Genetic Characterization of Bull Trout from the Wenaha River Basin

by

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Abstract

Collections of bull trout from the Wenaha River watershed were analyzed to determine the relationship among reaches in these areas, and to neighboring basins. Sixteen nuclear microsatellite DNA loci that are included in the standardized suite of loci were used to examine the levels and patterns of genetic variation. Tests of population subdivision, factorial correspondence analysis, and the neighbor-joining tree suggested the collections of bull trout from upper North Fork Wenaha are different to samples from the lower N.F. Wenaha River (upstream of the Oregon Border). The other collections in the Wenaha River basin are not differentiated from one another, (S.F. Wenaha, lower N.F. Wenaha, and the Butte Creek Basin). Comparison of bull trout from the Wenaha Basin to the Asotin, Tucannon and Walla Walla populations identify significant differences. Four major groupings (upper N.F. Wenaha, all other Wenaha Basin collections, Asotin and Tucannon, and Walla Walla Basin) were identified using multiple analysis approaches. The factorial correspondence plot and the radial tree identify separation of the same four groups, but only has bootstrap support for the Walla Walla Basin and the upper N.F. Wenaha.

Introduction

Bull trout populations have been analyzed throughout WA state (Hawkins and Von Bargen 2006; Small and Bowman 2007) including watersheds in the Southeastern portion of the state (Kassler and Mendel 2007 and 2008; Small et al. 2012). Life history differences in bull trout and isolation of populations has resulted in genetic structure among the different populations of bull trout. Spruell et al. (2003) evaluated 65 populations of bull trout from the Northwestern part of the United States and concluded that there was little genetic variation within bull trout populations but substantial divergence among populations. Bull trout in many areas of southeast Washington have been shown to be genetically distinct. Kassler and Mendel (2007) analyzed bull trout within the Walla Walla River basin finding significant differences among populations within those basins and in comparison to each other. The Walla Walla River basin stream reaches included in this study consists of the Walla Walla River, Touchet River, Wolf Fork of the Touchet River (Wolf Fork), and Mill Creek (Figure 1). The Walla Walla River flows directly into the Columbia River, upstream of McNary Dam. The Tucannon River is a Washington tributary of the Snake River that enters downstream of both the Asotin Creek and Grande Ronde Rivers. WDFW previously collected bull trout samples from seven different areas of the Tucannon River basin. The WDFW and the US Fish and Wildlife Service (USFWS) genetic laboratories collaborated to conduct preliminary genetic analysis and determined that there were at least six distinct populations in the Tucannon Basin (personal communication from Pat DeHann, USFWS, 2006). Kassler and Mendel (2008) compared samples from both the Wenaha River drainage and the Asotin Creek Basin. The Asotin Creek Basin (located near the boundary waters of the Snake River with Idaho) and the Wenaha River Basin (tributary to the Grande Ronde River that flows into the Snake River upstream of Asotin Creek) are significantly differentiated from each other.

This report combines new genetic analyses (upper N.F. Wenaha and Butte Creek Basin) with previous analyses to determine if there are distinct populations of bull trout

within each basin, and to make comparisons of bull trout across all watersheds within southeast Washington. These genetic evaluations should be useful for understanding bull trout populations and the interactions to assist in the recovery and management of bull trout throughout southeastern WA. A complete analysis of bull trout in the region will provide managers evidence of mixing and/or reproductive isolation of bull trout within these basins and with neighboring basins. Bull trout samples were collected and analyzed from the N.F. Wenaha River above the falls, N.F. Wenaha (from the falls downstream to the Oregon border), S.F. Wenaha (from USFS), Butte Creek, E.F. and W.F. Butte Creek and compared with collections from the Walla Walla River basin, Tucannon River and Asotin Creek to address the following management goals:

- How genetically differentiated are the two collections from the N.F. Wenaha River? There is a barrier between the areas where the two collections were taken; therefore there is potential for the two areas to be differentiated.

- How differentiated are the samples from the W.F. Butte Creek to samples from E.F. Butte Creek and the Butte Creek mainstem? Are bull trout in each of the branches differentiated and represent different populations?

- How differentiated are the bull trout in the Wenaha drainage (upper N.F. Wenaha, N.F. Wenaha, Butte Creek and S.F. Wenaha)?

- On a larger basin-wide scale of bull trout in SE Washington, how different are the collections from the Wenaha River basin, the Tucannon River, Asotin Creek, and the Walla Walla Basin (including the N.F. Touchet River and Wolf Fork juveniles) from one another.

