Genetic Characterization of Bull Trout from the Asotin and North Fork Wenaha River Basins

by

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December 2008

Abstract

Collections of bull trout from the upper and lower Asotin Creek were analyzed to determine the relationship between these areas within the Asotin Creek. Bull trout samples from the North Fork Wenaha River, Walla Walla River basin and Tucannon River were also compared to the samples from the Asotin Creek. 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 the upper and lower Asotin Creek are genetically differentiated; however there are some samples from the upper Asotin Creek that appear in the lower Asotin Creek. Bull trout in both the upper and lower Asotin Creek are differentiated from samples of bull trout in the North Fork Wenaha River, the Walla Walla River basin, and the Tucannon River. Bull trout from the North Fork Wenaha were different than bull trout in both Asotin Creek and the Tucannon River.

Introduction

Bull trout populations have historically occurred from northern California to Alaska and from the Pacific coast inland to Idaho by the Columbia and Snake Rivers. 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. Kassler and Mendel (2007) analyzed bull trout within the Walla Walla River basin and found significant differences among populations within the basin. The area upstream of the Tucannon River on the Snake River has not been extensively studied to determine the genetic relatedness of populations in that region. Specifically, the Asotin Creek basin (located in the southwest corner of Washington State and a tributary of the Snake River) and the Wenaha River (tributary to the Grande Ronde River that flows into the Snake River upstream of Asotin Creek) have not been analyzed and compared at a southeast Washington scale. 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, and Mill Creek. The Walla Walla River flows directly into the Columbia River. The Tucannon River is a Washington tributary of the Snake River that enters downstream of both the Asotin Creek and Grande Ronde Rivers. Current information on the identification and status of bull trout populations is inconsistent given the difference between the bull trout recovery plan and recent genetic analyses by Kassler and Mendel (2007) and Spruell et al. (2003).

Recovery and management of bull trout in the Asotin Creek and surrounding basins requires better information and planning. Managers need to know if there is evidence of mixing and/or reproductive isolation of bull trout within the upper and lower Asotin Creek Basin and the relationships of Asotin Creek bull trout to nearby basins. Bull trout samples were collected from Asotin Creek and the North Fork Wenaha River, analyzed and compared with a component of the bull trout samples from the Walla Walla River

basin (Kassler and Mendel 2007) and a separate study in the Tucannon River (USFWS and WDFW ongoing study) with a microsatellite DNA analysis to address the following management goals:

- Document and describe the genetic composition of bull trout collected in the upper part of the Asotin Creek versus bull trout collected in the lower portion of the Asotin Creek. Specifically, are there significant genetic differences among upper and lower areas within the Asotin Creek; and are lower Asotin bull trout likely a migratory component of the upper Asotin population?

- Compare the genetic characteristics and stock structure of bull trout in Asotin Creek, N.F. Wenaha River, and Walla Walla River basin and the Tucannon River.

Methods

Collections

Washington Department of Fish and Wildlife (WDFW) staff collected fin tissue samples from bull trout in the upper North Fork Asotin Creek and lower North Fork Wenaha River (within WA) during electrofishing surveys in 2005 (plus nine samples collected in 2006 in upper Asotin Creek). WDFW staff operating an adult steelhead trap and an out-migrant (smolt) trap in lower Asotin Creek (Mayer et al. 2008) provided tissue samples of subadult and adult bull trout captured in 2005-2008. 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. Our general sampling protocol for collecting genetic samples while electrofishing applied a preferred tissue sampling protocol that was developed for the upper Tucannon River Basin to emphasize collection of juvenile bull trout (preferably less than 121 mm FL) from their natal production areas (Mendel et al. 2006). One pass, upstream electrofishing surveys were conducted in July and August at randomly selected sites of approximately 15-46 m in length. Each captured bull trout was measured and length (mm of FL) was recorded. We attempted to avoid 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. Tissue samples collected from bull trout at the lower Asotin Creek trap included all sizes captured.

Comparable genetic data were obtained from bull trout samples collected from other bull trout studies in the Walla Walla Basin (Kassler and Mendel 2007) and the Tucannon River Basin (USFWS and WDFW on-going collaborative study). WDFW analyzed adult bull trout samples from lower Asotin Creek and juvenile samples from the North Fork Wenaha River. We compared these results with data from juvenile bull trout collected by WDFW for upper (North Fork) Asotin Creek and upper Tucannon River (obtained from the USFWS-Abernathy Genetics Lab). WDFW data from a portion of the adult and juvenile bull trout from the Walla Walla River basin were also used for comparison.

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. 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.

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.

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).

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.

Ancestry for individuals in Asotin Creek was evaluated using a Bayesian analysis implemented in the program STRUCTURE 2.1 (Pritchard et al. 2000). Five independent runs were computed allowing admixture with 50,000 burn-ins and 450,000 iterations. Analyses were conducted using all individuals with K (number of possible populations) set from 1 to 2, depending on the particular test. When K = 1 was used we were testing for a single ancestral group of bull trout, with K = 2, we were testing for differences between upper and lower groups of bull trout in the Asotin Creek.

We used GENECLASS2 (version 2.0.g, Piry et al. 2004) to perform maximum likelihood jackknife assignments. In the jackknife procedure, each individual fish is removed from the dataset, the allele frequencies of the baseline subpopulations are recalculated, and the fish is assigned to the most likely group. Jackknife assignments were used to evaluate the reliability of the assignments of the temporal collections, and to determine the relationships among subpopulations. Correct jackknife assignment relies upon a robust baseline as well as true distinctions among groups.

Results and Discussion

Collections

A total of 375 individuals were analyzed from lower Asotin Creek, upper Asotin Creek, North Fork Wenaha River (within WA), Walla Walla River basin (Walla Walla River, Mill Creek, Touchet River, and Wolf Fork of the Touchet River), and Tucannon River (Table 1). Three individuals from the lower Asotin Creek collections were dropped from analysis because they failed to amplify DNA at eight or more loci.

Table 1. Collections of bull trout from Asotin Creek, N.F. Wenaha River (within WA), Walla Walla River Basin, and Tucannon River. Collection code, year, sampling location, size/age designation as adult or adult, and number analzyed for each collection (samples with data for nine or more loci) were included in the analysis.

