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Moongeun Yoon[1], Noriko Azuma[1], Shunpei Sato[2], James E. Seeb[3], Richard L. Wilmot[4], Shigehiko Urawa[2], Akihisa Urano[5], Syuiti Abe[1]

[1]*Division of Marine Biosciences, Graduate School of Fisheries Sciences, Hokkaido University, Japan*
[2]*Gene Conservation Section, National Salmon Resources Center, Japan*
[3]*Gene Conservation Laboratory, Alaska Department of Fish and Game, U.S.A.*
[4] *Auke Bay Laboratory, Alaska Fisheries Science Center, NOAA, U.S.A.*
[5]*Division of Biological Science, Graduate School of Sciences, Hokkaido University, Japan*

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Genetic variation among Pacific Rim chum salmon populations inferred from the microsatellite DNA analysis

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[3]Gene Conservation Laboratory, Alaska Department of Fish and Game, U.S.A.
[4] Auke Bay Laboratory, Alaska Fisheries Science Center, NOAA, U.S.A.
[5]Division of Biological Science, Graduate School of Sciences, Hokkaido University, Japan

ABSTRACT

The genetic variation and population structure of chum salmon in the Pacific Rim were investigated using allelic variation at four polymorphic microsatellite DNA (msDNA) loci of more than 3,300 individuals from 76 populations representing Japan, Russia and North America. Genetic variation was greater in the populations of Japan than those of Russia and North America, although allelic variation was different among the examined loci. Pairwise population F_{ST} estimates revealed substantial genetic differentiation among the three regional groups of populations. The analysis of molecular variance demonstrated clear structuring among the three geographic groups of populations and within Russian and North American populations but weak to moderate structuring within Japanese populations. These results suggest that the observed geographic pattern of the three regions is congruent with the patterns obtained by the previous mtDNA analysis, and hence the two DNA markers will become useful for construction of a better baseline for genetic stock identification of chum salmon in high seas.

INTRODUCTION

Due to their wide geographic distribution in the Pacific Rim (Quinn 2005) and high commercial importance, chum salmon (*Oncorhynchus keta*) have received considerable attention. Information of the genetic variation and population structure of Pacific Rim chum salmon is therefore important for addressing the population history, the patterns of ocean migration and the stock composition of mixed populations in high seas. Recently developed mitochondrial (mt) and nuclear DNA markers are expected to provide a powerful means to estimate the genetic variation and population structure in salmon with an increased accuracy and resolution (e.g. Park et al. 1993, Taylor et al. 1994, Bickham et al. 1995, Sato et al. 2001, 2004, Beacham and Candy 2005, Habicht et al. 2005). However, sufficient information has not yet been accumulated to conclude which DNA markers improve discrimination among populations and whether any variation in the utility of different markers exists at different geographic scale of analysis.

mtDNA with a single genetic locus may not explain historical event like genetic drift in lower hierarchical level of populations. Genetic drift in populations involved random change in gene frequencies, and these changes will not occur in the same way in independent loci (Slatkin and Hudson 1991). In addition, mtDNA data allow only the reconstruction of maternal lineages (Wilson et al. 1985, Avise et al. 1987). Species may have a difference in the mechanism or independent behaviors in the dispersal of males and females. These mean that population structure estimated with maternally inherited mtDNA may differ from that assessed with biparentally inherited DNA markers.

Microsatellite (ms) DNA with tandem repeats of 2 to 4 base short motifs is a class of highly polymorphic nuclear DNA markers that are suitable for studies on the level of intraspecific genetic diversity and population structure (Bruford and Wayne 1993). A variety of polymorphic msDNA loci have so far been developed for the population analysis of Pacific salmon, with a hope to utilize them as a tool of genetic stock identification (GSI) (e.g. Beacham and Candy 2005, Habicht et al. 2005).

In an attempt to improve estimation of the genetic diversity and populations structure of Pacific Rim chum salmon, msDNA analysis at four polymorphic loci (OKM4, OKM5, OKM7 and Okm8) (Abe et al. 2002) was performed using more than 3,300 fish from 76 of 96 Asian and North American populations previously analyzed for mtDNA (Yoon et al. 2005).

MATERIALS AND METHODS

Sampling profile

Liver, fins, or muscle samples of chum salmon were collected from 2,179 individuals from 76 populations from Japan (14), Korea (1), Russia (16) and North America (45) for the msDNA analysis (Table 1) were used, all of which were the same samples as those used for the mtDNA analysis (Yoon et al. 2005). Liver samples were stored at –80°C, and fins and muscle samples were kept in ethanol at room temperature until DNA extraction.

Mitochondrial and microsatelliteDNA analyses

DNA was isolated with conventional phenol-chloroform method (Sambrook et al. 1989) from the stored specimens as in our previous studies (Sato et al. 2001, 2004). Four polymorphic msDNA loci (OKM4, OKM5, OKM7 and OKM8) (see also Abe et al. 2002) were examined by polymerase chain reaction (PCR) amplification, in which one primer of each primer set was labeled with the fluorescent dyes: FAM, NED, NIC or PET. PCR conditions included one min pre-cycling denaturation at 95℃, followed by 30 cycles of denaturation at 95℃ for 15 sec, annealing at 57℃ (at 55℃ for OKM5) for 15 sec, and extension at 72℃ for 60 sec. PCR products were analyzed using an ABI PRISM 3130XL Genetic analyzer (Applied Biosystems).

