. Since the John Day River has never been stocked with hatchery-reared fish, this study provided the opportunity to evaluate the genetic integrity and structure of Chinook salmon in a wilderness area amid many hatchery-supported populations in the Columbia River. No tests for bottlenecks (Wilcoxon tests for heterozygosity excess) were significant, and the temporal variation was slight and not significant within any spawning reach except for the collections from the Middle Fork John Day River. Overall, the genetic distance estimates suggest that there are three distinct subpopulations in the John Day River, namely, those in (1) the North Fork John Day River (including Granite Creek), (2) the Middle Fork John Day River, and (3) the upper mainstem John Day River. These genetic relationships were supported by results from a neighbor-joining dendrogram. Assignment tests indicate that out-of-basin straying occurs throughout the John Day River, the largest percentage of strays going to the North Fork John Day River. Immigration may have acted to avert genetic bottlenecks and maintain genetic diversity in populations with fluctuating census size. Yet the genetic substructure of the Chinook salmon in the John Day River indicates natural reproduction from philopatric individuals, possibly with higher reproductive success than immigrants. The evidence presented here elucidates the balance of philopatry and dispersal acting to maintain genetic diversity and localized structure among the Chinook salmon of the John Day River.

In highly philopatric species such as Chinook salmon *Oncorhynchus tshawytscha*, natural populations are typically distinct, with regional genetic structure at selectively neutral loci (Beacham et al. 2006), and local adaptation may act to strengthen structure (i.e., Heath et al. 2006). Anthropogenic disturbances have caused severe declines in Chinook salmon abundance in a large part of the species' range (Gustafson et al. 2007), leading to increased genetic drift in small populations. However, genetic drift can quickly be counterbalanced by modest levels of gene flow from neighboring populations. Since Chinook salmon generally function under regional metapopulation dynamics, dispersal among populations can act to negate genetic effects of demographic bottlenecks (e.g., Narum et al. 2007).

The Chinook salmon in the John Day River are part of the mid-Columbia River spring-run evolutionarily significant unit (ESU) and, along with most of the spring Chinook salmon stocks in the interior Columbia River, they have experienced dramatic fluctuations in abundance over the last several decades (Figure 1). While the John Day River is one of the few subbasins in the Columbia River drainage that have yet to receive direct transplants of hatchery-reared stocks, dispersal and straying from outside populations may occur. Artificial propagation of Chinook salmon in the Columbia River is intense, and concerns about supplementation of natural populations include the loss of local genetic structure (Hindar et al. 1991), decreases in genetic diversity and effective population size (Waples 1994), and alterations to stochastic genetic drift (Utter et al. 1995). Further, transplants and straying from hatchery-reared stocks may disrupt locally coadapted gene complexes (see Utter 2001 for a review). Since straying from outside stocks into distinct native populations can lead to loss of interpopulation variation (Miller and Kapuscinski 2003), information

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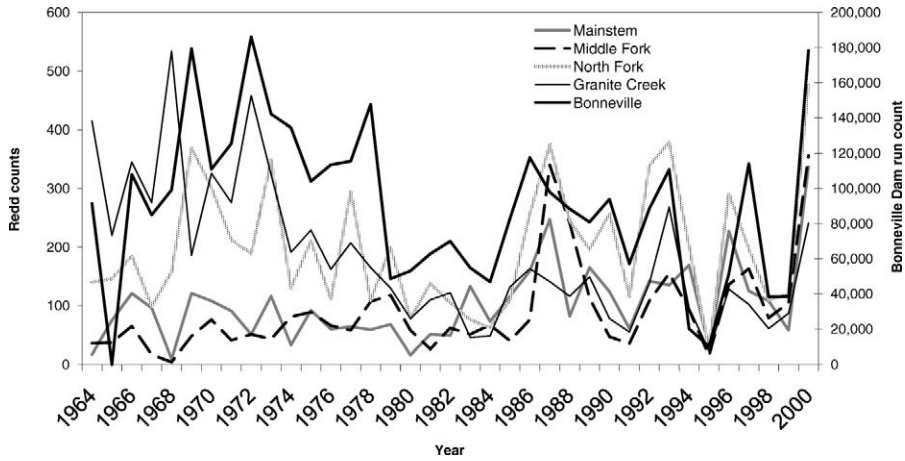

