The use of minisatellite DNA variation for stock identification of chum salmon, *Oncorhynchus keta*

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Abstract.—Geographic variation in minisatellite DNA was examined in 42 stocks of chum salmon, *Oncorhynchus keta*, from the eastern and western Pacific by restricting genomic DNA with *Hae*III and hybridizing it with two minisatellite probes. Regional differentiation in allelic frequencies at the two minisatellite loci was observed; Japanese stocks were distinct from Russian and Yukon River stocks, and stocks from those two regions were distinct from stocks in southeast Alaska and British Columbia. No significant annual variation was observed in allelic frequencies at the two loci for three stocks. Simulated mixed fishery samples of 100 Japanese chum salmon were resolved with a baseline of nine Japanese stocks with an average error of 0.5% per mixture stock. Similarly, simulated mixtures of Yukon River chum salmon resolved with a baseline of five stocks resulted in an average error of 0.6% per mixture stock. Fraser River chum salmon mixtures were resolved with an average error of 3% per mixture stock. With Asian and North American stocks pooled into two groups, accuracy of classification for individual fish to continent of origin was 78% for 323 Asian fish, and 94% for 797 North American fish. Minisatellite DNA variation can be successfully applied to problems of stock discrimination in chum salmon.

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Chum salmon, *Oncorhynchus keta*, have the widest natural geographic distribution of all Pacific salmon species, ranging in Asia from Korea to the Arctic coast of Russia, and in North America from California to the Arctic coast of Canada (Salo, 1991). Chum salmon support major fisheries in both Asia and North America, and management of the fisheries in North America usually requires that stock composition of the catches be determined in order to prevent excessive exploitation of particular stocks. A variety of methods have been applied to discriminate among chum salmon populations and to estimate stock composition in fisheries. Early work consisted in applying physical tags to individual fish (Pritchard, 1931; Anderson and Beacham, 1983), but quantitative estimates of stock composition were generally unable to be derived from these studies. Variation in scale characters has also been used for stock identification in chum salmon (Kovtun, 1983; Nikolayeva and Semenets, 1983; Ishida et al., 1989), but the ability to discriminate among stocks in a localized area is limited.

Genetic methods of stock identification for chum salmon have been evaluated by using both protein- and DNA-level variation. Stock identification techniques based upon genetic variation at protein-coding loci have been shown to provide information on stock structure (Okazaki, 1982; Omelchenko et al., 1992; Kondzela et al., 1994; Phelps et al., 1994; Winans et al., 1994) and stock composition in chum salmon fisheries (Beacham et al., 1985, 1987). By screening genetic variation at the DNA level, using mitochondrial DNA variation (Ginatulina and Mashkin, 1990; Ginatulina, 1992; Cronin et al., 1993; Park et al., 1993) and minisatellite DNA variation (Taylor et al., 1994), the population structure of a species can be determined. Variation in minisatellite DNA has been used to provide accurate and precise estimates of stock composition in simulated mixtures in a number of *Oncorhynchus* species (Beacham et al., 1995, 1996; Miller et al., 1996. Minisatellite DNA consists of tandemly repeated arrays of nucleotides where each array contains a core sequence (Jarman and Wells, 1989). Variable numbers of the tandem repeats (VNTR) occur between restriction sites, resulting in allelic variation at the minisatellite locus. Heterozygosity at these loci can be substantially greater than that commonly observed with protein electrophoresis. Average heterozygosity at protein electrophoretic loci is less than 10% in chum salmon (Winans et al., 1994); heterozygosities at minisatellite loci in salmonids are reported to be between 35 and 80% (Taggart and Ferguson, 1990; Bentzen and Wright, 1993; Taylor et al., 1996). The high level

of variation at minisatellite loci offers the potential for increased population differentiation in contrast with other methods of stock identification.

Initial screening of Asian and North American chum salmon populations with a single-locus minisatellite probe suggested that there were three regional groups of populations, namely Japanese, Russian and Yukon River, and southeast Alaska and British Columbia (Taylor et al., 1994). However, detection of population structure or differentiation among populations within the three major regional groups of chum salmon was limited, even though previous surveys of variation at protein-coding loci suggested that there was at least regional structuring within the British Columbia populations (Okazaki, 1982; Beacham et al., 1987). In the current study, variation at two additional minisatellite DNA loci is reported, and the efficacy of using variation at three minisatellite loci to discriminate among chum salmon populations in local areas is evaluated.

Materials and methods

Samples, laboratory procedures, and probes

The stocks surveyed, their geographic locations, and the methods of DNA extraction, restriction enzyme digestion (*Hae*III), Southern blotting, and membrane preparation have been outlined by Taylor et al. (1994). Summarized briefly, 4 μg of *Hae*III-digested DNA were loaded onto agarose gels containing 30 wells. For the 30 lanes on each gel, 4 lanes were used for λDNA restricted with *Hind*III and *Eco*RI as a molecular weight-size marker, and 26 lanes were used for genomic DNA, one of which was a control fish run. DNA was size-fractionated by electrophoresis in 0.8% agarose gels in 0.5× TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0). After electrophoresis, the agarose gels were depurinated, alkali-denatured, and then the DNA was transferred under vacuum to nylon hybridization membranes and fixed to the membranes by crosslinking under ultraviolet illumination for 3 min.

The probes used in the laboratory analysis were p*Ssa*-A33 and p*Ssa*-A34, hypervariable minisatellite single locus probes in salmonids (Taggart and Ferguson, 1990; Prodöhl et al., 1994). Prior to hybridization with the target probe, the previous probe used was stripped from the membrane by the high temperature method outlined by Noppinger et al. (1992). Following hybridization with each probe, the membranes were washed twice in 2× SSC/0.1% SDS at room temperature for 15 min, once in 2× SSC/0.1% SDS at 65°C for 30 min, once in 1× SSC/0.1% SDS at 65°C for 30 min, and were given a final wash in 1× SSC at room temperature for 15 min. The probes were labelled with ^{32}P, and the membranes exposed to X-ray film. Up to 42 stocks of chum salmon were surveyed in the analysis (the term "stock" was used according to the sense of Ricker (1972), where a stock represents a discrete breeding unit spawning at a given time or place in a river).

Analysis of autoradiographs

The autoradiographs were analyzed with Bioimage software (Millipore Corp. Imaging Systems, Ann Arbor, MI) and scanned with a Kodak high-resolution two-dimensional charge coupled device (CCD) camera at a 1,024 × 1,024 pixel density. The images were translated into digital file images and stored at a workstation for later analysis. Lambda DNA-size markers (21.23, 5.15, 4.97, 4.27, 3.53, and 2.03 kilo base pairs [kbp]) were run on four lanes of each gel and used to create a size standard network that spanned the whole image. Molecular sizes were then assigned by the software to each fragment in each lane on the basis of their relationship to the size standard network. When the p*Ssa*-A33 and p*Ssa*-A34 probes were used, each lane contained either one or two bands; fish with a single band were considered homozygous and two comigrating bands of the same size were scored for those fish. The two probes detect alleles in chum salmon at a single locus where the alleles are inherited in a Mendelian fashion (unpublished data), as was observed for p*Ssa*-A34 in sockeye salmon, *O. nerka* (Taylor et al., 1996), and chinook salmon, *O. tshawytscha* (Stevens et al., 1993), and for both p*Ssa*-A33 and p*Ssa*-A34 in brown trout, *Salmo trutta* (Prodöhl et al., 1994).

Band assignment

Fish from one stock could be analyzed on multiple gels, and thus it was necessary to determine measurement error of band size among gels in order to combine the data. Precision of the estimation of band size was determined by analyzing a standard fish over all gels and by determining the relationship between precision of the estimate and fragment size as outlined by Taylor et al. (1994). As measurement error was present in the estimation of fragment size, a binning procedure (Gill et al., 1990; Galbraith et al., 1991) was used to assign each observed fragment to a particular size class or bin. Distribution of fragment sizes at the *Ssa*-A33 locus ranged from about 4 to 22 kbp, whereas the distribution at the *Ssa*-A34 locus ranged from 2.5 to 8.5 kbp, with very few fragments observed greater than 6.0 kbp (Fig. 1). Bin

