Genetic Characterization of Adult Chinook Trapped in lower Asotin Creek

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The Washington Department of Fish and Wildlife (WDFW) and the National Marine Fisheries Service (NMFS) consider Asotin Creek Spring Chinook to be functionally extirpated because annual redd counts were near zero for many years (Table 1, and Snake River Salmon Recovery Plan – Technical Document 2006). In recent years the numbers of adult spring Chinook and redds observed in Asotin Creek have increased to as high as 13 redds per year in 2004. WDFW staff were able to access upstream migrating Chinook at a lower Asotin Creek trap that was operated to sample and enumerate adult steelhead (Mayer et al. 2008). Tissue samples, fish lengths, scales, and tag or mark information were collected from 31 adult or jack Chinook in 2005 and 2007(Table 2). Adult Chinook were sampled during May through early October in 2005, and during May and June in 2007.

Our goal was to determine the likely origin of the adult spring Chinook in Asotin Creek through the use of genetic analyses. This information would then be used to inform management decisions regarding potential reintroduction efforts for spring Chinook in Asotin Creek (either for fisheries enhancement or ESA recovery efforts) and for making funding decisions regarding habitat enhancement projects in Asotin Creek. Funding for genetic analyses was provided by WDFW.

We performed standard population genetic tests on this collection (31 samples from 2005 and 2007). The genetic equilibrium (within and among loci) for the Asotin collection was investigated. The allele frequencies of the Asotin collection were compared to references collection from the GAPS baseline (i.e., genic test). The relative genetic differences were evaluated using factorial correspondence analysis (FC). Two FC analyses were performed, one comprising all GAPS collections listed in Table 3, and a second analysis consisting only of Snake River spring/summer Chinook. The possible origin of individual Asotin Chinook was estimated using individual assignment methods and the compatibility of each Asotin genotype to Tucannon reference collections (i.e., the potential introduction source) was documented (baseline = Table 3). See APPENDIX 1 for description of methods.

Association of alleles, within or among loci was not observed (data not shown). This information suggests the Asotin samples represent a collection from a single

underlying population. The allele frequencies of the Asotin collection are generally distinct from all other GAPS collections (Table 3), as the genic tests showed statistically significant differences between all but two reference collections (data not shown). The two non-statistically different comparisons regarded Pahsimeroi River and Tucannon Hatchery reference collections. The p-value values were small for these two tests (Pahsimeroi=0.00017 and Tucannon=0.00034), but when p-values were adjusted for multiple comparison the values were not statistically significant. The relative genetic differences between the Asotin collection and other upper Columbia River su/fa, Snake River fall, and Snake River spring/summer reference collections are shown in Figure 1. Figure 2 shows a similar FC analysis except Columbia River su/fa and Snake River fall collections were removed. Asotin and Tucannon collections appear differentiated from other Snake River spring/summer collections.

Individual assignment was performed to assess further the genetic composition of the Asotin collection (Table 4). The Asotin collection appears to be composed mostly of Snake spring/summer fish, although some fall fish were detected. Most assignments were to the Tucannon and Minam reference collections. Yet, the exclusion probabilities suggested that it was statistically possible that most of the spring/summer fish could have arisen from the Tucannon collections. Formerly, the only known coded-wire tag recovery, after years of WDFW spawning surveys and recent adult trapping, was from a 74 cm female that was a 1999 Tucannon Hatchery release (cwt = 636132). Three additional Chinook were captured with detected CWTs during the sampling reported on here (individuals 05AY0061, 07AD0008, and 07AD0011), but CWTs were not extracted from these fish. These fish were excluded from the general analyses described above because they were not technically "unknowns". Yet, for completeness, we did conduct individual assignments on them, where 05AY0061 assigned to Snake River fall with probability=1.00 and both 07AD0008 and 07AD0011 assigned to Snake River sp/su with probability=1.00 (Table 4).