Population genetic data analysis

Departure from Hardy-Weinberg equilibrium (HWE) and genotype frequency at each locus were assessed in each population using Genepop version 3.4 program packages. Gene diversity according to Nei (1987) and pairwise population F_{ST} estimation were performed using the Arlequin version 2.000 program package (Schneider et al. 2000). In order to assess the extent of genetic divergence at different levels of geographic hierarchy, the overall molecular variance was partitioned into components corresponding to the population divergence within and among regions by the analysis of molecular variance model (AMOVA; Excoffier et al. 1992) using the above Arlequin program.

RESULTS

Considerable variation in the allele frequency was observed at the four microsatellite loci examined. A total number of 90 alleles were observed across the four loci, which ranged from 19 alleles at the OKM7 to 31 alleles at the OKM8 over all populations, and the observed heterozygosity for all populations ranged from 0.206 at the OKM7 to 0.689 at the OKM8 (Table 2), reflecting the large difference in number of alleles among these loci.

Eighty of the 304 tests for Hardy-Weinberg equilibrium showed significant deviation from observed allele frequencies with P-value of the probability test, with mostly decreased heterozygsities (Table 2). Significant heterozygosity excess was occasionally observed at the OKM5 or OKM8 in small fraction of populations from Japan, Russia and North America (Table 2). Significant disequilibrium with insufficient heterozygosity was found in the Pymta River from Russia, and Chiginagak River, Port Beauclere in the Kuiu Island and Ecstall River from North American populations at all of the examined loci (Table 2).

The number of alleles per locus across all populations was highest at the OKM8 locus with 16 in the Chitose River and lowest at the OKM7 with 1 in the Noatak, Seenjek River, Donjek River, Upper Nushagak, Kitoi Hatchery and Sawmil Creek, Berner’s Bay. The expected heterozygosity within Japanese populations (0.262-0.889) was higher than Russia (0.065-0.851) and North American populations (0-0.822) (Table 2). Also, gene diversity of Japanese populations (0.216 to 0.887) was higher than Russian (0.05-0.851) and North American populations (0-0.824) (Table 2). These finding suggest greater genetic variation in the populations of Japan than those of Russia and North America.

As shown in Table 3, pairwise population F_{ST} estimates were greater between Japan and North America (0.018-0.071) than between Japan and Russia (0.010-0.032) or between Russia and North America (0.008-0.050). AMOVAs (Table 4) indicated that most variation occurred within populations with the high variability (range from 88.9 to 92.5%) of all the microsatellite loci used. Even though regional groups in Japan showed weak to moderate structuring (P<0.05), there was significant structuring among Japan, Russia and North America, and among regional groups within Russia and North America (P<0.001)

DISCUSSION

Population genetic analyses with allelic variation of four msDNA loci using more than 3,300 individuals from 76 populations of Japan and Korea (15), Russia (16) and North America (45) disclosed a greater heterozygosity and gene diversity in the populations of Japan than those of Russia and North America, which was in keeping with our previous observations by mtDNA analysis (Yoon et al, 2005). These findings suggest that genetic variation of Japanese population is higher than that of Russian and North American populations.

Heterozygosity decrease in the large number of Russian and North American populations may be associated with the occurrence of null alleles, non-random mating, or a demographically unstable condition that had not yet reached genetic equilibrium. In any case, it is unknown at present why such a heterozygosity insufficiency occurs in these geographic regions.

The AMOVAs and pairwise F_{ST} estimates with the present msDNA data revealed clear geographic structuring in the Pacific Rim chum salmon populations among and within regions, although the extent of divergence was lower than that estimated by mtDNA variation (Yoon et al. 2005). Interestingly, AMOVA showed an increased resolution in the geographic differentiation among three regional groups of North American populations (3.7%, P<0.001, Table 4) as compared with the previous estimation (2.1%, P<0.001) using mtDNA sequence analysis (Yoon et al. 2005). These results suggest that msDNA may be useful for within-region analysis or discrimination at lower hierarchical level, whereas mtDNA may be useful for higher hierarchical level analysis such as intercontinental comparisons.

Taken together, estimates of the extent of population differentiation using msDNA and mtDNA data are mostly congruent, although F_{ST} values by msDNA analysis (Table 3) seem generally low compared with those by mtDNA analysis (see Abe et al. 2002). The observed congruence of population genetic profiles suggests that these two DNA markers will become useful for construction of a better baseline for chum salmon GSI in high seas.

