Determination of population structure and stock composition of chum salmon (*Oncorhynchus keta*) in Russia determined with microsatellites

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Abstract—Variation at 14 microsatellite loci was examined in 34 chum salmon (*Oncorhynchus keta*) populations from Russia and evaluated for its use in the determination of population structure and stock composition in simulated mixed-stock fishery samples. The genetic differentiation index (F_{st}) over all populations and loci was 0.017, and individual locus values ranged from 0.003 to 0.054. Regional population structure was observed, and populations from Primorye, Sakhalin Island, and northeast Russia were the most distinct. Microsatellite variation provided evidence of a more fine-scale population structure than those that had previously been demonstrated with other genetic-based markers. Analysis of simulated mixed-stock samples indicated that accurate and precise regional estimates of stock composition were produced when the microsatellites were used to estimate stock compositions. Microsatellites can be used to determine stock composition in geographically separate Russian coastal chum salmon fisheries and provide a greater resolution of stock composition and population structure than that previously provided with other techniques.

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In Asia, there are two distinct types of chum salmon (*Oncorhynchus keta* Walbaum). The early-maturing or "summer" chum salmon generally returns to spawn from June through August in streams bordering Kamchatka, the Sea of Okhotsk, the east coast of Sakhalin Island, and the Amur River. Later-maturing or "autumn" chum salmon generally return to spawn from September through November in streams in Japan, the southern Kuril Islands, the west coast of Sahkalin Island, and the Amur River (Sano, 1966). In general, summer chum salmon spawn in areas where egg incubation occurs in subsurface stream flow, whereas autumn chum salmon spawn in areas of groundwater upwelling (Volobuyev et al., 1990). In major river drainages, autumn chum salmon generally migrate further up the drainage to spawn than do summer chum salmon, and are larger, younger, and more fecund than the summer-run fish (Sano, 1966).

Determination of the origin of salmon in mixed-stock fisheries is important for effective management. For chum salmon in Asia, scale pattern variation has provided a technique for the determination of origin of individuals to large geographic areas (Tanaka et al., 1969; Ishida et al., 1989), and in some cases reportedly to a specific river drainage (Nikolayeva and Semenets, 1983). Trace elements in otoliths have also been reported to be effective for stock identification of Korean populations (Sohn et al., 2005). Stock identification techniques based on scale pattern analysis have generally been replaced by applications based on genetic variation, owing to the increased resolution that is possible by applying genetic variation (see example outlined by Wilmot et al. [1998]). Analyses of genetic variation have been demonstrated to be effective in determining salmonid population structure, as well as determining origins of salmon in mixed-stock fisheries. For Russian chum salmon, analyses of allozyme variation have indicated differentiation among populations on the east and west coasts of Kamchatka (Winans et al., 1994), and either marignal (Salmenkova et al., 2007) or some level of differentiation between populations on Sakhalin Island and populations on the mainland Russian coast (Efremov, 2001). Populations in the far northeastern

portions of mainland Russia were distinct from populations in western Alaska (Wilmot et al., 1994). Surveys of allozyme variation have generally indicated regional population differentiation among Russian populations.

DNA-level markers have substantially increased the number of polymorphic loci that are available to be included in analyses of genetic variation. Initial surveys of mitochondrial (mt) DNA variation indicated regional differentiation between Sakhalin Island and mainland populations (Ginatulina, 1992). Later analyses of additional mtDNA variation indicated marked differentiation between Japanese and Russian populations (Sato et al., 2004), and some differentiation among Russian populations (Brykov et al., 2003; Polyakova et al., 2006). Limited examinations of minisatellite variation have indicated some level of differentiation between Japanese and Russian populations, but have yielded little evidence of regional structure for Russian populations (Taylor et al., 1994; Beacham, 1996).

Analyses of microsatellite variation have been effective for determining salmonid population structure in local areas (Small et al., 1998; Banks et al., 2000; Beacham et al., 2004), as well as broad-scale differences across the Pacific Rim (Beacham et al., 2005, 2006). Microsatellites have also been of considerable value in estimating stock composition in mixed-stock salmon fisheries, on both a population-specific (Beacham et al., 2003) and regional basis (Beacham et al., 2006). Microsatellite variation in chum salmon provides the means to examine fine-scale population structure (Chen et al., 2005), as well as the means for fine-scale estimation of stock composition in mixed-stock fisheries (Beacham et al., in press). Analyses of microsatellite variation in Russian chum populations would likely be of value by providing increased resolution of population structure compared with that provided by previous techniques, and would likely aid in increasing accuracy and precision of estimates of stock composition in mixed-stock fishery samples.

Our objectives were to analyze the variation at 14 microsatellite loci to evaluate population structure of Russian chum salmon populations from the far north eastern coast of Russia to the more southern areas of Primorye and Sakhalin Island, and then to evaluate the use of these loci for the practical purpose of providing accurate and precise estimates of stock composition in mixed-stock fishery samples. Stock composition evaluation was accomplished by the analysis of simulated mixed-stock fishery samples.

