Genetic structure of *Oncorhynchus mykiss* populations in the Grande Ronde River, Imnaha River, and adjacent regions of the Snake River Basin[1]

Final Report

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Introduction

This study was a collaborative effort with the Oregon Department of Fish & Wildlife (ODFW), Washington Department of Fish & Wildlife (WDFW), Idaho Department of Fish & Game (IDFG), Nez Perce Tribe (NPT), Confederated Tribes of the Umatilla Indian Reservation (CTUIR), Confederated Tribes of the Warm Springs Reservation (CTWSR), and U.S. Fish & Wildlife Service (USFWS) to characterize genetic variation within and among natural populations of *Oncorhynchus mykiss* in northeast Oregon and southwest Washington. Allele frequencies at 16 nuclear DNA loci (microsatellites), were used to characterize temporal and spatial genetic variation among 112 population[3] samples from 46 field localities (natural populations), five hatchery stocks, and one sample of marked strays. Most natural populations were sampled from the same field locations for three consecutive years to distinguish temporal and spatial components of genetic variation within and among populations (a 4th year of samples has been collected but was not included for analysis under this contract). In all, more than 5,000 fish were analyzed for this study. The principal goal of this work was to provide a detailed description of the population genetic structure of *O. mykiss* throughout the Grande Ronde and Imnaha river basins. Reference samples were also included from other watersheds in the Snake and mid-Columbia rivers. The purpose of this report is to provide the comanaging agencies and tribes in the Snake and Columbia river basins an overall summary of the available results with detailed materials and methods used to generate those data. Only cursory population genetic analyses for quality control were conducted for this report; however, even these preliminary

[3]The term "population" in the context of sampling locations is used here for consistency with scientific, peer-reviewed genetics literature. This sampling terminology should not be taken as implicit inference of population boundaries in either a biological or management sense. The first null hypothesis is that individuals at all locations are panmictic, or freely interbreeding, and there are no population boundaries within the Snake River basin.

results will likely be of interest to managers and are therefore included in this report. In general, the results showed strong concordance between geographic proximity and genetic similarity among populations. Temporal variation among samples within streams was substantially less than spatial variation among streams. This pattern was clearly evident in the consistent clustering of replicate samples among years from the same population in a neighbor joining dendrogram derived from pair wise F_{ST} estimates. The biological implications of these observations will be explored more fully with tribal, state, and Federal collaborators as these data are prepared for publication in the peer-reviewed scientific literature. A full quantitative analysis of the result obtained in this study will be presented at that time.

MATERIALS AND METHODS

Population sampling

State and tribal biologists conducted the population sampling for this study. Sample sites and study design were developed by state (IDFG, ODFW, and WDFW) and tribal (NPT, CTUIR, and CTWSR) biologists working jointly with Federal scientists (USFWS and NMFS). A small amount of fin tissue (typically < 5 mm^2) was collected non-lethally from each fish. Most of the tissue samples used in this study represented young-of-the-year (age 0+) parr sampled in streams throughout the region over three consecutive years (Fig. 1). Also included were five selected hatchery stocks and representative outgroup populations from major river systems in Idaho (Clearwater and Salmon rivers, including the Dworshak and Pahsimeroi hatchery stocks). In a few cases, samples were taken from adults returning to various trapping facilities. To augment available collections and address various subsidiary questions, some additional samples, beyond those contracted, were drawn from the *Conservation Biology Division Tissue Archive* at NMFS'

Northwest Fisheries Science Center. Nearly all tissues were preserved and stored in 95% ethanol, although seven collections from the Deschutes River were delivered to the laboratory in an aqueous lysis buffer. A total of 5,268 individuals were genotyped for this study. Collections including 46 field sampling locations, five hatchery stocks, and one sample of hatchery strays (marked fish trapped at the Warm Springs National Fish Hatchery in the Deschutes River basin).

Each sampling location was represented by sub-samples of approximately 6 to 10 individual fish taken from each of 10 to 15 sites spread over several kilometers within each stream. Forty-eight individuals per site per year were drawn randomly from all the tissues collected, stratified among the sub-samples described above. Fish from a total of 112 individual collections were examined (Table 1). Field collections of juvenile *O. mykiss* were designed to target the progeny of anadromous parents (i.e., steelhead parr), both temporally and spatially, by taking fin tissue from age 0+ fish in putative steelhead rearing areas. Nevertheless, it is entirely possible that parr samples could have included the progeny of non-anadromous *O. mykiss* in unknown proportions (i.e., resident rainbow trout). One sample of 35 anadromous adults trapped at the Lightning Cr. weir was compared to a sample of parr collected upstream from the weir. This allowed evaluation of the genetic similarity of the two samples in this one system.[4]

[4]Additional Lightning Creek and Cow Creek samples have been genotyped as well as adult samples from Catherine Creek, Lookingglass Creek, and upper Grande Ronde River.

Microsatellite genotyping

DNA was isolated from 2-10 mg of rayed fin tissue from each individual fish. The Qiagen DNeasy 96 Tissue Kit[5] was used following the manufacturer's instructions for mouse-tail DNA isolation. DNA was quantified by using PicoGreen dye (Molecular Probes, Eugene, Oregon) and fluorescence spectroscopy. In some cases, batches of samples were given only cursory checks of DNA concentration, and working dilutions were made based on average expected yield. However, problem samples that failed to amplify were often quantified before rerunning.

This study employed multi-locus genotyping of simple-sequence repeat (microsatellite), nuclear DNA markers. Sixteen microsatellite loci were selected based on reliability of PCR amplification and genotyping, as well as breadth of use among laboratories (e.g., University of Idaho, Washington State University, WDFW, and especially IDFG, which is conducting a similar study in collaboration with U.S. Geological Survey focused on *O. mykiss* populations in Idaho (Table 2). We also considered allele frequency distributions observed in a preliminary survey of steelhead variation, prioritizing loci with relatively uniform distributions and several common alleles. There was no *a priori* consideration of geographic relationships among survey populations in this selection process. These initial survey populations spanned much of the North American range of *O. mykiss*, but were pooled for consideration of allele frequency distribution. This approach was used to obtain highly informative DNA markers that would most accurately measure the average genetic structure in the Snake River basin without introducing any bias derived from predetermined expectations.

[5]Reference to trade names does not indicate endorsement by the National Marine Fisheries Service, the US Fish and Wildlife Service, nor any of the collaborators.

