Genetic characterization of coho salmon (*Oncorhynchus kisutch)* in Agency Creek, a tributary to the South Yamhill River (Willamette Basin, OR)

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Introduction

As described in the Oregon Department of Fish and Wildlife (ODFW) Coast Range Subbasin Fish Management Plan (Wevers et al. 1992), the Coast Range subbasin drains approximately 1,800 square miles of the midwestern side of the Willamette basin. The major streams of the subbasin, from north to south, are the Yamhill River, Rickreall Creek, Luckiamute River and Marys River. These streams originate in the steep and deeply dissected slopes of the upper elevations of the Coast Range (average elevation 2,000 feet) and flow eastward, emptying into the Willamette River. Agency Creek, the study site for this project, is a tributary to the South Yamhill River.

ODFW began stocking programs for steelhead and coho salmon in the South Yamhill watersheds in the 1960s and continued until the late 1980s. Anecdotal information from The Confederated Tribes of Grand Ronde Tribal Elders and Oregon State reports (as cited in Wevers et al. 1992) indicate the presence of steelhead before stocking programs began. Coho salmon, however, were not native above Willamette Falls, and were not present in the Reservation until introduced by the stocking programs. Stocking records indicate that several stocks were outplanted into the South Yamhill tributaries, including Agency Creek, in varying numbers and at various life history stages (Table 1). Coho salmon are now reproducing naturally and have been documented in several streams on and around the Reservation. Tribal weir counts indicate that 907 coho salmon returned to Agency Creek in 2009 and 992 coho salmon returned in 2010. Due to operational constraints, accurate counts were not feasible in 2011, however, a minimum of 66 coho salmon were counted passing the weir. Fish passage counts conducted at Willamette Falls indicate fewer coho salmon likely returned to the basin in 2011. There were 5,362 coho salmon passing Willamette Falls in 2011 compared to over 20,000 in 2009 and 2010.

In 2007, Van Doornik et al. published a study of 84 populations of coho salmon from the Oregon and Washington coasts using 10 microsatellites in common with the Agency Creek project. Included in this study were samples from four of the ten hatcheries used by ODFW to stock South Yamhill River tributaries (Big Creek, Bonneville, Sandy, and Trask), as well as two natural-origin samples from rivers where hatchery coho salmon were also produced (Alsea and Siletz; Table 1). At the time that coho salmon were released into the South Yamhill tributaries, most of the source hatcheries were using broodstock originally derived from the Toutle River in southwest Washington. It is important to note that current broodstock lines propagated at these

source hatcheries have transitioned from Toutle River fish towards more local source populations (Table 2). The contemporary genetic characteristics of these hatchery populations, consequently, may differ somewhat from the genetic characteristics of the stocks released into the South Yamhill tributaries in the 1960s through the late 1980s. To address this concern, we have included a collection from the Washington Department of Fish and Wildlife (WDFW) North Toutle River Hatchery in addition to the contemporary collections. This facilitates the comparison of the current Agency Creek population not only to the stocking hatcheries but to the original broodstock line. We have also included additional hatchery and natural-origin populations from the Lower Columbia River which may also have contributed to the founding of the Agency Creek population.

The majority of coho salmon from the Lower Columbia River return to spawn as three-year-olds, with successful spawning of coho jacks (two-year-olds) facilitating geneflow among lineages or broodlines. In the absence of jacks, some coho salmon populations may exhibit high levels of divergence among broodlines. Therefore, our analysis included collections made from Agency Creek in four consecutive return years (2008-2011) to fully assess the genetic variability of the naturally spawning population. This report contains analysis and conclusions from the final year of a study started in 2009 which was designed to examine the genetic variability of Agency Creek coho salmon, and evaluate their relationship to coho salmon populations in the region. Our specific goal of this study was to address three main objectives:

1) Within population genetic diversity of Agency Creek coho salmon: Is there evidence at selectively neutral genetic markers of multiple stocks in Agency Creek? The level of allelic diversity will be assessed and degree of genetic divergence between years will be determined.

2) Genetic population structure: What is the genetic relationship of Agency Creek coho salmon to other Oregon and Washington populations previously characterized?

3) Genetic similarity to planted hatchery stocks: What is the relationship of naturally produced coho salmon in Agency Creek to the stocks previously planted?

Methods

Sample collections

Agency Creek coho salmon samples included in this study were collected by biologists of The Confederated Tribes of Grand Ronde (CTGR). Our sampling goal for this study was 95 individuals from each of three consecutive return years. This goal was met in 2009 and 2010, but only 63 samples were collected in 2011 due to power outages occurring at the weir that year. We supplemented the 2011 collection with 30 samples from the 2008 return year which represents the same broodline.

Data generated at the USFWS Abernathy Fish Technology Center (AFTC) for Agency Creek coho salmon were combined with additional genotype data from both AFTC and data obtained from the coho salmon baseline published in Van Doornik et al. (2007). We selected 14 reference collections to be used for this project: four hatchery populations used to stock South Yamhill tributaries (Big Creek, Bonneville, Sandy, Trask hatcheries), two natural-origin populations from rivers where a hatchery was used for stocking but for which no hatchery sample was available (Alsea River, Siletz River), a Toutle River Hatchery collection which represents the original broodstock line used at most of the source hatcheries, and seven additional collections to more fully capture the genetic variability of Lower Columbia River coho salmon (Table 3).

