Population structure of Fraser River chinook salmon (*Oncorhynchus tshawytscha*): an analysis using microsatellite DNA markers

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Abstract—Microsatellite DNA analysis was applied in a genetic study of 20 chinook salmon populations from four regions within the Fraser River drainage of British Columbia, Canada. Twelve populations were represented by samples collected in different years. A total of 2612 fish were examined at three microsatellite loci. Each locus was highly polymorphic, with 30 alleles at *Ots*101, 28 alleles at *Ots*100, and 35 alleles at *Ots*102. Average observed heterozygosities were 86%, 88%, and 71%, respectively. In a dendrogram analysis of pairwise genetic distances, four geographically based groups were observed consisting of the lower Fraser River, the middle Fraser River, the upper Fraser River, and the Thompson River. An analysis of molecular variance showed that 97.57% of the genetic variance was within populations and 1.80% of the genetic variance was partitioned among populations. We detected significantly different allele frequencies among populations within regional groupings and temporal stability in allele frequencies in populations for which multiple years of samples were analyzed. Regional divergence may reflect colonization patterns following the last ice age, and divergence among populations within regions may reflect local adaptation. The elucidation of population structure of chinook salmon of the Fraser River watershed will be useful information for management designed to conserve genetic biodiversity.

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The Fraser River watershed produces greater numbers of Pacific salmon than any other river system in British Columbia (B.C.). Approximately 65 tributaries of the Fraser River are used as spawning and rearing habitat for chinook salmon (*Oncorhynchus tshawytscha),* and these streams produce up to one third of the commercial catch of chinook salmon from Brirish Columbia (Fraser et al., 1982). Although chinook salmon account for only 1% to 5% of the total escapements of salmon within the watershed (Northcote and Atagi, 1997), these fish are an important cultural, sporting, and food resource. Chinook salmon populations in the Fraser watershed have been negatively impacted by a variety of forces, in some cases reducing (Bradford, 1994) or completely eliminating (Slaney et al., 1996) local populations.

Historical efforts to maintain and enhance salmon runs through transplantation have had mixed results, illustrating that the characteristics of a population influence its ability to thrive in a given environment (Wood, 1995). Also, transplantation of fish and hatchery production practices may alter genetic composition of wild stocks (Waples, 1994). In fisheries, it is important not to over harvest small populations that may contain unique adaptive traits. For the above reasons it is advantageous to understand how population structures evolve in order to protect individual salmon runs and to preserve biodiversity.

Most of the chinook salmon populations of B.C. were founded after the ice of the Wisconsin glaciation retreated approximately 10,000 to 15,000 year ago (McPhail and Lindsey, 1986). If chinook salmon recolonization is similar to that of sockeye and coho salmon (Wood et al., 1994; Small et al., 1998a), reestablishment of the present day B.C. chinook salmon populations may have occurred from at least two different sources, Beringia to the north and Cascadia to the south (Gharrett et al., 1987), and possibly from a refuge in the Queen Charlotte Islands (Warner et al., 1982). The genetic character of the founding fish may be reflected in present day genetic structure, but because Pacific salmon return to their natal streams to spawn (Scheer, 1939; Quinn, 1984), reproductive isolation can lead to divergence of phenotypic and genotypic characters. Neutral genetic markers can be used to measure the degree of reproductive isolation and potential for local adaptation.

Over the years, a variety of methods have been used to examine population structure. Allozyme analysis has long been a mainstay in fish genetics research and has been used to determine population structure in chinook salmon of Alaska (Gharrett et al., 1987), from California to Alaska (Utter et al., 1989), of the Yukon River (Beacham et

al., 1989; Wilmot et al., 1992), of California and Oregon (Bartley et al. 1992), and in British Columbia (Teel et al. 2000). These studies suggest that chinook salmon populations were genetically heterogeneous and that populations could be placed into genetically defined groups corresponding to geographic regions. In some of the earlier studies there was little genetic distinction between geographically separate groups because the allozyme markers showed low polymorphism. However, using 25 polymorphic allozymes, Teel et al. (2000) detected strong population divergence within the Fraser River and among major rivers in British Columbia.