GSI Code	Year	Collection Location	adult/juvenile	# Analyzed
05GB	2005	upper N.F. Asotin Creek**	juvenile	20
N/A	2006	upper N.F. Asotin Creek**	juvenile	8
N/A	2006	upper N.F. Asotin Creek tributary (Cougar Cr.)**	juvenile	1
05OD	2005	lower Asotin Creek trap	adult	10
06IS	2006	lower Asotin Creek trap	adult	7
07ME	2007	lower Asotin Creek trap	adult	7
08IF	2008	lower Asotin Creek trap	adult	3
05GM	2005	upper N.F Wenaha River	juvenile	53
99AL	1999	Touchet River trap*	adult	10
00AN	2000	Touchet River trap*	adult	10
03LC	2003	Touchet River trap*	adult	39
03LM	2003	Touchet River trap*	adult	15
98LS	1998	Walla Walla River trap*	adult	7
99AM	1999	Walla Walla River trap*	adult	2
00AO	2000	Walla Walla River trap*	adult	14
00AU	2000	Mill Creek trap*	adult	42
03LO	2003	Wolf Fork*	juvenile	38
04DG	2004	Wolf Fork*	juvenile	41
05GF	2005	Tucannon River**	juvenile	26
N/A	2006	Tucannon River**	juvenile	22

* Data were taken from Kassler and Mendel 2007

** Data were provided by Pat DeHaan at USFWS-Abernathy

Locus Statistics

Tests of Hardy-Weinberg equilibrium for each locus and population did not reveal any loci 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 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 0.152. The remaining loci had between 5 – 29 alleles and observed heterozygosity was between 0.615 – 0.873. 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.

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

Multiplex	Locus	Annealing temp ^oC	# Alleles/ Locus	Allele Size Range (bp)	Heterozygosity H_o	Heterozygosity H_e	
Sco-A	*Sco-107**	57	15	249 - 319	0.747	0.835	WDFW unpublished
	*Sco-109**	57	29	254 - 392	0.873	0.898	WDFW unpublished
Sco-B	*Sco-106**	57	19	131 - 240	0.818	0.864	WDFW unpublished
	*Sfo-18**	53	1	145	0.000	0.000	Angers and Bernachez 1996
	*Smm-22**	53	27	194 - 302	0.828	0.926	Crane et al. 2004
Sco-C	*Omm-1130**	57	21	246 - 336	0.830	0.918	Rexroad et al. 2001
	*Sco-102**	57	5	166 - 181	0.127	0.131	WDFW unpublished
None	*Sco-212**	60	16	241 - 300	0.595	0.635	DeHaan & Ardren 2005
Sco-E	*Omm-1128**	57	16	265 - 351	0.696	0.789	Rexroad et al. 2001
	*Sco-105**	57	14	154 - 210	0.718	0.772	WDFW unpublished
Sco-I,1	*Sco-200**	60	8	126 - 155	0.633	0.715	DeHaan & Ardren 2005
	*Sco-202**	47	5	110 - 134	0.615	0.638	DeHaan & Ardren 2005
	*Sco-218**	60	18	190 - 269	0.733	0.788	DeHaan & Ardren 2005
Sco-I,2	*Sco-220**	60	15	294 - 359	0.699	0.788	DeHaan & Ardren 2005
Sco-J	*Sco-215**	47	2	289 - 293	0.152	0.195	DeHaan & Ardren 2005
	*Sco-216**	57	9	217 - 265	0.652	0.680	DeHaan & Ardren 2005

Population Statistics

The estimates of genetic diversity, including heterozygosity and allelic richness, within these bull trout groups ranged from 0.572 to 0.661 and from 4.9 to 6.6, respectively (Table 3).

Table 3. Collection location and population statistics [heterozygosity (expected (H_e) and observed (H_o), allelic richness (A_o), F_{IS}, and number of loci with significant linkage disequilibrium before and after Bonferroni correction of P-values (Rice 1989)

Asotin Creek

	Heterozygosity				
Collection location	H_o	H_e	A_o	F_{IS}	Linkage Disequilbrium
N.F. Asotin Creek - upper	0.584	0.596	5.6	0.020	31 / 4
Asotin Creek - lower	0.602	0.559	5.4	-0.078	64 / 16

N.F. Wenaha River

	Heterozygosity				
Collection location	H_o	H_e	A_o	F_{IS}	Linkage Disequilbrium
N.F. Wenaha River	0.661	0.654	6.4	-0.010	10 / 1

Walla Walla River Basin

	Heterozygosity				
Collection location	H_o	H_e	A_o	F_{IS}	Linkage Disequilbrium
Touchet River - Dayton Dam	0.596	0.609	5.3	0.002	23 / 3
Mill Creek, OR - city intake dam	0.574	0.573	4.9	-0.001	6 / 1
Walla Walla River, OR - nursery bridge	0.572	0.575	5.0	0.006	3 / 0
Wolf Fork	0.599	0.600	5.2	0.002	14 / 2

Tucannon River

	Heterozygosity				
Collection location	H_o	H_e	A_o	F_{IS}	Linkage Disequilbrium
Tucanon River above bear Cr.	0.628	0.643	6.6	0.024	23 / 2

alpha p-value for Linkage Disequilibrium: 0.05/105 = 0.00048

alpha p-value for F_{IS}: 0.05/128 = 0.00039

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 3). 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 3 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 does 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 3). Linkage disequilibrium can be caused by genetic drift, inclusion of family groups within collections, assortative mating and/or analysis of an

admixed collection. One collection (lower Asotin Creek) had the highest levels of disequilibrium suggesting this collection is comprised of bull trout from multiple areas. The lower Asotin Creek samples are a mixture of bull trout from upper and lower Asotin Creek (or include individuals from outside the Asotin Creek Basin).

Genetic Differences Among Groups

Several statistical tests were conducted to examine the interrelationships among these populations of adult and juvenile bull trout. Tests of genetic differentiation among the multiple collections indicated all collections were highly significantly different from each other (Table 4). Tests of genotypic differences 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 be too sensitive because all collections appear to be significantly different from each other. Using this test along with pairwise F_{ST} will provide a better understanding of the genetic relationships among collections.

Table 4. P-values for genotypic differentiation tests (above diagonal) for each of the collections sites. Pairwise F_{ST} values (below diagonal) for comparison.