Laboratory analysis

DNA was extracted from all samples using QIAGEN DNeasy tissue kits following manufacturer's protocols (Qiagen Inc., Valencia, CA). Polymerase chain reaction (PCR) was used to amplify 11 microsatellite loci (Table 4) from template DNA. PCRs were carried out in 10µl volumes containing 2µl of template DNA, 5µl of 2X QIAGEN Multiplex PCR Master Mix (final concentration of 3mM $MgCl_2$), and 0.2µl of oligonucleotide PCR primer mix. PCR conditions were as follows: initial denaturation at 95°C for 15 minutes, then 29 cycles of 95°C for 30 seconds, 90 seconds at the multiplex specific annealing temperature (Table 4) and 60 seconds primer extension at 72°C, followed by a final extension at 60°C for 20 minutes. Following PCR, capillary electrophoresis was carried out on an ABI 3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) following the manufacturer's protocols. The G5 filter set was used to produce electropherograms, and electrophoresis data were analyzed using

the program GENEMAPPER v4.0 (Applied Biosystems Inc.). Genotypes were scored by two independent readers (double-scoring). Following completion of the data collection, 10% of all samples were re-analyzed as part of AFTC's QA/QC protocol.

Statistical analysis

Individuals missing data at more than two loci and individuals with duplicate genotypes were screened for and removed in R (R Development Core Team 2010).

Evidence of multiple populations and/or family structure was evaluated in all collections using GENEPOP v4.0.1 (Rousset 2008) to test for departures from Hardy-Weinberg Equilibrium (HWE) and for evidence of linkage disequilibrium (LD) between all pairs of loci. Significance values (α=0.05) for HWE and LD were corrected for multiple tests using a sequential Bonferroni correction (Rice 1989). The 2008 and 2011 Agency Creek collections were compared using a correspondence analysis computed in GENETIX version 4.05.2 (Belkhir et al. 2004). They were then pooled for all subsequent analyses based on significant genetic homogeneity indicated by exact tests performed in GENEPOP v4.0.1 (data not shown).

Genetic diversity within all collections was assessed by calculating observed (H_O) and expected heterozygosity (H_E) in GENALEX v6.41 (Peakall and Smouse 2006), and by determining allelic richness (A_R) within collections using a rarefaction method implemented in HP-Rare v1.0 (Kalinowski 2005). Large genetic samples are expected to have more alleles than small samples. Rarefaction is a statistical technique to deal with this problem which allows the number of alleles in large samples to be compared with the number of alleles in small samples.

To compare the genetic divergence among years in Agency Creek, and among all collections, pairwise F_{ST} values were calculated using FSTAT, which also tests the statistical significance of the results by permuting the data. Divergence among return years (2009, 2010, and 2008/11) in Agency Creek was also assessed using correspondence analysis computed in GENETIX version 4.05.2. Genetic divergence among Agency Creek and all other collections was then analyzed using principal component analysis (PCA) in PCAGEN (Goudet 2005). Correspondence analysis and PCA are useful tools for exploring the genetic connectedness of individuals and populations because of their ability to use high-dimensional data (e.g., multilocus genetic data) to reveal the structure of variation among individuals or populations in a smaller number of dimensions which are easy to visualize. Finally, a neighbor-joining tree based on

Cavalli-Sforza and Edwards (Cavalli-Sforza and Edwards 1967) chord distances was calculated in POPULATIONS (Langella 2001). Bootstrap values were calculated for 1000 replicates of the dataset as a measure of the confidence in the observed branching patterns.

Results

Laboratory analysis

Two individuals collected in 2008 and one individual each from the 2010 and 2011 Agency Creek collections failed to genotype successfully. All individuals collected in 2009 were successfully genotyped. No duplicate genotypes were detected among the Agency Creek samples. Genotyping error rates were 0%, with no genotype discrepancies occurring between the original and re-analyzed samples.

Statistical analysis

One locus out of 11 (*Ots103*) was out of HWE in 8 collections and was removed from further analyses, leaving 10 microsatellite loci. Deviations from linkage equilibrium were generally scattered among collections and loci. An exception was the juvenile collection from Eagle Creek Hatchery which showed some evidence for LD at 4 out of the possible 45 pairs of loci. This is likely a result of family groups being over represented in the sample. No pattern of widespread deviations from HWE or evidence of LD was observed in the other collections, which suggests that each collection represents a single, randomly mating population.

Tests for genetic homogeneity indicated that the 2008 and 2011 Agency Creek samples were indistinguishable from each other (data not shown). Prior to pooling the collections for subsequent analyses, however, a correspondence analysis was produced to demonstrate this genetic similarity among years (Figure 1). In a correspondence analysis the genetic profile of each individual is shown graphically in relation to all other individuals, enabling an examination of variation both within and among collections. The figure shows consistent overlap of individuals from the 2008 and 2011 collection years and that the population centers for the collections are indistinguishable. There is also similar dispersion of individuals within each of the collection years, indicating similar levels of genetic diversity within the collections. Combined with the tests for homogeneity, this indicates that the two collections are genetically

indistinguishable, and they were therefore pooled into a single collection (Agency2008/2011) for all subsequent analyses.

