

Genetic assignments for migratory bull trout captured in the Walla Walla and Umatilla rivers: assessing connectivity through genetic analyses

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Background

Bull trout (*Salvelinus confluentus*) spawn in cold, headwater streams and express a variety of life histories categorized by migration strategy. In the Columbia Basin, residents remain in their natal stream; fluvial and adfluvial bull trout, after rearing a year or two in their natal stream, migrate out to larger tributaries and lakes, respectively, for feeding and rearing then return to spawn in their natal stream. Migration is thus a critical component of bull trout life history and directly impacts their growth and reproductive success. Because of severe population declines, some related to migration impediments, bull trout were listed as threatened throughout the coterminous United States in 1999 (USFWS 2002). Studies are now underway to assess migration patterns, distribution, and abundance of bull trout as baseline information for recovery planning.

Several tributaries to the Snake and Columbia rivers contain migratory bull trout populations. The Walla Walla River (Figure 1), a tributary of the Columbia River, supports five bull trout local populations within two core areas (USFWS 2002). These include three local populations in the Touchet River Core Area (North Fork and South Fork Touchet River, and Wolf Creek), and two in the Walla Walla Core Area (South Fork Walla Walla River and Mill Creek). In the Walla Walla basin, migratory adult bull trout return to headwater spawning areas in September and October. Following spawning, most individuals migrate downstream to overwintering areas from October through December. While juveniles remain in their natal tributary for at least a year, migratory subadult and adult bull trout use the lower Walla Walla

River and the mainstem Columbia River during fall, winter, and spring for rearing and overwintering (Anglin et al. 2009a, 2009b, 2010, 2010a; Barrows et al. 2012a, 2012b).

Anthropogenic changes to migratory corridors (eg. irrigation diversions, dikes, and hydroelectric dams) potentially impact connectivity among bull trout spawning and rearing areas as well as connectivity among local populations and between core areas (metapopulations). Migratory corridors lacking sufficient passage and with poor riverine habitat conditions (eg. low streamflow and high temperature) may impede migration and isolate historically connected populations (Barrows 2012b). In addition to affecting migration timing, these altered habitats may foster exotic predators and novel competitors (Williams et al. 2005; Ferguson et al. 2005).

This genetic analysis is a component of a broader effort to describe connectivity between bull trout populations within the Walla Walla Basin as well as to neighboring basins (i.e., Umatilla and Tucannon basins). Following a metapopulation model, connectivity between bull trout populations relies on migratory corridors that allow occasional gene flow as fish from one population disperse downstream and subsequently ascend non-natal streams for spawning.

Objectives

1. Determine the population of origin for migratory bull trout that leave the Columbia River and ascend the adult fish ladder at Three Mile Falls Dam on the Umatilla River.
2. Determine the population of origin for migratory bull trout sampled in mid- and lower basin portions of the Walla Walla River.
3. Determine the population of origin for bull trout sampled in the North Fork Walla Walla River and compare them to bull trout from South Fork Walla Walla River.

Materials and Methods

Sampling: A total of 74 tissue samples were collected over three years (Table 1) from bull trout encountered at the Three Mile Falls Dam fish ladder in the Umatilla River (N = 8) and throughout the Walla Walla River (N = 66). Sample information (date, river kilometer, capture method, size, tag number) is detailed in Table 2.

Genotyping: For each tissue sample DNA was extracted using silica membrane based kits (Macherey-Nagel) following standard protocol. Bull trout were genotyped with 16 microsatellite loci used for the range-wide bull trout study (Ardren et al. 2011). Microsatellite alleles were amplified by PCR (polymerase chain reaction) following PCR conditions detailed in Small et al. (2009). PCR products were visualized using an ABI-3730 DNA Analyzer with internal size standards (GS500LIZ 3730) and GeneMapper 3.7 software. To keep data compatible with the range-wide bull trout database, allele nomenclature was standardized to nomenclature for data in Ardren et al. (2011). We confirmed that each sample was unique by testing the data set for matching genotypes using the Microsatellite Toolkit, an Excel Add-In (available online from Stephen Park, UC Dublin, <http://animalgenomics.ucd.ie/sdepark/ms-toolkit/>).

Statistical analyses: Bull trout population origins were examined using three analytical methods. In the first method, we used GENETIX (Belkhir et al. 2001) to conduct factorial correspondence analyses (FCA) to visualize relationships between the bull trout of unknown origins and bull trout collections from the WDFW and range-wide (RWDB) bull trout databases. A FCA generates composite axes from combinations of allele frequencies in the data set that explain the maximum genetic variance and plots individual genotypes within the space defined by the axes. The first three axes generally define most of the genetic variance. Individuals that are genetically similar plot near each other and individuals that are genetically different plot distantly from each other.

In the second method, we used a Bayesian clustering analysis implemented in STRUCTURE 2.2 (Pritchard et al. 2000) to estimate individual and population ancestry. STRUCTURE sorts individuals (or portions of individuals if they are hybrids) into hypothetical population clusters (K), minimizing Hardy-Weinberg disequilibrium and linkage disequilibrium in the clusters. These clusters are composed of individuals with common ancestry shared through contemporary or recent gene flow. Bull trout populations are spatially segregated and natal fidelity is high such that contemporary gene flow is minimal. Thus, all members of a baseline population would occupy the same cluster and unknown migratory individuals would occupy the same genetic cluster as members of their home population and be identified back to origin. We conducted analyses in 5 independent runs that allowed admixture with 50,000 burn-ins (a randomization procedure that prevents starting conditions from influencing outcomes) and 200,000 iterations and observed solution stabilities at each K. For each run, the program outputs a likelihood value for the number of clusters, given the data. When the likelihood value reaches a maximum or asymptote the program has resolved the identifiable genetic groups in the data set.

In the third method, we conducted an assignment test using the program GeneClass (Piry et al. 2004). Baseline collections consisted of the 9 populations listed in Table 1, including four populations from the Walla Walla basin, three populations from the Snake basin and one from the Umatilla. We first tested the baseline to confirm that fish in baseline collections assigned to their home collection (supporting that unknown fish would assign to their home collection) and then assigned USFWS bull trout of unknown origins to the baseline. In the baseline test, using the algorithm from (Rannala and Mountain 1997), each fish was assigned a likelihood of being observed in each baseline collection based on the genotype of the fish and allele frequencies of baseline collections (each fish was removed from its home collection during estimation). The fish is assigned to the collection with the highest likelihood. The migratory USFWS bull trout were assigned as unknown fish to the baseline. The procedure is the same for unknown fish (uses the same algorithm to calculate likelihoods of assignment to each baseline collection) and the highest likelihood suggests the collection of origin for the fish. Because baseline individuals assigned back to their home collection there was sufficient power in the genotypic data to assign unknown fish to their population of origin.

