Population structure of chum salmon (*Oncorhynchus keta*) across the Pacific Rim, determined from microsatellite analysis

Terry D. Beacham (contact author)
John R. Candy
Khai D. Le
Michael Wetklo

Email address for contact author: Terry.Beacham@dfo-mpo.gc.ca

Fisheries and Oceans Canada,
Pacific Biological Station,
3190 Hammond Bay Road
Nanaimo, B. C., Canada V9T 6N7

Abstract—The Pacific Rim population structure of chum salmon (*Oncorhynchus keta*) was examined with a survey of microsatellite variation to describe the distribution of genetic variation and to evaluate whether chum salmon may have originated from two or more glacial refuges following dispersal to newly available habitat after glacial retreat. Variation at 14 microsatellite loci was surveyed for over 53,000 chum salmon sampled from over 380 localities ranging from Korea through Washington State. An index of genetic differentiation, F_{ST}, over all populations and loci was 0.033, with individual locus values ranging from 0.009 to 0.104. The most genetically diverse chum salmon were observed from Asia, particularly Japan, whereas chum salmon from the Skeena River and Queen Charlotte Islands in northern British Columbia and those from Washington State displayed the fewest number of alleles compared with chum salmon in other regions. Differentiation in chum salmon allele frequencies among regions and populations within regions was approximately 18 times greater than that of annual variation within populations. A regional structuring of populations was the general pattern observed, with chum salmon spawning in different tributaries within a major river drainage or spawning in smaller rivers in a geographic area generally more similar to each other than to populations in different major river drainages or geographic areas. Population structure of chum salmon on a Pacific Rim basis supports the concept of a minimum of two refuges, northern and southern, during the last glaciation, but four possible refuges fit better the observed distribution of genetic variation. The distribution of microsatellite variation of chum salmon on a Pacific Rim basis likely reflects the origins of salmon radiating from refuges after the last glaciation period.

Manuscript submitted 28 August 2008.
Manuscript accepted 22 January 2009.
Fish. Bull. 107:244–260 (2009).

Delineation of phylogenetically and adaptively distinct groups in the distribution of chum salmon around the Pacific Rim may lead to conservation of genetic diversity through an understanding of the origin and the evolutionary processes promoting and maintaining genetic differentiation. An evaluation of genetic variation in describing the population structure of salmonids, is a key component in the elucidation of management units or conservation units in a species and can be applied to manage fisheries exploiting specific stocks of salmon. Several methods of surveying genetic variation have been used to investigate regional and Pacific Rim variation in chum salmon (*Oncorhynchus keta* Walbaum). Allozymes have been used for a number of years to describe chum salmon population differentiation and structure (Okazaki, 1982a; Kijima and Fujio, 1982; Wilmot et al., 1994; Efremov, 2001; Salmenkova et al., 2007). Variation in mitochondrial (mt) DNA has also been investigated (Park et al., 1993; Sato et al., 2001, 2004), as has minisatellite variation (Taylor et al., 1994; Beacham, 1996). Non-mtDNA single nucleotide polymorphisms have been examined (Smith and Seeb, 2008), as have microsatellites (Chen et al. 2005; Beacham et al. 2008a, 2008b, 2008c, 2009). Microsatellites are useful for evaluating fine-scale population structure in salmonids (Banks et al., 2000), and for investigating population structure around the Pacific Rim (Beacham et al., 2006a, 2006b).

Chum salmon display one of the widest spawning distributions of Pacific salmon. In Asia, chum salmon are distributed from Korea and Japan in the south to the Arctic Ocean coast of Russia in the north; in North America, the distribution has historically ranged from California in the south to the Beaufort Sea coast in the north, and as far east as the Mackenzie River in the Arctic (Salo, 1991). After fry emerge from the gravel nest in the spring or are released from hatcheries, they generally move directly to marine residence, first to estuaries, and later in the year to nearshore and offshore waters. Most individuals reside three to five years in the marine environment and then undertake spawning migrations generally to their natal river beds.

Chum salmon were likely fairly widely distributed along the Pacific Rim before the last major glaciation (McPhail and Lindsey, 1970). The advent of glaciation restricted the distribution of chum salmon to some major and perhaps minor refuges. Existing chum salmon population structure has been associated with colonization events following the last glaciation (Seeb and Crane, 1999). Modern populations were thought to have originated largely from a Bering Sea refuge in the north and a Columbia River refuge in the south (McPhail and Lindsey, 1970). In Asia,

local refuges may also have been present in the Kamchatka region (Varnavskaya et al., 1994), and in British Columbia, on the Queen Charlotte Islands and perhaps on coastal islands in the central coast region (Warner et al., 1982; Wood, 1995). Seeb and Crane (1999) indicated that existing populations from the Alaska Peninsula south to Washington may have derived primarily from the southern refuge, whereas Asian and western Alaskan populations may have derived from a northern refuge. Microsatellite variation can be used to examine relationships between existing Pacific Rim population structure and proposed patterns of dispersal from glacial refuges.

In the current study, we evaluated chum salmon dispersal pathways from glacial refugia after glacial retreat. In addition, we examined regional differentiation in allelic frequencies and levels of allelic diversity to evaluate whether local enhancement activities have had any effect on genetic diversity or population structure. These objectives were accomplished by analyzing variation at 14 microsatellite loci to evaluate relationships among Pacific Rim populations of chum salmon. The distribution of genetic diversity among regions, populations, and sampling years was estimated in the study.

Materials and methods

More than 53,000 chum salmon from 381 populations from Korea, Japan, Russia, Alaska, Canada, and Washington were analyed from 59 geographic regions (Table 1, Fig. 1), with the specific populations and sample sizes outlined by Beacham et al.[1] Tissue samples were collected from mature chum salmon, preserved in 95% ethanol, and analyzed at the Molecular Genetics Laboratory at the Pacific Biological Station (Fisheries and Oceans Canada, Nanaimo, BC). DNA was extracted from the tissue samples using a variety of methods, including a chelex resin protocol outlined by Small et al. (1998), a Qiagen 96-well Dneasy® procedure (Mississauga, Ontario), or a Promega Wizard SV96 Genomic DNA Purification system (Promega, Madison, WI). Once DNA was extracted, surveys of variation at 14 microsatellite loci were conducted: *Ots3* (Banks et al., 1999), *Oke3* (Buchholz et al., 2001), *Oki2* (Smith et al., 1998), *Oki100* (Beacham et al., 2008a), *Omm1070* (Rexroad et al., 2001), *Omy1011* (Spies et al., 2005), *One101, One102, One104, One111,* and *One114* (Olsen et al., 2000), *Ots103* (Nelson and Beacham, 1999), *Ssa419* (Cairney et al., 2000), and *OtsG68* (Williamson et al., 2002).

In general, polymerase chain reaction (PCR) DNA amplifications were conducted using DNA Engine Cycler Tetrad2 (BioRad, Hercules, CA) in 6μL volumes consisting of 0.15 units of Taq polymerase, 1 μL of extracted DNA, 1× PCR Hotstar buffer (Qiagen, Mississauga, Ontario, Canada), 60 μM each nucleotide, 0.40 μM of each primer, and deionized water. The thermal cycling profile involved one cycle of 15 minutes at 95°C, followed by 30–40 cycles of 20 seconds at 94°C, 30 to 60 seconds at 47–65°C and 30 to 60 seconds at 68–72°C (depending on the locus). Specific PCR conditions for a particular locus could vary from this general summary as outlined by Beacham et al. (in press). PCR fragments were initially size fractionated in denaturing polyacrylamide gels using an ABI 377 automated DNA sequencer (Applied Biosystems, Foster City, CA), and genotypes were scored by Genotyper 2.5 software (Applied Biosystems, Foster City, CA) using an internal lane sizing standard. Later in the study, microsatellites were size fractionated in an ABI 3730 capillary DNA sequencer (Applied Biosystems, Foster City, CA), and genotypes were scored by GeneMapper software 3.0 (Applied Biosystems, Foster City, CA) using an internal lane sizing standard. Allele identification between the two sequencers were standardized by analyzing approximately 600 individuals on both platforms and converting the sizing in the gel-based data set to match that obtained from the capillary-based set.

Data analysis

All annual samples available for a location were combined to estimate population allele frequencies, as was recommended by Waples (1990). Each population at each locus was tested for departure from Hardy-Weinberg equilibrium by using the computer software Genetic Data Analysis (GDA) (Univ. of Connecticut, Storrs, CT). Critical significance levels for simultaneous tests were evaluated using sequential Bonferroni adjustment (Rice 1989). Weir and Cockerham's (1984) F_{ST} estimates for each locus over all populations were calculated with FSTAT version 2.9.3.2 (Goudet, 1995). The significance of the multilocus F_{ST} value over all samples was determined by jackknifing the F_{ST} value over loci. The 59 geographic regions outlined in Table 1 were combined into 15 larger regional groups as outlined in Table 3 in order to display mean pairwise F_{ST} values between regions, but the two additional continental reporting groups (Asia, North America) incorporated in Table 3 were not used in the analysis of regional F_{ST} variation. Cavalli-Sforza and Edwards (CSE) (1967) chord distance was used to estimate genetic distances among all populations. An unrooted neighbor-joining tree based upon CSE was generated using NJPLOT (Perriere and Gouy, 1996). Bootstrap support (by sampling loci) for the major nodes in the tree was evaluated with the CONSENSE program in PHYLIP software, based upon 1000 replicate trees (Felsenstein, 1993). FSTAT was used to measure the "allelic richness" (allelic diversity standardized to a sample size of 911 fish) for each regional group of populations evaluated. The distribution of genetic variation in chum salmon was evaluated among regions, among populations within regions, and among sampling years within populations. In order to

[1] Beacham, T. D., J. R. Candy, S. Urawa, S. Sato, N. V. Varnavskaya, K. D. Le, and M. Wetklo. 2008. Microsatellite stock identification of chum salmon on a Pacific Rim basis and a comparison with single nucleotide polymorphisms (SNPs). Manuscript in review.

