

GENETIC ANALYSIS OF WILD COHO SALMON POPULATIONS BEFORE AND AFTER COHO SALMON ENHANCMENT

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Introduction

Concern has increased about the genetic effects of cultured salmonid fishes on natural populations. Avoidance of extreme negative outcomes was one reason for the establishment of a genetic management policy for the State of Alaska. However, founder effect, subsequent genetic drift, domestication selection, inbreeding, or a combination may still cause genetic divergence of a hatchery stock from its wild donor population. If these processes are occurring in the hatchery in combination with straying and its resultant introgression of hatchery salmon into wild Alaskan systems, there may be negative impacts on wild salmon stocks.

Our Approach

In the late 1960's and early 1970's before coho salmon (*Oncorhynchus kisutch*) enhancement activities were developed on Southern Baranof Island, personnel from the National Marine Fisheries Service Little Port Walter (LPW) research station collected adult coho salmon scales from Sashin Creek and Nakvassin Creek. Research on coho enhancement at Little Port Walter, using Sashin Creek broodstock, started in the mid 1970's and ended in the early 1980's. The information gained from coho enhancmnet research at LPW lead to the development of the Deer Lake coho enhancement project and coho enhancement at Port Armstrong hatchery in the mid-1980's. These hatcheries are run by private nonprofit organizations and produce ~ 1million coho smolts annually. Broodstock for both hatcheries originated from two years of Sashin Creek broodstock and one year of Deep Creek broodstock. Nakvassin Creek coho have not been used as broodstock for any enhancement projects and remain a pristine wild population. The present study uses a combination of 15 microsatellite and 11 SNP markers to compare the population structure between Sashin and Nakvassin creek coho before and after local enhancement, and Deer lake and Armstrong hatchery coho. Coho samples from Auke creek (located 140 mile north in Juneau, Ak) are use as an out group in this study.



Figure 1. Right map of study sites on southern Baranof Island in Southeast Alaska. Left map indicating southern Baranof Island in Alaska.

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Table 1. Sample information for coho sampled, and summary of genetic variation within samples.

Label	Site	Location	Year	Stock	Age	Tissue	N	N _a	N _e	N _{r(16)}	N _p	H _O	H _E	f
Arm07	Armstrong Hat.	Raceway	2007	Deep Cove Cr.	F	F	182	4.81	2.83	3.89	0.00	0.48	0.48	0.01
Auk09	Auke Cr.	Weir	2009	Wild	A	Ax	149	5.42	2.83	3.97	0.38	0.48	0.49	0.02
Deer08	Deer Lk. H.	Net Pens	2008	Deep Cove Cr.	S	Ad	62	4.46	2.75	3.89	0.00	0.48	0.48	-0.01
Deer09	Deer Lk. H.	Net Pens	2009	Sashin Cr.	S	Ad	84	4.08	2.62	3.60	0.00	0.49	0.47	-0.02
Nak69	Nakvassin Cr.	Creek Mouth	1967	Wild	A	S	24	3.88	2.65	3.68	0.00	0.46	0.49	0.07
Nak70	Nakvassin Cr.	Creek Mouth	1970	Wild	A	S	40	3.65	2.35	3.33	0.00	0.46	0.46	0.01
Nak74	Nakvassin Cr.	Creek Mouth	1974	Wild	A	S	44	3.62	2.33	3.40	0.04	0.46	0.46	0.04
Nak75	Nakvassin Cr.	Creek Mouth	1975	Wild	A	S	32	3.58	2.35	3.27	0.00	0.44	0.47	0.08
Nak07	Nakvassin Cr.	Creek Mouth	2007	Wild	A	Ax	25	4.12	2.55	3.58	0.00	0.47	0.49	0.05
Nak08	Nakvassin Cr.	Creek Mouth	2008	Wild	A	Ax	45	3.77	2.32	3.44	0.00	0.47	0.46	-0.01
Sashin67	Sashin Cr.	Weir	1967	Wild	A	S	53	4.38	2.58	3.85	0.00	0.45	0.47	0.06
Sashin71	Sashin Cr.	Weir	1971	Wild	A	S	27	4.77	2.76	4.11	0.00	0.48	0.47	0.00
Sashin73	Sashin Cr.	Weir	1973	Wild	A	S	41	5.27	2.94	4.24	0.15	0.49	0.50	0.04
Sashin74	Sashin Cr.	Weir	1974	Wild	A	S	43	4.81	2.96	4.34	0.00	0.53	0.50	-0.04
Sashin07	Sashin Cr.	Weir	2007	Wild	A	S	39	5.08	3.01	4.27	0.08	0.49	0.50	0.03
Sashin10	Sashin Cr.	Weir	2010	Wild	A	S	42	5.15	2.99	4.27	0.08	0.51	0.50	0.00