Genetic diversity varied among years in Agency Creek and among collections in the region. Within Agency Creek, the pooled 2008/2011 collection had the highest allelic richness (mean A_R = 12.25) followed by the 2010 collection (mean A_R = 12.01) and the 2009 collection (mean A_R = 11.17); however, all were below the overall average among all collections of 12.36 alleles (Table 3).

Pairwise F_{ST} values were significantly different from zero after 2720 permutations for all but one comparison (Cowlitz and Elochoman late run hatcheries). Agency Creek collections were distinct from all other collections and were significantly different between years (Table 5). Pairwise F_{ST} values between Agency Creek collections ranged from 0.015 (Agency Creek 2009 and Agency Creek 2010) to 0.020 (Agency Creek 2010 and Agency Creek 2008/2011). F_{ST} values among all collections ranged from 0.007 (Cowlitz and Elochoman late run hatcheries) to 0.078 (Agency Creek 2009 and Trask Hatchery), and although the collections from Agency Creek were significantly different from each other, the magnitude of the differences was smaller than most pairwise differences between Agency Creek and other collections.

The genetic divergence among Agency Creek and the other collections is shown graphically by the PCA (Figure 2) and the neighbor-joining dendrogram (Figure 3). The PCA is a graphical representation of the variation in the genetic data among populations; of all the variation in allele frequencies among populations, the plot shown in Figure 2 explains 35.5%. Although the degree of separation in the PCA is not high, no collection from Agency Creek appears clustered closely with any of the other collections in the region. A similar pattern of genetic structure is seen in the neighbor-joining dendrogram. Although many of the branching patterns are unsupported, high bootstrap support is evident for some clusters of populations. There is strong bootstrap support for the relationships among late run (Type S) populations (Clackamas late run, Cowlitz Hatchery, and Elochoman Hatchery late run), and for the relationships among coastal Oregon populations (Siletz River, Alsea River, and Trask Hatchery). Agency Creek collections cluster on the opposite side of the figure with 69% bootstrap support, and appear more closely related to early run (Type N) populations from the Lower Columbia River. Agency Creek collections are clustered together but with relatively long branch lengths which suggests that they are different from one another, but more similar to each other than to

other groups. The Agency Creek collections also appear divergent in the PCA, and the relationship between years of Agency Creek coho salmon is shown more specifically by the correspondence analysis (Figure 4). The figure shows that while there is overlap of individuals from different collection years, the population centers for each annual collection of individuals are not identical. The greatest separation observed is between the Agency Creek 2010 collection and the Agency Creek 2008/2011 collection which is supported by the pairwise F_{ST} values observed among the Agency Creek collections. The correspondence analysis also shows greater dispersion of individuals from the 2010 and 2008/2011 collections when compared to the 2009 collection. This greater genetic diversity within the 2010 and 2008/2011 collections was supported by our analysis of allelic richness.

Discussion

Preliminary results reported in 2009 and 2010 indicated that Agency Creek coho salmon were genetically distinct from the hatchery stocks that were planted in the South Yamhill River tributaries by ODFW in the 1960's and 70's (McGlauflin and Hawkins 2011). Here we have presented analyses of four consecutive return years (2008-2011) of coho salmon in Agency Creek that support those previous results. Some of the original stocking populations were not available for comparison (Cascade, Klaskanine, Oxbow, and Elk River hatcheries), but given the divergence between Agency Creek and available hatcheries, as well as among hatchery collections, it seems unlikely that the addition of these populations would change the results presented here.

While our results indicate that coho salmon in Agency Creek are distinct from the hatchery stocks planted there, we are not able to definitively determine their origin or rule out the hatchery stocks as the founding populations. The comparison between contemporary samples from the stocking populations and Agency Creek is not straight forward. The move towards local broodstock sources and integrated hatchery programs in the past decade makes it likely that contemporary hatchery populations are genetically different from the populations produced there forty years ago. In fact, most of the hatchery plantings were comprised mainly of Toutle River lineage broodstock, while today all of the original source hatcheries still in existence produce only local fish (data from ODFW). Based on this information, a sample of North Toutle River Hatchery coho salmon from 2002 was obtained and also included in this analysis to facilitate

comparisons to the original broodstock line. Our results indicate that there is also genetic differentiation between Agency Creek and the Toutle River population, however, this does not necessarily rule out the ODFW hatchery stocks as originators of the Agency Creek spawning population. The 2002 Toutle River sample may not resemble the original natural population, or the various broodstock lineages that it founded. Furthermore, even if the source populations have not changed, thirty-three years (11 coho salmon generations) have passed since the last coho salmon were planted in the South Yamhill tributaries, enough time for populations to diverge significantly if there is no gene flow between them as would have been the case in Agency Creek.

Coho salmon spawning in Agency Creek also appear to be divergent from proximate populations that were included in this report. The Elochoman River Hatchery and Cowlitz River Hatchery were added to broaden the baseline to include the entire Lower Columbia River. The Elochoman Hatchery early run is still produced from a broodstock lineage founded from Toutle River stock, making it an especially interesting comparison. Natural-origin fish in the Clackamas River and two samples representing the Eagle Creek Hatchery population (also on the Clackamas) were included because they are the closest spawning populations of coho salmon to Agency Creek, and could be a potential source of strays in Agency Creek. The Eagle Creek Hatchery population was derived from a mixture of broodstock from the Sandy River, Toutle River, and Big Creek, and is genetically distinct among broodlines making it another especially interesting comparison. The results of the PCA, neighbor-joining dendrogram, and population differentiation tests clearly show that Agency Creek is genetically distinct from all of these collections. There also is no evidence in tests of LD or HWE of multiple populations in any of the Agency Creek collections, which would indicate large proportions of strays from nearby populations.