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Table 1. Sampling location, date of collection, geographical coordinates, number of chum salmon samples (N) in 76 populations of chum salmon

Sampling location	Date of collection	Latitude	Longitude	N
Japan				
Hokkaido Island				
Chitose River	14 Oct. 1996	43° 07′ 16” N	141° 33′ 23” E	54
Tokushibetsu River	23 Sep. 1997	44° 50′ 30” N	142° 39′ 23” E	31
Tokoro River (late run)	20 Nov. 1998	44° 07′ 25” N	144° 04′ 52” E	43
Tokoro River (early run)	13 Oct. 1999	44° 07′ 25” N	144° 04′ 52” E	41
Nishibetsu River	25 Sep. 1997	43° 23′ 02” N	145° 17′ 23” E	58
Kushiro River	22 Oct. 1998	42° 59′ 57” N	144° 21′ 34” E	45
Tokach River	17 Oct. 1996	42° 41′ 42” N	143° 39′ 55” E	57
Yurappu River	17 Nov. 1998	42° 16′ 02” N	140° 16′ 45” E	48
Honshu Island				
Tsugaruishi River (late run)	10 Dec. 1997	39° 35′ 12” N	141° 56′ 51” E	64
Tsugaruishi River (early run)	Oct. 1999	39° 35′ 12” N	141° 56′ 51” E	44
Koizumi River	21 Nov. 1996	38° 45′ 00” N	141° 31′ 00” E	24
Gakko River	10 Dec. 1996	39° 03′ 09” N	139° 53′ 11” E	48
Uono River	23 Oct. 1996	37° 16′ 28” N	138° 51′ 08” E	49
Jintsu River	7 Nov.1995	36° 45′ 38” N	137° 13′ 18” E	50
Korea				
Namdae River	13 Nov. 2000	38° 09′ 00” N	128° 38′ 00” E	43
Russia				
Primorye				
Avakumovka River	1994	43° 44′ 00” N	131° 29′ 00” E	32
Sakhalin Island				
Kalininka River	1994	47° 00′ 00” N	142° 05′ 00” E	46
Kalininka River (early run)	2003	47° 00′ 00” N	142° 05′ 00” E	19
Belaya River	2003	47° 15′ 00” N	142° 47′ 00” E	22
Taranay River	2003	46° 37′ 00” N	142° 26′ 00” E	25
Udarnitza River	2003	59° 20′ 00” N	143° 00′ 00” E	23
Tym River	2003	51° 05′ 00” N	142° 41′ 00” E	24
Kamchatka				
Tigil River	2002	58° 02′ 00” N	158° 13′ 00” E	42

Table 1. *(Continued)*

Sampling location	Date of collection	Latitude	Longitude	N
Hairusova River	1993	57° 02′ 00” N	156° 40′ 00” E	46
Vorovskaya River	1990	54° 15′ 00” N	155° 14′ 00” E	40
Pymta River	2002	53° 41′ 00” N	155° 59′ 00” E	47
Bolshaya Hatchery	1999	52° 56′ 00” N	157° 13′ 00” E	45
Bistraya River	2001	53° 20′ 00” N	157° 28′ 00” E	48
Apuka River	2002	60° 28′ 00” N	169° 39′ 00” E	47
Olutorsky Bay	2002	60° 19′ 00” N	168° 30′ 00” E	43
Kamchatka River (early run)	1991	56° 15′ 00” N	162° 30′ 00” E	48
North America				
Northwest Alaska				
Noatak River	1991	67° 02′ 00” N	162° 29′ 00” W	38
Salmon River	1991	66° 31′ 00” N	141° 54′ 00” W	48
Unalakleet River	1992	63° 51′ 00” N	160° 41′ 00” W	48
Andreafsky River (summer run)	1993	62° 02′ 00” N	163° 13′ 00” W	48
Sheenjek River (fall run)	1992	66° 36′ 00” N	145° 17′ 00” W	42
Tanana River	1993	65° 11′ 00” N	151° 57′ 00” W	49
Toklet River	1992	64° 44′ 00” N	143° 42′ 00” W	47
Pelly River	1993	62° 55′ 00” N	137° 15′ 00” W	45
Donjek River	1994	61° 25′ 00” N	139° 58′ 00” W	42
Togiak River	1993	59° 04′ 00” N	160° 20′ 00” W	46
Upper Nushagak	1993	59° 03′ 00” N	158° 22′ 00” W	49
Kwethluk River	1994	60° 50′ 00” N	161° 24′ 00” W	46
South Fork Kuskokwin River	1995	60° 41′ 00” N	161° 59′ 00” W	47
Alaska Peninsula				
Joshu Green River	1992	55° 38′ 26” N	162° 51′ 11” W	42
Frosty Creek	1992	55° 19′ 33” N	163° 26′ 04” W	44
St. Catherine’s Cove	1992	55° 01′ 12” N	163° 52′ 41” W	46
Belkofski River	1992	55° 05′ 00” N	162° 03′ 00” W	46
Chiginagak River	1991	57° 02′ 00” N	156° 47′ 00” W	45
Southcentral Alaska				
Kitoi Hatchery	1993	58° 11′ 00” N	152° 21′ 00” W	45
Kizhuyak River	1992	57° 05′ 00” N	152° 50′ 00” W	40