Methods

Collections

Washington Department of Fish and Wildlife (WDFW) staff collected fin tissue samples from bull trout in the Washington portion of the Wenaha River basin (Table 1; Figure 1). The US Fish and Wildlife Service (USFWS) provided data from samples from the South Fork Wenaha that had been collected by US Forest Service (USFS) staff (courtesy of Phil Howell, USFS, and Pat DeHann, USFWS). A tissue sample from each fish was placed in a separate vial of 100% ethanol for preservation immediately after collection and uniquely labeled to correspond with fish length and other data for that individual fish. The general sampling protocol used by WDFW for collecting genetic samples while electrofishing applied a preferred tissue sampling protocol that was developed to emphasize collection of juvenile bull trout (preferably less than 121 mm FL) from their natal production areas (Mendel et al. 2006) to minimize incorporation of bull trout potentially from other drainages. This protocol was generally used in the Butte Creek, Wenaha, and Tucannon basins, but adult samples were included in many of the others areas of SE WA.

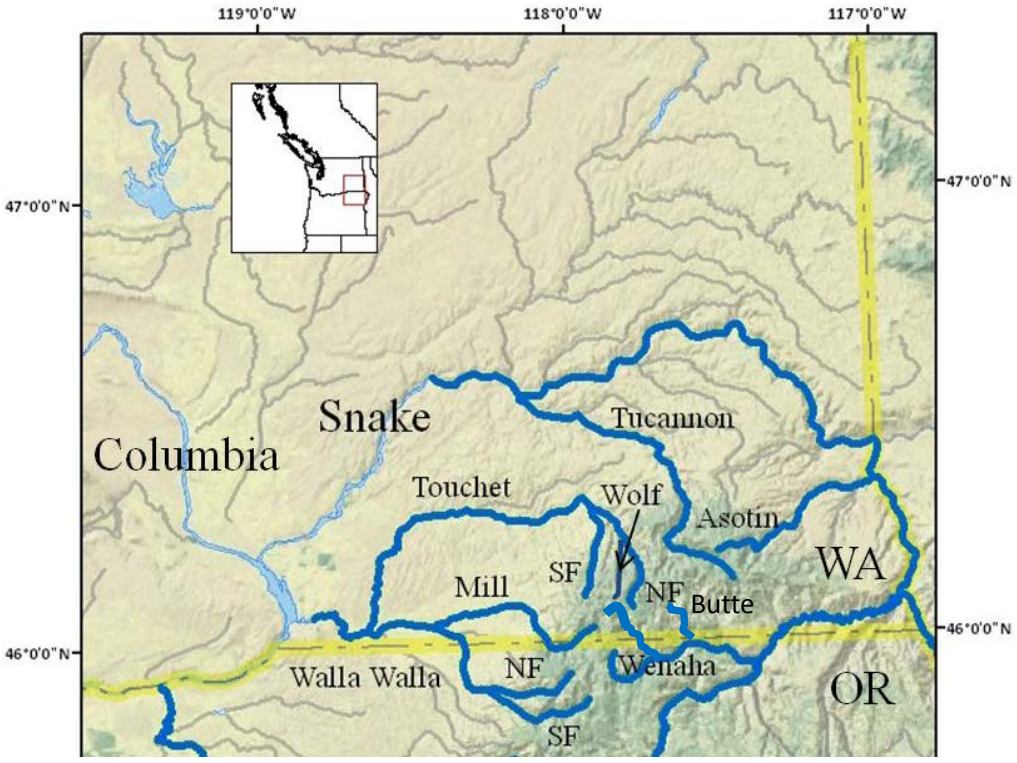


Table 1. Collections of bull trout from locations in SE Washington. Total number of individuals that were analyzed / individuals with data for 9 or more loci that were included in the analysis. Size range of fish included in the analysis or number of individuals lesser or greater than the size listed. Collection statistics (allelic richness, F_{IS}, heterozygosity (H_O and H_E), and linkage disequilibrium (before and after Bonferroni correction)) and p-values for deviations from Hardy-Weinberg equilibrium (HWE). P-values were defined as significant after implementation of Bonferroni correction for multiple tests (Rice 1989).

