

Abstract—Population structure of pink salmon (*Oncorhynchus gorbuscha*) from British Columbia and Washington was examined with a survey of microsatellite variation to describe the distribution of genetic variation. Variation at 16 microsatellite loci was surveyed for approximately 46,500 pink salmon sampled from 146 locations in the odd-year broodline and from 116 locations in the even-year broodline. An index of genetic differentiation, F_{ST} , over all populations and loci in the odd-year broodline was 0.005, with individual locus values ranging from 0.002 to 0.025. Population differentiation was less in the even-year broodline, with a F_{ST} value of 0.002 over all loci, and with individual locus values ranging from 0.001 to 0.005. Greater genetic diversity was observed in the odd-year broodline. Differentiation in pink salmon allele frequencies between broodlines was approximately 5.5 times greater than regional differentiation within broodlines. A regional structuring of populations was the general pattern observed, and a greater regional structure in the odd-year broodline than in the even-year broodline. The geographic distribution of microsatellite variation in populations of pink salmon likely reflects a distribution of broodlines from separate refuges after the last glaciation period.

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Population structure of pink salmon (*Oncorhynchus gorbuscha*) in British Columbia and Washington, determined with microsatellites

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Pink salmon (*Oncorhynchus gorbuscha*) spawn in more than 700 rivers in British Columbia (Aro and Shepard, 1967), and the distribution of spawning populations varies in odd-numbered years and even-numbered years. Spawning occurs primarily only in odd years in the Fraser River drainage, mainly in even years on the Queen Charlotte Islands, and in both years in central and northern coastal areas of British Columbia (Neave, 1952). Because virtually all pink salmon mature at two years of age (Bilton and Ricker, 1965), two distinct broodlines in the species have developed (odd-year and even-year), with virtually no gene flow between the broodlines. This reproductive isolation of the broodlines has resulted in reported differences in body size (Godfrey, 1959), morphological characteristics (Ricker, 1972; Beacham, 1985), timing of spawning (Aro and Shepard, 1967; Dyagilev and Markevich, 1979), and genetic differentiation (Aspinwall, 1974; Beacham et al., 1988). Evaluation of the genetic population structure both between and within broodlines in British Columbia and Washington form the basis of the current study.

Estimation of genetic population structure has been a key area of research in salmon assessment and management. Identification of a genetically distinct group of populations in the distribution of a species is a key step in conserving and maintaining genetic diversity. Genetically distinct populations or regional groups of populations (stocks) were determined through surveys of genetic variation to evaluate the population structure of a species (Shaklee and Bentzen, 1998). Identification of genetically distinct groups in the distribution of pink salmon in British Columbia and Puget Sound may lead to the conservation of genetic diversity by fisheries and resource management. An evaluation of genetic variation is effective in describing the population structure of salmonids, is a key part in the elucidation of management units or conservation units for a species and can be applied to manage fisheries exploiting specific stocks of salmon.

Determination of genetic population structure is an important part in developing a genetically based method for estimation of stock composition. Allozymes were initially the key set of genetic markers used in evaluat-

ing population structure in pink salmon in British Columbia (Beacham et al., 1988; Shaklee et al., 1991) and elsewhere in the Pacific Rim distribution of the species (Aspinwall, 1974; Varnavskaya and Beacham, 1992; Shaklee and Varnavskaya, 1994; Noll et al., 2001; Hawkins et al., 2002). Analysis of allozyme variation provided the general pattern of marked differentiation between the broodlines, but identification of fine-scale regional structure was limited, although estimation of stock composition of samples from mixed-stock fisheries was conducted (Beacham et al., 1985; Shaklee et al., 1991). Development of DNA markers has led to new avenues of research for using genetic variation in defining population structure. Mitochondrial DNA variation again showed strong differentiation between the broodlines, but differentiation among regional groups of pink salmon was limited (Brykov et al., 1996, 1999; Churikov and Gharrett, 2002). However, Golovanov et al. (2009) noted that differentiation among even-year populations was higher than among odd-year populations in the northern Sea of Okhotsk region.

Microsatellites are reported to be useful for evaluating fine-scale population structure in salmonids (Banks et al., 2000) and have been used to evaluate large-scale and regional variation in chum salmon (*O. keta*) (Beacham et al., 2009). Initial applications of microsatellite variation to evaluate individual identification and population structure were reported by Olsen et al. (1998, 2000a). However, surveys of population variation were quite limited in these studies, and no comprehensive evaluation of variation at microsatellites has been conducted for pink salmon. A survey of microsatellite variation over a broader geographic range of pink salmon distribution would likely be valuable for evaluating population structure.

In the current study, we outline the microsatellite-based population structure of pink salmon in British Columbia and Washington as an initial step in evaluating whether higher resolution in estimation of stock composition may be possible when compared with estimates previously derived with allozymes. This objective was accomplished by analyzing variation at 16 microsatellite loci to evaluate relationships in population structure of pink salmon, as well as by analyzing regional differences in allelic variation. The distribution of genetic diversity among broodlines, regions, and populations was estimated in the study, as well as the stability of population structure.

Materials and methods

Collection of DNA samples and laboratory analysis

Tissue samples were collected from mature pink salmon. Samples were preserved in 95% ethanol, and sent to the Molecular Genetics Laboratory at the Pacific Biological Station of Fisheries and Oceans Canada. DNA was extracted from the tissue samples by using a variety of methods, including a chelex resin protocol outlined

Table 1

Microsatellite loci surveyed in pink salmon (*Oncorhynchus gorbuscha*) and their associated annealing and extension temperatures and times (seconds), as well as the number of cycles used in polymerase chain reaction amplifications.

Locus	Annealing	Extension	Cycles
<i>Oki10</i>	53°C/30s	70°C/30s	39
<i>Oki101</i>	53°C/45s	68°C/30s	40
<i>One101</i>	50°C/30s	70°C/30s	39
<i>One102</i>	50°C/30s	70°C/30s	39
<i>One104</i>	50°C/30s	70°C/30s	36
<i>One109</i>	55°C/30s	70°C/30s	34
<i>One111</i>	55°C/30s	70°C/30s	34
<i>One114</i>	50°C/30s	70°C/45s	38
<i>Ots213</i>	52°C/45s	72°C/60s	38
<i>Ots7e</i>	51°C/30s	72°C/30s	35
<i>OtsG253b</i>	60°C/45s	72°C/45s	35
<i>OtsG311</i>	50°C/45s	68°C/45s	34
<i>OtsG68</i>	50°C/30s	70°C/30s	36
<i>Ssa407</i>	60°C/30s	70°C/30s	39
<i>Ssa408</i>	60°C/45s	70°C/45s	40
<i>Ssa419</i>	50°C/30s	70°C/30s	40

by Small et al. (1998), a Qiagen 96-well DNeasy[®] procedure (Qiagen, Mississauga, Ontario), or a Promega Wizard SV96 Genomic DNA Purification system (Promega, Madison, WI). Once extracted DNA was available, surveys of variation at 16 microsatellite loci were conducted: *Oki10* (Smith et al., 1998), *Oki101* (Beacham et al., 2011), *OtsG68*, *OtsG253b*, *OtsG311* (Williamson et al., 2002), *Ots213* (Greig et al., 2003), *Ots7e* (Wright et al., 2008), *One101*, *One102*, *One104*, *One109*, *One111*, *One114* (Olsen et al., 2000b), *Ssa407*, *Ssa408*, *Ssa419* (Cairney et al., 2000)