Table 1. *(Continued)*

Sampling location	Date of collection	Latitude	Longitude	N
McNeil River	1994	59° 10′ 00” N	154° 14′ 00” W	49
Chunlina, Sustina River	1993	61° 16′ 00” N	150° 30′ 00” W	45
WHN hatchery2002	2002	61° 20′ 27” N	148° 08′ 58” W	45
WHN hatchery1992	1992	61° 20′ 27” N	148° 08′ 58” W	41
Olsen Creek	1992	60° 51′ 00” N	146° 05′ 00” W	48
Olsen Creek 2004	8 Jun. 2004	60° 51′ 00” N	146° 05′ 00” W	40
Southeast Alaska				
Little Port walter	8 Aug. 2004	56° 23′ 00” N	134° 48′ 00” W	40
Sawmil Creek, Berner’s Bay	28 July. 1993	58° 52′ 00” N	134° 55′ 00” W	45
Long Bay, Chichigof Island	25 Aug. 1991	57° 51′ 00” N	135° 31′ 00” W	48
Whale Bay, Baranof Island	12 Aug. 1993	56° 34′ 00” N	134° 59′ 00” W	46
Port Beauclere, Kuiu Island	20 Aug. 1995	56° 11′ 00” N	133° 56′ 00” W	45
Fish Creek, Portland Canal	25 Sep. 1988	55° 04′ 00” N	130° 11′ 00” W	49
Disappearance Creek, POW Island	25 Sep. 1998	54° 59′ 00” N	132° 13′ 00” W	50
Blossom River	1986	55° 40′ 28” N	131° 01′ 13” W	50
Marten River	1986	55° 16′ 23” N	130° 53′ 22” W	50
British Columbia				
Ecstall River, Skeena River area	12 Sep. 1988	54° 08′ 00” N	129° 56′ 00” W	48
Bag Harbor, QCI	Mid-Oct. 1989	52° 09′ 00” N	131° 05′ 00” W	47
Nekite Channel	15 Sep. 1989	51° 23′ 00” N	127° 07′ 00” W	48
Vedder River	2002	49° 08′ 00” N	122° 06′ 00” W	43
Nitinet River	1992	48° 49′ 00” N	124° 37′ 00” W	41
Harrison River	2002	49° 13′ 00” N	121° 57′ 00” W	25
Cowichan Bay	1997	48° 42′ 00” N	123° 32′ 00” W	37
Washington				
Nooksack River	1998	48° 46′ 00” N	122° 35′ 00” W	49
Quilcene River	1998	47° 49′ 00” N	122° 52′ 00” W	46
Hamilton Creek	1998	45° 34′ 00” N	122° 10′ 00” W	48

Table 2. Range of allele size (S), total number of alleles (A_T), observed (H_O), expected (H_E) heterozygosity, and gene diversity (H_S) by msDNA locus for 76 chum salmon populations

Population		Locus			
		OKM4	OKM5	OKM7	OKM8
Chitose River	S	99-119	99-127	67-91	102-160
	A_T	10	12	8	16
	H_O	0.889	0.703*	0.407*	0.889*
	H_E	0.833	0.714	0.572	0.882
	H_S	0.883	0.713	0.562	0.882
Tokushibetsu River	S	103-141	101-117	75-105	106-164
	A_T	11	8	8	12
	H_O	0.903	0.806	0.548	0.839
	H_E	0.819	0.754	0.506	0.872
	H_S	0.817	0.756	0.505	0.872
Tokoro River (late run)	S	101-125	99-119	75-85	106-160
	A_T	12	9	5	14
	H_O	0.651*	0.605	0.372	0.791
	H_E	0.708	0.682	0.347	0.861
	H_S	0.709	0.667	0.327	0.853
Tokoro River (early run)	S	101-125	101-127	75-83	106-160
	A_T	10	10	4	13
	H_O	0.610	0.805	0.293	0.781
	H_E	0.705	0.751	0.262	0.889
	H_S	0.691	0.750	0.261	0.887
Nishibetsu River	S	101-121	101-123	75-105	106-132
	A_T	10	11	9	11
	H_O	0.586	0.741	0.603	0.672*
	H_E	0.671	0.798	0.545	0.870
	H_S	0.671	0.792	0.544	0.868
Kushiro River	S	105-125	101-125	75-85	106-162
	A_T	10	10	5	13
	H_O	0.733	0.578	0.244	0.822
	H_E	0.750	0.672	0.264	0.864
	H_S	0.739	0.673	0.264	0.861
Tokachi River	S	101-121	101-123	75-101	106-164
	A_T	10	12	8	15
	H_O	0.737	0.719	0.298	0.825
	H_E	0.804	0.729	0.301	0.885
	H_S	0.804*	0.720	0.301	0.885
Yurappu River	S	105-119	99-123	71-91	106-160
	A_T	8	11	7	11
	H_O	0.708	0.708	0.438	0.771
	H_E	0.785	0.740	0.553	0.844
	H_S	0.786	0.731	0.540	0.845
Tsugaruishi River (late run)	S	105-117	101-123	75-91	106-160
	A_T	7	10	7	10
	H_O	0.862	0.759	0.483	0.759
	H_E	0.784	0.751	0.447	0.833
	H_S	0.779	0.751	0.447	0.833

Table 2. (continued)