Aggregate Pop #	Code[A]	Collection Location	Life Stage	N =	Fish sizes (mm FL)	Allelic Richness[B]	F_{IS} (p-value)[C]	H_e	H_o	Linkage Dis[D]
1	05GB	NF Asotin Creek	mixed	20 / 20	4 > 145	4.2	0.020 (0.264)	0.5955	0.5841	30 / 4
1	no code	NF Asotin Creek[E]	mixed	9	3 > 140					
2	05OD	lower Asotin Creek	mixed	10 / 10	3 > 145	4.0	-0.078 (0.996)	0.5593	0.6021	69 / 12
2	06IS	lower Asotin Creek	adult	10 / 7	163-323					
2	07ME	lower Asotin Creek	adult	7 / 7	153-390					
2	08IF	lower Asotin Creek	adult	3 / 3	366-407					
3	05GM	N.F. Wenaha	juvenile	53 / 53	< 121	4.8	-0.010 (0.695)	0.6545	0.6610	9 / 0
4	06JX	N.F. Wenaha (above falls)	juvenile	26 / 26	< 145	2.1	0.046 (0.196)	0.3060	0.2923	9 / 1
5	no code	S.F. Wenaha[E]	juvenile	28	1 > 145	4.6	-0.071 (0.998)	0.6300	0.6741	10 / 2
6	00AO	Walla Walla	adult	14 / 14	> 330	3.9	0.006 (0.419)	0.5750	0.5717	2 / 0
6	98LS	Walla Walla	adult	7 / 7	> 330					
6	99AM	Walla Walla	adult	2 / 2	> 379					
7	00AU	Mill Creek	adult	43 / 42	282-698	3.9	-0.001 (0.505)	0.5739	0.5744	7 / 2
8	03LO	Wolf Fork	mixed	39 / 38	54-553	4.1	0.003 (0.428)	0.5996	0.5981	15 / 2
8	04DG	Wolf Fork	juvenile	41 / 41	< 133					
9	06JY	E.F. Butte Creek	juvenile	7 / 7	57-143	4.0	0.069 (0.121)	0.6119	0.5725	6 / 0
9	06JZ	Butte Creek	juvenile	1 / 1	73					
10	06KC	W.F. Butte Creek	juvenile	29 / 29	< 145	4.3	0.022 (0.227)	0.6201	0.6066	3 / 0
11	03LC	Touchet River - Dayton Trap	adults	40 / 39	> 252	4.1	0.037 (0.004)	0.5918	0.5700	50 / 12
11	03LM	Touchet River - Dayton Trap	adults	19 / 15	> 240					
11	03LP	N.F. Touchet River	mixed	21 / 20	9 > 145					
11	04DF	N.F. Touchet River	juveniles	45 / 45	< 141					
12	no code	Tucannon R.[E] (Buckley to Bear)	juveniles	26	1 > 123	4.8	0.024 (0.117)	0.6426	0.6276	24 / 1
12	no code	Tucannon R.[E] (Buckley to Bear)	juveniles	22	< 120					

[A] - Year that samples were collected is identifed by the two numbers in the WDFW GSI code

[B] - based on a minimum of 7 diploid individuals

[C] - adjusted alpha p-value = 0.00026

[D] - adjusted alpha p-value = 0.00042

[E] - Data provided by USFWS

One pass, upstream electrofishing surveys were conducted in July and August at randomly selected sites of approximately 15-46 m in length to collect juvenile samples in the two N.F. Wenaha reaches, upper Asotin Creek, Butte Creek reaches, and the upper Tucannon River. Each captured bull trout was measured and fork length (mm) was recorded. We generally avoided collecting more than five fish samples per site, or more than three fish samples per size class (< 70 mm, 71-99 mm and 100-120 mm). Sites were widely separated. The limitation on the numbers of fish samples collected per site and wide separation of sites was intended to minimize the collection of siblings. Where we were unable to collect at least 30-40 samples per stream reach or tributary using these criteria we were compelled to include larger bull trout to provide adequate sample sizes for analysis.

Comparable genetic data from the Walla Walla River Basin (Kassler and Mendel 2007), Asotin Creek, and the N.F. Wenaha River (Kassler and Mendel 2008) were used in addition to data provided by the USFWS-Abernathy genetics lab from the S.F. Wenaha River and from samples collected by WDFW in the Tucannon River.

Laboratory Analyses

Genomic DNA was extracted by digesting a small piece of fin tissue using the nucleospin tissue kits obtained from Macherey-Nagel following the recommended conditions in the user manual. Extracted DNA was eluted with a final volume of 100 µL.

A total of 16 microsatellite loci were assessed in this study (Table 2). Twelve of the loci were selected by a group of five participating laboratories for standardization with an additional four loci to be used for regional studies. Microsatellite alleles were sized using an internal size standard. GENEMAPPER (Version 3.7) software (Applied Biosystems) was used to collect and analyze the microsatellite data. Data from USFWS has been standardized for allele naming with the WDFW Molecular Genetics

Laboratory; therefore we were able to include data without having to conduct any data conversions.