FIGURE 1.—Redd counts of Chinook salmon in four spawning reaches of the John Day River from 1964 to 2000, along with the corresponding run counts at Bonneville Dam. Data were compiled from Appendix B of Carmichael et al. (2002).

regarding gene flow and its influence to genetic structure of endemic populations is critical for conservation of genetic diversity in this species.

This study provided the opportunity to evaluate the genetic integrity and structure of Chinook salmon in the John Day wilderness area. Samples collected from multiple spawning areas in the John Day River were genotyped with 13 microsatellite loci to test for bottlenecks and temporal stability within sites as well as genetic differentiation among sites, and to estimate gene flow from other Columbia River populations. Because the populations in the John Day River are part of the broader Columbia River metapopulation of Chinook salmon, natural dispersal into the John Day River is expected from other nearby populations in the mid-Columbia River ESU, as is limited migration from more distant populations throughout the basin and the straying of poorly acclimated hatchery-reared fish. Under a scenario of moderate immigration but predominance of philopatric spawners, migrants may have lower reproductive success than native fish (e.g., McLean et al. 2004), and gene flow is expected to have a greater effect on genetic diversity than localized genetic structure of wild populations.

Methods

A total of 330 tissue samples were collected from spawned-out carcasses of Chinook salmon over four reaches of the John Day River (Figure 2), including the North Fork John Day River, Granite Creek, Middle Fork John Day River, and upper main-stem John Day River (hereafter, John Day River will be abbreviated as JDR when referencing specific sites and spelled out when referring to the drainage in general). Samples were collected in each of 3 years (2004, 2005, and 2006) for each spawning reach except for Granite Creek, which was only sampled in 2005 and 2006. All samples were collected from carcasses with intact adipose fins, and therefore the samples were assumed to be naturally produced since most hatchery-reared spring-run Chinook salmon in the interior Columbia River have adipose fins clipped as juveniles prior to release. Sample size varied by reach and year (Table 1), and tissues were preserved in a 95% solution of nondenatured ethanol.

We extracted DNA from all samples using Qiagen DNeasy protocols in conjunction with a Qiagen 3000 BioRobot. The DNA was arrayed in 96-well plates for high-throughput genotyping. Template DNA was amplified via the polymerase chain reaction (PCR) at 13 microsatellite loci following the standardized protocols discussed in Seeb et al. (2007). This included nine tetranucleotide microsatellite loci—*Oki100* (K. Miller, Canada Department of Fisheries and Oceans, 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 Applied Biosystems 3100 or 3730 genetic analyzers and genotyped with GeneMapper software.

Deviation from Hardy–Weinberg equilibrium was evaluated at each locus and population by means of the Markov chain–Monte Carlo algorithm implemented in GENEPOP version 3.3 (Raymond and Rousset 1995). Tests for linkage disequilibrium between all pairs of