Materials and methods

Tissue samples were collected from mature chum salmon at a number of rivers during previous analyses of genetic variation (Winans et al., 1994). Additional tissue samples were sent to the Molecular Genetics Laboratory at the Pacific Biological Station. The geographic area of the 34 populations sampled ranged from Primorye in the south to northeastern Russia (Fig. 1) and encompassed eight geographic regions (Table 1). DNA was extracted from the tissue samples by a variety of methods, including that with chelex resin outlined by Small et al. (1998), a Qiagen 96-well Dneasy® procedure (Qiagen, Mississauga, Ontario, Canada), or a Promega Wizard SV96 Genomic DNA Purification system (Promega, Madison, WI). Once extracted DNA was available, analyses of variation at 14 microsatellite loci were conducted: *Ots3* (Banks et al., 1999), *Oke3* (Buchholz et al., 2001), *Oki2* (Smith et al., 1998), *Oki100* (primer sequence 5′ to 3′ F: GGTGTTTTAATGTTGTTTCCT, R: GTTTCCAGAGTAGTCATCTCTG), *Omm1070* (Rexroad et al., 2001), *Omy 1011* (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, PCR DNA amplifications were conducted by using DNA Engine Cycler Tetrad2 (BioRad, Hercules, CA) in 6-μL volumes consisting of 0.15 units of Taq polymerase, 1 μL (25–50 ng) of extracted DNA, 1× PCR buffer (Qiagen, Mississauga, Ontario, Canada),

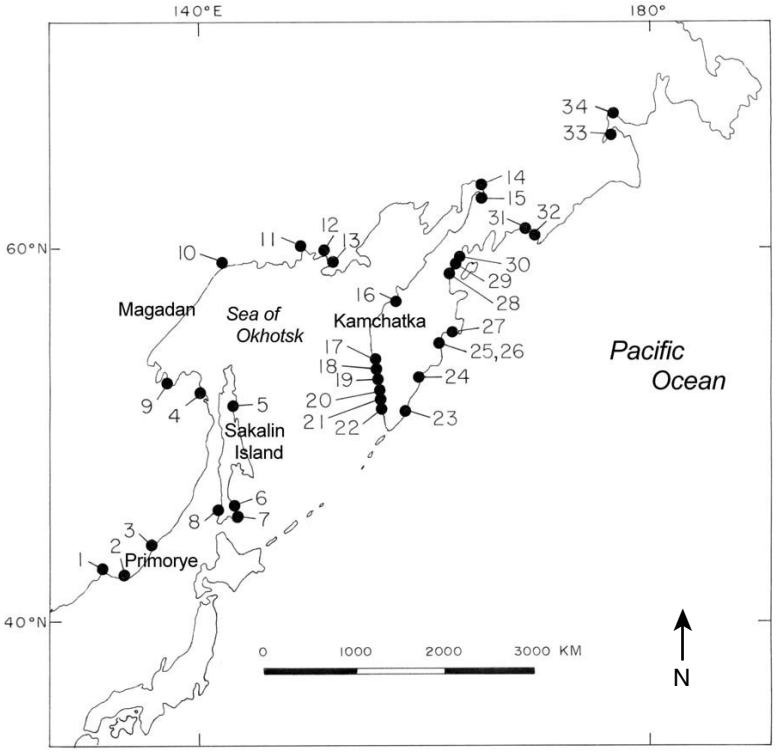

Figure 1

Map indicating the locations in Russia where chum salmon (*Oncorhynchus keta*) from 34 populations or sampling sites were collected. Numbers for and locations of populations are indicated in Table 1.

Table 1

Population, sample collection years, number of fish sampled per year, and total number of fish sampled for 34 populations of chum salmon (*Oncorhynchus keta*) in eight geographic regions from Russia. Eight regions have been defined, and populations (numbered in brackets) were sampled in each region listed. *N* = population size.

Region and population	Years	Annual sample size	*N*
1 Primorye			
Narva [1]	1994	17	17
Ryazanovka [2]	1994	49	49
Avakumovka [3]	1994	35	35
2 Amur River			
Amur River [4]	1994, 2001, 2004	43, 97, 198	338
3 Sakhalin Island			
Tym [5]	1995	55	55
Naiba [6]	1994, 1995	50, 99	149
Udarnitsa [7]	1994	50	50
Kalininka [8]	1994	49	49
4 Magadan			
Tugur [9]	2004	98	98
Okhota [10]	2004	94	94
Magadan [11]	1991	79	79
Tauy [12]	1990	55	55
Ola [13]	1990, 1992	80, 40	120
5 Northern Sea of Okhotsk			
Oklan [14]	1993	76	76
Penzhina [15]	1993	43	43
6 West Kamchatka			
Hairusova [16]	1990, 1993	138, 48	186
Vorovskaya [17]	1991, 1993	79, 170	249
Kol [18]	1991	79	79
Pymta [19]	1992, 1993	40, 59	99
Kikchik [20]	1992, 2005	20, 86	106
Utka [21]	1992	40	40
Bolshaya [22]	2004	96	96
Plotnikova [23]	2001	69	69
7 East Kamchatka			
Zhypanova [24]	2004	46	46
Kamchatka [25]	1990	76	76
Ivashka [26]	2005	48	48
Nerpichi [27]	1992	39	39
Karaga [28]	2005	42	42
Ossora [29]	1990, 1996, 2005	39, 41, 48	128
Dranka [30]	2005	44	44
Apuka [31]	2002	47	47
Olutorsky Bay [32]	2002	49	49
8 Northeast Russia			
Anadyr [33]	1991, 1992	79, 15	94
Kanchalan [34]	1991	79	79