DNA markers can be more polymorphic than allozyme markers and thus may be more sensitive to population structure; with higher levels of polymorphism, there is an increased likelihood for populations to contain unique alleles or to have frequency differences in alleles that are shared among populations. Among the DNA-based markers, mitochondrial DNA has been used to examine genetic structure in chinook salmon populations of the West Coast of North America (Wilson et al., 1987; Cronin et al., 1993). These studies suggest that there is structuring among West Coast chinook salmon populations. However, the low resolution of this method limits its utility. Minisatellite DNA has been used to study Canadian chinook salmon populations. Beacham et al. (1996) found that chinook salmon formed two major regional groups in British Columbia: a southern group consisting of populations of the Fraser River, Vancouver Island, and the southern mainland; and a northern group consisting of populations of the Skeena River, the Yukon River, and the northern mainland. However, owing to technical complexity, the technique is unsuitable for studies involving large numbers of individuals.

Microsatellite DNA loci are highly polymorphic and technically easy to use (Nelson et al., 1998) and provide powerful markers for elucidating population structure. Microsatellite loci have provided information regarding population divergence in chinook salmon (Banks et al., 1996) and other salmonids (Angers et al., 1995; Scribner et al., 1996; Nelson et al., 1998; Small et al., 1998a, 1998b). In our study we exploited the ease of analysis and the highly polymorphic nature of microsatellite DNA loci to study population structure of chinook salmon. We surveyed variation at three microsatellite loci within 20 Fraser River chinook salmon populations and examined temporal stability of microsatellite allele frequencies. We used this information to hypothesize the genetic structure of chinook salmon populations within the Fraser River watershed.

Materials and methods

DNA extraction

Liver or scale samples were analyzed from 2612 individual chinook salmon from 20 populations of the Fraser and Thompson River watersheds (Fig. 1.). Sample sizes ranged from 30 to 347 fish (Table 1). Liver samples were obtained from spawning wild adults. Hatchery adults were sources for the Chehalis-red and Chilliwack-red samples. The nomenclature "-red" refers to the red flesh color of the fish in the population. DNA was extracted from liver and scales archived on scale cards according to the methods of Nelson et al. (1998). Liver samples taken prior to 1994 were subjected to DNA extractions as described in Beacham et al. (1996). Each 25 µL of polymerase chain reaction (PCR) required either 100 ng of genomic DNA, 0.1 to 1 µL of liver extracts, or 5 to 10 µL of scale extract.

PCR amplification

The loci amplified in this work were *Ots*100 (Nelson et al., 1998), *Ots*101 (Small et al., 1998a) and *Ots*102 (Nelson and Beacham, 1999). PCR amplification was carried out in 96-well microtiter plates with a MJ PTC-100 thermal cycler (MJ research, Watertown, MA). 25-µL PCR reactions contained 10 pmol (0.4 µM) of each primer, 80 µM of each nucleotide, 20 mM tris-pH 8.8, 2 mM $MgSO_4$, 10 mM KCl, 0.1% triton ×–100, 10 mM $(NH_4)SO_4$, and 0.1 mg/mL of bovine serum albumin. Primer set *Ots*100 required a 10% final volume of glycerol in the PCR. PCR temperature cycles were preceded by a denaturation incubation of 3 min at 94°C; samples then were held at 80°C while 1 unit of DNA polymerase was added. PCR cycle parameters and primer sequences for each locus are presented in Table 2. Three µLs of 10× loading dye (50 mM EDTA pH 8.0, 30% glycerol, 0.25% bromphenol blue) were added to each reaction and ten µLs of this solution was loaded on each gel lane for electrophoresis.

Gel electrophoresis

Microsatellite alleles were size-fractionated on nondenaturing polyacrylamide gels 17 cm wide by 14.5 cm long. Gels consisted of a 19:1 ratio of acrylamide to bis-acrylamide. Gel contained 2× TAE buffer (Maniatis et al., 1982) as did the gel box reservoirs. Electrophoretic conditions are described in Table 2. Each gel included three 20 base-pair (bp) marker lanes (GenSura Labs Inc., Del Mar, CA) to create a molecular size grid for sizing amplified microsatellites, 24 population samples, and one "standard fish" to estimate the precision of allele sizes (Table 3). Standard deviations were calculated for alleles from two different standard fish for each primer set. Gels were stained with 0.5 µg/mL of ethidium bromide in water and visualized with ultraviolet light (Fig. 2).