It is important to compare multiple brood years from a population of coho salmon to capture all the genetic variation, since brood years can vary greatly even within a population (e.g. Eagle Creek Hatchery). Nearly all individuals of the species mature at three years of age, so the only gene flow that occurs between brood years is through the small proportion of jacks. This is clearly the case for Agency Creek coho salmon; return years 2009, 2010, and 2008/2011 were significantly distinct from one another, although still most similar to each other compared to the other included populations. This result was also evident in the Neighbor-joining tree with the

three Agency Creek collections clustered together with 69% bootstrap support, yet separated by relatively long branch lengths indicating genetic differences between the collections. The factorial correspondence analysis clearly shows both the overlap among individuals from each collection and the difference between the centers of the distributions. The percentage of jacks in the population was less than 10% in all of the collections (data from The Confederated Tribes of Grand Ronde), so brood years in Agency Creek essentially represent populations with less than 10% gene flow between them. Interestingly, the 2008 collection represents the parental generation of the 2011 collection; therefore, the genetic homogeneity between these two collections suggests reproductive success from the naturally spawning population in Agency Creek. We would not expect to observe this similarity between two collection years of the same broodline if the collections were composed of random strays from other populations.

Historical evidence suggests it is unlikely that a natural population of coho salmon existed in Agency Creek before stocking began in 1962 but although stocking ended in 1987 coho salmon continue to return each year. Whether stocking was the origin of the current spawning population or not, evidence presented here indicates that the population is now genetically distinct from the contemporary North Toutle River Hatchery coho salmon population, hatchery stocks from which planted fish were obtained, as well as coho salmon from nearby spawning populations in the Clackamas River. While these results are interesting, and suggest that the population is now unique, they do not enable us to draw any strong conclusions about the origin of coho salmon in Agency Creek because it is difficult to make a direct comparison between the current population and the original stocked fish. What is clear is that four consecutive years of coho salmon returning to Agency Creek are genetically more similar to each other than they are to all other populations in the region.

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Table 1. Stocking history of the South Yamhill River tributaries (data from The Confederated Tribes of Grand Ronde).

Source hatchery	Year	Number	Lifestage
Alsea	1969	200	Adults
Big Creek	1969	300	Adults
	1970	1,226,997	Fry
	1972	397,240	Fry
Bonneville	1962	402,052	Fry
	1963	462,907	Fry
	1966	150	Adults
	1968	140	Adults
	1972	208	Adults
	1973	435,226	Yearling
	1974	484,769	Yearling
	1987	41,088	Fry
Cascade	1964	600	Adults
	1976	124,869	Yearling
Elk River	1973	196,100	Fry
Klaskanine	1965	1,827,209	Fry
	1967	806	Adults
	1968	306,000	Fry
Oxbow	1965	64,152	Fry
Sandy	1962	63,158	Yearling
	1963	44,979	Yearling
	1964	61,814	Yearling
	1965	69,793	Yearling
	1966	14,329	Yearling
	1982	31,388	Fry
Siletz	1967	100	Adults
Trask	1967	104,250	Fry
STEP*	1983	262,388	Fry
	1984	200,000	Fry
	1985	202,740	Fry
	1986	236,314	Fry
	1987	274,125	Fry

*Volunteer based Salmon and Trout Enhancement Program which hatches and rears salmon eggs from several hatcheries to rehabilitate native fish stocks (http://www.dfw.state.or.us/fish/STEP/).

Table 2. Broodstock lineage information for each hatchery used by Oregon Department of Fish and Wildlife to stock the South Yamhill River tributaries between 1962 and 1976 (data from Oregon Department of Fish and Wildlife)

Hatchery	Previous Broodstock	Current Broodstock
Alsea	Unknown	None
Big Creek	Toutle River	Big Creek
Bonneville	Toutle River	Sandy River, Tanner Creek
Cascade	Toutle River, Cowlitz River*	Sandy River, Tanner Creek
Elk River	Toutle River	None
Klaskanine	Toutle River	Big Creek, Eagle Creek
Oxbow	Toutle River	Sandy River, Tanner Creek
Sandy	Toutle River	Sandy River
Siletz	Unknown	Siletz River
Trask	Toutle River	Trask

*Only used in the 1976 Cascade Hatchery stocking of S. Yamhill Tributaries

Table 3. Collection data for coho salmon from Agency Creek and coho salmon populations from the Oregon Coast and Lower Columbia River. Population ID, collection year, lifestage, and samples included in analyses (n) are shown. Estimates of genetic variation include: expected heterozygosity (H_E), observed heterozygosity (H_O), and allelic richness (A_R).