Conclusion: The Asotin collection appears to be mostly Snake River spring/summer Chinook, with a few fall Chinook mixed in. We are unable to explain the presence of three fall Chinook during June and July in 2007, based on genetic analyses, but the genetic results from the October sample is consistent with the timing of fall Chinook in the Snake River Basin. FC analysis, assignment tests, and exclusion suggest the Asotin collection is most similar to Tucannon River reference collections, but the collection is not composed completely of "Tucannon-like" Chinook.

Literature Cited: Mayer, K., M. Schuck, and D. Hathaway. 2008. Assess Salmonids in the Asotin Creek Watershed: 2007 Annual Report. WDFW Report to BPA. http://wdfw.wa.gov/fish/papers/se_wash_reports/asotin_final07.html

Table 1. Unexpanded Asotin Creek spring Chinook redd counts from the WDFW SASSI database. Area surveyed included U.S. Forest Service boundary to Lick Creek on the north fork of Asotin Creek. (The past couple of years surveys have been from the US Forest Service boundary to Headgate Dam.)

Year	*Number of Observed Redds*	*Year*	*Number of Observed Redds*
1986	*1*	*2001*	*4*
1987	*3*	*2002*	*4*
1988	*1*	*2003*	*1*
1989	*0*	*2004*	*13*
1990	*2*	*2005*	*2*
1991	*0*	*2006*	*11*
1992	*0*	*2007*	*3*
1993	*2*	*2008*	*6*
1994	*0*	*2009*	*6*
1995	*0*		
1996	*0*		
1997	*1*		
1998	*0*		
1999	*0*		
2000	*1*		

Table 2. Sampling information for adult spring Chinook included in genetic analyses.

Sample ID	Year	Date	Origin	Sex	Length (cm)	FLOY#	Scale	Age Fresh	Total Age
05AY0051	2005	21-May	w	f	72	424	4j3	w1.2	3
05AY0052	2005	21-May	w	m	57	425	4j4	w1.2	3
05AY0053	2005	22-May	w	f	69	426	4j5	w1.2	3
05AY0054	2005	23-May	w		61.5	427	4j6	w1.2	3
05AY0055	2005	24-May	w	m	71	428	4k1	w1.2	3
05AY0056	2005	25-May	w		72	429	4k2	w1.2	3
05AY0057	2005	29-May	w	f	68	431	4k5	w1.2	3
05AY0058	2005	18-Jun	h	f	80		4l2	h1.3	4
05AY0059	2005	27-Jun	w	m	70	432	4l4	wR.2	-
05AY0060	2005	14-May	w	f	63	421	4i3	r	-
05AY0061[a]	2005	13-Jul	h	m	50	433	4m1	h0.2	2
05AY0062	2005	18-Jul	w	m	54	435	4m2	w1.2	3
05AY0063	2005	12-Sep	h	m	72		4m3	h1.2	3
05AY0064	2005	02-Oct	w	f	72		4m4	u	-
05AY0101	2005	18-May	w	m	69		4J4	w1.2	3
05AY0102	2005	19-May	w		66	423	4J2	w1.2	3
07AD0001	2007	13-May	w		69	852	c1-1	1	3
07AD0002	2007	15-May	w		70	853	c1-2	1	3
07AD0003	2007	17-May	w		70	854	c1-3	1	3
07AD0004	2007	18-May	w		74	855	c1-4	1	3
07AD0005	2007	19-May	w		67	856	c1-5	1	3
07AD0006	2007	20-May	w		81	857	c1-6	1	4
07AD0007	2007	21-May	w		48	858	c2-1	1	2

07AD0008[a]	2007	24-May	h		69	859	c2-2	1	3
07AD0009	2007	25-May	w		67	860	c2-3	1	3
07AD0010	2007	27-May	w		66	861	c2-4	1	3
07AD0011[a]	2007	29-May	h		74	862	c2-5	1	3
07AD0012	2007	05-Jun	w		73	863	c4-1	1	3
07AD0013	2007	05-Jun	w	F	74	864	c4-2	r	r
07AD0015	2007	11-Jun	w		40	865	c4-3	1	2
07AD0016	2007	27-Jun	w		72	869	c4-6	1	3

[a] These fish may have had coded-wire tags, based on electronic detection. The shaded samples indicate these were fall Chinook based on DNA analyses (Table 4).

