Genetic Analysis of Willamette River Steelhead of Unknown Run Origin

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Introduction

The Willamette River has historically been populated by native winter-run steelhead (*Oncorhynchus mykiss*). Non-native summer-run steelhead have been introduced into the system as part of the Willamette River segregated harvest summer steelhead program (HSRG 2009), and are believed to be spawning naturally within the system. Because numerous winter-run steelhead populations in the Willamette River are listed as “Threatened” under the Endangered Species Act, there is concern about potential genetic and ecological effects of non-native summer-run steelhead on threatened winter-run populations. However, at most life-stages and in most Willamette River habitats it is not straightforward to identify summer run fish. As a consequence it is generally unknown when and where naturally produced summer-run steelhead are in the system.

One technique that could provide information about the reproductive success of naturally spawning summer-run steelhead in the Willamette River is genetic stock identification (GSI) (see Manel et al. 2005 for a review of genetic identification methods). If significant genetic differentiation exists between the winter-run and summer-run steelhead populations, it will be possible to estimate the proportion of each run in a mixed-sample of fish of unknown run origin, by collecting genetic data from those fish. GSI techniques require a genetic database of allele frequencies representing populations that may be present in a mixture of fish whose origins are of interest. Genotypes of fish of unknown origin are then compared to the database and are assigned to the population or group of genetically similar populations (a reporting group) from which it most likely originated.

The results presented within this report are the result of a project undertaken by National Oceanic and Atmospheric Administration (NOAA) Fisheries personnel at the request of Oregon Department of Fish and Wildlife (ODFW) with funding from the US Army Corps of Engineers. The goals of this study were to, 1) Genotype Willamette River steelhead of known origin for 15 microsatellite loci and add the resulting data to a pre-existing baseline of genetic data; 2) Genotype Willamette River steelhead smolts of unknown origin for the same loci; 3) Use GSI techniques to estimate the run of origin (summer or winter) and population group membership of each smolt sample.

Methods

Sample collection

Willamette River steelhead samples were collected by ODFW, and included smolts of unknown run origin from a trap located at Willamette Falls, and presumed winter-run adult steelhead captured at Foster trap on the South Santiam River and Minto trap on the North Santiam River (Table 1). Samples of archived scales collected from 1986 – 1988 from several winter and summer run populations were also included in order to gain a cursory view of the temporal stability of allele frequencies for some populations. Microsatellite data for 17 Willamette steelhead populations that had previously been collected by NOAA Fisheries personnel for earlier work on Willamette River steelhead, (Maureen Hess, Columbia River Inter-Tribal Fish Commission, unpublished data) were used as baseline for the GSI estimates (Table 1).

Data collection

Genomic DNA was isolated from fin tissue or scales using the Promega Wizard DNA Purification Kit (Promega Corp.), following the manufacturer’s instructions. Samples were genotyped for 15 microsatellite loci–*Ocl1* (Condrey and Bentzen 1998), *Ogo4* (Olsen et al. 1998), *Oke4* (Bucholz et al. 1999) *Oki23* (Smith et al. 1998), *Omy1001*, *Omy1011* (Spies et al. 2005), *Omy7* (Stephenson et al. 2009), *Omy77 (*Morris et al. 1996), *One14* (Scribner et al. 1996), *Ots3, Ots4* (Banks et al. 1999), *Ots100* (Nelson and Beacham 1999), *Ssa289* (McConnell et al. 1995), *Ssa407, Ssa408* (Cairney et al. 2000). Polymerase chain reactions (PCR) were conducted to amplify the loci of interest, and the resulting PCR products were analyzed via capillary gel electrophoresis using an Applied Biosystems 3100 genetic analyzer. Individual genotypes were determined using GeneScan and Genotyper software (Applied Biosystems Inc.). A total of 250 smolts captured at Willamette Falls, 61 adults from Foster and Minto traps, and 318 adults from scale samples were genotyped.

Data analyses

We tested all loci at each sample for departures from expected Hardy-Weinberg proportions using the program GENEPOP (Raymond and Rousset 1995) and applied sequential Bonferroni corrections to the critical significant values to correct for multiple tests (Rice 1989). Significant departures from expected Hardy-Weinberg proportions can indicate the presence of multiple populations, highly related individuals, or high levels of inbreeding within a sample.