Polymerase chain reaction (PCR) amplifications were conducted in 10 uL reaction volumes as follows: 1.75 mM $MgCl_2$, 200 uM each dNTP, 0.5 U *Taq* polymerase (Promega), 2 - 20 ng genomic DNA. Primer concentrations varied among loci as listed in Table 2. Amplifications were carried out in thermal cyclers configured for 96-well plates (PTC-0225 DNA Engine Tetrad™, MJ Research, Inc., Waltham, Massachusetts). Thermal cycler programs were similar among loci except for differences in annealing temperature and final extension temperature. In general, a single denaturation cycle of 2 min at 95° was followed by 32 cycles with 40 sec at 94°, 40 sec at the locus-specific annealing temperature (Table 2), 40 sec at 72°, and concluded with a single 5 min to 1 hr extension cycle at the final temperature given in Table 2. A prolonged annealing/extension cycle at the end of the amplification facilitated consistent genotyping by driving all the PCR product to the "plus A" state, thus eliminating an adenylation problem that otherwise produced a fragment 1 bp smaller than the primary product from each allele. Even with this method, however, nonadenylated products sometimes competed with the desired product. Differential adenylation sometimes complicated automated genotyping, but in no case did it result in uncertain genotypes when scored manually.

Electrophoretic separation and genotyping of PCR products were carried out using fluorescent capillary electrophoresis on the Applied Biosystems Incorporated (ABI) 3100 genetic analyzer. Dye set G5 was used, providing four discrete labels in addition to a size standard labeled with LIZ. The loci were assayed as two multiplex sets; Set 1: *Ocl1*(VIC), *Ogo4* (NED), *Omy71* (PET), *Omy1001* (PET), *Oneu14* (PET), *Ots1* (NED), *Ots3* (FAM), *Ots4* (VIC), *Ots100* (FAM); Set 2: *Oke4* (VIC), *Oki23*MMBL (FAM), *Omy77* (NED), *Omy1011* (VIC), *Ssa289* (VIC),

Ssa407 (PET), *Ssa408* (NED). The multiplex sets are groups of microsatellite loci that differ in either size or fluorescent label (Fig. 2) and can be assayed simultaneously for a single individual in a single electrophoretic separation (Olsen et al 1996). The term "multiplex" has also been used to indicate simultaneous PCR amplification as well as electrophoresis. Here we refer specifically to the latter. Although, some amplifications in this study were done for pairs of loci in a single reaction, most amplifications targeted a single locus at a time and were pooled subsequently for electrophoretic separation and genotyping. Primer sequences and literature citations are given in Table 2.

In preparation for multiplex genotyping on the ABI 3100, PCR products were diluted and combined in such a way that roughly balanced peak heights were obtained across alleles and loci within each multiplex set (dilutions varied widely among loci and to a lesser extent over time, but typically ranged between 1:100 and 1:400). One microliter of the diluted and pooled products was added to 10 uL formamide containing 0.1 uL LIZ 500 molecular weight size standard (ABI). The samples were injected for 22 sec and electrophoresed through a 36 cm capillary (POP4 polymer) for 40 min at 15 kV and 100 uA at 60°C. Genescan 3.7™ software (ABI) was used to interpret raw fluorescent data and estimate relative fragment mobilities. The Local Southern method of regression analysis (Genescan default) was employed with 250- and 340-bp fragments omitted from consideration due to inconsistent mobilities of these fragments (ABI Technical Support, per. comm.). Genotyper 3.7™ (ABI) was used to label alleles and export tables of genotypes. Samples analyzed multiple times were reconciled to assure consistent genotyping. Size ranges associated with specific allele designations are provided in Table 3 (Genotyper files and raw ABI injection files available on request). Multi-locus

genotypes for all fish representing each population sample were compiled, and allele frequencies for each sample calculated. Although some DNA preparations were recalcitrant to amplification and analysis, nearly every population sample included at least 20 genotypes at every locus (Appendix A).

Error checking and data quality

Microsatellite allele frequencies for each sample were compared to genotypic frequencies as a check of internal consistency and quality control (test of Hardy-Weinberg-Castle expectations using the program FSTAT, Goudet 2001). FSTAT was also used to estimate F_{ST} as a genetic distance metric between all population pairs. To visualize the overall temporal and spatial patterns of allele frequency variation among samples, the program NTSYS (Rohlf 2000) was used to construct a neighbor joining tree from the matrix of pairwise F_{ST} values. Consideration of genetic relationships in the context of error checking sought only to evaluate the internal consistency of the data. There was no *a priori* assumption regarding the structure of relationships, rather the results from each multiplex set was analyzed individually and compared.

Tests for population differentiation were carried out using row-by-column contingency testing of allele frequencies (simulated Fisher's Exact Test). Some investigators have opted for confidence limits on F_{ST} to make this evaluation. The former method is used here because confidence limits on F_{ST} are thought to be overly conservative, e.g., a highly significant result from the contingency test may show an F_{ST} estimate that substantially overlaps zero. This phenomenon is a general attribute of confidence limits on derived statistics.

RESULTS

The most striking result from this study was the high degree of concordance between genetic and geographic distance (Fig. 3). In nearly every case, temporal genetic variation among samples within streams was less than the spatial genetic variation among streams, as evident in the consistent clustering of repeat samples from the same population in the neighbor joining tree (although statistical tests of this observation were not part of this contract and will await peer-reviewed publication). Higher-order geographic structure differentiated major basins of the Snake River drainage (e.g., Clearwater, South Fork Salmon, Middle Fork Salmon rivers). Northeast Oregon populations further grouped into several major clusters largely consistent with their geographic proximities: (a) the lower Grande Ronde River, which included Joseph Creek, Mud Creek, Indian Creek, South Fork Wenaha River, Little Minam River, and a sub-cluster from the upper Wallowa River (Whiskey Creek, Prairie Creek, and Lostine River); (b) a second cluster from the lower Grande Ronde River was represented by streams in southeast Washington (Cottonwood Creek, Cougar Creek, Menatchee Creek, Rattlesnake Creek), Lookingglass Creek, the Wallowa Hatchery stock and a sample of marked hatchery strays trapped at Warm Springs National Fish Hatchery; (c) the upper Grande Ronde River upstream of La Grande, Oregon, (d) mid- and upper Imnaha River, (e) lower Imnaha River (Cow and Lightning creeks). Notable genetic outliers included Dry Creek (Grande Ronde River) and the Pahsimeroi Hatchery stock (upper Salmon River).

One exception to overall geographic concordance was seen in the samples from Cougar Creek and other populations in southeast Washington. In the first versions of Figure 3, used to evaluate potential errors, Cougar Creek samples taken in different years did not cluster together, and one

sample was dramatically different from all others in the study (data not shown). Discussions with field biologists indicated that several of the samples from the lower Grande Ronde River in southeast Washington were thought to be derived from very few returning adults. Although accurate enumeration of summer steelhead is difficult or impossible in these streams, adult returns were known to be small in some of those years, and parr densities were extremely low. It was assumed that the odd results for the lower Grande Ronde River were due to random genetic drift and subsequent population sampling errors associated with collections primarily or exclusively from single brood years (i.e., age 0+ parr); that is, those parr samples genetically over-represented the few adult spawners that returned in a particular year and, thus, did not represent the genetic constitution of the population as a whole (including individuals still out in the ocean). Based on that assumption, samples from each stream in southeast Washington were pooled across years so that the pooled samples would better represent all adult spawners at those sites. This small modification of the primary data (pairwise F_{ST} among all individual sites and years) resulted in the grouping of all the southeast Washington samples in a single cluster, except for the Crooked Creek samples in the Wenaha River basin. The Crooked Creek population had allele frequencies most similar to those from Indian Creek, South Fork Wenaha River, and other middle and lower Grande Ronde River populations.

