Abstract—We surveyed variation at 13 microsatellite loci in approximately 7400 chinook salmon sampled from 52 spawning sites in the Fraser River drainage during 1988–98 to examine the spatial and temporal basis of population structure in the watershed. Genetically discrete chinook salmon populations were associated with almost all spawning sites, although gene flow within some tributaries prevented or limited differentiation among spawning groups. The mean F_{ST} value over 52 samples and 13 loci surveyed was 0.039. Geographic structuring of populations was apparent: distinct groups were identified in the upper, middle, and lower Fraser River regions, and the north, south, and lower Thompson River regions. The geographically and temporally isolated Birkenhead River population of the lower Fraser region was sufficiently genetically distinctive to be treated as a separate region in a hierarchial analysis of gene diversity. Approximately 95% of genetic variation was contained within populations, and the remainder was accounted for by differentiation among regions (3.1%), among populations within regions (1.3%), and among years within populations (0.5%). Analysis of allelic diversity and private alleles did not support the suggestion that genetically distinctive populations of chinook salmon in the south Thompson were the result of postglacial hybridization of ocean-type and stream-type chinook in the Fraser River drainage. However, the relatively small amount of differentiation among Fraser River chinook salmon populations supports the suggestion that gene flow among genetically distinct groups of postglacial colonizing groups of chinook salmon has occurred, possibly prior to colonization of the Fraser River drainage.

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The geographic basis for population structure in Fraser River chinook salmon (*Oncorhynchus tshawytscha*)

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The Fraser River drainage supports the largest group of chinook salmon (*Oncorhynchus tshawytscha*) populations in North America, and the most abundant individual chinook salmon population in British Columbia (the Harrison River in the lower Fraser River drainage). Chinook salmon are distributed throughout the entire Fraser River drainage, and spawning populations exist in approximately 65 tributaries (Fraser et al., 1982). There is substantial variation among and within populations for life history traits such as length of juvenile freshwater residence, size and age at maturity, and timing of adult migration and spawning. Juveniles (largely from the Harrison River population) can migrate to the marine environment immediately after fry emerge in the spring, or during the first summer after developing in fresh water for several months ("ocean-type"), or they can remain in fresh water for a year or longer ("stream-type") before migrating to the ocean (Fraser et al., 1982; Healey, 1983, 1991).

Migrating adult chinook salmon returning to spawn in Fraser River tributaries enter the drainage from April to October—individual populations having characteristic migration times (Fraser et al., 1982). Spring or "early run" populations are designated as those in which at least 50% of the fish pass through the lower Fraser by 15 July and include populations of the mid and upper Fraser areas, the lower Thompson area, and a small lower Fraser population that spawns in the upper reaches of the Harrison drainage (Birkenhead River). Summer or "mid run" populations are those in which the majority of the fish pass through the lower Fraser after 15 July, but before 31 August, and include populations of the mid Fraser area, and the north and south Thompson areas. Chinook salmon of the large Harrison River population are fall or "late run" fish and enter the lower Fraser in September and October. Thus, in the Fraser drainage, it is the late-run Harrison population of the lower Fraser that is characterized by ocean-type juveniles, whereas the earlier migrating spring and summer populations of the interior Fraser drainage are characterized by stream-type juveniles. Exceptions are

several interior populations of the South Thompson River system, which produce substantial proportions of both stream- and ocean-type juveniles (DFO[1]).

Variability in chinook salmon flesh pigmentation levels exceeds that of other Pacific salmonids, resulting from a phenotypic dichotomy of "red-fleshed" and "white-fleshed" forms that is under relatively simple genetic control (Withler, 1986; McCallum et al., 1987). Chinook salmon that return to spawn in the Fraser River are mixtures of red- and white-fleshed fish (Godfrey, 1975). The large Harrison River population is considered to be entirely white-fleshed; whereas red-fleshed fish predominate in populations of the upper Fraser and Thompson drainages. Red-fleshed chinook salmon are also abundant in mid-Fraser tributaries, but a number of populations are polymorphic for flesh color (e.g. the Quesnel River population consists of approximately equal proportions of the two flesh colors).

Similar variation in migration timing and juvenile fresh water residence times is observed in chinook salmon of the Columbia River drainage (Myers et al., 1998). In that system, adult migration time is not simply correlated with juvenile life history type; spring-, summer-, and fall-run populations in the lower and mid Columbia regions can all be characterized by ocean-type juveniles. Genetic analysis has indicated that, in the Columbia drainage, juvenile life history type is a better indicator of genetic relationships among populations than is adult migration time and supports the suggestion that the juvenile life history types might represent separate "races" in that area (Myers et al., 1998). In British Columbia, which has been recolonized by chinook and other salmon species in the last 10,000 years since the Wisconsin glacial period from as many as four possible refugial areas (Gharrett et al., 1987; Wilson et al., 1987; Utter et al., 1989; Cronin et al., 1993), the phylogenetic relationships of ocean- and stream-type populations have yet to be determined and may vary on a geographic basis dependent on both adaptation and the history of colonization.

In the Fraser River, a genetic demarcation between fish of the lower and interior Fraser watersheds observed in coho and sockeye salmon and attributed to independent postglacial colonization of the two regions (Wehrhahn and Powell, 1987; Wood et al., 1994; Small et al., 1998; Withler et al., 2000), is also evident in chinook salmon (Beacham et al., 1996; Teel et al., 2000; Nelson et al., 2001). Factors underlying the substantial genetic differentiation among chinook salmon occupying different areas of the interior Fraser watershed, a region postulated to have been colonized from the Columbia River refuge (Utter et al., 1989), are less clear (Teel et al., 2000; Nelson et al., 2001).

Differentiation among areas within the Thompson drainage, and between the interior Fraser and Thompson drainages, has also been observed in coho and sockeye salmon. Of the three species, the chinook salmon is the most extensively and continuously distributed throughout the interior Fraser region. The variability in age of maturity, both within and among populations, is greater in chinook salmon than in coho or sockeye salmon—a factor that may increase the effective number of spawning fish each year and reduce genetic variability due to drift both within and among populations of chinook salmon. On the other hand, the development of population-specific anatomical structures and life history traits indicate that recent gene flow has been sufficiently restricted among populations to enable strong adaptation (Taylor, 1991).

The presence of ocean-type juveniles in the Eagle and Shuswap River populations of the relatively warm and productive South Thompson River drainage may represent a relatively recent adaptive response to environmental conditions enabling attainment of sufficient size in the first spring of freshwater residence for juveniles to undertake seaward migration. Conversely, Teel et al. (2000) suggested that chinook salmon of the south Thompson region were genetically intermediate to those of the mid–upper and lower Fraser areas and might represent hybridization of stream- and ocean-type races that independently colonized the lower and interior portions of the Fraser drainage.

Conservation of chinook salmon genetic diversity within the Fraser River requires delineation of the phylogenetically and adaptively distinct groups within the drainage, an understanding of their origins and the evolutionary processes promoting and maintaining their differentiation, and the ability to manage them on an independent basis. Similar zoogeographic factors will have influenced the coho, chinook, and sockeye populations recolonizing the Fraser drainage, although the differing utilization of glacial refugia, environmental requirements, and propensities for homing and straying may have resulted in quite different evolutionary responses among them.

The objective of the present study was to analyze variation at 13 microsatellite loci in 52 samples of chinook salmon from the Fraser and Thompson River drainages to determine population structure. By detecting differentiation in allelic frequencies, levels of allelic diversity, and the presence of unique alleles, we were able to use the high levels of polymorphism and heterozygosity at the microsatellite loci to indicate the relationships among chinook salmon occupying different regions of the Fraser drainage. The distribution of genetic diversity in the Fraser River drainage among regions, populations, and sampling years is estimated, as well as the stability of population structure within major tributaries of the Fraser River. We also examined the patterns of divergence within the drainage with respect to ocean- and stream-type life histories.