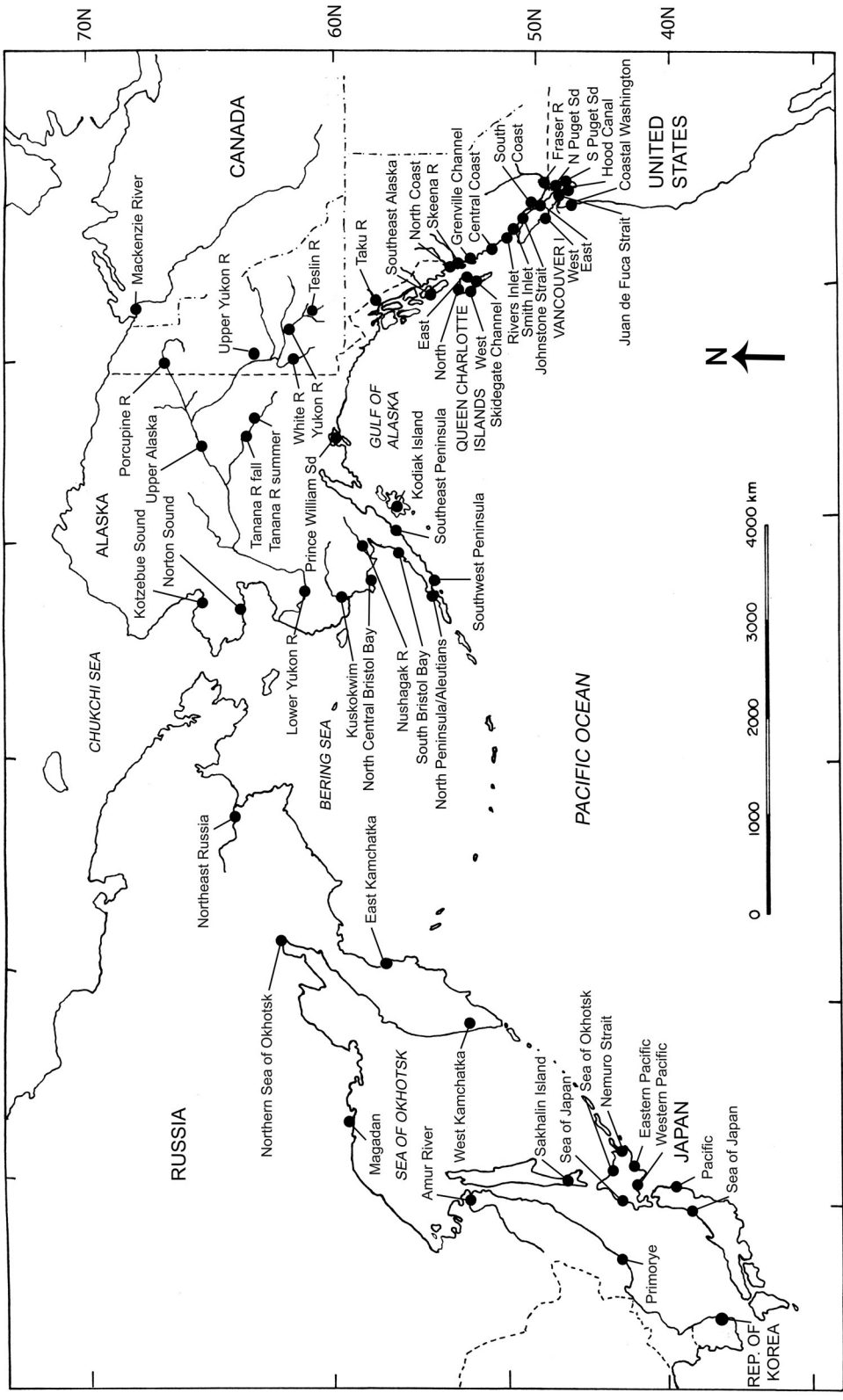

Figure 1

Map of the Pacific Rim indicating the general geographic regions where chum salmon (*Oncorhynchus keta*) from 381 populations were surveyed. The regions are listed in Table 1.

Table 1

Summary of the number of sampling sites or populations of chum salmon (*Oncorhynchus keta*) within each geographic region listed in Figure 1. A complete listing of the populations is outlined by Beacham et al.[1] in their Appendix Table 1. *n* is the number of populations sampled within regions. The range of population sample sizes within regions is given in parentheses.

Geographic area	Reporting region	*n*	Mean population sample size
Korea	Korea	1	100 (100–100)
Japan	Honshu Island, Sea of Japan Coast	5	106 (80–160)
	Hokkaido Island, Sea of Japan Coast	3	147 (60–280)
	Hokkaido Island, Sea of Okhotsk Coast	5	108 (50–160)
	Hokkaido Island, Nemuro Strait	2	95 (80–110)
	Hokkaido Island, eastern Pacific Coast	2	105 (80–130)
	Hokkaido Island, western Pacific Coast	4	120 (80–160)
	Honshu Island, Pacific Coast	5	68 (19–80)
Russia	Primorye	3	34 (17–49)
	Amur River	1	338 (338–338)
	Sakhalin Island	4	76 (49–149)
	Magadan	5	89 (55–120)
	Northern Sea of Okhotsk	2	60 (43–76)
	West Kamchatka	8	116 (40–249)
	East Kamchatka	9	58 (39–128)
	Northeast Russia	2	87 (79–94)
Arctic Canada	Mackenzie River	1	33 (33–33)
Yukon River	Lower river summer run (United States)	11	185 (92–347)
	Tanana River summer run (United States)	2	211 (185–236)
	Tanana River fall run (United States)	3	160 (80–241)
	Upper Alaska (United States)	4	149 (73–229)
	Porcupine River (Canada)	2	463 (329–597)
	White River (Canada)	3	207 (62–486)
	Mainstem Yukon River (Canada)	4	144 (83–175)
	Teslin River (Canada)	1	143 (143–143)
	Upper Yukon River early fall (Canada)	1	120 (120–120)
Western Alaska	Kotzebue Sound	6	155 (45–374)
	Norton Sound	10	278 (50–474)
	Kuskokwim River and bay	6	94 (68–171)
	Nushagak River	2	78 (74–82)
	North Central Bristol Bay	4	77 (64–92)
	South Bristol Bay	4	83 (57–97)
	North Peninsula and Aleutians	3	122 (93–179)
Central Alaska	Southwest Peninsula	4	83 (70–104)
	Southeast Peninsula	3	91 (87–94)
	Kodiak Island	3	89 (71–100)
	Prince William Sound	4	98 (92–100)
Southeast Alaska	Southeast Alaska	14	119 (50–333)
Queen Charlotte Islands	West Coast	11	209 (42–393)
	North Coast	4	132 (80–221)
	East Coast	11	161 (17–376)
	Skidegate Channel	8	181 (79–232)

Continued

Table 1 (continued)

Geographic area	Reporting region	*n*	Mean population sample size
Northern British Columbia	Taku River	5	34 (12–65)
	North Coast	18	117 (28–242)
	Skeena River	13	95 (12–182)
	Grenville Channel	6	122 (40–242)
	Central Coast	52	190 (13–419)
	Rivers Inlet	8	79 (40–153)
	Smith Inlet	2	363 (226–499)
Southern British Columbia	Johnstone Strait	13	134 (20–409)
	South Coast	14	137 (15–344)
	Vancouver Island east coast	9	227 (167–285)
	Vancouver Island west coast	10	133 (43–243)
	Fraser River	23	151 (24–427)
Washington	North Puget Sound	7	85 (50–100)
	South Puget Sound	3	100 (100–100)
	Hood Canal	2	95 (88–102)
	Strait of Juan de Fuca	2	100 (100–100)
	Coast of Washington	4	91 (61–106)

maintain a balanced design, regions included in the analysis required two or more populations each with two or more years of samples available. Regions were distributed around the Pacific Rim and were a subset of the 59 geographic regions outlined in Table 1 and Figure 1. The specific populations included from each region are in shown parentheses: West Kamchatka (Hairusova, Vorovskaya), Western Alaska (Snake, Eldorado), Yukon River summer run (Gisasa, Tozitna), Southeast Alaska (DIPAC hatchery, Disappearance), Queen Charlotte Islands west coast (Clapp Basin, Mace), Queen Charlotte Islands east coast (Lagoon, Pallant), Northern British Columbia (Ensheshese, Kateen), Grenville Channel (Markle, Wilson), British Columbia central coast (Bullock Channel, Quaal, Salmon), Smith Inlet (Walkum, Nekite), Johnstone Strait (Viner Sound, Nimpkish), Vancouver Island east coast (Big Qualicum, Cowichan), and Fraser River (Inch, Stave). Estimation of variance components of river drainage or region differentiation, among populations within drainages or regions, and among years within populations was determined with Genetic Data Analysis.

Results

Variation within populations

Substantial variation was observed in the number of alleles at the 14 microsatellite loci surveyed in the study. The fewest number of alleles was observed at *Oke3* (26 alleles), and the greatest number of alleles observed at *One111* (149 alleles) (Table 2). Lower heterozygosity was observed at loci with fewer than 40 alleles. The genotypic frequencies at each locus conformed to those expected under Hardy-Weinberg equilibrium (HWE).