Table 3. Reference collections used in comparative analysis of Asotin Chinook samples. Factorial correspondence analysis included either spring/summer and fall collections (figure 1) or just spring/summer (figure 2). Individual assignment tests included all collections shown.

Referece Collection	Regional Aggregate
ClearwaterRFa	Snake_R_fa
LyonsFerryH	Snake_R_fa
NezPerceTH	Snake_R_fa
BearValleyCr	Snake_R_sp/su
BigCr	Snake_R_sp/su
CamasCr	Snake_R_sp/su
CapehornCr	Snake_R_sp/su
CatherineCr	Snake_R_sp/su
ChamberlainCr	Snake_R_sp/su
CrookedFkCr	Snake_R_sp/su
DworshakH	Snake_R_sp/su
EFSalmonR	Snake_R_sp/su
ImnahaR	Snake_R_sp/su
JohnsonCr	Snake_R_sp/su
JohnsonH	Snake_R_sp/su
LochsaR	Snake_R_sp/su
LoloCr	Snake_R_sp/su
LookingGlassH	Snake_R_sp/su
MinamR	Snake_R_sp/su
NewsomeCr	Snake_R_sp/su
PahsimeroiR	Snake_R_sp/su
RapidRH	Snake_R_sp/su
RedR	Snake_R_sp/su
SawtoothH	Snake_R_sp/su
SeceshR	Snake_R_sp/su
TucannonH	Snake_R_sp/su
TucannonR	Snake_R_sp/su
WenahaCr	Snake_R_sp/su
WFYankeeFrk	Snake_R_sp/su
HanfordReach	U_Columbia_R_su/fa
PriestRapidH	U_Columbia_R_su/fa
PriestRapidsHFa	U_Columbia_R_su/fa
UmatillaH	U_Columbia_R_su/fa
UmatillaHFa	U_Columbia_R_su/fa