Materials and methods

Collection of baseline DNA samples and laboratory analysis

Genomic DNA was extracted from either liver, scales, operculum punches, or fin clips from chinook salmon sampled between 1987 and 1998 by using the phenol-chloroform

[1] DFO (Department of Fisheries and Oceans). 1995. Fraser River chinook salmon. Fraser River Action Plan, Fishery Management Group, Vancouver, British Columbia, Canada, 24 p. [Available from Fisheries and Oceans, 555 West Hasting St., Suite 1220, Vancouver, British Columbia, Canada V6B 5G3.]

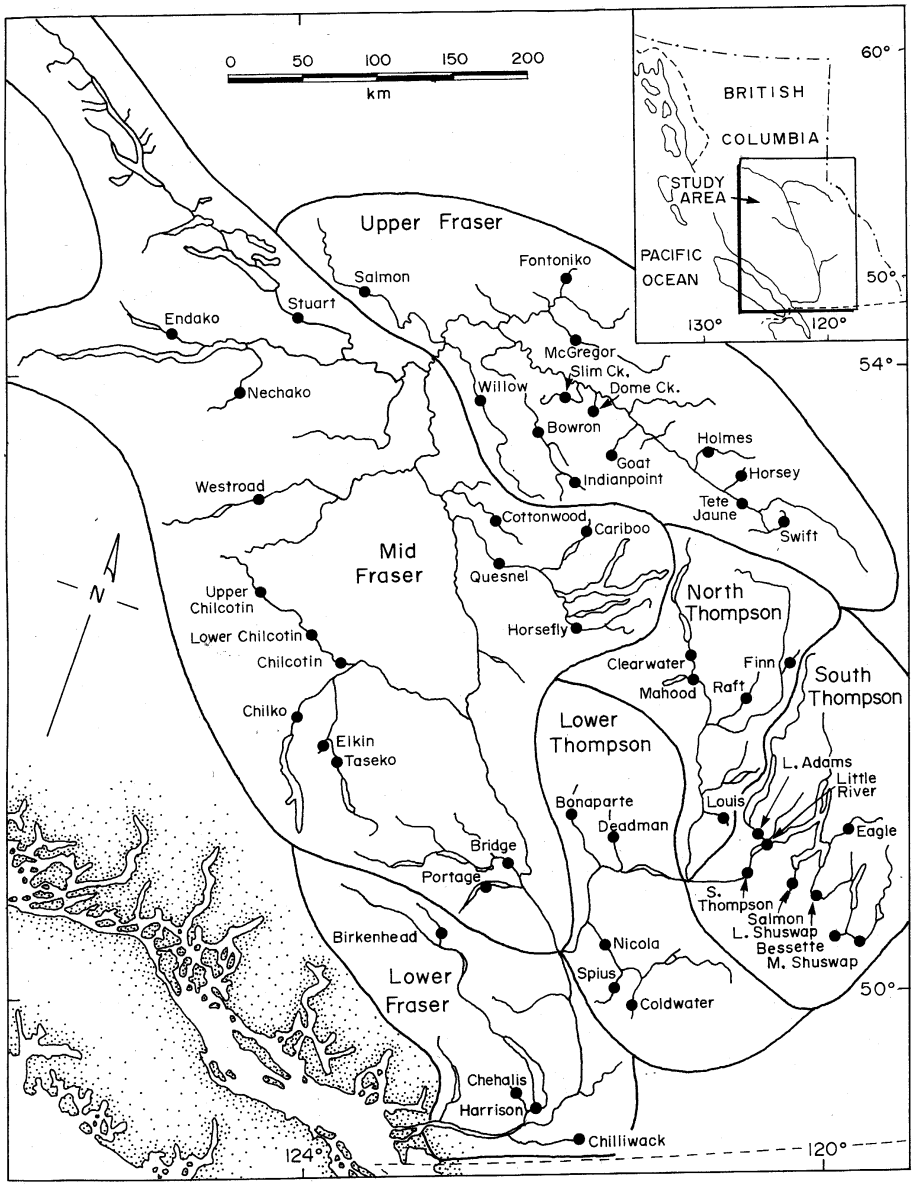

Figure 1

Locations in the Fraser River drainage where 52 chinook salmon spawning aggregates were sampled at least once during 1988–98.

protocol of Miller et al. (1996) (early extractions) or the chelex resin protocol of Small et al. (1998) (later extractions). Samples were derived from adults in all areas except the McGregor River where, because of the difficulty of obtaining adults, juveniles were sampled (Fig. 1). Samples of adults were obtained from hatcheries during egg collections, from wild spawning fish or carcasses on the spawning grounds, or in the case of the Chilcotin River sample, from a mixed sample of angled fish from the river.

For the survey of baseline populations, PCR products at six microsatellite loci—*Ots100*, *Ots101*, *Ots102*, *Ots104*, *Ots107* (primers outlined by Nelson and Beacham, 1999) and *Ssa197* (O'Reilly et al., 1996)—were size-fractionated on nondenaturing polyacrylamide gels by staining with 0.5 mg/mL ethidium bromide in water and illuminating with ultraviolet light. Nelson et al. (1998, 2001) have provided a more complete description of gel electrophoretic conditions. Three 20-bp marker lanes (Gensura Labs Inc., Del Mar, CA), 24 population samples, and one standard fish were run on each gel. The size of the amplified alleles were determined from the molecular size grid created with the 20-bp markers. Digital images of the resulting gels were analyzed by using BioImage Whole Band software (Millipore Corp. Imaging Systems, Ann Arbor, MI). Beacham and Wood (1999) provided a more complete description of the methods used to identify alleles with this technology. Precision of estimation of allele size with this technology has been outlined for *Ots100*, *Ots101*, and *Ots102* by Nel-

son et al. (2001), and for loci in other species by Beacham and Wood (1999) and Beacham et al. (2000). Precision of estimation of allele size for *Ots104*, *Ots107*, and *Ssa197* was similar to that for other loci with alleles spanning a similar size range. For example, estimated allele size for a standard fish analyzed 515 times at *Ots107* was within a 4-bp interval 98% of the time for an estimated allele size of 171 bp, and 85% of the time for an allele size of 300 bp. As outlined by Nelson et al. (2001), bin widths were expanded at larger allele sizes to account for less precise estimation of allele size as determined from the standard fish, and alleles were defined on the basis of bin width.

With the acquisition of an automated DNA sequencer (the ABI Prism 377) in our laboratory, PCR products at seven additional loci—*Ogo2*, *Ogo4* (Olsen et al., 1998), *Oke4* (Buchholz et al., 1999), *Omy325* (O'Connell et al., 1997), *Oki100* (K. M. Miller, unpubl. data), and *Ots2*, *Ots9* (Banks et al., 1999)—were size fractionated (96 samples on a gel) on 4.5% denaturing polyacrylamide gels run at 3000 V for 2.25 h at a gel temperature of 51°C. PCR products at *Ots2* and *Ots9* were amplified with a single PCR reaction, as were *Ogo4* and *Omy325*, and *Ogo2* and *Oke4*. Primer concentrations in the multiplex PCR reactions were adjusted to ensure equal amplification of both loci. Allele sizes were determined with Genescan 3.1 and Genotyper 2.5 software (PE Biosystems, Foster City, CA). Allele frequencies for all location samples surveyed in this study are available at http://www-sci.pac.dfo-mpo.gc.ca/aqua/pages/bgsid.htm.