The number of alleles observed displayed considerable variation across regional groups of chum salmon. Asian chum salmon were considerably more diverse than those in North America, with Asian populations displaying the greatest number of alleles at all 14 loci examined (P=0.0001) (Table 3). With sample sizes standardized to 911 fish per region, Japanese chum salmon were the most genetically diverse set of populations examined with 581 alleles observed, greater than in all other regional groups of populations. The least diverse groups of populations were observed in the Queen Charlotte Islands, the Skeena River, the east coast of Vancouver Island, and Washington State, with an average of 414 alleles observed in chum salmon from these regions. Japanese chum salmon displayed 40% more alleles and Russian chum salmon 35% more alleles than did chum salmon from the four regions of lower genetic diversity. The greatest difference in diversity was observed at locus *One111,* with the greatest number of observed alleles, and Asian chum salmon displayed 80% more alleles than did chum salmon from the four regions of lower genetic diversity. Even with *One111* removed from the analysis, Asian chum salmon were still more diverse than chum salmon in all regions in North America (P=0.0002).

Distribution of genetic variance

Gene diversity analysis of the 14 loci surveyed was used to evaluate the distribution of genetic variation

Table 2

Number of alleles per locus, an index of gentic differentiation F_{ST} (SD in parentheses), expected heterozygosity (H_e), observed heterozygosity (H_o), and percent significant Hardy-Weinberg equilibrium (HWE) test for 14 microsatellites (n=381 tests) among 381 chum salmon (*Oncorhynchus keta*) populations.

Locus		Number of alleles	F_{ST}	H_e	H_o	HWE
1	*Oke3*	26	0.104 (0.005)	0.67	0.65	3.7
2	*Oki100*	31	0.039 (0.002)	0.83	0.83	0.3
3	*Ots3*	31	0.097 (0.005)	0.76	0.75	4.7
4	*Oki2*	42	0.062 (0.005)	0.86	0.85	0.8
5	*Omy1011*	44	0.027 (0.001)	0.90	0.89	0.3
6	*One104*	48	0.027 (0.001)	0.93	0.92	1.6
7	*Ots103*	54	0.019 (0.001)	0.94	0.93	1.1
8	*Ssa419*	54	0.028 (0.001)	0.84	0.83	0.5
9	*One101*	56	0.058 (0.002)	0.87	0.86	1.6
10	*Omm1070*	60	0.009 (0.000)	0.95	0.94	1.3
11	*One114*	60	0.017 (0.001)	0.92	0.91	1.6
12	*One102*	69	0.011 (0.001)	0.92	0.90	2.1
13	*OtsG68*	69	0.017 (0.001)	0.95	0.94	1.8
14	*One111*	149	0.036 (0.003)	0.94	0.93	3.9
Total			0.033 (0.007)	0.88	0.87	

Table 3

Mean number of alleles observed per locus at 14 microsatellite loci for chum salmon (*Oncorhynchus keta*) from 15 geographic areas standardized to a sample size of 911 fish per geographic area. Geographic areas, listed in Table 1, were: Japan (includes Korea), Russia, Western Alaska (WAK), Yukon River (includes Arctic Canada), Central Alaska (CAK), Southeast Alaska (SeAK), Queen Charlotte Islands (QCI), northern British Columbia (NBC), Skeena River, Central Coast British Columbia (CBC) (includes Grenville Channel, Rivers Inlet, and Smith Inlet), Southern British Columbia (includes Johnstone Strait), east coast Vancouver Island (ECVI), west coast Vancouver Island (WCVI), Fraser River, Washington (Wash), and North America (NA).

Area	*Oke 3*	*Oki 100*	*Oki 2*	*Omm 1070*	*Omy 1011*	*One 101*	*One 102*	*One 104*	*One 111*	*One 114*	*Ots 3*	*Ots 103*	*Ots G68*	*Ssa 419*	Total
Japan	16.6	25.0	23.8	51.0	39.5	37.8	42.3	37.4	122.0	34.5	25.8	46.8	53.1	25.2	580.8
Russia	14.9	27.1	18.9	45.1	31.3	40.2	28.4	34.4	130.2	40.6	22.4	47.6	51.2	26.6	558.9
Total Asia	15.8	26.1	21.4	48.1	35.4	39.0	35.4	35.9	126.1	37.6	24.1	47.2	52.2	25.9	569.9
WAK	9.9	24.2	18.2	37.2	28.1	33.0	21.7	27.7	110.7	39.9	19.2	40.4	41.7	17.9	469.8
Yukon R.	12.9	22.8	20.5	37.7	28.6	35.9	28.2	29.3	112.1	35.0	21.2	38.1	41.3	16.7	480.3
CAK	9.7	25.2	20.0	36.3	25.7	29.5	27.0	30.6	106.4	35.4	18.9	39.7	40.6	20.2	465.2
SeAK	8.6	21.6	20.4	40.9	24.3	37.6	28.9	28.5	94.7	35.5	18.6	43.3	43.1	22.8	468.8
QCI	11.8	17.2	20.8	38.3	21.1	36.6	29.1	28.5	69.7	26.1	18.1	39.7	44.0	23.1	424.1
NBC	14.5	19.2	20.3	40.4	26.5	41.0	31.3	31.3	102.0	31.1	18.2	42.5	45.8	25.2	489.3
Skeena R.	7.5	15.8	18.0	37.3	22.7	35.9	25.9	28.0	65.3	25.2	16.1	37.5	42.0	17.7	394.9
CBC	13.9	19.3	20.2	41.7	26.2	39.0	31.4	28.6	93.8	33.7	21.4	42.1	45.2	22.7	479.2
SBC	16.2	17.9	20.2	38.8	26.0	40.6	28.8	33.0	76.1	31.2	22.0	39.1	44.8	20.5	455.2
ECVI	8.0	15.7	23.0	39.8	21.8	37.0	24.9	25.9	73.9	27.0	19.6	36.9	44.9	19.0	417.4
WCVI	11.0	17.8	25.8	35.9	24.7	39.8	28.2	29.8	74.6	31.7	22.4	39.0	47.4	16.8	444.9
Fraser R.	13.0	20.8	18.1	42.2	25.0	38.8	23.4	31.1	89.6	34.3	20.6	38.7	56.0	15.4	467.0
Washington	10.5	17.6	18.0	38.8	18.8	36.3	26.6	29.0	71.0	34.0	15.8	40.5	45.5	15.6	418.0
Total NA	11.3	19.6	20.3	38.9	24.6	37.0	27.3	29.3	87.7	32.3	18.2	39.8	44.8	19.5	451.8

Table 4

Hierarchical gene-diversity analysis of 27 populations of chum salmon (*Oncorhynchus keta*) within 13 regions for 14 microsatellite loci. Regions had a Pacific Rim distribution, and the time difference between the earliest and latest samples included for specific populations ranged from 1 to 21 years. Ratio is the sum of the variance components of among populations within regions and among regions divided by the variance component among years within populations. * $P<0.05$ ** $P<0.01$.

Locus	Within populations	Among years within populations	Among populations within regions	Among regions	Ratio
Oke3	0.9204	0.0004	0.0056**	0.0736**	198.0
Oki100	0.9673	0.0008	0.0070**	0.0249**	39.9
Ots3	0.9254	0.0018*	0.0044**	0.0685**	40.5
Oki2	0.9625	0.0065**	0.0044*	0.0266**	4.8
Omy1011	0.9783	0.0016	0.0013	0.0187**	12.5
One104	0.9783	0.0004	0.0030**	0.0183**	53.3
Ots103	0.9837	0.0007	0.0029**	0.0126**	22.1
Ssa419	0.9785	0.0018*	0.0054**	0.0143**	10.9
One101	0.9635	0.0015	0.0088**	0.0262**	23.3
Omm1070	0.9918	0.0011	0.0031**	0.0040*	6.5
One114	0.9881	0.0009	0.0042**	0.0068*	12.2
One102	0.9941	0.0008	0.0018	0.0033*	6.4
OtsG68	0.9854	0.0015	0.0036**	0.0095**	8.7
One111	0.9727	0.0016*	0.0030	0.0227**	16.1
Total	0.9722	0.0015	0.0041**	0.0222**	17.5

among regions, among populations within regions, and among years within populations. Within populations, the time difference between the earliest and latest samples included in the analysis ranged from 21 years (1986–2007) for Disappearance Creek (Southeast Alaska), 18 years (1986–2004) for Lagoon Creek (Queen Charlotte Islands), 16 years (1989–2005) for Nekite River in Smith Inlet, 15 years (1988–2003) for Gisasa River (Lower Yukon River), down to 1–3 year differences for populations in a number of regions. For 13 regions ranging from west Kamchatka to the Fraser River, the amount of variation within populations ranged from 92% (*Oke3*) to 99% (*Omm1070*), with the average for an individual locus 97% (Table 4). Variation among the 13 regions included in the analysis accounted for 2.2% of total observed variation. Variation among populations within regions accounted for 0.4% of observed variation, with differences among regions over five times greater than differences among populations within regions. The variation among sampling years within populations was the smallest source of variation observed, accounting for 0.2% of all variation. Differentiation among regions and populations within regions was approximately 18 times greater than that of annual variation within populations. For the time intervals surveyed in our study, annual variation in microsatellite allele frequencies was relatively minor compared with differences among populations within regions and among regions on a geographically diverse scale of distribution of the populations analyzed.

Population structure

Significant genetic differentiation was observed among chum salmon populations sampled in the different geographic regions surveyed. The F_{ST} value over all populations and loci was 0.033, with individual locus values ranging from 0.009 (*Omm1070*) to 0.104 (*Oke3*) (Table 2). Chum salmon from Japan and the Yukon River were among the most distinct regional groups of stocks included in the survey (Table 5). Greatest genetic differentiation (greatest difference in F_{ST} values) was observed in comparisons between Japanese, Russian, western Alaskan, and Yukon River chum salmon compared with those in other regions in North America to the south and east. In Asia, chum salmon from Japan were generally distinct from those in Russia. In North America, significant regional differentiation was generally observed, with chum salmon in more northern regions distinct from those in more southern regions.