We performed several analyses to examine the genetic population structure that currently exists among Willamette steelhead populations, to aid in the identification of reporting groups for GSI. First, we produced a dendrogram of genetic distances using the allele frequencies of the pre-existing database for Willamette steelhead and of the scale samples. We calculated Cavalli-Sforza Edwards (1967) chord distances over 1000 bootstrap replicates using the program PHYLIP (Felsenstein 2005). The results were then visualized by constructing a consensus neighbor-joining tree. Secondly, we calculated the amount of variation among four putative reporting groups (identified from the dendrogram) with an AMOVA test as implemented in the program Arlequin (Excoffier et al. 2005). A high degree of variation among groups would indicate substantial differentiation among the groups, a prerequisite for making accurate GSI estimates.

In order to estimate the level of accuracy we could expect from the existing data when assigning unknown fish to a reporting group, we performed a leave-one-out test using the program ONCOR (Kalinowski 2008). This test removes an individual from the baseline and assigns it to a reporting group as if it were a fish of unknown origin. After this is completed for every individual in the baseline, the assignments are compared to the known origin of each fish to measure the accuracy of the assignments.

Based on the results of the genetic population structure analyses and simulations using the baseline, we grouped samples into four reporting groups to make stock of origin estimates – Winter-run eastern tributaries, Winter-run western tributaries, Summer-run, and Resident. After individuals were assigned to a group of origin, we compared the distribution of the assignments by collection date using a Chi-square test, to test for the possibility that the different groups were outmigrating at different times. Additional GSI estimates were conducted on two sets of samples collected at Foster and Minto traps to confirm that they were winter-run steelhead, as they were presumed to be. These samples were independent of the baseline used to make assignments.

Results and Discussion

Over all samples, 7.1% of the tests for departures from expected Hardy-Weinberg proportions are significant ($P < 0.05$), however, none of the test are significant after the sequential Bonferroni correction is applied. The AMOVA results indicate that a significant ($P < 0.001$) amount of variation exists among the four putative reporting groups. The relative amount of variation among groups (4.4%) is slightly greater than the amount attributable to among–population variation within reporting groups (3.5%).

The dendrogram of genetic distances shows that the samples cluster into three distinct groups– Summer-run, Winter-run western tributaries, and Winter-run eastern tributaries, and an additional sub-group within the Winter-run eastern tributaries cluster - Residents (Figure 1). The genetic relationships in our analysis are generally consistent with those reported in previous studies (e.g., Myers et al. 2006). Eagle Creek National Fish Hatchery (NFH) is geographically located on the eastern side of the Willamette River; however, it most closely clusters with samples from western tributaries. This most likely reflects the fact that the stock used at Eagle Creek NFH originated from the lower Columbia River, the same stocks believed to be the ancestral source of at least some of steelhead currently spawning in western tributaries (Myers et al. 2006). Thus, we included Eagle Creek NFH in the "winter-run western tributaries" reporting group for GSI analyses. The Molalla winter-run 1986 and Clackamas R. winter-run 1986 more closely cluster with the western tributary samples than with other eastern tributary samples. This indicates that significant genetic changes have occurred in these populations over time, and may warrant further analyses in the future.

The leave-one-out tests show that the baseline can be used to accurately estimate the stock of origin for the winter-run eastern tributaries populations, the summer-run populations and the resident populations (Table 2). The winter-run western tributaries populations (mainly Canyon Cr. and Luckiamute R.) did not reassign with as high of accuracy as the other groups, perhaps because the steelhead spawning in the western tributaries of the Willamette River may be of mixed ancestry (Myers et al. 2006). Given that these populations are in Hardy-Weinberg equilibrium, it is most likely that these populations have become established independent of their founding lineages, as opposed to the populations being an admixture, where multiple independent populations that have not become fully introgressed with each other are residing in the same location. All of the individuals from that group that do not reassign correctly to the winter-run western tributaries group assign instead to the winter-run eastern tributaries group. Thus, the simpler problem of estimating whether an individual has a summer- or winter-run origin was even more accurate. There is no evidence of widespread introgression of summer-run stock into winter-run populations as only one individual from a winter-run population (Molalla, North Fork) assigns to the summer-run group in the leave-one-out test. All other individuals correctly assign to the winter-run group.

A total of 88.3% of the smolts collected at Willamette Falls assign to the winter-run eastern tributaries reporting group, 7.5% to the summer-run reporting group, and 4.2% to the winter-run western tributaries reporting group (Table 3). Assignment probability was high, as 94% of the assignments have P values > 0.90 and 90% of the assignments have P values > 0.95. Chi-square test results provide no evidence that the different groups were outmigrating at different times (summer-run $P = 0.478$, winter-run eastern tributaries $P = 0.997$, winter-run western tributaries $P = 0.213$).