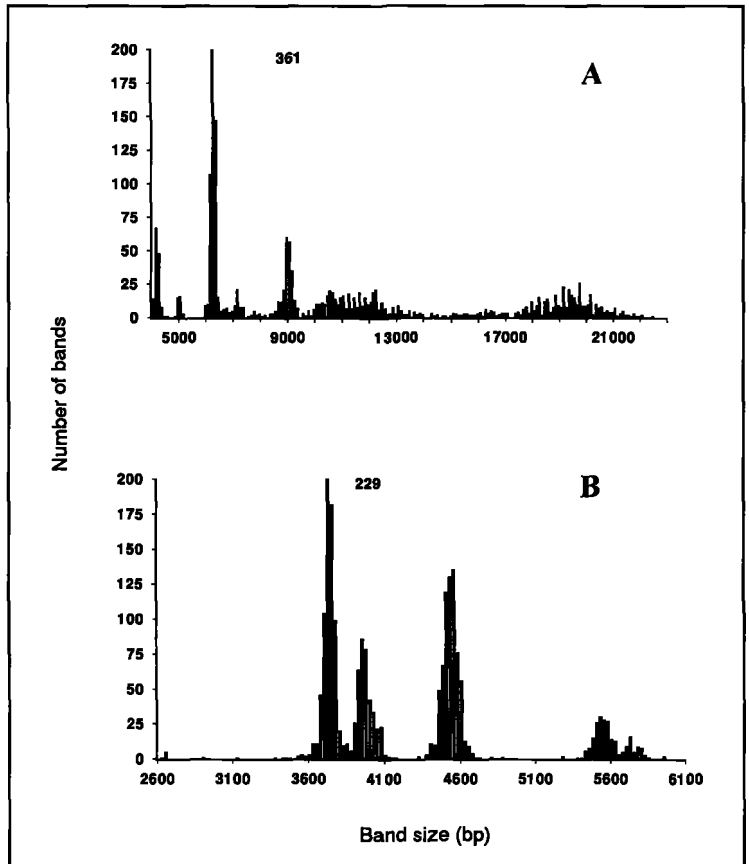

Figure 1

Observed frequency distribution of DNA band size over all chum salmon, *Oncorhynchus keta*, populations surveyed for variation with the probes p*Ssa*-A33 (**A**) and p*Ssa*-A34 (**B**). Frequencies of any band over 200 were truncated and the limits are indicated in the figure (i.e. 361 and 229).

intervals for definition of alleles at the *Ssa*-A33 locus were defined by 15 classes as outlined in Table 1, and bin intervals for definition of alleles at the *Ssa*-A34 locus were defined by 18 classes as outlined in Table 2. The average bin width was 9.8 standard deviations (SD) for bins containing bands <5,000 bp and 8.3 SD's for bins containing bands >5,000 bp. Variation previously observed with the *Ssa*1 probe was reanalyzed from the 44-bin characterization of Taylor et al. (1994) to a 31-bin characterization, with bin limits outlined by Beacham et al. (1996).

Data analysis

Mean allele frequencies were calculated by stock for *Ssa*-A33 and *Ssa*-A34 loci with the bins considered as alleles. Differences in heterozygosity among stocks in different areas were evaluated by the log-likelihood ratio or *G*-test statistic (Sokal and Rohlf, 1981). Annual variation in the allele frequency distributions was evaluated for each probe for three stocks that had been sampled over two years. The chi-square (χ^2) test with 1,000 Monte Carlo simulations of the distribution of the χ^2 statistic (Roff and Bentzen, 1989) was used to evaluate significance of annual variation in allele frequency distributions (McElroy et al., 1992). Genotype frequencies within populations were compared with those expected under Hardy-Weinberg equilibrium by 500 Monte Carlo simulations of the distribution of the χ^2 statistic. As the data set included both allele frequencies (*Ssa*-A33 and *Ssa*-A34) and band counts (*Ssa*1), a generalized or chord distance value (Pimentel, 1979) was calculated between each pair of stocks. A dendrogram was obtained by clustering the distance estimates by means of the neighbor-joining algorithm (Felsenstein, 1990). Classification of individual fish to specific stocks was conducted by using the DISCRIM procedure of SAS (SAS Institute Inc., 1989) with a jackknife sampling procedure, whereby classification

Table 1

Allele frequencies at the *Ssa*-A33 locus for 42 stocks of chum salmon. Genomic DNA was restricted with *Hae*III and hybridized with the p*Ssa*-A33 probe. Bin intervals for definition of alleles were: 4100–4400 bp (1), 4401–4700 bp (2), 4701–5000 bp (3), 5001–5500 bp (4), 5501–6000 bp (5), 6001–6500 bp (6), 6501–7000 bp (7), 7001–7700 bp (8), 7701–8500 bp (9), 8501–9300 bp (10), 9301–10,000 bp (11), 10,001–11,000 bp (12), 11,001–13,000 bp (13), 13,001–16,000 bp (14), >16,001 bp (15). *n* is sample size. North = two stocks from southeast Alaska and two from northern British Columbia; WCVI = west coast of Vancouver Island; ECVI = east coast of Vancouver Island; and Mainland = southern coastal mainland of British Columbia.

Stock	*n*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Chitose	46	0.217	0.011	0.000	0.043	0.000	0.391	0.043	0.152	0.011	0.000	0.000	0.000	0.000	0.076	0.054
Tokachi	47	0.245	0.000	0.000	0.074	0.000	0.223	0.053	0.128	0.021	0.011	0.000	0.000	0.000	0.106	0.138
Miomoto	16	0.281	0.000	0.000	0.031	0.000	0.281	0.000	0.063	0.063	0.000	0.000	0.000	0.063	0.094	0.125
Ohkawa	8	0.188	0.063	0.000	0.125	0.000	0.250	0.000	0.063	0.000	0.063	0.000	0.000	0.063	0.125	0.063
Shibetsu	14	0.214	0.000	0.000	0.000	0.000	0.357	0.036	0.036	0.000	0.107	0.000	0.000	0.071	0.071	0.107
Teshio	8	0.250	0.000	0.000	0.063	0.000	0.313	0.000	0.000	0.188	0.000	0.000	0.000	0.125	0.063	0.000
Tokoro	10	0.350	0.000	0.000	0.000	0.000	0.150	0.050	0.300	0.000	0.000	0.000	0.000	0.000	0.050	0.100
Honoari	48	0.229	0.000	0.010	0.115	0.000	0.354	0.000	0.073	0.052	0.000	0.010	0.000	0.010	0.031	0.115
Abashiri	48	0.281	0.000	0.010	0.052	0.000	0.281	0.010	0.073	0.021	0.010	0.000	0.000	0.042	0.063	0.156
Total for Japan	245	0.247	0.004	0.004	0.063	0.000	0.304	0.024	0.102	0.031	0.012	0.002	0.000	0.024	0.071	0.110
Anadyr	34	0.147	0.000	0.000	0.000	0.000	0.485	0.000	0.000	0.000	0.088	0.029	0.000	0.103	0.029	0.118
Kanchalan	38	0.145	0.013	0.000	0.000	0.000	0.408	0.013	0.000	0.000	0.079	0.013	0.026	0.079	0.066	0.158
Ola	16	0.000	0.000	0.000	0.000	0.000	0.844	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.094
Kamchatka	9	0.111	0.000	0.000	0.000	0.000	0.389	0.000	0.000	0.000	0.056	0.056	0.056	0.000	0.167	0.167
Total for Russia	97	0.119	0.005	0.000	0.000	0.000	0.505	0.005	0.000	0.000	0.067	0.021	0.015	0.077	0.052	0.134
Andreafsky	35	0.043	0.000	0.000	0.000	0.000	0.529	0.000	0.043	0.000	0.000	0.014	0.000	0.014	0.000	0.358
Kluane	29	0.017	0.000	0.000	0.000	0.000	0.483	0.000	0.103	0.000	0.086	0.000	0.000	0.000	0.000	0.310
Fishing Branch	29	0.034	0.000	0.000	0.000	0.000	0.552	0.000	0.000	0.000	0.155	0.000	0.000	0.034	0.000	0.224
Sheenjek	28	0.071	0.000	0.000	0.000	0.000	0.589	0.018	0.000	0.000	0.214	0.000	0.000	0.000	0.000	0.107
Tatchun	28	0.000	0.000	0.000	0.000	0.000	0.714	0.018	0.000	0.000	0.125	0.000	0.000	0.000	0.000	0.143
Total for Yukon River	149	0.034	0.000	0.000	0.000	0.000	0.570	0.007	0.030	0.000	0.111	0.003	0.000	0.010	0.000	0.234
Fish Creek	46	0.000	0.000	0.000	0.000	0.000	0.402	0.120	0.000	0.000	0.033	0.000	0.011	0.087	0.000	0.347
Breezy Bay	42	0.000	0.000	0.000	0.000	0.000	0.405	0.012	0.000	0.000	0.048	0.012	0.012	0.036	0.000	0.476
Atnarko	24	0.000	0.000	0.000	0.000	0.000	0.583	0.000	0.000	0.000	0.000	0.000	0.042	0.042	0.000	0.333
Pallant	50	0.000	0.000	0.000	0.000	0.000	0.630	0.000	0.000	0.000	0.000	0.010	0.020	0.040	0.000	0.300
Total for North	162	0.000	0.000	0.000	0.000	0.000	0.500	0.037	0.000	0.000	0.022	0.006	0.019	0.052	0.000	0.364
Conuma	23	0.000	0.000	0.000	0.000	0.043	0.478	0.000	0.000	0.022	0.043	0.000	0.043	0.065	0.043	0.261
Nitinat	48	0.000	0.000	0.000	0.000	0.000	0.615	0.042	0.000	0.000	0.010	0.021	0.052	0.125	0.021	0.115
Demamiel	24	0.000	0.000	0.000	0.000	0.000	0.417	0.000	0.000	0.000	0.063	0.000	0.000	0.125	0.000	0.396
Total for WCVI	95	0.000	0.000	0.000	0.000	0.011	0.532	0.021	0.000	0.005	0.032	0.011	0.037	0.111	0.021	0.222

continued

Table 1 (continued)

