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Population Structure and Stock Identification of Steelhead in Southern British Columbia, Washington, and the Columbia River Based on Microsatellite DNA Variation

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Abstract.—The purpose of this study was to describe population structure and determine the potential for genetic stock identification for steelhead *Oncorhynchus mykiss* in British Columbia using microsatellite DNA markers. Variation at eight microsatellite DNA loci (*Oki200, Omy77, Ots1, Ots3, Ssa85, Ots100, Ots103,* and *Ots108*) was surveyed in approximately 1,500 steelhead from 22 populations in southern British Columbia, Washington, and the Columbia River drainage as well as in more than 450 steelhead from two commercial salmon fisheries conducted off the southwest coast of Vancouver Island. Nine populations were sampled for two or more years, and variation in allele frequencies among populations and regions was, on average, about 3.7 times greater than annual variation within populations. Regional structuring of populations was apparent, with Thompson River, upper Fraser River, and Columbia River populations forming distinct groups. Significant differences in allele frequencies were observed among regional stock groups at all loci. After variation within populations was accounted for, variation among regions was the greatest source of the remaining variation (4.4%), followed by variation among populations within regions (3.1%) and variation among years within populations (2.0%). The overall classification accuracy of single individuals to five regional groups using a jackknifed discriminant analysis was 80%. Simulated mixed-stock samples suggested that variation at the eight microsatellite DNA loci surveyed should provide relatively accurate and precise estimates of stock composition for fishery management applications. Analyses of commercial marine fisheries samples indicated that during 1994–1996 more than 85% of the steelhead sampled in a directed chum salmon fishery off the mouth of the Nitinat River originated in the Fraser River drainage with the majority of steelhead from the Thompson River. However, in 1997, steelhead of U.S. origin were estimated to have composed 60% of the samples, and the Canadian component was largely of Fraser River steelhead, possibly reflecting anomalies associated with climatic variation. Estimated stock composition of samples from the 1997 sockeye salmon fishery in Barkley Sound indicated that the majority (71%) of steelhead was of Vancouver Island origin with the remainder being of U.S. origin.

Steelhead *Oncorhynchus mykiss* (anadromous rainbow trout) are found in all major coastal river systems in British Columbia. Three distinct seasonal run timings are observed: summer-run steelhead enter freshwater between May and September, fall-run steelhead arrive between August and November, and winter-run steelhead between November and May. Run timing reflects hydrology of the natal stream and generally corresponds to geography, with summer and winter runs occurring in coastal systems (e.g., Vancouver Island and lower Fraser River) and fall runs in interior systems (e.g., Thompson River and upper Fraser River; Parkinson 1984a). No commercial fisheries are directed at steelhead in British Columbia, and this species is highly regarded by the freshwater sport fishing sector. Efforts to reduce bycatch of steelhead are significant in commercial salmon fisheries because this species is considerably less abundant naturally than most other Pacific salmon species.

Understanding population structure is a key factor in the management of many Pacific salmonids, and surveys of genetic variation to determine pop-

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ulation structure have been conducted extensively for several of these species. However, only two studies assessing the population structure of steelhead in British Columbia have been conducted. Parkinson (1984b) examined variation at five allozyme loci in primarily fry and parr from assumed steelhead and rainbow trout populations. He observed significant differences in allele frequencies among some adjacent streams as well as regional structuring of populations where Skeena River, coastal, and southern interior groups were distinct. Taylor (1995) used two minisatellite DNA loci to evaluate genetic variation among steelhead and rainbow trout populations mainly in British Columbia. He observed significant variation among populations within watersheds and among regions, but sampling was restricted to eight broadly distributed populations.

More recently, the development of highly polymorphic microsatellite loci has improved the resolution of detectable genetic variation among salmonid populations. Nonlethal sampling and the abundance of loci make this technology a very effective method in describing population structure in Pacific salmon (Beacham et al. 1998; Seeb et al. 1998; Small et al. 1998) and steelhead (Nielsen et al. 1994, 1997b; Wenburg et al. 1996). Microsatellite DNA loci can also be very useful in estimation of stock composition in mixed-stock salmon fisheries (Beacham and Wood 1999).

Identification of specific stocks is a key tool in the fisheries management of mixed-stock salmonid fisheries. In British Columbia, the origin of steelhead intercepted in commercial salmon fisheries has been a contentious issue among fisheries management agencies, in particular where species' migration timing and routes overlap significantly. Using three allozyme loci, Parkinson (1984a) was able to distinguish coastal from interior steelhead stocks and assess contributions in commercial fisheries in southern British Columbia. Additional biological information including size differences, coded wire tags, and in-river timing studies has also been used to estimate stock composition (R. Bison, British Columbia Ministry of Environment, Lands, and Parks, personal communication). However, some management applications required more stock-specific information than was available using these techniques. We chose to evaluate genetic variation among steelhead populations in southern British Columbia with microsatellite DNA technology in hopes of addressing this issue and improving the accuracy and precision of estimated stock compositions.

Specifically, the objective of the present study was to analyze variation at eight microsatellite DNA loci for mainly summer- and fall-run steelhead populations in southern British Columbia to describe population structure and evaluate potential applications for stock identification. We then applied microsatellite DNA technology to estimate stock composition of steelhead intercepted in two salmon fisheries off the southwest coast of Vancouver Island. To account for the possible interception of steelhead of U.S. origin in these two fisheries, we also included representative steelhead populations from coastal Washington and the Columbia River in our survey.

Methods

Collection of DNA samples and polymerase chain reaction.—The DNA was extracted either from scales, from previously collected frozen samples stored at −20°C, or from a punch of operculum tissue preserved in 95% ethanol for 14 populations from southern British Columbia and 8 populations from Washington State and the Columbia River drainage (Figure 1). The DNA samples from Canadian populations were derived from adults, whereas samples from most U.S. populations were derived from juveniles; exceptions were the Clearwater, Lower Salmon, and Kalama river populations, sampled as adults. All Canadian populations were summer and fall run except that of the Salmon River on the east coast of Vancouver Island. All U.S. populations were summer and fall run except that of Bogachiel River in the Columbia River drainage. Approximately 1,500 fish were sampled for the analysis. Within each population, samples analyzed spanned a maximum range of 23 years, with 1–6 brood years sampled (Table 1). The DNA was extracted from scales as outlined by Nelson et al. (1998). For the tissue samples, approximately 0.3 g of tissue was placed in each well of a 96-well plate containing 0.2 mL of 5% chelex in TE buffer (10 mM tris pH 7.4, 1 mM EDTA pH 8.0, 0.10 mg/mL proteinase K, and 0.1% sodium dodecyl sulfate), incubated for 15 min at 50°C, and then incubated for an additional 15 min at 95°C. The supernatant from each well was collected and placed in a fresh 96-well plate and stored at −20°C. About 1 μL of this extract was required for each polymerase chain reaction (PCR).

Loci amplified via PCR were the dinucleotide repeats *Omy77* (Morris et al. 1996), *Ots1* (Banks et al. 1999), *Ots3* (Banks et al. 1999), *Ssa85* (O'Reilly et al. 1996), and a new locus, *Oki200.*