Estimates of the run of origin for the samples collected at Foster and Minto traps confirmed that they were winter-run steelhead. All individuals except one assign to the winter-run eastern tributaries reporting group with high probabilities ($P > 0.99$). The remaining individual assigns to the winter-run western tributaries reporting group, but with a low probability ($P = 0.52$).

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Table 1. Samples used in this project and their run type/location, the life stage and year they were collected, and the number of individuals that were genotyped.

Name	Run/Source	Life stage	Collection year	N
Samples genotyped for genetic stock ID				
Foster Trap, South Santiam R.	unknown	Adult	2009	50
Minto Trap, North Santiam R.	unknown	Adult	2009	11
Willamette Falls Trap	unknown	Smolt	2009	250
Samples genotyped for temporal analysis				
Clackamas R.	Summer-run	Adult	1986	88
Molalla R.	Summer-run	Adult	1988	46
North Santiam R.	Summer-run	Adult	1986	23
North Santiam R.	Summer-run	Adult	1987	16
Molalla R.	Winter-run E. tribs	Adult	1986	66
North Santiam R.	Winter-run E. tribs	Adult	1986	39
Clackamas R.	Winter-run E. tribs	Adult	1986	40
Samples from previous work				
Clackamas H.	Summer-run	Adult	2006	50
South Santiam H.	Summer-run	Adult	2007	47
Calapooia R.	Winter-run E. tribs	Juvenile	1997	38
Clackamas R., North Fork Dam	Winter-run E. tribs	Adult	2005	42
Clackamas R.	Winter-run E. tribs	Juvenile	2000	80
Eagle Cr., Wild	Winter-run E. tribs	Adult	2000	63
Molalla R., North Fork	Winter-run E. tribs	Juvenile	1996	50
North Santiam R., Bennet Dam	Winter-run E. tribs	Adult	2005	45
North Santiam R., Marion Forks H.	Winter-run E. tribs	Juvenile	1998	45
South Santiam R., Foster Dam	Winter-run E. tribs	Adult	2005	49
South Santiam R., Wiley Cr.	Winter-run E. tribs	Juvenile	1997	39
Canyon Cr.	Winter-run W. tribs	Juvenile	1997	34
Eagle Creek National Fish H.	Winter-run W. tribs	Juvenile	2000	62
Luckiamute R.	Winter-run W. tribs	Juvenile	1997	31
Willamina R.	Winter-run W. tribs	Juvenile	1997	34
Deer Cr.	Resident	Juvenile	1998	40
Willamette R., North Fork of the Middle Fork	Resident	Juvenile	1998	31

Table 2. Results of the leave-one-out test for the baseline used for GSI estimates, showing the percentage of individuals from each population that correctly assigned to their reporting group.

Population	Reporting Group	%
Calapooia R.	Winter-run E. tribs	100.0%
Clackamas R., North Fork Dam	Winter-run E. tribs	100.0%
Clackamas R., Wild	Winter-run E. tribs	100.0%
Eagle Cr. Wild	Winter-run E. tribs	98.1%
Molalla R., North Fork	Winter-run E. tribs	97.3%
North Santiam., Bennet Dam	Winter-run E. tribs	100.0%
North Santiam R., Marion Forks H.	Winter-run E. tribs	100.0%
South Santiam R., Foster Dam	Winter-run E. tribs	93.3%
South Santiam R., Wiley Cr.	Winter-run E. tribs	100.0%
Deer Cr.	Resident	100.0%
Willamette R., North Fork of the Middle Fork	Resident	100.0%
Clackamas H.	Summer-run	100.0%
South Santiam H.	Summer-run	100.0%
Canyon Cr.	Winter-run W. tribs	79.4%
Eagle Cr. National Fish H.	Winter-run W. tribs	100.0%
Luckiamute R.	Winter-run W. tribs	78.3%
Willamina R.	Winter-run W. tribs	92.3%

Table 3.Estimated origin, the probability of assignment (*P*), and collection date for240 steelhead smolts collected at Willamette Falls.