Age A, adult; S, smolt; F, fry; Ax, axillary process; Ad, adipose fin; S, scale; N, sample size; N_a, average number of alleles at 26 loci; N_e, effective number of alleles; N_{r(16)}, average allelic richness for 16 individuals; N_p, average number of private alleles; H_O, average observed heterozygosity; H_E, average expected heterozygosity; f, divergence between observed and expected heterozygosities.

Table 2. Loci names, PCR conditions, and primer citation.

Microsatellite Locus	Dye	Range in basepairs	# alleles	H _e	Source
Panel A					
Ok11	FAM TM	81-88	4	0.361	Smith et al. (1998)
Ogo4	TAMRA TM	127-131	3	0.117	Ohlen et al. (1998)
On1	TAMRA TM	189-193	3	0.622	Banks et al.1999
On5M	FAM TM	120-158	13	0.696	Banks et al.1999
Onet5	ROX TM	173-185	4	0.649	Schriener et al. (1996)
Panel B					
P53	ROX TM	162-188	12	0.777	de Fromental et al. (1992)
One111	FAM TM	182-210	5	0.611	Schriener et al. (1996)
On4	VIC TM	121-138	6	0.471	Banks et al. (1999)
Omp1011	VIC TM	161-217	13	0.811	Spies et al. (2005)
Ok1	FAM TM	89-139	13	0.791	Smith et al. (1998)
Panel C					
Ok123	FAM TM	116-219	27	0.839	Smith et al. (1998)
Ssa407	ROX TM	156-236	19	0.854	Carney et al. (2000)
On212	VIC TM	113-125	4	0.506	Greig et al. (2003)
On213	VIC TM	150-312	27	0.801	Greig et al. (2003)
Ogo2	FAM TM	237-257	9	0.651	Ohlen et al. (1998)
SNPs					
Okierf15	VIC TM FAM TM	NA	2	0.167	Smith et al. (2006)
OkIE287	VIC TM FAM TM	NA	2	0.391	Smith et al. (2006)
Okieffibp258	VIC TM FAM TM	NA	2	0.163	Smith et al. (2006)
OkKDPDH146	VIC TM FAM TM	NA	2	0.155	Smith et al. (2006)
OkHGFA311	VIC TM FAM TM	NA	2	0.455	Smith et al. (2006)
Okms167	VIC TM FAM TM	NA	2	0.342	Smith et al. (2006)
Okms323	VIC TM FAM TM	NA	2	0.128	Smith et al. (2006)
OkLWSsp554	VIC TM FAM TM	NA	2	0.278	Smith et al. (2006)
OkSWCF232120	VIC TM FAM TM	NA	2	0.311	Smith et al. (2006)
Oksemp328	VIC TM FAM TM	NA	2	0.219	Smith et al. (2006)
OkSWS1op38	VIC TM FAM TM	NA	2	0.491	Smith et al. (2006)

The number of alleles and expected heterozygosity (H_e) were tabulated for each locus across all populations using FSTAT (Goudet 2001).