Conversion of allele sizes between manual and automated sizing systems

With the acquisition of an automated DNA sequencer, we switched the survey of variation at *Ots100, Ots101, Ots102*, *Ots104, Ots107,* and *Ssa197* from the previous manual gel method to the automated sequencer, although most of the alleles surveyed in this study at these loci would have been analyzed on manual gels. Estimated allele sizes at these loci differed between those derived from nondenaturing gels stained with ethidium bromide and those derived from the denaturing gels and flourescent tags on the automated sequencer. In order to convert allele sizes between the two systems, we analyzed approximately 600 fish on both systems and determined the distributions of allele frequencies. By inspection of the allele frequencies, we were able to match specific allele sizes obtained from the sequencer to specific allele sizes from the manual gels and then convert the sizing in the automated sequencer data set to match that obtained from the manual gels. Estimated allele sizes from both systems were very highly correlated (r^2>0.987 for all loci). In general, sizes for the same allele from the sequencer were larger than those estimated from manual gels, with the differential increasing with allele size.

Data analysis

Each population at each locus was tested for departure from Hardy-Weinberg equilibrium (HWE) by using GENEPOP (Raymond and Rousset, 1995). The dememorization number was set at 1000, and 50 batches were run for each test with 1000 iterations/batch (Raymond and Rousset, 1995). Annual samples within populations were tested separately, and 106 tests were conducted at each locus (Table 1). Linkage disequilibrium between loci in each population was also evaluated by using GENEPOP; 106 tests were conducted for each two-locus combination. With 13 loci, there were 78 different two-locus combinations to evaluate. Critical significance levels for simultaneous tests were evaluated by using sequential Bonferroni adjustment (Rice, 1989). F_{ST} estimates for each locus were calculated with FSTAT (Goudet, 1995), and the standard deviation of the estimate for an individual locus was determined by jackknifing over populations and for all loci combined by bootstrapping over loci. All annual samples available for a location were combined to estimate population allele frequencies, as was recommended by Waples (1989). Computation of allelic diversity (the average number of alleles observed per locus), and identification of unique alleles was carried out with genetic data analysis (GDA) (Lewis and Zaykin, 2001). To minimize the effects of varying sample sizes on estimates of allelic diversity, values were reported only for population samples consisting of at least 50 fish. The number of unique alleles observed per region was standardized by calculating the number observed for each one-hundred fish sampled within each region. A neighbor-joining analysis illustrating genetic relationships among populations was also conducted with GDA incorporating TreeView (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html) presentation.

Estimation of variance components of regional differences, population differences within regions, and annual variation within populations was determined with GDA. Six regions were defined based upon observed population structure: lower, middle, and upper Fraser River, south, north, and lower Thompson River. One highly distinctive population, the Birkenhead River, was handled in two ways. It was either included in the analysis as a separate (seventh) region, or excluded from the analysis of regional structure. Balanced experimental designs were required for variance component analysis, and thus two or more populations in each region were required (each population sampled in at least two years). When the Birkenhead River was treated as a region, two populations were defined from the four annual samples available for the regional analysis. The 1993 and 1996 samples were treated as one population, and the 1997 and 1998 samples as the second population. Samples included in the variance components analysis were the 28 locations from which at least two annual samples were available and at least 25 fish in total were sampled (Table 1, Horsey and Goat excluded). Population structure within tributaries of the Fraser River and Thompson River was evaluated with variance components analysis for seven tributaries. These were major tributaries of either the Fraser River or Thompson River for which we had surveyed populations in the tributary for two or more years and included the Harrison, Chilcotin, Quesnel, Nechako rivers in the Fraser River drainage and the Nicola and Shuswap rivers in the Thompson River drainage (Fig. 1). Variance components due to sampling locations within tributaries were compared with variance components due to sampling

Table 1

Population sample, collection years, number of fish sampled per year, total number of fish sampled, expected heterozygosity (H_e), observed heterozygosity (H_o), and percentage of tests significant for pairwise linkage disequilibrium (n=106 tests per locus pair combination) for 52 samples of Fraser River chinook salmon in seven regions of the Fraser River drainage.

Population	Years	Annual n	Total n	H_e	H_o	Linkage
Birkenhead						
Birkenhead River[1,3]	1993, 1996, 1997,1 998	43, 31, 20, 27	121	0.75	0.68	0.6
Lower Fraser						
Harrison (white)[2,3]	1988, 1992, 1994	134, 99, 100	333	0.86	0.83	25.6
Chehalis (red)[2,3]	1994	25	25	0.82	0.78	1.3
Chilliwack (white)[2,3]	1994, 1995	83,89	172	0.85	0.81	3.2
Chilliwack (red)[2,3]	1994	28	28	0.83	0.81	0.0
Mid Fraser						
Cottonwood[1]	1995	53	53	0.81	0.78	0.0
Quesnel[1,3]	1990, 1994, 1995, 1996, 1997	14, 69, 91, 226, 73	473	0.83	0.78	2.8
Cariboo[1]	1996	12	12	0.77	0.74	0.0
Stuart[1]	1991, 1992, 1994, 1995, 1996	58, 56, 73, 99, 172	458	0.83	0.81	1.3
Nechako[1]	1991, 1992, 1994, 1995, 1996	58, 63, 61, 69, 137	388	0.84	0.78	3.1
Horsefly[1]	1996, 1997	14, 15	29	0.82	0.76	0.0
Westroad[1]	1997	31	31	0.85	0.83	5.1
Chilko[1]	1994, 1995	43, 79	122	0.82	0.78	0.6
Upper Chilcotin[1]	1995, 1998	22, 21	43	0.82	0.77	1.3
Lower Chilcotin[1]	1996	74	74	0.83	0.76	0.0
Chilcotin[1]	1997	47	47	0.85	0.80	0.0
Taseko[1]	1997, 1998	37, 27	64	0.79	0.75	0.0
Endako[1]	1997, 1998	25, 32	57	0.83	0.81	9.6
Elkin[1]	1995, 1996	19, 216	235	0.83	0.77	3.8
Portage[1]	1996	27	27	0.84	0.80	0.0
Bridge[1,3]	1994, 1995, 1996	23, 35, 326	384	0.84	0.78	0.0
Upper Fraser						
Tete Jaune[1]	1993, 1994, 1995	66, 94, 88	248	0.83	0.80	18.8
Dome Creek[1,3]	1991, 1994, 1995, 1996	34, 51, 94, 148	327	0.83	0.80	5.8
Horsey[1]	1995, 1997	13, 11	24	0.83	0.76	0.0
Goat[1]	1995, 1997	12, 12	24	0.84	0.81	0.0
Holmes[1]	1995, 1996	43, 54	97	0.84	0.79	1.3
Swift[1]	1995, 1996	63, 164	227	0.82	0.78	2.6
Slim Creek[1]	1995	65	65	0.82	0.80	0.0

continued

years within locations. In the Harrison River–Lillooet River drainage, the Birkenhead River was treated as a single population with four years of samples in this analysis.

Results

Variation within populations

The number of alleles observed per locus ranged from 12 to 54, and fewer alleles were observed at the loci with dinucleotide repeats (*Ogo2*, *Ogo4*, *Oke4*, *Omy325*, *Ots2*, and *Ots9*) than at the loci with tetranucleotide repeats. Heterozygosities were generally lower for the loci with dinucleotide repeats (mean H_e=0.71) than for the other loci (mean H_e=0.91) (Table 2). Genotypic frequencies at each locus within sampling location and year generally conformed to Hardy-Weinberg equilibrium expectations, with the notable exception of *Ots102*. At this locus, 57% of the HWE tests were statistically significant, and in all cases there was an excess of observed homozygotes. One or more nonamplifying allele(s) was likely present at this locus in many of the Fraser River populations surveyed. Observed heterozygosities were less than expected heterozygosities for all loci analyzed on the manual gels. Unequal amplification of alleles (Wattier et al., 1998) may have resulted in the failure to detect large alleles in some heterozygous individuals with ethidium bromide staining. There was no evidence of linkage between any of the microsatellite loci used in this study, but four of the 52 samples surveyed in the study exhibited significant linkage disequilibrium in more than 10% of the pairwise comparisons between loci (Table 1). These sample locations were Harrison River, Tete Jaune, Fontoniko, and Bessette Creek.