Population		Locus			
		OKM4	OKM5	OKM7	OKM8
Tsugaruishi River (early run)	S	103-129	99-123	75-91	106-148
	A_T	10	12	7	3
	H_O	0.727	0.659*	0.386	0.886
	H_E	0.789	0.758	0.387	0.866
	H_S	0.790	0.749	0.387	0.865
Koizumi River	S	107-125	99-119	75-91	106-124
	A_T	6	9	5	8
	H_O	0.895	0.737	0.632	0.684*
	H_E	0.802	0.784	0.542	0.804
	H_S	0.795	0.785	0.539	0.782
Gakko River	S	101-119	101-127	75-91	106-160
	A_T	10	12	7	12
	H_O	0.830	0.681	0.617	0.702*
	H_E	0.830	0.635	0.618	0.868
	H_S	0.830	0.622	0.606	0.865
Uono River	S	101-123	101-123	73-83	104-160
	A_T	12	11	5	13
	H_O	0.816	0.551*	0.592	0.776
	H_E	0.857	0.678	0.488	0.839
	H_S	0.852	0.655	0.487	0.839
Jintsu River	S	103-129	99-127	75-87	106-160
	A_T	9	12	8	15
	H_O	0.735	0.796	0.510	0.816
	H_E	0.799	0.777	0.557	0.876
	H_S	0.800	0.777	0.558	0.869*
Namdae River	S	103-121	101-119	77-83	100-126
	A_T	10	9	4	13
	H_O	0.953	0.767	0.186*	0.930*
	H_E	0.846	0.709	0.215	0.832
	H_S	0.844	0.708	0.216	0.830
Avakumovoka River	S	109-117	101-113	67-93	92-160
	A_T	4	3	8	13
	H_O	0.406	0.375	0.531	0.718*
	H_E	0.442	0.369	0.643	0.824
	H_S	0.442	0.343	0.625	0.819
Kalinika River	S	95-121	101-119	61-83	98-126
	A_T	11	10	7	11
	H_O	0.913	0.696	0.370*	0.739*
	H_E	0.823	0.672	0.509	0.851
	H_S	0.822	0.671	0.509	0.851
Kalinika River (early run)	S	97-121	101-119	61-83	100-120
	A_T	9	9	4	6
	H_O	0.631	0.737	0.368	0.895
	H_E	0.680	0.859	0.543	0.791
	H_S	0.651	0.857	0.538	0.788

Table 2. (continued)

Population		Locus			
		OKM4	OKM5	OKM7	OKM8
Belaya River	S	99-125	99-105	65-119	98-128
	A_T	5	9	6	6
	H_O	0.636	0.591	0.500*	0.727
	H_E	0.703	0.582	0.707	0.770
	H_S	0.705	0.582	0.703	0.771
Taranay River	S	109-125	99-115	73-87	102-126
	A_T	6	7	7	8
	H_O	0.440	0.480	0.320*	0.960
	H_E	0.470	0.523	0.474	0.752
	H_S	0.441	0.495	0.448	0.748
Udamitza River	S	109-121	99-117	73-83	104-120
	A_T	7	6	6	5
	H_O	0.348	0.783	0.217*	0.913
	H_E	0.450	0.682	0.388	0.780
	H_S	0.449	0.680	0.356	0.777
Tym River	S	99-117	101-121	65-83	98-120
	A_T	8	7	3	5
	H_O	0.500	0.583	0.167	0.750
	H_E	0.492	0.666	0.268	0.752
	H_S	0.461	0.640	0.232	0.735
Tigil River	S	109-121	97-113	79-83	104-120
	A_T	5	4	3	5
	H_O	0.500	0.643	0.071	0.810
	H_E	0.514	0.636	0.093	0.755
	H_S	0.496	0.636	0.070	0.754
Hairusova River	S	109-121	99-117	79-85	102-130
	A_T	6	7	3	7
	H_O	0.630	0.500*	0.044	0.696
	H_E	0.572	0.491	0.065	0.641
	H_S	0.571	0.476	0.043	0.628
Vorovskaya River	S	109-121	101-117	79-83	104-120
	A_T	5	7	3	7
	H_O	0.625	0.375	0.050	0.675
	H_E	0.554	0.481	0.074	0.619
	H_S	0.553	0.482	0.050	0.618
Pymta River	S	97-121	99-117	67-83	100-124
	A_T	8	8	3	10
	H_O	0.468*	0.426*	0.043*	0.553*
	H_E	0.563	0.619	0.104	0.642
	H_S	0.562	0.606	0.084	0.630
Bolshaya Hatchery	S	103-115	99-117	79-83	106-126
	A_T	7	9	3	9
	H_O	0.400*	0.689	0.044	0.689*
	H_E	0.690	0.626	0.066	0.826
	H_S	0.693	0.625	0.044	0.822

Table 2. (continued)