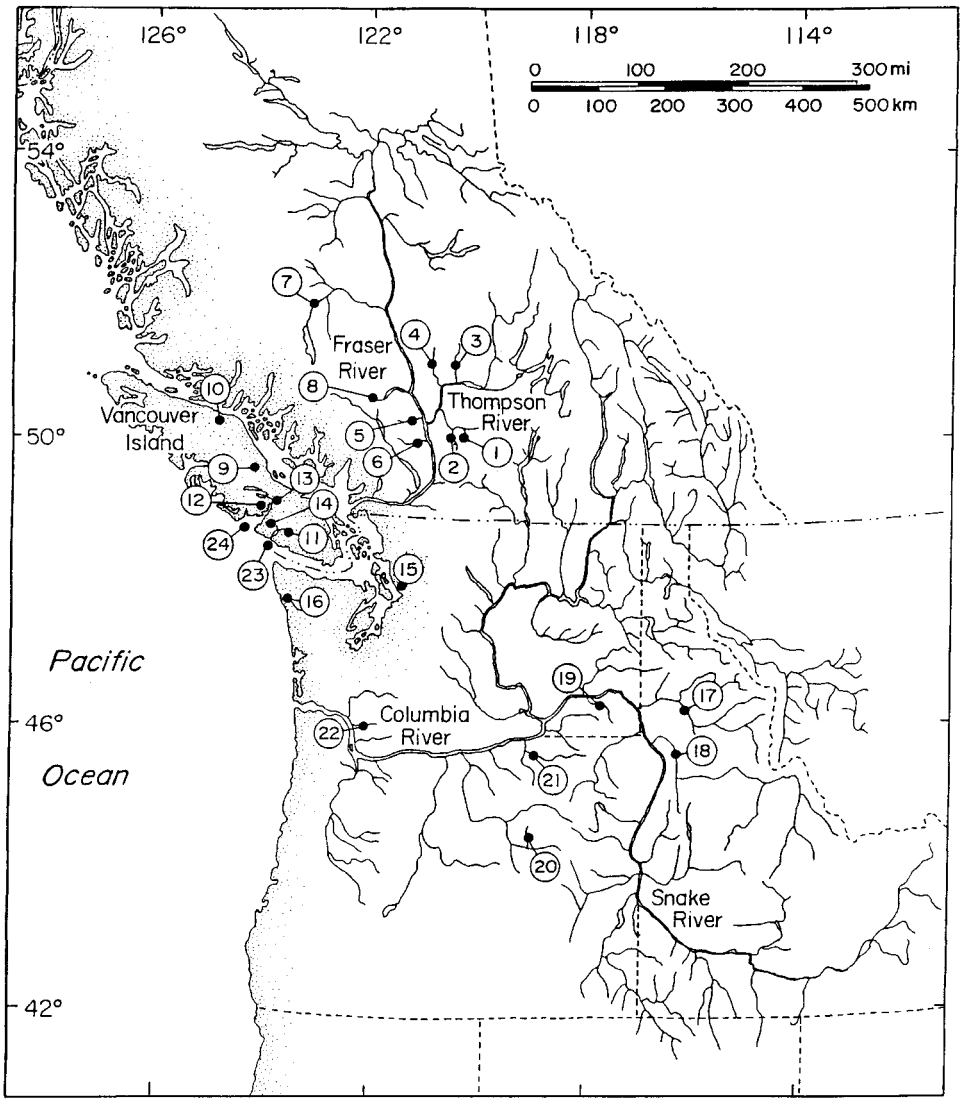

FIGURE 1.—Locations of steelhead populations and commercial salmon fisheries sampled in the survey, 1976–1997. Numbered locations are identified in Table 1.

Primer sequences for *Oki200* were forward 5′-ACC-CTC-GGC-CCA-ACG-TAA-TT-3′ and reverse 5′-ACA-GCA-GCT-GGA-GTG-AAT-CTT-3′ (A = adenine, C = cytosine, G = guanine, T = thymine). The tetranucleotide repeat loci surveyed were *Ots100* (Nelson et al. 1998), *Ots103* (Beacham et al. 1998), and *Ots108* (Nelson and Beacham 1999). We used revised primers for the *Ots108* locus: forward 5′-TCT-GTT-TAT-CTT-TCT-ATT-AG-3′ and reverse 5′-TGG-CAA-GGA-GAG-ACA-GAG-GG-3′. For all primer sets used in this study, PCR was conducted in 25-μL reactions containing 12 pmol (0.48 μM) each primer, 80 μM each nucleotide, 20 mM tris pH 8.8, 2 mM MgSO4, 10 mM KCl, 0.1% Triton X-100, 10 mM $(NH_4)SO_4$, and 0.1 mg/mL nuclease-free bovine serum albumin. All PCR analyses in this study were preceded by an initial denaturation step of 3 min at 94°C. All cycle extension (30 cycles for all loci) steps were for 60 s at 72°C and all cycle denaturation steps were for 20 s at 94°C. The PCR of loci *Oki200*, *Omy77*, *Ots1*, *Ots3*, *Ssa85*, *Ots100*, *Ots103*, and *Ots108* was accomplished with annealing temperatures of 55°C, 50°C, 50°C, 54°C, 55°C, 55°C, 55°C, and 42°C, respectively. Annealing times were 15 s for *Ssa85*, 20 s for *Ots1*,

TABLE 1.—Steelhead samples collected from 22 populations in five regions or drainages of British Columbia, Washington coast, and Columbia River, as well as from two commercial fisheries off the west coast of Vancouver Island. Numbers of fish sampled (*N*) correspond with each year.

Population[a]	Years sampled	*N* for each year	Total *N*
	Thompson River		
(1) Spius Creek	1986, 1987, 1988, 1989, 1995, 1996	16, 17, 15, 20, 50, 1	119
(2) Coldwater River	1995, 1996, 1997	32, 3, 31	66
(3) Deadman River	1995, 1997	84, 75	159
(4) Bonaparte River	1994, 1995	95, 250	345
	Upper Fraser River		
(5) Stein River	1976, 1978, 1980, 1997	1, 1, 2, 13	17
(6) Nahatlatch River	1976, 1979, 1997	10, 3, 30	43
(7) Chilko River	1995, 1996	27, 22	49
(8) Bridge River	1980, 1996, 1997	8, 9, 5	22
	Vancouver Island		
(9) Puntledge River	1996, 1997	18, 35	53
(10) Salmon River	1997	26	26
(11) Caycuse River	1997	47	47
(12) Nahmint River	1997	44	44
(13) Robertson Creek	1995, 1996	50, 24	74
(14) China Creek	1997	12	12
	Washington State		
(15) Deer Creek	1993, 1995	52, 50	102
(16) Bogachiel River	1994	51	51
	Columbia River		
(17) Clearwater River	1996	50	50
(18) Lower Salmon River	1996	50	50
(19) Upper Tucannon River	1991	99	99
(20) Beech River	1996	21	21
(21) Umatilla River	1994, 1996	21, 28	49
(22) Kalama River	1989, 1991, 1996	5, 5, 9	19
	Commercial fisheries		
(23) Nitinat River mouth	1994, 1995, 1996, 1997	140, 89, 143, 26	398
(24) Barkley Sound	1997	58	58

[a] Numbers refer to map location on Figure 1.

30 s for *Oki200* and *Ots100*, and 60 s for the other loci.

Gel electrophoresis and band analysis.—The PCR products were size fractionated on 16 × 17-cm nondenaturing polyacrylamide gels visualized by staining with ethidium bromide in water (0.5 mg/mL) and illuminating with ultraviolet light. Nelson et al. (1998) provided a more complete description of gel electrophoretic conditions. All gels were run for 14–18 h at 65–70 V, with gels at 8% acrylamide for analysis of *Ots1* and 10% acrylamide for analysis of the other loci. Twenty-nine lanes per gel were loaded: one outside lane containing 1 kb (kilobase) ladder DNA (Gibco BRL), three 20-bp (base pair) marker lanes (Gensura Labs, Inc., Del Mar, California), one lane for a standard fish to determine precision of estimation of allele size, and 24 lanes for individual fish analyzed. Standard fish were run no closer than four lanes to a 20-bp marker lane.

Gels were scanned at a 1,024 × 1,024-pixel density with a Kodak charge-coupled device camera with low-light capability and a yellow filter. Images were analyzed using BioImage Whole Band software (Millipore Corp. Imaging Systems, Ann Arbor, Michigan). The size of the amplified microsatellite alleles was reported to the nearest 1 bp based on the molecular size grid created with the 20-bp markers.

Because some uncertainty existed in estimation of allele size as determined from the 20-bp grid due to variation in electrophoretic conditions between gels, we identified alleles on the basis of a binning procedure (Gill et al. 1990). Peaks in the

estimated allele size-frequency distribution by base pair were used to identify alleles empirically, and bin widths generally corresponding to a repeat unit were set with the peak occurring in the middle of the bin. Precision of estimation of allele size was evaluated with the standard fish analyzed for each locus. There were two instances where bin width was not based on the repeat unit. Although dinucleotide repeats constitute the basis of variation at the *Ssa85* and *Ots1* loci, the level of precision of estimated allele sizes from the standard fish indicated that alleles varying in 2-bp increments could not be consistently resolved at these loci. Therefore, alleles were usually defined on the basis of a 4-bp bin, which has the practical effect of pooling alleles of adjacent size. For all loci, alleles were designated by the lower limit of the allele bin. For example, the 79-bp allele at *Ots103* was defined as all allele sizes estimated at 79, 80, 81, and 82 bp, but in practice nearly all estimated sizes of this allele were observed at either 80 or 81 bp.