Because adult bull trout migrate throughout waterways and may have been sampled in the Walla Walla system but originated outside the system, we also calculated exclusion probabilities for each fish using a Monte Carlo simulation (Paetkau et al. 2004) to estimate whether the fish's population of origin was excluded from the baseline. The simulation created 10,000 individuals for each baseline collection to create genotypes likely to be encountered for that collection. Then

it computed assignment likelihoods for simulated individuals and compared the likelihood of the genotype of the fish in question to the distribution of the likelihoods for simulated baseline collections. If probabilities of being sampled in each baseline collection were all below 1%, then the individual likely arose from a population outside the baseline.

Results

Genotyping was successful for most USFWS bull trout. All genotypes were unique: no fish had matching genotypes. One fish (07NX0099) had insufficient data (5 loci in its genotype, likely due to low DNA quantity) to allow confident assignment for that fish and was excluded from analyses.

In exploratory tests, we included a bull trout population from the Yakima, but it was so different that it added no information beyond the fact that no USFWS unknown bull trout came from the Yakima basin (data not shown). Yakima bull trout were excluded from further analyses. The baseline bull trout data plotted in three distinct clusters along the first two axes in the factorial correspondence analysis (FCA) plots (Figure 2a): one cluster consisted of South Fork Walla Walla River and Mill Creek bull trout, one cluster consisted of North Fork Touchet River and Wolf Creek bull trout, and one cluster consisted of Wenaha, Asotin and Tucannon rivers bull trout. The Umatilla River bull trout cluster was superimposed on the South Fork Walla Walla and Mill cluster but separated from them along the third axis (Figure 2b). Most of the USFWS bull trout clustered with the South Fork Walla Walla bull trout (Figure 2c), but three fish, 07NX0067, 07NX0074 and 07NX0095, clustered with the Mill Creek bull trout and one fish, 10PV0002, clustered with the Tucannon River bull trout.

The STRUCTURE analysis supported genetic distinction among baseline collections and the identities of unknown-origin USFWS bull trout (Figure 3). In the STRUCTURE plot, each fish's ancestry is represented by a bar of color, with the color representing a genetic cluster or population. Where fish have a single color in their bar, all of their ancestry was in one cluster and where fish have more than one color in their bar, their ancestry may be mixed. Fish may also appear to have small amounts of mixture (small amount of different color in the bar) if by chance they have an allele in their genotype that is more common in a different population. Most baseline collections occupied their own genetic cluster (had a single color represented in all of their individual fish's color bars) and Asotin and Wenaha bull trout separated at higher K value (not shown). Most of the USFWS bull trout were in the South Fork Walla Walla cluster (had a dark blue color bar), two fish (07NX0074 and 07NX0095) were in the Mill cluster (had a red color bar), one fish (07NX0067) had roughly even ancestry in the South Fork Walla Walla and Mill clusters (had roughly 50:50 dark blue and red color bar), and one fish (10PV0002) was in the Tucannon cluster (had a green color bar).

The GeneClass assignment results concurred with the origins of unknown USFWS fish suggested by cluster in the FCA and STRUCTURE analysis (Table 2). Most USFWS bull trout assigned to South Fork Walla Walla, two fish, 07NX0074 and 07NX0095, assigned to Mill, two fish, 07NX0026 and 07NX0067 had roughly equal assignment likelihoods to South Fork Walla Walla and Mill, and one fish, 10PV0002, assigned to Tucannon. In this analysis, the fish

07NX0026 appeared to have mixed ancestry because it assigned with nearly equal likelihood to two collections (Appendix I). However, the fish had 8 loci in its genotype which is minimal genetic information for an assignment test and likely contributed to this result. The exclusion analysis supported that all USFWS unknown-origin bull trout originated from populations in the baseline. There was no indication that any bull trout came from populations absent from the baseline.

We use these three methods because they analyze the data from different perspectives and we are testing for congruence among the methods as support for conclusions. The FCA is not an assignment test, but plots genotypes according to genetic similarities and differences in a genetic space. The STRUCTURE analysis has no *a priori* knowledge of the baseline populations and finds genetic clusters in the data set based on principles of population structure. In the GeneClass assignments, we construct an *a priori* baseline (tested to confirm its utility) and calculate likelihoods that unknown fish came from each of the baseline collections, with the highest likelihood indicating the population of origin.

In summary, congruence among results from the three methods confirm that bull trout ascending the fish ladder in the Umatilla River originated in the South Fork Walla Walla River (N = 7) and the Tucannon River (N = 1); bull trout collected in the North Fork Walla Walla River originated in the South Fork Walla Walla River; most of the bull trout migrating within the Walla Walla River were from the South Fork Walla Walla River and two or three were from Mill Creek (one individual appeared to be mixed ancestry between Walla Walla and Mill populations).

Discussion

Our results indicate that all eight bull trout trapped since 2007 at Three Mile Falls Dam in the Umatilla River originated outside the Umatilla Basin. Telemetry work conducted by USFWS demonstrated that one of these fish successfully navigated the mainstem Umatilla River and reached known spawning grounds in the North Fork Umatilla River during bull trout spawning period (P. Sankovich, personal communication). The remaining bull trout ascended the Umatilla River but did not reach the spawning grounds. The confirmed influx of migratory bull trout from the Walla Walla and Tucannon rivers into the Umatilla River and tracking of one individual to spawning grounds, demonstrate the possibility for biological interaction between bull trout from the Walla Walla, Tucannon, and Umatilla rivers. However, because migratory bull trout have been observed entering adjacent tributaries not known to be utilized by bull trout for spawning (Mahoney et al. 2006; Ratliff et al. 1996; M. Barrows, unpublished data), the out-of-basin bull trout tracked to a spawning site may have been there for reasons other than spawning, such as feeding. A closer genetic relationship between the Walla Walla and Umatilla rivers bull trout than between the Walla Walla and Tucannon rivers bull trout (M. Small, unpublished data), suggests that bull trout from the Walla Walla River do occasionally spawn with Umatilla River bull trout. The population of bull trout in the North Fork Umatilla River has declined severely in recent years and may be at risk of extirpation. Dispersal of bull trout from the Walla Walla and Tucannon rivers into the Umatilla Basin may contribute to the continued persistence and potential recovery of bull trout in the basin.