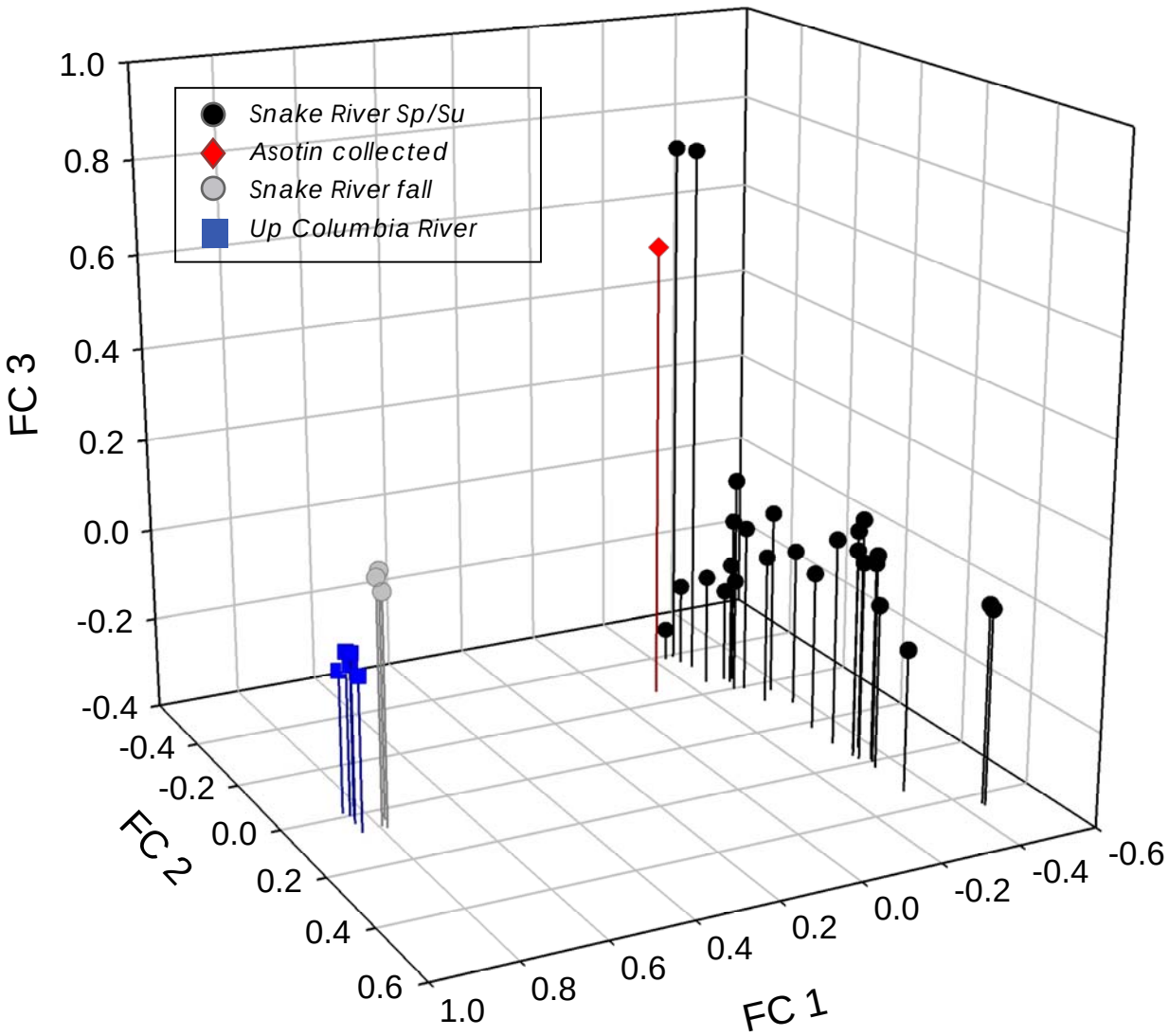

Figure 1. Factorial correspondence analysis on genetic data showing relative genetic differences among collections.