Table 2. Microsatellite locus information (number alleles/locus and allele size range) for multiplexed loci used in the analysis of bull trout from the following drainages: Asotin Creek, Wenaha River, Tucannon River and Walla Walla River Basin. Also included are the observed (H_o) and expected (H_e) heterozygosity for each locus.

					Heterozygosity		
Multiplex	Locus	Annealing temp ^oC	# Alleles/ Locus	Allele Size Range (bp)	H_o	H_e	
Sco-A	*Sco-107**	57	15	249 - 319	0.734	0.848	WDFW unpublished
	*Sco-109**	57	29	254 - 392	0.824	0.914	WDFW unpublished
Sco-B	*Sco-106**	57	20	131 - 240	0.783	0.880	WDFW unpublished
	*Sfo-18**	53	2	145 - 153	0.002	0.002	Angers and Bernachez 1996
	*Smm-22**	53	28	194 - 302	0.836	0.932	Crane et al. 2004
Sco-C	*Omm-1130**	57	23	246 - 336	0.792	0.923	Rexroad et al. 2001
	*Sco-102**	57	5	166 - 181	0.105	0.108	WDFW unpublished
None	*Sco-212**	60	16	241 - 300	0.583	0.623	DeHaan & Ardren 2005
Sco-E	*Omm-1128**	57	16	265 - 351	0.642	0.757	Rexroad et al. 2001
	*Sco-105**	57	14	154 - 210	0.669	0.775	WDFW unpublished
Sco-I,1	*Sco-200**	60	9	122 - 155	0.600	0.703	DeHaan & Ardren 2005
	*Sco-202**	47	5	110 - 134	0.548	0.613	DeHaan & Ardren 2005
	*Sco-218**	60	18	190 - 269	0.752	0.796	DeHaan & Ardren 2005
Sco-I,2	*Sco-220**	60	16	290 - 359	0.684	0.816	DeHaan & Ardren 2005
Sco-J	*Sco-215**	47	2	289 - 293	0.201	0.270	DeHaan & Ardren 2005
	*Sco-216**	57	10	213 - 265	0.624	0.697	DeHaan & Ardren 2005

Statistical Analyses

Tests for Hardy-Weinberg proportions between all pairs of loci within each subpopulation were performed using GENEPOP (version 3.4; Raymond and Rousset 1995). Allele frequencies were calculated using CONVERT (version 1.3; Glaubitz 2003).

Observed and expected heterozygosity was computed for each subpopulation using GDA (Lewis and Zaykin 2001). Allelic richness and inbreeding coefficient (F_{IS} from Weir and Cockerham 1984) were computed for each subpopulation with FSTAT (version 2.9.3.2; Goudet 1995). Linkage disequilibrium was compared between each locus for each collection using GENEPOP v 3.4 (10,000 dememorizations, 100 batches, and 5,000 iterations per batch). Statistical significance for the linkage disequilibrium analysis was evaluated using a Bonferroni correction of p-values (Rice 1989). The Bonferroni correction is a procedure that is employed to minimize Type I errors (declaring a significant difference due to chance) by dividing the 0.05 significance level by the total number of tests being conducted. Values that are significant after correction can then be evaluated based on their true significance and not by chance alone.

Pairwise estimates of genotypic differentiation and F_{ST} were computed to examine population structure using GENETIX (version 4.03, Belkhir et al. 2001). These estimates use allelic and genotypic frequency data to assess differences between pairs of populations being analyzed.

We used GENETIX (version 4.03, Belkhir et al. 2001) to provide a factorial correspondence analysis and a graphical representation of the genetic variation among all individual samples in multi-dimensional space. Genotypic data for an individual sample is transformed into a value and plotted using the value. The multi-dimensional data space represents all the individual values. Each axis (three-dimensional in this case) is derived from the individual values where the first axis (x) is a line, analogous to a least squares regression, which encompasses the maximum amount of variation present among all loci and populations. The second and subsequent axes are derived from a decreasing amount of observed variation.

Genetic distance between pairs of subpopulations was estimated using Cavalli-Sforza and Edwards (1967) chord distance as performed in PHYLIP (version 3.5c, Felsenstein 1993). Bootstrap calculations were performed using SEQBOOT followed by

calculations of genetic distance using GENDIST. The NEIGHBOR-JOINING method of Saitou and Nei (1987) was used to generate the dendrograms and CONSENSE to generate a final consensus tree from the 1,000 replicates. The dendrogram generated in PHYLIP was plotted as a radial tree using TREEVIEW (version 1.6.6, Page 1996).