Much of the detailed data on bull trout movement within the Walla Walla Basin and connectivity between local populations and core areas has been obtained from PIT-tagging efforts and PIT-tag detection arrays deployed throughout the Walla Walla Basin. Although detection histories of bull trout PIT-tagged in Mill Creek have revealed occasional movements into the South Fork Walla Walla River, realized gene flow had not been previously verified. By further assessing connectivity through genetic analyses, we can now confirm gene flow between the Mill Creek and South Fork Walla Walla River local populations as evidenced by the apparent mixed ancestry of at least one of the individuals captured in the South Fork Walla Walla River.

Recent PIT-detection data verifies that migratory adult bull trout regularly move into and out of the North Fork Walla Walla River (M. Barrows, unpublished data). The genetic assignments presented in these analyses confirm that individuals captured in the North Fork Walla Walla River do not differ genetically from individuals in the South Fork Walla Walla local population. Because bull trout spawning has not been confirmed in the North Fork Walla Walla River, it is likely that migratory fish originating in the South Fork Walla Walla River use the North Fork Walla Walla River for foraging and overwintering habitat.

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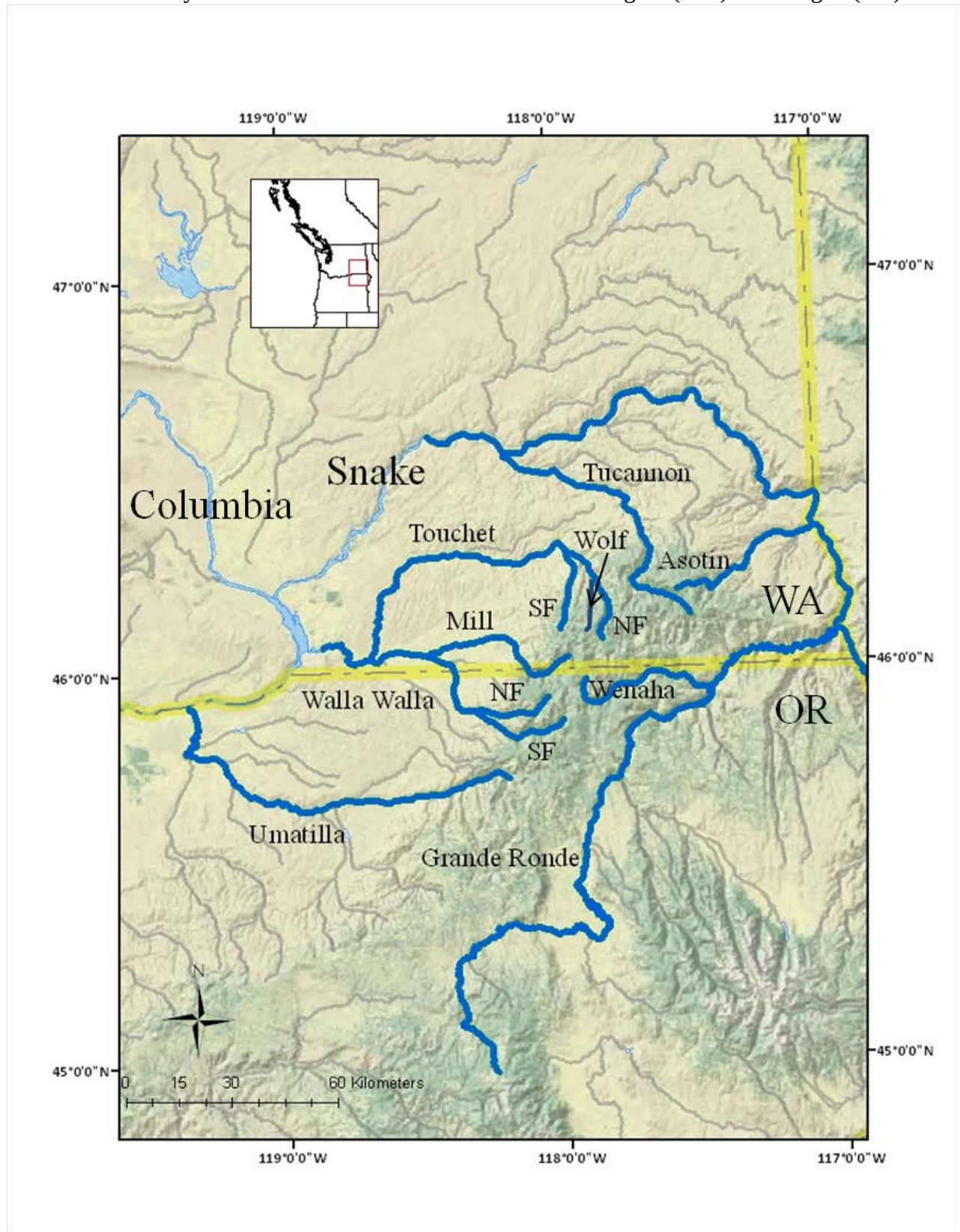
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Figure 1. Map of tributaries where bull trout were collected for this study and locations for baseline population data. The horizontal yellow line marks the border between Washington (WA) and Oregon (OR).



WDFW report – migratory bull trout in the Walla Walla basin

Figure 2a. Factorial correspondence analysis plot of individual bull trout genotypes along axes 1 and 2. The unknown USFWS bull trout are labeled “USFWS”.