Table 3. Pairwise test results																
Collection Lineage	Arm07 D.C.	Auk09 Auk.C	Deer08 D.C.	Deer09 Sash.C	Nak69 Nak.C	Nak70 Nak.C	Nak74 Nak.C	Nak75 Nak.C	Nak07 Nak.C	Nak08 Nak.C	Sashin67 Sash.C	Sashin71 Sash.C	Sashin73 Sash.C	Sashin74 Sash.C	Sashin07 Sash.C	Sashin10 Sash.C
Arm07	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Auk09	0.110	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Deer08	0.012	0.107	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Deer09	0.106	0.102	0.169	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005
Nak69	0.106	0.113	0.074	0.150	-	0.005	0.031	0.293	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000
Nak70	0.111	0.116	0.114	0.152	0.022	-	0.010	0.251	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nak74	0.095	0.124	0.107	0.129	0.020	0.017	-	0.114	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nak75	0.093	0.121	0.110	0.142	0.011	0.006	0.007	-	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Nak07	0.082	0.070	0.106	0.053	0.046	0.049	0.036	0.037	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nak08	0.113	0.131	0.093	0.144	0.019	0.027	0.013	0.014	0.035	-	0.000	0.000	0.000	0.000	0.000	0.000
Sashin67	0.062	0.057	0.066	0.056	0.102	0.092	0.077	0.092	0.028	0.097	-	0.432	0.000	0.000	0.000	0.000
Sashin71	0.075	0.070	0.071	0.054	0.112	0.102	0.086	0.109	0.028	0.101	0.001	-	0.046	0.134	0.000	0.001
Sashin73	0.067	0.056	0.068	0.063	0.098	0.101	0.089	0.101	0.044	0.097	0.007	0.007	-	0.001	0.000	0.000
Sashin74	0.085	0.077	0.095	0.044	0.116	0.121	0.093	0.113	0.025	0.112	0.013	0.011	0.015	-	0.000	0.000
Sashin07	0.087	0.096	0.090	0.010	0.129	0.125	0.098	0.115	0.046	0.116	0.028	0.028	0.044	0.025	-	0.061
Sashin10	0.068	0.088	0.075	0.015	0.123	0.118	0.096	0.110	0.042	0.112	0.025	0.019	0.025	0.025	0.005	-

Pairwise D_{st} test results from DEIMEX (Gerlach et al. 2010) in lower triangular matrix. Non-significant D_{st} values are in bold. P values from pairwise allelic test results from GENEPOP4.1 (Rousset, 2008) are in the upper triangular matrix. Non-significant (>0.05) P values after Bonferroni correction are in bold. Linage abbreviations: 1) D.C. = Deep Cove Cr., 2) Auk.C. = Auke Creek, 3) Nak.C. = Nakvassin Creek, and 4) Sash.C. = Sashin Creek.

Table 4. AMOVA results from ARLEQUIN 3.5.1.1 (Laurent Excoffier and Heidi Lischer, 2009)

		Var. components	% Var.	F _{ct}	F _{sc}	F _{st}	P
1 Group	Among collections	0.305	4.591			0.046	0.000
	Within collections	6.329	95.409				
4 Groups (Auk09, Hatchery (Arm & Deer), Nak collections, Sashin collections)	Among groups	0.213	3.184			0.052	0.000
	Among collections	0.133	1.987		0.021		0.000
	within groups						
	Within collections	6.329	94.828	0.032			0.000
4 Groups (Auk09, Arm07+Deer08, Nak collections, Sashin collections + Deer09)	Among groups	0.271	4.049			0.053	0.000
	Among collections	0.086	1.280		0.013		0.000
	within groups						
	Within collections	6.329	94.671	0.040			0.000
5 Groups (Auk09, Arm07, Deer08 + Deer09, Nak collections, Sashin collections)	Among groups	0.205	3.073			0.050	0.000
	Among collections	0.125	1.877		0.019		0.000
	within groups						
	Within collections	6.329	95.050	0.031			0.000
6 Groups (Auk09, Arm07, Deer08, Deer09, Nak collections, Sashin collections)	Among groups	0.252	3.777			0.038	0.000
	Among collections	0.080	1.206		0.013		0.000
	within groups						
	Within collections	6.329	95.016	0.038			0.000

Collections were grouped in different arrangements and data were analyzed for partitioning of molecular variance. P is the probability that a random value would be larger than the observed variance (Var) and F values.