Population		Locus			
		OKM4	OKM5	OKM7	OKM8
Bistraya River	S	109-121	101-117	79-85	102-120
	A_T	6	4	3	6
	H_O	0.500	0.813*	0.083	0.833*
	H_E	0.469	0.543	0.101	0.644
	H_S	0.469	0.529	0.081	0.642
Apuka River	S	103-125	99-117	67-83	100-122
	A_T	6	10	5	10
	H_O	0.575	0.638*	0.043*	0.511*
	H_E	0.561	0.685	0.144	0.658
	H_S	0.561	0.678	0.125	0.660
Olutorsky Bay	S	105-121	99-121	67-91	106-124
	A_T	7	10	6	8
	H_O	0.558	0.628*	0.163	0.651*
	H_E	0.537	0.697	0.219	0.613
	H_S	0.536	0.687	0.198	0.613
Kamchatka	S	81-121	99-115	69-85	102-122
River	A_T	6	6	4	8
(early run)	H_O	0.500	0.146*	0.063	0.542
	H_E	0.547	0.299	0.121	0.627
	H_S	0.548	0.283	0.102	0.616
Noatak River	S	99-125	99-117	79	106-120
	A_T	7	8	1	4
	H_O	0.421	0.316*	Monomorphic	0.342*
	H_E	0.498	0.413		0.607
	H_S	0.480	0.393	0.000	0.585
Salmon River	S	99-125	101-117	75-79	106-126
	A_T	8	7	2	5
	H_O	0.438	0.354	0.020*	0.417
	H_E	0.499	0.351	0.120	0.454
	H_S	0.484	0.334	0.101	0.440
Unalakleet	S	109-125	79-117	65-79	100-120
	A_T	7	10	3	4
	H_O	0.625	0.333*	0.020*	0.271*
	H_E	0.613	0.508	0.121	0.549
	H_S	0.613	0.509	0.102	0.551
Andreafsky	S	109-121	101-115	75-79	106-120
River	A_T	5	4	2	4
	H_O	0.479	0.354	0.021	0.354*
	H_E	0.450	0.327	0.042	0.516
	H_S	0.449	0.327	0.021	0.518
Seenjak River	S	109-121	101-119	79	106-120
	A_T	5	5	1	3
	H_O	0.500	0.214	Monomorphic	0.548
	H_E	0.481	0.429		0.486
	H_S	0.481	0.410	0.000	0.485

Table 2. (continued)

Population		Locus			
		OKM4	OKM5	OKM7	OKM8
Tanana River	S	109-127	101-119	79-85	106-118
	A_T	8	6	2	5
	H_O	0.347*	0.286	0.000	0.592
	H_E	0.527	0.311	0.061	0.573
	H_S	0.511	0.294	0.041	0.563
Toklet River	S	109-121	101-127	75-79	106-112
	A_T	4	10	2	2
	H_O	0.426	0.723	0.064	0.404
	H_E	0.493	0.679	0.083	0.454
	H_S	0.489	0.679	0.062	0.447
Pelly River	S	109-123	101-119	75-79	106-112
	A_T	5	4	2	2
	H_O	0.711	0.089*	0.022	0.600
	H_E	0.586	0.190	0.044	0.512
	H_S	0.585	0.170	0.022	0.488
Donjek River	S	107-121	101-113	79	106-112
	A_T	5	3	1	3
	H_O	0.500	0.333	Monomorphic	0.571
	H_E	0.566	0.336		0.503
	H_S	0.545	0.316	0.000	0.479
Togiak River	S	109-125	101-117	79-85	106-120
	A_T	7	5	3	5
	H_O	0.587	0.370	0.044	0.696
	H_E	0.566	0.444	0.065	0.596
	H_S	0.565	0.445	0.043	0.585
Upper Nushagak River	S	103-119	101-117	79	106-120
	A_T	6	6	1	4
	H_O	0.531	0.265	Mononorphic	0.469
	H_E	0.530	0.296		0.541
	H_S	0.530	0.278	0.000	0.542
Kwethluk River	S	99-125	101-119	65-83	100-120
	A_T	10	9	3	8
	H_O	0.457	0.457*	0.000*	0.435*
	H_E	0.533	0.588	0.185	0.611
	H_S	0.533	0.588	0.165	0.592
South Fork Kuskokwin River	S	107-121	101-117	75-79	100-120
	A_T	5	6	2	6
	H_O	0.596	0.213	0.021	0.511*
	H_E	0.623	0.291	0.042	0.618
	H_S	0.611	0.273	0.021	0.606
Joshu Green River	S	109-129	101-117	79-83	106-126
	A_T	7	6	2	7
	H_O	0.442	0.372	0.047	0.581
	H_E	0.470	0.461	0.069	0.620
	H_S	0.470	0.445	0.046	0.609

Table 2. (continued)