Results and Discussion

Collections

A total of 63 individuals were analyzed from the upper N.F. Wenaha (above falls), E.F. Butte Creek, W.F. Butte Creek, and mainstem Butte Creek (Table 1). Sample size for the collection from Butte Creek was only one; therefore it was included with the samples from E.F. Butte for the analysis.

Locus Statistics

Tests of Hardy-Weinberg equilibrium for each locus and population revealed only one locus (Smm-22) in the collection from the Touchet River that did not meet Hardy-Weinberg expectations after Bonferroni correction (Rice 1989). Deviation from Hardy-Weinberg expectation at several loci and populations could indicate several things; non random mating of individuals (inbreeding or assortative mating) in the population (evident by an increase in homozygotes, known as a Walhund effect), the populations are small and subject to genetic drift, or there have been errors in the scoring the locus (null alleles). Any locus or population that is not in equilibrium for multiple collections or loci would be dropped from analysis.

Allele frequencies for all collections analyzed are in Appendix 1 (available upon request) and information for each locus is shown in Table 2. Observed and expected heterozygosity was also calculated for all loci. Three loci (*Sfo-18**, *Sco-102**, and *Sco-215**) had five or fewer alleles scored and observed heterozygosity of less than 0.201. The remaining loci had between 5 – 29 alleles and observed heterozygosity was between 0.548 – 0.836. Heterozygosity is a measure of the molecular variation at a

given locus and is utilized in statistical analyses to determine if the variation meets the expected values in Hardy Weinberg proportion to describe the population and locus.

Population Statistics

The estimates of genetic diversity, including heterozygosity and allelic richness, within these bull trout groups ranged from 0.2923 to 0.6741 and from 2.1 to 4.8, respectively (Table 1).

Overall, genetic diversity was quite similar among all collections and comparable to other analysis of bull trout (Bettles et al. 2005, Hawkins and Von Bargen 2006, Kassler and Mendel 2007, and Small and Bowman 2007). Genetic diversity (heterozygosity and allelic richness) is a measure of the diversity detected in a population sample and is affected by the number of individuals contributing to that population (e.g. populations with few individuals or populations with related individuals will have low genetic diversity). Observed heterozygosity was not significantly different than expected for samples from any collection site and therefore did not indicate few, or related, parents for the progeny sampled.

Estimates of within population variation, or the inbreeding coefficient (F_{IS}), were also assessed to determine the level of variation within each population to determine if the individuals were potentially inbred (Table 1). F_{IS} values can range from negative 1.0 – 1.0 and p-values for F_{IS} will determine if a value is significantly different from zero. Any significant value is an indicator that there are lower heterozygosity values within that population (because of small sample size or that the population is inbred) than would be expected in Hardy-Weinberg equilibrium. All F_{IS} values shown in Table 1 are not significantly different than zero after Bonferroni correction was applied. If a population were inbred the heterozygosity and allelic richness values would be low because there are fewer individuals mating and therefore fewer possible allele combinations. The values for F_{IS} would be high and contrast with the genetic diversity values. F_{IS} is a measure of the heterozygosity within a population; therefore a higher value indicates

fewer heterozygotes implying that more closely related individuals were breeding together. The low genetic diversity values along with the low F_{IS} values for all collections do not support a conclusion that the bull trout populations are comprised of siblings, but is the result of small population size from each collection site.

Tests for linkage disequilibrium revealed varying levels of disequilibrium in these collections of bull trout (Table 1). Linkage disequilibrium can be caused by genetic drift, inclusion of family groups within collections, assortative mating and/or analysis of an admixed collection. Two collections (lower Asotin Creek and Touchet River) had 50 or more significant locus comparisons of linkage disequilibrium. These collections are comprised of individuals from the lower portions of these drainages and therefore they may include a mixture of bull trout from a large geographic area within each basin.

Genetic Differences Among Groups

Several statistical tests were conducted to examine the interrelationships among these populations of bull trout. Tests of population differentiation among the multiple collections indicated all collections were highly significantly different from each other, with exception of the collections from W.F. Butte Creek and E.F. Butte Creek (Table 3). Tests of population differentiation reveal a significant difference between collections if there are measurable allele frequency differences among the collections. Separation of bull trout into different basins will result in allele differentiation among basins and therefore all collections will be significantly different. If all the collections are significantly different we will be unable to determine if some of the collections are less differentiated from each other. Using this test along with pairwise F_{ST}; therefore provides a better understanding of the genetic relationships among collections.