In general, polymerase chain reaction (PCR) DNA amplifications were conducted by using a DNA Engine Cycler Tetrad2 (BioRad, Hercules, CA) in 6- μ L volumes consisting of 0.15 units of Taq polymerase, 1- μ L of extracted DNA, 1 \times PCR buffer (Qiagen), 60 μ M each nucleotide, 0.40 μ M of each primer, and deionized water. The thermal cycling profile involved one cycle of Taq activation for 15 minutes at 95°C, followed by a denaturation cycle of 30 seconds at 94°C, with annealing and extension conditions for each locus as outlined in Table 1. PCR fragments were initially size fractionated in denaturing polyacrylamide gels with an ABI 377 automated DNA sequencer, and genotypes were scored by Genotyper, vers. 2.5 software (Applied Biosystems, Foster City, CA) by using an internal lane sizing standard. Later in the study, microsatellites were size fractionated in an ABI 3730 capillary DNA sequencer, and

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Table 2

Pink salmon (*Oncorhynchus gorbuscha*) spawning regions, number of populations sampled, years when sampling occurred, average number of fish sampled per population per year over all populations within a region, and average total number of fish sampled (N) per population within the region for 146 odd-year and 116 even-year populations in 15 geographic regions (Fig. 2). Listing of populations in each region as well as allele frequencies for all population samples surveyed in this study are available at the Molecular Genetics Laboratory website <http://www.pac.dfo-mpo.gc.ca/science/facilities-installations/pbs-sbp/mgl-lgm/data-donnees/index-eng.htm>. Values shown are averages with the ranges of samples sizes in parentheses.

Region	Populations	Years	Average annual population sample size	Average population size (N)
Washington (odd)	12	1995–2009	110 (44–498)	257 (98–755)
Fraser River-upper (odd)	9	1987–2009	95 (69–102)	201 (100–463)
Fraser River-lower (odd)	6	1987–2009	87 (50–100)	245 (98–463)
East Coast Vancouver Island (odd)	10	1987–2009	108 (85–219)	173 (85–397)
South Coast British Columbia (odd)	13	1987–2009	105 (25–200)	169 (38–390)
Central Coast British Columbia (odd)	59	2003–2009	111 (2–227)	164 (32–394)
Skeena River (odd)	10	2003–2007	163 (67–229)	228 (123–393)
North Coast British Columbia (odd)	25	2003–2009	151 (9–233)	211 (87–381)
Queen Charlotte Islands (odd)	2	2005	210 (200–219)	210 (200–219)
East Coast Vancouver Island (even)	2	2006–2008	83 (50–113)	124 (85–163)
South Coast British Columbia (even)	11	2002–2010	119 (27–237)	162 (47–452)
Central Coast British Columbia (even)	50	2002–2010	96 (5–202)	133 (18–312)
Skeena River (even)	7	2002–2006	162 (63–228)	208 (100–381)
North Coast British Columbia (even)	25	2002–2010	113 (3–215)	190 (24–425)
Queen Charlotte Islands (even)	21	2002–2006	135 (32–200)	176 (32–364)

genotypes were scored by GeneMapper software, vers. 3.0 (Applied Biosystems) by using an internal lane sizing standard. Allele identification between the two sequencers was standardized by analyzing approximately 600 individuals on both platforms and converting the sizing in the gel-based data set to match that obtained from the capillary-based set.

Data analysis

All annual samples available for a location were combined to estimate population allele frequencies, as is recommended by Waples (1990). Each population in each broodline at each locus was tested for departure from Hardy-Weinberg equilibrium of genotypic frequencies by using the software Genetic Data Analysis (GDA; Univ. of Connecticut, Storrs, CT). Critical significance levels for simultaneous tests were evaluated by using Bonferroni adjustment for each broodline separately (odd-year broodline: $0.05/146=0.00034$, even-year broodline $0.05/116=0.00043$; Rice, 1989). Weir and Cockerham's (1984) F_{ST} estimates for each locus over all populations were calculated with FSTAT, vers. 2.9.3.2 (Goudet, 1995). The significance ($P<0.05$) of the multilocus F_{ST} value over all samples was determined by jackknifing over loci. Populations were combined into 14 regional groups in order to develop a practical method to display mean pairwise F_{ST} values between regions, as well as the mean number of alleles observed per locus in each region. These 14 regional groups were constructed from the 15 regional groups outlined in Table 2 by combining the upper and lower Fraser River regions into a

single region, with other regions remaining as outlined in Table 2. Broodlines were separated in odd-year and even-year spawning lines. Geographic areas were outlined in Figure 1. The 14 regional groups correspond to the geographic areas and broodlines as follows: 1) Washington odd-year; 2) Fraser River (upper-lower) odd-year; 3) east coast Vancouver Island odd-year; 4) south coast British Columbia odd-year; 5) central coast British Columbia odd-year; 6) Skeena River odd-year; 7) north coast British Columbia odd-year; 8) Queen Charlotte Islands odd-year; 9) east cCoast Vancouver Island even-year; 10) south coast British Columbia even-year; 11) central coast British Columbia even-year; 12) Skeena River even-year; 13) north coast British Columbia even-year; 14) Queen Charlotte Islands even-year. Individual populations remained discrete within these larger regional groups for determination of pairwise F_{ST} values.

Genotypic disequilibrium and potential genetic linkage among loci were tested with GDA with 1500 iterations per test. The number of pairs of loci exhibiting potential linkage was summed for the 146 populations sampled in the odd-year broodline and 116 populations sampled in the even-year broodline (Table 2). Statistical significance was evaluated by using a Bonferroni adjustment as outlined previously. FSTAT was used to measure the "allelic richness" (allelic diversity standardized to a sample size of 240 fish per region) for the 14 regional groups of populations. Computation of the number of alleles observed per locus was carried out with GDA. Cavalli-Sforza and Edwards (CSE) chord distance (1967) was used to estimate