Table 1 (continued)

Population	Years	Annual *n*	Total *n*	H_e	H_o	Linkage
Upper Fraser (cont.)						
Indianpoint[1]	1995	42	42	0.82	0.81	1.3
Willow[1]	1995, 1996	53, 16	69	0.83	0.75	0.0
Fontoniko[1]	1996	57	57	0.80	0.77	19.7
McGregor[1]	1997	119	119	0.81	0.80	2.6
Salmon River[1]	1996, 1997	95, 131	226	0.82	0.76	3.8
Bowron[1]	1995, 1997, 1998	54, 16, 39	109	0.82	0.78	6.4
Lower Thompson						
Nicola[1,3]	1992, 1994, 1995, 1997	54, 73, 75, 49	251	0.81	0.77	1.0
Coldwater[1,3]	1994, 1995, 1996, 1997	27, 31, 75, 43	176	0.82	0.77	0.0
Spius[1,3]	1996	58	58	0.80	0.80	2.6
Deadman[1]	1996, 1997	132, 61	193	0.82	0.77	1.9
Bonaparte[1]	1996	306	306	0.82	0.79	5.1
North Thompson						
Mahood[1]	1995	17	17	0.82	0.74	0.0
Raft[1]	1995, 1996	14, 115	129	0.83	0.80	0.6
Finn[1]	1996	101	101	0.80	0.74	0.0
Louis[1]	1996, 1997	32, 107	139	0.78	0.74	1.9
Clearwater[1]	1997	169	169	0.82	0.76	5.1
South Thompson						
Little River[2]	1996	53	53	0.83	0.73	0.0
Lower Shuswap[2]	1994, 1995, 1996, 1997	130, 73, 90, 42	335	0.82	0.77	2.6
Middle Shuswap[2]	1994, 1995, 1997	109, 86, 118	313	0.81	0.76	0.9
Salmon[1]	1996, 1997	72, 56	128	0.82	0.77	1.3
Eagle[1,2]	1995	36	36	0.80	0.69	1.3
Lower Adams[2,3]	1996	103	103	0.83	0.78	3.8
South Thompson[2]	1996	157	157	0.84	0.80	5.1
Bessette[1]	1998	17	17	0.81	0.79	17.9

[1] Stream-type population.
[2] Ocean-type population.
[3] Hatchery-enhanced population.

Population structure

The mean F_{ST} value over 52 samples and 13 loci surveyed was 0.039 (0.040 with *Ots102* excluded), and the dinucleotide loci had higher mean F_{ST} values (0.067) than the other loci (mean=0.026) (Table 2). With the exception of several sampling locations within tributaries affected by transplantation (discussed below), the geographically distinct sampling sites of this study possessed individual spawning populations of chinook salmon. Neighbor-joining clustering based on F_{ST} values for the 12 loci in HWE indicated a regional population structure which corresponded broadly to six groups: lower, middle, and upper Fraser River, and south, north, and lower Thompson River. An exception was the Birkenhead River population of the lower Fraser region, which was distinct from all other populations (Fig. 2). Thompson River populations were well differentiated from those of the Fraser River, and within the Thompson River drainage, the populations of the south, north, and lower regions were distinctive. Only the Louis Creek population of the north Thompson failed to cluster geographically, instead grouping rather distantly with the lower Thompson populations. The mid and upper Fraser River populations each formed distinctive groups, although the Portage Creek and Bridge River populations of the mid Fraser showed similarities to lower Fraser and lower Thompson populations, respectively. The transplanted red-fleshed "Chilliwack" and "Chehalis" River populations in the lower Fraser drainage clustered with source populations Slim Creek and Bowron River from the upper Fraser drainage. Mean F_{ST} values both within and among regions were statistically significant (all $P<0.05$) (Table 3).

The distinctive lower Fraser region, providing the Harrison and Chilliwack River samples, was differentiated from all other regions, although F_{ST} values indicated a somewhat closer relationship of lower Fraser with mid Fraser populations than with populations of other regions (Table 3). The distinctiveness of the Birkenhead River population was apparent in the F_{ST} values obtained in comparisons with all regional groups. The upper Fraser

and north Thompson regions were both closely related to mid Fraser populations, but less similar to each other. The lower and south Thompson regions were distinctive from other regions (with the exception of the Louis Creek population from the north Thompson which resembled lower Thompson populations), and from each other. Removal of the Louis Creek population from the analysis resulted in the F_{ST} value among the North Thompson populations decreasing from 0.043 to 0.026 and the F_{ST} value between the north and lower Thompson regions increasing from 0.047 to 0.058 (Table 3).

Heterozygosity was highest in the lower Fraser region but similar among all interior Fraser regions (Table 4). Allelic diversity was also high in the lower Fraser samples, but on a regional basis, allelic diversity was highest in the mid Fraser region followed by the south Thompson and lower Fraser regions. More unique alleles were observed per hundred fish sampled in the Birkenhead and Lower Fraser region than elsewhere in the drainage (Table 4). Among the interior regions, the mid Fraser and south Thompson regions each had more than one unique allele recorded per hundred fish sampled, whereas the remaining regions had less than one.

Table 2

Number of alleles, expected heterozygosity (H_e), observed heterozygosity (H_o), percent significant Hardy-Weinberg equilibrium tests (HWE, n=106 tests), and F_{ST} among 52 chinook salmon spawning locations (standard deviation in parentheses) for 13 microsatellite loci.

Locus	Alleles	H_e	H_o	HWE	F_{ST}
Ogo2	18	0.71	0.70	1.9	0.077 (0.011)
Ogo4	20	0.80	0.80	3.7	0.076 (0.014)
Oke4	14	0.66	0.63	1.9	0.074 (0.014)
Oki100	39	0.92	0.92	7.4	0.026 (0.003)
Omy325	31	0.77	0.75	8.4	0.081 (0.012)
Ots2	18	0.71	0.71	6.5	0.042 (0.009)
Ots9	12	0.58	0.58	0.0	0.051 (0.009)
Ots100	34	0.91	0.87	10.3	0.022 (0.002)
Ots101	33	0.91	0.86	13.1	0.016 (0.003)
Ots102	54	0.89	0.61	57.0	0.036 (0.003)
Ots104	33	0.92	0.89	8.4	0.022 (0.003)
Ots107	47	0.90	0.86	13.1	0.036 (0.004)
Ssa197	33	0.92	0.90	4.7	0.024 (0.003)
All loci					0.039 (0.006)

Distribution of genetic variation

Gene diversity analysis of the 12 microsatellite loci in HWE was used to determine the magnitudes of variation among annual samples within populations and of variation among chinook salmon populations of seven regions (Birkenhead treated as a region) or six regions (the Birkenhead River population excluded). For seven regions, the hierarchical analysis indicated that 95.0% of the variation occurred within populations (Table 5). Variation among regions was the greatest source of the remaining variation (3.1%), followed by variation among populations within regions (1.3%), and variation among years within populations (0.5%). With the Birkenhead population excluded, regional variation accounted for 2.6% of the total, whereas the variation attributed to populations and sampling year was unchanged. The differences observed among regions, among populations within regions, and among years within populations were significant (all P<0.01). On average, differences among populations (includes regional and population components) were about 8.5 times greater than annual variation within populations.