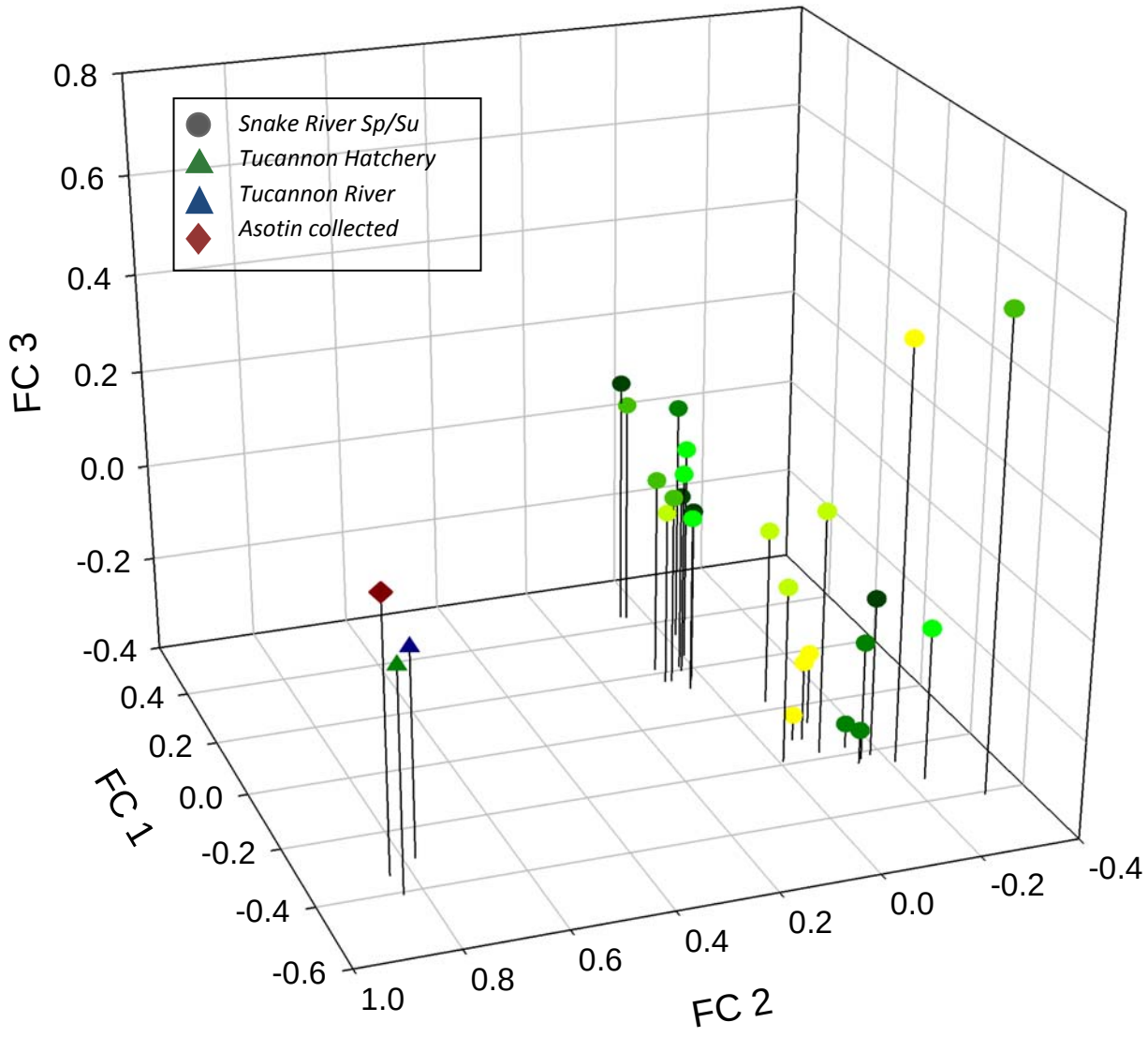

Figure 2. Factorial correspondence analysis on genetic data showing relative genetic differences among collections.

Table 4. Individual assignment analysis summary. The reference collection with the highest assignment probability is labeled Best Collection and the associated posterior probability value is shown in Prob1 column. The reference collection with second highest assignment probability is labeled 2nd Best. The Aggregate column shows the regional reporting group the assigned Best Collection belongs within and the associated posterior probability (Prob3). The TucannonH and TucannonR columns show the exclusion probability test results for each genotype given the allele frequencies of Tucannon Hatchery and Tucannon River collections. A probability below 0.05 (bolded) means the genotype for that individual is statistically unlikely to has arisen from those reference collections (i.e., could be excluded).