Population		Locus			
		OKM4	OKM5	OKM7	OKM8
Frosty Creek	S	109-119	101-117	79-83	106-126
	A_T	6	8	2	8
	H_O	0.523	0.614	0.023	0.523
	H_E	0.498	0.616	0.045	0.561
	H_S	0.436	0.527	0.174	0.569
St. Catherine's Cove	S	109-125	101-121	79-83	106-120
	A_T	7	9	2	5
	H_O	0.523	0.614	0.023	0.523
	H_E	0.498	0.616	0.045	0.561
	H_S	0.482	0.616	0.023	0.550
Belkofski River	S	105-125	101-121	79-83	106-126
	A_T	8	9	2	5
	H_O	0.457*	0.696*	0.022	0.717*
	H_E	0.535	0.775	0.043	0.646
	H_S	0.521	0.776	0.022	0.643
Chiginagak River	S	105-125	95-119	71-83	102-132
	A_T	9	10	6	11
	H_O	0.378*	0.489*	0.067*	0.689*
	H_E	0.672	0.767	0.357	0.718
	H_S	0.673	0.768	0.339	0.707
Kitoi Hatchery	S	109-123	101-121	79	106-120
	A_T	7	8	1	5
	H_O	0.822	0.600	Monomorphic	0.689
	H_E	0.799	0.637		0.709
	H_S	0.791	0.633	0.000	0.709
Kizhuyak River	S	103-125	101-121	65-79	98-126
	A_T	11	11	4	10
	H_O	0.500	0.625*	0.050*	0.775*
	H_E	0.638	0.822	0.145	0.636
	H_S	0.625	0.824	0.122	0.634
McNeil River	S	101-119	101-121	79-83	104-120
	A_T	7	9	3	6
	H_O	0.273*	0.523*	0.159	0.636
	H_E	0.328	0.812	0.229	0.654
	H_S	0.310	0.803	0.229	0.653
Chunlina, Sustina River	S	105-125	101-119	69-79	106-112
	A_T	7	7	2	3
	H_O	0.735	0.633	0.020	0.612
	H_E	0.757	0.721	0.041	0.549
	H_S	0.752	0.714	0.002	0.548
WHN hatchery 2002	S	109-127	99-117	79-85	104-120
	A_T	7	8	2	6
	H_O	0.400	0.711	0.022	0.622
	H_E	0.401	0.761	0.044	0.666
	H_S	0.386	0.753	0.022	0.661

Table 2. (continued)

Population		Locus			
		OKM4	OKM5	OKM7	OKM8
WHN hatchery 1992	S	109-125	101-121	79-83	106-120
	A_T	6	9	2	5
	H_O	0.439	0.537*	0.049	0.585
	H_E	0.455	0.770	0.072	0.601
	H_S	0.437	0.771	0.048	0.594
Olsen Creek	S	109-123	101-121	79-85	106-120
	A_T	6	9	4	5
	H_O	0.292	0.542	0.104*	0.750
	H_E	0.353	0.738	0.159	0.637
	H_S	0.352	0.739	0.140	0.636
Olsen Creek 2004	S	109-121	101-115	73-79	104-124
	A_T	5	6	2	7
	H_O	0.350	0.700	0.025	0.575
	H_E	0.373	0.687	0.050	0.570
	H_S	0.353	0.687	0.025	0.544
Little Port Walter	S	109-123	99-119	69-87	106-126
	A_T	7	9	4	9
	H_O	0.175*	0.650	0.025*	0.750*
	H_E	0.357	0.787	0.234	0.819
	H_S	0.357	0.782	0.212	0.820
Sawmil Creek, Berner's Bay	S	109-125	107-119	79	106-120
	A_T	6	7	1	5
	H_O	0.400	0.556*	Monomorphic	0.578
	H_E	0.434	0.727		0.545
	H_S	0.417	0.729	0.000	0.545
Long Bay, Chichigog Island	S	109-121	79-117	71-83	102-120
	A_T	5	8	4	7
	H_O	0.250	0.458	0.063	0.667*
	H_E	0.248	0.497	0.102	0.631
	H_S	0.248	0.483	0.082	0.630
Whale Bay, Baranof Island	S	109-123	101-119	79-81	104-120
	A_T	7	8	2	7
	H_O	0.587	0.652	0.000*	0.435
	H_E	0.538	0.680	0.065	0.576
	H_S	0.522	0.664	0.043	0.564
Port Beauclere, Kuiu Island	S	105-125	101-125	67-81	102-120
	A_T	6	11	3	5
	H_O	0.408*	0.469*	0.020*	0.633*
	H_E	0.578	0.604	0.255	0.668
	H_S	0.567	0.592	0.237	0.656
Fish Creek	S	103-123	101-119	65-83	98-122
	A_T	9	8	4	9
	H_O	0.367*	0.612	0.061	0.674*
	H_E	0.446	0.657	0.119	0.722
	H_S	0.431	0.645	0.100	0.723

Table 2. (continued)

Population		Locus			
		OKM4	OKM5	OKM7	OKM8
Disappearance	S	109-123	107-117	79-83	106-120
Creek, POW	A_T	7	7	2	4
Island	H_O	0.474	0.632	0.132	0.605
	H_E	0.532	0.700	0.149	0.624
	H_S	0.515	0.695	0.124	0.610
Blossom River	S	109-123	107-117	79-83	106-126
	A_T	6	6	3	6
	H_O	0.457	0.457*	0.087	0.609
	H_E	0.470	0.679	0.145	0.636
	H_S	0.454	0.667	0.125	0.625
Marten River	S	101-127	107-119	73-83	104-124
	A_T	9	7	4	9
	H_O	0.544	0.609	0.174	0.609
	H_E	0.530	0.629	0.164	0.591
	H_S	0.530	0.616	0.164	0.591
Ecstall River	S	103-129	101-119	65-83	100-120
	A_T	9	8	3	6
	H_O	0.208*	0.583*	0.083*	0.646
	H_E	0.270	0.774	0.178	0.670
	H_S	0.270	0.767	0.159	0.670
Bag Harbor	S	109-129	101-121	79-83	106-120
	A_T	8	10	2	5
	H_O	0.383	0.447	0.085	0.702
	H_E	0.438	0.558	0.103	0.628
	H_S	0.421	0.541	0.082	0.627
Nekite Channel	S	105-123	101-117	71-83	106-126
	A_T	8	7	3	7
	H_O	0.271*	0.542	0.167	0.813
	H_E	0.368	0.620	0.244	0.806
	H_S	0.368	0.608	0.226	0.799
Vedder River	S	103-119	107-121	75-83	104-120
	A_T	5	7	4	6
	H_O	0.093	0.581	0.256	0.767
	H_E	0.114	0.597	0.309	0.766
	H_S	0.091	0.597	0.307	0.766
Nitinet River	S	101-129	101-117	73-83	98-124
	A_T	8	6	5	7
	H_O	0.414	0.512	0.317	0.732
	H_E	0.385	0.505	0.308	0.711
	H_S	0.385	0.505	0.287	0.699
Harrison River	S	109-127	105-117	77-83	106-120
	A_T	4	4	3	6
	H_O	0.120	0.600	0.200	0.720
	H_E	0.155	0.585	0.255	0.753
	H_S	0.118	0.585	0.220	0.753