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, 30–60 seconds at 47–65°C, and 30–60 seconds at 68–72°C (depending on the locus). Specific PCR conditions for a particular locus could vary from this general outline. PCR fragments were initially size fractionated in denaturing polyacrylamide gels by using an ABI 377 automated DNA sequencer, 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, and genotypes were scored by GeneMapper software 3.0 (Applied Biosystems, Foster City, CA) by using an internal lane-sizing standard. Allele identification between the two sequencers was standardized by analyzing the same 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

Each population at each locus was tested for departure from Hardy-Weinberg equilibrium (HWE) by using genetic data analysis (GDA). Critical significance levels for simultaneous tests (34 populations, Table 1) were evaluated using Bonferroni adjustment (0.05/34=0.0015) (Rice, 1989). All annual samples available for a location were combined to estimate population allele frequencies, as was recommended by Waples (1990). F_{st} estimates for each locus were calculated with FSTAT (Goudet, 1995), individual locus values were determined by jackknifing over populations, and the overall F_{st} estimate was determined by jackknifing over loci (Goudet, 1995). Inter-regional comparisons of F_{st} estimates were determined by calculation of all appropriate pairwise point estimates of F_{st} values, and then determining the mean and standard deviation of these values. The Cavalli-Sforza and Edwards (CSE) (1967) chord distance was used to estimate distances among 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 dendrogram was evaluated with the CONSENSE program from PHYLIP (Univ. Washington, Seattle, WA) and based on 500 replicate trees. Computation of the number of alleles observed per locus, as well as allelic diversity standardized to a common sample size, was carried out with FSTAT.

Estimation of stock composition

Genotypic frequencies were determined for each locus in each population, and the Statistical Package for the Analysis of Mixtures software program (SPAM, vers. 3.7, Debevec et al., 2000) was used to estimate stock composition of simulated mixtures. The Rannala and Mountain (1997) correction to baseline allele frequencies was used in the analysis in order to accommodate the occurrence of fish in the mixed sample that were from a specific population having an allele not observed in the baseline samples from that population. All loci were considered to be in Hardy-Weinberg equilibrium, and expected genotypic frequencies were determined from the observed allele frequencies. Reported stock compositions for simulated fishery samples were the bootstrap mean estimate of each mixture of 150 fish analyzed, and mean and variance estimates were derived from 100 bootstrap simulations. Both the baseline population and the simulated single-population were sampled with replacement in order to simulate random variation involved in the collection of the baseline and fishery samples.

Results

Variation within and among populations

The observed number of alleles observed at a locus ranged from 21 alleles at *Oke3* and *Oki2* to 138 alleles at *One111* (Table 2). Lower expected heterozygosities were generally observed at loci with fewer alleles. The genotypic frequencies observed at the 14 loci generally conformed to those expected under Hardy-Weinberg equilibrium (HWE) after Bonferroni correction. For the *Oke3* and *OtsG68* loci, a minor HWE nonconformance of genotypic frequencies was observed, and observed heterozygosities were 2–6% less than those expected (Table 2).

Genetic diversity, with respect to the number of alleles observed, was evident among regional groups of chum salmon. Chum salmon populations from Primorye, the northern Sea of Okhotsk, and northeast Russia displayed fewer alleles (mean 320 alleles) than did populations in Magadan, west Kamchatka, and east Kamchatka (mean 370 alleles) (Table 3). Chum salmon from the latter regions displayed approximately 16% more alleles than did those from the former regions. The greatest differentiation in allelic diversity was observed at those loci with greater numbers of alleles, particularly at locus *One111*.

Population structure

Genetic differentiation was evident among chum salmon populations from the different geographic regions surveyed. The F_{st} value over all 34 populations and 14 loci surveyed was 0.017, and individual locus values ranged from 0.003 (*One102*) to 0.054 (*Ots3*) (Table 2). Chum salmon populations from Primorye and the Amur River were well defined compared with other regional populations (Table 4). Populations from the southwestern portion of Russia (Primorye, Amur River, Sakhalin Island) were most distinct from those in more northern and eastern regions (Magadan, Sea of Okhotsk, Kamchatka, northeast Russia).

Regional clustering of population samples was observed in the analysis of population structure. Strong clustering of population samples from the Primorye region was observed; the three population samples included in the analysis clustered together in 100% of the trees examined (Fig. 2). Similarly, strong clustering was observed in most of the population samples from Sakhalin Island, as well as the two population samples from the northern coast of the Sea of Okhotsk. The two population samples from northeast Russia clustered together in 100% of the trees examined, together with the Utka River population sample from west Kamchatka. Although there was a general clustering of population samples from east and west Kamchatka, these regional

Table 2

Number of alleles per locus, F_{st}, expected heterozygosity (*He*), observed heterozygosity (*Ho*), and percent significant Hardy-Weinberg equilibrium tests (HWE) for 14 microsatellite loci examined in 34 populations of chum salmon (*Oncorhynchus keta*) listed in Table 1. Standard deviation of F_{st} is shown in parentheses.