IND	Best Collection	Prob1	2nd Best	Prob2	Aggregate	Prob3	TucannonH	TucannonR
Asotin05-0051	TucannonR	0.986	MinamR	0.013	Snake_R_sp/su	1.000	0.061	0.304
Asotin05-0052	MinamR	0.875	TucannonR	0.124	Snake_R_sp/su	1.000	**0**	**0.005**
Asotin05-0053	MinamR	0.506	TucannonR	0.364	Snake_R_sp/su	1.000	0.394	0.458
Asotin05-0054	MinamR	0.994			Snake_R_sp/su	1.000	0.067	0.111
Asotin05-0055	MinamR	1.000			Snake_R_sp/su	1.000	**0.002**	**0.02**
Asotin05-0056	TucannonH	0.765	TucannonR	0.127	Snake_R_sp/su	1.000	0.873	0.754
Asotin05-0057	TucannonH	0.941	TucannonR	0.060	Snake_R_sp/su	1.000	0.442	0.243
Asotin05-0058	TucannonR	1.000			Snake_R_sp/su	1.000	**0.011**	0.25
Asotin05-0059	PahsimeroiR	0.881	TucannonR	0.062	Snake_R_sp/su	1.000	0.056	0.298
Asotin05-0060	MinamR	1.000			Snake_R_sp/su	1.000	**0.038**	**0.007**
Asotin05-0061[a]	NezPerceTH	1.000			Snake_R_fa	1.000	**-**	-
Asotin05-0062	MinamR	0.764	LochsaR	0.230	Snake_R_sp/su	1.000	**0.043**	0.283
Asotin05-0063	LochsaR	0.776	MinamR	0.196	Snake_R_sp/su	1.000	**0.01**	0.132
Asotin05-0064	ClearwaterRFa	0.858	HanfordReach	0.131	Snake_R_fa	0.858	**0**	**0**
Asotin05-0101	TucannonR	0.995			Snake_R_sp/su	1.000	0.489	0.806
Asotin05-0102	Unassigned[b]						-	-
Asotin07-0001	TucannonR	0.934	TucannonH	0.066	Snake_R_sp/su	1.000	0.153	0.265
Asotin07-0002	TucannonH	0.992			Snake_R_sp/su	1.000	0.282	0.148
Asotin07-0003	TucannonR	0.980	TucannonH	0.015	Snake_R_sp/su	1.000	0.221	0.424
Asotin07-0004	TucannonR	1.000			Snake_R_sp/su	1.000	0.43	0.914
Asotin07-0005	TucannonR	1.000			Snake_R_sp/su	1.000	0.188	0.805
Asotin07-0006	BigCr	0.891	TucannonR	0.108	Snake_R_sp/su	1.000	0.192	0.455
Asotin07-0007	TucannonR	0.926	LochsaR	0.059	Snake_R_sp/su	1.000	**0.03**	0.236
Asotin07-0008[a]	TucannonR	1.000			Snake_R_sp/su	1.000	-	-
Asotin07-0009	TucannonR	0.984	TucannonH	0.016	Snake_R_sp/su	1.000	0.399	0.652
Asotin07-0010	TucannonH	0.924	TucannonR	0.076	Snake_R_sp/su	1.000	0.983	0.9
Asotin07-0011[a]	TucannonR	1.000			Snake_R_sp/su	1.000	**-**	**-**

Asotin07-0012	HanfordReach	0.875	UmatillaHFa	0.125	U_Columbia_su/fa	1.000	**0**	**0**
Asotin07-0013	HanfordReach	0.996			U_Columbia_su/fa	0.998	**0**	**0**
Asotin07-0015	MinamR	0.866	TucannonH	0.091	Snake_R_sp/su	1.000	0.452	0.201
Asotin07-0016	TucannonR	0.975	ClearwaterRFa	0.019	Snake_R_sp/su	0.976	**0.01**	0.092

[a] These fish may have had coded-wire tags, based on electronic detection.
[b] This sample could not be assigned, defective.

APPENDIX 1

Methods and Materials

Assessing within collection genetic diversity - For each locus and collection GENETIX version 4.03 (Belkhir et al. 1996) was used to assess Hardy-Weinberg equilibrium, where deviations from the neutral expectation of random associations among alleles (F_{IS}) were calculated following Weir and Cockerham, (1984). A permutation procedure implemented in GENETIX was employed to generate the rejection zone for the null hypothesis. Genotypic linkage disequilibrium was calculated following Weir (1979) using GENETIX version 4.03 (Belkhir et al. 1996). Statistical significance of linkage disequilibrium (LD) was assessed using a permutation procedure implemented in GENETIX for each locus by locus combination within each collection, where results are reported as the proportion of pairwise combinations significant at $\alpha = 0.05$ (p-values were adjusted for multiple comparisons).