Individual ID	Estimated origin	*P*	Collection Date
90357-001	Winter-run, E. tribs	1.0000	4/22/2009
90357-002	Winter-run, E. tribs	0.9663	4/22/2009
90357-003	Winter-run, W. tribs	0.8173	4/22/2009
90357-004	Winter-run, E. tribs	1.0000	4/22/2009
90357-005	Winter-run, E. tribs	1.0000	4/22/2009
90357-006	Summer	0.9995	4/22/2009
90357-007	Winter-run, E. tribs	1.0000	4/22/2009
90357-008	Winter-run, E. tribs	1.0000	4/22/2009
90357-009	Winter-run, E. tribs	1.0000	4/22/2009
90357-010	Winter-run, E. tribs	1.0000	4/22/2009
90357-011	Winter-run, E. tribs	1.0000	4/22/2009
90357-012	Summer	0.9998	4/22/2009
90357-013	Winter-run, E. tribs	1.0000	4/22/2009
90357-014	Winter-run, E. tribs	0.9995	4/22/2009
90357-015	Winter-run, W. tribs	0.9940	4/22/2009
90357-016	Winter-run, E. tribs	1.0000	4/22/2009
90357-017	Winter-run, E. tribs	1.0000	4/22/2009
90357-018	Winter-run, E. tribs	1.0000	4/22/2009
90357-019	Winter-run, E. tribs	1.0000	4/22/2009
90357-020	Winter-run, E. tribs	0.9901	4/22/2009
90357-021	Winter-run, E. tribs	1.0000	4/22/2009
90357-022	Winter-run, W. tribs	0.9987	4/22/2009
90357-023	Winter-run, E. tribs	1.0000	4/22/2009
90357-024	Winter-run, E. tribs	0.9426	4/22/2009
90357-025	Winter-run, E. tribs	0.9753	4/22/2009
90357-026	Winter-run, E. tribs	1.0000	4/22/2009
90357-027	Winter-run, E. tribs	1.0000	4/22/2009
90357-028	Winter-run, E. tribs	1.0000	4/22/2009
90357-029	Winter-run, E. tribs	0.9999	4/22/2009
90357-030	Winter-run, E. tribs	1.0000	4/22/2009
90357-031	Winter-run, E. tribs	0.7097	4/22/2009
90357-032	Winter-run, E. tribs	0.9994	4/22/2009
90357-033	Winter-run, E. tribs	1.0000	4/22/2009
90357-034	Winter-run, E. tribs	0.9989	4/22/2009
90357-035	Winter-run, E. tribs	0.9871	4/22/2009
90357-036	Winter-run, E. tribs	1.0000	4/22/2009

90357-037	Winter-run, E. tribs	0.9871	4/22/2009
90357-038	Winter-run, E. tribs	1.0000	4/22/2009
90357-039	Winter-run, E. tribs	1.0000	4/22/2009
90357-040	Winter-run, E. tribs	1.0000	4/22/2009
90357-041	Winter-run, E. tribs	1.0000	4/22/2009
90357-042	Winter-run, E. tribs	1.0000	4/22/2009
90357-043	Winter-run, E. tribs	0.9987	4/22/2009
90357-044	Winter-run, E. tribs	1.0000	4/22/2009
90357-045	Winter-run, E. tribs	1.0000	4/22/2009
90357-046	Winter-run, E. tribs	1.0000	4/22/2009
90357-047	Winter-run, E. tribs	1.0000	4/22/2009
90357-048	Winter-run, E. tribs	0.9101	4/22/2009
90357-049	Winter-run, E. tribs	0.9998	4/22/2009
90357-050	Winter-run, E. tribs	1.0000	4/22/2009
90357-051	Winter-run, E. tribs	1.0000	4/29/2009
90357-052	Winter-run, E. tribs	1.0000	4/29/2009
90357-053	Winter-run, E. tribs	1.0000	4/29/2009
90357-054	Winter-run, E. tribs	1.0000	4/29/2009
90357-055	Winter-run, E. tribs	1.0000	4/29/2009
90357-056	Winter-run, E. tribs	1.0000	4/29/2009
90357-057	Winter-run, E. tribs	1.0000	4/29/2009
90357-058	Winter-run, E. tribs	1.0000	4/29/2009
90357-059	Summer	1.0000	4/29/2009
90357-060	Winter-run, E. tribs	1.0000	4/29/2009
90357-061	Winter-run, E. tribs	0.9982	4/29/2009
90357-062	Winter-run, E. tribs	1.0000	4/29/2009
90357-063	Winter-run, E. tribs	1.0000	4/29/2009
90357-064	Winter-run, E. tribs	0.9680	4/29/2009
90357-065	Summer	0.9996	4/29/2009
90357-066	Winter-run, E. tribs	1.0000	4/29/2009
90357-067	Winter-run, W. tribs	0.6808	4/29/2009
90357-068	Winter-run, W. tribs	0.9709	4/29/2009
90357-069	Winter-run, E. tribs	1.0000	4/29/2009
90357-070	Winter-run, E. tribs	0.9999	4/29/2009
90357-071	Winter-run, E. tribs	1.0000	4/29/2009
90357-072	Winter-run, W. tribs	0.9772	4/29/2009
90357-073	Winter-run, E. tribs	1.0000	4/29/2009
90357-074	Winter-run, E. tribs	1.0000	4/29/2009
90357-075	Winter-run, E. tribs	0.9969	4/29/2009
90357-076	Winter-run, E. tribs	0.9998	4/29/2009
90357-077	Winter-run, E. tribs	1.0000	4/29/2009
90357-078	Winter-run, E. tribs	1.0000	4/29/2009