Digital images of gels were obtained as described in Nelson et al. (1998). Individual alleles were identified by using the procedure outlined in Small et al. (1998a). A four-bp bin was used for all *Ots*101 alleles. A four-bp bin was used to define smaller alleles of *Ots*100 and five- to eight-bp bins were used for larger alleles. A four-bp bin was used for the smaller alleles of *Ots*102 and five- to six-bp bins were used for larger alleles. These bin sizes (see Table 1 for bin designations) were four or more standard deviations wide according to estimates derived from the standard fish. Bins are referred to as "alleles" throughout the text.

Table 1

Allele frequencies, observed heterozygosity (H_0), and expected heterozygosity (H_e) at loci *Ots*101, *Ots*100, and *Ots*102 for 20 chinook salmon zygosity. The allele number (in basepairs) is the lower limit of the bin. The weighted mean of allele frequencies for regions (L Fr=lower Fraser;

***Ots*101**

Alleles	Harrison 326	Chilliwack White 181	L Fr 507	Quesnel 186	Stuart 294	Nechako 187	Chilko 120	Bridge 56	Cottonwood 51	Mid Fr 893	Tete Jaune 249	Chilliwack Red 29
142	0	0	0	0	0.002	0	0	0	0	0.001	0	0
146	0	0	0	0.008	0.003	0	0.004	0	0	0.003	0	0
150	0	0.003	0.001	0.054	0.02	0.045	0.008	0	0	0.029	0.002	0.017
154	0.003	0.003	0.003	0.056	0.066	0.048	0.042	0.027	0.029	0.053	0.008	0
158	0	0.008	0.003	0.062	0.029	0.059	0.025	0.045	0.127	0.048	0.028	0
162	0.005	0.003	0.004	0.016	0.02	0.064	0.017	0.089	0.078	0.036	0.06	0.052
166	0.035	0.017	0.029	0.046	0.02	0.027	0.017	0.089	0	0.029	0.014	0.034
170	0.014	0.025	0.018	0.054	0.01	0.003	0.017	0.027	0	0.019	0.004	0
174	0.015	0.039	0.024	0.024	0.01	0.019	0.05	0.009	0	0.02	0.036	0
178	0.046	0.028	0.039	0.019	0.015	0.019	0.071	0.071	0.059	0.03	0.042	0.017
182	0.067	0.014	0.048	0.03	0.029	0.037	0.025	0.027	0.049	0.031	0.032	0.034
186	0.069	0.05	0.062	0.054	0.066	0.08	0.038	0.045	0.118	0.064	0.034	0.069
190	0.075	0.11	0.088	0.032	0.097	0.061	0.117	0.089	0.108	0.079	0.112	0.017
194	0.112	0.066	0.096	0.129	0.075	0.04	0.146	0.098	0.118	0.092	0.078	0.121
198	0.126	0.155	0.136	0.097	0.138	0.123	0.117	0.063	0.108	0.117	0.104	0.121
202	0.118	0.08	0.105	0.081	0.109	0.107	0.083	0	0.088	0.091	0.08	0.138
206	0.09	0.072	0.084	0.056	0.068	0.061	0.083	0.045	0.059	0.064	0.058	0.069
210	0.063	0.077	0.068	0.048	0.071	0.04	0.025	0.134	0.049	0.057	0.098	0.034
214	0.044	0.075	0.055	0.038	0.048	0.029	0.004	0.018	0	0.031	0.026	0
218	0.015	0.003	0.011	0.022	0.024	0.053	0.021	0.036	0	0.028	0.042	0.086
222	0.026	0.022	0.025	0.016	0.01	0.021	0.008	0.036	0	0.015	0.06	0.138
226	0.026	0.041	0.032	0.022	0.017	0.011	0.004	0	0	0.013	0.04	0.017
230	0.017	0.03	0.022	0.011	0.015	0.013	0.021	0	0	0.013	0.014	0.017
234	0.014	0.014	0.014	0	0.01	0.011	0.013	0.009	0	0.008	0.012	0
238	0.005	0.008	0.006	0	0.005	0.011	0.021	0.027	0	0.008	0.012	0
242	0.006	0.017	0.01	0.005	0.007	0.008	0.017	0.018	0	0.008	0	0
246	0.003	0.019	0.009	0.008	0.007	0.005	0	0	0.01	0.006	0	0.017
250	0.003	0.011	0.006	0.008	0	0.003	0	0	0	0.002	0	0
254	0.002	0.008	0.004	0.005	0.005	0	0	0	0	0.003	0	0
259	0	0.003	0.001	0	0.002	0	0.008	0	0	0.002	0	0
Hobs	0.844*	0.889	0.86	0.855	0.881	0.877	0.933	0.839	0.686*	0.868	0.863*	0.897
Hexp	0.926	0.934	0.927	0.94	0.932	0.941	0.925	0.929	0.922	0.939	0.936	0.931