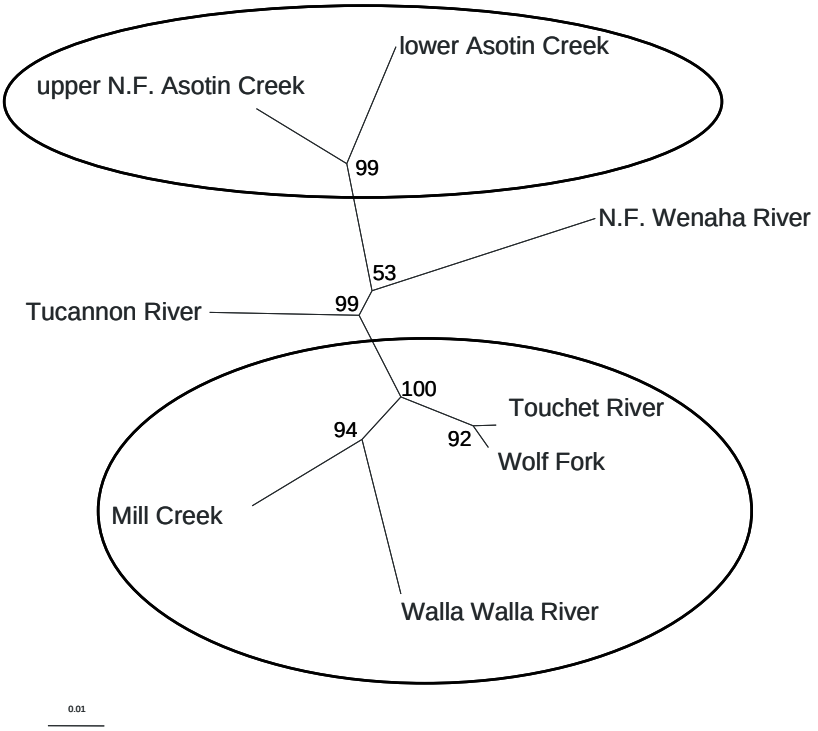
	upper N.F. Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf Fork	Tucannon
upper N.F. Asotin	---	0.000	0.000	0.000	0.000	0.000	0.000	0.000
lower Asotin	0.066	---	0.000	0.000	0.000	0.000	0.000	0.000
N.F. Wenaha	0.102	0.128	---	0.000	0.000	0.000	0.000	0.000
Touchet	0.105	0.145	0.104	---	0.000	0.000	0.000	0.000
Walla	0.147	0.160	0.140	0.067	---	0.000	0.000	0.000
Mill	0.153	0.168	0.135	0.094	0.071	---	0.000	0.000
Wolf Fork	0.113	0.142	0.108	0.010	0.071	0.097	---	0.000
Tucannon	0.099	0.074	0.081	0.082	0.087	0.102	0.085	---

Assessment of the pairwise F_{ST} estimates was conducted on the groups of fish from each sampling location (Table 4). The pairwise estimate between the upper and lower collections of Asotin Creek was 0.066 while values between the upper and lower Asotin Creek samples and Wenaha were 0.102 and 0.128. The pairwise results for the Wenaha River and Tucannon River were 0.081. These values indicate that bull trout in the upper and lower Asotin Creek are approximately as different to the Wenaha as they are to the Tucannon River. Variation in 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 genetic relationship among collection groups was examined by assessing the groups in the neighbor-joining tree (Figure 1). All groups were associated with over 90% bootstrap support with the exception of the N.F. Wenaha River. The upper and lower Asotin Creek collections grouped together with 99% bootstrap support. The collections from the Walla Walla River basin grouped together with high bootstrap support and the Tucannon River and N.F. Wenaha River grouped between the Asotin Creek collections and the Walla Walla River basin collections. Interestingly, the upper North Fork Asotin Creek and the upper Tucannon River samples were collected only about 4-4.8 aerial kilometers from one another as these streams drain the east and west sides, respectively, of the same ridge line. Similarly, the N. F. Wenaha and the upper Wolf Fork drain the south and north slopes, respectively, of the same ridgeline and the bull trout collections were made about 8-10 air km apart. The relationship of the collections in this radial diagram indicates that bull trout within Asotin Creek are more similar to each other than the other collections included in the analysis. The diagram also suggests that bull trout from upstream and downstream of Asotin Creek are more similar than bull trout in the Walla Walla River basin. Radio telemetry studies have suggested that some bull trout from the Wenaha River may overwinter in the Snake River not far from Asotin Creek (Baxter 2002, Hemmingsen et al. 2001). Recent reevaluation of these telemetry data suggest they may not document Wenaha River bull trout using the mainstem Snake River – contrary to previous reports (pers. comm. with

Steve Starcevich, ODFW, Nov. 2008). It is unknown at this time whether bull trout from the Wenaha River or other populations outside of Asotin Creek potentially overwinter or forage in lower Asotin Creek. Additional bull trout movement or genetic data are needed to determine the likely source of these lower Asotin Creek bull trout.

Figure 1. Relationship of bull trout from Asotin Creek (upper and lower), N.F. Wenaha River, Walla Walla River basin, and Tucannon River based on the genetic distance matrix using Cavalli-Sforza and Edwards (1967) chord distance. Bootstrap values for all clusters are shown.



The factorial correspondence analysis reveals four groups: Asotin Creek (upper and lower), N.F. Wenaha River, Tucannon River, and the Walla Walla River basin collections (Figure 2). The polygon surrounding the Asotin Creek bull trout has some overlap with the N.F. Wenaha River polygon; otherwise all polygons separate from each other. The plots of the upper Asotin Creek, lower Asotin Creek, and Tucannon River collections reveals separation of the Tucannon from the Asotin Creek collections and

separation of the upper Asotin Creek collection from the lower Asotin Creek. The polygon that encompasses the lower Asotin Creek individuals however overlaps the upper Asotin Creek polygon (Figure 3). The plot of upper and lower Asotin Creek with the N.F. Wenaha reveals the same overlap between the upper and lower Asotin collection, but also reveals separation between the Asotin Creek samples and the N.F. Wenaha River samples (Figure 4).

Figure 2. Factorial correspondance analysis conducted with GENETIX showing the distribution of individual adult bull trout from upper and lower Asotin Creek, N.F. Wenaha River, Tucannon River, and the Walla Walla River basin.