Table 3. P-values for test of population differentiation (below diagonal) for each of the collections sites and pairwise F_{ST} values (above diagonal) for comparison. Value highlighted in grey identifies a non significant comparison for the test of population differentiation and a value that was not significantly different for the pairwise F_{ST} analysis.

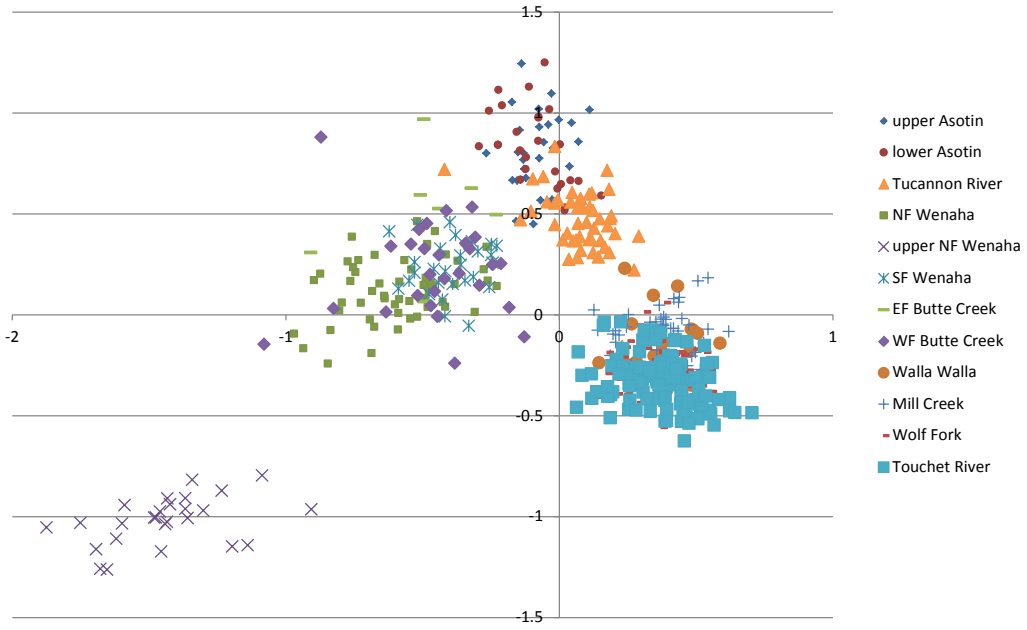
	upper Asotin Creek	lower Asotin Creek	NF Wenaha	upper NF Wenaha	SF Wenaha	Walla Walla	Mill Creek	Wolf Fork	EF Butte Creek	WF Butte Creek	Touchet River	Tucannon River
upper Asotin Creek	****	0.0660	0.1019	0.3637	0.0714	0.1466	0.1515	0.1129	0.1078	0.1255	0.1162	0.0991
lower Asotin Creek	0.0000	****	0.1283	0.4153	0.1077	0.1605	0.1663	0.1424	0.1435	0.1576	0.1624	0.0735
NF Wenaha	0.0000	0.0000	****	0.2404	0.0306	0.1403	0.1335	0.1084	0.0847	0.0575	0.1142	0.0813
upper NF Wenaha	0.0000	0.0000	0.0000	****	0.3118	0.3866	0.3845	0.3050	0.4025	0.3127	0.2976	0.3208
SF Wenaha	0.0000	0.0000	0.0000	0.0000	****	0.1267	0.1250	0.1002	0.0837	0.0628	0.1043	0.0715
Walla Walla	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.0707	0.0713	0.1752	0.1547	0.0879	0.0874
Mill Creek	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.0952	0.1764	0.1477	0.1175	0.1003
Wolf Fork	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.1337	0.1167	0.0315	0.0850
EF Butte Creek	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	**0.0301**	0.1290	0.1095
WF Butte Creek	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0009**	****	0.1184	0.0941
Touchet River	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****	0.1007
Tucannon River	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****

Assessment of the pairwise F_{ST} estimates was conducted on the groups of fish from each sampling location (Table 3). The pairwise estimate between all of the collections was significantly different from zero with the exception of the collections from W.F. Butte Creek and E.F. Butte Creek. Variation in pairwise F_{ST} values among collections depends on the overall genetic variation of the populations being analyzed and is therefore a reference to that difference.