Assessing among collection genetic differentiation - Differentiation of allele frequencies was assessed by the randomization chi-square test implemented in FSTAT version 2.9.3.2 (Goudet 1995). Multi-locus genotypes were randomized between collections (11900 permutations). The G-statistic for observed data was compared to G-statistic distributions from randomized datasets (i.e., the null distribution of no allelic differentiation between collections). P-values were adjusted for multiple comparisons at $\alpha = 0.05$. Population differentiation was assessed using factorial correspondence analysis (FC) on allele frequencies. In brief, genetic data are transformed into a contingency table, where each individual is described by its multi-locus genotype (i.e., contingency table is individual's X alleles). The relationship between any two individuals in n-dimensional space (n = number of alleles) is represented by their χ^2 distance. Specifically, the plot represents the ordination of individuals along three orthogonal vectors that represent the three largest eigenvalues derived from the weighted contingency table. Additionally, each individual was not shown, but rather the collection centroids, which are the "centers of mass" for each collection.

Individual Assignment – Population of origin for "unknown" Chinook collected from Asotin Creek were estimated by using a partial Bayesian procedure based on the likelihood of unknown-origin genotypes being derived from reference stocks/populations, given the allele frequencies for those reference stocks/populations. In brief, the analysis procedure is as follows. Within a mixture, we first generated the conditional probability of each genotype occurring in each reference population, based on the allele frequencies in the reference populations, using equation 10 of Rannala and Mountain (1997) (i.e., probability of the genotype, conditioned on the allele frequencies for each reference population). For each genotype in the mixture, we then calculated the probability (i.e., posterior probability) that the sample was from each reference population by taking the Rannala and Mountain (1997) conditional probability and multiplying it by a prior, and then dividing by a normalizing constant. Initially, the prior was uniform, 1/N, where N is the number of populations used for the reference baseline. The initial probability matrix provided information about the likely source population for each unknown individual, but more importantly, provided an estimate of which reference populations were contributing to the unknown mixture. If the reference populations did not contribute equally to the mixture, the initial use of a uniform prior can be improved. The mean probability for a reference baseline population in the mixture analyzed (i.e., mean posterior probability over all unknown individuals) is the estimated contribution of that reference population to the mixture. Therefore, the population composition of the mixture was represented by the mean posterior probabilities of all reference collections from the initial matrix. This newly gained information about the population composition of the mixture replaced the uniform prior

during an additional round of probability estimation to generate a second probability matrix. Once again, the mean posterior probabilities that represent estimates of baseline population contributions to the mixture were used as new priors. This iterative refinement of the probability matrix continued until the mean posterior probabilities change less than a predefined threshold from round to round. This procedure results in the maximum likelihood solution for stock composition (Millar 1985). The individual assignments are extracted from the mixture estimation and reported either by collection (e.g. TucannonR) or aggregate (e.g. Snake River sp/su). This procedure was implemented using the program ONCOR (ST Kalinowski unpublished).

We determined the compatibility of each genotype to Tucannon River reference collections, as these collections were the potential source of the unknown-origin fish captured in Asotin Creek. Similar to individual assigned described above (excluding iterative refinement), the Rannala and Mountain (1997) algorithm was used to calculate likelihoods that an individual fish originated in each of the two baseline collections**.** The probability that each individual genotype originated from each Tucannon reference collection was determined with a Monte Carlo simulation (Paetkau et al. 2004). The simulation creates 10,000 individuals for each baseline collection to simulate the genotypes likely to be encountered for that collection. Assignment likelihoods are computed for each simulated individual to generate an expected likelihood distribution. The likelihood of the actual fish is then used to define the rejection zone. The genotype of actual fish is hypothesized to have arisen from the reference collection if the assignment probability is greater than 0.05 (i.e., the null hypothesis is accepted).