***Ots*100**

Alleles	Harrison 347	Chilliwack White 168	L Fr 515	Quesnel 183	Stuart 226	Nechako 174	Chilko 122	Bridge 55	Cottonwood 53	Mid Fr 813	Tete Jaune 254	Chilliwack Red 30
150	0.001	0	0.001	0	0	0	0	0.018	0	0.001	0	0
207	0.01	0	0.007	0	0.011	0.011	0	0.018	0	0.007	0	0
211	0.016	0.009	0.014	0.008	0.018	0.009	0.004	0	0	0.009	0	0
215	0	0	0	0.003	0.004	0.003	0	0.018	0	0.004	0	0
219	0	0	0	0.003	0	0	0	0	0	0.001	0	0
227	0.001	0.009	0.004	0	0	0	0	0	0	0	0	0
231	0.001	0	0.001	0	0	0	0	0	0	0	0	0.017
235	0.004	0	0.003	0.003	0	0.006	0.078	0.009	0	0.014	0	0

Table 1

populations from the Fraser River drainage. Populations out of Hardy-Weinberg equilibrium are indicated by * next to the observed hetero-Mid Fr=middle Fraser; U Fr=upper Fraser) are given. Sample sizes are provided below population names.

Chehalis Red 30	Bowron 55	Holmes 49	Indianpoint 40	Slim 70	U Fr 522	L Shuswap 192	M Shuswap 195	Eagle 33	Coldwater 37	Nicola 233	Thompson 690
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0.003	0	0	0	0	0.001
0	0	0	0.013	0	0.003	0.023	0.008	0	0	0.019	0.015
0.033	0.018	0	0.013	0	0.009	0.023	0.023	0	0	0.028	0.022
0.017	0.036	0	0	0	0.018	0.013	0.015	0	0.027	0.004	0.011
0.017	0.036	0.02	0.063	0	0.043	0.029	0.005	0.03	0.027	0.026	0.021
0	0.009	0.02	0.025	0	0.013	0.013	0.021	0.121	0.054	0.028	0.028
0	0	0	0.013	0	0.003	0.003	0.023	0	0.014	0.039	0.021
0	0.027	0.02	0.013	0	0.023	0.005	0.008	0.015	0.014	0.002	0.006
0.05	0.064	0.092	0.013	0.057	0.048	0.008	0.003	0.106	0.054	0.019	0.017
0.017	0.036	0	0.025	0.057	0.032	0.063	0.085	0.106	0.068	0.105	0.086
0.15	0.091	0.051	0.05	0.007	0.048	0.044	0.074	0.091	0.122	0.06	0.064
0.15	0.082	0.153	0.087	0	0.093	0.065	0.095	0.242	0.054	0.026	0.068
0.05	0.091	0.143	0.138	0.007	0.081	0.182	0.215	0.121	0.041	0.099	0.153
0.133	0.136	0.061	0.175	0.164	0.12	0.195	0.208	0.015	0.068	0.09	0.148
0.1	0.036	0.143	0.1	0.029	0.08	0.068	0.044	0.03	0.068	0.058	0.056
0.067	0.091	0.092	0.05	0.1	0.071	0.109	0.054	0.03	0.054	0.071	0.074
0.067	0.091	0.102	0.063	0.121	0.093	0.094	0.046	0.045	0.108	0.073	0.072
0.017	0.018	0	0.025	0.093	0.03	0.042	0.026	0.015	0.095	0.097	0.057
0	0.018	0.01	0.025	0.007	0.031	0.005	0.018	0	0.068	0.06	0.03
0.033	0.027	0.01	0.063	0.136	0.065	0	0.008	0	0.027	0.026	0.012
0.067	0.045	0.031	0.025	0.114	0.049	0	0.021	0	0	0.021	0.013
0.017	0.036	0.041	0.025	0.05	0.025	0	0	0	0.014	0.009	0.004
0.017	0	0	0	0.036	0.011	0	0.003	0.015	0.014	0.013	0.007
0	0.009	0	0	0.014	0.009	0.008	0	0	0.014	0	0.003
0	0	0	0	0.007	0.001	0.003	0	0	0	0.015	0.006
0	0	0.01	0	0	0.002	0	0	0	0	0.011	0.004
0	0	0	0	0	0	0.003	0	0	0	0	0.001
0	0	0	0	0	0	0	0	0.015	0	0.002	0.001
0	0	0	0	0	0	0	0	0	0	0	0
0.867	0.964	0.776	0.975	0.814	0.86	0.865	0.841	0.636*	0.784	0.888	0.851
0.933	0.927	0.898	0.925	0.9	0.933	0.891	0.882	0.879	0.946	0.94	0.92