Stock	*n*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Alouette	23	0.000	0.000	0.000	0.000	0.000	0.283	0.000	0.000	0.000	0.087	0.000	0.130	0.196	0.022	0.282
Chehalis	24	0.000	0.000	0.000	0.000	0.000	0.354	0.021	0.000	0.000	0.042	0.000	0.063	0.250	0.000	0.271
Chilliwack	48	0.000	0.000	0.000	0.000	0.000	0.302	0.000	0.000	0.000	0.063	0.000	0.052	0.302	0.031	0.250
Inch	21	0.000	0.000	0.000	0.000	0.000	0.381	0.000	0.000	0.000	0.119	0.024	0.119	0.190	0.000	0.167
Stave	23	0.000	0.000	0.000	0.000	0.000	0.391	0.000	0.000	0.022	0.043	0.000	0.109	0.109	0.043	0.282
Wahleach	22	0.000	0.000	0.000	0.000	0.000	0.318	0.000	0.000	0.000	0.114	0.023	0.000	0.364	0.023	0.159
Total for Fraser River	161	0.000	0.000	0.000	0.000	0.000	0.332	0.003	0.000	0.003	0.075	0.006	0.075	0.245	0.022	0.240
Cowichan	22	0.000	0.000	0.000	0.000	0.000	0.159	0.000	0.000	0.000	0.023	0.000	0.045	0.386	0.000	0.387
Big Qualicum	59	0.000	0.000	0.000	0.000	0.000	0.246	0.008	0.000	0.000	0.051	0.000	0.085	0.237	0.042	0.331
Chemainus	24	0.000	0.000	0.000	0.000	0.000	0.229	0.000	0.000	0.000	0.167	0.000	0.125	0.208	0.000	0.271
Goldstream	24	0.000	0.000	0.000	0.000	0.000	0.333	0.000	0.000	0.000	0.104	0.000	0.021	0.333	0.021	0.188
L. Qualicum	36	0.000	0.000	0.000	0.014	0.000	0.194	0.014	0.056	0.000	0.056	0.042	0.028	0.153	0.139	0.306
Nanaimo	24	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.021	0.083	0.000	0.063	0.292	0.000	0.292
Puntledge	23	0.000	0.000	0.000	0.000	0.000	0.239	0.000	0.000	0.000	0.174	0.022	0.174	0.130	0.022	0.239
Total for ECVI	212	0.000	0.000	0.000	0.002	0.000	0.236	0.005	0.009	0.002	0.085	0.009	0.075	0.241	0.040	0.295
Tzoonie	23	0.000	0.000	0.000	0.000	0.000	0.239	0.000	0.000	0.000	0.043	0.022	0.043	0.283	0.065	0.304
Mamquam	28	0.000	0.000	0.000	0.018	0.000	0.214	0.000	0.000	0.000	0.125	0.018	0.018	0.304	0.071	0.232
Sliammon	6	0.000	0.000	0.000	0.000	0.000	0.500	0.083	0.000	0.000	0.167	0.000	0.083	0.083	0.000	0.083
Cheakamus	6	0.000	0.000	0.000	0.000	0.000	0.333	0.000	0.000	0.000	0.000	0.000	0.000	0.417	0.000	0.250
Total for Mainland	63	0.000	0.000	0.000	0.008	0.000	0.262	0.008	0.000	0.000	0.087	0.016	0.032	0.286	0.056	0.246

functions were developed with all fish sampled except the one to be classified. The accuracy of classification of individual fish was summarized both on a population and on a regional basis.

Estimation of stock composition

Baseline data

In this study, counts were available from 15 alleles at the *Ssa*-A33 locus, 18 alleles at the *Ssa*-A34 locus, and from 31 band counts at the *Ssa*1 locus for each fish. The maximum-likelihood model (Fournier et al., 1984) used in the estimation of stock composition requires that the characters used in the analysis be independent. When allele counts and band counts were used to characterize the chum salmon stocks, a principal-components analysis on the correlation matrix of the 64 variables in the study was conducted in order to obtain uncorrelated variables or principal components, as was described by Beacham et al. (1996). Summarized briefly, the PRINCOMP procedure in SAS was used in the principal-components analysis. The observed data (number of bands per bin) were standardized by subtracting the overall corresponding mean and by dividing by the corresponding bin standard deviation over all populations for the original variables. The original variables were then represented as factor scores for uncorrelated variables or principal components. The first 57 principal components accounted for 100% of the total observed variation. The input to the maximum-likelihood model required discrete frequency counts for each variable, and as outlined by Beacham et al. (1995), the continuous distributions of the principal component scores were represented as 12-bin histograms. Limits of the bins were as follows: ≤–2.50 (1), –2.49 to –1.50 (2), –1.49 to –1.00 (3), –0.99 to –0.60 (4), –0.59 to –0.30 (5), –0.29 to 0.00 (6), 0.00 to 0.29 (7), 0.30 to 0.59 (8), 0.60 to 0.99 (9), 1.00 to 1.49 (10), 1.50 to 2.49 (11), and ≥2.50 (12). Each stock was then characterized as an input matrix of 57 rows (principal components) and 12 columns (counts of principal component scores in each of the 12 bins).

Table 2

Allele frequencies at the *Ssa*-A34 locus for 42 stocks of chum salmon. Genomic DNA was restricted with *Hae*III and hybridized with the p*Ssa*-A34 probe. Bin intervals for definitions of alleles were as follows: 2600–2700 bp (1), 2701–2800 bp (2), 2801–2900 bp (3), 2901–3100 bp (4), 3101–3300 (5), 3301–3500 bp (6), 3501–3700 bp (7), 3701–3900 bp (8), 3901–4100 bp (9), 4101–4400 bp (10), 4401–4700 bp (11), 4701–5000 bp (12), 5001–5500 bp (13), 5501–6000 bp (14), 6001–6500 bp (15), 6501–7000 bp (16), 7001–7700 bp (17), 7701–8500 bp (18). *n* is sample size. Abbreviations for geographic regions are defined in Table 1.