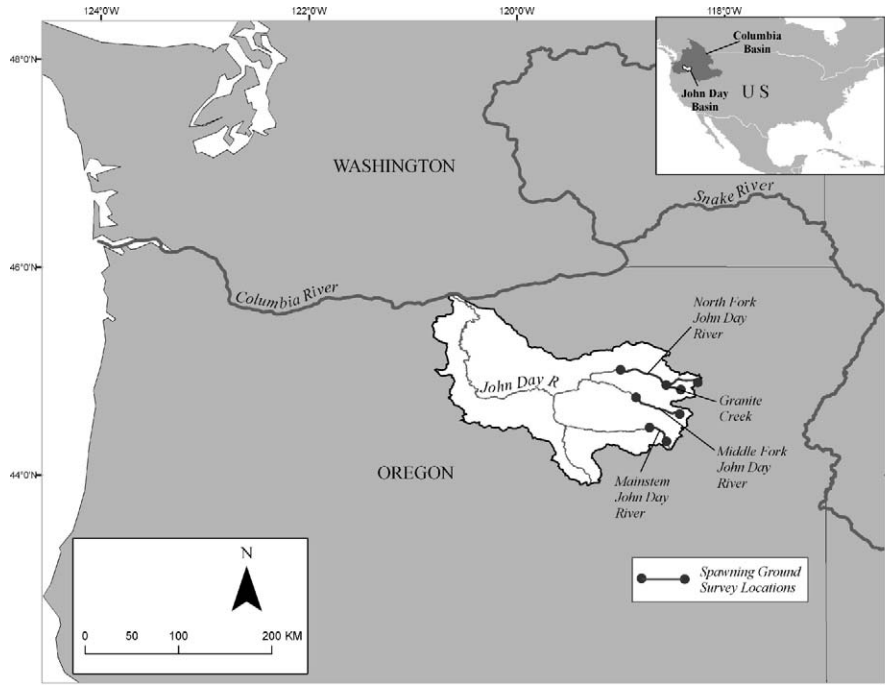

FIGURE 2.—Map of the John Day River drainage showing the spawning ground sample areas for Chinook salmon.

loci were also performed using simulated exact tests in GENEPOP. Because multiple comparisons were involved, Bonferroni corrections were made against type I error in both tests (Rice 1989). To estimate the genetic diversity of each collection, the unbiased heterozygosity (H_E), observed heterozygosity (H_O), total number alleles (A), and allelic richness (AR, i.e., the average alleles per locus corrected for a sample size of 18) were estimated with HP-Rare version 4.1 (Kalinowski 2005).

TABLE 1.—Descriptive statistics for 11 collections of Chinook salmon from the John Day River basin, including estimates of genetic diversity (A = total alleles, H_E = unbiased heterozygosity, H_O = observed heterozygosity, and AR = allelic richness), test for bottlenecks (Wilcoxon test for heterozygote excess), and percentages of individuals assigned to the John Day River (JDR) or Snake River.

							Assignment tests[a]		
Collection	n	A	H_E	H_O	AR	Wilcoxon P-value	JDR	Snake	Unknown
Middle Fork									
2004	23	147	0.771	0.767	7.8	0.89	65.2%	13.0%	21.7%
2005	37	172	0.764	0.753	8.1	0.89	62.2%	2.7%	35.1%
2006	28	158	0.759	0.775	7.9	0.73	32.1%	35.7%	32.1%[b]
Upper main stem									
2004	18	120	0.756	0.731	7.3	0.45	66.7%	5.6%	27.8%
2005	14	109	0.738	0.769	7.3	0.92	78.6%	14.3%	7.1%
2006	35	154	0.764	0.726	7.5	0.93	62.9%	11.4%	25.7%
Granite Creek									
2005	26	154	0.775	0.763	8.1	0.88	50.0%	7.7%	42.3%
2006	34	179	0.763	0.733	8.1	0.94	50.0%	5.9%	44.1%
North Fork									
2004	20	157	0.778	0.787	8.5	0.93	30.0%	30.0%	40.0%
2005	51	196	0.788	0.769	8.3	0.61	27.5%	17.6%	54.9%[b]
2006	44	195	0.780	0.770	8.4	0.68	18.2%	34.1%	47.7%

[a] Assignment at ≥80% stringency level from GENECLASS, otherwise unknown.
[b] One sample from each of these collections was assigned to the upper Columbia River with ≥80% confidence.