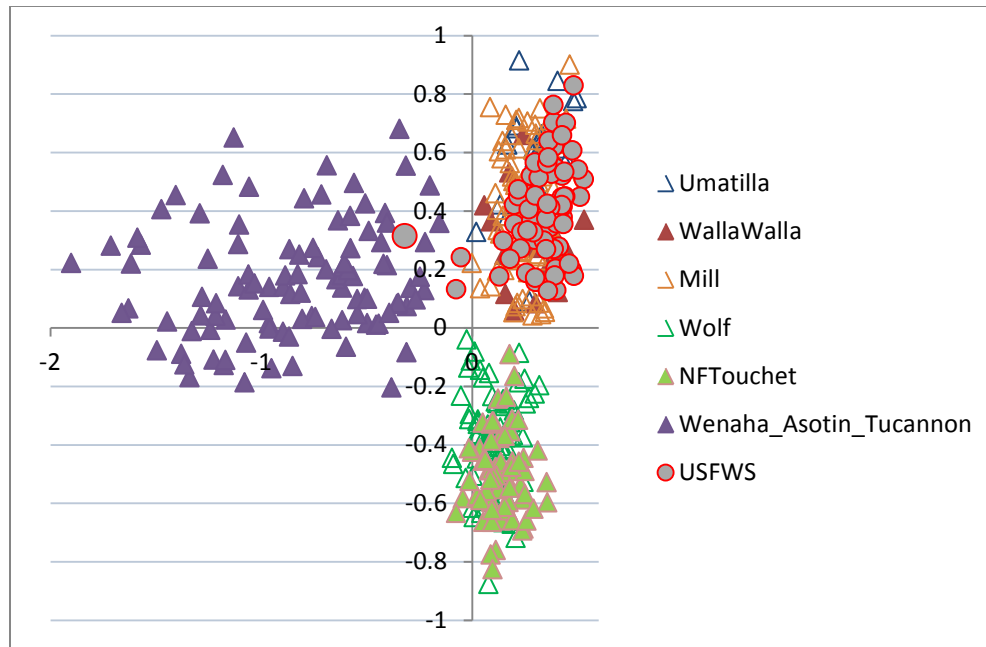


Figure 2b. Factorial correspondence analysis plot of individual bull trout genotypes along axes 1 and 3. Touchet and Wolf fish excluded to improve viewing.

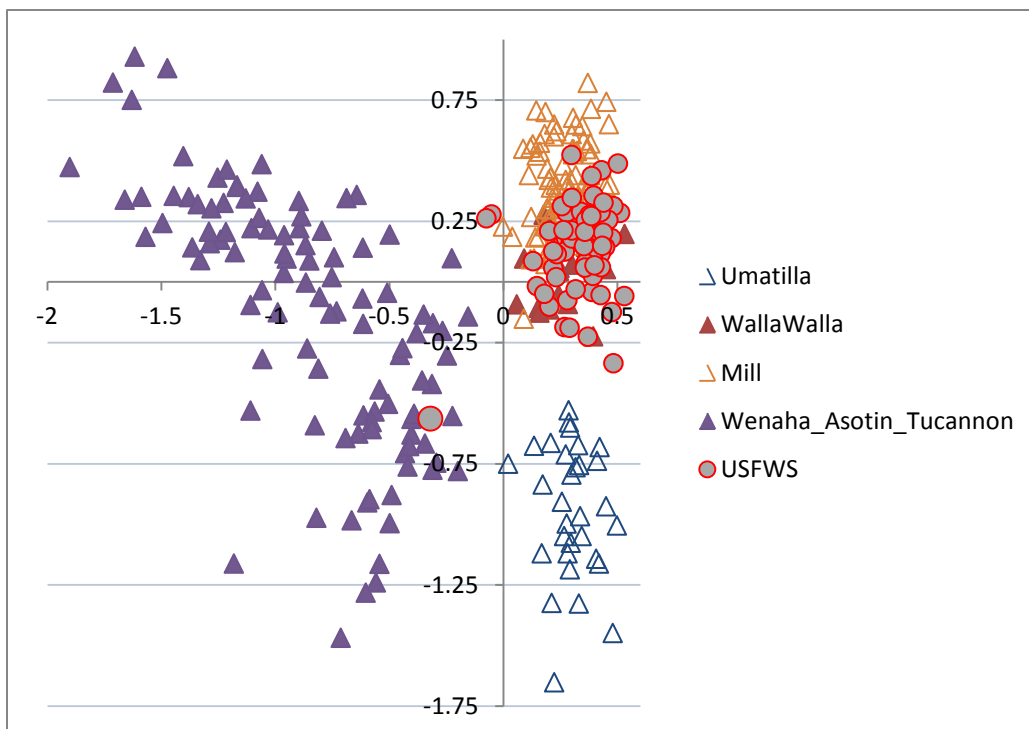
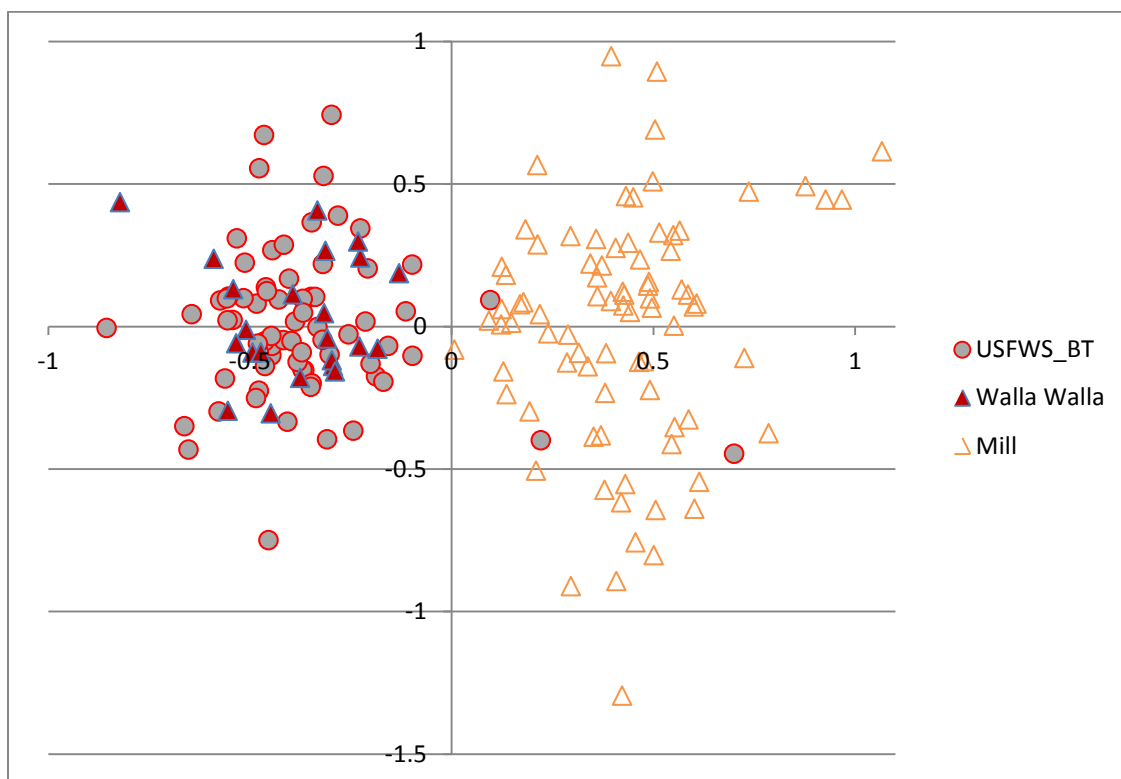
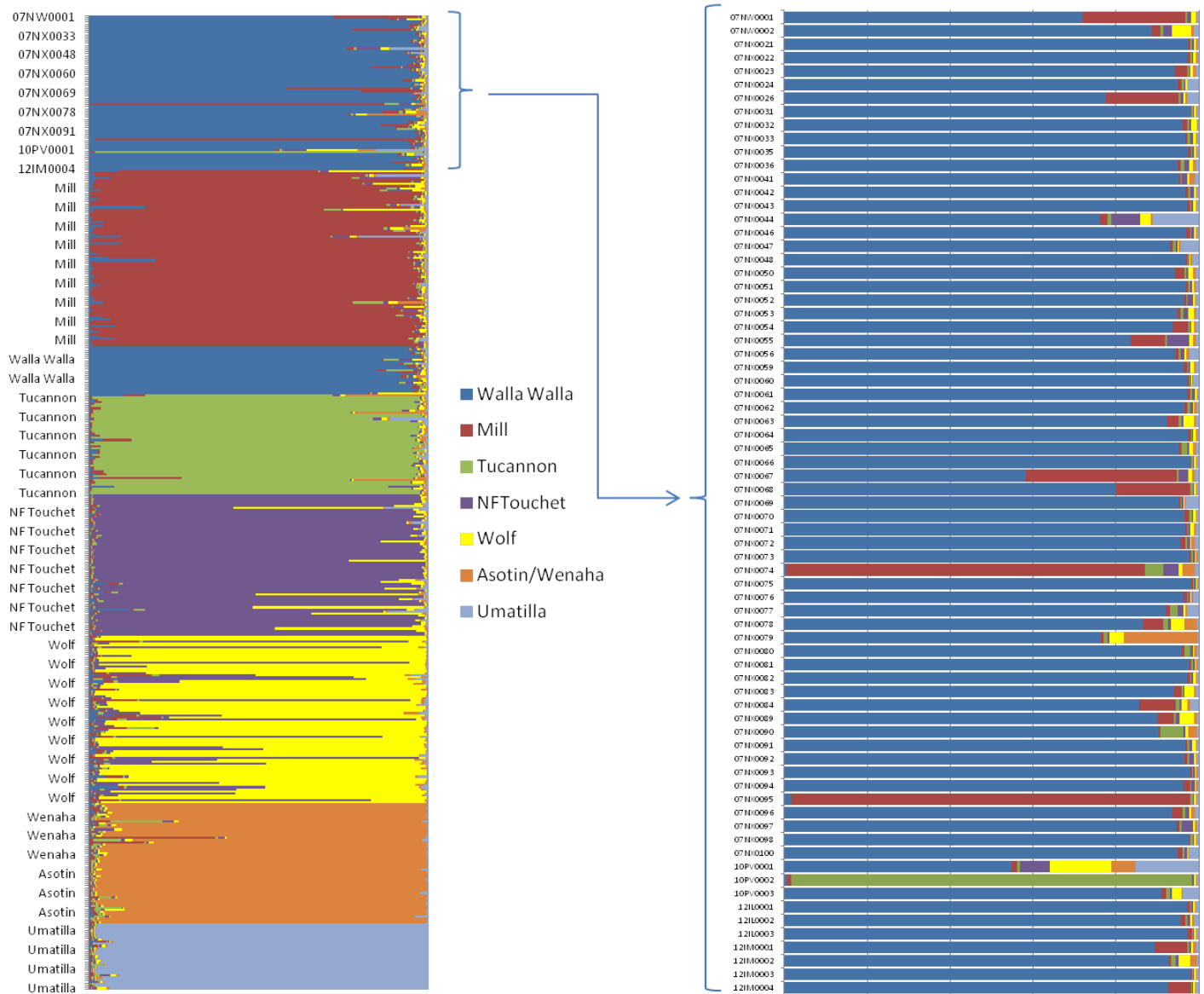