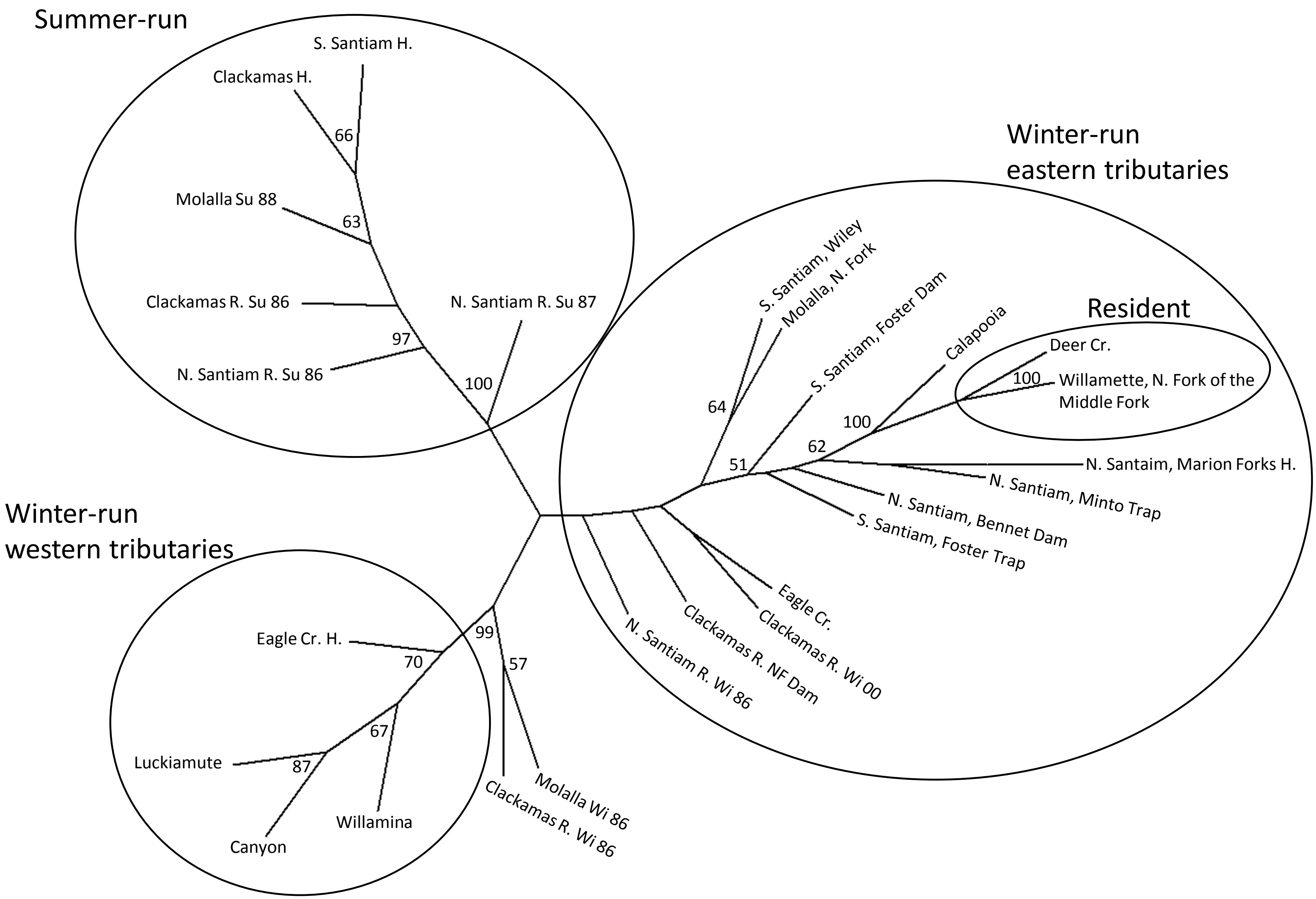
90357-079	Winter-run, E. tribs	1.0000	4/29/2009
90357-080	Winter-run, E. tribs	1.0000	4/29/2009
90357-081	Winter-run, E. tribs	0.9261	4/29/2009
90357-082	Winter-run, E. tribs	1.0000	4/29/2009
90357-083	Winter-run, W. tribs	1.0000	4/29/2009
90357-084	Winter-run, E. tribs	1.0000	4/29/2009
90357-085	Winter-run, E. tribs	1.0000	4/29/2009
90357-086	Winter-run, E. tribs	1.0000	4/29/2009
90357-087	Winter-run, E. tribs	0.6635	4/29/2009
90357-088	Winter-run, E. tribs	1.0000	4/29/2009
90357-089	Winter-run, E. tribs	1.0000	4/29/2009
90357-090	Winter-run, E. tribs	1.0000	4/29/2009
90357-091	Winter-run, E. tribs	1.0000	4/29/2009
90357-092	Winter-run, W. tribs	0.5705	4/29/2009
90357-093	Winter-run, E. tribs	1.0000	4/29/2009
90357-094	Winter-run, E. tribs	1.0000	4/29/2009
90357-095	Winter-run, E. tribs	0.9601	4/29/2009
90357-096	Winter-run, E. tribs	0.9943	4/29/2009
90357-097	Winter-run, E. tribs	1.0000	4/29/2009
90357-098	Winter-run, E. tribs	1.0000	4/29/2009
90357-099	Winter-run, E. tribs	0.9994	4/29/2009
90357-100	Winter-run, E. tribs	1.0000	4/29/2009
90357-102	Winter-run, E. tribs	0.9998	5/5/2009
90357-103	Summer	1.0000	5/5/2009
90357-104	Winter-run, E. tribs	1.0000	5/5/2009
90357-105	Summer	0.9853	5/5/2009
90357-106	Winter-run, E. tribs	1.0000	5/5/2009
90357-107	Winter-run, E. tribs	1.0000	5/5/2009
90357-108	Winter-run, E. tribs	1.0000	5/5/2009
90357-109	Winter-run, E. tribs	1.0000	5/5/2009
90357-110	Winter-run, E. tribs	1.0000	5/5/2009
90357-111	Winter-run, E. tribs	0.9999	5/5/2009
90357-112	Winter-run, E. tribs	1.0000	5/5/2009
90357-113	Winter-run, E. tribs	1.0000	5/5/2009
90357-114	Winter-run, E. tribs	1.0000	5/5/2009
90357-115	Summer	0.9819	5/5/2009
90357-116	Winter-run, E. tribs	1.0000	5/5/2009
90357-117	Winter-run, E. tribs	0.9949	5/5/2009
90357-119	Winter-run, E. tribs	1.0000	5/5/2009
90357-120	Winter-run, E. tribs	1.0000	5/5/2009
90357-121	Winter-run, E. tribs	1.0000	5/5/2009
90357-122	Summer	0.9949	5/5/2009