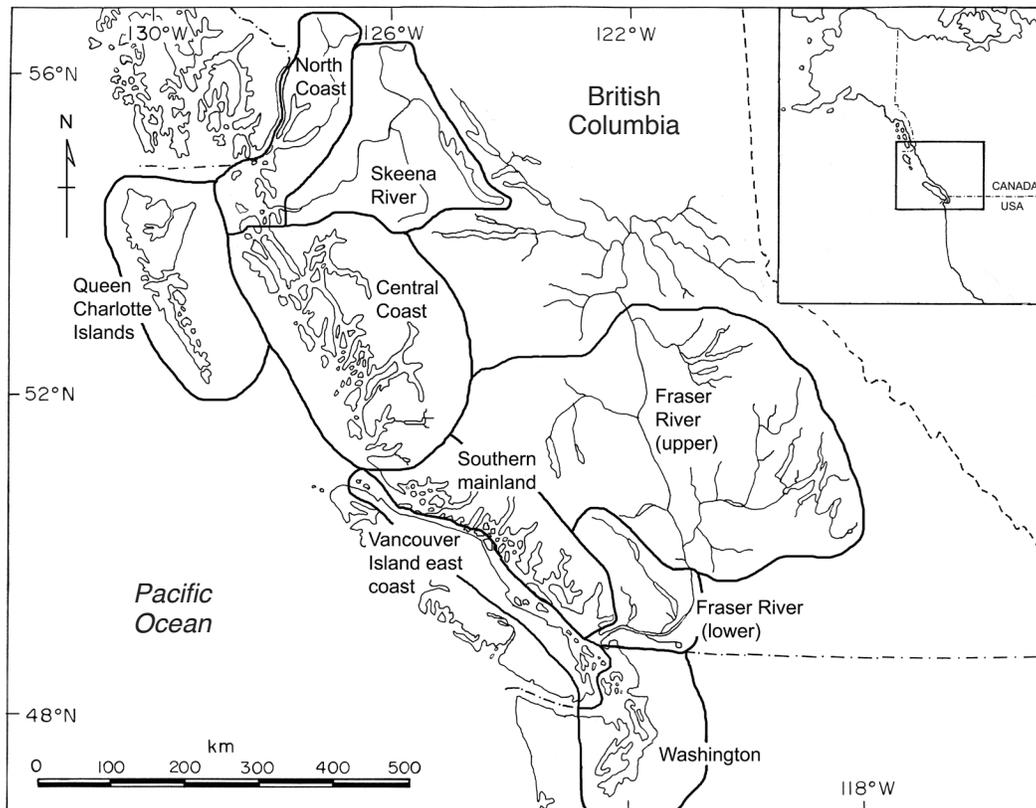


Figure 1

Map of British Columbia and northern Washington coasts indicating the general geographic regions where pink salmon (*Oncorhynchus gorbuscha*) from 146 odd-year and 116 even-year populations were surveyed, with the regions listed in Table 2. The regions depicted in the figure are the following: 1 Washington; 2 lower Fraser River; 3 upper Fraser River; 4 east coast Vancouver Island (ECVI); 5 southern British Columbia; 6 central coast; 7 Skeena River; 8 north coast; and 9 Queen Charlotte Islands. In 6 of these regions (ECVI, southern British Columbia mainland, central coast, Skeena River, north coast, Queen Charlotte Islands), pink salmon spawn in both even and odd years. Because 6 regions have both broodlines present, the map encompasses 12 regional groups of populations when even and odd populations are separated. The other 3 regional groups (upper and lower Fraser River, and Washington) have pink salmon only in odd years. This summary accounts for the 15 regional groups outlined in Table 2.

genetic distances among all populations. An unrooted neighbor-joining tree based upon CSE was generated with NJPLOT (Perriere and Gouy, 1996). Bootstrap support for the major nodes in the tree was evaluated with the CONSENSE program from PHYLIP based upon 500 replicate trees (Felsenstein, 1993). The distribution of genetic variation in pink salmon was evaluated with a gene diversity analysis with the analysis structured between broodlines, among regions within broodlines, and among populations within regions. The analysis was conducted with GDA, which will support a maximum of three nested levels of variation in addition to the error mean square. All populations outlined in Table 2 were included in the analysis. Allele frequencies for all location samples surveyed in this study are available at the Molecular Genetics Laboratory website <http://www.pac.dfo-mpo.gc.ca/science/facilities-installations/pbs-sbp/mgl-igm/data-donnees/index-eng.htm>.

Results

Variation within populations

Variation was displayed in the number of observed alleles at the 16 microsatellite loci surveyed in the study. The fewest number of alleles was observed at *Ots7e* (12 alleles odd-year broodline, 13 alleles even-year broodline), and the greatest number of alleles was observed at *Oki10* (85 alleles odd-year broodline, 83 alleles even-year broodline) (Table 3). Heterozygosities were generally above 90%, with notable exceptions observed at *Ots7e* (both broodlines) and *One111* (odd-year only). Genotypic frequencies at all 16 loci surveyed typically conformed to those expected under Hardy-Weinberg equilibrium (HWE) for populations in both broodlines. Greater overall population differentiation was observed in the odd-year broodline ($F_{ST} = 0.005$) than in the even-year broodline ($F_{ST} = 0.002$).

Table 3

Number of alleles per locus, an index of genetic differentiation F_{ST} (standard deviation in parentheses), expected heterozygosity (H_e), observed heterozygosity (H_o), and percent significant Hardy-Weinberg equilibrium (HWE) tests for 16 microsatellite loci among 146 odd-year and 116 even-year pink salmon (*Oncorhynchus gorbuscha*) populations.

Locus	Number of alleles	F_{ST}	H_e	H_o	HWE
Odd-year broodline					
<i>Oki10</i>	85	0.004 (0.000)	0.94	0.94	0.0
<i>Oki101</i>	83	0.002 (0.000)	0.97	0.96	0.7
<i>One101</i>	52	0.003 (0.000)	0.96	0.95	0.7
<i>One102</i>	32	0.006 (0.001)	0.92	0.91	0.0
<i>One104</i>	40	0.007 (0.001)	0.95	0.94	0.0
<i>One109</i>	32	0.005 (0.001)	0.90	0.90	0.0
<i>One111</i>	36	0.012 (0.001)	0.72	0.71	0.0
<i>One114</i>	49	0.003 (0.000)	0.96	0.95	2.1
<i>Ots213</i>	69	0.003 (0.001)	0.97	0.95	2.8
<i>Ots7e</i>	12	0.025 (0.003)	0.45	0.45	0.0
<i>OtsG253b</i>	46	0.005 (0.001)	0.95	0.94	1.4
<i>OtsG311</i>	34	0.009 (0.001)	0.89	0.88	1.4
<i>OtsG68</i>	34	0.004 (0.001)	0.93	0.93	0.0
<i>Ssa407</i>	73	0.006 (0.001)	0.94	0.94	0.0
<i>Ssa408</i>	69	0.002 (0.000)	0.97	0.95	6.2
<i>Ssa419</i>	66	0.003 (0.000)	0.96	0.93	7.5
Total		0.005 (0.001)			
Even-year broodline					
<i>Oki10</i>	83	0.001 (0.000)	0.94	0.93	1.8
<i>Oki101</i>	73	0.002 (0.000)	0.97	0.96	0.9
<i>One101</i>	65	0.001 (0.000)	0.96	0.95	1.8
<i>One102</i>	35	0.002 (0.000)	0.93	0.93	0.0
<i>One104</i>	36	0.004 (0.000)	0.92	0.92	0.0
<i>One109</i>	29	0.003 (0.000)	0.91	0.90	0.0
<i>One111</i>	38	0.004 (0.001)	0.92	0.91	2.6
<i>One114</i>	42	0.002 (0.000)	0.96	0.95	0.0
<i>Ots213</i>	71	0.002 (0.000)	0.97	0.97	5.1
<i>Ots7e</i>	13	0.005 (0.001)	0.58	0.58	1.8
<i>OtsG253b</i>	48	0.002 (0.000)	0.95	0.94	3.5
<i>OtsG311</i>	34	0.003 (0.000)	0.93	0.91	0.0
<i>OtsG68</i>	32	0.003 (0.000)	0.93	0.93	0.0
<i>Ssa407</i>	77	0.002 (0.000)	0.96	0.93	2.6
<i>Ssa408</i>	69	0.002 (0.000)	0.97	0.95	1.8
<i>Ssa419</i>	66	0.002 (0.000)	0.97	0.95	2.6
Total		0.002 (0.000)			

Individual locus F_{ST} values ranged from 0.002 to 0.012 for the odd-year broodline, and between 0.001 and 0.005 for the even-year broodline. For the odd-year broodline, individual pairs of loci displayed potential linkage of between 0% and 28% (*Ssa408* and *Ssa419*) of the populations surveyed. In the even-year broodline, individual pairs of loci displayed potential linkage of between 0% and 13% (*Ssa407* and *Ssa419*) of the populations surveyed. Potential linkage among loci was higher in the *Ssa* series of loci than that observed in the other microsatellites, but different patterns were observed within the broodlines. Potential linkage among the *Ssa* loci was not judged to

be at a level that required removal from subsequent analyses.