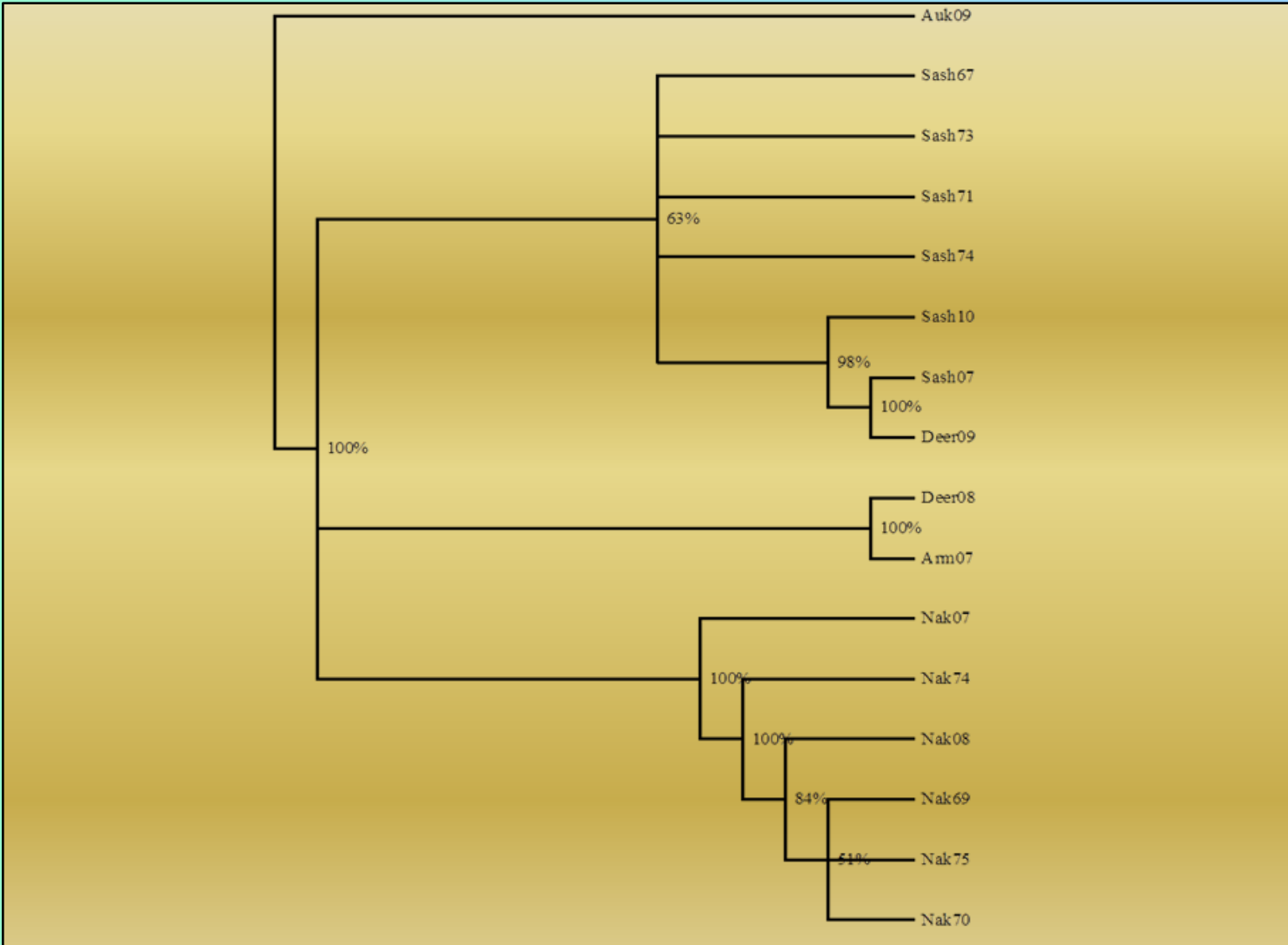


Figure 2. Unrooted neighbor-joining phenogram generated from CSE distance matrix for 16 coho salmon collections. Collection codes are as indicated in Table 1. Bootstrap support values are labeled as percentages at the nodes.