Figure 2c. Factorial correspondence analysis plot of USFWS bull trout (excluding 10PV0002) and baseline bull trout from the Walla Walla River and Mill Creek.



WDFW report – migratory bull trout in the Walla Walla basin

Figure 3. STRUCTURE plot showing genetic ancestries for USFWS bull trout and bull trout collections in the baseline. Each fish's ancestry is represented by a bar of color associated with a genetic group (groups listed next to color in middle). If a fish has all one color in its bar, it is likely pure ancestry and if it has more than one color in its bar, it could be mixed ancestry or have an allele that is common in another population. The USFWS bull trout are at the top of the plot and these are expanded to the right, showing that the majority of the USFWS bull trout are from the Walla Walla (South Fork) population, two are from Mill Creek, one is from the Tucannon and at least one has mixed ancestry. Please zoom to see individual ID codes.



WDFW report – migratory bull trout in the Walla Walla basin

Table 1. List of bull trout samples examined in the study and archived bull trout data used as a population genetic baseline. Data from the range-wide bull trout database are labeled as “RWDB” in the code column. The collection names in the “name” column are used throughout this document.

	Name	WDFW code	location	N
Project Data	USFWS	07NW	Umatilla	2
		10PV	Umatilla	3
		12IL	Umatilla	3
		07NX	Walla Walla	62
		12IM	North Fork Walla Walla	4
			Total	74
Archived Data	Umatilla	RWDB	Umatilla	31
Walla Walla Basin	Walla Walla	98LS, 99AM, 00AO	South Fork Walla Walla	23
	Mill	98AI, 00AU	Mill Cr	83
	Wolf	03LO, 04DG	Wolf Cr (Touchet)	80
	NFTouchet	03LP, 04DF	North Fork Touchet	66
Snake Basin	SFWenaha	RWDB	South Fork Wenaha R	28
	Asotin	RWDB	Asotin Cr	29
	Tucannon	RWDB	Tucannon R	47

WDFW report – migratory bull trout in the Walla Walla basin

Table 2. Detailed collection information for individual bull trout. The WDFW Sample ID is the individual code used by the WDFW genetics lab to identify each fish. The population name in the “Assignment” column is the group to which the fish assigned in the GeneClass assignment test (“?” indicates ambiguous assignment, see Appendix I). The “sample #” is the identification code used by USFWS collectors. The tag site is the river where the fish was intercepted, followed by the approximate river kilometer (km).