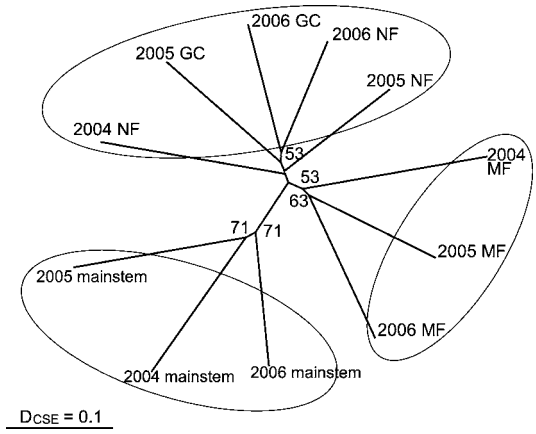

FIGURE 3.—Neighbor-joining dendrogram of 11 Chinook salmon collections from the John Day River basin based on Cavalli-Sforza and Edwards' (1967) chord distance (D_{CSF}). Population abbreviations are as follows: NF = North Fork John Day River, GC = Granite Creek, and MF = Middle Fork John Day River. The numbers at the branches indicate the percent bootstrap support for clades from 1,000 iterations; only numbers 50% or higher are shown.

To evaluate potential genetic bottlenecks, heterozygosity excess was tested under a two-phase mutation (TPM) model in BOTTLENECK version 1.2.02 (Piry et al. 1999). Generic parameters of 12% variance and 1,000 iterations were used for the TPM estimates. Proportion of stepwise mutations in TPM was set at 90% as recommended by Luikart and Cornuet (1998).

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 the false discovery rate referred to as the B-Y FDR, which provides a large increase in the power to differentiate populations relative to traditional Bonferroni methods (Narum 2006).

To show the degree of relatedness between sample collections, pairwise genetic distances (Cavalli-Sforza and Edwards 1967) were calculated between all populations with POPULATIONS version 1.2.30 software (Langella 2001). Genetic chord distances were then used to construct a neighbor-joining tree of sample populations with bootstrap replicates over 1,000 iterations. A consensus dendrogram was displayed with the program TREEVIEW version 1.6.6 (Page 1996).

Assignment tests calculate the probability that an individual's multilocus genotype derives from alternative groups (species or populations) and assigns membership to the most likely group (Paetkau et al. 1995). Assignment tests were performed with the partial Bayesian method (Rannala and Mountain 1997) implemented in GENECLASS version 2.0 (Piry et al. 2004) with the leave-one-out option. Individuals were classified a priori by collection site and assigned to reference populations that included each of the 11 JDR collections, along with 46 other populations of interior Columbia River basin spring-run Chinook salmon (genotypes from Narum et al. 2007 and Seeb et al. 2007). Partial Bayesian assignment is preferred when not all possible populations have been sampled to reduce misassignment error over fully Bayesian methods (Cornuet et al. 1999; Berry et al. 2004). To increase confidence in assignment test results, a stringency level of 80% or higher was utilized to identify population of origin as recommended by Berry et al. (2004). Samples with lower than 80% probability of assignment were classified as unknown.

Results and Discussion

No locus or population was found to deviate significantly from Hardy–Weinberg proportions after corrections for multiple tests (Bonferroni-adjusted critical value = 0.05/143 = 0.0003). Only one test for linkage disequilbrium was significant in the 2005 collection from the North Fork JDR (*Ots208b* versus *Ots201b*; $P < 0.00001$), and no other tests were significant. Estimates of genetic diversity that corrected for differences in sample size displayed a consistent relationship among spawning areas, as the North Fork JDR had the highest measures of H_E and AR, the Middle Fork JDR had intermediate values, and the upper main-stem JDR had the lowest diversity (Table 1). This relationship was also observed in total alleles and H_O, but differences in collection sample sizes likely influenced these estimates.