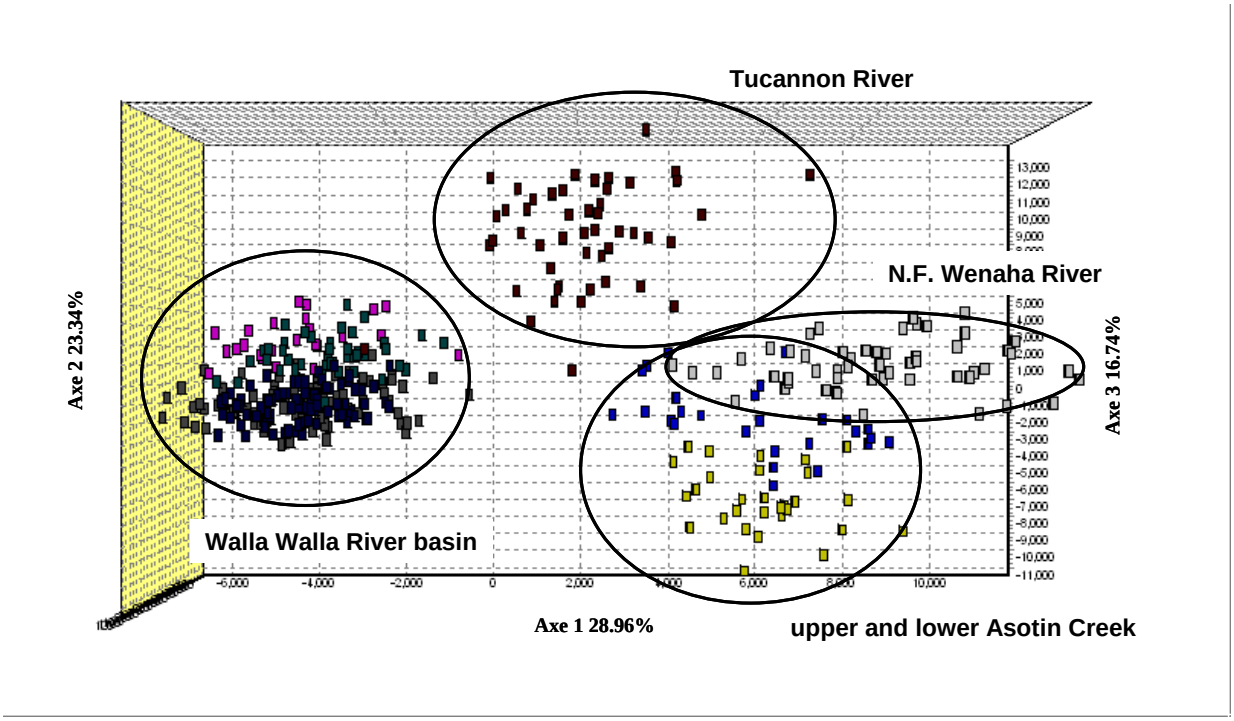


Figure 3. Factorial correspondance analysis conducted with GENETIX showing the distribution of individual adult bull trout from upper and lower Asotin Creek and the Tucannon River.

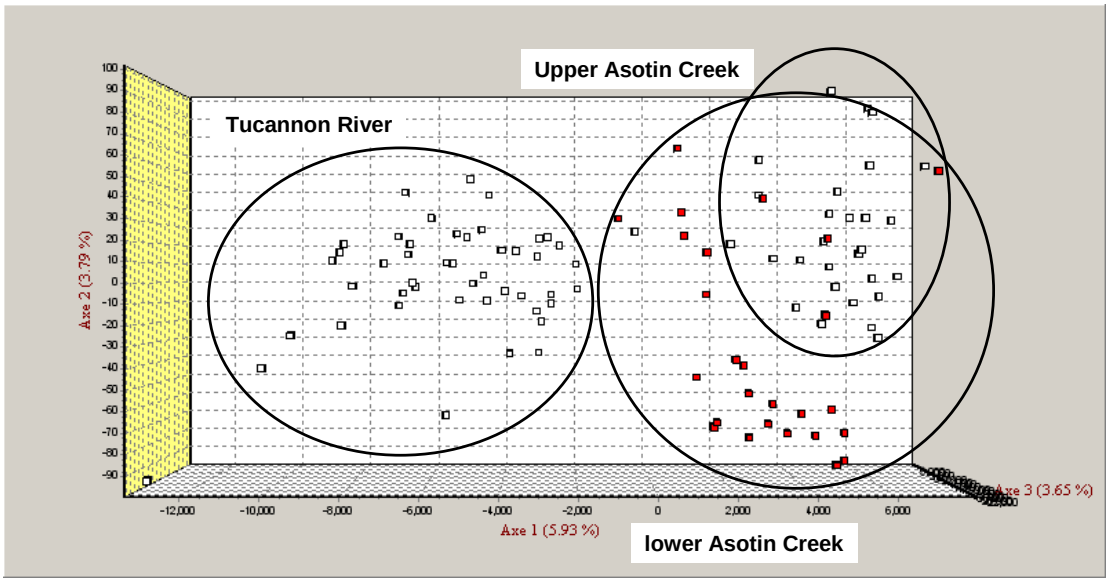
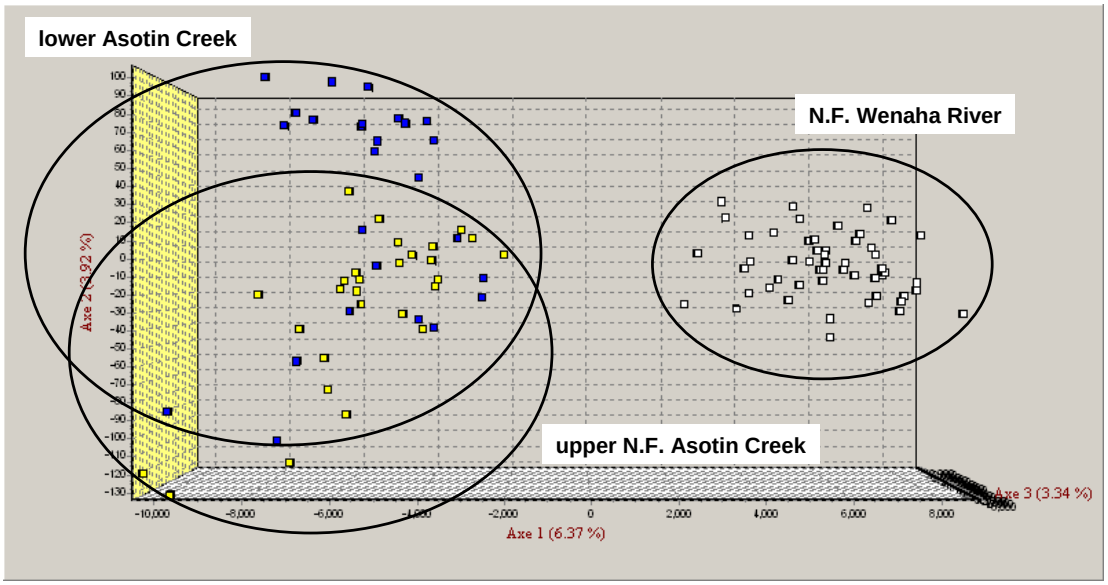
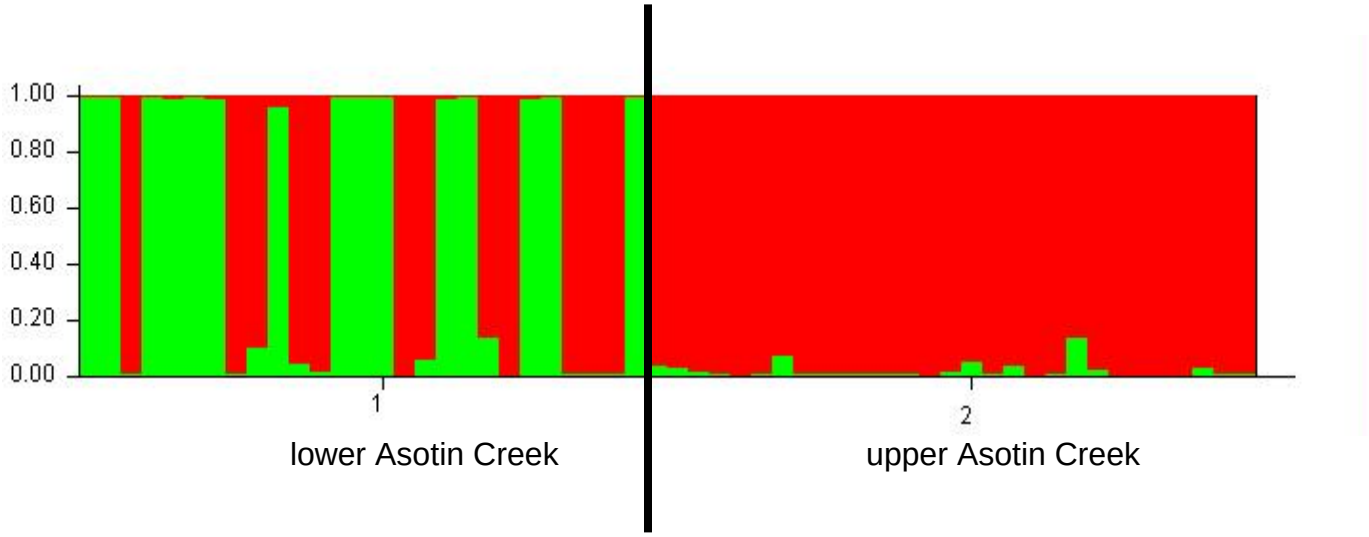