Two major lineages of chum salmon populations were identified in the cluster analysis. The first lineage included all populations sampled from Korea, Japan, Russia, the Mackenzie River, Kotzebue Sound, Norton Sound, the Yukon River, and northern and central Bristol Bay. Populations from southern Bristol Bay were intermediate between the two major lineages, and all populations from the Alaska Peninsula south and east to Washington State were identified as the second major lineage (Fig. 2). Within the first lineage, all Asian populations were distinct from all North American

Table 5

Mean pairwise F_{ST} values averaged over 14 microsatellite loci from 15 regional groups of chum salmon (*Oncorhynchus keta*) outlined in Table 3 that were sampled at 381 locations across the Pacific Rim. Comparisons were conducted between individual populations in each region. Values in bold are the diagonal, and are comparisons among populations within each region. F_{ST} values are listed below the diagonal, with standard deviations above the diagonal. Some of the reporting regions listed in Table 1 were combined as indicated in Table 3 in order to facilitate the analysis. RC is region code, and codes are as follows: 1) Japan, 2) Russia, 3) Western Alaska, 4) Yukon River, 5) Central Alaska, 6) Southeast Alaska, 7) Queen Charlotte Islands, 8) Northern British Columbia, 9) Skeena River, 10) Central British Columbia, 11) Southern mainland British Columbia, 12) West coast Vancouver Island, 13) East coast Vancouver Island, 14) Fraser River, 15) Washington.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	**0.019**	0.009	0.014	0.023	0.015	0.007	0.008	0.009	0.009	0.009	0.008	0.007	0.008	0.011	0.011
2	0.026	**0.017**	0.013	0.018	0.016	0.011	0.010	0.011	0.012	0.011	0.011	0.010	0.010	0.011	0.014
3	0.028	0.024	**0.012**	0.018	0.018	0.011	0.011	0.011	0.012	0.011	0.009	0.010	0.011	0.015	0.013
4	0.053	0.054	0.031	**0.018**	0.020	0.013	0.014	0.014	0.019	0.014	0.015	0.013	0.017	0.022	0.016
5	0.042	0.032	0.037	0.064	**0.027**	0.014	0.016	0.015	0.019	0.016	0.014	0.015	0.013	0.011	0.015
6	0.042	0.029	0.035	0.062	0.024	**0.007**	0.006	0.005	0.016	0.006	0.007	0.005	0.011	0.006	0.009
7	0.050	0.039	0.043	0.068	0.034	0.015	**0.012**	0.007	0.017	0.007	0.007	0.005	0.014	0.008	0.010
8	0.044	0.031	0.037	0.063	0.026	0.008	0.015	**0.008**	0.017	0.007	0.009	0.007	0.013	0.009	0.012
9	0.053	0.041	0.046	0.066	0.035	0.019	0.025	0.019	**0.014**	0.017	0.014	0.012	0.017	0.015	0.017
10	0.043	0.031	0.037	0.062	0.030	0.011	0.014	0.010	0.020	**0.008**	0.007	0.005	0.013	0.008	0.009
11	0.046	0.033	0.040	0.068	0.039	0.022	0.025	0.021	0.030	0.018	**0.014**	0.007	0.019	0.012	0.012
12	0.044	0.034	0.038	0.062	0.038	0.019	0.018	0.019	0.026	0.017	0.016	**0.008**	0.016	0.009	0.010
13	0.043	0.032	0.034	0.060	0.039	0.026	0.031	0.026	0.035	0.025	0.019	0.022	**0.022**	0.018	0.011
14	0.041	0.028	0.035	0.063	0.037	0.025	0.030	0.026	0.033	0.024	0.018	0.021	0.020	**0.013**	0.015
15	0.051	0.039	0.047	0.076	0.045	0.028	0.033	0.029	0.035	0.025	0.022	0.023	0.028	0.022	**0.022**

populations. Within the Asian portion of the lineage, Japanese, Korean, and Russian Primorye populations were distinct from other Asian populations. In the second lineage, populations from Washington and southern British Columbia were among the most distinct group of populations, along with populations from the Queen Charlotte Islands in northern British Columbia.

Chum salmon spawning in tributaries of different major river drainages generally clustered together in the analysis. For example, Fraser River populations clustered together in 39% of dendrograms evaluated, Skeena River populations clustered together in 97% of dendrograms evaluated, and Taku River populations clustered together in 96% of dendrograms evaluated (Fig. 2). The one exception was the Yukon River, where lower river summer-run populations did not form distinct clusters unique from neighboring populations in the Kuskokwim River and the Nushagak River.

A very distinct regional cluster of populations was observed in the Asian populations, with Korean, Japanese, and populations from the Primorye region in Russia clustering together in 100% of dendrograms evaluated. Within that cluster, populations from Primorye clustered together in 67% of dendrograms evaluated, indicative of genetic differentiation between populations from that region and those in Japan and Korea. Within Japan, a general regional structuring of populations was observed, with populations from the Pacific coast of Honshu Island forming a distinct group (92% of dendrograms evaluated), as did populations from the Nemuro Strait (89% of dendrograms) and the eastern Pacific coast of Hokkaido Island (50% of dendrograms). Within Russia, Magadan region populations clustered together in 41% of dendrograms evaluated, as did populations from the northern Sea of Okhotsk (100% of dendrograms). Although populations from east coast of Kamchatka and west coast of Kamchatka generally clustered as two distinct regional groups, the groupings were not strongly supported by the bootstrap analysis. Populations from northeast Russia were distinct from those in other regions, with the possible exception of the Utka River population from west Kamchatka.

In North America, some level of regional structuring of populations was observed in both Kotzebue Sound and Norton Sound (Fig. 2). Within the Yukon River drainage, there was clear separation between summer-run populations in the lower and mid- portions of the drainage and fall-run populations in the upper portion of the drainage. For the fall-run, populations in the White River in the Yukon Territory were quite distinct, clustering together in 100% of dendrograms evaluated. Similarly, fall-run populations in the Tanana River (upper portion of Yukon River drainage in Alaska) clustered together in 74% of the dendrograms evaluated, and summer-run populations in the Tanana River drainage clustered together in 96% of dendrograms. Summer-run populations in the lower Yukon River drainage did not cluster exclusively with each

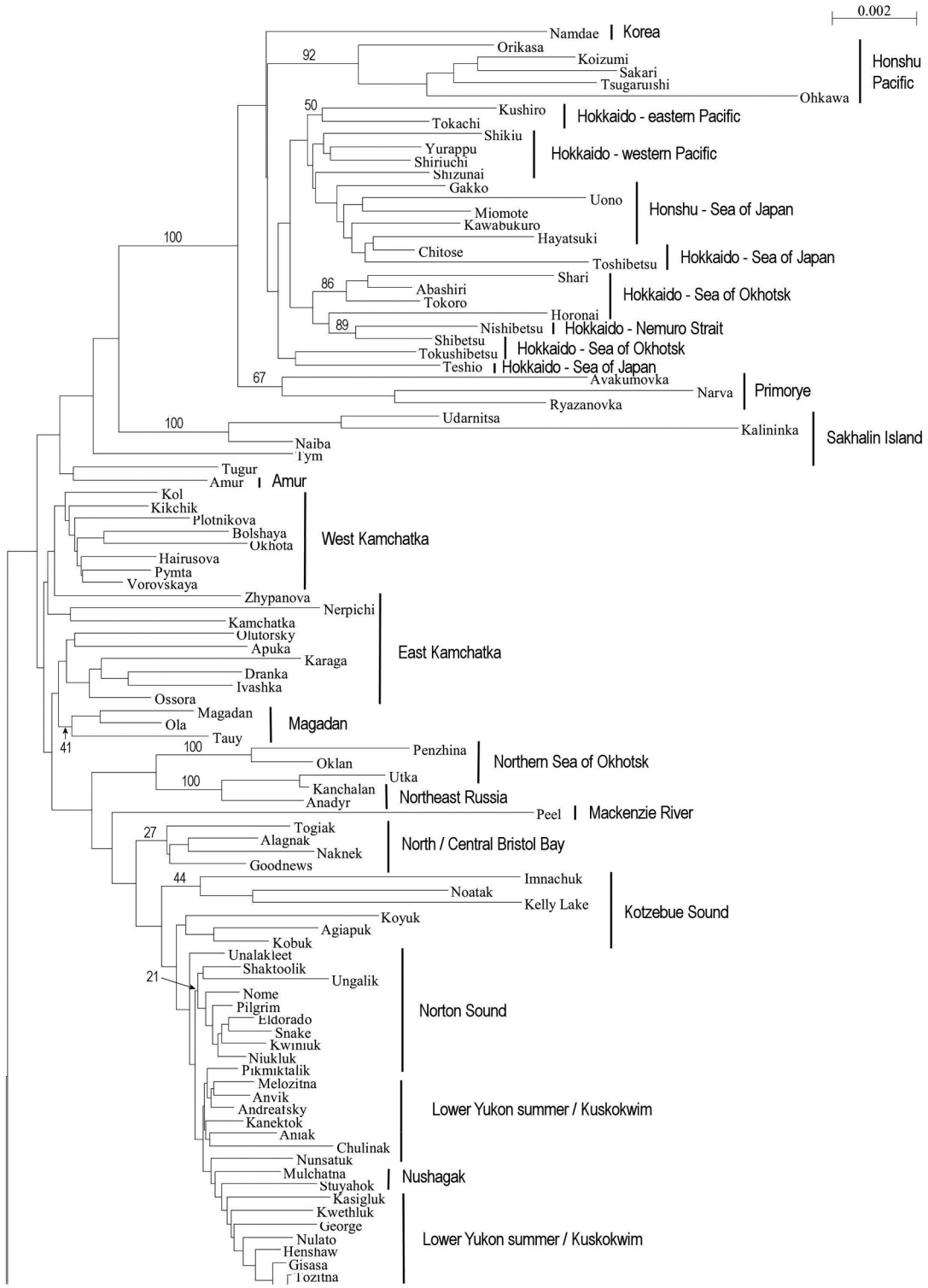

Figure 2

Neighbour-joining dendrogram of Cavalli-Sforza and Edwards (1967) chord distance for 381 Pacific Rim populations of chum salmon (*Oncorhynchus keta*) surveyed at 14 microsatellite loci. Bootstrap values at major tree nodes indicate the percentage of 1000 trees where populations beyond the node clustered together.