We conducted a pedigree study of the inheritance of microsatellite alleles at all loci surveyed. Ten male and 10 female steelhead were used to create 10 full-sibling families, and then both parents and 15 progeny from each family were subsequently screened at the eight loci surveyed in our study. Alleles were inherited in a Mendelian fashion. Because the use of PCR with nondenaturing electrophoresis typically produces allelic homoduplex bands (true alleles) and heteroduplex bands above the homoduplex bands (Haddad et al. 1997), we used the family crosses to distinguish the allelic homoduplex bands from the heteroduplex bands.

Data analysis.—Annual variation in allele frequencies within populations was tested with the program GENEPOP, version 3.1, by unbiased estimates of Fisher's exact test (Raymond and Rousset 1995). For all tests conducted with GENEPOP, the dememorization number was set at 1,000, and 50 batches were run for each test with 1,000 iterations/batch. Years in which fewer than 10 fish in the population were sampled were excluded from the analysis, and populations that were sampled in multiple years but had fewer than 10 fish sampled per year were also excluded. Each stock at each locus was tested for departure from Hardy–Weinberg equilibrium using GENEPOP. Tests of genetic differentiation using all pairwise comparisons were also conducted by determining unbiased estimates of Fisher's exact test using GENEPOP with parameters set as outlined previously. Critical significance levels for simultaneous tests were evaluated using sequential Bonferroni adjustment (Rice 1989). Cavalli-Sforza and Edwards (1967) chord distance was used to estimate distance among populations. An unrooted consensus neighbor-joining tree based on 500 replicate trees was generated with the CONSENSE program from PHYLIP (Felsenstein 1993). Estimation of variance components of regional differences, population differences within regions, and annual variation within populations was determined with BIOSYS (Swofford and Selander 1981). A hierarchical gene diversity analysis was conducted to assess geographic structuring of genetic variation incorporating regions, populations, and sampling years (Table 1). Identification of individuals to specific stocks was done with the DISCRIM procedure in SAS (SAS Institute 1989) with a jackknife sampling procedure. Classification functions were developed using all fish sampled except the one to be classified, with each fish tested individually in turn. Identification of individual fish was restricted to those fish for which data were available at all eight loci analyzed.

Estimation of stock composition in simulated mixed-stock fishery samples.—Genotypic frequencies were determined at each locus for each stock, and the model of Fournier et al. (1984) was used to estimate stock composition. Variation was scored at 7 alleles but subsequently condensed and summarized by 4 alleles (10 genotypes) at *Oki200,* and initially by 20 alleles and condensed to 10 alleles (55 genotypes) at *Omy77,* with adjacent alleles combined (Table 2). Variation at *Ots3* was scored at 12 alleles with no subsequent grouping of alleles. Variation at *Ots1* was initially scored at 21 allele bins but subsequently condensed to 11 alleles (66 genotypes), with no alleles observed between 179 and 222 bp. At *Ssa85,* variation was initially scored by 17 alleles and condensed to 13 alleles (91 genotypes). Variation at *Ots100* was scored at 14 alleles (105 genotypes) and 7 alleles (28 genotypes) at *Ots103,* with no subsequent grouping of allele numbers at either locus. Variation at *Ots108* was initially scored at 35 alleles and condensed to 25 alleles (325 genotypes). Combining low-frequency adjacent alleles reduced the number of genotypic frequencies to be estimated with the available samples, resulting in little or no loss in the ability to discriminate among stocks. All microsatellite DNA loci used to estimate stock compositions were assumed to be in Hardy–Weinberg equilibrium, and expected genotypic frequencies were determined from the observed allele

TABLE 2.—Method of pooling low-frequency alleles to reduce the number of genotypic frequencies to be estimated in baseline populations for mixed stock analysis.

Pooled alleles, renumbered	Microsatellite allele numbers pooled for each locus:							
	Oki200	*Omy77*	*Ots1*	*Ots3*	*Ssa85*	*Ots100*	*Ots103*	*Ots108*
1	1–2	1	1–2	1	1	1	1	1
2	3–4	2–3	3	2	2	2	2	2
3	5	4–5	4	3	3	3	3	3
4	6–7	6–7	5	4	4	4	4	4
5		8–9	6	5	5	5	5	5
6		10–11	7	6	6	6	6	6
7		12–13	8–16	7	7	7	7	7
8		14–15	17	8	8	8		8
9		16–17	18	9	9	9		9
10		18–20	19	10	10	10		10
11			20–21	11	11	11		11
12				12	12	12		12
13					13–17	13		13
14						14		14
15								15
16								16
17								17
18								18
19								19
20								20
21								21
22								22–30
23								31
24								32–33
25								34–35

frequencies. All 22 populations listed in Table 1 were included in the baseline used to estimate stock composition of the mixtures. Each baseline population at each locus was resampled with replacement to simulate random variation involved in the collection of the baseline samples during the estimation of stock composition of each mixture. Hypothetical fishery samples of 150 fish were generated by randomly resampling with replacement the baseline stocks at each locus and adding the derived multilocus genotypes for the appropriate number of fish from each stock to the mixture. Estimated stock composition of the mixture was then determined, with the whole process repeated 100 times to estimate the mean and standard deviation of the individual stock composition estimates.

Fishery sampling.—Incidental catches of steelhead were sampled in two marine salmon commercial fisheries, one near the mouth of the Nitinat River and one in Barkley Sound off the southwest coast of Vancouver Island (Figure 1). The fishery near the Nitinat River mouth is directed at chum salmon *O. keta* and occurs from late September through October. Steelhead catches in this fishery were sampled on an annual basis during1994–1997. The fishery in Barkley Sound is directed at sockeye salmon *O. nerka*, and in 1997 the fishery began in late June and terminated by mid-July. The estimated stock composition of each sample was determined as a point estimate using data from all fish in the sample and with the 22 populations listed in Table 1 as the baseline. Standard deviations of individual stock estimates were derived from bootstrap resampling of both the baseline stocks and the mixture sample.

Results

Precision of Allele Size Estimation

Standard deviations of the estimate of allele size for the heterozygous standard fish analyzed at each locus ranged from 0.25 to 0.89 bp, with the larger alleles estimated with the least precision (Table 3). For the dinucleotide-repeat loci *Ots3* and *Oki200*, all estimated sizes of a particular standard allele were within a 2-bp interval. Estimated sizes for the alleles of the standard fish at the tetranucleotide-repeat locus *Ots103* were all within a 4-bp interval. Estimated sizes of both alleles at the dinucleotide-repeat *Omy77* locus were within a 2-bp interval for more than 96% of observations. Similar levels of precision were observed at the tetranucleotide-repeat *Ots108* locus. At the *Ssa85* locus, estimated

TABLE 3.—Precision of estimates of allele size (and SD) in base pairs, bp, at each microsatellite locus for standard fish run only once per electrophoretic gel; *N* is the number of gels on which allele sizes for a standard fish were estimated.

		Allele size, bp		Allele size, bp	
Locus	*N*	Mean (SD)	Range	Mean (SD)	Range
Ots3	87	80.2 (0.38)	80–81	86.1 (0.25)	86–87
Ots103	94	76.2 (0.43)	76–77	87.8 (0.42)	87–89
Oki200	34	87.4 (0.49)	87–88	100.9 (0.25)	100–101
Omy77	85	101.2 (0.53)	100–102	120.4 (0.52)	119–121
Ots108	20	98.7 (0.49)	98–99	166.7 (0.80)	165–168
	45	98.2 (0.46)	97–99	167.3 (0.84)	165–169
Ssa85	39	106.4 (0.50)	106–107	128.5 (0.51)	128–129
	80	106.0 (0.46)	105–107	128.3 (0.50)	127–129
Ots100	71	165.5 (0.89)	163–167	173.2 (0.83)	171–175
Ots1	103	156.8 (0.60)	156–158	238.3 (0.80)	237–240

allele sizes for one of the standard fish were in a 2-bp bin for only 91% of occurrences, and thus we conservatively binned alleles of adjacent size into a 4-bp bin. Sizes for both the 166-bp and 174-bp alleles for the standard fish at *Ots100* were estimated within a 4-bp bin for 97% (69/71) of occurrences. Although variation at *Ots1* should be based on dinucleotide repeats, the precision of estimation of allele size of the standard fish did not allow for identification of dinucleotide alleles with a high degree of confidence (Table 3). Accordingly, alleles of adjacent size were binned, resulting in identification of alleles in 4-bp bins.