Locus	Number of alleles	F_{st}	*He*	*Ho*	HWE
Oke3	21	0.050 (0.022)	0.80	0.74	8.6
Oki2	21	0.027 (0.005)	0.87	0.87	2.9
Ots3	26	0.054 (0.014)	0.78	0.78	0.0
Oki100	29	0.015 (0.003)	0.91	0.91	0.0
Ssa419	30	0.010 (0.003)	0.87	0.87	0.0
One102	33	0.003 (0.001)	0.91	0.91	0.0
Omy1011	34	0.010 (0.003)	0.93	0.92	0.0
One104	41	0.013 (0.003)	0.94	0.94	0.0
One101	46	0.052 (0.009)	0.84	0.84	0.0
One114	46	0.015 (0.004)	0.93	0.93	5.7
Omm1070	50	0.005 (0.001)	0.95	0.95	0.0
Ots103	51	0.008 (0.002)	0.95	0.94	0.0
OtsG68	53	0.005 (0.001)	0.95	0.93	8.6
One111	138	0.007 (0.001)	0.98	0.98	0.0
Total		0.017 (0.004)			

Table 3

Mean number of alleles observed per locus at 14 microsatellite loci for 34 chum salmon (*Oncorhynchus keta*) populations from eight regions as outlined in Table 1. Regions were the following: 1) Primorye, 2) Amur River, 3) Sakhalin Island, 4) Magadan, 5) Northern Sea of Okhotsk, 6) West Kamchatka, 7) East Kamchatka, and 8) Northeast Russia. Allele numbers have been standardized to a sample size of 79 fish per region.

	1	2	3	4	5	6	7	8
Oke3	9.0	8.5	9.9	8.7	7.9	8.7	9.7	7.0
Oki100	16.0	18.5	22.3	21.4	17.7	19.6	21.4	19.2
Oki2	14.0	12.1	15.1	14.4	14.7	13.8	16.3	14.5
Omm1070	30.1	26.6	29.0	33.3	27.7	32.3	32.9	29.5
Omy1011	22.0	20.0	22.6	23.7	25.2	22.7	21.8	23.6
One101	20.7	28.3	21.9	25.0	16.6	22.8	23.3	23.1
One102	24.1	19.3	20.1	17.7	16.8	18.5	18.9	14.4
One104	23.8	27.6	23.5	23.9	23.4	24.6	24.9	22.7
One111	51.7	50.8	55.8	71.8	57.9	75.1	71.8	60.7
One114	23.1	24.6	24.0	28.6	19.2	27.3	26.4	25.5
Ots3	12.0	14.1	14.3	14.0	9.7	14.7	16.1	12.9
Ots103	30.0	35.3	38.4	34.4	27.3	35.1	34.4	29.1
OtsG68	32.9	34.2	39.3	35.6	28.7	36.3	36.6	32.7
Ssa419	16.5	14.8	16.0	16.7	14.4	18.7	16.2	11.9
Total	325.9	334.7	352.2	369.2	307.2	370.2	370.7	326.8

groupings were not strongly supported in the cluster analysis.

Stock identification

Genetic differentiation observed among the chum salmon in the regions surveyed was evaluated to determine if it was sufficient for a mixed-stock analysis with the objective of obtaining accurate regional stock compositions. Analysis of simulated single-region samples indicated that estimates of the contribution of chum salmon from that region were usually greater than 89% (Table 5), although there was the expectation that errors in estimation for the region in question would be maximized when the single region comprised 100% of the simulated sample. The level of accuracy observed in the estimated

Table 4

Mean pairwise population F_{st} values observed over 14 microsatellite loci for 34 chum salmon (*Oncorhynchus keta*) populations from eight regions. Regions were the following: 1) Primorye, 2) Amur River, 3) Sakhalin Island, 4) Magadan, 5) Northern Sea of Okhotsk, 6) West Kamchatka, 7) East Kamchatka, and 8) Northeast Russia. Boldface font indicates significant difference (P<0.05). A dash indicates that it was not possible to determine within-region pairwise population F_{st} values for the Amur River because only a single population was sampled.

Region	1	2	3	4	5	6	7	8
1	0.012							
2	**0.032**	—						
3	**0.031**	**0.035**	0.020					
4	**0.026**	**0.021**	**0.031**	0.004				
5	**0.036**	**0.036**	**0.046**	**0.010**	0.000			
6	**0.028**	**0.028**	0.027	**0.009**	**0.014**	0.006		
7	**0.026**	**0.024**	0.029	0.006	**0.011**	0.006	0.004	
8	**0.033**	**0.033**	**0.041**	**0.014**	**0.009**	**0.016**	**0.014**	0.000

Table 5

Mean estimated contribution (%) of populations of chum salmon (*Oncorhynchus keta*) in Russia for simulated mixtures. Estimates are for only a single region (correct regional estimate = 100%) and were calculated from the populations listed for each mixture, with each population comprising 50% (100% for Amur River) of each simulated regional mixture. Estimates were determined from 14 microsatellite loci for eight separate regional stocks. The region designation includes percentages allocated to all populations within a region. Simulations were conducted with a 34-population baseline, 150 fish in the mixture sample, and 100 resamplings in the mixture sample and baseline samples. Standard deviations are shown in parentheses.

Mixture	Region	Regional estimate	Population	Population estimate
1	Primorye	89.5 (2.5)	Avakumovka	43.9 (4.5)
			Ryazanovka	45.5 (4.5)
2	Amur River	98.2 (1.1)	Amur	98.2 (1.1)
3	Sakhalin Island	94.8 (1.8)	Naiba	55.1 (4.8)
			Tym	39.6 (4.7)
4	Magadan	90.0 (3.0)	Ola	44.3 (4.5)
			Okhota	44.0 (4.1)
5	Northern Sea of Okhotsk	86.9 (3.1)	Oklan	47.8 (4.7)
			Penzhina	39.1 (4.3)
6	West Kamchatka	95.9 (2.2)	Vorovskaya	49.2 (5.4)
			Hairusova	43.0 (5.2)
7	East Kamchatka	86.1 (3.2)	Ossora	43.9 (4.9)
			Kamchatka	41.2 (4.7)
8	Northeast Russia	89.8 (2.9)	Anadyr	46.5 (4.9)
			Kanchalan	43.3 (3.7)

stock compositions indicated that accurate regional estimates of stock composition should be obtained in samples containing individuals from multiple regions.