90357-123	Winter-run, E. tribs	0.6457	5/5/2009
90357-124	Winter-run, E. tribs	0.9999	5/5/2009
90357-126	Winter-run, E. tribs	1.0000	5/5/2009
90357-127	Winter-run, E. tribs	1.0000	5/5/2009
90357-129	Winter-run, E. tribs	0.9999	5/5/2009
90357-130	Winter-run, E. tribs	1.0000	5/5/2009
90357-131	Winter-run, E. tribs	1.0000	5/5/2009
90357-132	Winter-run, E. tribs	1.0000	5/5/2009
90357-133	Winter-run, E. tribs	1.0000	5/5/2009
90357-134	Winter-run, E. tribs	0.9811	5/5/2009
90357-135	Winter-run, E. tribs	1.0000	5/5/2009
90357-136	Winter-run, E. tribs	0.8627	5/5/2009
90357-137	Winter-run, E. tribs	0.9948	5/5/2009
90357-138	Winter-run, E. tribs	1.0000	5/5/2009
90357-139	Winter-run, E. tribs	0.6649	5/5/2009
90357-140	Winter-run, E. tribs	1.0000	5/5/2009
90357-141	Winter-run, E. tribs	0.9855	5/5/2009
90357-142	Winter-run, E. tribs	1.0000	5/5/2009
90357-143	Winter-run, E. tribs	1.0000	5/5/2009
90357-144	Winter-run, E. tribs	0.9989	5/5/2009
90357-145	Winter-run, E. tribs	1.0000	5/5/2009
90357-146	Winter-run, E. tribs	0.8031	5/5/2009
90357-147	Winter-run, E. tribs	0.9353	5/5/2009
90357-148	Winter-run, E. tribs	1.0000	5/5/2009
90357-149	Winter-run, E. tribs	0.9983	5/5/2009
90357-150	Winter-run, E. tribs	0.8954	5/5/2009
90357-151	Winter-run, E. tribs	1.0000	5/14/2009
90357-152	Winter-run, E. tribs	1.0000	5/14/2009
90357-153	Winter-run, E. tribs	1.0000	5/14/2009
90357-154	Winter-run, E. tribs	1.0000	5/14/2009
90357-155	Winter-run, E. tribs	0.9179	5/14/2009
90357-156	Winter-run, E. tribs	1.0000	5/14/2009
90357-157	Winter-run, E. tribs	0.9986	5/14/2009
90357-158	Winter-run, E. tribs	0.9858	5/14/2009
90357-159	Winter-run, E. tribs	0.9988	5/14/2009
90357-160	Winter-run, E. tribs	0.8807	5/14/2009
90357-161	Winter-run, E. tribs	0.7875	5/14/2009
90357-162	Winter-run, E. tribs	1.0000	5/14/2009
90357-163	Winter-run, E. tribs	1.0000	5/14/2009
90357-164	Winter-run, E. tribs	1.0000	5/14/2009
90357-165	Summer	0.7442	5/14/2009
90357-166	Winter-run, E. tribs	1.0000	5/14/2009