Population	Population ID	Collection year	Lifestage	n	H_E	H_O	A_R
Agency Creek collections							
Agency Creek	Agency09	2009	Adults	95	0.79	0.81	11.17
Agency Creek	Agency10	2010	Adults	94	0.83	0.83	12.01
Agency Creek	Agency08/11	2008 & 2011	Adults	90	0.82	0.80	12.25
Reference collections							
Alsea River*	Alsea	n/a	Adults	39	0.86	0.88	15.45
Big Creek Hatchery*	Big	2002	Juveniles	58	0.85	0.86	12.21
Bonneville Hatchery*	Bonneville	2002	Juveniles	61	0.83	0.83	12.17
Clackamas River (early run)	ClackamasE	2010	Adults	89	0.83	0.84	12.65
Clackamas River (late run)	ClackamasL	2010	Adults	76	0.81	0.81	10.46
Cowlitz Hatchery*	Cowlitz	2002 & 2003	Juveniles	110	0.84	0.84	13.21
Eagle Creek Hatchery (2007 brood year)	EagleBY07	2010	Adults	114	0.83	0.85	12.07
Eagle Creek Hatchery (2009 brood year)	EagleBY09	2010	Juveniles	98	0.84	0.84	12.67
Elochoman Hatchery (early run)*	ElochomanE	2003	Juveniles	40	0.81	0.84	12.09
Elochoman Hatchery (late run)*	ElochomanL	2003	Juveniles	33	0.81	0.84	12.96
Sandy Hatchery*	Sandy	2002	Juveniles	71	0.82	0.84	10.19
Siletz River*	Siletz	2000 & 2001	Adults	58	0.88	0.89	14.98
Toutle Hatchery	Toutle	2002	Adults	70	0.82	0.82	12.88
Trask Hatchery*	Trask	2002	Juveniles	57	0.81	0.84	10.65

*Data from Van Doornik et al. (2007)

Table 4. Annealing temperature and reference for each of 11 microsatellite markers used in this study. The number of alleles and heterozygosity across all considered populations are also given.

Locus	Annealing temperature	# of Alleles	Heterozygosity	Reference
Ocl8	61	22	0.880	Condrey and Bentzen 1998
Oki1	59	18	0.828	Smith et al. 1998
Oki10	61	37	0.919	Smith et al. 1998
Oki23	61	29	0.863	A. Spidle, unpublished data*
One13	61	21	0.841	Scribner et al. 1996
Ots3	50	16	0.733	Banks et al. 1999
Ots103	59	52	0.797	Small et al. 1998
Ots213	59	30	0.653	Greig et al. 2003
OtsG422	59	58	0.948	Williamson et al. 2002
P53	59	16	0.847	de Fromentel et al. 1992
Ots505	61	15	0.813	Naish and Park 2002

*Genbank accession number AF272822

Table 5. Pairwise divergence (F_{ST}) between coho salmon collections from Agency Creek and coho salmon populations from the Oregon Coast and Lower Columbia River. Significance levels are determined over 2720 permutations. All values were significant except for those in bold text. Darker shading indicates higher levels of divergence between populations.

	Agency09	Agency10	Agency08/11	Alsea	Big	Bonneville	ClackamasE	ClackamasL	Cowlitz	EagleBY07	EagleBY09	ElochomanE	ElochomanL	Sandy	Siletz	Toutle	Trask
Agency09	--																
Agency10	0.015	--															
Agency08/11	0.017	0.020	--														
Alsea	0.045	0.024	0.036	--													
Big	0.038	0.022	0.033	0.026	--												
Bonneville	0.028	0.020	0.030	0.024	0.016	--											
ClackamasE	0.032	0.024	0.026	0.028	0.024	0.013	--										
ClackamasL	0.047	0.032	0.046	0.035	0.041	0.028	0.034	--									
Cowlitz	0.028	0.019	0.027	0.026	0.025	0.016	0.020	0.024	--								
EagleBY07	0.037	0.024	0.035	0.032	0.025	0.010	0.016	0.028	0.018	--							
EagleBY09	0.032	0.019	0.031	0.024	0.019	0.021	0.021	0.029	0.021	0.015	--						
ElochomanE	0.032	0.028	0.025	0.029	0.024	0.019	0.015	0.037	0.023	0.028	0.020	--					
ElochomanL	0.027	0.023	0.020	0.034	0.033	0.023	0.023	0.026	**0.007**	0.018	0.021	0.022	--				
Sandy	0.043	0.030	0.040	0.026	0.025	0.007	0.022	0.037	0.024	0.017	0.028	0.038	0.035	--			
Siletz	0.049	0.027	0.034	0.010	0.023	0.029	0.028	0.041	0.027	0.035	0.027	0.030	0.035	0.036	--		
Toutle	0.039	0.026	0.033	0.028	0.022	0.028	0.024	0.037	0.027	0.034	0.021	0.013	0.028	0.042	0.027	--	
Trask	0.078	0.061	0.059	0.042	0.065	0.060	0.059	0.063	0.056	0.061	0.062	0.055	0.058	0.061	0.036	0.050	--