It was essential to pool the Washington samples, even in the beginning, because the individual annual replicates were so different, both from one another and from the rest of the sample sites, that they introduced a great deal of noise and confusion into any subsequent multivariate analyses (whether clustering or ordination). A full consideration of effective population size and

a more complete analysis of the southeast Washington samples will appear in a future peer-reviewed publication.

Data from the two multiplex sets were analyzed independently and compared in an effort to distinguish data processing errors from anomalous samples or tissue collection and DNA extraction errors. When pairwise F_{ST} values were analyzed for Multiplex 1, a handful of samples showed dramatic departures from the overall pattern of geographic consistency. That is, in most cases replicate samples taken across years from the same location clustered together, whereas a few samples clustered in the wrong river basin on a long branch and far from the other two samples taken in different years (again, most locations were represented by samples taken in each of 3 years). In the analysis of the second multiplex set those "problem" samples clustered in the appropriate basin along with their annual replicates, suggesting that our results were due to a few genotyping errors at a specific locus rather than a problem with the tissue sample itself. In this second analysis, a few other samples that were "well behaved" in the first analysis, now showed anomalous relationships. Therefore, the problem apparently lies in genotyping at specific loci in one multiplex or the other, rather than in field sampling or DNA extraction. The nature and degree of the disparity between multiplex sets virtually precludes simple stochastic variation among loci (data not shown). Again, these disparities reflect lack of internal consistency and are not related to any preconceived notion of expected relationships.

As a result of analyzing each multiplex set individually, we believe the following samples may include subtle genotyping errors and should be viewed with caution: (Multiplex Set 1) Camp Creek 1999 and 2001, Chesnimnus Creek 1999, Gumboot Creek 2000, Lookingglass Creek

2001; (Multiplex Set 2) Gedney Creek 1992, Indian Creek 2001, Meadow Creek 1999 and 2001. These samples are being re-analyzed in an attempt to resolve suspected errors.

In a final summary analysis, all samples were pooled across years within locations or sample sites (Fig. 4). This level of pooling resulted in 52 population samples that showed significant allele frequency variation and, again, strong concordance with geographic proximity and hierarchical basin/sub-basin structure. Again, it was not the intent of this report to present more than the most cursory analysis of these data. Recognizing the legitimacy imparted by scientific peer-review, as well as the large number of collaborators who played a critical role, the agreement among comanagers was that detailed analyses would be deferred to one or more coauthored publications.

DISCUSSION

The observed hierarchical population relationships and the high genetic similarity among temporally-separate samples from the same stream may reflect homing fidelity by anadromous fish, the genetic influence of local resident populations, or some combination of the two. Interestingly, very distinct populations like Prairie Creek and Mud Creek clustered with geographically proximate populations. This was not true, however, for Dry Creek, which proved to be one of the most distinctive populations sampled; although substantial variation existed among years for this population, all three replicate samples clustered together, distinct from all other samples. This apparent genetic divergence of Dry Creek may reflect a large proportion of resident fish. In general, the genetic relationships among sympatric resident and anadromous *O. mykiss* represent an interesting and important problem and clearly warrant further study. More

detailed analyses of samples like Dry Creek will evaluate the likelihood that they represent two discrete, genetically isolated populations collected together as a mixture. However, with an F_{IS} value of 0.03 (one measure of population admixture) and not significantly different from zero, there is little evidence that the Dry Creek parr represent two genetically isolated populations.

Our field sampling design of natural populations for this study was developed after detailed discussions among state, tribal, and Federal biologists familiar with watersheds in the lower Snake River and general sampling problems associated genetic studies of anadromous salmonid fishes. The biologists identified, *a priori,* a number of potential sources of sampling error, both spatial and temporal. The study design involved fin clips collected non-lethally from approximately 50 age 0+ parr from each population (i.e., stream) for three consecutive years. The resulting hierarchical sampling design accounted for (a) spatial variation among sub-basins and among streams within sub-basins (e.g., upper Grande Ronde River vs. lower Grande Ronde River), (b) temporal variation among adults spawning in different years in the same stream, and (c) genetic and field sampling errors associated with analyses of age 0+ parr. The fact that a sample of returning adults taken at the Lightning Creek weir in 2000 clustered as the nearest neighbor to the parr sample taken in the summer of the same year suggests, at least for this one stream, that parr samples can accurately represent anadromous natural populations, if appropriate field sampling precautions are taken (see Materials and Methods). Results from additional anadromous adult samples will be analyzed, to further evaluate the relationships between returning adults and the samples of age 0+ parr used in this study. Offering potential further insight, an ongoing pedigree study in the Imnaha River system includes resident and anadromous adults and all juvenile age classes.

Further analyses

This report presents only preliminary results obtained incidentally through an evaluation of the integrity and internal consistency of the data. With the help of several state, tribal, and Federal collaborators and coauthors, I will be conducting more detailed analyses and will draft a complete manuscript for publication in the peer-reviewed scientific literature. The manuscript will not address fishery management implications but will focus strictly on the scientific presentation of the data and the population genetics and evolutionary biology of *O. mykiss* in the lower Snake River.

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Table 1. *Oncorhynchus mykiss* tissue samples from locations in the Grande Ronde and Imnaha river basins with additional representatives from sub-basins throughout the Snake River. (Switch order of sub-basin and basin columns. Check sub-basin number. The Wenaha R. and Grande Ronde R descriptions need change columns to be consistent with reminder of table.