Patterns of genetic differentiation were apparent from pairwise F_{ST} estimates and exact tests, as the temporal variation was slight (average $F_{ST} = 0.005$) and not significant within any spawning reach except the Middle Fork JDR. Genetic similarity was observed in tests between geographically proximate collections from Granite Creek and North Fork JDR with low F_{ST} values (range = 0.001–0.009; average = 0.005) that generally were not significantly different. However, pairwise genetic distance was higher among all other spawning sites (average $F_{ST} = 0.019$) and were significantly different as *P*-values were less than the B-Y FDR corrected critical value of 0.011. Overall, the genetic distance estimates suggest that there are three distinct subpopulations in the John Day River, represented by the three tributaries, namely, (1) the North Fork JDR (including Granite Creek), (2) the

Middle Fork JDR, and (3) the upper main-stem JDR. These genetic relationships were supported by results from the neighbor-joining dendrogram (Figure 3), where three clusters of populations were concordant with the subpopulations identified in the F_{ST} analysis. In comparison with Chinook salmon stocks in the Salmon River drainage, the other major wilderness area in the Columbia River basin, population structure within the John Day River appears weaker as indicated by lower bootstrap support for branches in the neighbor-joining tree than for branches of a similar dendrogram for the Salmon River (Narum et al. 2007). This may indicate higher gene flow, less genetic drift, or both among the populations in the John Day River than among those in the Salmon River, which may be due to the relatively downstream location of the John Day River subbasin and therefore the higher potential for immigration from upstream stocks.

Interestingly, none of the collections offered significant evidence for genetic bottlenecks ($P > 0.05$; Table 1) despite fluctuating population sizes and very low redd counts as recently as 1995 (<30 redds per site). Similarly, lack of genetic bottlenecks were noted by previous studies of Chinook salmon populations in the spring- and summer-run Snake River ESU that have experienced known demographic bottlenecks (Narum et al. 2007; Neville et al. 2007). This result was attributed to dispersal and recolonization from neighboring populations in the Snake River. A comparable circumstance may exist with Chinook salmon in the John Day River as results from assignment tests (Table 1) indicated gene flow from outside sources is likely responsible for maintaining genetic variability in these populations. Across sites, the upper main-stem JDR collections had the lowest proportion of individuals assigned outside of the John Day River or as being of unknown origin, while the North Fork JDR had the highest (Table 1), suggesting that immigration from outside the subbasin is most common into the North Fork JDR. The majority of individuals that could be confidently assigned as out-of-basin origin were Snake River fish (55 of 57 individuals), and only two upper Columbia River samples. However, unknown assignments occurred at each site in the John Day River (ranging from 7.1% to 54.9%), and once again the most unknown origin fish occurred in the North Fork JDR, intermediate in the Middle Fork JDR, and least in the upper main-stem JDR (Table 1). This result may be at least partially attributable to the genetic similarity of other mid-Columbia River populations due to recent ancestry and lack of statistical power among the genetic markers to differentiate nearby populations from those in the John Day River. However, high proportions of unknown assignments would also be expected in the North Fork JDR if gene flow occurs at rates reflected by assignment tests.

Despite gene flow from outside sources, three distinct subpopulations exist in the John Day River. Immigration may have acted to avert genetic bottlenecks and maintain genetic diversity in populations with fluctuating census size. Yet genetic substructure of Chinook salmon in the John Day River indicates natural reproduction from philopatric individuals, possibly with higher reproductive success than immigrants. This balance of philopatry with moderate dispersal is consistent with the results of previous studies suggesting that the Chinook salmon populations within a region act as a larger metapopulation for long-term persistence (Isaak et al. 2003; Narum et al. 2007). This study further demonstrated that dispersal of naturally produced individuals into the John Day River may be more common than hatchery straying as there have been limited numbers of hatchery-reared fish (identified by adipose fin-clip) documented in this drainage (range = 0.7–4.6% of carcasses; T.L.S., unpublished data).

This study suggests that the John Day River is a wild refuge for Chinook salmon and that surrounding populations provide adequate dispersal in a metapopulation framework to maintain diversity. Evidence presented here elucidates the balance of philopatry and dispersal to maintain genetic diversity and localized structure in Chinook salmon. The John Day River is one of few subbasins in the Columbia River that has limited anthropogenic impacts with respect to hatcheries, habitat, hydropower, and harvest (also known as the "four Hs"), and this provides direct conservation benefits to Chinook salmon.

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