Variation within Populations

All eight microsatellite loci examined were quite polymorphic in all populations surveyed. Observed heterozygosities (H_O) of the loci examined for all populations were as follows: *Oki200* 0.60 (population range 0.58–1.00), *Omy77* 0.72 (0.44–0.86), *Ots1* 0.55 (0.32–0.90), *Ots3* 0.61 (0.49–0.74), *Ssa85* 0.71 (0.48–0.85), *Ots100* 0.76 (0.62–1.00), *Ots103* 0.40 (0.08–0.49), and *Ots108* 0.81 (0.22–0.95). Mean observed heterozygosity was 0.600 for the four Thompson River populations, 0.614 for the four upper Fraser River populations, 0.617 for the six Vancouver Island populations, 0.656 for the two Washington populations, and 0.653 for the six Columbia River populations (Table 4). There was little difference in observed heterozygosities among steelhead in the three regions in Canada, but Canadian populations were, on average, slightly less heterozygous than those from two regions in the United States.

Genotypic frequencies observed in 22 populations and 8 loci surveyed in our study were those expected for populations in Hardy–Weinberg equilibrium with a few exceptions. Significant departures (correction for 22 tests per locus, $\alpha = 0.0023$) from the expected Hardy–Weinberg distribution of genotypic frequencies were observed at six loci in the upper Tucannon River sample and at three loci in the Robertson Creek sample (Table 4). There was no evidence of a consistent departure of genotypic frequencies expected from Hardy–Weinberg distribution at any locus, indicating that null alleles were not in significant frequency.

Significant annual variation (correction for eight tests per population, $\alpha = 0.0063$) in allele frequencies was observed in at least one locus in five of the nine populations that had been sampled in multiple years and for which adequate sample sizes were available (Years in Table 4). Most of the variation was observed at the *Oki200*, *Omy77*, and *Ots100* loci. There was no discernable relationship between the length of time between first and last sampling years and the number of loci at which significant variation was observed. For example, significant differences at four loci were observed in the Deer Creek population with samples spanning 3 years. Significant differences at only three loci were observed in the Spius Creek population with samples spanning a 10-year period, but the power of detecting differences was likely to be reduced in this population, given the smaller annual sample sizes (Table 1).

Variation among Populations within and among Regions

Regional genetic differentiation at all loci was observed among the steelhead populations surveyed in our study. For example, all populations of Thompson River steelhead had relatively high frequencies of allele *100* at *Omy77* with an overall value of 0.28,and upper Fraser River populations had an overall observed frequency of 0.18, where-

TABLE 4.—Observed heterozygosities (H_O), probability of conformance to Hardy–Weinberg equilibrium (HWE), and, where applicable, probability of homogeneity of allele frequencies among sampling years within populations (Years). An asterisk (*) indicates significance at $P < 0.05$.

Population	Trait	*Oki200*	*Omy77*	*Ots1*	*Ots3*	*Ssa85*	*Ots100*	*Ots103*	*Ots108*
				Thompson River					
Spius Creek	H_O	0.526	0.800	0.463	0.635	0.630	0.620	0.389	0.793
	HWE	0.297	0.357	0.557	0.760	0.233	0.103	0.527	0.323
	Years	0.000*	0.002*	0.026	0.613	0.073	0.000*	0.101	0.030
Coldwater River	H_O	0.656	0.857	0.400	0.594	0.481	0.621	0.250	0.841
	HWE	0.520	0.329	0.009	0.448	0.089	0.060	0.578	0.501
	Years	0.370	0.913	0.241	0.324	0.127	0.012	0.906	0.259
Deadman River	H_O	0.522	0.671	0.597	0.667	0.746	0.691	0.494	0.784
	HWE	0.087	0.007	0.287	0.459	0.536	0.035	0.372	0.394
	Years	0.000*	0.000*	0.032	0.601	0.050	0.002*	0.673	0.124
Bonaparte River	H_O	0.720	0.735	0.505	0.667	0.746	0.691	0.494	0.784
	HWE	0.280	0.001*	0.004*	0.140	0.028	0.475	0.973	0.020
	Years	0.744	0.965	0.435	0.974	0.544	0.002*	0.871	0.210
				Fraser River					
Stein River	H_O	0.688	0.800	0.538	0.688	0.647	1.000	0.313	0.938
	HWE	0.533	0.663	0.201	0.594	0.058	0.090	1.000	0.782
Nahatlatch River	H_O	0.732	0.703	0.487	0.500	0.524	0.813	0.308	0.814
	HWE	0.670	0.438	0.395	0.680	0.437	0.375	0.550	0.451
	Years	0.104	0.002*	0.422	0.620	0.005*	0.170	0.312	0.472
Chilko River	H_O	0.250	0.592	0.324	0.551	0.543	0.723	0.408	0.870
	HWE	0.456	0.106	1.000	0.723	0.905	0.006	0.409	0.614
	Years	1.000	0.570	0.132	0.458	0.330	0.007	0.805	0.993
Bridge River	H_O	0.409	0.684	0.450	0.529	0.810	0.800	0.250	0.950
	HWE	0.006	0.034	1.000	0.197	0.113	0.170	1.000	0.934
				Vancouver Island					
Puntledge River	H_O	0.731	0.810	0.850	0.577	0.717	0.851	0.353	0.891
	HWE	0.898	0.913	0.611	0.174	0.008	0.231	1.000	0.367
	Years	0.660	0.062	0.175	0.037	0.115	0.147	0.446	0.291
Salmon River	H_O	0.808	0.654	0.478	0.680	0.667	0.727	0.462	0.824
	HWE	0.130	0.132	0.226	0.775	0.263	0.274	0.310	0.430
Caycuse River	H_O	0.630	0.532	0.500	0.489	0.739	0.652	0.277	0.851
	HWE	0.310	0.000*	0.044	0.164	0.999	0.004	1.000	0.000*
Nahmint River	H_O	0.744	0.463	0.395	0.455	0.791	0.791	0.159	0.535
	HWE	0.045	0.000*	0.037	0.641	0.650	0.699	0.032	0.004
Robertston Creek	H_O	0.647	0.657	0.576	0.736	0.708	0.694	0.100	0.785
	HWE	0.472	0.010	0.000*	0.201	0.095	0.000*	1.000	0.000*
China Creek	H_O	0.727	0.750	0.900	0.250	0.727	0.727	0.333	0.222
	HWE	0.184	0.155	0.242	1.000	0.361	0.538	0.305	0.000*
				Washington State					
Deer Creek	H_O	0.707	0.778	0.532	0.680	0.598	0.857	0.250	0.857
	HWE	0.379	0.150	0.394	0.313	0.220	0.716	0.598	0.310
	Years	0.001*	0.022	0.005*	0.007	0.027	0.000*	0.091	0.000*
Bogachiel River	H_O	0.725	0.692	0.649	0.714	0.816	0.730	0.080	0.840
	HWE	0.759	0.167	0.680	0.386	0.551	0.044	1.000	0.095
				Columbia River					
Clearwater River	H_O	0.653	0.755	0.820	0.580	0.633	0.816	0.082	0.767
	HWE	0.871	0.012	0.746	0.496	0.426	0.353	1.000	0.000*
Salmon River	H_O	0.596	0.800	0.653	0.580	0.531	0.721	0.085	0.767
	HWE	0.970	0.000*	0.011	0.385	0.371	0.117	1.000	0.464
Tucannon River	H_O	0.478	0.811	0.609	0.648	0.589	0.847	0.184	0.869
	HWE	0.001*	0.000*	0.000*	0.001*	0.000*	0.000*	0.400	0.008
Beech River	H_O	0.700	0.811	0.609	0.648	0.589	0.847	0.143	0.850
	HWE	0.666	0.472	0.301	0.602	0.208	0.678	1.000	0.377
Umatilla River	H_O	0.563	0.827	0.725	0.600	0.776	0.731	0.155	0.833
	HWE	0.539	0.194	0.078	0.033	0.440	0.093	1.000	0.004
	Years	0.806	0.179	0.187	0.621	0.460	0.147	0.706	0.832
Kalama River	H_O	0.526	0.632	0.692	0.579	0.846	0.895	0.368	0.917
	HWE	0.256	0.073	0.930	0.049	0.481	0.497	0.354	0.875