The number of alleles observed displayed variation among the regional groups of pink salmon surveyed. With the number of alleles observed standardized to a sample size of 240 individuals per region for both broodlines, the odd-year broodline populations with the fewest number of observed alleles originated from Washington (486 alleles), whereas populations with the greatest number of alleles originated from the northern coastal region of British Columbia (551 alleles) (Table 4). In the even-year broodline, populations with the fewest numbers of alleles originated from the east

Table 4

Mean number of alleles observed per locus at 16 microsatellite loci for pink salmon (*Oncorhynchus gorbuscha*) from 14 geographic broodline areas standardized to a sample size of 240 fish per geographic area. Regional groups (see Table 2), are as follows: 1) Washington odd-year, 2) Fraser River (upper+ lower) odd-year, 3) east coast Vancouver Island odd-year, 4) south coast British Columbia odd-year, 5) central coast British Columbia odd-year, 6) Skeena River odd-year, 7) north coast British Columbia odd-year, 8) Queen Charlotte Islands odd-year, 9) east coast Vancouver Island even-year, 10) south coast British Columbia even-year, 11) central coast British Columbia even-year, 12) Skeena River even-year, 13) north coast British Columbia even-year, 14) Queen Charlotte Islands even-year. The two Fraser River regions in Table 2 were combined for the analysis.

Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Odd	Even
<i>Oki10</i>	30.77	33.14	41.59	40.84	47.21	40.53	48.42	44.81	26.93	31.14	38.21	36.03	43.31	35.67	40.91	35.21
<i>Oki101</i>	50.95	53.09	54.73	55.30	55.83	51.38	54.65	55.79	49.88	48.04	51.90	52.11	55.65	48.74	53.96	51.05
<i>One101</i>	35.30	37.33	35.93	37.70	36.39	36.18	36.36	35.78	35.78	36.66	39.15	36.55	39.41	39.42	36.37	37.83
<i>One102</i>	22.73	22.25	23.22	23.45	24.44	20.79	24.40	23.99	23.94	25.73	25.53	26.08	26.16	25.68	23.16	25.52
<i>One104</i>	30.29	31.81	30.85	31.78	30.64	28.23	31.10	30.49	29.89	27.95	30.04	24.63	30.40	27.40	30.65	28.39
<i>One109</i>	17.31	18.00	17.51	19.13	19.62	20.18	20.23	18.90	16.00	18.00	18.60	18.54	19.33	16.98	18.86	17.91
<i>One111</i>	19.79	21.45	21.52	21.81	21.01	19.73	21.24	20.54	21.97	24.05	25.27	22.29	23.51	22.40	20.88	23.25
<i>One114</i>	37.24	39.89	38.61	38.84	37.30	34.99	36.19	35.26	30.00	32.23	34.74	35.46	35.78	34.58	37.29	33.80
<i>Ots213</i>	43.25	42.92	45.32	49.90	51.23	50.38	51.77	50.54	43.91	50.37	50.34	48.99	51.27	46.15	48.16	48.51
<i>Ots7e</i>	5.79	5.90	7.00	6.43	7.68	7.12	7.80	6.48	4.00	5.84	5.82	6.17	7.27	4.92	6.77	5.67
<i>OtsG311</i>	16.65	15.91	17.97	18.32	20.50	20.44	20.24	21.25	20.00	21.41	21.06	18.75	21.49	19.69	18.91	20.40
<i>OtsG68</i>	24.95	25.11	25.12	24.44	26.38	27.07	26.01	23.80	20.98	21.94	21.99	20.99	25.23	21.14	25.36	22.04
<i>OtsG253b</i>	33.96	33.88	34.08	35.64	33.92	31.77	34.03	32.57	32.83	34.17	35.74	34.44	36.26	33.12	33.73	34.43
<i>Ssa407</i>	27.87	27.35	31.41	31.74	33.37	31.83	32.28	25.33	47.90	48.86	52.42	51.36	51.01	49.25	30.15	50.13
<i>Ssa408</i>	52.54	53.04	54.75	51.62	52.89	49.94	54.09	52.31	49.90	48.73	54.88	52.09	55.73	51.32	52.65	52.11
<i>Ssa419</i>	36.33	40.19	49.00	46.13	53.07	47.58	52.36	50.93	48.00	46.94	47.97	44.46	50.95	44.42	46.95	47.12
Total	485.69	501.24	528.60	533.04	551.46	518.14	551.17	528.78	501.90	522.06	553.65	528.92	572.73	520.90	524.76	533.36

coast of Vancouver Island (502 alleles), and those populations with the greatest number of alleles originated from the northern coastal region of British Columbia (573 alleles). The greatest difference in number of alleles between the broodlines was observed at the locus *Ssa407*, with an average of 30 alleles observed at the locus in the odd-year populations, and 50 alleles observed in the even-year populations ($P < 0.01$). Within the odd-year broodline, lower numbers of observed alleles of Washington populations, and to some extent Fraser River populations, compared with other regional groups of populations, were concentrated in the *Oki10* and *Ssa419* loci (Table 4).

Distribution of genetic variance

Gene diversity analysis of the 16 microsatellites surveyed was used to evaluate the distribution of genetic variation between two broodlines, among regions (15 regions, Table 2) within broodlines, and among populations within regions (146 odd-year, 116 even-year populations). The amount of variation within populations ranged from 85.1% (*One111*) to 99.8% (*Oki101*, *One101*), and averaged 98.1% across all loci (Table 5). Variation between the two broodlines accounted for 1.5% of total observed variation and was the largest source of variation after within-population variation. Variation among regions within broodlines was the next largest source of variation and accounted for 0.3% of total observed

variation. Variation among populations within regions accounted for 0.2% of total observed variation. Differentiation between the broodlines was approximately three times greater than any combined regional or population source of variation. Significant broodline differentiation in allele frequencies was observed in 13 of the 16 loci surveyed, with the greatest difference observed at *One111*, which was nine times larger than that observed at any other locus (Table 5). For the geographic range of populations surveyed in the study, broodline differences contributed more to differentiation of allele frequencies than any regional or population source of differentiation.

Population structure

Regional genetic differentiation was observed among pink salmon populations sampled in the different geographic regions. As expected, the largest differences in genetic differentiation were observed between regional groups of populations when compared with populations in the alternate broodline (regional F_{ST} values ranging from 0.014 to 0.036) (Table 6). In the odd-year broodline, the largest average population differentiation was observed in comparisons of populations originating from Washington compared with populations from British Columbia (regional F_{ST} values ranging from 0.011 to 0.016) (Table 6). Within British Columbia, pink salmon in the odd-year broodline originating from the Fraser River were the most genetically distinct

Table 5

Hierarchical gene-diversity analysis of regional F_{ST} values for 262 populations of pink salmon (*Oncorhynchus gorbuscha*) within 15 regions (9 regions in odd-year broodline, 6 regions in even-year broodline) for 16 microsatellite loci, with the regions outlined in Table 2. ** $P < 0.01$ * $P < 0.05$