Structure within major tributaries

In many cases, samples from separate locations within tributary systems clustered together (Fig. 2), indicating that a higher rate of gene flow may occur among chinook salmon spawning aggregates within tributaries. Gene diversity analysis confirmed that chinook salmon sampled from separate spawning locations within tributaries tended

Table 3

Mean pairwise F_{ST} averaged over 12 loci for 50 locations of chinook salmon for seven regional groups in the Fraser River and Thompson River drainages. Comparisons were conducted between individual populations in each region (the numbers of populations in each region are listed in Table 1). The transplanted red Chehalis and red Chilliwack populations were not included in the analysis. Standard deviation is shown in parentheses. Boldface font indicates the diagonal.

	Lower Fraser	Middle Fraser	Upper Fraser	North Thompson	South Thompson	Lower Thompson
Birkenhead	0.107 (0.023)	0.125 (0.014)	0.132 (0.007)	0.137 (0.017)	0.111 (0.017)	0.138 (0.008)
Lower Fraser	**0.005 (—)**	0.067 (0.022)	0.069 (0.021)	0.080 (0.023)	0.068 (0.021)	0.073 (0.027)
Middle Fraser		**0.025 (0.012)**	0.028 (0.013)	0.043 (0.018)	0.049 (0.011)	0.047 (0.013)
Upper Fraser			**0.017 (0.008)**	0.048 (0.016)	0.045 (0.009)	0.042 (0.008)
North Thompson				**0.043 (0.021)**	0.052 (0.014)	0.047 (0.011)
South Thompson					**0.022 (0.009)**	0.051 (0.008)
Lower Thompson						**0.011 (0.005)**

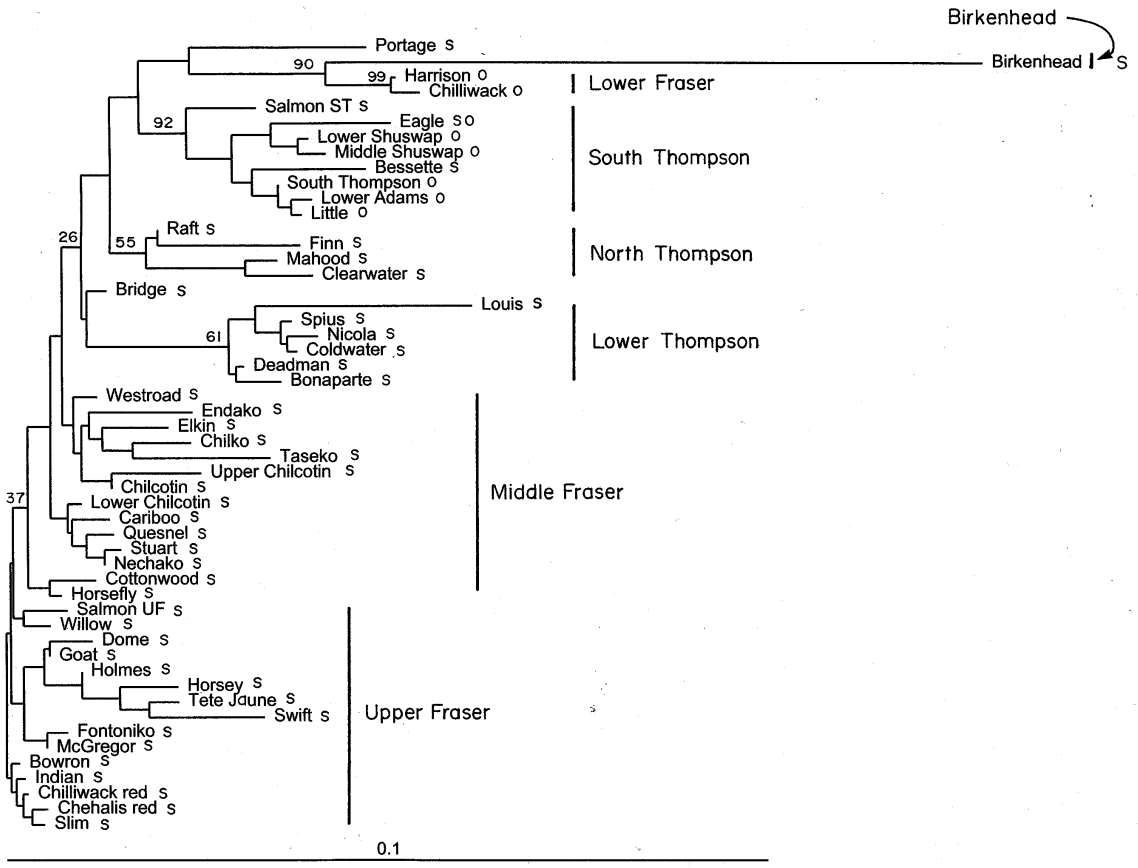

Figure 2

Neighbor-joining dendrogram of F_{ST} differences for 52 spawning groups of Fraser River chinook salmon surveyed at 12 microsatellite loci in Hardy-Weinberg equilibrium. Bootstrap values at the tree nodes indicate the percentage of 100 trees where populations beyond the node occurred together. Populations have been designated as ocean type (O) or stream type (S) after the name of the population.

to be more similar to each other but that the degree of differentiation among populations within tributaries varied among tributary systems (Table 6). For example, in the Nicola River drainage, temporal variation in allele frequencies within the Nicola and Coldwater samples was about three times larger than differences between them (Table 6). There was no significant difference in allele frequencies at any locus between the two locations in relation to annual variation within location (all 13 $P>0.10$). Thus it seems likely that there is sufficient gene flow between these two sites (and likely the Spius Creek site) that the Nicola River drainage can be considered to contain a single chinook salmon population. Significant differentiation was observed between the lower and middle Shuswap River samples at *Ogo2* ($F=7.82$, $P<0.05$), indicating that there has been some restriction of gene flow between the two spawning locations. Structure was observed among the three sites sampled from the Nechako River system, with significant differences observed at *Oke4*, *Ogo2*, and *Ots9* (all $F>6.0$, $P<0.05$). Similarly, after accounting for annual variation, significant differentiation between the Quesnel and Horsefly sites in the Quesnel River drainage was observed at *Ogo2* ($F=6.63$, $P<0.05$), and among four Chilko River sites at *Ots107*, *Ogo4*, *Ots9*, and *Omy325* (all >6.6, all $P<0.05$). The most pronounced allele frequency differentiation was observed between the Harrison and Birkenhead River populations in the Harrison River–Lillooet River drainage. Interpopulation differentiation was 24 times greater than intrapopulation variation; the two populations differed in allele frequencies at all loci except *Ssa197* and *Ots101* (all $F>6.6$, all $P<0.05$).