Stock	*n*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Chitose	46	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.054	0.446	0.022	0.152	0.000	0.022	0.250	0.000	0.000	0.000	0.043
Tokachi	48	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.104	0.302	0.021	0.240	0.010	0.010	0.250	0.000	0.010	0.021	0.000
Miomoto	16	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.094	0.563	0.000	0.125	0.000	0.031	0.156	0.000	0.000	0.000	0.000
Ohkawa	16	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.656	0.000	0.094	0.000	0.000	0.219	0.000	0.031	0.000	0.000
Shibetsu	14	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.321	0.000	0.357	0.000	0.071	0.214	0.000	0.000	0.000	0.000
Teshio	8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.188	0.375	0.000	0.188	0.000	0.063	0.188	0.000	0.000	0.000	0.000
Tokoro	10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.350	0.000	0.250	0.000	0.100	0.250	0.000	0.000	0.000	0.000
Honoari	48	0.000	0.000	0.000	0.000	0.000	0.010	0.031	0.115	0.344	0.000	0.240	0.010	0.094	0.156	0.000	0.000	0.000	0.000
Abashiri	48	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.083	0.375	0.000	0.240	0.000	0.042	0.219	0.010	0.000	0.000	0.000
Total for Japan	254	0.000	0.000	0.000	0.000	0.000	0.004	0.020	0.083	0.394	0.008	0.213	0.004	0.043	0.215	0.002	0.004	0.004	0.008
Anadyr	25	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.100	0.060	0.040	0.640	0.000	0.000	0.120	0.000	0.000	0.000	0.000
Kanchalan	39	0.000	0.000	0.000	0.000	0.013	0.013	0.013	0.282	0.064	0.000	0.590	0.000	0.000	0.026	0.000	0.000	0.000	0.000
Ola	15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.067	0.000	0.600	0.000	0.000	0.100	0.000	0.033	0.000	0.000
Kamchatka	13	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.269	0.038	0.000	0.538	0.000	0.000	0.115	0.000	0.000	0.000	0.000
Total for Russia	92	0.000	0.000	0.000	0.000	0.005	0.005	0.022	0.217	0.060	0.011	0.598	0.000	0.000	0.076	0.000	0.005	0.000	0.000
Andreafsky	36	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.236	0.069	0.000	0.611	0.000	0.000	0.069	0.000	0.000	0.000	0.000
Kluane	30	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.217	0.300	0.017	0.467	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fishing Br	29	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.224	0.241	0.000	0.448	0.000	0.000	0.086	0.000	0.000	0.000	0.000
Sheenjek	28	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.286	0.125	0.000	0.571	0.000	0.000	0.018	0.000	0.000	0.000	0.000
Tatchun	28	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.268	0.286	0.000	0.393	0.000	0.000	0.054	0.000	0.000	0.000	0.000
Total for Yukon River	151	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.245	0.199	0.003	0.503	0.000	0.000	0.046	0.000	0.000	0.000	0.000
Fish Creek	48	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.563	0.125	0.000	0.271	0.010	0.000	0.031	0.000	0.000	0.000	0.000
Breezy Bay	46	0.000	0.000	0.000	0.000	0.000	0.011	0.054	0.413	0.098	0.000	0.348	0.000	0.011	0.065	0.000	0.000	0.000	0.000
Atnarko	24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.396	0.083	0.000	0.417	0.000	0.000	0.104	0.000	0.000	0.000	0.000
Pallant	50	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.510	0.110	0.000	0.260	0.010	0.000	0.110	0.000	0.000	0.000	0.000
Total for North	168	0.000	0.000	0.000	0.000	0.000	0.003	0.015	0.482	0.107	0.000	0.310	0.006	0.003	0.074	0.000	0.000	0.000	0.000
Conuma	23	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.522	0.130	0.000	0.304	0.000	0.000	0.022	0.000	0.000	0.000	0.000
Demamiel	23	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.391	0.174	0.000	0.370	0.000	0.022	0.043	0.000	0.000	0.000	0.000
Nitinat	48	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.354	0.115	0.000	0.417	0.000	0.010	0.063	0.000	0.000	0.000	0.000
Total for WCVI	94	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.404	0.133	0.000	0.378	0.000	0.011	0.048	0.000	0.000	0.000	0.000

continued

Table 2 (continued)

Stock	*n*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Alouette	23	0.000	0.000	0.000	0.000	0.000	0.000	0.152	0.348	0.130	0.065	0.130	0.000	0.043	0.130	0.000	0.000	0.000	0.000
Chehalis	24	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.333	0.208	0.000	0.354	0.000	0.021	0.021	0.000	0.000	0.000	0.000
Chilliwack	48	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.417	0.208	0.000	0.281	0.000	0.010	0.073	0.000	0.000	0.000	0.000
Inch	21	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.381	0.095	0.000	0.381	0.000	0.000	0.095	0.000	0.000	0.000	0.000
Stave	23	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.543	0.174	0.022	0.130	0.022	0.000	0.065	0.000	0.000	0.000	0.000
Wahleach	22	0.000	0.000	0.000	0.000	0.000	0.000	0.068	0.477	0.068	0.000	0.318	0.000	0.000	0.068	0.000	0.000	0.000	0.000
Total for Fraser River	161	0.006	0.000	0.000	0.000	0.000	0.000	0.050	0.416	0.158	0.012	0.267	0.003	0.012	0.075	0.000	0.000	0.000	0.000
Cowichan	22	0.023	0.000	0.000	0.000	0.000	0.000	0.045	0.591	0.045	0.000	0.250	0.000	0.000	0.045	0.000	0.000	0.000	0.000
Big Qualicum	58	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.612	0.121	0.017	0.181	0.000	0.000	0.043	0.000	0.009	0.000	0.000
Chemainus	24	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.458	0.229	0.000	0.208	0.000	0.021	0.042	0.000	0.000	0.000	0.000
Goldstream	23	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.457	0.196	0.022	0.217	0.000	0.000	0.043	0.000	0.000	0.000	0.000
L. Qualicum	36	0.000	0.000	0.000	0.014	0.000	0.000	0.028	0.625	0.069	0.014	0.236	0.000	0.014	0.014	0.000	0.000	0.000	0.000
Nanaimo	24	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.417	0.146	0.000	0.292	0.000	0.021	0.042	0.000	0.000	0.000	0.000
Puntledge	23	0.000	0.000	0.000	0.000	0.000	0.000	0.152	0.261	0.043	0.000	0.413	0.000	0.065	0.065	0.000	0.000	0.000	0.000
Total for ECVI	210	0.007	0.000	0.000	0.002	0.000	0.000	0.048	0.517	0.119	0.010	0.243	0.000	0.014	0.040	0.000	0.002	0.000	0.000
Tzoonie	24	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.479	0.167	0.000	0.208	0.000	0.000	0.083	0.000	0.042	0.000	0.000
Mamquam	29	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.397	0.172	0.017	0.345	0.000	0.000	0.052	0.000	0.000	0.000	0.000
Sliammon	13	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.692	0.077	0.000	0.192	0.000	0.000	0.038	0.000	0.000	0.000	0.000
Cheakamus	13	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.462	0.115	0.000	0.346	0.000	0.000	0.077	0.000	0.000	0.000	0.000
Total for Mainland	79	0.006	0.000	0.000	0.000	0.000	0.000	0.006	0.481	0.146	0.006	0.278	0.000	0.000	0.063	0.000	0.013	0.000	0.000

Mixture data

A series of hypothetical fishery samples that contained fish from a specific region or stock were analyzed in order to evaluate the use of minisatellite DNA for stock composition. Test samples of 100 fish were generated 50 times by randomly resampling with replacement stocks from the appropriate river or region; then the mean and standard deviation of the 50 estimates were computed. Stock compositions of the mixture were determined under the assumption that the baseline data were perfectly representative of the stocks (fixed-frequency distribution) and by simulating random variation involved in the collection of baseline samples (resampled distribution). Each baseline stock was resampled with replacement to obtain new distributions of the principal components with the same sample size as in the original sample (Millar, 1990).

Results

Geographic variation in *Ssa*-A33 allele frequencies

Allele size varied from 4.1 to 21.3 kbp (Fig. 2). Major alleles were observed at the 4.1 to 4.4 kbp (bin 1), 6.0 to 6.5 kbp (bin 6), and 8.5 to 9.3 kbp (bin 10) (Fig. 1; Table 1). Variation in allele frequencies was observed among Japanese, Russian, and Yukon rivers, and other North American stocks. An allele between 4.1 and 4.4 kbp was fairly common in Japanese stocks (mean frequency 0.25), less common in Russian stocks (0.12), and absent in North American stocks south of the Yukon River (Table 1). Similarly, alleles greater than 16.0 kbp were fairly rare in Asian stocks (Japanese stock mean frequency: 0.11; Russian: 0.13) but were more common in North American stocks (regional frequencies: 0.24–0.26). Clear differences in allele frequencies were observed in chum salmon stocks in broadly separated geographic areas.

In North American stocks, regional differences in allelic frequencies were observed. Mean frequency of the 11.0–13.0 kbp allele was 0.01 for Yukon River stocks, 0.05 for southeast Alaska and northern British Columbia stocks, 0.11 for west coast of Vancouver Island (WCVI) stocks, and 0.24