Code	Site	Basin	Sub-basin	Coll'n yr	Ad vs juv	BY	N	Total	Collector	CBD Acc. #
Hatchery Samples										
Dwo97	Dworshak Hatchery	Clearwater R.	NF Clearwater	1997	J	1996	48	60	IDFG	32371
Lsh01	Little Sheep Hatchery	Imnaha R.	Imnaha R.	2001	A	2001	48	779	ODFW	32804
Pah90	Pahsimeroi Hatchery	Salmon R.	Salmon R.	1990	J	1988	48	48	IDFG	30548
Rou90	Round Butte Hatchery	Deschutes R.	Deschutes R.	1990	A	N/A	21	21	CTWSR	32759
Wal95	Wallowa Hatchery	Grande Ronde R.	Wallowa R.	1995	J	1993	48	80	ODFW	32110
Was99	Strays to Warm Springs NFH	Deschutes R.	Deschutes R.	1999	A	N/A	48	50	CTWSR	32758
Wild Samples										
Pel98	Pelton Trap	Deschutes R.	Deschutes R.	1998	A	N/A	48	50	CTWSR	32734
Shi00	Shitike Cr. weir	Deschutes R.	Deschutes R.	2000	A	N/A	11	11	CTWSR	32735
Shi01	Shitike Cr. weir	Deschutes R.	Deschutes R.	2001	A	N/A	48	48	CTWSR	32736
War99	Warm Springs NFH	Deschutes R.	Deschutes R.	1999	A	N/A	33	33	CTWSR	32737
War01	Warm Springs NFH	Deschutes R.	Deschutes R.	2001	A	N/A	18	18	CTWSR	32738
Aso95	Asotin Cr.	Snake R.	Snake R.	1995	J		48	53	WDFW	32902
Aso00	Asotin Cr.	Snake R.	Snake R.	2000	J		48	99	WDFW	32950
Aso01	Asotin Cr.	Snake R.	Snake R.	2001	J		48	99	WDFW	32951
Tuc91	Tucannon R.	Snake R.	Snake R.	1991	J	1990	40	40	WDFW	30677
Tuc95	Tucannon R.	Snake R.	Snake R.	1992	J	1991	40	40	WDFW	30772
Tuc92	Tucannon R.	Snake R.	Snake R.	1995	J	1994	48	48	WDFW	32131
Pot00	EF Potlatch R.	Clearwater R.	Clearwater R.	2000	J		48	48	IDFG	33006
Ten00	Ten Mile Cr.	Clearwater R.	Clearwater R.	2000	J		48	48	IDFG	33003
Can00	Canyon Cr.	Clearwater R.	Lochsa R.	2000	J	2000	48	48	IDFG	33004
Fis90	Fish Cr.	Clearwater R.	Lochsa R.	1990	J	1989	48	54	IDFG	30564
Fis91	Fish Cr.	Clearwater R.	Lochsa R.	1991	J	1990	48	90	IDFG	30673

Fis93	Fish Cr.	Clearwater R.	Lochsa R.	1993	J	1992	48	64	IDFG	32002
Bea00	Bear Cr.	Clearwater R.	Selway R.	2000	J		48	48	IDFG	33000
Ged90	Gedney Cr.	Clearwater R.	Selway R.	1990	J	1989	48	83	IDFG	30563
Ged91	Gedney Cr.	Clearwater R.	Selway R.	1991	J	1990	48	80	IDFG	30672
Ged92	Gedney Cr.	Clearwater R.	Selway R.	1992	J	1991	48	80	IDFG	30774
Rap00	Rapid R.	Salmon R.	Little Salmon R.	2000	J		48	48	IDFG	33005
Bar99	Bargamin Cr.	Salmon R.	Salmon R.	1999	J		48	48	NMFS	31178
Hos00	Horse Cr.	Salmon R.	Salmon R.	2000	J		48	48	IDFG	33008
Big00	Big Cr. (lower)	Salmon R.	MF Salmon R.	2000	J		48	48	IDFG	33002
Loo99	Loon Cr.	Salmon R.	MF Salmon R.	1999	J		48	48	NMFS	31179
Mar00	Marsh Cr.	Salmon R.	MF Salmon R.	2000	J		48	48	IDFG	33001
Joh00	Johnson Cr.	Salmon R.	SF Salmon R.	2000	J		48	48	IDFG	32999
Sec99	Secesh R.	Salmon R.	SF Salmon R.	1999	J		30	30	NMFS	32650
Sto00	Stolle Meadows	Salmon R.	SF Salmon R.	2000	J		48	48	IDFG	33007
Cat99	Catherine Cr.	Grande Ronde R.	Grande Ronde R.	1999	J	1999	48	117	CTUIR/ODFW	32809
Cat00	Catherine Cr.	Grande Ronde R.	Grande Ronde R.	2000	J	2000	34	34	CTUIR/ODFW	32824
Cat01	Catherine Cr.	Grande Ronde R.	Grande Ronde R.	2001	J	2001	48	79	CTUIR/ODFW	32879
Cot00	Cottonwood Cr.	Grande Ronde R.	Grande Ronde R.	2000	J		48	60	WDFW	32947
Cot01	Cottonwood Cr.	Grande Ronde R.	Grande Ronde R.	2001	J		48	60	WDFW	32948
Cou99	Cougar Cr.	Grande Ronde R.	Grande Ronde R.	1999	J		40	40	WDFW	32936
Cou00	Cougar Cr.	Grande Ronde R.	Grande Ronde R.	2000	J		48	58	WDFW	32941
Cou01	Cougar Cr.	Grande Ronde R.	Grande Ronde R.	2001	J		20	20	WDFW	32944
Dry99	Dry Cr.	Grande Ronde R.	Grande Ronde R.	1999	J	1999	48	100	CTUIR/ODFW	32808
Dry00	Dry Cr.	Grande Ronde R.	Grande Ronde R.	2000	J	2000	48	100	CTUIR/ODFW	32826
Dry01	Dry Cr.	Grande Ronde R.	Grande Ronde R.	2001	J	2001	48	55	CTUIR/ODFW	32889
Fly99	Fly Cr.	Grande Ronde R.	Grande Ronde R.	1999	J	1999	48	100	CTUIR/ODFW	32806
Fly00	Fly Cr.	Grande Ronde R.	Grande Ronde R.	2000	J	2000	48	100	CTUIR/ODFW	32819
Fly01	Fly Cr.	Grande Ronde R.	Grande Ronde R.	2001	J	2001	48	79	CTUIR/ODFW	32882
Ind99	Indian Cr.	Grande Ronde R.	Grande Ronde R.	1999	J	1999	48	73	CTUIR/ODFW	32811
Ind00	Indian Cr.	Grande Ronde R.	Grande Ronde R.	2000	J	2000	48	100	CTUIR/ODFW	32821
Ind01	Indian Cr.	Grande Ronde R.	Grande Ronde R.	2001	J	2001	48	69	CTUIR/ODFW	32887