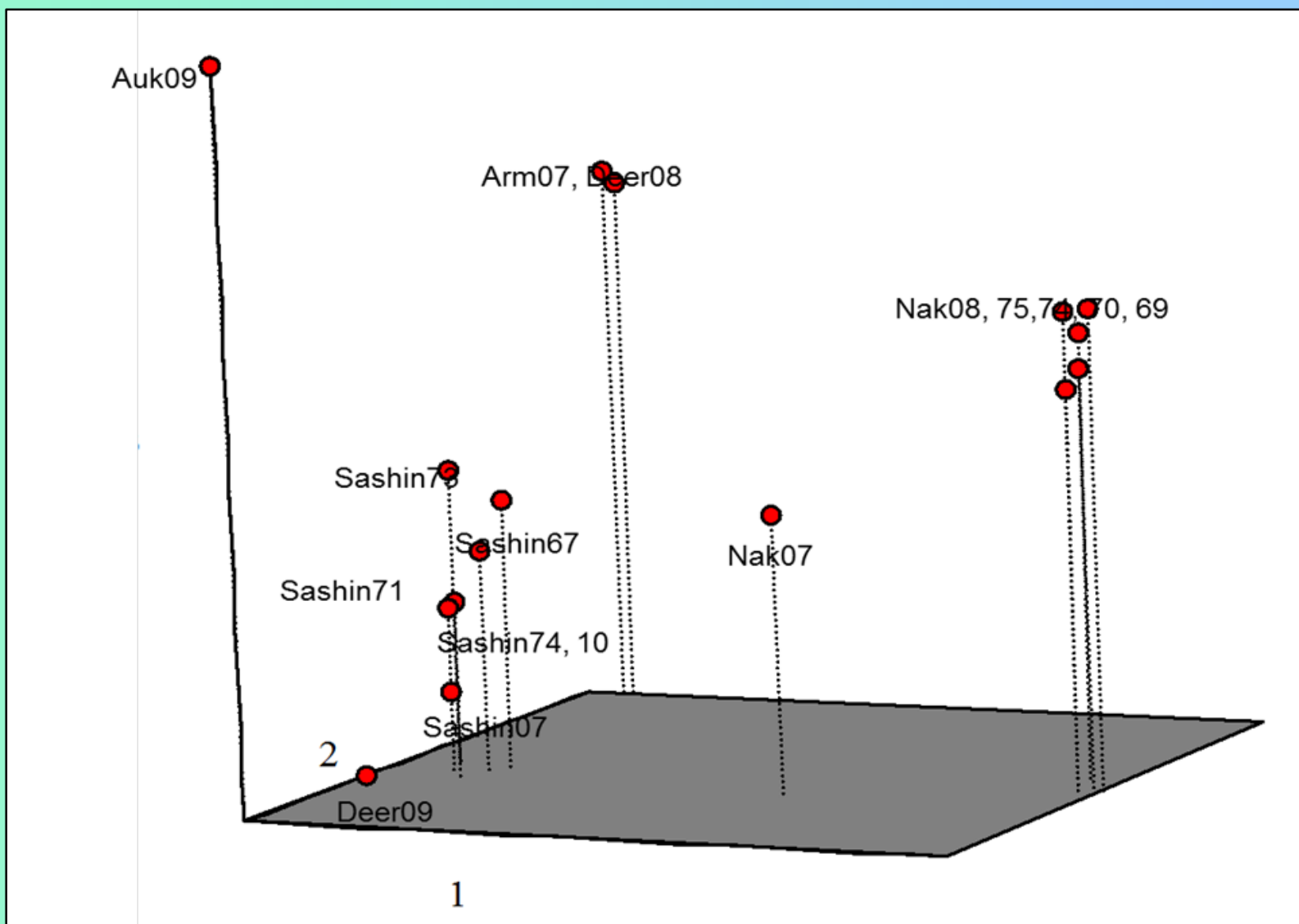


Figure 3. Plot of first three PC's of the PCA analysis of 16 coho salmon collections. Collection codes are as described in Table 1.