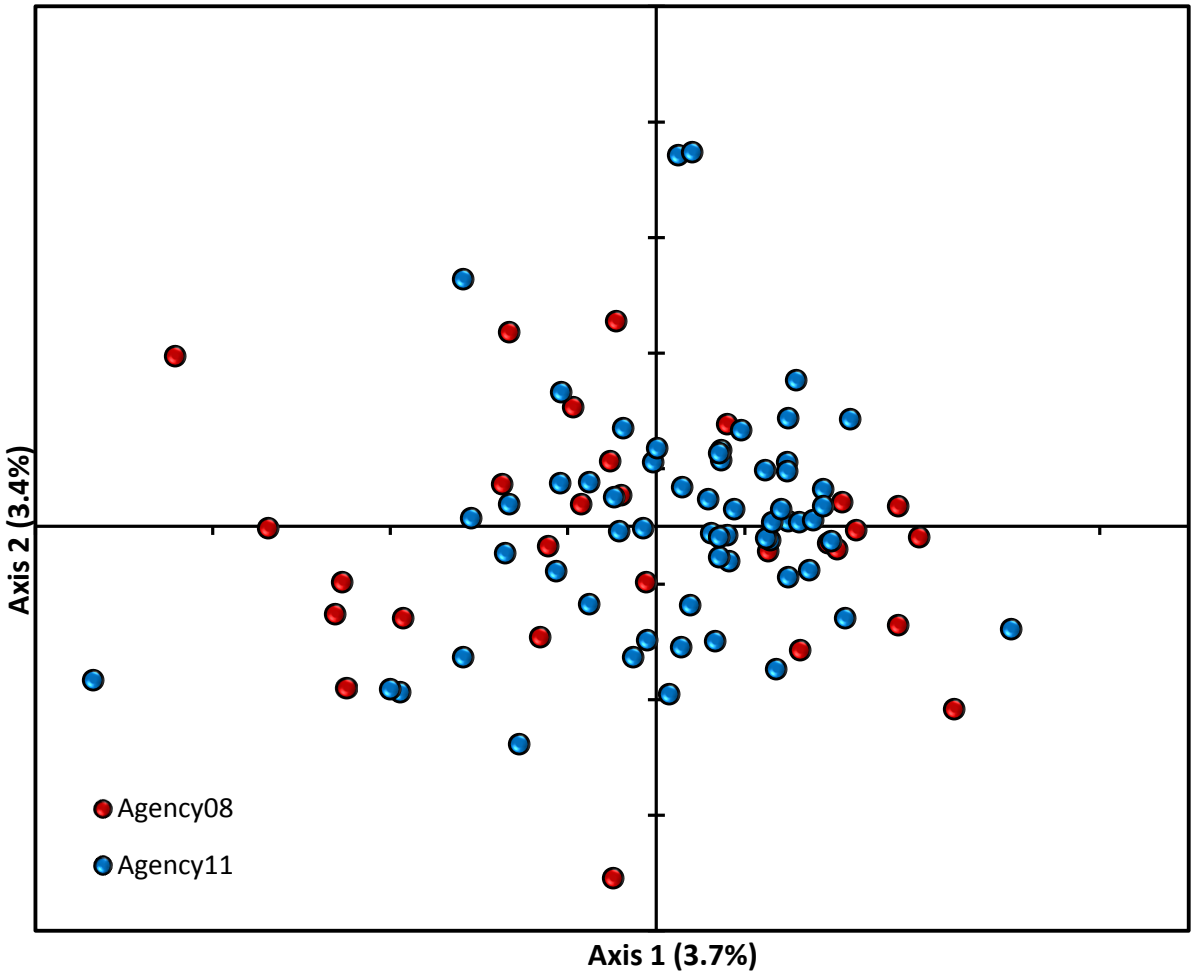

Figure 1. Factorial correspondence analysis of genotype data for the 2008 and 2011 Agency Creek coho salmon collections. Each point represents one individual, and the distance between points corresponds to the amount of genetic divergence.