The factorial correspondence analysis identifies four separate groups representing genetic differences (Figure 2). The separation among individuals into the groups was almost complete and included the following four groups: 1) Walla Walla Basin (Touchet River, Walla Walla River, Mill Creek, and Wolf Fork); 2) upper Asotin Creek, lower Asotin Creek, and Tucannon River; 3) S.F. Wenaha, N.F. Wenaha, and all the Butte Creek Basin collections (E.F. Butte Creek, and W.F. Butte Creek); and 4) upper N.F.

Wenaha River. The collection from the Tucannon does have some separation to the individuals from the Asotin Creek watershed and are not as genetically similar as the collections in the Asotin. The pairwise F_{ST} values and genotypic differentiation between the Asotin and Tucannon suggest they are significantly different; however the differences between them are less than the other collections in the analysis.

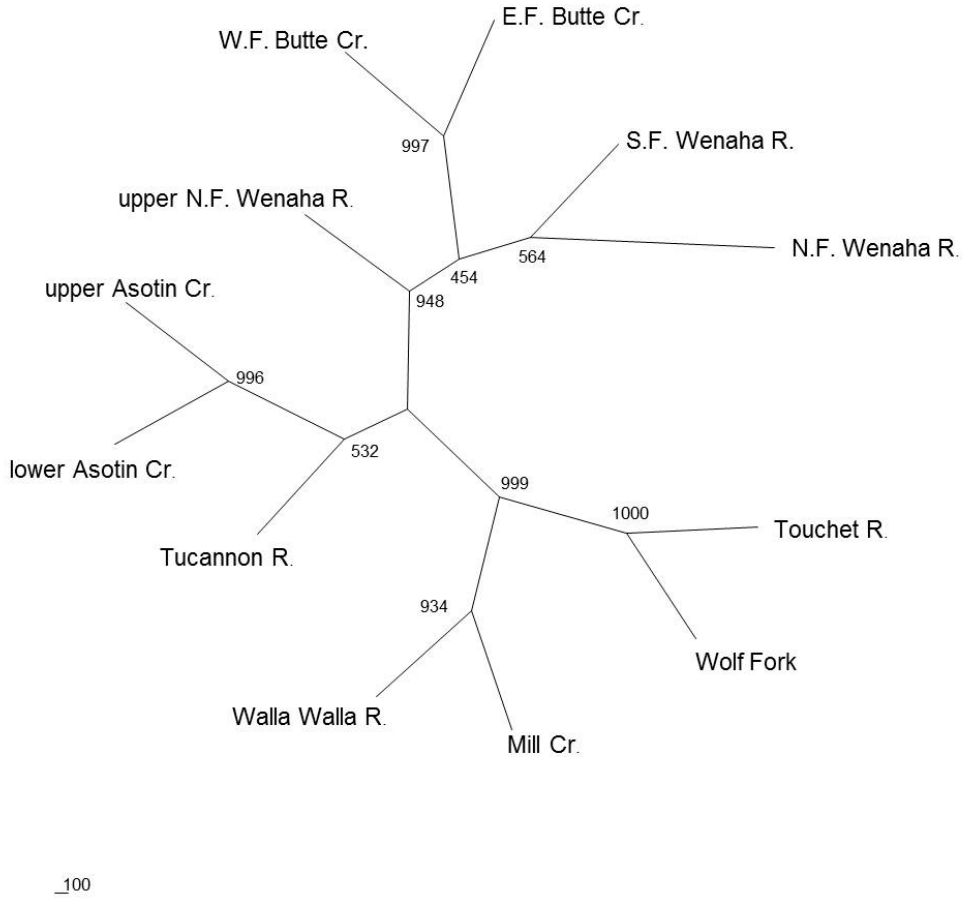
Figure 2. Factorial correspondence analysis conducted with GENETIX showing the distribution of individual bull trout from Asotin Creek, Wenaha River, Tucannon River, and the Walla Walla River basins.



The genetic relationship among collection groups was also examined by assessing the groups in the neighbor-joining tree (Figure 3). The four collections within the Walla Walla River basin, the upper and lower Asotin Creek collections, the N.F. Wenaha (above falls), and the collections from Butte Creek (E.F. and W.F.) had over 90%

bootstrap support. The relationship of the collections in this radial diagram indicates that bull trout within the Wenaha River basin group together, but do not have strong bootstrap support for the position on the tree.

Figure 3. Genetic relationship of bull trout collections from areas in southeastern WA using Cavalli-Sforza and Edwards (1967) chord distance. Bootstrap values are shown at each node.



Conclusions

Evaluation of the genetic analysis was performed to address specific management questions:

1. How genetically differentiated are the two collections from the N.F. Wenaha River? There is a natural barrier falls (about 10-12 feet high, located at N46.0472269 W117.8871524) between the areas where the two collections were taken; therefore there is potential for the two areas to be differentiated.