Chehalis Red 30	Bowron 50	Holmes 46	Indianpoint 39	Slim 70	U Fr 519	L Shuswap 210	M Shuswap 192	Eagle 31	Coldwater 62	Nicola 268	Thompson 713
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0.01	0	0	0	0.001	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0.024	0	0.002
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0.001	0	0	0	0	0	0
0	0.04	0	0.013	0	0.005	0	0	0	0	0.002	0.001

continued

Table 1 (continued)

***Ots*100 (continued)**

Alleles	Harrison 347	Chilliwack White 168	L Fr 515	Quesnel 183	Stuart 226	Nechako 174	Chilko 122	Bridge 55	Cottonwood 53	Mid Fr 813	Tete Jaune 254	Chilliwack Red 30
239	0.001	0.003	0.002	0.098	0.027	0.032	0.115	0.027	0.009	0.056	0.002	0.1
243	0	0.006	0.002	0.049	0.051	0.029	0.078	0.027	0.085	0.05	0.016	0.067
247	0.006	0.006	0.006	0.022	0.02	0.02	0.012	0	0	0.017	0.033	0.033
251	0.006	0.009	0.007	0.005	0.029	0.009	0	0	0	0.011	0.081	0.033
255	0.004	0	0.003	0.044	0.027	0.055	0.012	0.045	0.009	0.034	0.039	0.017
260	0.019	0.03	0.022	0.101	0.093	0.055	0.012	0.018	0.019	0.065	0.063	0.117
266	0.039	0.024	0.034	0.063	0.1	0.069	0.004	0.027	0.028	0.061	0.039	0.05
271	0.058	0.083	0.066	0.022	0.044	0.023	0.045	0.091	0.057	0.039	0.059	0.1
276	0.032	0.068	0.044	0.06	0.024	0.034	0.004	0.027	0.047	0.033	0.065	0.05
281	0.13	0.083	0.115	0.137	0.08	0.083	0.07	0.073	0.198	0.099	0.091	0.067
288	0.101	0.134	0.112	0.145	0.053	0.075	0.078	0.055	0.057	0.082	0.106	0.017
293	0.111	0.089	0.104	0.046	0.058	0.124	0.053	0.118	0.142	0.078	0.128	0.133
299	0.118	0.077	0.105	0.057	0.097	0.055	0.102	0.1	0.094	0.08	0.089	0.067
305	0.086	0.092	0.088	0.06	0.066	0.057	0.07	0.082	0	0.06	0.057	0.067
311	0.13	0.119	0.126	0.038	0.084	0.069	0.131	0.027	0.075	0.073	0.053	0
318	0.084	0.107	0.091	0.025	0.075	0.095	0.119	0.1	0.17	0.082	0.02	0.033
326	0.026	0.045	0.032	0.003	0.035	0.08	0.012	0.091	0.009	0.036	0.033	0.033
334	0.007	0.003	0.006	0.005	0.004	0.003	0	0.027	0	0.005	0.026	0
342	0.006	0	0.004	0.003	0	0.006	0	0	0	0.002	0	0
358	0.003	0.003	0.003	0	0	0	0	0	0	0	0	0
Hobs	0.876	0.869	0.868	0.907	0.947	0.908	0.893	0.8*	0.849	0.905	0.882	0.867
Hexp	0.911	0.917	0.913	0.923	0.938	0.937	0.918	0.927	0.887	0.939	0.929	0.933