Lok99	Lookingglass Cr.	Grande Ronde R.	Grande Ronde R.	1999	J	1999	48	151	CTUIR/ODFW	32807
Lok00	Lookingglass Cr.	Grande Ronde R.	Grande Ronde R.	2000	J	2000	48	54	CTUIR/ODFW	32823
Lok01	Lookingglass Cr.	Grande Ronde R.	Grande Ronde R.	2001	J	2001	48	85	CTUIR/ODFW	32877
Mea99	Meadow Cr.	Grande Ronde R.	Grande Ronde R.	1999	J	1999	48	100	CTUIR/ODFW	32815
Mea00	Meadow Cr.	Grande Ronde R.	Grande Ronde R.	2000	J	2000	48	100	CTUIR/ODFW	32818
Mea01	Meadow Cr.	Grande Ronde R.	Grande Ronde R.	2001	J	2001	48	51	CTUIR/ODFW	32885
Men99	Menatchee Cr.	Grande Ronde R.	Grande Ronde R.	1999	J		40	40	WDFW	32938
Men00	Menatchee Cr.	Grande Ronde R.	Grande Ronde R.	2000	J		48	60	WDFW	32939
Men01	Menatchee Cr.	Grande Ronde R.	Grande Ronde R.	2001	J		48	60	WDFW	32945
Mud99	Mud Cr.	Grande Ronde R.	Grande Ronde R.	1999	J	1999	48	100	CTUIR/ODFW	32814
Mud00	Mud Cr.	Grande Ronde R.	Grande Ronde R.	2000	J	2000	48	73	CTUIR/ODFW	32820
Mud01	Mud Cr.	Grande Ronde R.	Grande Ronde R.	2001	J	2001	48	67	CTUIR/ODFW	32886
Rat99	Rattlesnake Cr.	Grande Ronde R.	Grande Ronde R.	1999	J		39	39	WDFW	32937
Rat00	Rattlesnake Cr.	Grande Ronde R.	Grande Ronde R.	2000	J		48	60	WDFW	32940
Rat01	Rattlesnake Cr.	Grande Ronde R.	Grande Ronde R.	2001	J		48	60	WDFW	32946
Che99	Chesnimnus Cr./Devil's Run	Grande Ronde R.	Joseph Cr.	1999	J	1999	48	70	NPT	32798
Che00	Chesnimnus Cr./Devil's Run	Grande Ronde R.	Joseph Cr.	2000	J	2000	48	85	NPT	32837
Che01	Chesnimnus Cr./Devil's Run	Grande Ronde R.	Joseph Cr.	2001	J	2001	48	100	NPT	32930
Elk99	Elk Cr.	Grande Ronde R.	Joseph Cr.	1999	J	1999	48	92	NPT	32799
Elk00	Elk Cr.	Grande Ronde R.	Joseph Cr.	2000	J	2000	48	94	NPT	32834
Elk01	Elk Cr.	Grande Ronde R.	Joseph Cr.	2001	J	2001	48	82	NPT	32931
Min99	Little Minam R.	Grande Ronde R.	Wallowa R.	1999	J	1999	48	64	CTUIR/ODFW	32812
Min00	Little Minam R.	Grande Ronde R.	Wallowa R.	2000	J	2000	48	91	CTUIR/ODFW	32827
Min01	Little Minam R.	Grande Ronde R.	Wallowa R.	2001	J	2001	48	86	CTUIR/ODFW	32890
Los99	Lostine R.	Grande Ronde R.	Wallowa R.	1999	J	1999	48	59	CTUIR/ODFW	32805
Los00	Lostine R.	Grande Ronde R.	Wallowa R.	2000	J	2000	48	66	CTUIR/ODFW	32828
Los01	Lostine R.	Grande Ronde R.	Wallowa R.	2001	J	2001	48	74	CTUIR/ODFW	32881
Pra99	Prairie Cr.	Grande Ronde R.	Wallowa R.	1999	J	1999	48	65	CTUIR/ODFW	32813
Pra00	Prairie Cr.	Grande Ronde R.	Wallowa R.	2000	J	2000	48	100	CTUIR/ODFW	32822
Pra01	Prairie Cr.	Grande Ronde R.	Wallowa R.	2001	J	2001	48	75	CTUIR/ODFW	32878
Whi99	Whiskey Cr.	Grande Ronde R.	Wallowa R.	1999	J	1999	48	100	CTUIR/ODFW	32810

Whi00	Whiskey Cr.	Grande Ronde R.	Wallowa R.	2000	J	2000	48	100	CTUIR/ODFW	32817
Whi01	Whiskey Cr.	Grande Ronde R.	Wallowa R.	2001	J	2001	48	100	CTUIR/ODFW	32880
Bsh99	Big Sheep Cr.	Imnaha R.	Imnaha R.	1999	J	1999	48	66	NPT	32800
Bsh00	Big Sheep Cr.	Imnaha R.	Imnaha R.	2000	J	2000	48	85	NPT	32838
Bsh01	Big Sheep Cr.	Imnaha R.	Imnaha R.	2001	J	2001	48	74	NPT	32929
Cam99	Camp Cr.	Imnaha R.	Imnaha R.	1999	J	1999	48	100	NPT	32801
Cam00	Camp Cr.	Imnaha R.	Imnaha R.	2000	J	2000	48	99	NPT	32836
Cam01	Camp Cr.	Imnaha R.	Imnaha R.	2001	J	2001	48	77	NPT	32928
Cow99	Cow Cr.	Imnaha R.	Imnaha R.	1999	J	1999	48	96	NPT	32832
Cow00	Cow Cr.	Imnaha R.	Imnaha R.	2000	J	2000	48	100	NPT	32835
Cow01	Cow Cr.	Imnaha R.	Imnaha R.	2001	J	2001	48	93	NPT	32926
Gum99	Gumboot Cr.	Imnaha R.	Imnaha R.	1999	J	1999	48	72	NPT	32797
Gum00	Gumboot Cr.	Imnaha R.	Imnaha R.	2000	J	2000	48	92	NPT	32840
Gum01	Gumboot Cr.	Imnaha R.	Imnaha R.	2001	J	2001	48	90	NPT	32927
Hor00	Horse Cr.	Imnaha R.	Imnaha R.	2000	J	2000	48	100	NPT	32803
Hor99	Horse Cr.	Imnaha R.	Imnaha R.	1999	J	1999	48	94	NPT	32833
Hor01	Horse Cr.	Imnaha R.	Imnaha R.	2001	J	2001	48	70	NPT	32925
Lig99	Lightning Cr.	Imnaha R.	Imnaha R.	1999	J	1999	48	90	NPT	32831
Lig00	Lightning Cr.	Imnaha R.	Imnaha R.	2000	J	2000	48	77	NPT	32839
Lig01	Lightning Cr.	Imnaha R.	Imnaha R.	2001	J	2001	48	81	NPT	32924
Liw00	Lightning Cr. Weir	Imnaha R.	Imnaha R.	2000	A	2000	35	35	NPT	32802
Lsc00	Little Sheep Cr.	Imnaha R.	Imnaha R.	2000	A	2000	48	58	ODFW	32720
Cro99	Crooked Cr.	Wenaha R.	Grande Ronde R.	1999	J		48	60	WDFW	32935
Cro00	Crooked Cr.	Wenaha R.	Grande Ronde R.	2000	J		48	60	WDFW	32942
Cro01	Crooked Cr.	Wenaha R.	Grande Ronde R.	2001	J		48	60	WDFW	32943
Wen99	SF Wenaha R.	Wenaha R.	Grande Ronde R.	1999	J	1999	48	100	CTUIR/ODFW	32816
Wen00	SF Wenaha R.	Wenaha R.	Grande Ronde R.	2000	J	2000	48	99	CTUIR/ODFW	32825
Wen01	SF Wenaha R.	Wenaha R.	Grande Ronde R.	2001	J	2001	48	100	CTUIR/ODFW	32888
Total	112 samples, 46 field sites, 5 hatchery stocks, 1 sample of marked strays	7 river basins	14 sub-basins				5153	8609	7 agencies	

Table 2. Polymerase chain reaction primers used to amplify and genotype steelhead microsatellite loci (unpublished primers are referenced to Genbank accession number where appropriate).