Testing of accuracy of regional estimates of stock composition in multiregion mixtures was conducted by evaluating four simulated fishery samples. Estimated stock composition of a simulated mixture containing chum salmon from Primorye, Sakhalin Island, the Amur River, and Magadan was within 3% of the actual population composition and 4% of the regional composition (Table 6, mixture 1). Similar results were observed for a simulated mixture comprising chum salmon from Sakhalin Island, Magadan, the northern Sea of Okhotsk, and west Kamchatka, with population estimates within 3% of actual composition and regional estimates within 3% (Table 6, mixture 2). Estimated stock compositions of a simulated mixture of chum salmon from west Kamchatka, east Kamchatka, and northeast Russia were within 6% of actual population composition and within 5% for regional contributions (Table 6, mixture 3). Regional estimated stock compositions of a more complex simulated sample of fish from

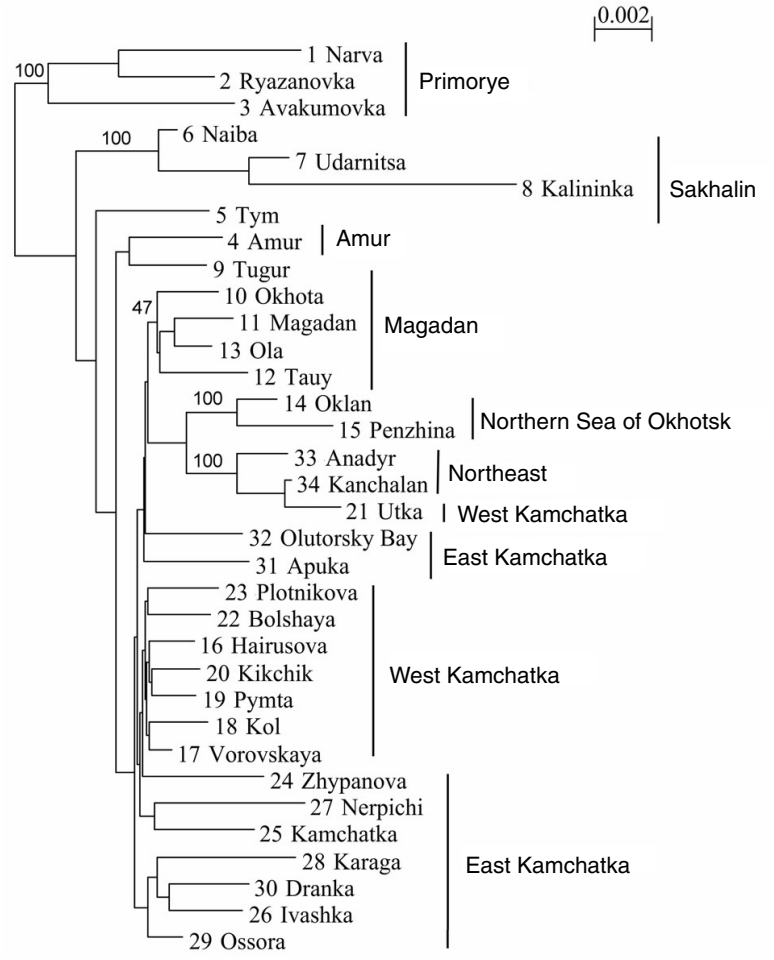

Figure 2

Neighbour-joining dendrogram of the Cavalli-Sforza and Edwards (1967) chord distance for 34 populations of chum salmon (*Oncorhynchus keta*) analyzed at 14 microsatellite loci. Bootstrap values at major tree nodes indicate the percentage of 500 trees where populations beyond the node clustered together. Numbers for and locations of populations are indicated in Table 1.

multiple regions were within 3% of actual regional contributions (Table 6, mixture 4). Accurate regional estimates of stock composition should be obtained when the current baseline is applied to mixed-stock samples of chum salmon taken from Russian coastal waters, provided that the individuals in the mixture originate entirely from Russian populations.

Discussion

Population structure

The range of populations sampled in the study required a concerted sampling effort, and in some locations collection of appropriate samples proved to be difficult. The number of fish sampled from a site or population ranged from 17 to 338 individuals. Estimated population-level allele frequencies will be subject to relatively larger sampling error at smaller population sample sizes, particularly for loci with large numbers of alleles such as *One111*. Small sample size may have contributed to errors on allele-frequency estimates for some populations. Sampling error may obscure genetic relationships among related populations, or conversely genetic relationships among some populations may be falsely inferred. However, the available evidence indicates that variation in population sample sizes did not obscure relationships among related populations. For example, we analyzed chum salmon from three sampling sites in Primorye with sample sizes ranging between 17 and 49 fish per site,. Although these sample sizes were small,

Table 6

Estimated contribution (%) of individual populations and regional estimates of chum chum salmon (*Oncorhynchus keta*) in Russia in simulated mixed-population samples. Each mixture of 150 fish was generated 100 times from baseline allele frequencies, and stock compositions of the mixtures were estimated with parametric resampling of each of the 34 baseline populations to obtain a new distribution of allele frequencies on each iteration. Actual= 100% accurate. Standard deviations are shown in parentheses. Fourteen microsatellites were used to estimate stock compositions of the simulated mixtures.