Locus	Within populations	Among populations within regions	Among regions within broodlines	Between broodlines
<i>Oki10</i>	0.9967	0.0014**	0.0017**	0.0002
<i>Oki101</i>	0.9976	0.0013**	0.0009**	0.0002
<i>One101</i>	0.9976	0.0012**	0.0009**	0.0003
<i>One102</i>	0.9942	0.0021**	0.0023**	0.0014*
<i>One104</i>	0.9782	0.0021**	0.0045**	0.0152**
<i>One109</i>	0.9902	0.0021**	0.0025**	0.0052**
<i>One111</i>	0.8507	0.0022**	0.0057**	0.1414**
<i>One114</i>	0.9944	0.0013**	0.0013**	0.0030**
<i>Ots213</i>	0.9952	0.0019**	0.0012**	0.0017**
<i>Ots7e</i>	0.9207	0.0056**	0.0111**	0.0626**
<i>OtsG253b</i>	0.9955	0.0019**	0.0023**	0.0003
<i>OtsG311</i>	0.9772	0.0023**	0.0047**	0.0158**
<i>OtsG68</i>	0.9896	0.0019**	0.0020**	0.0065**
<i>Ssa407</i>	0.9930	0.0018**	0.0034**	0.0018*
<i>Ssa408</i>	0.9942	0.0013**	0.0009**	0.0036**
<i>Ssa419</i>	0.9949	0.0016**	0.0012**	0.0023**
Total	0.9806	0.0019**	0.0027**	0.0148**

Table 6

Mean pairwise F_{ST} values averaged over 16 microsatellite loci from 14 regional groups of pink salmon (*Oncorhynchus gorbuscha*) outlined in Table 2 that were sampled at 262 locations (146 odd-year, 116 even-year) in British Columbia and Washington. Comparisons were conducted between individual populations in each region. Values in bold on the diagonal represent comparisons among populations within each region. F_{ST} values are listed above the diagonal, and standard deviations are shown below the diagonal. RC is region code, and codes are as follows: 1) Washington odd-year, 2) Fraser River odd-year, 3) east coast Vancouver Island odd-year, 4) southern coast British Columbia odd-year, 5) central coast British Columbia odd-year, 6) Skeena River odd-year, 7) north coast British Columbia odd-year, 8) Queen Charlotte Islands odd-year, 9) east coast Vancouver Island even-year, 10) southern Coast British Columbia even-year, 11) central coast British Columbia even-year, 12) Skeena River even-year, 13) north coast British Columbia even-year, 14) Queen Charlotte Islands even-year.

RC	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0.011	0.011	0.013	0.011	0.013	0.016	0.014	0.015	0.036	0.034	0.032	0.032	0.031	0.034
2	0.010	0.001	0.009	0.006	0.007	0.010	0.008	0.010	0.029	0.027	0.026	0.025	0.025	0.028
3	0.009	0.003	0.006	0.006	0.006	0.008	0.006	0.008	0.025	0.023	0.021	0.021	0.020	0.023
4	0.009	0.002	0.003	0.004	0.003	0.006	0.004	0.006	0.025	0.023	0.021	0.020	0.020	0.024
5	0.009	0.002	0.003	0.002	0.001	0.003	0.001	0.003	0.019	0.017	0.015	0.015	0.014	0.018
6	0.009	0.003	0.003	0.003	0.002	0.001	0.004	0.007	0.019	0.017	0.015	0.015	0.014	0.018
7	0.008	0.003	0.003	0.003	0.002	0.002	0.001	0.003	0.019	0.017	0.015	0.015	0.014	0.018
8	0.009	0.001	0.003	0.003	0.001	0.003	0.002	0.003	0.022	0.020	0.018	0.019	0.018	0.021
9	0.010	0.003	0.006	0.004	0.003	0.003	0.003	0.003	0.008	0.005	0.004	0.006	0.005	0.007
10	0.009	0.003	0.006	0.004	0.003	0.003	0.003	0.003	0.002	0.003	0.002	0.004	0.002	0.005
11	0.009	0.003	0.006	0.004	0.002	0.002	0.002	0.002	0.002	0.003	0.001	0.003	0.001	0.004
12	0.008	0.002	0.005	0.004	0.002	0.002	0.002	0.002	0.002	0.003	0.002	0.002	0.002	0.006
13	0.009	0.002	0.006	0.004	0.002	0.002	0.002	0.002	0.002	0.002	0.001	0.002	0.001	0.004
14	0.009	0.002	0.006	0.004	0.002	0.002	0.002	0.002	0.003	0.002	0.001	0.002	0.001	0.002

group of populations (regional F_{ST} values ranging from 0.006 to 0.010). The least differentiation was observed between populations from the northern and central coastal regions of British Columbia ($F_{ST}=0.001$). In the even-year broodline, the largest regional genetic differentiation was observed between populations from the east coast of Vancouver Island and those from the Queen Charlotte Islands ($F_{ST}=0.007$), whereas the least differentiation between regional groups of populations was observed between the northern and central coastal regions of British Columbia ($F_{ST}=0.001$) (Table 6).

Two distinct lineages of pink salmon were observed in the cluster analysis, and they were clearly based on whether pink salmon spawned in odd-numbered or even-numbered years. All odd-year populations clustered together with 100% bootstrap support, as did all even-year populations (Fig. 2). Within the odd-year broodline, a Washington group of populations was well supported (Washington populations clustered together in 83% of dendrograms evaluated). Within Washington, further geographic subdivision was observed, with populations from the Strait of Juan de Fuca (Gray Wolf River, Dungeness River) clustering with the Hood Canal hatchery population in 100% of dendrograms evaluated. The Hood Canal hatchery population was the most genetically distinct population included in the survey (Fig. 2). The three remaining populations from Hood Canal (Dosewallips River, Hamma Hamma River, and Duckabush River), in addition to the Gray Wolf River, Dungeness River, and Hood Canal hatchery populations, were well separated from other populations in Washington, clustering together in 100% of dendrograms evaluated. Pink salmon populations from the Fraser River in southern British Columbia were a well-defined geographic cluster— all 15 populations clustered together in 98% of dendrograms evaluated. Furthermore, populations in the upper portion of the drainage were separated from those populations in the lower portion of the drainage, with upper populations clustering together in 98% of dendrograms evaluated. Populations from the central portion of the east coast of Vancouver Island (Quinsam River, Puntledge River, Oyster River, Big Qualicum River, and Nanaimo River) clustered together in 100% of dendrograms evaluated, as did 98% of populations from the northern portion of the east coast of Vancouver Island (Keogh River, Quatse River, Cluxewe River). Populations from the northern portion of the South Coast of British Columbia (Kakweiken River, Lull Creek, Ahta Creek, Heydon River, Glendale River) constituted a well-defined group (96% of dendrograms evaluated). Those in the southern portion of the south coast (Cheakamus River, Ashlu River, Mamquam River, Squamish River, Indian River) were not well supported, but displayed some affinity to each other in the cluster analysis (Table 6). All 10 odd-year populations sampled from the Skeena River formed a distinct regional group (50% bootstrap support). Populations sampled from the central coast and north coast regions of British Columbia did not cluster into distinct geographic units. Although some sepa-

ration was observed, genetic differentiation between populations in the two regions was limited, and this was reflected in the lack of consistency in population clustering in the dendrograms evaluated.

Cluster analysis of the populations sampled in the even-year broodline revealed a general lack of consistency in geographically based clustering of the populations. The only exception was observed for the Skeena River drainage, where all seven populations sampled clustered together in 97% of dendrograms evaluated. There was some evidence for a weak association for 19 of 21 populations from the Queen Charlotte Islands, but the cluster was not well supported (22% bootstrap support). As with the odd-year broodline, populations sampled from the central coast and north coast regions of British Columbia did not cluster into distinct geographic units (Fig. 2), which again reflected the overall lack of genetic differentiation ($F_{ST}=0.001$) between populations in the two regions.