Figure 4. Factorial correspondance analysis conducted with GENETIX showing the distribution of individual adult bull trout from upper and lower Asotin Creek and the N.F. Wenaha River.



Analyses using STRUCTURE (Pritchard et al. 2000) was used to determine individual ancestry of the collections in Asotin Creek. Average Ln score at K = 1 was –2393.5 and average Ln at K = 2 was –2199.6. The point where the average Ln scores reaches a plateau defines which K describes the number of ancestral groups in the data being analyzed (Pritchard 2000). The plateau occurs at K = 2 and indicates that there were two ancestral groups detected in the lower Asotin Creek (one group represented the upper Asotin and other the lower Asotin; Figure 5). Bull Trout from the lower Asotin had 57.0% ancestry in the lower Asotin group and 43.0% ancestry in the upper Asotin group while the samples from the upper Asotin had approximately 98.0% ancestry in the upper Asotin group. The mixture of ancestry from the lower Asotin Creek with the upper Asotin Creek is based on the presence of the upper Asotin Creek ancestry that was detected in individuals in the lower Asotin Creek. This result suggests the upper and lower Asotin Creek are differentiated, but there is movement of bull trout from the upper Asotin Creek into lower Asotin Creek.

Figure 5. STRUCTURE plot for bull trout in the Asotin River drainage with 2 possible populations: ancestry of each individual fish is represented by a single bar of color with grey (green) corresponding to lower Asotin Creek ancestry and black (red) corresponding to upper Asotin Creek ancestry. Mixed ancestry is indicated by both black (red) and grey (green) for each individual. The vertical black line delineates separation of individuals from lower Asotin Creek and upper Asotin Creek.



Jackknife Analysis

Jackknife tests were used to assess the differentiation between collections by calculating how well an individual would assign back to their original population of origin (Table 5). Results of the jackknife analysis revealed that each collection assigned back to their population of origin with very high probability. The only collections where individuals assigned to an incorrect population of origin were between the upper and lower Asotin Creek and the Touchet and Wolf Fork. The geographic separation and genetic differentiation between the upper and lower Asotin Creek and the Touchet and Wolf Creek are the lowest; therefore it is not surprising that mis-assignments would occur between these pairs of collections.

Table 5. Results of the jacknife analysis for four collections of bull trout in Asotin Creek, Wenaha River, Walla Walla River Basin, and Tucannon River. Shading indicates correct assignment back to stock-of-origin in the jacknife analysis.

	upper Asotin	lower Asotin	Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
upper Asotin	27	1						
lower Asotin	4	21						
Wenaha			53					
Touchet				47	1		19	
Walla					23			
Mill						40		
Wolf				10			37	
Tucannon								46

Conclusions

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

1. Document and describe the genetic composition of bull trout collected in the upper N.F. of Asotin Creek versus bull trout collected in the lower mainstem of the Asotin Creek. Specifically, are there significant genetic differences among upper and lower areas within Asotin Creek?