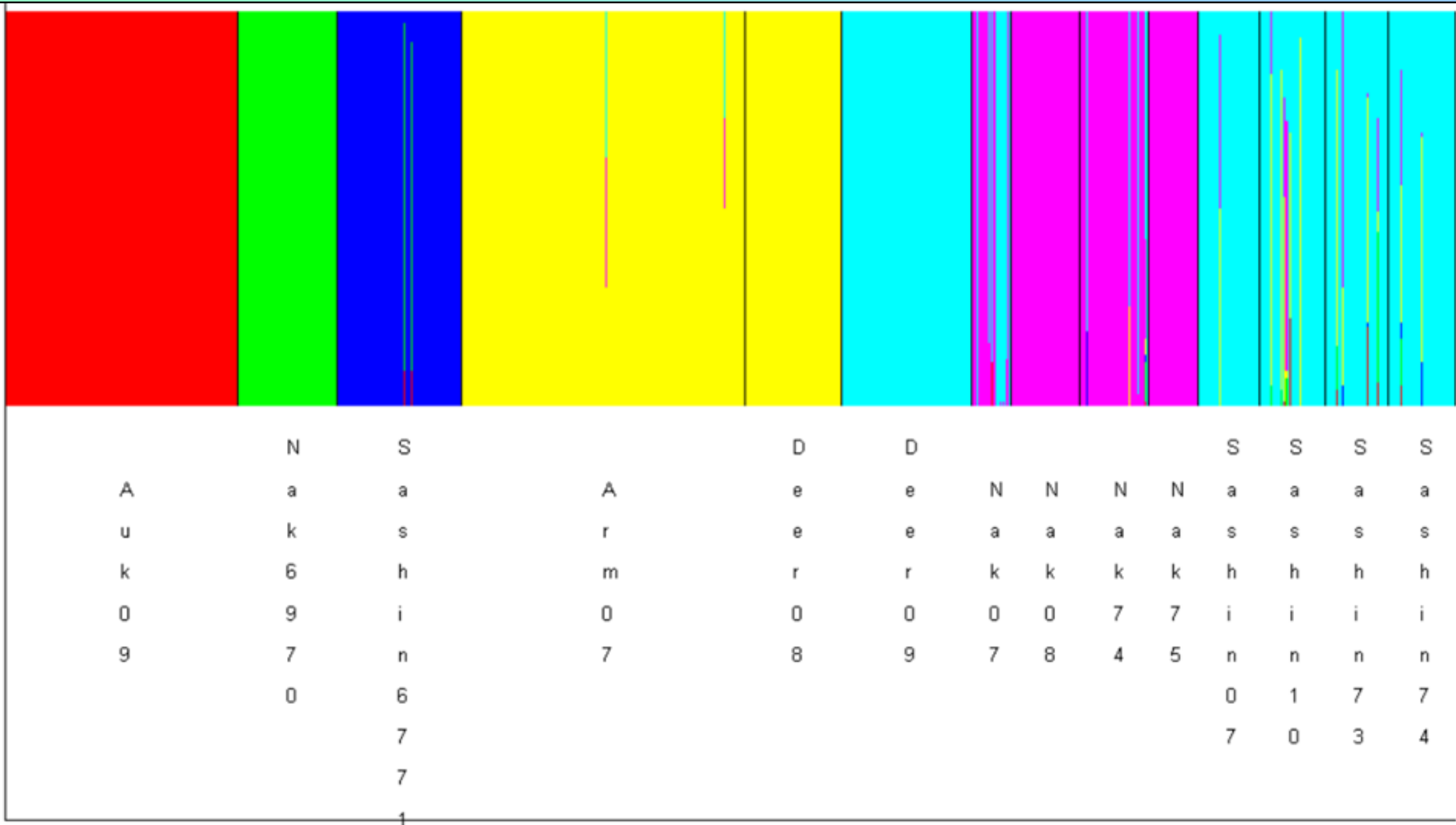


Figure 4. Individual assignment based on BAPS trained genotypic clustering analysis. Histograms demonstrate the proportion of each individual's genome that originated from each of 16 collections. Each individual is represented by a vertical line. Collections are separated by black lines.

Summary

Genetic analysis revealed five results: 1) The loci used in this study demonstrate an ability to differentiate between all lineages by collection site, 2) All lineages cluster into distinct groups which suggest little gene flow between lineages, 3) Temporal collections cluster within lineages, with the exception of one Nakvassin creek collection, 4) There is some evidence of an admixture of samples within one Nakvassin creek collection which we believe is a result of collecting samples at the mouth of the creek; 5) There is evidence of admixed individuals within all Sashin creek collections which we believe resulted from operation of a permanent one way weir structure at the mouth of Sashin creek since the 1940's.