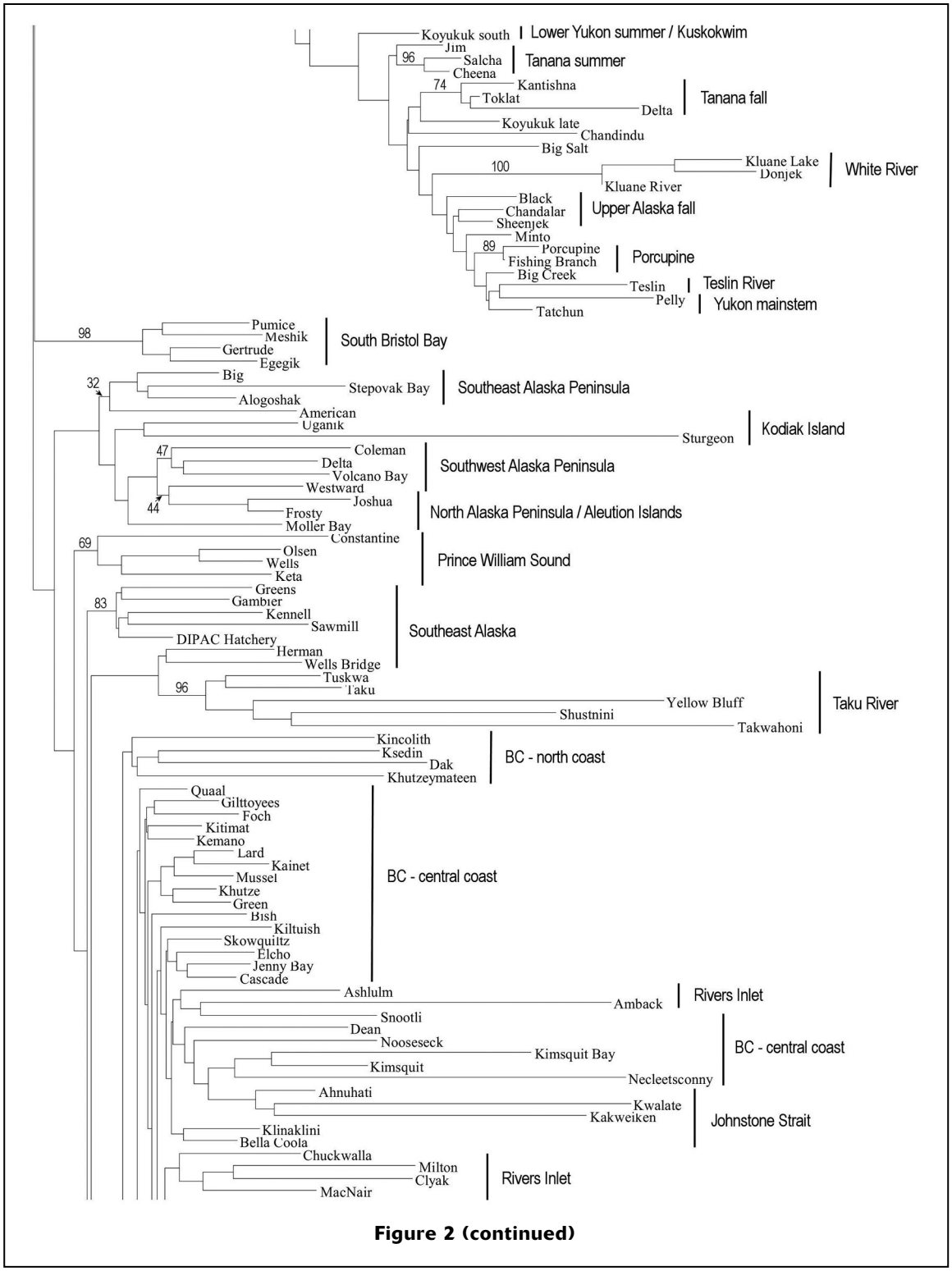

Figure 2 (continued)

other, including populations from the Kuskokwim River in western Alaska and Nushagak River from northern Bristol Bay in the dendrogram cluster.

Geographically-based regional clustering was observed in the populations surveyed south and east of northern Bristol Bay. Populations from southern Bristol Bay formed a distinct cluster in 98% of dendrograms evaluated, with bootstrap support observed for populations from the western south coast of the Alaska Peninsula, eastern south coast of the Alaska Peninsula, Kodiak

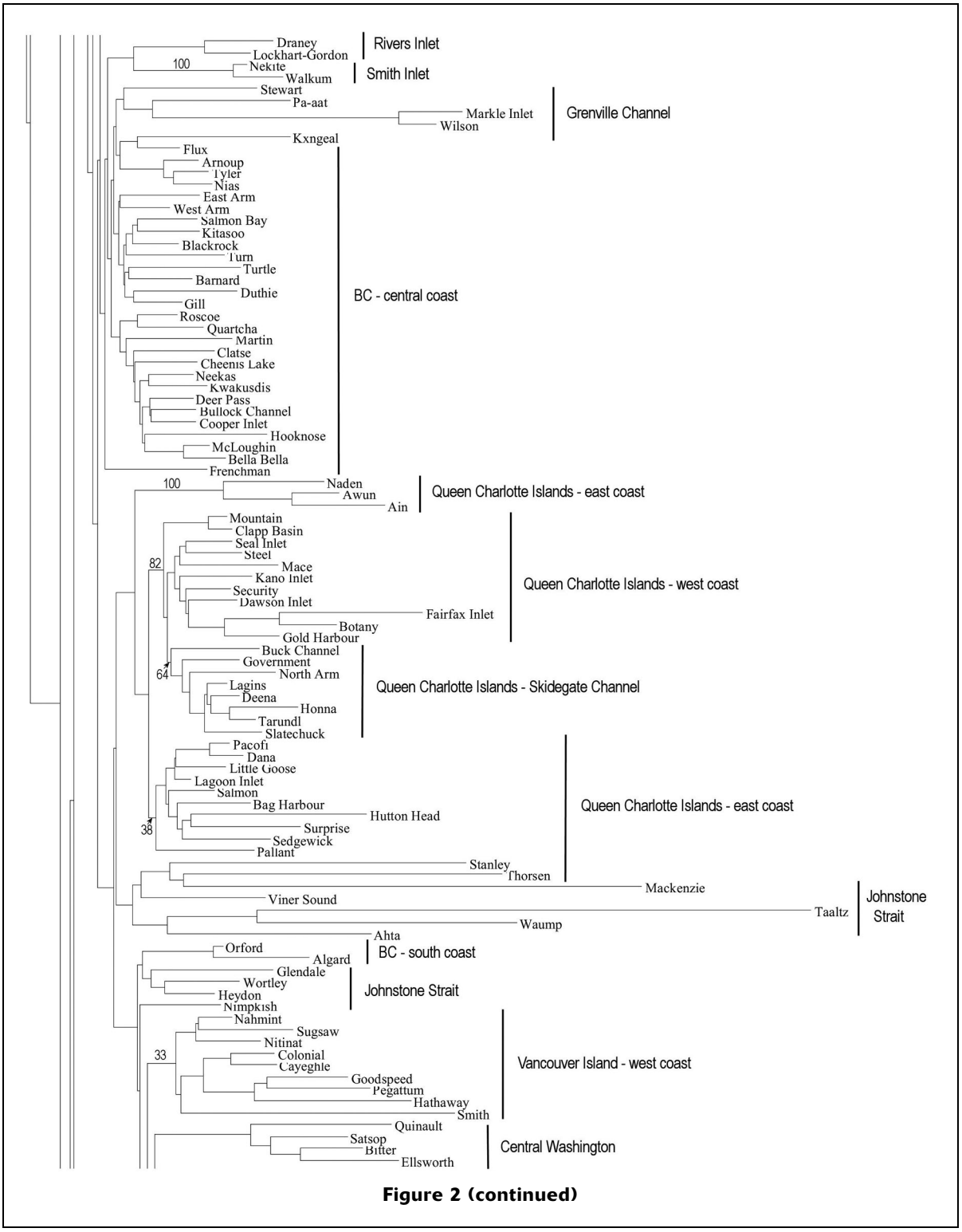

Figure 2 (continued)

Island, and Prince William Sound. Populations from northern southeast Alaska formed a distinct cluster in the analysis, but populations from southern southeast Alaska were less distinct than those in the northern portion of the region. Some clusters in the dendrogram included populations from both southern southeast Alaska and northern British Columbia (Fig. 2).