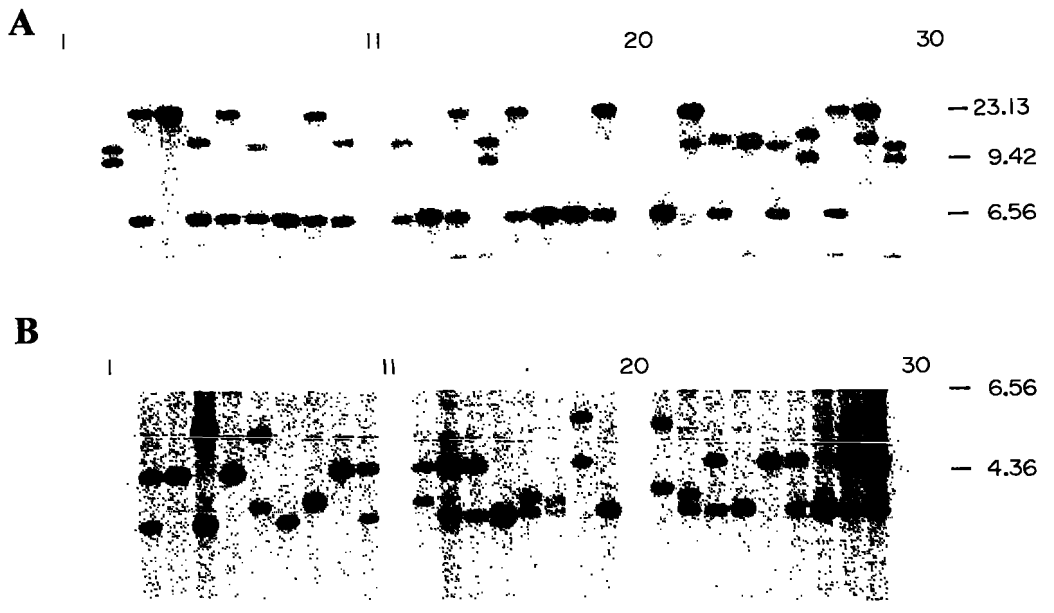

Figure 2

Autoradiographs of chum salmon DNA restricted with *Hae*III and hybridized with the probe p*Ssa*-A33 (**A**) or p*Ssa*-A34 (**B**). In panel A, lanes 1, 11, 20, and 30 represent size-standard fish; lanes 2 and 29 represent standard fish; lanes 3–8, 16–19, and 21–22 represent Atnarko fish; and 9–10, 12–15, and 23–28 represent Chilliwack fish. In panel B, the lanes are similar; Chilliwack chum salmon DNA is seen in lanes 3–8, 16–19, and 21–22, and Nitinat chum salmon DNA in lanes 9–10, 12–15, and 24–29.

or greater for east coast of Vancouver Island (ECVI), mainland British Columbia, or Fraser River stocks (Table 1). However, within a region, marked differences in allelic frequencies could be observed among stocks. For example, frequency of the allele greater than 16.0 kbp ranged between 0.16 and 0.28 for six Fraser River stocks, and frequency of the 11.0–13.0 kbp allele ranged between 0.13 and 0.39 for seven ECVI stocks. This variation in allele frequencies among stocks within regions may allow discrimination among stocks within the region but it increases the complexity of discriminating among stocks between regions.

Genotypic frequencies at the *Ssa*-A33 locus were in Hardy-Weinberg equilibrium in all 42 stocks sampled. On a regional basis, heterozygosity ranged from 54 to 72% for the stocks sampled, with Japanese stocks significantly more heterozygous than the pooled aggregate of North American stocks (G=6.03, df=1, P<0.05)(Table 1).

Geographic variation in *Ssa*-A34 allele frequencies

Allele size varied from 2.6 to 8.5 kbp (Fig. 2), with major alleles at 3.7–3.9 kbp (bin 8), 3.9–4.1 kbp (bin 9), and 4.4–4.7 kbp (bin 11) (Fig. 1; Table 2). As with the *Ssa*-A33 locus, differences in allele frequencies were observed among Japanese, Russian, and North American stocks. Of the Russian stocks surveyed, all had a relatively high frequency (mean 0.60) of the 4.4–4.7 kbp allele, significantly different from Japanese stocks (mean 0.21)(χ^2=89.6, df=1, P<0.01)(Table 2). Mean frequency of the 3.7–3.9 kbp allele was 0.08 in Japanese stocks, 0.22–0.25 in Russian and Yukon River stocks, and 0.40–0.52 in southeast Alaska and British Columbia stocks. Similar patterns of the genetic distinctiveness of the major stock groups were observed at both the *Ssa*-A33 and *Ssa*-A34 loci.

In British Columbia, regional differences in allele frequencies were fairly minor in comparison with the variation among stocks within regions (Table 2). For example, in the six Fraser River stocks, frequencies of the 4.4–4.7 kbp allele ranged from 0.13 to 0.38 (mean 0.27), in the seven ECVI stocks from 0.18 to 0.41 (mean 0.24), and in the four mainland stocks from 0.19 to 0.35 (mean 0.28). The variation in allele frequencies among stocks within regions may be used effectively for localized stock discrimination, but as with the *Ssa*-A33 locus, makes discrimination among regional groups of stocks more difficult.

Table 3

Observed heterozygosities for chum salmon, *Oncorhyncus keta*, from eight regions at the *Ssa*-A33 and *Ssa*-A34 loci. Stocks sampled within each region are listed in Tables 1 and 2. *n* = number of fish sampled in each region. Stocks sampled in each region are outlined in Table 1. Abbreviations for geographic regions are defined in Table 1.

Region	*n*	*Ssa*-A33	*n*	*Ssa*-A34
Japan	245	0.718	254	0.768
Russia	97	0.629	92	0.620
Yukon	149	0.557	151	0.642
North	162	0.568	168	0.655
WCVI	95	0.537	94	0.713
Fraser	161	0.720	161	0.658
ECVI	212	0.703	210	0.579
Mainland	63	0.651	79	0.633

As with the *Ssa*-A33 locus, genotypic frequencies at the *Ssa*-A34 locus were in Hardy-Weinberg equilibrium in all of the 42 stocks sampled. Mean heterozygosity of the Japanese stocks (0.77) was higher than that for any other regional group of stocks (Table 3) and significantly higher than that for North American (G=15.0, df=1, $P<0.05$) or Russian stocks ($G=6.50$, df=1, $P<0.05$). Heterozygosity of Japanese chum salmon was consistently higher than that for other regional groups of chum salmon when both the *Ssa*-A33 and *Ssa*-A34 loci were considered.

Annual variation in allele frequencies

Samples from two Vancouver Island (Big Qualicum, Nitinat) stocks and one Fraser River (Chilliwack) stock were obtained in both 1991 and 1992. No significant annual variation was observed in allele frequencies at either the *Ssa*-A33 or *Ssa*-A34 loci for any stock ($P>0.05$)(6 tests conducted).

Stock structure

Neighbor-joining analysis of the distances between stocks derived from allele frequencies at the *Ssa*-A33 and *Ssa*-A34 loci and from band counts observed with the *Ssa*1 probe indicated that Japanese chum salmon stocks were distinct from all other groups of stocks (Fig. 3). Regional stock structure was also examined with principal-components analysis, and all nine Japanese stocks sampled were clearly separated from all other stocks (Fig. 4). Russian and Yukon River stocks were more similar to each other than to more distant stocks in Japan and British Columbia (Figs. 3 and 4). There was weak regional structuring among stocks of British Columbia chum salmon, and there was some evidence that southeast Alaska and northern British Columbia stocks were more similar to each other than to stocks in southern British Columbia (Fig. 3) and some evidence that there was separation between ECVI and Fraser River stocks (Fig. 4).

Estimation of stock composition

The potential use of minisatellite DNA variation for estimation of stock composition of stocks from broad geographic areas was evaluated for two main groupings of chum salmon, namely mixtures of Japanese and Russian chum salmon and mixtures of Russian and Yukon River chum salmon. On the basis of stock structure, relatively accurate and precise estimates of stock composition should be derived from samples containing Japanese and Russian chum salmon (Table 4). Similarly, in applications where estimation of Russian and Yukon River chum salmon compositions is of interest, relatively accurate and precise estimates of stock composition of the two target groups should be derived (Table 4). The Andrefsky River stock was in a group with Russian stocks in the neighbor-joining analysis (Fig. 3) but seemed separable from Russian stocks in the principal-components analysis (Fig. 4). The simulations suggested that even if the Andrefsky River comprised all the Yukon River component, separation of Russian and Yukon River stock components should still be possible (Table 4).

The application of minisatellite DNA variation to local stock composition issues was evaluated for three situations; i.e. mixtures of Japanese, Yukon River, and southern British Columbia chum salmon stocks were evaluated separately. The simulations suggested that both discrimination among all of the Japanese stocks sampled and relatively accurate estimates of stock composition observed in both single-stock and multistock simulated mixtures were possible (Table 5). Because less than 20 fish per stock were sampled for five of the nine stocks surveyed (Table 1), increased baseline sampling, however, would be required to confirm these results before application to actual fishery samples. The simulations of Yukon River chum salmon mixtures suggested that all five stocks were distinct from each other and that accurate estimates of stock composition may be possible with minisatellite DNA variation (Table 6). The fall-run Sheenjek River stock was distinguishable from fall-run stocks spawning in the Yukon Territory. The simulations of southern British Columbia chum salmon mixtures suggested that minisatellite DNA variation may be applicable for estimation of stock composition in fishery samples. Estimates of stock composition for