Discussion

Population structure

The genetic structure of Fraser drainage chinook salmon populations has a strong geographic basis, indicating that isolation by distance is a major component of population structure. Regional differentiation accounts for approximately twice the variation in allele frequencies as does

Table 4

Regional and population level allelic diversity (mean number of alleles per locus) and expected heterozygosity (H_e) at microsatellite loci for Fraser River chinook salmon. *n* indicates the sample number of fish included for regional values and the number of populations included for population values. The number of unique alleles observed per hundred fish sampled is shown for each region; the total number of unique alleles is shown in parentheses.

	n	Allelic diversity	H_e	Unique alleles
Birkenhead	1 (52 fish)	13.3	74.0	3.85 (2)
Lower Fraser				1.95 (5)
population	2	22.2	85.2	
region	256	24.2	85.5	
Mid Fraser				1.16 (16)
population	5	19.2	82.3	
region	1380	27.2	83.5	
Upper Fraser				0.75 (5)
population	5	16.7	82.2	
region	670	23.1	83.4	
Lower Thompson				0.21 (1)
population	4	17.4	81.0	
region	480	21.3	81.7	
South Thompson				1.79 (10)
population	5	18.7	81.5	
region	558	24.8	82.2	
North Thompson				0.98 (2)
population[1]	2	19.2	82.1	
region	204	22.5	82.7	
Louis Creek	68	14.6	76.7	

[1] Louis Creek population excluded.

Table 5

Hierarchical gene diversity analysis for 28 populations (pops) of Fraser River chinook salmon for the 12 microsatellite loci in HWE; only populations sampled for at least two years were included in the analysis. Regions, populations within regions, and years within populations are outlined in Table 1.

	Absolute diversity		Relative diversity			
Locus	Total	Within populations	Within populations	Among years within populations	Among populations within regions	Among regions
Ogo2	0.7465	0.7007	0.9386	0.0023	0.0155	0.0436
Ogo4	0.8788	0.8071	0.9184	0.0089	0.0109	0.0618
Oke4	0.7288	0.6822	0.9360	0.0042	0.0128	0.0470
Oki100	0.9513	0.9259	0.9733	0.0017	0.0140	0.0110
Omy325	0.8477	0.7770	0.9166	0.0047	0.0181	0.0606
Ots2	0.7364	0.7083	0.9619	0.0032	0.0133	0.0216
Ots9	0.6139	0.5962	0.9712	0.0008	0.0146	0.0134
Ots100	0.9492	0.9279	0.9776	0.0053	0.0075	0.0096
Ots101	0.9364	0.9173	0.9796	0.0073	0.0058	0.0073
Ots104	0.9475	0.9240	0.9751	0.0054	0.0083	0.0112
Ots107	0.9423	0.9053	0.9607	0.0042	0.0098	0.0253
Ssa197	0.9456	0.9216	0.9746	0.0060	0.0079	0.0115
All			0.9499	0.0053[1]	0.0134[1]	0.0314[1]

[1] $P<0.01$.

Table 6

Hierarchical gene-diversity analysis of spawning aggregations within six tributaries of the Fraser River for 12 microsatellite loci. sites within the tributary divided by the variance component of sampling years within sites.

	Nicola, Coldwater (Lower Thompson)		Lower, Middle Shuswap (South Thompson)		Harrison, Birkenhead (Lower Fraser)	
Locus	Among years within sites	Between sites within tributary	Among years within sites	Between sites within tributary	Among years within sites	Between sites within tributary
Ogo2	0.0000	0.0000	0.0002	0.0063	0.0000	0.1171
Ogo4	0.0147	0.0000	0.0017	0.0040	0.0003	0.0559
Oke4	0.0093	0.0030	0.0055	0.0000	0.0064	0.0559
Oki100	0.0003	0.0062	0.0017	0.0062	0.0025	0.0289
Omy325	0.0260	0.0006	0.0008	0.0020	0.0000	0.2452
Ots2	0.0042	0.0000	0.0053	0.0000	0.0034	0.0814
Ots9	0.0004	0.0000	0.0010	0.0004	0.0002	0.0153
Ots100	0.0080	0.0034	0.0048	0.0044	0.0033	0.0423
Ots101	0.0042	0.0033	0.0082	0.0000	0.0090	0.0291
Ots104	0.0030	0.0000	0.0094	0.0000	0.0018	0.0182
Ots107	0.0036	0.0045	0.0037	0.0030	0.0086	0.0550
Ssa197	0.0056	0.0080	0.0101	0.0117	0.0026	0.0135
All	0.0090	0.0026	0.0057	0.0040	0.0040	0.0939
Sites/Years	0.29		0.70		23.5	

intraregional variation among populations, and spawning sites within a tributary system may or may not support genetically discrete populations. Time of adult migration also appears to promote genetic differentiation.

The Birkenhead River population, the most distinctive of the 52 individual chinook salmon spawning aggregates surveyed, occupies the upper portion of the Harrison River–Lillooet River drainage and is characterized by a very early adult migration time. Historical recreational fisheries on this population occurred in the Birkenhead River during April and May (Fraser et al., 1982). Conversely, the Harrison River population in the lower reaches of the same drainage is a late run, entering the Harrison River just prior to spawning in October and November. The Birkenhead River population has low levels of polymorphism and heterozygosity at both allozyme loci (Teel et al., 2000) and microsatellite loci, likely reflecting small population size at least in recent history and low levels of gene flow because of its spatial and temporal isolation from other populations. Another example of a spawning population isolated temporally from neighboring sites is the Louis Creek population of the north Thompson region. The migration time of Louis Creek chinook salmon is early or "spring run," whereas other north Thompson populations are later "summer run" migrants. The distant clustering of the Louis Creek chinook salmon with the lower Thompson populations may reflect either a common origin or more recent gene flow due to common migration times. The low level of allelic diversity observed in the Louis Creek population and its distinction from the lower Thompson populations indicate that current levels of gene flow are low, and the genetic similarity may be due to common ancestry.

Significant linkage disequilibrium was detected in samples from four populations: Harrison River, Tete Jaune (main stem Fraser River), Fontoniko Creek, and Bessette Creek. Linkage disequilibrium may reflect sample admixture (Waples and Smouse, 1990) in the Harrison River and Tete Jaune samples. The Harrison River samples were obtained from broodstock collections at a hatchery on the Chehalis River, a tributary of the Harrison River. Initial broodstock for the hatchery was derived from chinook salmon collected from the Harrison River, and over time broodstock has been developed from fish returning to the hatchery. Chinook salmon returning to the Chehalis hatchery were also used to found the Chilliwack River population, which is maintained by production in the Chilliwack hatchery and spawning in the Chilliwack River. During the 1990s, chinook salmon were transplanted back from the Chilliwack hatchery to the Chehalis hatchery. Thus, the samples examined in our study, collected between 1988 and 1994, may reflect some mixing of genetically related but heterogeneous groups of fish from the Harrison, Chehalis, and Chilliwack rivers in the Chehalis hatchery broodstock. The Tete Jaune samples were obtained at Tete Jaune Cache in the extreme headwaters of the Fraser River. Because the samples were collected from the mainstem Fraser River, there is potential for admixture of populations, although it is thought that there are few chinook salmon spawning sites upstream from this location. Significant linkage disequilibrium was detected in single-year samples from Fontoniko Creek (a tributary of the McGregor River) and Bessette Creek (a tributary of the Shuswap River). Population admixtures would not typically be expected in such terminal locations, and the cause of the disequilibrium is unknown.

Table 6

Spawning sites within each tributary are indicated in the first row of the table. "Sites/Years" is the ratio of the variance components of

Nechako, Stuart, Endako (Mid Fraser)		Quesnel, Horsefly (Mid Fraser)		Chilko, Upper Chilcotin, Elkin, Taseko (Mid Fraser)	
Among years within sites	Between sites within tributary	Among years within sites	Between sites within tributary	Among years within sites	Between sites within tributary
0.0005	0.0073	0.0019	0.0411	0.0014	0.0080
0.0111	0.0000	0.0064	0.0233	0.0028	0.0418
0.0031	0.0127	0.0000	0.0031	0.0147	0.0211
0.0008	0.0041	0.0000	0.0094	0.0013	0.0197
0.0042	0.0016	0.0041	0.0146	0.0046	0.0319
0.0023	0.0057	0.0000	0.0177	0.0000	0.0122
0.0000	0.0041	0.0027	0.0396	0.0000	0.0224
0.0030	0.0026	0.0028	0.0068	0.0070	0.0174
0.0030	0.0027	0.0020	0.0009	0.0117	0.0000
0.0030	0.0025	0.0014	0.0019	0.0052	0.0156
0.0022	0.0043	0.0020	0.0111	0.0000	0.0244
0.0046	0.0047	0.0017	0.0025	0.0085	0.0102
0.0039	0.0049	0.0232	0.0170	0.0057	0.0232
1.25		7.40		4.07	

The disequilibrium observed in the Bessette Creek sample may simply reflect small sample size (17 fish).