Discussion

In the current study of microsatellite variation in pink salmon, approximately 46,500 individuals were surveyed from 146 odd-year and 116 even-year populations, 16 microsatellites were analyzed encompassing 812 alleles, and 12–85 alleles were identified per locus. Sample size ranged from 18 to 755 individuals per population, with at least 100 individuals sampled in 127 of the 146 odd-year populations, and 92 of the 116 even-year populations. Only six odd-year and seven even-year populations had fewer than 40 individuals surveyed. With a range in the number of individuals sampled per population, sampling errors may have influenced the estimated allele frequencies within populations, particularly for populations with fewer than 40 individuals sampled. If sampling errors are large in estimation of allele frequencies, there is a potential for these errors to obscure genetic relationships among related populations. Kalinowski (2005) reported that loci with larger numbers of alleles produced estimates of genetic distance with lower coefficients of variation than loci with fewer numbers of alleles, without requiring larger sample sizes from each population. Given the results from the cluster analysis, variation in the number of individuals sampled per population likely did not result in misidentification of genetic relationships among populations.

Inferences from the genetic relationships of populations surveyed in our study were dependent upon accurate determination of population allele frequencies. Microsatellite alleles differ in size, but alleles of the same size at a locus in geographically separate populations may not have the same origin as a result of size homoplasy. Convergent mutations in different lineages may produce alleles of the same size, with the result that there may be greater differentiation among lineages than revealed by analysis of size variation alone. However, with over 800 alleles observed across all loci in the study, the large amount of variation present

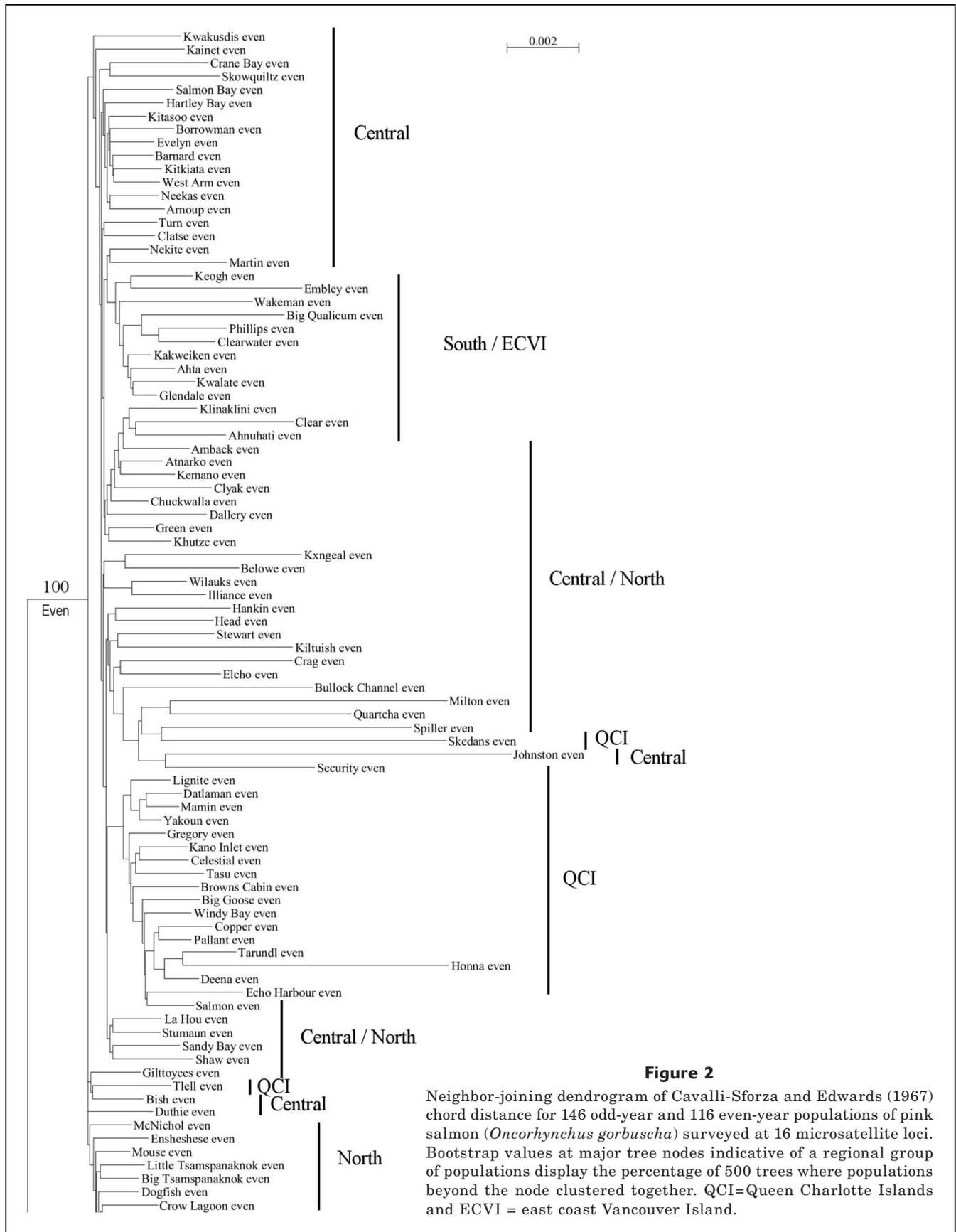
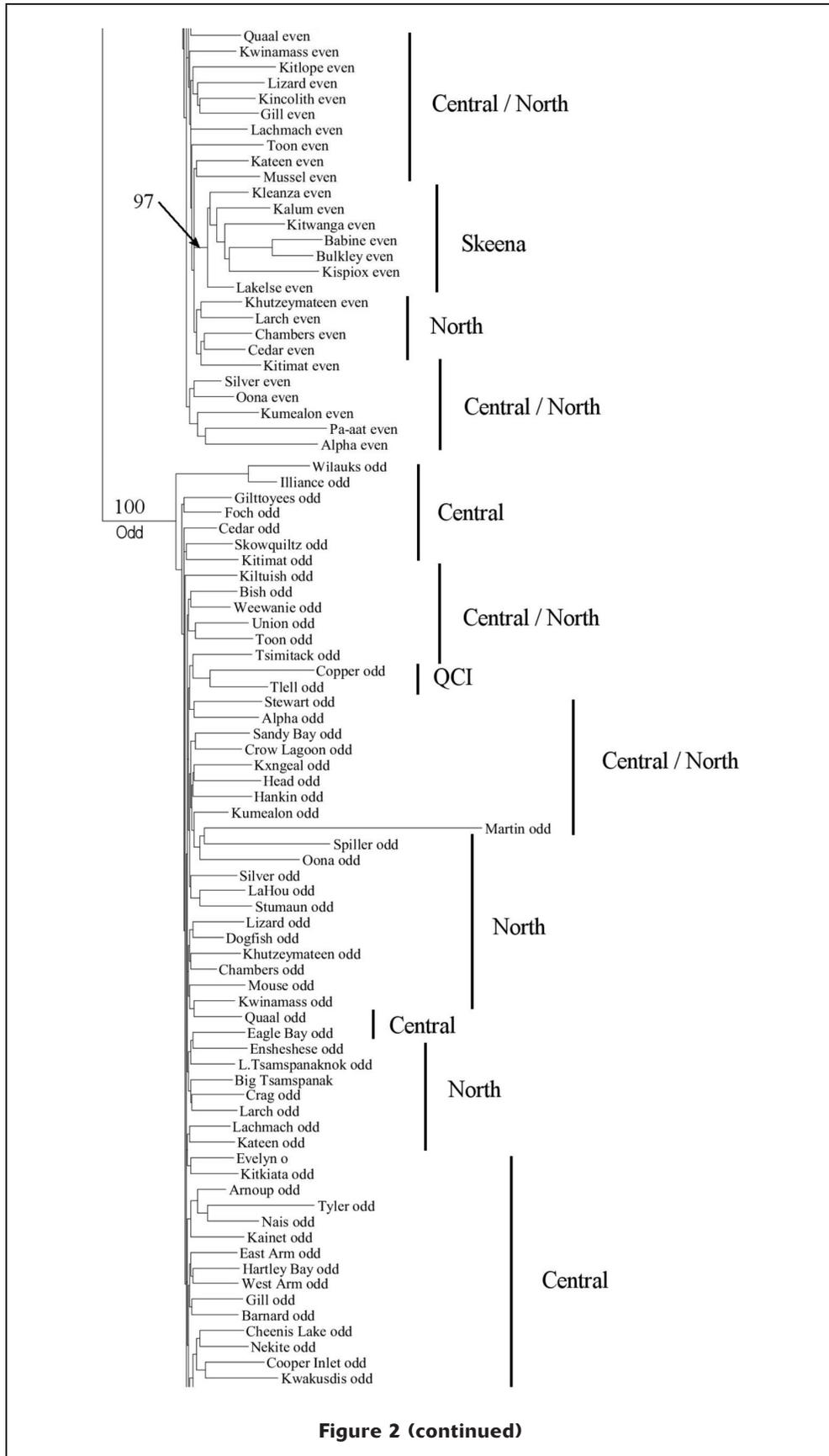