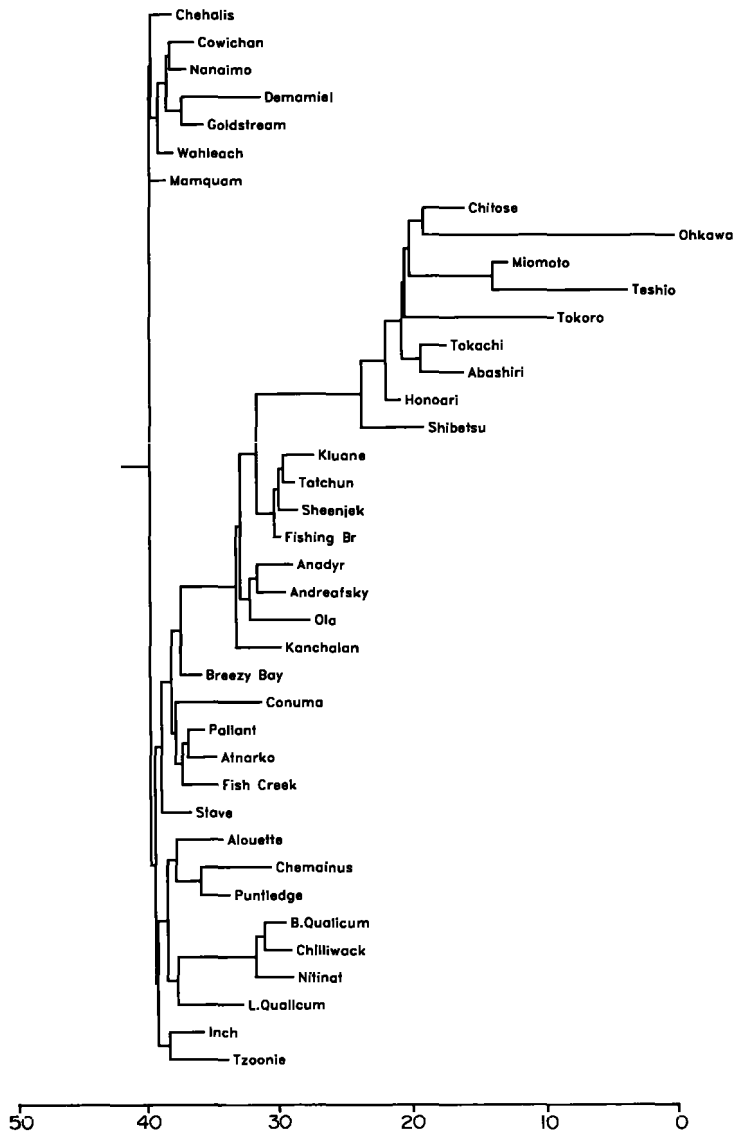

Figure 3

A neighbor-joining dendrogram of relationships among 39 stocks of chum salmon based on allele frequencies at the *Ssa*-A33 and *Ssa*-A34 loci and on band counts of occurrence for the probe *Ssa*1.

the six individual Fraser River stocks, derived from a baseline for Fraser River only, indicate that discrimination among the stocks was possible (Table 7). Similar results were also observed when an ECVI-only or WCVI-only baseline was used and when ECVI and WCVI fish were in the simulated mixtures as appropriate. When an 18-stock regional baseline was used, there was a clear indication of separation among Fraser River, ECVI, and WCVI stocks (Table 7).

Identification of individuals

The accuracy of classifying individuals to specific stocks or regions was dependent upon the specific stock under consideration; the greatest accuracy was observed for the more distinctive stocks or regional groups. With a 39-stock baseline (Sliammon, Cheakamus, and Kamchatka were removed owing to lack of *Ssa*1 band counts), classification accuracy

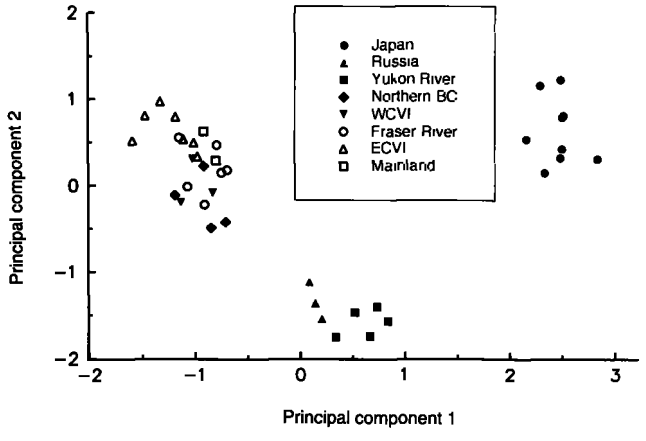

Figure 4

Plot of first two principal components (PC) incorporating variation at the *Ssa*-A33 and *Ssa*-A34 loci and including *Ssa*-1 band counts for 39 stocks indicated in Figure 2. North = two stocks from southeast Alaska and two stocks from northern British Columbia. WCVI = west coast of Vancouver Island; ECVI = east coast of Vancouver Island; Mainland = southern coastal mainland of British Columbia.

for Japanese chum salmon to specific stocks averaged 15%, but 82% were correctly identified as Japanese in origin (Table 8). Similarly, classification accuracy for Russian chum salmon to stock of origin was 30%, and to region of origin, 47%. Yukon River chum salmon were fairly distinctive and the origin of about 75% of the fish was correctly identified. Accurate classification for individual southern British Columbia chum salmon to region of origin ranged from about 9 to 44% (Table 8), presumably reflecting less separation among these groups compared with that among Japanese, Russian, and Yukon River chum salmon. With Asian and North American stocks pooled into two groups, accuracate classification to continent of origin for 323 Asian fish was 78%, and that for 797 North American fish was 94%. The minisatellite DNA variation provided a reasonable measure of accuracy in classifying individuals to continent of origin.

Discussion

Allele frequencies at the *Ssa*-A33 and *Ssa*-A34 loci indicated that there were three regional groups of chum salmon stocks: the Japanese, Russian and Yukon River, and southeast Alaska and British Columbia stocks. The greatest differences were observed between stocks from Japan and British Columbia. Similar results were also observed in a survey of minisatellite DNA variation with the *Ssa*1 probe (Taylor et al., 1994). The differentiation between stocks from Japan and British Columbia has also been demonstrated in studies of variation at protein-coding loci (Okazaki, 1982) and more recently in mitochondrial DNA in the region coding for NADH dehydrogenase subunits 5 and 6 (Park et al., 1993). These results are consistent with the conclusion that stocks that are more distant geographically also show more distinct genetic differentiation (Taylor et al., 1994). These patterns presumably reflect the dispersal of salmon from different refuges after the retreat of glaciers at the end of the Wisconsinian glacial period (Lindsey and McPhail, 1986). The separation between Japanese and Russian stocks reported by Winans et al. (1994) in a survey of variation at protein-coding loci was also observed in the current study with variation at minisatellite loci. A high degree of genetic differentiation has been consistently observed between Japanese and North American chum salmon, regardless of

Table 4

Estimated percentage composition of a variety of mixtures of chum salmon. Each mixture of 100 fish was generated 50 times with replacement, and a stock composition of the mixtures estimated by using either a fixed distribution of the allele frequencies or band counts in the baseline stocks, or by randomly resampling each baseline stock with replacement to obtain a resampled distribution of the frequencies or counts for each baseline stocks, with the same sample size in the new distribution as in the original one. Band frequencies used included those derived from genomic DNA restricted with *Hae*III and hybridized with *Ssa*-1, p*Ssa*-A33, and p*Ssa*-A34. Twelve (9 Japanese, 3 Russian) baseline stocks were used to resolve the Japanese and Russian mixtures, and 8 (3 Russian, 5 Yukon) baseline stocks for Russian and Yukon River chum salmon mixtures. Standard deviations are given in parentheses.

	Distribution (%)		
Origin	Actual	Fixed	Resampled
Japan vs. Russia			
Japan	0	0.0 (0.0)	5.2 (4.2)
Japan	25	24.8 (0.4)	28.3 (4.3)
Japan	50	49.5 (0.6)	51.3 (5.4)
Japan	75	74.7 (0.05)	74.5 (4.8)
Japan	100	99.6 (0.6)	99.6 (0.9)
Russia vs.Yukon			
Russia	0	0.1 (0.4)	3.6 (3.5)
Russia	25	24.1 (1.4)	28.2 (3.6)
Russia	50	48.3 (1.5)	49.9 (4.7)
Russia	75	72.4 (1.9)	72.5 (3.9)
Russia	100	97.5 (1.8)	96.0 (3.7)
Andreafsky	100	100.0 (0.0)	94.5 (5.0)

Table 5

Estimated percentage composition of 100-fish mixtures of Japanese chum salmon stocks resolved with a 9-stock baseline in which distributions of the allele frequencies or band counts in each baseline stock were fixed or resampled as outlined in Table 4. Standard deviations are given in parentheses.