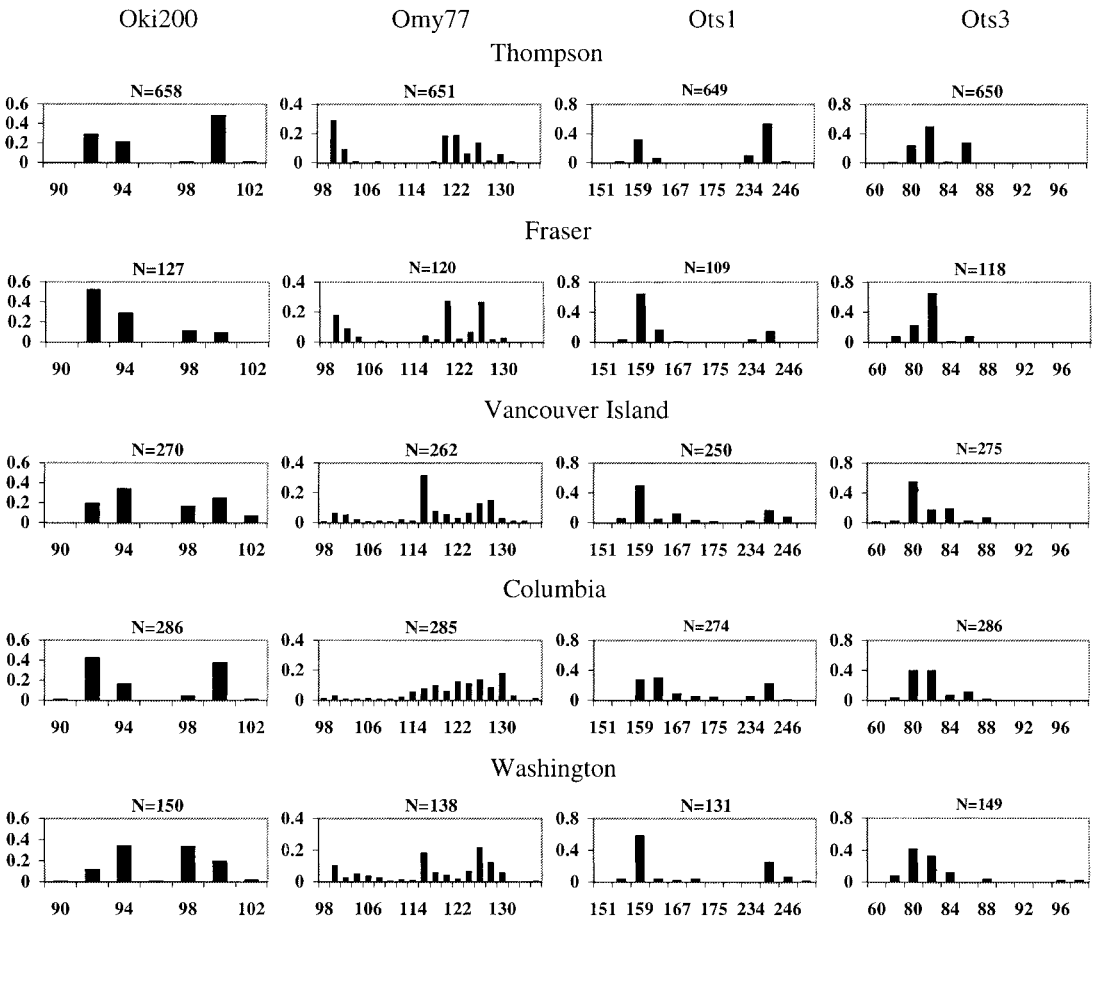

FIGURE 2.—Regional allele frequencies of steelhead in the Thompson River, upper Fraser River, Vancouver Island, Columbia River, and Washington State regions at eight microsatellite loci. For all loci, alleles were designated by the lower limit (base pairs) of the allele bin used to define the alleles; N = number of steelhead sampled in each region.

as the overall frequency of the allele in Columbia River steelhead populations was 0.02 (Figure 2). Thompson River steelhead were also distinguished by higher frequencies of allele *140* at *Ots108* (0.40) than steelhead in all other regions (<0.10). Upper Fraser River steelhead had higher frequencies of allele *92* at *Oki200* (0.52) than did steelhead in other regions (0.13–0.42) and also had higher frequencies of allele *82* at *Ots3* (0.64) than did steelhead in other regions (0.17–0.49). Vancouver Island steelhead were characterized by relatively high frequencies of allele *116* at *Omy77* (0.31) compared with steelhead in other regions (0.00–0.18) as well as higher frequencies of allele *126* at *Ssa85* (0.40) than steelhead in other regions (0.03–0.14). Columbia River steelhead had higher frequencies of allele *96* at *Ots108* (0.20) than did other regional groups (0.02–0.09) and had a virtual absence of alleles of less than 112 bp at *Omy77*. The two Washington populations differed from steelhead in other regions by displaying higher frequencies of allele *167* at *Ots100* (0.22) than steelhead in other regions (0.05–0.13).

Pairwise comparisons of population allele frequencies within regions were conducted to evaluate the level of population separation within regions, with the populations compared derived from those having the most similarity (Figure 3).