Samples of Fraser River chinook salmon of the Harrison and Chilliwack rivers were little differentiated (F_{ST}=0.004), in accordance with their common origin from the Harrison River system. In addition, eggs from early migrating red-fleshed chinook salmon from Bowron River and Slim Creek in the upper Fraser and from the Quesnel and Chilko rivers of the mid Fraser were transplanted to the lower Fraser hatcheries between 1985 and 1988. Fish from these transplants returned to the lower Fraser hatcheries, maintaining an early migration time and red flesh coloration that distinguishes them from the native fall-run population. Fish returning from the initial releases were chosen as brood stock for succeeding generations only if they carried a coded wire tag indicating that they were of Slim Creek or Bowron River origin. Transplantation from the Chilliwack to the Chehalis hatchery of the red-fleshed fish occurred during years in which returns to the Chehalis hatchery were low. The observed strong genetic affinity of the Chehalis and Chilliwack red-fleshed fish to each other and to upper Fraser chinook salmon reflects this transplantation record.

Although the lower Fraser was the most genetically distinct of the six geographic regions examined, the genetic distinctiveness of the upper Fraser, lower Thompson, and south Thompson populations from each other was almost as great as their differentiation from the lower Fraser populations. Differentiation among regions within the smaller Thompson drainage was greater than that between the mid and upper Fraser regions of the larger interior Fraser drainage. The mean pairwise F_{ST} value within the Thompson drainage was 0.050, whereas within the interior Fraser drainage it was 0.028. Relatively strong differentiation among salmonids of the north, south, and lower Thompson regions has been noted previously not only for chinook salmon (Teel et al., 2000), but also coho (Small et al., 1998) and sockeye (Withler et al., 2000) salmon. The consistent population structure of the region across species, with quite distinct patterns of spatial variability (Myers et al., 1998), indicates that colonization of the Thompson and interior Fraser drainages may have been episodic, rather than a single event. If this is the case, the current affinities among the interior Fraser and Thompson regions may represent some combination of related but distinct founding populations and subsequent gene flow.

Origin of chinook salmon in the Fraser River

During the Wisconsin glaciation, ice-free refugia existed to the south of British Columbia in the Columbia River drainage and Pacific coastal regions. Extant chinook salmon populations in these areas display a genetic dichotomy that is well correlated with juvenile life history type (Myers et al., 1998). The presence of genetically distinct chinook salmon in the lower and interior Fraser River drainages has led to the suggestion that the drainage was colonized independently by stream-type chinook salmon from the Columbia refuge and ocean-type chinook salmon from a Pacific coastal (Teel et al., 2001) or northern Beringial (Utter et al., 1989) refuge. In a large survey of chinook salmon populations, including those of ocean- and stream-type populations from nonglaciated southern regions, Utter et al. (1989) found that 87% of genetic variation was contained within populations, and the remainder was partitioned among populations. In

five genetically distinct groups of chinook salmon in the Sacramento and San Joaquin rivers of California, 8.2% of genetic variation determined from a microsatellite survey was not contained within populations (Banks et al., 2000). In our present study of Fraser River chinook salmon, 95% of genetic variation was present within populations, where 3.0% was due to differences among regions (including the lower Fraser, putatively colonized independently) and 1.3% was due to differences among populations within regions.

Pairwise F_{ST} values between geographic regions of the Fraser drainage (Birkenhead excluded) did not exceed 0.080. Thus, if indeed Fraser River chinook salmon are descendants of genetically distinct chinook salmon "races" from two (or more) glacial refugia, the observed relatively low levels of differentiation suggest that introgression has occurred among the races before or since their colonization of the Fraser drainage. Support for the idea of the Fraser drainage as a chinook salmon "melting pot" comes from allozyme data (Fig. 19 in Myers et al., 1998) which indicate that chinook salmon of the both the lower and interior Fraser drainages are genetically intermediate to chinook salmon sampled from three postulated glacial refugia (the Columbia drainage, the Pacific coast and Beringia, the northern refuge). Minisatellite DNA variation has also indicated that Fraser River chinook salmon are genetically intermediate to populations in southern and northern British Columbia, groups putatively derived from southern and northern refugial areas (Beacham et al., 1996).

Teel et al. (2000) suggested that the presence of populations in the south Thompson region that have high proportions of ocean-type juveniles, and the genetic distinctiveness of the region, indicate that hybridization of ocean- and stream-type chinook salmon occurred within this region of the Fraser drainage. However, our study revealed that chinook salmon of the lower Thompson and south Thompson regions are the most genetically distinct of the interior chinook salmon groups, apparently strongly isolated from each other as well as from chinook salmon from all other regions. The south Thompson populations were not more closely related to the ocean-type chinook salmon of the lower Fraser than were the predominantly stream-type populations in other interior regions. Moreover, whereas the high level of allelic diversity observed in the south Thompson region may support the idea of historical hybridization, the presence in the south Thompson samples of a relatively large number of unique alleles (i.e. alleles not observed in other Fraser regions) does not support the idea that the hybridization occurred between the ocean-type chinook salmon that colonized the lower Fraser and the stream-type populations elsewhere in the drainage. The genetic distinction of the south Thompson chinook salmon seems more likely due to a unique colonization history than to recent gene flow within the Fraser drainage. South Thompson chinook salmon may have resulted from a historical admixture of refugial ocean- and stream-type races, but their genetic similarity to other interior Fraser chinook salmon groups, and the intermediate genetic position of all Fraser River chinook populations, indicate that all groups of Fraser chinook salmon may possess similar racial admixture. The ocean-type chinook salmon populations of the south Thompson may have originated from stream-type fish and thus reflect adaptation to environmental conditions conducive to the production of large juveniles capable of smolting in their first year of life.

Our data provide some evidence of gene flow among chinook salmon in different regions of the Fraser. Mid Fraser chinook salmon were characterized by a high level of allelic diversity but were notable in their low level of differentiation from chinook salmon of both the upper Fraser and north Thompson regions. Mid Fraser fish were also the most closely related of all the interior groups to lower Fraser chinook salmon. As for the south Thompson region, a high number of unique alleles in the mid Fraser region indicated that the high level of diversity was unlikely to result solely from hybridization of postglacial founding populations from the lower and upper Fraser regions.

Further genetic analysis of chinook salmon in British Columbia and refugial areas will reveal the nature of genetic variation in the Fraser River and other regions of southern British Columbia that likely represent "contact zones" of postglacial recolonization. Although extensive introgression may complicate the identification of conservation units based on "important phylogeographic subdivisions within species, those based on historical separations or fluctuations that are still evident in the gene pool" (Moritz, 1994), it may also have endowed the chinook salmon of southern British Columbia with a high level of adaptive diversity and evolutionary potential. It is thus important to determine if the regional structure evident within the Fraser drainage represents an intermediate step in the erosion of genetic differentiation among refugial groups by ongoing gene flow, or in the differentiation of introgressed founding groups adapting to environmental variability. Comparison of the distribution of variation at neutral and adaptive loci, such as that conducted for sockeye salmon of the Fraser River drainage (Miller et al., 2001), may help determine the geographic scale of adaptation for Fraser River chinook salmon.