WDFW Sample ID	Assignment	sample #	tag/release date	tag site	river km	length	tag id	capture method
07NW0001	Walla Walla	6	4/29/07	Umatilla	6.4	385	3D9.1BF1FC832D	TRAP
07NW0002	Walla Walla	7	5/3/07	Umatilla	6.4	325	3D9.1BF1B2F5EC	TRAP
10PV0001	Walla Walla	9	5/13/2010	Umatilla	6.4	365	3D9.1BF1FD0EB5	TRAP
10PV0002	Tucannon	10	6/9/2010	Umatilla	6.4	375	3D9.1BF1B2DA44	TRAP
10PV0003	Walla Walla	11	6/12/2010	Umatilla	6.4	395	3D9.1BF1B2E8E4	TRAP
12IL0001	Walla Walla	31	5/6/2012	Umatilla	6.4	345	3D9.1BF1FDCEF7	TRAP
12IL0002	Walla Walla	32	5/10/2012	Umatilla	6.4	380	384.1B795B26A9	TRAP
12IL0003	Walla Walla	33	5/15/2012	Umatilla	6.4	305	3D9.1BF1B29947	TRAP
07NX0021	Walla Walla	477-021	6/7/2007	Walla	509.076	280	3D9.1C2C550609	HOOK
07NX0022	Walla Walla	477-022	6/6/2007	Walla	509.076	139	3D9.1C2C54F76A	FYKNET
07NX0023	Walla Walla	477-023	6/6/2007	Walla	509.076	145	none	FYKNET
07NX0024	Walla Walla	477-024	6/6/2007	Walla	509.076	150	none	HOOK
07NX0026	Walla Walla?	477-026	5/24/2007	Walla	509.074	318	3D9.1C2C54F9C1	HOOK
07NX0031	Walla Walla	477-031	9/13/2007	Walla	509.074	240	3D9.1C2C5505A8	HOOK
07NX0032	Walla Walla	477-032	6/19/2007	Walla	509.076	216	3D9.1C2C54F658	HOOK
07NX0033	Walla Walla	477-033	6/19/2007	Walla	509.076	234	3D9.1C2C54FDBC	HOOK
07NX0035	Walla Walla	477-035	6/19/2007	Walla	509.074	201	3D9.1C2C55004D	HOOK
07NX0036	Walla Walla	477-036	6/7/2007	Walla	509.074	196	3D9.1C2C54FC7E	HOOK
07NX0041	Walla Walla	477-041	10/23/2007	Walla	509.076	304	3D9.1C2C54FCA5	FYKNET
07NX0042	Walla Walla	477-042	10/23/2007	Walla	509.074	308	3D9.1C2C54F335	HOOK
07NX0043	Walla Walla	477-043	10/24/2007	Walla	509.076	248	3D9.1C2C5501FC	FYKNET
07NX0044	Walla Walla	477-044	10/24/2007	Walla	509.076	200	3D9.1C2C54F400	FYKNET
07NX0046	Walla Walla	477-046	10/24/2007	Walla	509.074	360	3D9.1C2C5502E9	HOOK
07NX0047	Walla Walla	477-047	10/24/2007	Walla	509.076	340	3D9.1C2C54FAC6	HOOK
07NX0048	Walla Walla	477-048	10/25/2007	Walla	509.082	302	3D9.1C2C54FFCC	HOOK
07NX0050	Walla Walla	477-050	10/26/2007	Walla	509.082	337	3D9.1C2C550377	HOOK
07NX0051	Walla Walla	477-051	10/26/2007	Walla	509.082	270	3D9.1C2C5504F1	HOOK
07NX0052	Walla Walla	477-052	10/26/2007	Walla	509.082	263	3D9.1C2C55041F	HOOK
07NX0053	Walla Walla	477-053	10/26/2007	Walla	509.082	204	3D9.1C2C55005F	HOOK
07NX0054	Walla Walla	477-054	10/26/2007	Walla	509.082	225	3D9.1C2C54FC69	HOOK
07NX0055	Walla Walla	477-055	11/5/2007	Walla	509.076	310	3D9.1C2C55005A	HOOK
07NX0056	Walla Walla	477-056	11/5/2007	Walla	509.076	288	3D9.1C2C54F989	HOOK
07NX0059	Walla Walla	477-059	11/6/2007	Walla	509.076	550	3D9.1C2C550311	HOOK
07NX0060	Walla Walla	477-060	11/6/2007	Walla	509.076	330	3D9.1C2C54F4D4	HOOK
07NX0061	Walla Walla	477-061	11/7/2007	Walla	509.061	325	3D9.1C2C54F7B3	HOOK
07NX0062	Walla Walla	477-062	11/7/2007	Walla	509.074	277	3D9.1C2C54FF8B	HOOK
07NX0063	Walla Walla	477-063	11/7/2007	Walla	509.074	285	3D9.1C2C55031F	HOOK
07NX0064	Walla Walla	477-064	11/7/2007	Walla	509.076	251	3D9.1C2C54FADF	HOOK