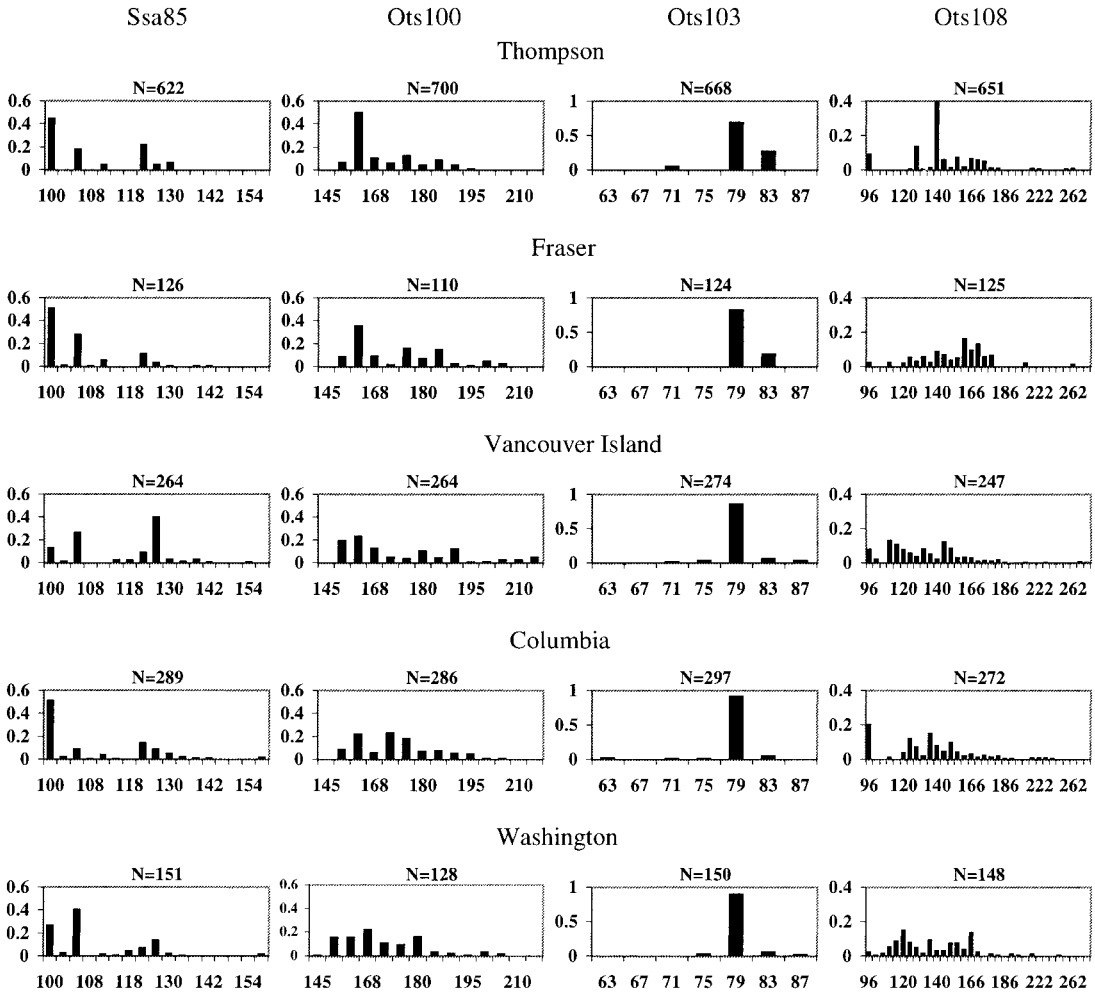

FIGURE 2.—Extended.

For the Thompson River populations, there were no significant differences in allele frequencies at any locus between the Coldwater River and Spius Creek populations (four populations, six tests per locus, $\alpha = 0.0083$), which is not surprising because they are both in tributaries of the same river drainage (Nicola River). Allele frequencies for the Deadman and Bonaparte river populations were different at four loci, indicative of some level of population separation. In the upper Fraser River region, allele frequencies of the Stein and Nahatlatch river populations were significantly different at only one locus (*Ots108*; four populations, six tests per locus), and allele frequencies of the Chilko and Bridge river populations were significantly different at three loci. For the Vancouver Island region, allele frequencies of the Nahmint and Caycuse river populations were significantly different at four loci (six populations, 15 tests per locus, $\alpha = 0.0033$), which is reasonable given that the populations are in different watersheds on the west coast of Vancouver Island. Allele frequencies of the Puntledge River and Robertson Creek populations were significantly different at seven loci, indicative of their locations on the east and west coasts of Vancouver Island, respectively. In the Columbia River region, there were no significant differences in allele frequencies at any locus between the Beech and Umatilla river populations (15 tests per locus, $\alpha = 0.0033$), but three signif-

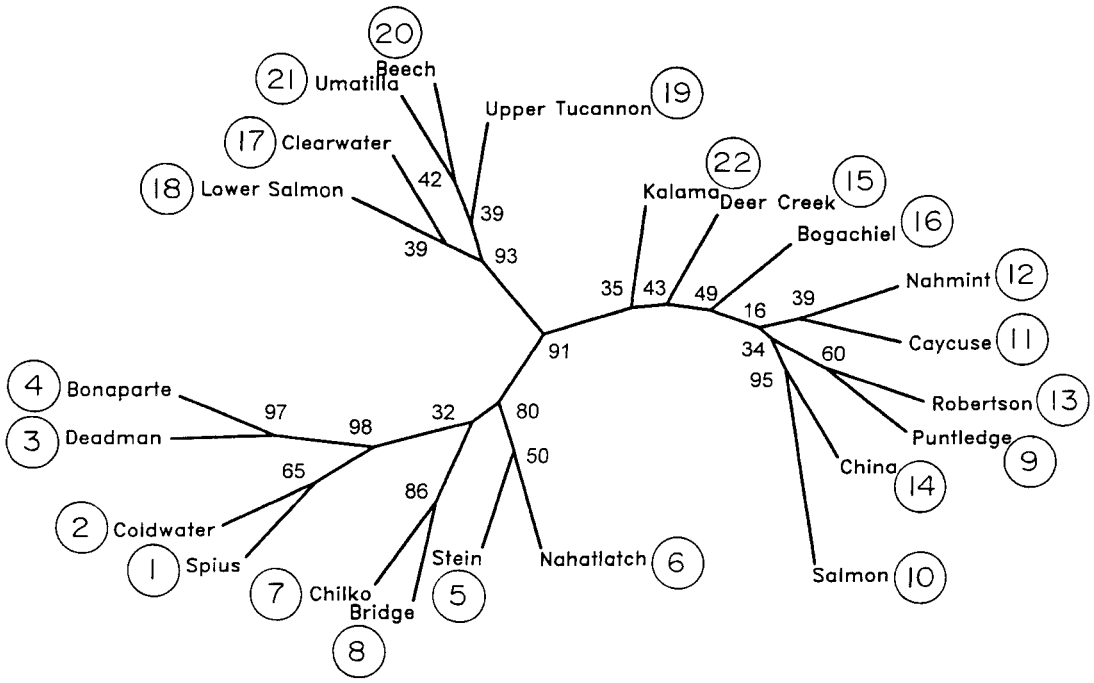

FIGURE 3.—Unrooted neighbor-joining tree outlining relationships of 22 steelhead populations. Bootstrap values at the tree nodes indicate the percentage of 500 trees where populations beyond the node occurred together. Numbers next to the population names correspond with locations in Figure 1 and Table 1.

icantly different frequencies were observed between the Lower Salmon and Clearwater river populations.

Hierarchical Population Structure

Because there was annual variation in allele frequencies for some populations at some of the loci surveyed, we compared the relative level of annual variability to the differentiation observed among populations and regions. Hierarchical gene diversity analysis of steelhead from 22 spawning populations indicated that 90.5% of variation occurred within populations (Table 5). Variation among regions was the greatest source of the remaining variation (4.4%), followed by variation among populations within regions (3.1%) and variation among years within populations (2.0%). The greatest structuring among regions was observed at *Ots1* (8.7%). The magnitude of the differentiation among populations within regions was usually greater than the annual variation within populations (Table 5). The ratio of the sum of the variance components of population and regional differentiation divided by the annual component within

TABLE 5.—Hierarchical gene diversity analysis of 22 populations of steelhead for eight microsatellite DNA loci. Regions, populations with regions, and years within populations were outlined in Table 1.

	Absolute diversity		Relative diversity			
Locus	Total	Within populations	Within populations	Among years within populations	Among populations within regions	Among regions
Oki200	0.7314	0.6428	0.8789	0.0138	0.0666	0.0432
Omy77	0.8939	0.8191	0.9163	0.0407	0.0098	0.0331
Ots1	0.7233	0.6199	0.8571	0.0028	0.0529	0.0873
Ots3	0.6851	0.6138	0.8959	0.0124	0.0556	0.0434
Ssa85	0.7714	0.7027	0.9110	0.0047	0.0419	0.0425
Ots100	0.8343	0.7704	0.9233	0.0470	0.0000	0.0296
Ots103	0.3063	0.2869	0.9367	0.0246	0.0064	0.0324
Ots108	0.9308	0.8641	0.9284	0.0126	0.0211	0.0379
All			0.9047	0.0203	0.0313	0.0437

populations was 8.0, 1.1, 50.1, 8.0, 18.0, 0.6, 1.6, and 4.7 for *Oki200*, *Omy77*, *Ots1*, *Ots3*, *Ssa85*, *Ots100*, *Ots103*, and *Ots108*, respectively. The average ratio for all loci was 3.77.

Regional structuring of populations was evident. Thompson River populations tended to form a relatively distinct group, with the four populations clustering in a well-defined group 98% of the time in the 500 trees used to create the consensus tree (Figure 3). The Columbia River populations (except for Kalama River, possibly due to small sample size) were distinct from populations in other regions and were well defined from those in the Thompson River or upper Fraser River drainages (consensus value 93%). Vancouver Island populations were less well differentiated but reasonably distinct from other populations.