	Distribution (%)		
Stock	Actual	Fixed	Resampled
Single-stock mixtures			
Chitose	100	100.0 (0.0)	94.1 (4.5)
Tokachi	100	100.0 (0.0)	94.4 (5.2)
Miomoto	100	100.0 (0.0)	91.0 (9.6)
Ohkawa	100	100.0 (0.0)	91.8 (10.4)
Shibetsu	100	100.0 (0.0)	92.3 (7.8)
Teshio	100	100.0 (0.0)	87.6 (13.5)
Tokoro	100	100.0 (0.0)	97.2 (5.8)
Honoari	100	100.0 (0.0)	94.2 (4.8)
Abashiri	100	100.0 (0.0)	93.2 (5.2)
Multistock mixture			
Chitose	10	9.7 (0.5)	11.0 (4.1)
Okachi	20	20.3 (0.6)	19.4 (3.0)
Honoari	30	30.2 (0.3)	29.7 (4.4)
Abashiri	40	39.8 (0.6)	39.8 (4.5)

Table 6

Estimated percentage composition of 100-fish mixtures of Yukon River chum salmon stocks resolved with a 5-stock baseline in which distributions of the allele frequencies or band counts in the baseline stock were fixed or resampled as outlined in Table 4. Standard deviations are given in parentheses.

	Distribution (%)		
Stock	Actual	Fixed	Resampled
Single-stock mixtures			
Andreafsky	100	100.0 (0.0)	97.0 (3.3)
Sheenjek	100	98.2 (1.7)	97.5 (3.1)
Fishing Branch	100	96.5 (2.0)	95.1 (4.1)
Tatchun	100	100.0 (0.0)	98.7 (3.4)
Kluane	100	100.0 (0.0)	95.9 (4.6)
Multistock mixture			
Kluane	10	9.3 (0.8)	10.0 (2.4)
Tatchun	20	20.3 (0.7)	19.8 (3.7)
Sheenjek	30	30.3 (0.4)	30.8 (4.0)
Fishing Branch	40	39.1 (1.1)	38.8 (4.5)

whether the survey has used protein electrophoretic, mitochondrial DNA, or minisatellite DNA variation.

In the current study, DNA bands were grouped in bins or "alleles" on the basis of the estimated size of the DNA fragment. The precision of estimated fragment size was based on analyzing a single standard fish on all gels, on obtaining repeated estimates of fragment size, and on determining a relationship between estimated fragment size and precision or standard deviation of the estimate (Galbraith et al., 1991; Taylor et al., 1994). The bin width was set at 8 to 10 SD, less than the 16 SD suggested by Galbraith et al. (1991) as necessary before the chances of assigning a band to the wrong bin falls below 0.05. However, because most of the source of variation in the Galbraith et al. (1991) study was due to digitizing bands that were manually marked on an acetate overlay, the computerized estimation system employed in the present study should result in greater precision of estimation of fragment size. Taylor et al. (1994) used a bin width of 6 SD, based upon the measurement precision of the fragment size of a standard fish, but a more conservative bin width of 8 to 10 SD was used in the current study.

The variation observed at the minisatellite loci examined in the current study was probably less than that actually present at the loci. Given the level of precision of the measurements of fragment size, closely spaced true alleles could not be distinguished reliably and were therefore combined in the binning process. It was assumed that there was no consistent bias in the assignment of fragments to specific bins. Additionally, differences in DNA sequence variation of two alleles of the same size were not determined. It is possible, for example, that an allele occurring in both Japanese and North American populations will show the same length for both populations but will reveal a considerably different DNA sequence.

The simulations conducted for mixtures containing localized stocks (Japan, Yukon River, and Fraser River) suggested that the minisatellite DNA variation surveyed may provide relatively accurate and precise estimates of individual stocks in the mixtures. Although the simulations were encouraging, the performance of the minisatellite DNA variation for estimation of stock composition needs to be verified by actual applications in field situations, where fish in the mixture can originate from stocks not found in the baseline, and where novel genotypes can be observed. In the present study, sample sizes of the baseline stocks were usually less than 50 fish; therefore, increased sampling of individuals in the stocks would be required in order to obtain representative distributions of allele frequencies or band counts.

The simulations suggested that accurate estimates of stock composition of individual stocks may be possible for mixtures of either Fraser River or Yukon River chum salmon. A previous survey of variation at seven protein-coding loci indicated that there was

Table 7

Estimated percentage composition of 100-fish mixtures of southern British Columbia chum salmon resolved with either an area-specific baseline (Fraser: 6 stocks; ECVI: 7 stocks; WCVI: 3 stocks) or an 18-stock baseline of southern stocks in which distributions of the allele frequencies or band counts in the baseline stocks were fixed or resampled as outlined in Table 4. The area sum is the total percentage allocated to all area stocks when the 18-stock regional baseline was used to resolve the mixtures. Standard deviation are given in parentheses. Abbreviations for geographic regions are defined in Table 1.

		Fixed			Resampled		
	Actual	Area baseline	Regional baseline	Area sum	Area baseline	Regional baseline	Area sum
Fraser River							
Single-stock mixtures							
Wahleach	100	95.5 (2.4)	95.5 (2.0)	100.0 (0.0)	93.9 (6.3)	87.1 (9.3)	93.1 (7.3)
Alouette	100	95.5 (2.4)	95.5 (2.4)	100.0 (0.0)	96.6 (3.6)	87.7 (7.9)	91.2 (7.9)
Chehalis	100	95.0 (2.9)	95.6 (2.0)	100.0 (0.0)	91.3 (6.7)	82.3 (8.6)	87.9 (8.6)
Chilliwack	100	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	98.4 (3.1)	85.4 (7.6)	87.4 (6.4)
Inch	100	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	95.1 (6.2)	93.0 (9.3)	93.8 (8.3)
Stave	100	92.0 (2.3)	92.0 (2.6)	100.0 (0.0)	88.6 (6.2)	82.2 (9.9)	91.8 (8.4)
Multi-stock mixture							
Wahleach	10	9.5 (0.7)	9.7 (0.5)	100.0 (0.0)	10.0 (2.6)	9.0 (2.8)	88.8 (5.1)
Alouette	20	19.1 (0.9)	18.9 (0.8)		17.5 (3.4)	16.0 (2.8)	
Chehalis	30	27.7 (1.4)	27.6 (1.3)		26.9 (4.7)	23.7 (3.9)	
Chilliwack	40	43.7 (1.8)	43.7 (1.6)		45.3 (4.8)	39.3 (5.3)	
ECVI							
Multi-stock mixture							
Cowichan	10	10.0 (0.0)	9.5 (0.6)	94.0 (2.8)	9.1 (2.1)	8.7 (2.1)	92.8 (3.8)
Nanaimo	20	20.0 (0.0)	18.8 (1.1)		18.2 (3.4)	16.4 (3.8)	
Little Qualicum	30	29.3 (0.7)	28.9 (0.8)		26.2 (3.6)	26.6 (4.2)	
Big Qualicum	40	40.7 (0.7)	36.8 (2.1)		46.3 (4.8)	42.0 (5.5)	
WCVI							
Multi-stock mixture							
Demamiel	20	20.0 (0.0)	19.3 (0.9)	98.2 (1.2)	18.4 (3.2)	17.0 (3.6)	89.0 (5.0)
Conuma	30	30.0 (0.0)	30.0 (0.0)		27.5 (4.3)	24.5 (3.2)	
Nitinat	50	50.0 (0.0)	48.9 (0.9)		54.1 (5.1)	47.6 (5.4)	

no marked distinction among Fraser River stocks (Beacham et al., 1987). Minisatellite DNA variation may provide the means to discriminate among Fraser River stocks if necessary in management applications. In the Yukon River drainage, summer-run stocks were well differentiated from fall-run stocks by analysis of variation at protein-coding loci (Wilmot et al., 1992, 1994). However, in the fall-run stocks, which spawn in rivers in both Alaska and the Yukon Territory, some stocks spawning in Alaskan rivers (Sheenjek and Chandalar rivers) were more similar to stocks spawning in the Yukon Territory rivers in Canada than they were to other Alaskan fall-run stocks. The addition of minisatellite DNA variation to techniques of stock discrimination may provide more accurate estimates of stock composition in mixed stocks (e.g. Canadian and American stocks).

Compared with some other methods of stock identification that are based upon biological tags, genetic-based methods of stock identication generally have the advantage of temporal stability of the characters used. For example, variation at protein-coding loci has usually been found to be temporally stable (Beacham et al., 1985, 1987; Kondzela et al., 1994; Wilmot et al., 1994), and similar results have been reported for minisatellite loci (Taylor et al., 1994; Beacham et al., 1996). The two-year temporal stability of allelic frequencies at the *Ssa*-A33 and *Ssa*-A34 loci observed in the current study suggests that annual sampling of contributing baseline stocks would not be required in order to estimate stock composition of appropriate mixtures. Because the populations sampled in this study tended to be major ones (escapement of British Columbia populations is usually >10,000 fish), the ratio of sample size to effective population size was less than 0.1, and the probability of a significant test to detect temporal variation is similar to the presumed level of significance (Waples, 1989).