90357-167	Winter-run, E. tribs	1.0000	5/14/2009
90357-168	Winter-run, E. tribs	1.0000	5/14/2009
90357-169	Winter-run, E. tribs	1.0000	5/14/2009
90357-170	Winter-run, E. tribs	0.9204	5/14/2009
90357-172	Winter-run, E. tribs	1.0000	5/14/2009
90357-173	Winter-run, E. tribs	1.0000	5/14/2009
90357-174	Winter-run, E. tribs	1.0000	5/14/2009
90357-175	Winter-run, E. tribs	1.0000	5/14/2009
90357-176	Winter-run, E. tribs	0.9916	5/14/2009
90357-177	Summer	0.9530	5/14/2009
90357-178	Summer	1.0000	5/14/2009
90357-179	Winter-run, E. tribs	0.9941	5/14/2009
90357-180	Winter-run, E. tribs	0.9996	5/14/2009
90357-181	Winter-run, E. tribs	0.9347	5/14/2009
90357-182	Winter-run, E. tribs	0.8218	5/14/2009
90357-183	Winter-run, E. tribs	1.0000	5/14/2009
90357-184	Winter-run, W. tribs	0.7264	5/14/2009
90357-185	Winter-run, E. tribs	1.0000	5/14/2009
90357-186	Winter-run, E. tribs	0.9999	5/14/2009
90357-187	Winter-run, E. tribs	0.9999	5/14/2009
90357-188	Winter-run, E. tribs	0.9998	5/14/2009
90357-189	Winter-run, E. tribs	0.9997	5/14/2009
90357-190	Winter-run, E. tribs	1.0000	5/14/2009
90357-192	Winter-run, E. tribs	1.0000	5/14/2009
90357-193	Winter-run, E. tribs	1.0000	5/14/2009
90357-194	Summer	1.0000	5/14/2009
90357-195	Winter-run, E. tribs	1.0000	5/14/2009
90357-196	Winter-run, E. tribs	1.0000	5/14/2009
90357-197	Winter-run, E. tribs	0.9997	5/14/2009
90357-198	Winter-run, E. tribs	1.0000	5/14/2009
90357-199	Winter-run, E. tribs	1.0000	5/14/2009
90357-200	Winter-run, E. tribs	0.9908	5/14/2009
90357-201	Winter-run, E. tribs	1.0000	5/21/2009
90357-202	Winter-run, E. tribs	1.0000	5/21/2009
90357-203	Winter-run, E. tribs	1.0000	5/21/2009
90357-204	Winter-run, E. tribs	1.0000	5/21/2009
90357-205	Winter-run, E. tribs	1.0000	5/21/2009
90357-206	Winter-run, E. tribs	0.9663	5/21/2009
90357-208	Winter-run, E. tribs	0.9997	5/21/2009
90357-209	Winter-run, E. tribs	1.0000	5/21/2009
90357-210	Winter-run, E. tribs	0.9999	5/21/2009
90357-211	Winter-run, E. tribs	0.9900	5/21/2009