Estimation of Stock Composition

We tested the utility of microsatellite DNA loci to estimate of stock composition in mixed-stock fisheries by estimating the accuracy and precision of stock composition estimates in simulated fishery samples. Two simulated test fishery mixtures were developed that span the range of likely regional abundances of steelhead in west coast Vancouver Island fisheries. Thompson River steelhead were estimated with a high degree of accuracy and precision (Table 6), reflecting the relative genetic distinctiveness of this stock. Estimated proportions of steelhead from the Fraser River watershed (combining Thompson and upper Fraser rivers) were accurate and precise. Estimated proportions of Canadian and U.S. stock composition were well estimated in the ranges examined. Each simulation used fish from different populations in each region, yet the estimates of regional contributions to the mixtures were relatively stable, indicative of the relative similarity of steelhead populations within regions. The simulations indicated that the eight microsatellite DNA loci surveyed could be used to provide relatively accurate and precise estimates of stock composition for fishery management applications given that no unsampled stocks contribute significantly to the mixture.

TABLE 6.—Estimated percentage composition of two simulated mixtures of steelhead incorporating variation at eight microsatellite DNA loci. Each mixture of 150 fish was generated 100 times with replacement, and stock compositions of the mixtures were estimated by resampling each baseline stock with replacement to obtain a new distribution of allele frequencies (sample size was constant). The individual estimates for all populations within a region have been summed (bold) to provide regional estimates (Σ) of stock composition.

Population or region	Actual %	Estimated % (SD)
Mixture 1		
Canada		
Deadman River	10	9.6 (4.8)
Bonaparte River	20	22.4 (4.6)
Coldwater River	20	15.0 (3.4)
ΣThompson River	50	50.8 (1.9)
Chilko River	20	18.3 (1.9)
ΣFraser River	20	19.6 (1.4)
Caycuse River	10	8.9 (1.3)
ΣCanada	**80**	**79.9 (1.9)**
United States		
Umatilla River	10	8.2 (1.8)
ΣColumbia River	10	10.9 (1.6)
Bogachiel River	10	8.3 (1.4)
ΣUnited States	**20**	**20.1 (1.9)**
Mixture 2		
Canada		
Spius Creek	10	8.4 (2.0)
Robertson Creek	20	19.2 (2.3)
Nahmint River	20	17.6 (2.2)
ΣCanada	**50**	**51.8 (2.9)**
United States		
Tucannon River	10	10.4 (1.6)
Kalama River	10	5.8 (1.8)
Bogachiel River	20	17.5 (2.7)
Deer Creek	10	12.5 (1.9)
ΣUnited States	**50**	**48.2 (2.9)**

Identification of Individuals

Determination of the origin of a single steelhead can sometimes be of interest in enforcement. The overall classification accuracy of single individuals to the five regional groups using a jackknifed discriminant analysis was 80%, with the highest accuracy (92%) observed in Thompson River steelhead, a distinctive stock group (Table 7). Thompson River steelhead were identified as originating from the Fraser River drainage in 97% of individuals, and upper Fraser River steelhead were identified as originating from the Fraser River drainage in 86% of individuals. When the analysis was restricted to only Fraser River drainage populations, Thompson River steelhead were correctly classified to region in 96% of individuals, and the upper Fraser River steelhead were correctly classified to region in 80% of individuals. The microsatellite loci surveyed in our study provide the basis to identify fish to specific regions with a fairly high level of accuracy.

Applications to Fisheries

During 1994–1996, more than 85% of the steelhead sampled in the directed chum salmon fishery off southwestern Vancouver Island (Figure 1, Table 1) were estimated to have originated in the

TABLE 7.—Percent correct classification (in bold italics) of individual fish using jackknifed discriminant analysis for five regional groupings of steelhead. Variation at eight microsatellite loci was used to classify individual fish, and only steelhead scored at all eight loci (*N*) were used in the analysis.

		Classified region				
Actual region	*N*	Thompson River	Fraser River	Vancouver Island	Columbia River	Washington State
Thompson River	467	***91.7***	5.4	0.2	2.6	0.2
Fraser River	80	15.0	***71.3***	1.3	5.3	6.3
Vancouver Island	193	1.6	2.1	***81.4***	3.1	11.9
Columbia River	212	8.5	3.3	2.4	***84.4***	1.4
Washington State	107	0.0	6.5	14.6	8.4	***70.1***

Fraser River drainage, with the majority of the steelhead originating from the Thompson River (Table 8). Only about 5% of the steelhead were estimated to have originated from Vancouver Island, with the remaining 10% of U.S. origin. There was little annual variation in the estimated stock composition in these three years. However, in 1997, although sample size was quite small (26 fish) and there was a large amount of uncertainty in the estimated stock composition, steelhead of U.S. origin were estimated to have composed the majority of the samples, and the Canadian component was largely of Fraser River steelhead.

Estimated stock composition of samples from the 1997 directed sockeye salmon fishery in Barkley Sound and Alberni Inlet indicated that the majority (71%) of steelhead was of Vancouver Island origin and the remainder was of U.S. origin. No Fraser River steelhead were estimated to have been present in this fishery.

Discussion

In the current study, we focussed on sampling adult steelhead when possible to derive the DNA samples. Sampling adults lessens the possiblity of nonrepresentative sampling or ''family sampling'' that may occur when juveniles are sampled (Hansen et al. 1997; Wenburg et al. 1998), avoids the potential of mixing samples of juvenile rainbow trout with juvenile steelhead (Parkinson 1984b), and avoids the potential of obtaining biased samples if juveniles rear in nonnatal streams. In the upper Tucannon River population, genotypic frequencies were not in Hardy–Weinberg equilibrium in six of eight loci surveyed, and this may reflect that a nonrepresentative sample of juveniles was obtained from this population.

Significant annual variation in allele frequencies was observed in at least one locus in five of nine populations in which annual comparisons were possible. Annual variation in allele frequencies at allozyme loci has also been observed previously (Chilcote et al. 1980; Parkinson 1984b; Reisenbichler and Phelps 1989; Reisenbichler et al. 1992). Higher annual variation in allele frequencies relative to population differentiation was observed at the eight microsatellite DNA loci surveyed in steelhead (population and region differentiation 3.7 times greater than variation within populations) than has been observed at six micro-

TABLE 8.—Estimated percentage stock compositions (and SD) for steelhead sampled (*N*) in commercial chum salmon fisheries off the mouth of Nitinat River (1994–1997) and commercial sockeye salmon fisheries in Barkley Sound (1997) off the southwest coast of Vancouver Island.

	Nitinat River mouth				Barkley Sound,
Region	1994 (N = 104)	1995 (N = 89)	1996 (N = 143)	1997 (N = 26)	1997 (N = 58)
Thompson River	56.6 (5.3)	44.5 (6.6)	49.9 (4.5)	22.8 (8.9)	0.0 (1.7)
Upper Fraser River	29.7 (5.6)	43.9 (6.6)	37.2 (4.9)	13.8 (8.1)	0.0 (1.4)
ΣFraser River	86.3 (5.0)	88.4 (6.5)	87.1 (4.4)	36.6 (8.2)	0.0 (2.4)
Vancouver Island	3.6 (3.2)	0.7 (0.5)	4.7 (2.0)	4.3 (5.4)	70.7 (11.4)
ΣCanada	**89.9 (4.3)**	**89.1 (4.4)**	**91.8 (3.4)**	**40.9 (12.8)**	**70.7 (11.6)**
Columbia River	5.7 (1.5)	1.3 (1.5)	3.4 (1.8)	37.2 (14.4)	7.8 (5.9)
ΣUnited States	**10.1 (4.3)**	**10.8 (4.4)**	**8.1 (3.4)**	**59.1 (12.8)**	**29.3 (11.6)**

satellite loci in sockeye salmon (population differentiation 7.4–11.8 times greater than variation within populations; Beacham and Wood 1999; Beacham et al., in press). Significant annual variation in allele frequencies can be a result of a real biological process or a result of unrepresentative or inadequate samples. Steelhead populations are generally characterized by relatively small population sizes, certainly considerably smaller than the sockeye salmon populations surveyed. Therefore, the higher levels of annual variation observed in steelhead compared with sockeye salmon may reflect smaller effective population size. However, because the number of steelhead surveyed in most years in the comparisons was relatively small, unrepresentative or inadequate sampling cannot be discounted and may in fact have contributed substantially to the observed annual variation.