Locus name	Annealing temp. (°C)	Final ext. temp. (°C)	Primer name*	Conc. (uM)	Primer sequence	Reference
Ocl1	60	72	*Ocl1*-F (NED) *Ocl1*-R	0.15	5'-ACT ACT AAC CAG CCC ACC ACC C-3' 5'-AGA CAG AGA GGG AGG GAA GC-3'	Condrey and Bentzen 1998
Oke4	54	54	*Oke4*-F(VIC) *Oke4*-R	0.4	5'-AGG CCC AAA GTC TGT AGT GAA GG-3' 5'-GAT GAA TCG AGA GAA TAG GGA CTG AAT-3'	Buchholz et al. 1999
Ogo4	48	72	*Ogo4*-F (HEX) *Ogo4*-R	0.6	5'-GTC GTC ACT GGC ATC AGC TA-3' 5'-GAG TGG AGA TGC AGC CAA AG-3'	Olsen et al. 1998
Oki23MMBL	54	72	*Oki23MMBL*-F (FAM) *Oki23MMBL*-R	0.6	5'-TGT GCT ATA GGG TGA ATG TGC-3' 5'-AAC AAC GGC ATC CCC ACT AA-3'	#AF272822
Omy7INRA	56	72	*Omy7*-F(PET) *Omy7*-R	0.4	5'-TTA AGT TTT GCC TAG ATA AGG G-3' 5'-CAA GGA ATG GCA CAG CTT G-3'	K. Gharbi, pers. comm.
Omy77	48	48	*Omy77*-F(NED) *Omy77*-R	0.6	5'-CGT TCT CTA CTG AGT CAT-3' 5'-GGG TCT TTA AGG CTT CAC TGC A-3'	Morris et al. 1996
Omy1001	60	60	*Omy1001*-F(PET) *Omy1001*-R	0.4	5'-GAT TCC ATA ACC TCG CCT TC-3' 5'-GTC CTT GTG CTG CCT GCT-3'	P. Bentzen pers. comm.
Omy1011	54	72	*Omy1011*-F(VIC) *Omy1011*-R	0.4	5'-AAC TTG CTA TGT GAA TGT GC-3' 5'-GAC AAA AGT GAC TGG TTG GT-3'	P. Bentzen pers. comm.
Oneu14	60	72	*Oneu14*-F(PET) *Oneu14*-R	0.3	5'-AGA AAC ATG AGA AAC GTC TAG GT-3' 5'-CCT TAT GAG TTT GGT CTC CAT GT-3'	Scribner et al. 1996
*Ots*1	52	72	*Ots1*-F (FAM) *Ots1*-R	0.3	5'-GGA AAG AGC AGA TGT TGT TAA-3' 5'-GAA GCA GCA GAT AAA GCA-3'	Banks et al. 1999

Ots3	47	72	*Ots3*-F (FAM) *Ots3*-R	0.3	5'-CAC ACT CTT TCA GGA G-3' 5'-AGA ATC ACA ATG GAA G-3'	Banks et al. 1999
Ots4	60	60	*Ots4*-F (HEX) *Ots4*-R	0.15	5'-GAC CCA GAG CAC AGC AAC A-3' 5'-GGA GGA CAC ATT TCA GCA G-3'	Banks et al. 1999
Ots100	54	54	*Ots100*-F (FAM) *Ots100*-R	0.3	5'-TGA AAC TGA GCT GTG TGA G-3' 5'-ACG GAC GTG CCA GTG AG-3'	Nelson and Beacham 1999
Ssa289	54	54	*Ssa289*-F (HEX) *Ssa289*-R	0.4	5'-CTT TAC AAA TAG AAC GAC T-3' 5'-TCA TAC AGT CAC TAT CAT C-3'	McConnell et al. 1995
Ssa407	60	60	*Ssa407*-F(PET) *Ssa407*-R	0.4	5'-TGT GTA GGC AGG TGT GGA C-3' 5'-CAC TGC TGT TAC TTT GGT GAT TC-3'	Cairney et al. 2000
Ssa408	54	54	*Ssa408*-F(NED) *Ssa408*-R	0.4	5'-AAT GGA TTA CGG GTA CGT TAG AAC-3' 5'-CTC TTG TGC AGG TTC TTC ATC TGT-3'	Cairney et al. 2000

*Labeled microsatellite primers are identified by a fluorescent dye given after the primer name.

Figures:

Figure 1. Map of locations (Please see Adobe Acrobat file “SRSH02_Fig1.pdf”)

Figure 2. Multiplex sets (Excel file “SRSH02_Fig2.pdf”)

Figure 3. Neighbor joining dendrogram (Adobe Acrobat file “SRSH02_Fig3&4.pdf”)

Figure 4. Simplified dendrogram (Adobe Acrobat file “SRSH02_Fig3&4.pdf”)

Appendices:

Appendix A. Allele frequencies (Excel file “SRSH02_AppA&B.xls”)

Appendix B. Allele sizes and designations (Excel file “SRSH02_AppA&B.xls”)

Appendix C. Microsatellite genotypes (Text file “SRSH02_AppC.gen” formatted for Genepop input)