- There are genetic differences that exist between bull trout in the upper and lower Asotin Creek as evidenced by the F_{ST} values and genotypic tests. The radial tree diagram groups them together demonstrating the upper and lower Asotin Creek are more similar to each other than other bull trout in the Columbia River and Snake River basins. The factorial correspondence plot reveals separation of upper Asotin Creek and lower Asotin to the other collections, but some overlap exists between the lower Asotin Creek and upper Asotin Creek. The STRUCTURE results then show that individuals with a genetic ancestry similar to upper Asotin Creek appear in lower Asotin Creek suggesting movement from the upper Asotin into the lower Asotin. The jackknife analysis did identify one sample from upper Asotin Creek that reassigned to lower Asotin Creek. Possibly a result of the presence of upper Asotin Creek genotypes present in the lower Asotin Creek collection. Neraas and Spruell (2001) found evidence of genetic differentiation between bull trout above and below barriers. They concluded that migratory bull trout from above barriers rear below the barrier and are then unable to return to natal sites to spawn. This same scenario may exist in Asotin Creek by the presence of upper Asotin genetic ancestry appearing in the lower Asotin Creek samples, with no genetic evidence of lower Asotin Creek ancestry found in upper Asotin Creek.

2. Compare the genetic characteristics and stock structure of bull trout in Asotin Creek, N.F. Wenaha River, the Walla Walla River basin and upper Tucannon River.

- Significant genetic differences were documented in bull trout populations from Asotin Creek, N.F. Wenaha River, Walla Walla River basin and the Tucannon River. The Walla Walla River basin has the largest genetic differences compared with the other groups. Asotin Creek is as different to both the N.F. Wenaha River and Tucannon River. The N.F. Wenaha River is closer to the Tucannon River in the radial tree and factorial correspondence plots, but the pairwise F_{ST} values between the N.F. Wenaha River and Tucannon River are lower than to the upper and lower Asotin Creek.

Acknowledgements

US Fish and Wildlife Service (ESA Section 6 funds) and WA State General Funds provided financial support for this study. We thank Jim Uehara of WDFW for his cooperation and assistance by providing Section 6 funding. WDFW staff in Dayton that contributed to collecting and cataloging tissue samples included Jeremy Trump, Mike Gembala, Carl Fulton, Derek Gloyn, Larabee Miller, Stephen Jeffers, plus Richard French from Lyons Ferry Hatchery. Kent Mayer and his staff provided the samples from the mainstem Asotin Creek trap. Pat DeHann at the USFWS Genetics Laboratory – Abernathy, WA shared genetic data from upper Asotin and the Tucannon River Basin. We appreciate assistance from Jennifer Von Bargen (WDFW) for technical contributions in the genetics laboratory. We thank Mo Small (WDFW) for discussion of data analysis and interpretation. Ken Warheit provided editorial comments and review.

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Appendix 1. Allele frequencies of bull trout from upper and lower Asotin Creek, N.F. Wenaha River, populations in the Walla Walla River basin, and Tucannon River.

Sco-107

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
249	---	---	---	---	0.0455	---	---	0.0326
253	---	---	---	---	0.0682	---	---	0.0109
257	---	---	---	0.0154	0.0909	0.0233	---	---
269	---	---	---	0.0615	---	---	0.0513	---
273	---	---	---	0.0385	---	---	0.0128	---
277	0.3276	0.2407	0.1887	0.0692	0.0227	---	0.0256	0.0109
281	0.2069	0.1667	0.0755	0.2077	0.5	0.2209	0.141	0.0435
285	0.0517	0.0185	0.066	0.2692	0.1364	0.2442	0.2115	0.1413
289	0.3448	0.537	0.1038	0.1231	---	0.0814	0.141	0.4239
293	0.0345	---	0.4811	0.1615	0.0909	0.3488	0.359	0.1413
297	0.0172	---	0.0377	0.0538	0.0455	0.0814	0.0449	0.0652
301	---	0.037	0.0377	---	---	---	0.0128	---
306	0.0172	---	---	---	---	---	---	0.1196
315	---	---	---	---	---	---	---	0.0109
319	---	---	0.0094	---	---	---	---	---

Sco-109

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
254	---	---	---	0.0139	---	---	---	---
258	0.0172	0.0833	---	0.0278	---	---	0.0513	0.0349
262	0.1379	0.2292	---	0.0694	0.0476	---	0.1282	0.1047
266	---	---	---	0.1042	0.2143	---	0.141	---
274	---	---	0.01	---	---	---	---	---
278	---	---	---	---	---	---	---	0.0116
286	0.069	---	---	---	---	0.0682	---	---
292	0.0517	---	0.16	---	---	---	---	---
296	0.0517	0.0417	0.11	0.3125	0.3571	0.2955	0.2949	0.2558
300	0.1207	---	0.07	0.0833	---	0.0227	0.0641	0.0349
304	0.2414	0.0833	0.27	0.0347	---	0.0455	0.0128	0.0116
308	0.0172	0.2292	0.03	0.0139	---	0.4318	0.0577	0.0581
312	0.1034	0.0625	0.1	---	0.0476	0.0682	0.0064	0.0116
316	0.0172	---	---	0.0764	---	---	0.0513	0.0116
330	0.069	---	---	---	---	---	---	---
334	---	---	0.01	---	---	---	---	---
342	0.0172	---	---	---	---	---	---	---
346	---	---	---	---	---	---	---	0.0465
350	---	---	---	0.0417	0.0238	---	0.0449	0.0349
352	---	---	0.01	---	---	0.0455	---	---
356	---	0.0417	---	0.0139	---	---	---	0.1047
360	---	---	0.05	0.0417	---	---	0.0064	---
364	---	---	---	---	---	0.0227	---	0.0814
368	---	---	0.01	---	---	---	---	---
372	---	---	0.07	---	---	---	---	---
376	0.0862	0.2083	0.02	---	---	---	---	0.0349
380	---	0.0208	0.04	0.1389	0.1905	---	0.1282	0.1628
384	---	---	0.04	0.0278	0.119	---	0.0064	---
392	---	---	---	---	---	---	0.0064	---

Appendix 1 continued.