In British Columbia (BC), four geographically based regional groups of populations were observed in the Queen Charlotte Islands (QCI). North coast QCI popu-

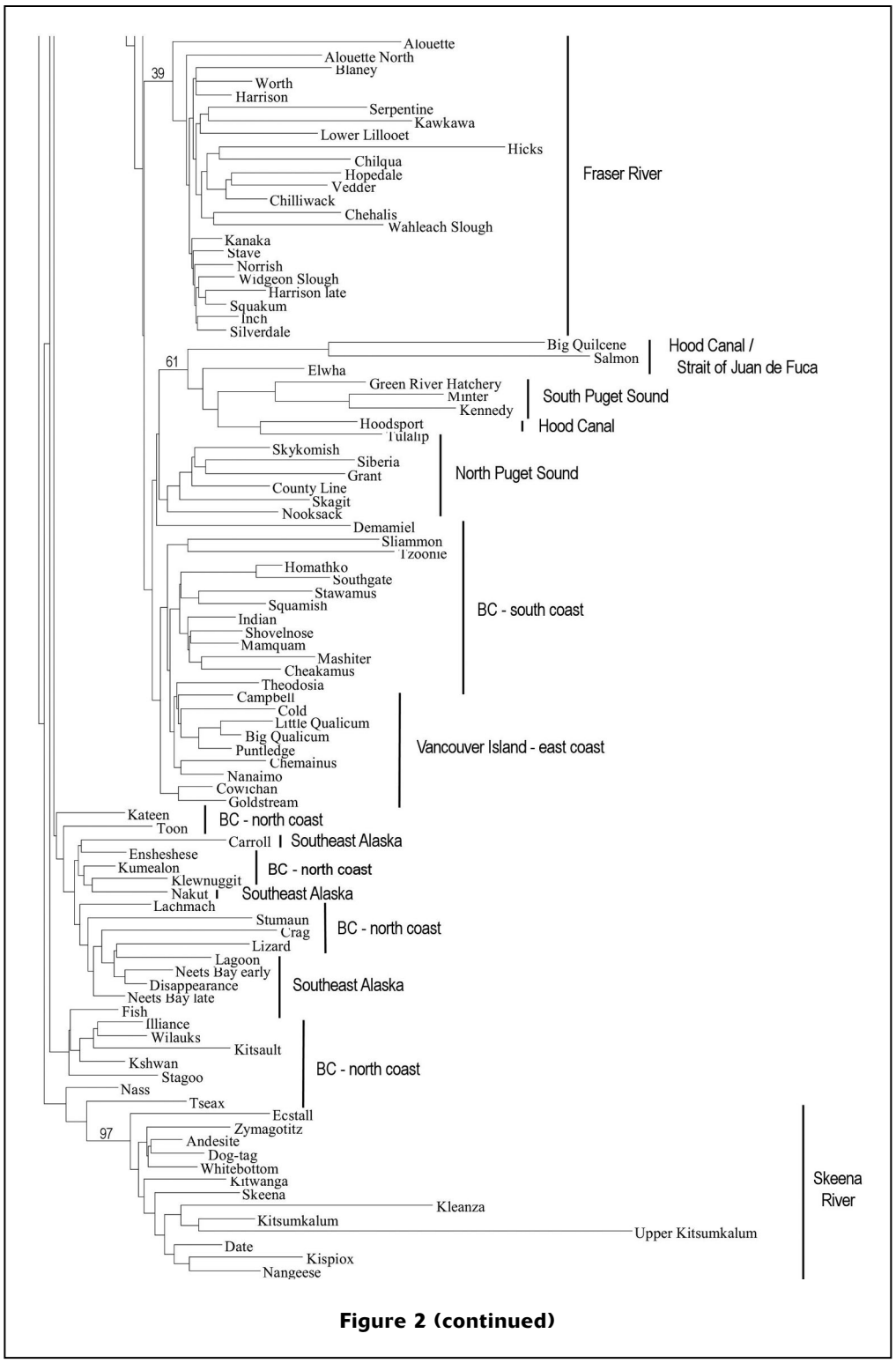

Figure 2 (continued)

lations were the most distinct clustering together in 100% of dendrograms evaluated. Regional populations were also observed along the east and west coasts of the QCI. Populations adjacent to Skidegate Channel, the body of water separating the major QCI components of Graham Island (north) and Moresby Island (south), clustered together with 64% bootstrap support. On the northern mainland, populations north of the Skeena River mouth were distinct from those south of the Skeena River. North of the Skeena River, there

were not distinct clusters observed between northern coastal British Columbia populations and those from southeast Alaska. Populations immediately south of the Skeena River in the Grenville Channel area clustered separately from those further south in the central coastal region of British Columbia. Yet farther south, populations from Rivers Inlet and Smith Inlet clustered together in geographically based groups, and this result was confirmed by 100% boostrap support observed for Smith Inlet populations (Fig. 2).

In southern BC, five geographically based groups of populations were revealed. East coast and west coast of Vancouver Island populations were regionally separate from each other, and also from other regional populations in southern BC. On the mainland, Johnstone Strait populations were separate from those in southern coastal areas, and the demarcation point between the two groups is Bute Inlet, at the northeast limit of the Strait of Georgia. Fraser River populations were distinct from other regional groups in southern BC.

In Washington, regional structuring of chum populations was observed. The most distinct regional group comprised populations from the outer Pacific coast, with populations clustering together with 100% bootstrap support. In more inside waters, populations from north Puget Sound were generally distinct from those in south Puget Sound, Hood Canal, and the Strait of Juan de Fuca. South Puget Sound populations were distinct from those in Hood Canal and the Strait of Juan de Fuca.

Discussion

The survey of microsatellite variation included an examination of variation at 14 loci encompassing approximately 800 alleles, with 26 to 149 alleles recognized per locus. The number of fish surveyed per population ranged from 12 to 597 individuals (Beacham et al.[1]). With a variable number of individuals surveyed per population, there was a potential for sampling error in estimated allele frequencies and in obscuring genetic relationships among related populations, particularly if sample sizes were small for some populations in a lineage. For example, for the Primorye populations from Russia, population sample sizes ranged from 17 to 49 individuals, and it was possible that estimates of genetic distances among populations were not determined satisfactorily for populations of smaller sample size, particularly for those loci with larger numbers of alleles. However, Kalinowski (2005) reported that loci with larger numbers of alleles (higher mutation rates) produced estimates of genetic distance with lower coefficients of variation than loci with fewer numbers of alleles, without requiring larger sample sizes from each population. Population structuring based upon geographic differences were observed for populations from Primorye, and all populations clustered together in 67% of dendrograms evaluated. Therefore, it seems likely that variation in the number of individuals surveyed within a population in our study did not generally result in misidentification of genetic relationships among populations.

Size homoplasy of microsatellite alleles may have some effect on the estimate of genetic differentiation observed among populations. Inferences about the genetic relationships of populations surveyed in our study were dependent upon accurate determination of population allele frequencies. Microsatellite alleles differ in size, but alleles of the same size at a locus in geographically disparate populations may not have the same origin as a result of size homoplasy. Convergent mutations in different lineages may produce alleles of the same size, with the result that there may be greater differentiation among lineages than revealed by analysis of size variation. However, with approximately 800 alleles observed across all loci in the study, the large amount of variation present at these loci largely compensates for size homoplasy (Estoup et al., 2002).

In this study, population allele frequencies were estimated by combining all samples collected over time for a population, regardless of the length of time that occurred between samples. In practice, the maximum length of time between samples for a population was 21 years, and up to six annual samples were combined for a population. Analysis of the distribution of genetic variation indicated that differentiation among regions and populations within regions was approximately 18 times greater than that of annual variation within populations, indicating that pooling of annual samples over time is a practical approach to estimate population allele frequencies. Relative stability of microsatellite allele frequencies over time is not unique to chum salmon; similar relative stability has been reported for sockeye salmon (*O. nerka*) (Beacham et al., 2006a) and Chinook salmon (*O. tshawytscha*) (Beacham et al., 2006b).

Surveys of genetically based population structure in chum salmon were initially conducted with allozymes. Okazaki (1982b), in a study evaluating allozyme variation in Asian and North American populations, concluded that there were 11 geographically based regional groups of populations across the Pacific Rim. The regional groups consisted of adjacent river populations that were genetically similar within one region. Many allozyme-based studies of regional population structure were subsequently reported. For example, Winans et al. (1994) provided additional details concerning population structure of Asian populations, Wilmot et al. (1994) compared population structure of chum salmon from western Alaska and northeast Russia, Kondzela et al. (1994) compared population structure of chum salmon from southeast Alaska and northern British Columbia, Beacham et al.(1987) evaluated population structure of chum salmon in British Columbia, and Phelps et al. (1994) evaluated population structure in the Pacific Northwest. Seeb and Crane (1999) again investigated chum salmon population structure throughout the Pacific Rim by examining variation at 40 allozymes, and reported that two major lineages of populations were observed. The northern lineage occurred in areas north of the Alaska Peninsula and into Russia and Japan,

whereas the southern lineage was observed in the Alaska Peninsula, Kodiak Island, and areas to the south and east. The two lineages were reported to overlap in the northern Alaska Peninsula.

Development of DNA-level markers provided additional markers for genetic evaluation of population structure of chum salmon, and surveys of mitochondrial DNA variation have been reported. Differentiation among Russian populations has been reported (Ginatulina, 1992; Brykov, 2003; Polyakova et al., 2006), as well as in Japanese populations (Sato et al., 2001). In an analysis of mtDNA variation across the Pacific Rim, Sato et al. (2004) reported that there were three major lineages of chum salmon, with populations from Japan, Russia, and North America comprising three distinct regional groups. Chum salmon from Japan were observed to be the most distinct, with less divergence between populations from Russia and western Alaska.

Minisatellite variation was used by Taylor et al. (1994) and Beacham (1996) to survey variation in 42 chum salmon populations across the Pacific Rim. Three regional groups of populations showed that those from Japan were the most distinct, followed by a second (less distinct) group comprising Russian and Yukon River populations, and a third group comprising southeast Alaska and British Columbia populations.