90357-212	Summer	1.0000	5/21/2009
90357-213	Winter-run, E. tribs	0.9996	5/21/2009
90357-214	Winter-run, E. tribs	1.0000	5/21/2009
90357-215	Winter-run, E. tribs	1.0000	5/21/2009
90357-216	Winter-run, E. tribs	1.0000	5/21/2009
90357-217	Winter-run, E. tribs	1.0000	5/21/2009
90357-218	Winter-run, E. tribs	1.0000	5/21/2009
90357-219	Summer	1.0000	5/21/2009
90357-220	Summer	0.9491	5/21/2009
90357-221	Winter-run, E. tribs	1.0000	5/21/2009
90357-222	Winter-run, E. tribs	0.9999	5/21/2009
90357-223	Winter-run, E. tribs	1.0000	5/21/2009
90357-224	Winter-run, E. tribs	1.0000	5/21/2009
90357-225	Winter-run, E. tribs	1.0000	5/21/2009
90357-226	Winter-run, E. tribs	1.0000	5/21/2009
90357-227	Winter-run, E. tribs	0.9744	5/21/2009
90357-228	Winter-run, W. tribs	0.9780	5/21/2009
90357-229	Winter-run, E. tribs	1.0000	5/21/2009
90357-230	Winter-run, E. tribs	1.0000	5/21/2009
90357-231	Summer	0.9995	5/21/2009
90357-232	Winter-run, E. tribs	1.0000	5/21/2009
90357-233	Winter-run, E. tribs	1.0000	5/21/2009
90357-234	Winter-run, E. tribs	1.0000	5/21/2009
90357-235	Winter-run, E. tribs	0.9991	5/21/2009
90357-236	Winter-run, E. tribs	0.9921	5/21/2009
90357-237	Summer	1.0000	5/21/2009
90357-238	Winter-run, E. tribs	0.9831	5/21/2009
90357-239	Winter-run, E. tribs	0.9998	5/21/2009
90357-240	Winter-run, E. tribs	0.9994	5/21/2009
90357-242	Winter-run, E. tribs	1.0000	5/21/2009
90357-243	Winter-run, E. tribs	1.0000	5/21/2009
90357-244	Summer	0.9973	5/21/2009
90357-245	Winter-run, E. tribs	1.0000	5/21/2009
90357-246	Winter-run, E. tribs	1.0000	5/21/2009
90357-247	Winter-run, E. tribs	0.9996	5/21/2009
90357-248	Winter-run, E. tribs	1.0000	5/21/2009

Figure 1. Neighbor-joining dendrogram of Cavalli-Sforza Edwards genetic distances among Willamette River steelhead populations. Bootstrap values (%) greater than 50% are shown. The last two digits of the brood year for the earliest samples are included in the sample names. Major groupings, which also correspond to the reporting groups used for GSI analyses, are circled.


Summer-run
S. Santiam H.
Clackamas H.
66
Molalla Su 88
63
Clackamas R. Su 86
N. Santiam R. Su 87
97
N. Santiam R. Su 86
100
Winter-run
eastern tributaries
S. Santiam, Wiley
Molalla, N. Fork
S. Santiam, Foster Dam
Calapooia
Resident
Deer Cr.
100
Willamette, N. Fork of the
Middle Fork
64
100
62
51
N. Santaim, Marion Forks H.
N. Santiam, Minto Trap
N. Santiam, Bennet Dam
S. Santiam, Foster Trap
Eagle Cr.
Clackamas R. Wi 00
Clackamas R. NF Dam
N. Santiam R. Wi 86
Winter-run
western tributaries
Eagle Cr. H.
99
70
57
67
Luckiamute
87
Willamina
Canyon
Molalla Wi 86
Clackamas R. Wi 86