Populations	Actual %	Estimated %	Populations	Actual %	Estimated %
Mixture 1			Mixture 3		
Amur	30	30.5 (4.0)	Plotnikova	20	14.0 (3.3)
Avakumovka	10	8.7 (2.0)	Kol	10	6.7 (2.5)
Ryazanovka	10	9.1 (2.4)	Utka	10	5.5 (1.9)
Naiba	20	21.0 (3.3)	Kamchatka	10	8.2 (2.4)
Udarnitsa	10	7.6 (2.2)	Ossora	10	10.6 (2.8)
Tauy	10	7.1 (2.5)	Olutorsky Bay	10	6.0 (2.6)
Ola	10	8.9 (2.5)	Anadyr	20	17.9 (3.4)
Regions			Kanchalan	10	12.3 (3.1)
Amur	30	30.5 (4.0)	Regions		
Primorye	20	17.9 (3.2)	West Kamchatka	40	40.8 (4.7)
Sakhalin Island	30	28.7 (3.8)	East Kamchatka	30	25.4 (3.9)
Magadan	20	16.5 (3.6)	Northeast Russia	30	30.2 (4.1)
West Kamchatka	0	4.0 (1.9)	Magadan	0	1.4 (1.2)
East Kamchatka	0	1.6 (1.2)	Northern Sea of Okhotsk	0	1.1 (0.8)
Mixture 2			Mixture 4		
Naiba	20	19.0 (3.2)	Avakumovka	10	8.2 (2.3)
Magadan	10	7.8 (2.6)	Naiba	10	10.1 (2.3)
Ola	10	9.7 (2.8)	Tym	10	8.1 (2.5)
Okhota	10	9.0 (2.5)	Ola	15	13.0 (2.8)
Oklan	10	7.8 (2.5)	Okhota	10	8.7 (2.6)
Bolshaya	20	16.5 (3.3)	Hairusova	15	13.7 (3.1)
Hairusova	10	10.0 (3.0)	Utka	10	5.5 (2.2)
Kikchik	10	7.5 (2.6)	Kamchatka	10	8.1 (2.5)
Regions			Kanchalan	10	11.6 (2.6)
Sakhalin Island	20	19.2 (3.3)	Regions		
Magadan	30	27.1 (4.0)	Primorye	10	8.3 (2.3)
Northern Sea of Okhotsk	10	8.0 (2.6)	Sakhalin Island	20	18.3 (3.1)
West Kamchatka	40	42.1 (4.7)	Magadan	25	22.7 (4.0)
East Kamchatka	0	2.9 (1.6)	West Kamchatka	25	26.6 (3.8)
			East Kamchatka	10	10.9 (2.9)
			Northeast Russia	10	12.2 (2.7)

and thus the estimation of allele frequencies would be subject to sampling error, the clustering of these populations was well supported by our bootstrap calculations (100%). Regional clustering of samples or populations is typically observed in chum salmon (Beacham et al., 1987; Winans et al., 1994), and thus it is unlikely that close genetic relationships among these populations from Primorye were inferred incorrectly.

If populations spawn in remote areas, opportunities to collect samples may be limited. In our study, all samples that were available for a specific sampling site or population were combined in order to estimate genetic differentiation among populations. Annual variation in allele frequencies within a population is typically less than the geographic and population differences observed; therefore pooling annual samples over time is a reasonable approach to estimate population-level allele frequencies. Relative annual stability of microsatellite allele frequencies is a general feature of microsatellite loci in salmonids (Tessier and Bernatchez, 1999; Beacham et al., 2006).

The population structure of chum salmon in Russia has been investigated previously. For example, Winans et al. (1994) after examining 35 allozyme loci, indicated that there were four groups of Russian chum salmon populations, and that one group generally comprised populations from west Kamchatka, a second group comprised populations from Magadan, the Sea of Okhotsk, and east Kamchatka, the third group was solely from east Kamchatka, and a fourth group comprised the

populations from the Utka River in west Kamchatka and the Anadyr River in northeast Russia. In our study, some similarities were observed between Magadan and the Sea of Okhotsk regional population groups, as well as between the groups from east and west Kamchatka. Winans et al. (1994) did not include populations from Primorye or Sakhalin Island in their survey, but Ginatulina (1992) had previously demonstrated clear differentiation of mitochondrial genotypes between the populations from these two regions. The results of our study revealed regional separation of populations between these two areas. Our analysis supports the concept of regional groups of populations, and generally supports the concordance in patterns of population differentiation derived from analysis of allozymes and mitochondrial DNA variation. Allendorf and Seeb (2000) reported a concordance between results from allozyme and microsatellite analyses of population structure for sockeye salmon (*O. nerka*).

Genetic differentiation of Russian chum salmon generally follows a regional structure because proximate populations are generally more similar to each other than to more distant populations. However, there were some cases of populations from one region clustering with populations from another region. One example was the Utka River population from west Kamchatka clustering with populations from northeast Russia. An association between the Utka River population and the Anadyr River population was also reported by Winans et al. (1994) in an analysis of allozyme variation. Because Winans et al. (1994) and authors of the present study analyzed the same sample from the Utka River population, concurrence between the allozyme and microsatellite analyses was not unexpected. However, the number of fish sampled for the Utka River population was the fewest for any of the west Kamchatka populations (Table 1), and it may be that an increase in sample size for this population would result in estimated allele frequencies that would be more similar to those of other populations in west Kamchatka. Additionally, the Tugur River population was most closely associated with the population from the Amur River. In recent geologic history, the Tugur River may have been once part of the Amur River drainage, but now flows into Tugur Bay on the Sea of Okhotsk. A common origin between the Amur River and Tugur River populations may account for the current association between the two populations.