Microsatellites have been used to evaluate chum salmon population differentiation and structure on a local and regional basis (Chen et al., 2005; Beacham et al., 2008a, 2008b, 2008c, in press). In those studies, as in the previous allozyme-based studies, regional groups of populations were observed, with the regional groups consisting of adjacent river populations or local populations that were genetically similar within one region. The results from the current study were remarkably similar to the results of the allozyme-based study reported by Seeb and Crane (1999), with populations from Korea, Japan, Russia, Kotzebue Sound, Norton Sound, the Yukon River, and northern Bristol Bay determined to be in one major lineage. Populations from southern Bristol Bay and the northern Alaska Peninsula were intermediate, and populations on the south side of the Alaska Peninsula, Kodiak Island, and areas to the south and east to Washington State were determined to be in a second major lineage.

Successful transplantation of salmon within the range of a species has the potential to alter genetic population structure. Population structure of chum salmon has been influenced to some degree by transplantations within its range. For example, due to frequent transplantations associated with hatcheries, most Japanese populations have received some level of transplantation of non-natal fish. Although initial studies indicated that the effect of transplantations were minimal in Japanese populations (Okazaki, 1982a), more recent work has shown that some current run-timing variation in populations may be a result of transplantations. Beacham et al. (2008b) reported that allozyme monitoring indicated that successful introduction and establishment of broodstock from the Chitose River on the Sea of Japan coast of Hokkaido Island to the Gakko River on the Sea of Japan coast of Honshu Island accounts for observed temporal differentiation in the existing Gakko River population. Transplantations have also occurred in Russian and North American populations, but there is little evidence for a demonstrable change in population structure as a result of transplantations.

Although most production of Japanese chum salmon is currently derived from hatcheries, there is little evidence that hatchery production has resulted in reduced genetic variation of the populations, in relation to chum salmon in other portions of the range. Initially, Kaeriyama (1999) indicated that, on the basis of allozymes, Japanese populations were less variable than Russian wild populations. In our study, on the basis of 14 microsatellites, we found no evidence that Japanese chum salmon populations were less genetically variable than Russian or North American chum salmon. In fact, the opposite result was observed, with higher levels of genetic variation observed in Japanese populations compared with chum salmon from other regions across the Pacific Rim.

Population structure of chum salmon across the Pacific Rim was demonstrated to have a regional basis. A regionally based population structure is generally required for genetic stock identification estimation because an important assumption is that the portion of the mixed-stock sample derived from unsampled populations is allocated to sampled populations from the same region. This assumption reduces the cost and complexity of developing a baseline for stock composition analysis. Chum salmon population structure thus meets the important condition that unsampled populations contributing to mixed fishery samples will likely be allocated to sampled populations in the same region.

Populations in the major river drainages surveyed all clustered together within a drainage, with the exception of the Yukon River, where lower river summer-run populations clustered with populations from the Kuskokwim River in western Alaska and the Nushagak River in northern Bristol Bay. Similar results were also reported in the allozyme survey conducted by Seeb and Crane (1999), who suggested that genetic exchange may have occurred between the Kuskokwim and Nushagak rivers during the last glaciation because both rivers were headwaters to a Bering Sea Land Bridge river that drained into the Bering Sea (Hopkins, 1967; Lindsay and McPhail, 1986). The ancient mouth of the Yukon River was farther south than at present (Hopkins, 1967; Knebel and Creager, 1973), increasing the probability of genetic exchange among ancestral populations of the Yukon, Kuskokwim, and Nushagak rivers.

Chum salmon likely had a different pattern of dispersal from refuges after the last glaciation ended in the Pleistocene Era some 10,000 years ago than did either sockeye salmon or Chinook salmon. For example, evaluation of genetic diversity in Asian and North American populations of sockeye salmon and Chinook salmon have indicated that there were similar levels of genetic diversity between populations from these

two continents (Beacham et al., 2006a, 2006b). This is in marked contrast to the pattern observed in chum salmon, with Asian chum salmon displaying significantly greater genetic diversity than that observed in chum salmon populations in North America. Surveys of mtDNA variation have also indicated that Japanese populations have the highest genetic diversity among Pacific Rim chum salmon (Sato et al., 2004). Chum salmon in Asia display a wider geographic distribution than either sockeye salmon or Chinook salmon, with most populations of these two species restricted to a Russian distribution, whereas chum salmon range as far south as South Korea. The distinctive nature of Korean, Japanese, and Primorye chum salmon, coupled with the higher diversity observed in Asian populations, indicates an Asian refuge from which chum salmon dispersed after the retreat of glaciers during the Pleistocene, either on the southern Asian mainland or the islands of Japan. The fact that Asian chum salmon display more genetic diversity than North American chum salmon reflects either that either higher population sizes were present in this refuge, allowing more genetic variation to be retained, or that dispersal from this refuge preceded those in North America, allowing more time for genetic mutations to accumulate. The concept of a glacial refuge near Japan was also suggested by Taylor et al. (1994).

In North America, the observed population structure of chum salmon would support the concept at a minimum of a Bering Sea refuge in the north (unglaciated areas of western Alaska or Russia) and a Columbia River refuge in the south (unglaciated area west of the Continental Divide) as suggested by McPhail and Lindsey (1970). Present day populations in Korea, Japan, and Primorye may be derived from the southern Asian (Japanese) refuge, populations from the Amur River through to southern Bristol Bay may be derived from the northern Bering refuge, and populations from the Alaska Peninsula to Washington may have been derived from the southern refuge. In British Columbia, an additional refuge may also have been present on the Queen Charlotte Islands (Warner et al., 1982). Queen Charlotte Islands chum salmon populations were distinct and also displayed lower genetic variation, very similar to sockeye salmon populations from the region (Beacham et al., 2006a). Wood (1995) suggested that sockeye salmon population structure on the central coast region of British Columbia was consistent with colonization from two different refugia, and therefore it is possible that present day populations in British Columbia are derived from chum salmon originating from a Queen Charlotte Islands refuge and that other portions of the coast were colonized by chum salmon that originated from a southern refuge.

Acknowledgments

A very substantial effort was undertaken to obtain samples from chum salmon sampled in this study. In North America, starting from the south, we thank J. B. Shaklee, various staff of Fisheries and Oceans Canada (DFO) for baseline sample collection, as well as First Nations staff, R. L. Wilmot, L. W. Seeb, S. Johnston, P. Milligan, J. Wenburg, and A. J. Gharrett. For Asia, samples were provided by V. V. Efremov, N. V. Varnakskaya, G. Winans, S. Urawa, S. Sato, and J. Park. L. Fitzpatrick drafted the map. C. Wallace assisted in the analysis. Funding for the study was provided by Fisheries and Oceans, Canada.

Literature cited

Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship, and D. Hedgecock.
1999. Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tshawytscha*). J. Hered. 90:281–288.

Banks, M. A., V. K. Rashbrook, M. J. Calavetta, C. A. Dean, and D. Hedgecock.
2000. Analysis of microsatellite DNA resolves genetic structure and diversity of chinook salmon (*Oncorhynchus tshawytscha*) in California's Central Valley. Can. J. Fish. Aquat. Sci. 57:915–927.

Beacham, T. D.
1996. The use of minisatellite DNA variation for stock identification of chum salmon, *Oncorhynchus keta*. Fish. Bull. 94:611–627.

Beacham, T. D., A. P. Gould, R. E. Withler, C. B. Murray, and L. W. Barner.
1987. Biochemical genetic survey and stock identification of chum salmon (*Oncorhynchus keta*) in British Columbia. Can. J. Fish. Aquat. Sci. 44:1702–1713.

Beacham, T. D., K. D. Le, M. Wetklo, B. McIntosh, T. Ming, and K. M. Miller.
In press. Population structure and stock identification of chum salmon from western Alaska determined with microsatellite and major histocompatibility complex variation. *In* Pacific salmon: ecology and management of western Alaska's populations (C. C. Krueger, and C. E. Zimmerman, eds.). Am. Fish. Soc., Symp., Bethesda, MD.

Beacham, T. D., S. Urawa, K. D. Le, and M. Wetklo.
2008b. Population structure and stock identification of chum salmon from Japan determined with microsatellite DNA variation. Fish. Sci. 74:983–994.

Beacham, T. D., B. Spilsted, K. D. Le, and M. Wetklo.
2008c. Population structure and stock identification of chum salmon *Oncorhynchus keta* from British Columbia determined with microsatellite DNA variation. Can. J. Zool. 86:1002–1014.

Beacham, T. D., B. McIntosh, C. MacConnachie, K. M. Miller, R. E. Withler, and N. V. Varnavskaya.
2006a. Pacific Rim population structure of sockeye salmon as determined from microsatellite analysis. Trans. Am. Fish. Soc. 135:174–187.

Beacham, K. L. Jonsen, J. Supernault, M. Wetklo, L. Deng, and N. Varnavskaya.
2006b. Pacific Rim population structure of Chinook salmon as determined from microsatellite variation. Trans. Am. Fish. Soc. 135:1604–1621.

Beacham, T. D., N. V. Varnavskaya, K. D. Le, and M. Wetklo.
2008a. Determination of population structure and stock identification of chum salmon (*Oncorhynchus keta*) in

Russia determined with microsatellites. Fish. Bull. 106:245–256.

Brykov, V. A., N. E. Poliakova, and A. V. Prokhorova.
2003. Phylogenic and geographic analysis of chum salmon *Oncorhynchus keta* (Walbaum) in Asian populations based on mitochondrial DNA variation. Russ. J. Genet. 39:61–67.

Buchholz, W. G., S. J. Miller, and W. J. Spearman.
2001. Isolation and characterization of chum salmon microsatellite loci and use across species. Anim. Genet. 32:160–165.