Table 8

Percentage of correct classification of individual fish with discriminant analyses for 39 stocks of chum salmon. The percentage of fish that were correctly classified to stock is indicated, as is the percentage of fish classified to other stocks in the same and other regions. Results listed are for jackknife samples, in which the fish tested were not included in the sample used to derive the discriminant functions. The regional percentage correct is the sum of the percentage of fish correctly classified to stock and the percentage classified to other stocks within the same region. n = the number of fish classified in each stock. Abbreviations for geographic regions are defined in Table 1.

Stock	n	Correct	Japan	Russia	Yukon	North	ECVI	WCVI	Fraser	Mainland	Regional
Chitose	46	19.6	58.7	2.2	6.5	6.5	0.0	0.0	4.3	2.2	78.3
Tokachi	47	10.6	76.7	2.1	2.1	2.1	2.1	0.0	2.1	2.1	87.3
Miomoto	16	0.0	87.5	6.3	6.3	0.0	0.0	0.0	0.0	0.0	87.5
Ohkawa	8	0.0	62.5	0.0	25.0	0.0	0.0	0.0	0.0	12.5	62.5
Shibetsu	14	14.3	50.0	0.0	21.4	7.1	7.1	0.0	0.0	0.0	64.3
Teshio	8	37.5	50.0	0.0	12.5	0.0	0.0	0.0	0.0	0.0	87.5
Tokoro	10	30.0	60.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	90.0
Honoari	48	14.6	66.7	0.0	4.2	4.2	4.2	0.0	6.2	0.0	81.3
Abashiri	47	17.0	66.0	4.2	6.4	2.1	2.1	0.0	2.1	0.0	83.0
Japan	244	15.2	66.4	2.0	6.6	3.3	2.0	0.0	2.8	1.6	81.6
Anadyr	25	40.0	0.0	12.0	20.0	12.0	0.0	0.0	16.0	0.0	52.0
Kanchalan	39	25.6	5.1	18.0	25.6	7.7	10.2	0.0	7.7	0.0	43.6
Ola	15	26.7	20.0	20.0	20.0	0.0	6.7	0.0	6.7	0.0	46.7
Russia	79	30.4	6.3	16.5	22.8	7.6	6.3	0.0	10.1	0.0	46.9
Andreafsky	28	39.3	7.1	21.4	21.4	3.6	3.6	0.0	0.0	3.6	60.7
Kluane	27	44.4	7.4	7.4	37.0	0.0	0.0	0.0	3.7	0.0	81.4
Fishing Branch	28	10.7	3.6	14.3	64.3	7.1	0.0	0.0	0.0	0.0	75.0
Sheenjek	28	17.9	7.1	21.4	50.0	0.0	0.0	0.0	3.6	0.0	67.9
Tatchun	28	14.3	0.0	7.1	75.0	0.0	0.0	0.0	3.6	0.0	89.3
Yukon	139	25.2	5.0	14.4	49.6	2.1	0.7	0.0	2.2	0.7	74.8
Pallant	50	18.0	0.0	4.0	4.0	32.0	8.0	12.0	20.0	2.0	50.0
Fish Creek	46	10.9	4.4	0.0	10.9	37.0	8.7	10.9	10.9	6.1	47.9
Breezy Bay	40	27.5	2.5	5.0	12.5	10.0	10.0	12.5	10.0	10.0	37.5
Atnarko	24	29.2	0.0	4.2	8.3	41.7	0.0	4.2	12.5	0.0	70.9
North	160	20.0	1.9	3.1	8.9	29.4	7.5	10.6	13.8	4.9	49.4
Big Qualicum	58	8.5	0.0	1.7	1.7	16.9	37.3	8.5	23.7	1.7	45.8
Chemainus	24	16.7	0.0	0.0	0.0	4.2	37.5	4.2	37.5	0.0	54.2
Cowichan	22	4.6	4.6	4.6	4.6	13.7	31.8	4.6	22.7	9.1	36.4
Goldstream	23	13.0	4.4	0.0	8.7	13.1	30.5	13.1	13.1	4.4	43.5
Little Qualicum	24	37.5	0.0	0.0	0.0	12.5	16.7	0.0	33.3	0.0	54.2
Nanaimo	24	0.0	4.2	4.2	4.2	20.8	29.2	0.0	20.8	16.7	29.2
Puntledge	25	16.0	4.0	0.0	0.0	12.0	24.0	16.0	20.0	8.0	40.0
ECVI	200	13.0	2.0	1.5	2.5	13.9	30.8	7.0	24.4	5.0	43.8
Demamiel	23	17.4	0.0	0.0	0.0	4.4	26.1	17.4	34.8	0.0	34.8
Nitinat	47	23.4	0.0	4.3	6.4	23.4	19.2	2.1	14.9	6.4	25.5
Conuma	22	0.0	4.6	0.0	9.1	31.8	9.1	13.6	27.3	4.6	13.6
WCVI	92	16.3	1.1	2.2	5.4	20.7	18.5	8.7	22.8	4.4	25.0
Alouette	23	21.7	0.0	0.0	8.7	8.7	21.7	4.4	21.7	13.0	43.4
Chehalis	24	8.3	4.2	4.2	8.3	16.7	20.9	8.3	20.8	8.3	29.1
Chilliwack	48	16.7	0.0	2.1	18.8	12.5	18.8	6.3	16.7	8.3	33.4
Inch	21	0.0	0.0	4.8	14.3	4.8	47.6	4.8	19.1	4.8	19.1
Stave	21	4.8	4.8	0.0	0.0	28.6	28.6	0.0	33.1	0.0	37.9
Wahleach	22	4.6	0.0	13.7	0.0	7.6	27.3	18.2	18.2	13.6	22.8
Fraser	159	10.7	1.3	3.8	10.1	12.6	25.8	6.9	20.7	8.2	31.4
Mamquam	24	0.0	4.2	0.0	8.3	25.0	16.7	4.2	33.3	8.3	8.3
Tzoonie	22	9.1	9.1	0.0	0.0	18.2	9.1	9.1	45.5	0.0	9.1
Mainland	46	4.5	6.7	0.0	4.1	21.6	12.9	6.6	39.4	4.2	8.7

The simulations suggest that relatively accurate estimates of stock composition may be possible should minisatellite DNA variation be applied to practical fisheries management issues but that identification of individual fish to specific regions, particularly in North American stocks surveyed, is less accurate. For example, estimated Fraser River stock composition of pure samples of Fraser River chum salmon ranged from 87 to 93% when resolved with an 18-stock resampled southern British Columbia baseline, but only about 31% of individual Fraser River fish were correctly identified as of Fraser River origin when a 39-stock North Pacific baseline was used. About 26% of the Fraser River individuals were classified as originating from the east coast of Vancouver Island, a result which may seem in conflict with the greater separation observed between Fraser River and ECVI stocks in the estimation of mixture composition. However, estimation of stock composition of a mixture and classification of individual fish in the mixture are separate problems, with classification of individual fish the more difficult problem. Data from only a single fish that is to be identified are considered in classification problems, but data from all fish in the mixture as a whole are considered when estimates of stock composition are determined. Higher levels of accuracy (99%) have been observed previously in estimation of chum salmon composition by using the entire mixture sample rather than by classifying individual fish in the same mixture to region of origin (<60%) (Fournier et al., 1984).

Although analysis of minisatellite DNA variation may be an effective method for estimation of stock composition of chum salmon, a number of improvements would be required before it could be applied (either by itself or as an enhancement) to existing stock identification techniques for chum salmon. Determination of the allelic frequency distributions or band counts at the minisatellite loci for all stocks contributing significantly to fisheries, as well as ensurance that sampling of the contributing populations is adequate to represent samples of the stock that have been collected, would be required.

Genetic variation provides a powerful means to estimate stock composition of chum salmon catches. The particular method used to screen variation will depend on the issue to be resolved. Estimation of stock composition to large regional groups could be conducted with analysis of variation at protein-coding loci, in mitochondrial DNA, in minisatellite DNA, or in the major histocompatability complex (MHC) genes (Miller and Withler, in press). Estimation of local stock composition to a fine scale, such as to tributaries of a major river system, will likely require direct measurement of DNA variation. Surveys of variation at minisatellite loci provide one possibility of achieving fine-scale estimation of stock composition, but analysis of variation at other minisatellite loci, in addition to those utilized in the current manuscript, will be required. No surveys of population variation at microsatellite loci for chum salmon have been conducted yet, although there may be great potential in their application (e.g. Brooker et al., 1994). Given the relative ease of laboratory analysis of variation at microsatellite loci, compared with that at minisatellite loci, microsatellite loci may be more practical than minisatellite loci, on a routine basis, for stock-identification analysis.

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