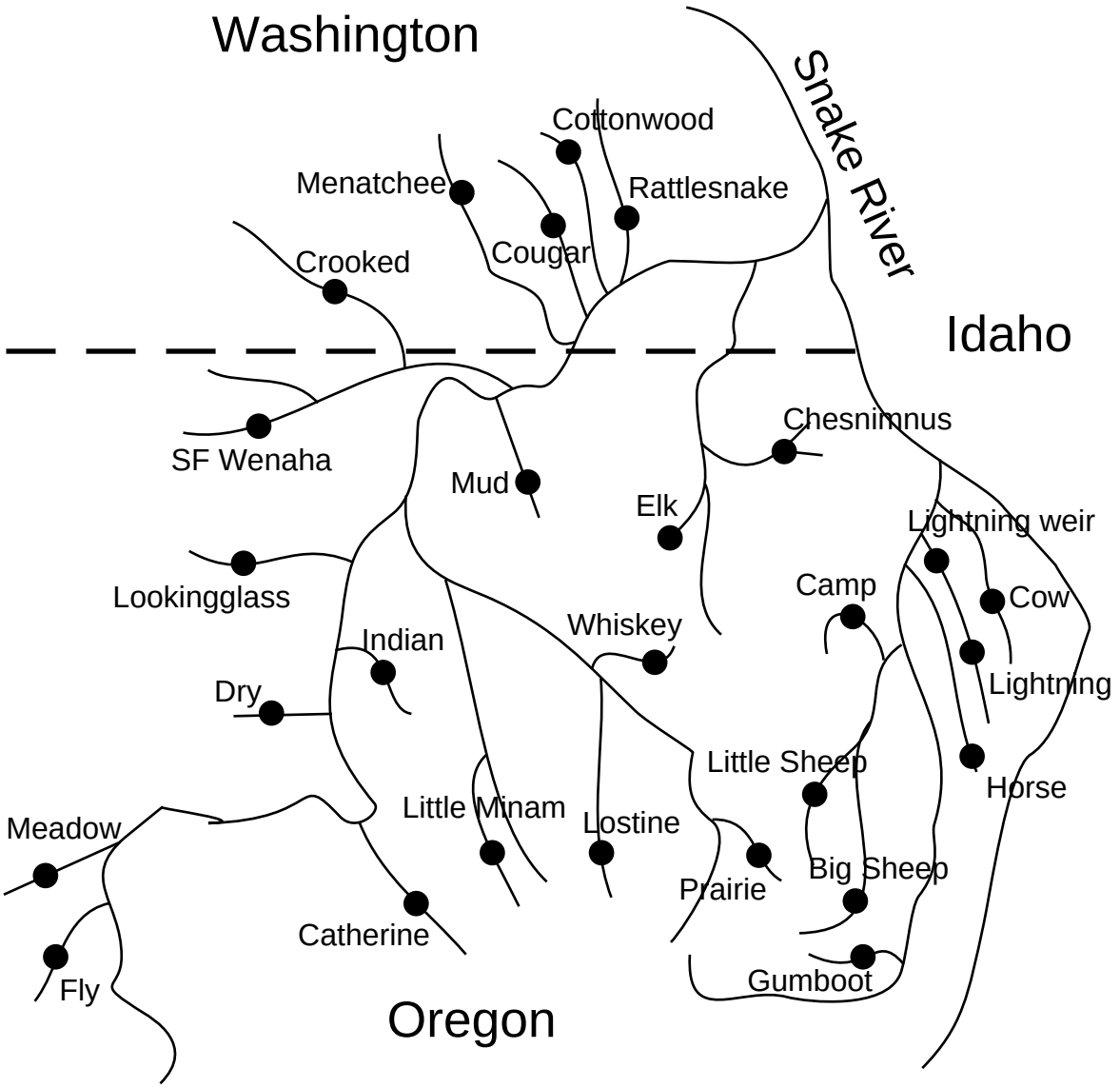

Figure 1. Sampling locations for study of *O. mykiss* population genetics in the Grande Ronde and Imnaha river basins (sample sites in the Salmon, Clearwater, and Deschutes river basins are not shown).

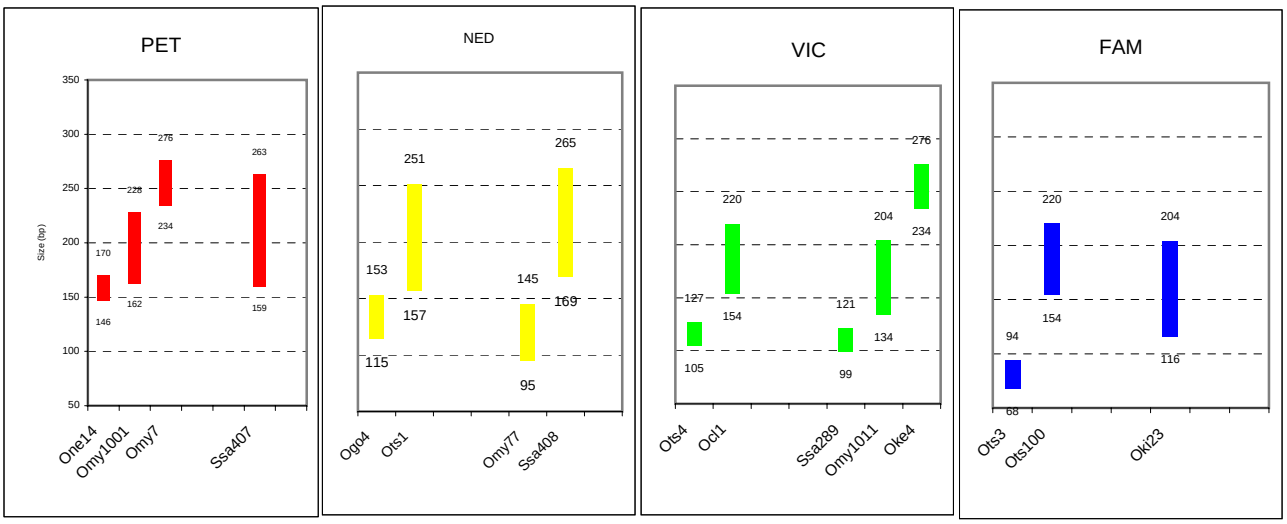

Figure 2. Multiplex sets showing size ranges of 16 microsatellite loci used for characterization of Snake River *O. mykiss*. Multiplex 1 is shown on the left of each panal, whereas Multiplex 2 loci appear on the right.