Cairney, M., J. B. Taggart, and B. Hoyheim.
2000. Characterization of microsatellite and minisatellite loci in Atlantic salmon (*Salmo salar* L.) and cross-species amplification in other salmonids. Mol. Ecol. 9:2175–2178.

Cavalli-Sforza, L. L., and A. W. F. Edwards.
1967. Phylogenetic analysis: models and estimation procedures. Am. J. Hum. Genet. 19:233–257.

Chen, J.-P., D.-J. Sun, C.-Z. Dong, B. Liang, W.-H.Wu, and S.-Y. Zhang.
2005. Genetic analysis of four wild chum salmon *Oncorhynchus keta* populations in China based on microsatellite markers. Environ. Biol. Fishes 73:181–188.

Efremov, V. V.
2001. Genetic variation and differentiation of populations of chum salmon *Oncorhynchus keta* (Walbaum) from Southern Russian Far East. Russ. J. Genet. 37:283–289.

Estoup, A., P. Jarne, and J. M. Cornuet.
2002. Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. Mol. Ecol. 11:1591–1604.

Felsenstein J.
1993. PHYLIP: Phylogeny Inference Package. Univ. Washington, Seattle, WA.

Ginatulina, L. K.
1992. Genetic differentiation among chum salmon, *Oncorhynchus keta* (Walbaum), from Primorye and Sakhalin. J. Fish Biol. 40:33–38.

Goudet, J.
1995. FSTAT (version 1.2): A program for IBM PC compatibles to calculate Weir and Cockerham's (1984) estimators of F-statistics. J. Hered. 86:485–486.

Hopkins, D. M.
1967. The Cenozoic history of Beringia-a synthesis. *In* The Bering land bridge. (D. M. Hopkins, ed.) p. 451–484. Stanford Univ. Press, Stanford, CA.

Kaeriyama M.
1999. Hatchery programmes and stock management of salmonid populations in Japan. *In* Stock enhancement and sea ranching (B. R. Howell, E. Moksness, and T. Svasand, eds.). p.153–167. Blackwell Science, Oxford, U.K.

Kalinowski, S. T.
2005. Do polymorphic loci require large sample sizes to estimate genetic distances? Heredity 94:33–36.

Kijima A, and Y. Fujio.
1982. Correlation between geographic distance and genetic distance in populations. Bull. Jap. Soc. Sci. Fish. 48:1703–1709.

Knebel, H. J., and J. S. Creager.
1973. Yukon River: evidence for extensive migration during the Holocene transgression. Science 179:1230–1232.

Kondzela, C. M., C. M. Guthrie, S. L. Hawkins, C. D. Russell, J. H. Helle, and A. J. Gharrett.
1994. Genetic relationships among chum salmon populations in southeast Alaska and northern British Columbia. Can. J. Fish. Aquat. Sci. (suppl. 1):50–64.

Lindsey, C. C., and J. D. McPhail.
1986. Zoogeograpy of fishes of the Yukon and Mackenzie basins. *In* The zoogeography of North American freshwater fishes (C. H. Hocutt and E. O. Wiley, eds.) p. 639–674. Wiley, New York.

McPhail, J. D., and C. C. Lindsey.
1970. Freshwater fishes of northwestern Canada and Alaska. Bull. Fish. Res. Board Can. 173:1–381.

Nelson, R. J., and T. D. Beacham.
1999. Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. Anim. Genet. 30:228–229.

Okazaki, T.
1982a. Geographical distribution of allelic variation of enzymes in chum salmon *Oncorhynchus keta*, river populations of Japan and the effects of transplantation. Bull. Jap. Soc. Sci. Fish. 48:1525–1535.

Okazaki, T.
1982b. Genetic study on population structure in chum salmon (*Oncorhynchus keta*). Bull. Far Seas Fish. Res. Lab. 19:25–116.

Olsen, J. B., S. L. Wilson, E. J. Kretschmer, K. C. Jones, and J. E. Seeb.
2000. Characterization of 14 tetranucleotide microsatellite loci derived from sockeye salmon. Mol. Ecol. 9:2185–2187.

Park, L. K, M. A. Brainard, D. A. Dightman, and G. A. Winans.
1993. Low levels of intraspecific variation in the mitochondrial DNA of chum salmon (*Oncorhynchus keta*). Mol. Mar. Biol. Biotech. 2:362–370.

Perriere, G., and M. Gouy.
1996. WWW-query: An on-line retrieval system for biological sequence banks. Biochimie 78:364–369.

Phelps, S. R., L. L. LeClair, S. Young, and H. L. Blankenship.
1994. Genetic diversity patterns of chum salmon in the Pacific Northwest. Can. J. Fish. Aquat. Sci. (suppl. 1):65–83.

Polyakova, N. E., A. V. Semina, and V. A. Brykov.
2006. The variability in chum salmon *Oncorhynchus keta* (Walbaum) mitochondrial DNA and its connection with the paleogeological events in the northwest Pacific. Russ. J. Genet. 42:1164–1171.

Rexroad, C. E., R. L. Coleman, A. M. Martin, W. K. Hershberger, and J. Killefer.
2001. Thirty-five polymorphic microsatellite markers for rainbow trout (*Oncorhynchus mykiss*). Anim. Genet. 32:317–319.

Rice, W. R.
1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Salo, E. O.
1991. Life history of chum salmon (*Oncorhynchus keta*). *In* Life history of Pacific salmon (C. Groot and L. Margolis, eds.), p. 231–309. UBC Press, Vancouver, BC.

Salmenkova, E. A., V. T. Omelchenko, D. V. Politov, K. I. Afanasyev, and G. A. Rubtsova.
2007. Genetic diversity of northern Okhotsk Sea coast populations of chum salmon with natural and artificial reproduction. Biologiya Morya 33:299–308.

Sato, S., J. Ando, H. Ando, S. Urawa, A. Urano, and S. Abe.
2001. Genetic variation among Japanese populations of chum salmon inferred from the nucleotide sequences of the mitochondrial DNA control region. Zool. Sci. (Tokyo) 18: 99–106.

Sato, S., H. Kojima, J. Ando, H. Ando, R. L. Wilmot, L. W. Seeb, V. Efremov, L. LeClair, W. Buchholz, D.-H. Jin, S. Urawa, M. Kaeriyama, A. Urano, and S. Abe.
2004. Genetic population structure of chum salmon in the Pacific Rim inferred from mitochondrial DNA sequence variation. Environ. Biol. Fishes 69:37–50.

Seeb, L. W., and P. A. Crane.
1999. High genetic heterogeneity in chum salmon in Western Alaska, the contact zone between northern and southern lineages. Trans. Am. Fish. Soc. 128:58–87.

Small, M. P., T. D. Beacham, R. E. Withler, and R. J. Nelson.
1998. Discriminating coho salmon (*Oncorhynchus kisutch*) populations within the Fraser River, British Columbia using microsatellite DNA markers. Mol. Ecol. 7:141–155.

Smith, C. T., B. F. Koop, and R. J. Nelson.
1998. Isolation and characterization of coho salmon (*Oncorhynchus kisutch*) microsatellites and their use in other salmonids. Mol. Ecol. 7:1613–1621.

Smith, C. T., and L. W. Seeb.
2008. Number of alleles as a predictor of the relative assignment accuracy of short tandem repeat (STR) and single-nucleotide-polymorphism (SNP) baselines for chum salmon. Trans. Am. Fish. Soc. 137:751–762.

Spies, I. B., D. J. Brasier, P. T. L. O'Reilly, T. R. Seamons, and P. Bentzen.
2005. Development and characterization of novel tetra-, tri-, and dinucleotide microsatellite markers in rainbow trout (*Oncorhynchus mykiss*). Mol. Ecol. Notes 5:278–281.

Taylor, E. B., T. D. Beacham, and M. Kaeriyama.
1994. Population structure and identification of North Pacific Ocean chum salmon (*Oncorhynchus keta*) revealed by an analysis of minisatellite DNA variation. Can. J. Fish. Aquat. Sci. 51:1430–1442.

Varnavskaya, N. V., C. C. Wood, and R. J. Everett.
1994a. Genetic variation in sockeye salmon (*Oncorhynchus nerka*) populations of Asia and North America. Can. J. Fish. Aquat. Sci. 51(suppl. 1):132–146.

Waples, R. S.
1990. Temporal changes of allele frequency in Pacific salmon populations: implications for mixed-stock fishery analysis. Can. J. Fish. Aquat. Sci. 47:968–976.

Warner, B. C., R. W. Mathews, and J. J. Clague.
1982. Ice-free conditions on the Queen Charlotte Islands, British Columbia, at the height of the late Wisconsin glaciation. Science 218:675–677.

Weir, B.S., and C. C. Cockerham.
1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.

Williamson, K. S., J. F. Cordes, and B. P. May.
2002. Characterization of microsatellite loci in chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. Mol. Ecol. Notes 2:17–19.

Wilmot, R. L., R. J. Everett, W. J. Spearman, R. Baccus, N. V. Varnavskaya, and S. V. Putivkin.
1994. Genetic stock structure of Western Alaska chum salmon and a comparison with Russian Far East stocks. Can. J. Fish. Aquat. Sci. 51 (suppl. 1):84–94.

Winans, G. A., P. B. Aebersold, S. Urawa, and N. V. Varnavskaya.
1994. Determining continent of origin of chum salmon (*Oncorhynchus keta*) using genetic identification techniques: status of allozyme baseline in Asia. Can. J. Fish. Aquat. Sci. 51(suppl. 1):95–113.

Wood, C. C.
1995. Life history variation and population structure in sockeye salmon. Am. Fish. Soc. Symp. 17:195–216.