Population structure of steelhead in British Columbia was investigated previously by Parkinson (1984b) who reported, based on the analysis of five allozyme loci, that there were three main regional groups: (1) Skeena River populations in northern British Columbia, (2) all coastal populations including those in the Fraser River (Stein and Nahatlatch rivers), and (3) an interior Fraser River group that included Thompson River and the upper Fraser River (Chilko and Bridge rivers). Our survey based on eight microsatellite DNA loci indicated a finer scale structure at the regional scale in southern British Columbia, with the Thompson River populations distinct from all upper Fraser River and upper Columbia River populations.

Similar patterns in genetic differentiation between Thompson River and other Fraser River populations has been observed in coho salmon *O. kisutch* (Small et al. 1998), chinook salmon *O. tshawytscha,* and sockeye salmon (T. D. Beacham, unpublished). This similarity among species suggests that separate founding populations from possibly two glacial refugia colonized the Fraser and Thompson rivers rather than divergence in a single postglacial founding population (Hewitt 1996). All upper Fraser River populations were also differentiated from coastal populations on Vancouver Island, and this distinction was not observed using allozymes (Parkinson 1984b). About 10% of total genetic variation was not observed within populations, indicative of genetic differentiation among populations and comparable with values observed in other salmonids (Wenburg et al. 1998).

Steelhead populations from the Columbia River constituted a very distinct group except for the lower Kalama River population, which was more similar to coastal populations in Washington State. Previous studies have noted that *O. mykiss* in the upper portions of both the Fraser River and Columbia River drainages were genetically distinct from the lower portions of these systems or coastal populations (Okazaki 1984; Reisenbichler et al. 1992), again reflecting the isolation of *O. mykiss* in two refugia during the last glaciation (McPhail and Lindsey 1986).

Representative sampling of the stocks contributing to a mixed-stock fishery is critical to ensure reliable estimation of stock composition. Given the logistical difficulties in obtaining samples from mature steelhead, it is highly desirable that the characters used in stock identification be stable over time or that annual variation is less than the differentiation among stocks to allow samples to be pooled over several years. This is an issue because temporal changes in allele frequencies can affect mixed-stock fishery analyses (Waples 1990). The choice of technique to use for estimation of stock composition in mixed-stock fisheries depends largely upon the relative differentiation among stocks of interest, the level of year-to-year variation within stocks, and the cost of analysis. In an analysis of microsatellite DNA variation over some 60 years in one population of Atlantic salmon *Salmo salar,* Nielsen et al. (1997a) reported some shifts in allele frequencies with time. However, individuals from samples 60 years apart clustered together when compared with the closest neighboring population and another reference population. Temporal variation within a population was less than the differentiation among populations, similar to the general pattern observed among the steelhead populations in our study. In the case of the steelhead populations surveyed, the genetic variation attributable to population and regional differentiation was about 3.7 times greater than the variation attributable to annual variation within stocks and could increase after judicious selection of microsatellite loci surveyed. Given the reasonable temporal stability of these loci, estimation of microsatellite allele frequencies in baseline populations would not be required annually, thus reducing costs considerably. However, some level of monitoring of allele frequencies with time is prudent to ensure that no dramatic changes have occurred.

The simulated mixtures evaluated for steelhead indicated that microsatellite DNA variation could be used to provide accurate and reasonably precise estimates of regional stock groups in the catch

mixtures. For example, under the assumption that baseline contributing stocks have been adequately sampled, it was possible to estimate the proportion of steelhead originating from Canada and the United States in a 150-fish simulated sample within an accuracy of 2%. It was also possible to differentiate between upper Fraser River and Thompson River steelhead within an accuracy of 1%. In applications in the Fraser River drainage, individual fish can be identified as originating from the upper Fraser or Thompson rivers with a fairly high degree of accuracy.

The stock composition results of this study are similar to those provided by Parkinson (1984a) with a predominance of interior fall-run steelhead occurring in the chum salmon fishery off the southwest coast of Vancouver Island. Our study was able to provide greater detail of interior stock composition and confirm that a majority of these interior fish were of Fraser River, and not Columbia River, origin in most years. The estimated stock composition of the steelhead bycatch in the Nitinat River mouth chum salmon fishery was relatively constant from 1994 to 1996 at approximately 85% Fraser River drainage populations, 5% Vancouver Island populations, and 10% U.S. populations, with Thompson River populations comprising the majority of the Fraser River component. These results are based on the assumption that stocks contributing to this fishery have been adequately represented in the baseline used to estimate stock composition and that unsampled stocks present in the fishery would have genetic characteristics most similar to geographically nearby sampled stocks. If steelhead originating from east coast of Vancouver Island, Oregon, or California occur in significant abundance in this fishery and are genetically distinct from the existing baseline populations, then there may be an unknown degree of bias in the estimated stock compositions. Ocean migration patterns suggest a distinction between steelhead populations north and south of Cape Blanco in Oregon (Busby et al. 1996). Steelhead from south of Cape Blanco tend to be south-migrating rather than north-migrating (Pearcy et al. 1990), so it is unlikely that California-origin steelhead would contribute to the steelhead bycatch in the Nitinat River mouth chum salmon fishery. In 1997, although sample sizes were very small (only 26 fish were sampled), the estimated proportion of steelhead of United States origin substantially increased, accounting for approximately 60% of the sample. Is this estimate reasonable given the results of the three previous years? The number of steelhead with adipose fin clips (indicating hatchery origin) was first recorded in this fishery in 1995. That year 4.5% of the steelhead sampled in this fishery had adipose fin clips, and 6.0% had them in 1996 (James Mitchell, Department of Fisheries and Oceans, personal communication). However, in 1997 50% (13/26) of the steelhead were fin-clipped, substantially higher than in the previous two years. In British Columbia populations sampled in this study, hatchery production of steelhead relative to wild production is quite limited with the exception of Robertson Creek, and there is no current hatchery enhancement of Thompson River or upper Fraser River populations. Thus it seems possible that the enhanced steelhead were of U.S. origin, and the DNA analysis was able to identify the higher proportion of U.S. steelhead in the sample. These results are consistent with the relative run timing anomalies of the Columbia River ''B'' run and interior Fraser River for this year. Upper Columbia River fish are usually earlier than interior Fraser River fish, but both groups were 2–3 weeks later than normal years, and the majority of Columbia River fish had adipose fin clips (89%) relative to interior Fraser River fish (<5%; Bison, personal communication)

The estimated stock composition of the steelhead bycatch in the 1997 Barkley Sound sockeye fishery indicated that approximately 71% of the sample was derived from Vancouver Island populations and 29% from U.S. populations, with no upper Fraser River or Thompson River steelhead present. No data were available on the proportion of steelhead sampled that had their adipose fins clipped. Given the timing of the fishery (late June to mid-July), it is unlikely that fall-run steelhead from the upper Fraser River or Thompson River would have been present. It would not generally be expected that steelhead originating from the United States should constitute much of the steelhead bycatch in the Barkley Sound sockeye salmon fishery. However, migration patterns in 1997 were unusual in other salmon species in British Columbia in 1997, presumably owing to ocean conditions, and the U.S. steelhead migration pattern in 1997 might have been in more inshore waters than normally expected. The elevated proportions of U.S. steelhead in the 1997 Barkley Sound sockeye salmon fishery would be consistent with the higher proportions observed in the Nitinat River mouth chum salmon fishery approximately 3 months later.

Although steelhead populations from the southeast coast of Vancouver Island have been sampled,

individual population sample sizes did not exceed 10 fish, and these populations were excluded in the development of the baseline used in estimation of stock composition. Future applications to estimation of steelhead stock composition in southern British Columbia marine fisheries could include additional populations from the east coast of Vancouver Island as well as from Puget Sound and the coastal regions of Washington, Oregon, and California.

Applications of microsatellite DNA variation to management of steelhead in the Fraser River drainage could include identification of individual steelhead to either Thompson River or upper Fraser River origin. Increased sampling of upper Fraser River populations will likely result in classification accuracy greater than the 80% level observed for that stock group in the current study. It should be possible to develop run timing curves for individual upper river and Thompson River populations as they pass through lower river test fisheries.

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