Figure 2

Neighbor-joining dendrogram of Cavalli-Sforza and Edwards (1967) chord distance for 146 odd-year and 116 even-year populations of pink salmon (*Oncorhynchus gorbuscha*) surveyed at 16 microsatellite loci. Bootstrap values at major tree nodes indicative of a regional group of populations display the percentage of 500 trees where populations beyond the node clustered together. QCI=Queen Charlotte Islands and ECVI = east coast Vancouver Island.



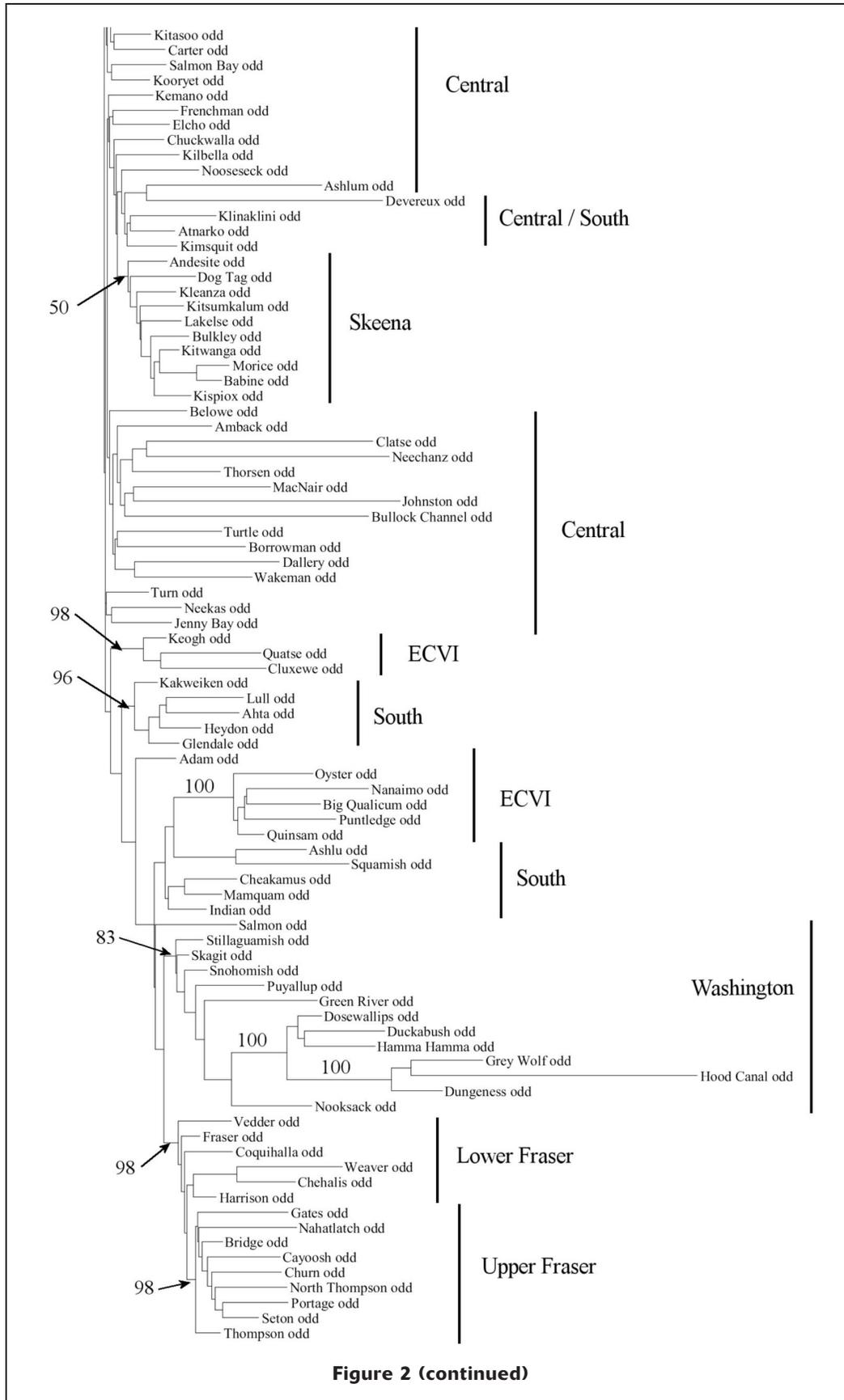


Figure 2 (continued)

at these loci largely compensates for size homoplasy (Estoup et al., 2002), and therefore the pattern of population structure identified was unlikely to be obscured or distorted by size homoplasy.

The distribution of genetic variance indicated that differences in allele frequencies between the broodlines were about three times greater than differences for all regional and population sources of variation, but that regional differences in allele frequencies were only about 1.4 times larger than differences among populations within regions. Previous analyses of annual variation in allele frequencies within populations have indicated that this source of variation can be as large as differences among populations within regions (Golovanov et al., 2009). Although not specifically illustrated in the current study because of limitations of the software to facilitate a four-level nested analysis of variance, similar results (not shown) were observed in the current study. The larger importance of annual variation within populations relative to population differentiation compared with other Pacific salmon species likely reflected the reduced population differentiation observed in pink salmon relative to that observed in other salmon species (Beacham et al., 2009, 2011).