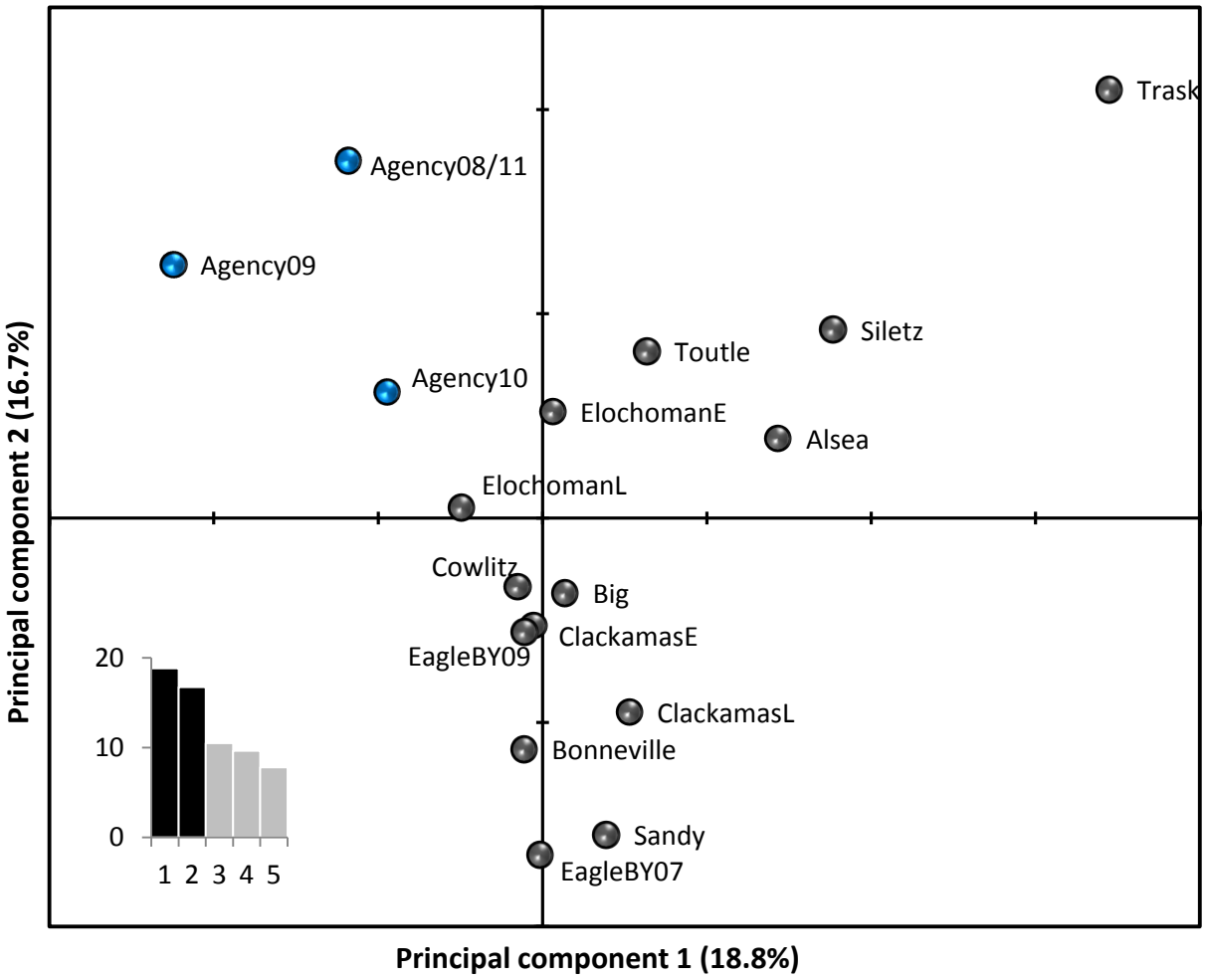

Figure 2. Principal component analysis (first two principal components shown) of coho salmon from the Oregon Coast and Lower Columbia River (Agency Creek collections shown in blue). The percentage of genetic variation explained by the first five principal components is shown in the lower left (plotted axes in black). Population IDs are from Table 3.