   There are genetic differences that exist between bull trout in the N.F. Wenaha River (upper N.F. Wenaha and lower N.F. Wenaha River). The radial tree diagram places the upper N.F. Wenaha River on a separate branch with strong bootstrap support while the other collections in the Wenaha R. Basin do not have strong bootstrap support. The factorial correspondence plot also reveals separation of samples from the upper N.F. Wenaha to the lower N.F. Wenaha. Studies of bull trout analyzing collections from above and below barriers to natural migration (Neraas and Spruell 2001) or from upper and lower reaches of rivers basins (Kassler and Mendel 2008) have found evidence of genetic differentiation. These studies have concluded that migratory bull trout from above barriers or in upper reaches are segregated and therefore genetically differentiated to the bull trout found below the barrier or from the lower reach of the basin. This is likely the same situation in the N.F. Wenaha River where bull trout in the upper portion of the river above a barrier are genetically differentiated from bull trout found below the barrier.

2. How differentiated are the samples from the W.F. Butte Creek to samples from E.F. Butte Creek and the Butte Creek mainstem? Are bull trout in each of the branches differentiated and represent different populations?

The collections from W.F. and E.F. Butte Creek are not genetically differentiated. The sample size for the E.F. Butte Creek collection was only eight individuals, and may not have represented the genetic diversity of the entire population. If those samples were however representative of the bull trout in the E.F. Butte Creek then they were not genetically differentiated from bull trout in W.F. Butte Creek in the test of population differentiation. The pairwise F_{ST} value between these two collection locations was also not significantly different from zero. The factorial correspondence plot shows the samples from E.F. and W.F. Butte Creek overlapping and the radial tree groups them with strong bootstrap support.

3. How differentiated are the bull trout in the Wenaha drainage (upper N.F. Wenaha, N.F. Wenaha, Butte Creek and S.F. Wenaha)?

   The collections from W.F. and E.F. Butte Creek were not significantly different from each other as noted above. The collections from the N.F. and S.F. Wenaha and Butte Creek basins were however significantly different from each other. The pairwise F_{ST} value for the upper N.F. Wenaha in comparison to the other collections in the Wenaha or Butte Creek basins was larger, indicating a greater genetic differentiation for this collection. The factorial correspondence plot also shows the upper N.F. Wenaha cluster is separated from all other collections. The radial tree did not identify strong bootstrap support for grouping of these collections, with exception of the collection from the upper N.F. Wenaha River. This suggests that the bull trout in the upper N.F. Wenaha River have been isolated from areas in the Wenaha River below the barrier and as a result are genetically differentiated. The heterozygosity and allelic richness levels were lower than other collections suggesting a smaller population size, but there was no indication that there were family groups or relatedness based on the F_{IS} or linkage disequilibrium.

4. On a larger basin-wide scale of bull trout in SE Washington, how different are the collections from the Wenaha River Basin, the Tucannon River, Asotin Creek, and the Walla Walla River Basin (including the N.F. Touchet River and Wolf Fork juveniles).

   Genetic differentiation exists among the basins in SE Washington as seen by the population differentiation and pairwise F_{ST} values. The factorial correspondence plot has four groups that were identified: 1) Asotin Creek and the Tucannon River; 2) Walla Walla River Basin (Touchet River, Walla Walla River, Mill Creek, and Wolf Fork); 3) Wenaha River Basin (including W.F. and E.F. Butte Creeks, S.F. and lower N.F. Wenaha rivers); and 4) upper N.F. Wenaha River. The radial tree shows separation of the same four groups, but only has bootstrap support for the collections in the Walla Walla River Basin and the upper N.F. Wenaha River. The bootstrap support is a measure of how many times the collections will group together when analyzed multiple times (1,000 iterations); therefore bootstrap support of over 800 is an indication that those collections are genetically differentiated from the other collections in the analysis. A group of collections with bootstrap support that is lower indicates the collections off of a branch will group together in a specific configuration fewer times. In the case of the collections within the Wenaha River Basin, where bootstrap support is between 454 – 564 (S.F. and N.F. Wenaha and E.F. and W.F. Butte Creek), it suggests that the configuration of these collections on this tree will be different approximately half of the time. This can be the result of the collections being genetically similar, and therefore the ability to separate them within the group is lower. The assessment of collections that are genetically the same, or different, is done by evaluating the results of several analyses. Collections that are genetically more similar may provide evidence that they are from a similar ancestral population or that individuals are interbreeding, but does not identify separate populations.

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