The current study indicates that the largest determinant of population structure of pink salmon in British Columbia and Washington was year of spawning (odd or even), with a distinct separation of the two broodlines. With the odd-year broodline, regional differentiation was stronger in the southern portion of the distribution of populations, with the greatest population differentiation within a region observed in Washington. The Hood Canal hatchery population (also known as Hoodsport hatchery 47°23'37"N, 123°08'54"W) was the most distinct, even though this population was derived from adults returning to the Dungeness River and Dosewallips River in 1953 (Hard et al., 1996). The distinctiveness of this population was reflected in allelic frequency differentiation. For example, the frequency of the *One102*³¹¹ allele was 0.54 in the Hood Canal hatchery population, 0.34 in the Gray Wolf River population, and <0.20 in all other Washington populations. Additionally, the frequency of *One109*¹³³ was 0.33 in the Hood Canal hatchery population, but <0.20 in all other Washington populations. All populations from drainages entering Hood Canal (Hood Canal hatchery, Dosewallips River, Duckabush River, Hama Hama River) or the Strait of Juan de Fuca (Dungeness River, Gray Wolf River) were distinct from those on the eastern side of Puget Sound (Snohomish River, Stillaguamish River, Skagit River, Green River, Puyallup River). Genetic separation of Strait of Juan de Fuca populations and Hood Canal populations from those in eastern Puget Sound was initially described by Shaklee et al. (1991) in an analysis of allozyme variation. However, as described by Shaklee et al. (1991), the Nooksack River population, located in the northeastern section of Puget Sound and nearest to the border with Canada, clustered with Hood Canal and Strait of Juan de Fuca populations, rather than with geographically closer populations on the east

side of Puget Sound. Similar results were observed in the current study. Shaklee et al. (1991) suggested that the genetic similarity of the Nooksack River population to that of Hood Canal populations was a consequence of a 1977 transfer of fertilized eggs from the Hood Canal hatchery to a tributary of the Nooksack River and a reduction of the native population due to habitat degradation. As Shaklee et al. (1991) outlined, this enhancement effort may have caused a genetic change in the characteristics of this population that has persisted over time (Hard et al., 1996).

Fraser River populations were separate from those in southern British Columbia (east coast of Vancouver Island, south coast mainland) and Washington, confirming the results from the previous analysis of allozyme variation reported by Beacham et al. (1988) and Shaklee et al. (1991). In the Fraser River drainage, some separation was observed between populations spawning upstream from the Fraser River canyon (southern limit approximately 175 km upstream from the mouth) from those spawning downstream of the canyon. Genetic separation between upriver and downriver populations had also been outlined previously by Beacham et al. (1988) and Shaklee et al. (1991). Similar genetic separation between upper drainage and lower drainage populations has been observed in coho salmon (*O. kisutch*) (Beacham et al., 2011) and reflects geographic separation between the two groups of populations.

In northern British Columbia, odd-year broodline populations in the Skeena River drainage were separate from those farther south in the central coastal region of British Columbia and from those farther north on the northern coastal region of British Columbia in a similar pattern to that outlined by Beacham et al. (1988). Similar differentiation was also observed in the even-year broodline, with Skeena River drainage populations distinct from other populations in northern British Columbia. Some differentiation was observed in the current study between even-year broodline pink salmon populations from the Queen Charlotte Islands and other regions in northern British Columbia (central coast, Skeena River, north coast) ($F_{ST} = 0.004\text{--}0.006$); differentiation of populations from the Queen Charlotte Islands had also been observed by Beacham et al. in 1988.

Studies of population structure in Pacific salmon are a useful initial step in developing and applying genetic variation to the problem of estimating stock composition in mixed-stock salmon fisheries. The key to successful application of genetic variation to estimation of stock composition centers around whether or not there is a regional basis to population structure. This is a key consideration because a regionally based population structure is generally required for genetic stock identification estimation, with the assumption that the portion of the mixed-stock sample derived from unsampled populations is allocated to sampled populations from the same region. With this assumption, the cost and complexity of developing a baseline for stock composition analysis is reduced, and refinements in estimated stock compositions are possible as the baseline is enhanced

in stages. For the odd-year broodline, applications in southern British Columbia would appear to be possible if fishery management objectives are to separate pink salmon of Washington, Fraser River, and southern British Columbia origin. Finer subdivision of stock composition estimation, particularly in the Washington region, may be possible, as separation of Hood Canal and Strait of Juan de Fuca populations from those in Puget Sound may be practical.

Studies of population structure in pink salmon have revealed some consistent patterns. The greatest differentiation observed in population structure has been consistently reported to occur between the two broodlines, whether in Asia or North America (Beacham et al., 1988; Kartavtsev, 1991; Varnavskaya and Beacham, 1992; Zhivotovsky et al., 1994; Salmenkova et al., 2006; Golovanov et al., 2009). In Asia, studies have indicated that genetic differentiation among populations is greater in the even-year broodline than in the odd-year broodline (Hawkins et al., 2002; Golovanov et al., 2009). In North America, the reverse situation occurs, with population differentiation among populations greater in the odd-year broodline than in the even-year broodline (Beacham et al., 1988; Gharrett et al., 1988; current study). These findings support the concept of two main refugia occupied by pink salmon during the most recent Pleistocene Era glaciation some 10,000 years ago (Aspinwall, 1974). The even-year broodline may have survived the glaciation in a northern refugium (Aspinwall, 1974). Once the glaciation ended, the even-year broodline dispersed from the northern refugium, colonizing southern regions more recently than northern ones. Conversely, the odd-year broodline may have occupied a southern refugium during the Pleistocene Era glaciation (McPhail and Lindsey, 1970), and dispersed northward, with northern populations derived more recently than southern ones. As populations closer to the refugium have had greater time to accumulate genetic mutations and thus display greater population differentiation, the current pattern of broodline and population differentiation is consistent with dispersal from a northern refugium for the even-year broodline (greater population genetic differentiation in even-year broodline) and dispersal from a southern origin for the odd-year broodline (greater population differentiation in odd-year broodline). Additionally, embryonic survival of the even-year broodline has been reported to be higher than that of the odd-year broodline in a cold (4°C) incubation environment, with higher alevin and fry growth of the even-year broodline also observed in the cold incubation environment (Beacham and Murray, 1988). Greater suitability of the even-year broodline to a colder environment is also illustrated by the spawning distributions of the broodlines in North America, with the even-year broodline in very low abundance from the southern portion of the range (Fraser River, Washington) and the odd-year broodline in low abundance in western Alaska. Alternatively, Krkošek et al. (2011) suggested that the distribution of

even-year and odd-year populations result from density-dependent mortality caused by interactions between the broodlines. However, it seems difficult to account for genetic population structure observed in pink salmon as a result of broodline interactions.

Conclusion

The level of differentiation observed among the pink salmon populations within broodlines surveyed in the current study was considerably less than in other species of Pacific salmon. Sockeye salmon (*O. nerka*) typically display high levels of genetic differentiation ($F_{ST}=0.097$, 14 loci, average 30 alleles per locus, 299 populations) (Beacham et al., 2006), with the other species displaying levels of genetic differentiation ranging between sockeye salmon and pink salmon. The low level of differentiation observed in pink salmon may be a result of a more recent colonization history (Hawkins et al., 2002), but may also be a result of straying among local populations within regions. As pink salmon juveniles spend little time in fresh water after fry emergence, imprinting on natal streams may not be as strong as in other species, and as a result may stray more upon returning spawning migrations (Quinn, 1993). Chum salmon (*O. keta*) juveniles spend similar amounts of time in fresh water as pink salmon, and population differentiation in the species is higher only than pink salmon ($F_{ST}=0.033$, 14 loci, average 57 alleles per locus, 380 populations) (Beacham et al., 2009). The low level of genetic differentiation observed in pink salmon population structure likely reflects higher levels of straying among populations during spawning than those observed for other Pacific salmon species.

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