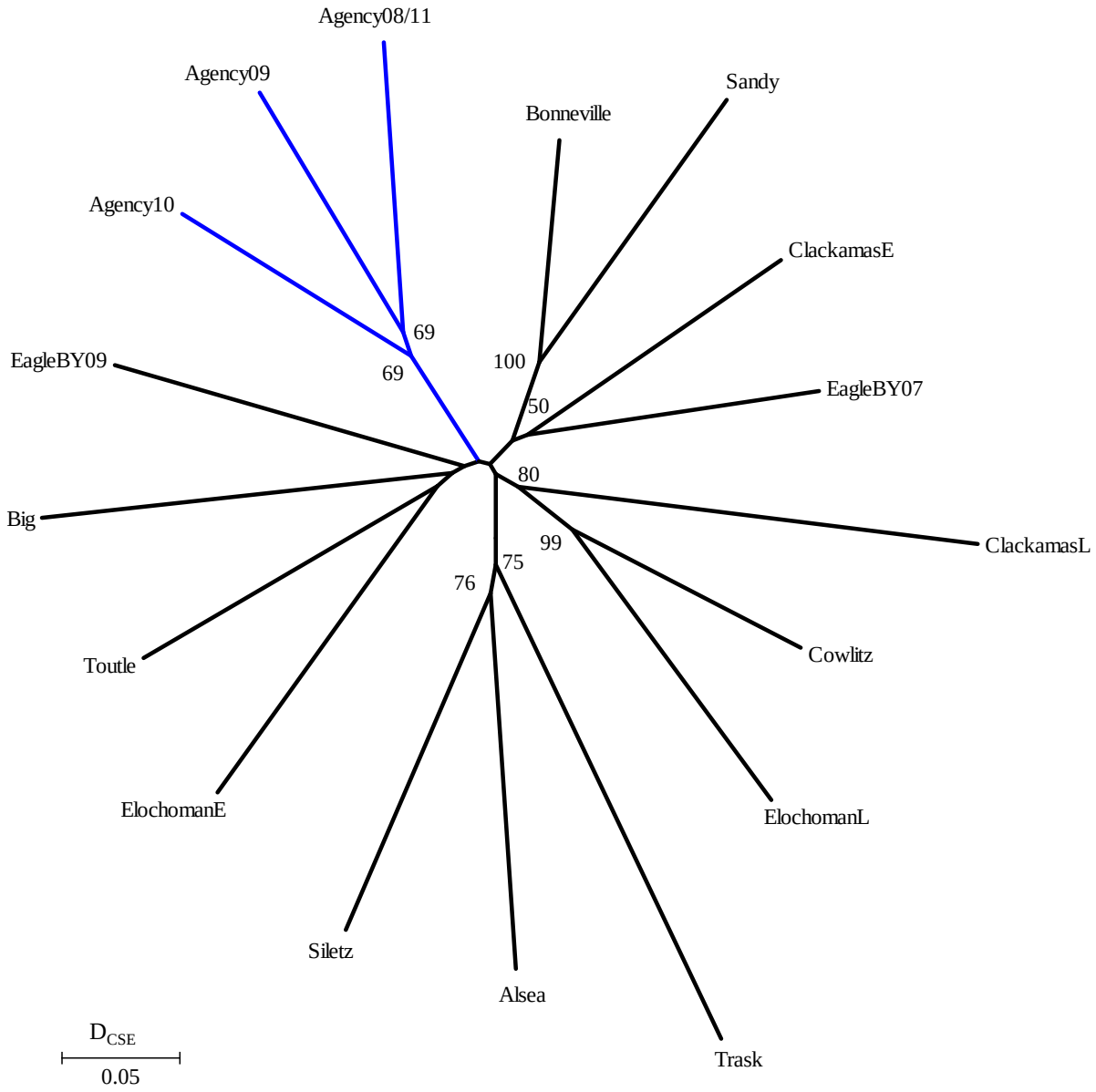

Figure 3. Neighbor-joining dendrogram using Cavalli-Sforza and Edwards (1967) chord distance (D_{CSE}) showing the relationships among populations of coho salmon from the Oregon Coast and Lower Columbia River (Agency Creek collections shown in blue). Bootstrap values represent the percentage of time out of 1000 bootstraps that each branching pattern was observed. Only values greater than or equal to 50% are shown. Population IDs are from Table 3.

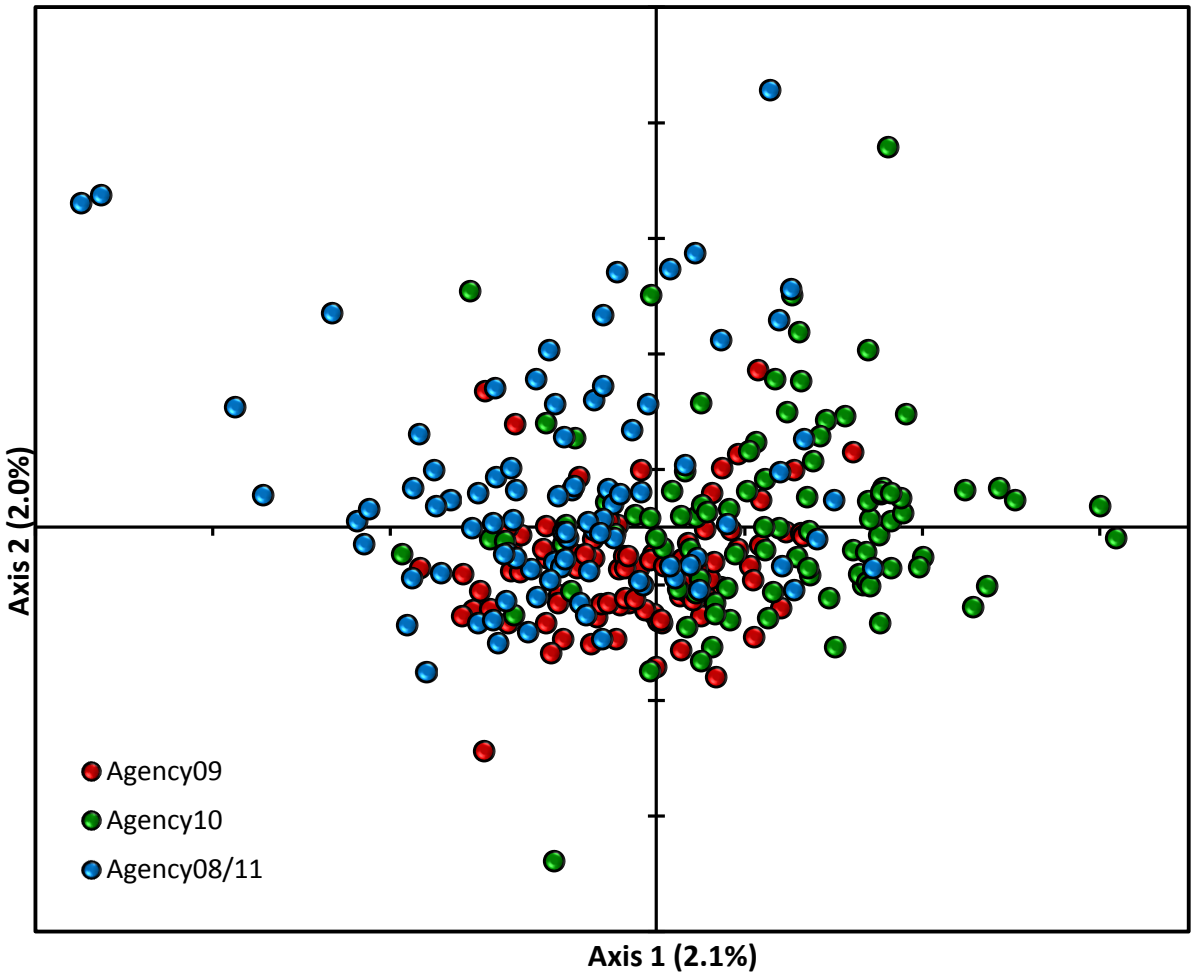

Figure 4. Factorial correspondence analysis of genotype data for all Agency Creek coho collections. Each point represents one individual, and the distance between points corresponds to the amount of genetic divergence.