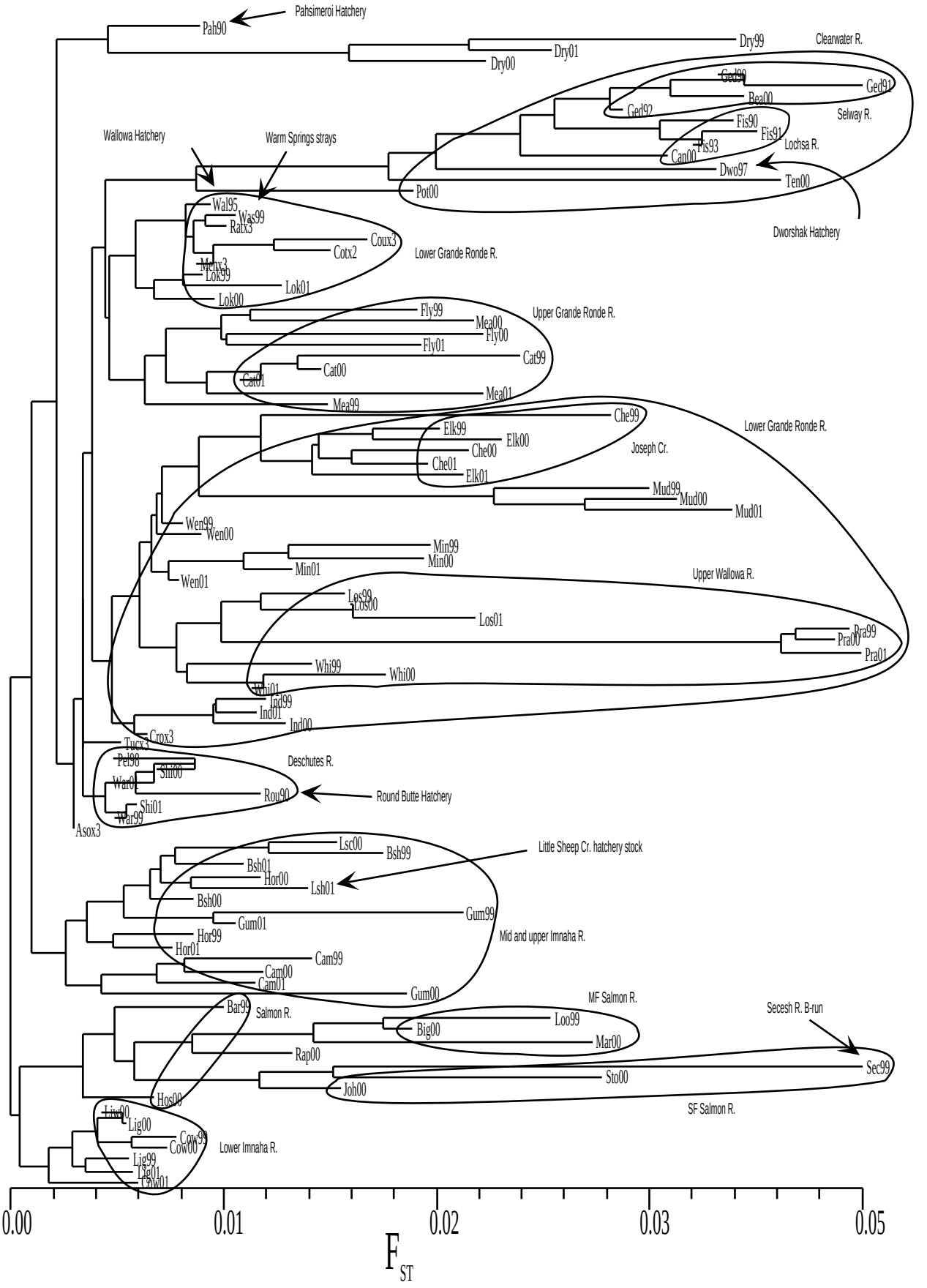

Fig. 3. Neighbor joining cluster analysis of pairwise F_{ST} values derived from 16 microsatellite loci (SE Washington samples pooled across years).

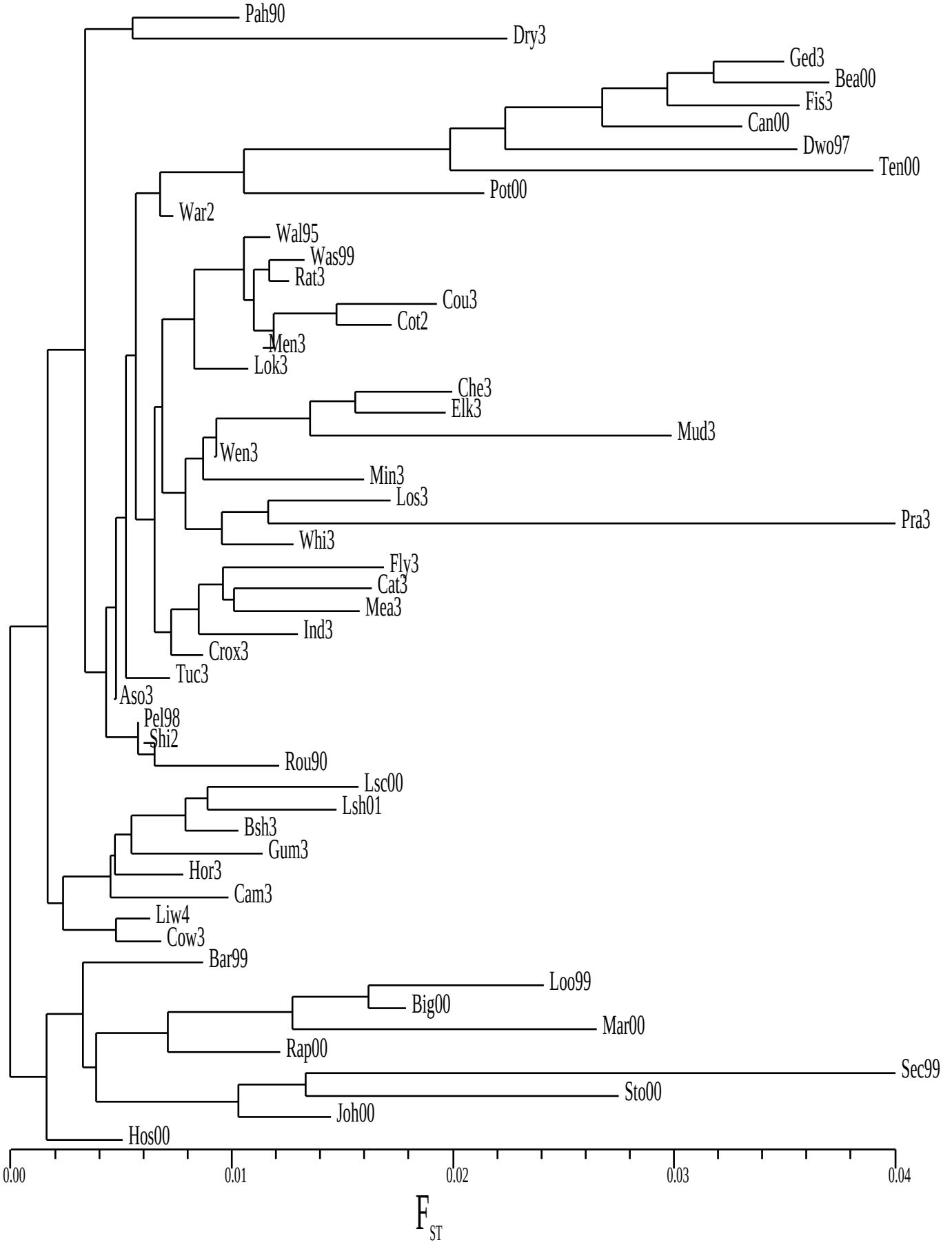

Fig. 4. Neighbor joining cluster analysis of pairwise F_{ST} values derived from 16 microsatellite loci (All samples pooled across years).