Distinctive groups of populations surveyed were those from the Primorye, Sahkalin Island, the northern Sea of Okhotsk, and northeast Russia, and a strong regional clustering of these populations was observed in the dendrogram analysis. Most of these population groups were characterized by slightly lower genetic variation compared with other populations surveyed in Russia. For other salmonids, populations from regions with reduced genetic variation have formed distinctive clusters in dendrogram analysis (Beacham et al., 2006).

Russian chum salmon populations displayed on average less genetic differentiation than did populations from western Alaska and adjacent areas. Despite the Russian populations being surveyed from a larger geographic area than were populations in western Alaska (Beacham et al., in press), and thus there was greater likelihood of differentiation due to isolation by distance, comparisons of locus-specific F_{st} values between the two groups indicated that Russian populations were less differentiated (lower values in 11 of 14 loci, $P=0.057$). This result indicates that there may be more straying among Russian populations than among those in western Alaska, possibly as a result of adaptation to harsh enviromental conditions or hatchery development and broodstock transfer in Russia. Alternatively, less differentiation would also be observed if Russian chum salmon had colonized available habitats more recently than had chum salmon in western Alaska.

Stock identification

Accurate, economical, and practical methods of stock identification are required to determine the migration pathways of juvenile and maturing salmon, and to manage fisheries that may intercept salmon during their migration to natal spawning grounds. Effective stock identification techniques are based on characters that display stable differentiation among groups to be discriminated, and these characters must be examined easily in a rapid and cost-effective manner. Allozymes provided the characters for initial genetically based population surveys and stock identification of Russian chum salmon (Winans et al., 1994, 1998). Later, single nucleotide polymorphisms (SNPs) were used to estimate the genetic structure of the population (Sato et al., 2001); therefore estimation of stock composition in mixed-stock samples can proceed (Sato et al., 2004). In an analysis of 30 haplotypes from mtDNA, Sato et al. (2004) were able to indicate some level of regional structure in populations in Russia, but the exact nature of the geographic structure was uncertain. In our analysis of microsatellite variation, clear differentiation was observed among regional groups of populations, and populations from Primorye were the most distinctive.

Accuracy of estimated stock compositions is directly influenced by the baseline used in the estimation procedure, and the level of genetic differentiation among regional groups of populations is a key component. However, sample sizes of populations in the baseline are also an important part because the accuracy of estimation is related to the population sample size (Beacham et al., 2006). Fewer than 60 fish were sampled for many of the populations sampled in our survey, and increasing sample sizes to approximately 150 fish per population would likely lead to all regional estimates of stock composition being in excess of 90% accurate in all simulated single-region mixture samples.

Surveys of genetic variation of salmon populations allow stock identification in mixed-stock fisheries, where the origins of fish contributing to mixed-stock fisheries are determined by comparing the genetic characteristics of fish in the fishery samples to the genetic characteristics of fish from potentially contributing populations.

Analysis of simulated mixed-stock samples of known origin is an initial practical method to evaluate the potential for applying genetic variation to mixed-stock fishery analysis. Our analysis of simulated mixtures indicated that microsatellite variation provides accurate estimates of regional contributions of chum salmon stocks from Russia, and in some cases provides reliable estimates of individual populations in simulated mixtures. Microsatellites have previously been reported to provide reliable estimates of stock composition in mixed-stock chum salmon samples of largely North American origin (Beacham et al., in press), and our results from simulated mixtures indicated that microsatellites should provide reliable estimates of stock composition for chum salmon in coastal waters in Russia. However, if Japanese or Korean chum salmon or potentially North American chum salmon are intercepted in coastal or nearshore fisheries in Russia, then clearly a larger baseline than the one examined in the current study would be required to provide reliable estimates of stock composition under these circumstances.

The application of microsatellites for the determination of population structure of Russian chum salmon will allow significant regional differentiation among these populations to be employed in estimating regional contributions to mixed-stock fishery samples from coastal waters. Microsatellites provide similar results for other Pacific salmon species (Beacham et al., 2005, 2006) and are likely to be effective in identifying the origin of Russian chum salmon in mixed-stock fisheries in nearshore and offshore waters.

The present analysis of microsatellite variation of Russian chum salmon provides evidence of a more fine-scale population structure than those that have previously been demonstrated with other genetic-based markers such as allozymes (Winans et al., 1994; Efremov, 2001) or mitochondrial based SNPs (Sato et al., 2004). This more fine-scale resolution of population structure was likely due to the larger number of alleles associated with the microsatellite loci than with either the allozyme or SNP loci. Because genetic-based markers generally exhibit annual stability in allele frequencies, they are generally more effective for stock identification applications than are techniques that rely on environmentally induced variation to discriminate among stocks, such as scale-pattern analysis of trace elements in otoliths. Once the baseline has been established for genetic applications, annual surveys of contributing stocks are not necessary, as is the case with for environmentally induced variation. Should greater resolution in stock composition estimates be required than that provided by the 14 microsatellites surveyed in the present study, the addition of markers specifically designed to provide the required resolution will be necessary. These markers could either be additional microsatellites, or perhaps single nucleotide polymorphisms (SNPs) (Smith et al., 2005). It is likely that a combination of microsatellites and SNPs can be employed to provide accurate population or regional estimates of stock composition of mixed-stock samples.