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Literature cited

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

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

Beacham, T. D., R. E. Withler, and T. A. Stevens.
1996. Stock identification of chinook salmon (*Oncorhynchus tshawytscha*) using minisatellite DNA variation. Can. J. Fish. Aquat. Sci. 53:380–394.

Beacham, T. D, and C. C Wood.
1999. Application of microsatellite DNA variation to estimation of stock composition and escapement of Nass River sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. 56:1–14.

Beacham, T. D, K. D. Le, M. R. Raap, K. Hyatt, W. Luedke, and R. E. Wither.
2000. Microsatellite DNA variation and estimation of stock composition of Barkley Sound, British Columbia sockeye salmon. Fish. Bull. 98:14–24.

Buchholz, W., S. J. Miller, and W. J. Spearman.
1999. Summary of PCR primers for salmonid genetic studies. U.S. Fish. Wild. Serv. Alaska Fish. Prog. Rep. 99-1.

Cronin, M. A., W. J. Spearman, R. L. Wilmot, J. C. Patton, and J. W. Bickham.
1993. Mitochondrial DNA variation in chinook (*Oncorhynchus tshawytscha*) and chum salmon (*O. keta*) detected by restriction enzyme analysis of polymerase chain reaction (PCR) products. Can. J. Fish. Aquat. Sci. 50:708–715.

Fraser, F. J., P. J. Starr, and A. Y. Federenko.
1982. A review of the chinook and coho salmon of the Fraser River. Can. Tech. Rep. Fish. Aquat. Sci. 1126.

Gharrett, A. J., S. M. Shirley, and G. R. Tromble.
1987. Genetic relationships among populations of Alaskan chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 44:765–774.

Godfrey, H.
1975. Review of the occurrence of red- and white-fleshed chinook salmon in British Columbia with particular reference to Fraser River fish present off the west coast of Vancouver Island. Fish. Res. Board Canada Man. Rep. 1359, 49 p.

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

Healey, M. C.
1983. Coastwide distribution and ocean migration patterns of stream- and ocean-type chinook salmon, *Oncorhynchus tshawytscha*. Can. Field-Nat. 97:427–433.
1991. The life history of chinook salmon (*Oncorhynchus tshawytscha*). *In* Life history of Pacific salmon (C. Groot and L. Margolis, eds.), p. 311–393. U.B.C. Press, Vancouver, B.C.

Lewis, P. O., and D. Zaykin.
2001. Genetic data analysis: computer program for the analysis of allelic data, version 1.0 (d16c). Free program distributed by the authors over the internet from http://lewis.eeb.uconn.edu/lewishome/software.html.

McCallum, I. M., K. M. Cheng, and B. E. March.
1987. Carotenoid pigmentation in two strains of chinook salmon (*Oncorhynchus tshawytscha*) and their crosses. Aquaculture 67:291–300.

Miller, K. M., K. H. Kaukinen, T. D. Beacham, and R. E. Withler.
2001. Geographic heterogeneity in natural selection on an MHC locus in sockeye salmon. Genetica 111:237–257.

Miller, K. M., R. E. Withler, and T. D. Beacham.
1996. Stock identification of coho salmon (*Oncorhynchus kisutch*) using minisatellite DNA variation. Can. J. Fish. Aquat. Sci. 53:181–195.

Moritz, C.
1994. Defining "evlutionarily significant units" for conservation. TREE 9:373–375.

Myers, J. M., R. G. Kope, G. J. Bryant, D. Teel, L. J. Lierheimer, T. C. Wainwright, W. S. Grant, F. W. Waknitz, K. Neely, S. T. Lindley, and R. S. Waples.
1998. Status review of chinook salmon from Washington, Idaho, Oregon, and California. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-NWFSC-35, 443 p.

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

Nelson, R. J, T. D. Beacham, and M. P. Small.
1998. Microsatellite analysis of the population structure of a Vancouver Island sockeye salmon (*Oncorhynchus nerka*) stock complex using non-denaturing gel electrophoresis. Mol. Mar. Biotech. 7:312–319.

Nelson, R. J., M. P. Small, T. D. Beacham, and K. J. Supernault.
2001. Population structure of Fraser River chinook salmon (*Oncorhynchus tshawytscha*): an analysis using microsatellite DNA markers. Fish. Bull. 99:94–107.

Olsen, J. B., P. Bentzen, and J. E. Seeb.
1998. Characterization of seven microsatellite loci derived from pink salmon. Mol. Ecol. 7: 1083–1090.

O'Connell, M., R. G. Danzmann, J. M. Cornuet, J.M. Wright, and M. M. Ferguson.
1997. Differentiation of rainbow trout populations in Lake Ontario and the evaluation of the stepwise mutation and infinite allele mutation models using microsatellite variability. Can. J. Fish. Aquat. Sci. 54:1391–1399.

O'Reilly, P. T., L. C. Hamilton, S. K. McConnell, and J. M. Wright.
1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Can. J. Fish. Aquat. Sci. 53: 2292–2298.

Raymond, M., and F. Rousset.
1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenism. Heredity 86:248–249.

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

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

Taylor, E. B.
1991. A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. Aquaculture 98:185–207.

Teel, D. J., G. B. Milner, G. A. Winans, and W. S. Grant.
2000. Genetic population structure and origin of life history types in chinook salmon in British Columbia, Canada. Trans. Am. Fish. Soc. 129:194–209.

Utter, F., G. Milner, G. Stahl, and D. Teel.
1989. Genetic population structure of chinook salmon, *Oncorhynchus tshawytscha*, in the Pacific Northwest. Fish. Bull. 87:239–264.

Waples, R. S.
1989. Temporal variaiton in allele frequencies: testing the right hypothesis. Evolution 43: 1236–1251.

Waples, R. S., and P. E. Smouse.
1990. Gametic disequilibrium analysis as a means of identifying mixtures of salmon populations. Am. Fish. Soc. Symp. 7:439–458.

Wattier, R., C. R. Engel, P. Saumitou-LaPrade, and M. Valero.
1998. Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis* (Rhodophyta). Mol. Ecol. 7:1569–1573.

Wehrhahn, C. F., and R. Powell.
1987. Electrophoretic variation, regional differences, and gene flow in the coho salmon (*Oncorhynchus kisutch*) of southern British Columbia. Can. J. Fish. Aquat. Sci. 44:822–831.

Wilson, G. M., W. K. Thomas, and A. T. Beckenbach.
1987. Mitochondrial DNA analysis of Pacific Northwest populations of *Oncorhynchus tshawytscha*. Can. J. Fish. Aquat. Sci. 44:1301–1305.

Withler, R. E.
1986. Genetic variation in carotenoid pigment deposition in the red-fleshed and white-fleshed chinook salmon (*Oncorhynchus tshawytscha*) of Quesnel River, British Columbia. Can. J. Gen. Cytol. 28:587–594.

Withler, R. E., K. D. Le, R. J. Nelson, K. M. Miller, and T. D. Beacham.
2000. Intact genetic structure and high levels of genetic diversity in bottlenecked sockeye salmon, *Oncorhynchus nerka*, populations of the Fraser River, British Columbia, Canada. Can. J. Fish. Aquat. Sci. 57:1985–1998.

Wood, C. C., B. E. Riddell, D. T. Rutherford, and R. E. Withler.
1994. Biochemical genetic survey of sockeye salmon (*Oncorhynchus nerka*) in Canada. Can. J. Fish. Aquat. Sci. 51 (suppl. 1):114–131.