Sco-106

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
131	0.069	0.1852	---	---	0.1304	0.0476	---	---
135	0.2069	0.2593	0.1132	0.0411	---	---	0.0253	0.0652
139	---	---	0.1887	---	---	---	---	---
164	---	0.037	---	---	---	---	---	---
168	---	---	0.0189	0.1986	---	---	0.1709	0.0217
172	0.0172	0.1111	---	0.0068	0.0652	---	---	0.2935
176	0.3621	0.2778	0.0849	0.137	0.1087	0.4405	0.1139	0.0543
180	---	0.0185	0.3208	0.0411	0.087	0.0595	0.0316	0.0435
184	---	---	0.0189	0.0548	---	0.0952	0.1456	---
192	0.0172	0.037	---	---	---	---	---	---
196	---	---	---	0.1027	---	---	0.0823	---
200	---	---	0.0094	---	---	0.0119	---	0.0217
204	---	---	---	---	0.0435	---	0.0063	0.1739
208	---	---	0.1038	0.3973	0.3913	0.3333	0.3797	0.3261
212	---	---	0.066	0.0205	0.1739	0.0119	0.0443	---
220	0.0517	---	---	---	---	---	---	---
224	0.2759	0.0741	---	---	---	---	---	---
232	---	---	0.0283	---	---	---	---	---
240	---	---	0.0472	---	---	---	---	---

Sfo-18

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
145	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Smm-22

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
194	---	---	0.0849	---	---	---	---	---
198	---	---	0.0566	---	---	---	---	---
202	---	---	0.0094	---	0.0217	---	---	---
206	---	---	0.0377	0.2411	0.0435	0.0128	0.3377	---
210	---	---	0.1132	0.0446	---	---	0.0519	0.0319
214	---	---	0.0472	0.0714	0.0217	0.1282	0.013	0.0106
218	0.0172	0.0385	---	---	0.087	0.0128	---	0.0319
222	---	---	0.0189	0.2143	0.2391	0.1538	0.1104	0.1596
226	0.0345	---	0.0094	0.0179	0.1739	0.141	0.013	0.0426
230	---	0.0192	0.0094	0.0179	0.087	0.0256	0.0519	0.0106
234	0.069	0.2885	0.0189	0.1071	0.0217	0.0641	0.2078	0.0745
238	0.0517	0.0192	0.0566	0.1518	0.0435	0.0256	0.0649	0.0957
242	0.2069	0.1923	0.0566	0.0893	0.0435	0.1795	0.0779	0.1596
246	0.1034	0.1154	0.0189	---	0.0217	0.1026	0.013	0.0426
250	0.1207	---	0.0283	0.0089	0.0217	0.0256	0.0065	0.0851
254	0.0517	---	0.0094	---	---	---	0.0065	0.0319
258	---	---	0.1132	0.0357	---	---	0.0195	0.0957
262	---	---	0.0943	---	0.0652	0.0513	---	0.0426
266	0.1034	0.0769	0.0094	---	0.0435	---	---	0.0532

Appendix 1 continued.

Smm-22

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
270	---	0.0192	0.1132	---	0.0217	---	0.0065	0.0213
274	0.0172	0.0385	0.0472	---	---	0.0513	---	0.0106
278	0.0345	---	---	---	0.0435	0.0256	0.0195	---
282	0.0862	---	0.0094	---	---	---	---	---
290	---	0.0385	0.0283	---	---	---	---	---
294	0.0172	---	0.0094	---	---	---	---	---
298	0.0345	0.1538	---	---	---	---	---	---
302	0.0517	---	---	---	---	---	---	---

Omm-1130

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
246	---	---	---	---	---	---	---	0.1778
258	0.0172	0.0217	---	---	---	---	---	---
262	---	---	---	---	---	---	---	0.0222
266	---	---	---	---	---	---	---	0.1222
270	---	---	0.1038	---	---	0.0357	---	---
274	---	---	0.0189	0.2500	---	0.1071	0.223	---
278	---	---	0.0094	0.1042	0.3611	0.1548	0.1081	0.0333
282	---	---	---	0.0104	---	---	---	---
286	---	---	0.0189	0.0625	0.25	0.1905	0.0878	---
290	0.1207	0.0435	0.0943	0.1146	0.0556	0.131	0.1689	0.0667
294	0.0172	0.3696	0.1604	0.1354	---	0.1786	0.0743	0.1444
298	0.3966	0.087	0.1698	0.0104	---	0.0119	0.0135	---
302	0.2586	0.2174	0.0094	---	---	---	---	---
306	0.0172	0.0652	0.0566	---	---	---	---	0.1889
312	0.1379	0.1087	---	---	---	---	---	0.0444
316	---	0.0217	0.0094	---	---	---	---	---
320	---	---	0.0094	---	---	---	---	---
324	0.0172	---	0.1321	---	---	---	---	---
328	---	---	0.0377	---	0.2778	---	---	0.1222
332	0.0172	0.0652	0.0849	0.1458	0.0556	0.0238	0.2027	0.0778
336	---	---	0.0849	0.1667	---	0.1667	0.1216	---

Sco-102

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
166	---	0.0370	---	---	---	---	---	---
169	0.9310	0.9074	0.9906	0.9464	1.0000	1.0000	0.8750	0.8854
173	---	---	0.0094	0.0536	---	---	0.1250	0.0521
177	0.0690	0.0556	---	---	---	---	---	0.0521
181	---	---	---	---	---	---	---	0.0104

Appendix 1 continued.

Omm-1128

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
265	---	---	---	---	---	---	0.0063	---
269	---	---	---	0.0070	---	---	0.0633	0.0667
273	0.0862	0.1852	0.1981	0.2042	0.0217	---	0.1772	0.0222
277	0.5000	0.0370	0.3396	0.4789	0.2826	0.4231	0.4114	0.1667
281	0.0690	0.5185	0.3302	0.0070	0.1087	0.0128	0.0063	0.3556
285	---	---	0.1226	---	---	---	---	---
289	---	0.0185	---	---	---	---	---	0.1111
293	---	---	---	---	---	---	---	0.0556
297	---	0.0185	---	---	---	---	---	---
327	0.0172	0.0185	---	---	---	---	---	---
331	---	---	---	---	---	---	---	0.0222
335	---	---	---	---	---	0.0256	---	---
339	0.1552	0.1481	0.0094	0.0986	0.3478	0.3077	0.1646	0.1556
343	0.1724	0.0556	---	0.1901	0.0652	0.2308	0.1709	0.0222
347	---	---	---	0.0141	0.0435	---	---	0.0222
351	---	---	---	---	0.1304	---	---	---