Table 2. (continued)

Population		Locus			
		OKM4	OKM5	OKM7	OKM8
Cowichan Bay	S	101-121	105-117	73-83	104-120
	A_T	5	6	4	7
	H_O	0.162	0.622	0.216	0.838
	H_E	0.181	0.629	0.311	0.754
	H_S	0.156	0.627	0.309	0.753
Nooksack River	S	101-121	101-119	61-85	100-122
	A_T	10	8	4	8
	H_O	0.429	0.694	0.041*	0.878
	H_E	0.498	0.639	0.100	0.780
	H_S	0.483	0.638	0.081	0.773
Quilcene River	S	105-121	109-117	79-83	104-122
	A_T	6	4	3	7
	H_O	0.217	0.522	0.174	0.609
	H_E	0.243	0.684	0.183	0.764
	H_S	0.224	0.686	0.163	0.753
Hamilton Creek	S	103-115	103-117	65-83	100-120
	A_T	5	8	4	7
	H_O	0.292*	0.583	0.271	0.792*
	H_E	0.348	0.588	0.316	0.780
	H_S	0.348	0.575	0.316	0.780
Total (all sites)	S	81-141	79-127	61-119	92-164
	A_T	20	20	19	31
	H_O	0.516	0.556	0.171	0.673
	H_E	0.541	0.608	0.206	0.689
	H_S	0.541	0.608	0.206	0.689

*Departure from Hardy-Weinberg equilibrium by Markov chain procedure ($P<0.01$)

Table 3. Pairwise F_{ST}.estimates for regional chum salmon populations based on msDNA data

	HOK	HON	KOR	PRI	SAK	KAM	NWA	AP	SCA	SEA	BCL	WST
Hokkaido (8)	0.001											
Honshu (6)	0.001	0										
Korea (1)	0.003	0.002	0									
Primorye (1)	0.015	0.019	0.019	0								
Sakhalin (6)	0.010	0.012	0.012	0.007	0.005							
Kamchtka (9)	0.029	0.032	0.031	0.018	0.024	0.017						
Northwest Alska(13)	0.063	0.071	0.070	0.025	0.043	0.050	0.008					
Alaska Peninsula (5)	0.034	0.039	0.038	0.008	0.022	0.030	0.023	0.010				
Southcentral Alaska (8)	0.018	0.021	0.021	0.008	0.013	0.020	0.038	0.018	0.010			
Southeast Alaska (9)	0.033	0.038	0.037	0.008	0.020	0.026	0.028	0.014	0.017	0.012		
British Columbia (7)	0.029	0.033	0.033	0.013	0.017	0.027	0.046	0.024	0.019	0.021	0.012	
Washington (3)	0.021	0.025	0.025	0.011	0.011	0.020	0.048	0.023	0.014	0.018	0.010	0.003

Table 4. Results of hierarchical analyses of molecular variance based on msDNA data of chum salmon. The percentage of variance (%), probability estimated from permutation (*P*), and the F-statistics (*Φ*) are given at each hierarchical level (Excoffier et al. 1992).

Variance component	%	*P*	*Φ*
Analysis I			
Among three regional groups (Japan, Russia, and North America)	5.6	<0.001	0.06
Among populations within groups	5.5	<0.001	0.06
Within populations	88.9	<0.001	0.11
Analysis II			
Among three regional groups in Japan (Hokkaido, Sea of Japan coast in Honshu, and Pacific Ocean coast in Honshu)	0.5	<0.05	0.01
Among populations within groups	1.4	<0.001	0.01
Within populations	98.1	<0.001	0.02
Analysis III			
Among four regional groups in Russia (Kamchtka, Sakhallin, Primorye, Megadan)	4.9	<0.001	0.05
Among populations within groups	6.0	<0.001	0.06
Within populations	89.1	<0.001	0.11
Analysis IV			
Among five regional groups in North America (Northwest Alaska, Alaska Peninsula/Southcentral Alaska, Southeast Alaska, British Columbia, and Washington)	3.7	<0.001	0.04
Among populations within groups	3.8	<0.001	0.04
Within populations	92.5	<0.001	0.08