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Literature cited

Allendorf, F. W., and L. W. Seeb.
2000. Concordance of genetic divergence among sockeye salmon populations at allozyme, nuclear DNA, and mitochondrial DNA markers. Evolution 54:640–651.

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., J. R. Candy, K. L. Jonsen, J. Supernault, M. Wetklo, L. Deng, K. M. Miller, R. E. Withler, and N. V. Varnavskaya.
2006. Estimation of stock composition and individual identification of Chinook salmon across the Pacific Rim by use of microsatellite variation. Trans. Am. Fish. Soc. 135:861–888.

Beacham, T. D., J. R. Candy, B. McIntosh, C. MacConnachie, A. Tabata, K. Kaukinen, L. Deng, K. M. Miller, R. E. Withler, and N. V. Varnavskaya.
2005. Estimation of stock composition and individual identification of sockeye salmon on a Pacific Rim basis using microsatellite and major histocompatibility complex variation. Trans. Am. Fish. Soc. 134:1124–1146.

Beacham, T. D., J. R. Candy, K. J. Supernault, M. Wetklo, B. Deagle, K. Labaree, J. R. Irvine, K. M. Miller, R. J. Nelson, and R. E. Withler.
2003. Evaluation and application of microsatellites for population identification of Fraser River chinook salmon (*Oncorhynchus tshawytscha*). Fish. Bull. 101:243–259.

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., M. Lapointe, J. R. Candy, B. McIntosh, C. MacConnachie, A.Tabata, K. Kaukinen, L. Deng, K. M. Miller, and R. E. Withler.
2004. Stock identification of Fraser River sockeye salmon (*Oncorhynchus nerka)* using microsatellites and major histocompatibility complex variation. Trans. Am. Fish. Soc. 133:1106–1126.

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* Sustainability of the Arctic-Yukon-Kuskokwim salmon fisheries: what do we know about salmon ecology, management, and fisheries? (C. C. Krueger, and C. E. Zimmerman, eds.). Am. Fish. Soc., Symp. Bethesda, MD.

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. 32:160–167.

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.

Debevec, E. M., R. B. Gates, M. Masuda, J. Pella, J. M. Reynolds, and L. W. Seeb.
2000. SPAM (version 3.2): Statistics program for analyzing mixtures. J. Hered. 91:509–510.

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

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

Ishida, Y., S. Ito, and K. Takagi.
1989. Stock identification of chum salmon *Oncorhynchus keta* from their maturity and scale characters. Bull. Jap. Soc. Sci. Fish. 55:651–656.

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.

Nikolayeva, Y., and N. I. Semenets.
1983. A contribution to stock differentiation of chum salmon, *Oncorhynchus keta* (Salmonidae), by scale structure in the first year of growth. J. Ichthyol. 23: 18–28.

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 Atlantic salmon. Mol. Ecol. 9:2155–2234.

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

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.

Rannala, B., and J. L. Mountain.
1997. Detecting immigration by using multilocus genotypes. Proc. Natl. Acad. Sci. USA 94: 9197–9201.

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. 32:283–319

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

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. Biol. Morya 33:299–308.

Sano, S.
1966. Salmon of the North Pacific Ocean—Part III. A Review of the Life History of North Pacific Salmon. 3. Chum salmon in the Far East. Int. N. Pac. Fish. Comm. Bull. 18:41–57.

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. Fish. 69:37–50.

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., W. D. Templin, J. E. Seeb, and L. W. Seeb.
2005. Single nucleotide polymorphisms provide rapid and accurate estimates of the proportions of U.S. and Canadian chinook salmon caught in Yukon River fisheries. N. Am. J. Fish. Manag. 25:944–953.

Sohn, D., S. Kang, and S. Kim.
2005. Stock identification of chum salmon (*Oncorhynchus keta*) using trace elements in otoliths. J. Oceanogr. 61: 305–312.

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.

Tanaka, S., M. P. Shepard, and H. T. Bilton.
1969. Origin of chum salmon (*Oncorhynchus keta*) in offshore waters of the North Pacific in 1956–1958 as determined from scale studies. Int. N. Pac. Fish. Comm. Bull. 26:57–155.

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.

Tessier, N., and L. Bernatchez.
1999. Stability of population structure and genetic diversity across generations assessed by microsatellites among sympatric populations of landlocked Atlantic salmon (*Salmo salar* L.). Mol. Ecol. 8:169–179.

Volobuyev, V. V., A. Yu. Rogatnykh, and K. V. Kuzishchin.
1990. Intraspecific forms of chum salmon, *Oncorhynchus keta*, along the continental coast of the Sea of Okhotsk. J. Ichthyol. 30:104–114.

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.

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.

Wilmot, R. L., C. M. Kondzela, C. M. Guthrie, and M. M. Masuda.
1998. Genetic stock identification of chum salmon harvested incidentally in the 1994 and 1995 Bering Sea trawl fishery. N. Pac. Anad. Fish Comm. Bull. 1: 285–299.

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.

Winans, G. A., P. B. Aebersold, Y. Ishida, and S. Urawa.
1998. Genetic stock identification of chum salmon in highseas test fisheries in the western North Pacific Ocean and Bering Sea. N. Pac. Anad. Fish Comm. Bull. 1:220–226.