Sco-105

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
154	---	---	0.0377	---	---	---	---	---
158	0.0172	0.0370	0.1509	0.0423	---	---	0.0190	0.0745
162	0.3448	0.5741	0.4434	0.2113	0.2609	0.4419	0.2342	0.5851
166	0.3276	0.0185	0.2170	0.2394	0.2391	0.1163	0.3544	0.1383
170	---	---	---	0.1408	0.3043	0.2791	0.1392	0.1277
178	---	0.0185	---	---	---	---	---	---
182	---	0.0370	---	---	---	---	---	---
186	0.0862	---	0.0094	---	---	---	---	---
190	0.2069	0.2963	0.1415	---	---	---	---	0.0106
194	---	---	---	---	---	---	---	0.0106
198	---	---	---	---	0.0217	---	---	---
202	---	---	---	---	0.0435	---	---	---
206	0.0172	0.0185	---	0.3662	0.1304	0.1512	0.2532	0.0532
210	---	---	---	---	---	0.0116	---	---

Sco-200

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
126	0.0345	---	---	---	---	---	---	0.0312
130	0.0690	0.0370	---	0.0486	0.2391	0.5875	0.0750	0.0729
134	0.0172	0.0370	0.0189	---	0.0652	---	---	0.0312
138	---	---	---	0.0139	0.0652	---	---	0.0104
142	0.1207	0.0926	0.3208	0.4444	0.2826	0.2625	0.3875	0.3646
147	0.7586	0.8333	0.5000	0.2847	0.1739	0.0750	0.2000	0.4896
151	---	---	0.1604	0.2083	0.1739	0.0750	0.3312	---
155	---	---	---	---	---	---	0.0063	---

Appendix 1 continued.

Sco-202

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
110	0.1552	---	0.0094	---	---	---	---	---
122	0.6034	0.6481	0.3491	0.5616	0.3913	0.1707	0.6500	0.3854
126	0.0862	0.1667	0.3491	0.1644	0.2609	0.2927	0.1375	0.4062
130	0.1552	0.1852	0.2925	0.2671	0.3478	0.5366	0.2062	0.2083
134	---	---	---	0.0068	---	---	0.0063	---

Sco-218

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
190	---	---	0.1792	---	---	---	---	---
205	---	---	0.0472	0.0145	---	---	0.0063	0.0111
209	0.1379	0.0370	---	---	---	---	0.0063	0.0111
213	0.4655	0.4074	0.2264	0.5435	0.4048	0.1765	0.5823	0.1667
217	---	0.0185	0.0472	0.0942	0.0952	0.0147	---	0.1333
221	0.1897	0.0741	0.1887	0.1304	---	0.0441	0.1013	0.1111
225	0.0690	0.0741	0.0472	0.0145	---	0.0147	0.0380	0.1889
229	0.0517	0.0185	---	0.0725	---	---	0.1456	0.1222
233	0.0345	0.2407	0.0849	0.0072	---	0.0147	0.0127	0.1111
237	---	0.0926	0.0189	0.0942	0.2143	0.4559	0.1076	0.0333
241	---	---	---	0.0290	0.2143	0.0882	---	---
245	---	---	0.0377	---	---	---	---	---
249	---	---	0.1226	---	---	---	---	---
253	0.0345	0.0185	---	---	---	---	---	0.0444
257	---	---	---	---	0.0238	0.1176	---	0.0556
261	---	---	---	---	0.0476	0.0441	---	---
265	0.0172	0.0185	---	---	---	---	---	0.0111
269	---	---	---	---	---	0.0294	---	---

Sco-220

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
294	---	---	0.0849	---	---	---	---	---
298	---	---	0.0094	---	---	---	---	0.0778
302	0.0345	0.0185	0.3113	0.1918	---	0.2162	0.1154	0.2000
306	0.1724	---	0.1792	0.2740	0.2273	0.1216	0.1474	0.0667
310	0.2414	0.0741	0.0566	0.1918	---	---	0.2308	0.0111
314	0.0862	0.4815	0.2075	0.2671	0.7727	0.4595	0.4487	0.4667
317	---	---	---	---	---	---	0.0064	---
318	0.3621	0.3519	0.0094	---	---	---	---	0.1556
322	0.0172	---	0.0283	0.0753	---	0.0135	0.0513	0.0111
326	---	---	---	---	---	0.1892	---	---
338	---	---	0.0094	---	---	---	---	---
347	---	---	0.0849	---	---	---	---	---
351	---	---	0.0094	---	---	---	---	---
355	---	0.0370	---	---	---	---	---	---
359	0.0862	0.0370	0.0094	---	---	---	---	0.0111

Appendix 1 continued.

Sco-215

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
289	0.8276	0.963	0.5	0.9841	1	0.9651	0.9812	0.883
293	0.1724	0.037	0.5	0.0159	---	0.0349	0.0187	0.117

Sco-216

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
217	---	---	0.3585	---	---	---	---	---
221	---	---	---	---	---	---	---	0.0106
237	---	---	0.0189	---	---	---	---	---
241	0.3276	0.2593	0.0472	0.3151	0.3571	0.2674	0.2562	0.1596
245	0.6552	0.6667	0.3208	0.3356	0.6190	0.6744	0.4313	0.5532
249	0.0172	0.0741	0.1132	0.2877	---	---	0.2375	0.2660
253	---	---	---	0.0479	---	---	0.0063	---
257	---	---	---	---	---	---	---	0.0106
265	---	---	0.1415	0.0137	0.0238	0.0581	0.0688	---

Sco-212

				Walla Walla River basin				
Allele Size	upper Asotin	lower Asotin	N.F. Wenaha	Touchet	Walla	Mill	Wolf	Tucannon
241	0.2414	0.2407	0.1698	0.0890	0.0652	0.1279	0.1437	0.1538
245	---	0.0741	0.0472	---	---	---	---	---
249	---	0.0185	---	---	---	---	---	---
253	---	---	---	---	---	0.2093	---	---
257	0.0517	---	---	0.2466	0.3696	0.0930	0.1313	0.0513
261	0.0172	---	---	---	---	---	---	---
269	0.0172	0.0370	---	---	---	---	---	0.0256
271	---	---	---	---	---	0.0349	---	0.0897
275	---	---	0.0566	---	---	---	---	0.0385
279	---	---	---	---	---	---	---	0.0385
281	---	---	0.0094	---	---	---	---	---
283	---	---	---	---	---	---	---	0.0385
287	---	---	---	---	---	---	---	0.0769
291	0.5345	0.3704	0.6604	0.5822	0.5217	0.5116	0.6562	0.4744
295	0.1379	0.2593	0.0094	0.0685	0.0435	---	0.0500	0.0128
300	---	---	0.0472	0.0137	---	0.0233	0.0187	---