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WDFW Sample ID	Assignment	sample #	tag/release date	tag site	river km	length	tag id	capture method
07NX0065	Walla Walla	477-065	11/7/2007	Walla	509.076	405	3D9.1C2C54FC61	HOOK
07NX0066	Walla Walla	477-066	11/7/2007	Walla	509.076	238	3D9.1C2C54FC1B	HOOK
07NX0067	Mill?	477-067	11/7/2007	Walla	509.076	266	3D9.1C2C54F963	HOOK
07NX0068	Walla Walla	477-068	11/7/2007	Walla	509.076	282	3D9.1C2C54FC4E	HOOK
07NX0069	Walla Walla	477-069	11/7/2007	Walla	509.061	305	3D9.1C2C54F773	HOOK
07NX0070	Walla Walla	477-070	11/7/2007	Walla	509.061	335	3D9.1C2C54FCF0	HOOK
07NX0071	Walla Walla	477-071	11/8/2007	Walla	509.061	285	3D9.1C2C54FEA1	HOOK
07NX0072	Walla Walla	477-072	11/8/2007	Walla	509.061	440	3D9.1C2C54FC65	HOOK
07NX0073	Walla Walla	477-073	11/8/2007	Walla	509.061	288	3D9.1C2C5505D0	HOOK
07NX0074	Mill	477-074	11/8/2007	Walla	509.061	300	3D9.1C2C5503B2	HOOK
07NX0075	Walla Walla	477-075	11/8/2007	Walla	509.061	300	3D9.1C2C550421	HOOK
07NX0076	Walla Walla	477-076	11/8/2007	Walla	509.076	560	3D9.1C2C54F538	HOOK
07NX0077	Walla Walla	477-077	11/8/2007	Walla	509.076	261	3D9.1C2C54FDCE	HOOK
07NX0078	Walla Walla	477-078	11/8/2007	Walla	509.076	338	3D9.1C2C54FF71	HOOK
07NX0079	Walla Walla	477-079	11/8/2007	Walla	509.076	254	3D9.1C2C550013	HOOK
07NX0080	Walla Walla	477-080	11/8/2007	Walla	509.076	254	3D9.1C2C54F3B7	HOOK
07NX0081	Walla Walla	477-081	11/8/2007	Walla	509.076	240	3D9.1C2C54FDD3	HOOK
07NX0082	Walla Walla	477-082	11/8/2007	Walla	509.076	210	3D9.1C2C54F62C	HOOK
07NX0083	Walla Walla	477-083	11/8/2007	Walla	509.076	223	3D9.1C2C550492	HOOK
07NX0084	Walla Walla	477-084	11/8/2007	Walla	509.061	315	3D9.1C2C55031B	HOOK
07NX0089	Walla Walla	477-089	11/9/2007	Walla	509.061	262	3D9.1C2C54FCE9	HOOK
07NX0090	Walla Walla	477-090	11/9/2007	Walla	509.082	262	3D9.1C2C54FC6E	HOOK
07NX0091	Walla Walla	477-091	11/9/2007	Walla	509.082	302	3D9.1C2C54F2EE	HOOK
07NX0092	Walla Walla	477-092	11/9/2007	Walla	509.082	270	3D9.1C2C54FAB8	HOOK
07NX0093	Walla Walla	477-093	11/27/2007	Walla	509.061	335	3D9.1C2C54F662	HOOK
07NX0094	Walla Walla	477-094	11/27/2007	Walla	509.061	285	3D9.1C2C550019	HOOK
07NX0095	Mill	477-095	11/27/2007	Walla	509.061	320	3D9.1C2C54F539	HOOK
07NX0096	Walla Walla	477-096	11/27/2007	Walla	509.061	280	3D9.1C2C54FE22	HOOK
07NX0097	Walla Walla	477-097	11/27/2007	Walla	509.076	337	3D9.1C2C54FE30	HOOK
07NX0098	Walla Walla	477-098	11/28/2007	Walla	509.061	280	3D9.1C2C54FADD	HOOK
07NX0099	no data	477-099	11/28/2007	Walla	509.061	285	3D9.1C2C55031E	HOOK
07NX0100	Walla Walla	477-100	11/28/2007	Walla	509.061	281	3D9.1C2C5505CA	HOOK
12IM0001	Walla Walla	1919-001	06/14/12	NFWW	509.081	481	384.1B795B270A	HOOK
12IM0002	Walla Walla	1919-002	06/14/12	NFWW	509.081	375	384.1B795B26F1	HOOK
12IM0003	Walla Walla	1919-009	06/28/12	NFWW	509.081	345	384.1B795B26B8	HOOK
12IM0004	Walla Walla	1919-010	06/28/12	NFWW	509.081	346	384.1B795B26C9	HOOK

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Appendix I. GeneClass likelihood values of assignment. The relative likelihood is the highest likelihood over the next most likely assignment (negative log likelihood of assignment “-log(L)”), values in red are ambiguous assignments (below 80%). The “N loci” is the number of loci in the individual’s genotype.

Assigned	Assign	score relative likelihood	N loci	Walla -log(L)	Mill -log(L)	Touchet -log(L)	Wolf -log(L)	NFTouchet -log(L)	Wenaha -log(L)	Asotin -log(L)	Tucannon -log(L)	Umatilla -log(L)
07NW0001	Walla	99.40	15	15.46	17.68	23.78	22.76	28.59	30.87	31.40	27.01	32.45
07NW0002	Walla	97.44	7	3.40	5.72	5.08	6.75	9.40	12.10	14.13	9.97	8.76
07NX0021	Walla	100.00	15	13.49	19.89	19.76	23.19	22.15	32.96	39.89	32.33	36.68
07NX0022	Walla	100.00	15	16.86	26.21	26.15	26.03	34.21	33.05	35.58	26.82	32.15
07NX0023	Walla	81.24	12	14.03	14.67	19.24	20.97	23.55	24.24	25.89	23.68	21.21
07NX0024	Walla	100.00	13	10.60	19.44	23.28	25.30	25.52	34.58	38.89	25.72	26.07
07NX0026	Walla	58.82	8	6.81	6.97	12.48	12.82	11.56	16.06	18.40	13.19	12.36
07NX0031	Walla	100.00	16	14.42	29.16	27.75	28.16	31.10	33.98	39.51	30.60	29.29
07NX0032	Walla	98.07	15	17.96	19.66	25.51	26.88	31.09	32.84	42.29	31.17	38.10
07NX0033	Walla	100.00	12	8.66	13.54	13.20	16.09	15.01	21.35	30.77	22.02	25.98
07NX0035	Walla	100.00	15	14.88	23.73	27.28	28.43	30.92	39.31	43.12	35.45	31.27
07NX0036	Walla	99.99	11	12.24	16.48	20.04	24.17	21.09	19.68	25.08	17.92	24.56
07NX0041	Walla	100.00	15	13.50	21.30	19.83	19.46	24.11	31.07	30.59	25.50	24.77
07NX0042	Walla	100.00	16	17.89	28.08	30.57	31.46	31.35	36.17	36.02	32.88	33.52
07NX0043	Walla	100.00	13	13.43	21.49	23.94	24.36	27.41	26.91	30.49	23.47	28.01
07NX0044	Walla	99.98	13	13.35	18.44	17.70	21.20	17.28	28.23	30.58	22.31	18.32
07NX0046	Walla	100.00	16	15.89	23.98	28.02	30.02	28.95	34.46	34.81	28.65	30.70
07NX0047	Walla	100.00	16	18.00	23.02	24.32	26.92	30.28	31.50	36.20	27.71	25.02
07NX0048	Walla	100.00	15	15.51	25.42	25.95	24.14	29.01	32.96	37.78	29.57	27.25
07NX0050	Walla	99.93	10	8.72	11.89	13.67	14.95	19.69	14.87	21.67	18.93	24.00
07NX0051	Walla	100.00	16	13.31	20.85	19.76	20.47	21.52	28.65	29.84	26.27	28.46
07NX0052	Walla	100.00	15	12.94	20.17	24.20	26.78	24.04	32.67	28.00	25.15	24.93
07NX0053	Walla	100.00	14	13.75	21.75	20.21	19.18	20.43	30.24	31.55	26.46	25.69
07NX0054	Walla	99.98	15	13.50	17.28	23.10	23.24	27.58	29.56	32.19	25.15	24.73
07NX0055	Walla	99.97	16	19.29	22.77	25.56	25.38	28.55	32.80	38.39	28.45	35.07
07NX0056	Walla	100.00	16	16.44	24.88	21.29	22.41	25.25	28.53	34.97	21.00	24.86
07NX0059	Walla	99.95	12	7.36	10.68	12.49	12.86	14.74	18.35	18.59	14.81	12.01
07NX0060	Walla	100.00	16	15.15	28.10	27.00	29.62	33.78	35.22	40.92	32.63	26.03
07NX0061	Walla	100.00	16	14.00	20.84	20.98	24.88	28.41	33.02	41.25	25.34	32.88
07NX0062	Walla	100.00	16	12.43	18.15	22.34	24.04	20.91	29.68	33.47	21.32	28.45
07NX0063	Walla	100.00	16	15.27	22.66	24.82	23.65	29.60	29.08	32.82	24.00	27.47
07NX0064	Walla	100.00	16	12.66	22.64	23.20	22.44	31.08	23.62	33.44	26.05	32.68
07NX0065	Walla	100.00	16	16.20	26.85	24.80	27.68	32.28	35.59	34.79	26.77	29.13
07NX0066	Walla	100.00	16	16.54	24.63	23.81	24.92	35.69	37.84	37.14	33.26	29.13
07NX0067	Mill	62.04	14	13.30	13.08	17.33	17.53	17.77	24.71	25.73	20.57	26.14
07NX0068	Walla	100.00	16	19.32	23.60	33.95	34.23	32.74	36.09	37.90	32.56	36.80
07NX0069	Walla	100.00	15	14.58	19.65	24.50	28.17	25.13	30.89	37.34	26.64	23.67
07NX0070	Walla	99.93	15	13.80	16.98	21.24	24.17	26.65	28.96	37.21	20.72	30.24
07NX0071	Walla	100.00	15	13.95	22.92	20.54	21.77	25.37	27.51	32.31	30.94	26.20
07NX0072	Walla	100.00	16	18.79	24.46	27.87	28.79	31.01	35.00	31.26	29.73	32.71
07NX0073	Walla	100.00	16	11.40	22.89	21.81	24.67	27.40	32.10	38.67	27.08	30.43
07NX0074	Mill	100.00	16	29.16	17.96	24.26	25.77	24.78	28.37	32.85	27.29	33.96
07NX0075	Walla	100.00	16	14.63	26.30	27.61	31.02	32.06	32.88	41.69	36.50	40.78
07NX0076	Walla	100.00	15	16.95	21.76	28.73	29.61	32.59	34.13	30.83	27.24	27.11
07NX0077	Walla	100.00	16	16.78	23.03	23.27	24.86	33.48	28.84	39.16	21.92	25.95
07NX0078	Walla	100.00	15	13.84	18.73	18.47	20.81	24.97	29.37	29.84	28.16	30.75

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Assigned	Assign	score relative likelihood	N loci	Walla '-log(L)	Mill '-log(L)	Touchet '-log(L)	Wolf '-log(L)	NFTouchet '-log(L)	Wenaha '-log(L)	Asotin '-log(L)	Tucannon '-log(L)	Umatilla '-log(L)
07NX0079	Walla	100.00	14	11.95	21.82	18.02	17.33	22.45	17.86	23.32	19.47	30.15
07NX0080	Walla	100.00	16	15.18	23.77	26.11	27.52	30.50	38.58	34.46	27.65	30.11
07NX0081	Walla	100.00	16	14.13	27.47	29.66	29.86	33.50	29.89	35.06	27.81	37.82
07NX0082	Walla	99.97	16	15.33	20.89	19.58	19.01	25.74	32.29	39.80	30.99	29.30
07NX0083	Walla	99.99	16	17.79	22.01	27.31	23.60	31.93	37.79	36.88	29.92	34.98
07NX0084	Walla	100.00	16	15.84	23.01	25.89	25.85	29.96	31.81	38.55	27.28	26.37
07NX0089	Walla	95.61	11	6.22	8.03	8.93	7.77	14.05	10.54	19.50	15.05	18.97
07NX0090	Walla	100.00	15	16.08	22.77	20.95	23.62	21.80	25.73	32.37	27.18	29.91
07NX0091	Walla	100.00	15	8.95	18.04	16.09	18.27	18.10	28.49	30.80	22.22	24.71
07NX0092	Walla	100.00	16	17.13	25.67	27.44	28.82	27.44	34.86	32.11	24.69	30.93
07NX0093	Walla	100.00	16	14.37	22.61	24.94	26.69	32.92	39.68	37.57	31.04	29.96
07NX0094	Walla	99.99	15	12.72	16.89	22.48	23.23	29.26	29.94	32.48	27.07	31.67
07NX0095	Mill	100.00	16	18.79	13.50	31.03	26.25	31.14	30.59	35.42	27.15	38.07
07NX0096	Walla	100.00	16	16.75	25.69	27.08	27.98	34.57	29.99	34.92	28.44	35.62
07NX0097	Walla	100.00	16	15.21	26.44	23.60	26.26	26.62	31.47	33.14	29.65	31.63
07NX0098	Walla	99.98	14	12.59	16.23	21.28	23.30	26.62	33.01	33.06	25.98	24.24
07NX0100	Walla	100.00	16	15.98	25.13	27.06	25.80	30.89	39.18	41.91	29.82	30.20
10PV0001	Walla	99.44	16	19.78	25.38	23.15	22.83	22.14	31.74	34.60	29.28	25.29
10PV0002	Tucannon	100.00	14	28.60	29.22	27.00	24.90	28.67	25.52	31.99	13.50	29.08
10PV0003	Walla	100.00	16	15.38	27.96	23.39	28.42	28.56	38.01	41.08	28.24	28.76
12IL0001	Walla	100.00	16	12.66	21.66	22.54	25.85	26.22	33.59	31.83	26.47	25.09
12IL0002	Walla	100.00	16	19.57	24.42	26.37	28.14	27.99	31.99	38.21	32.28	35.58
12IL0003	Walla	99.98	15	12.64	16.31	21.98	24.67	24.78	30.70	32.55	26.09	27.01
12IM0001	Walla	99.68	16	14.00	16.49	20.99	24.39	27.34	26.49	33.12	28.99	25.61
12IM0002	Walla	100.00	15	12.62	24.25	24.31	23.94	27.19	30.75	33.55	27.09	31.15
12IM0003	Walla	100.00	16	13.53	27.53	26.25	26.42	33.17	34.49	37.36	34.43	32.31
12IM0004	Walla	96.21	16	16.05	17.45	30.42	30.54	38.69	33.94	37.47	29.81	30.55