***Ots*102**

Alleles	Harrison 302	Chilliwack White 180	L Fr 482	Quesnel 174	Stuart 255	Nechako 129	Chilko 114	Bridge 47	Cottonwood 48	Mid Fr 767	Tete Jaune 262	Chilliwack Red 29
134	0	0	0	0	0	0	0	0	0	0	0	0
163	0	0	0	0	0	0	0	0	0	0	0	0
167	0	0	0	0	0	0.008	0.004	0.011	0	0.003	0	0
171	0.003	0	0.002	0	0.002	0	0	0	0.021	0.002	0	0
175	0	0.008	0.003	0.02	0.045	0.004	0.009	0	0.01	0.022	0	0.086
179	0.015	0.008	0.012	0.052	0.049	0.07	0.031	0.106	0	0.051	0.008	0.138
183	0.065	0.106	0.08	0.178	0.267	0.163	0.364	0.138	0.229	0.233	0.302	0.224
187	0.038	0.017	0.03	0.066	0.055	0.066	0.039	0.053	0	0.053	0.006	0.017
192	0.023	0	0.015	0	0.004	0.027	0.004	0	0.01	0.007	0	0
197	0.003	0.014	0.007	0.006	0.025	0.008	0.035	0.011	0	0.017	0	0
201	0.007	0.003	0.005	0.02	0.004	0.008	0.013	0	0.01	0.01	0	0.017
205	0.008	0.014	0.01	0.011	0.002	0.008	0	0	0	0.005	0	0
209	0.018	0.047	0.029	0.026	0.012	0.023	0.009	0	0.042	0.018	0.013	0.034
213	0.041	0.047	0.044	0.086	0.065	0.07	0.066	0.245	0.115	0.085	0.073	0.034
217	0.142	0.106	0.129	0.075	0.057	0.101	0.083	0.043	0.313	0.087	0.042	0.069
221	0.151	0.122	0.14	0.057	0.043	0.054	0.083	0.043	0.01	0.052	0.055	0.086
226	0.098	0.106	0.101	0.023	0.029	0.07	0.018	0.064	0	0.033	0.029	0.017
231	0.046	0.081	0.059	0.057	0.122	0.089	0.026	0.053	0.021	0.077	0.015	0.017

Table 1 (continued)

Chehalis Red 30	Bowron 50	Holmes 46	Indianpoint 39	Slim 70	U Fr 519	L Shuswap 210	M Shuswap 192	Eagle 31	Coldwater 62	Nicola 268	Thompson 713
0.033	0.08	0.011	0.09	0.057	0.032	0.002	0	0	0	0.007	0.003
0.033	0.08	0.033	0.103	0.057	0.039	0.002	0.01	0	0	0	0.004
0	0	0.011	0	0.007	0.02	0	0.003	0	0	0	0.001
0	0	0.033	0.051	0	0.048	0.007	0.003	0.016	0	0.007	0.006
0.017	0.02	0.022	0.013	0.007	0.027	0.055	0.044	0.016	0.008	0	0.029
0.167	0.12	0.087	0.141	0.164	0.099	0.048	0.036	0.016	0	0	0.025
0.017	0.03	0.065	0.026	0.029	0.038	0.083	0.065	0.065	0.073	0.083	0.076
0.15	0.04	0.054	0.051	0.1	0.069	0.138	0.094	0.065	0.016	0.018	0.076
0.017	0.11	0.098	0.051	0.121	0.075	0.136	0.096	0.258	0.016	0.014	0.083
0.133	0.15	0.185	0.064	0.093	0.104	0.155	0.201	0.081	0.056	0.023	0.115
0	0.03	0.054	0.077	0.007	0.067	0.067	0.07	0.032	0.073	0.087	0.073
0.183	0.09	0.087	0.09	0.136	0.122	0.112	0.188	0.065	0.073	0.14	0.135
0.1	0.1	0.12	0.064	0.114	0.093	0.076	0.063	0.097	0.065	0.08	0.074
0.017	0.06	0.054	0.077	0.05	0.056	0.069	0.065	0.065	0.145	0.158	0.102
0.017	0.02	0.022	0.038	0.036	0.039	0.026	0.052	0.065	0.032	0.08	0.052
0.05	0	0.043	0.026	0.014	0.022	0.01	0.005	0.032	0.242	0.131	0.067
0.05	0.01	0.011	0.026	0.007	0.026	0.005	0.005	0.097	0.153	0.106	0.053
0.017	0.01	0	0	0	0.014	0.007	0	0.032	0.024	0.062	0.025
0	0	0.011	0	0	0.001	0.002	0	0	0	0.002	0.001
0	0	0	0	0	0	0	0	0	0	0	0
0.933	0.86	0.826	0.923	0.814	0.871	0.914	0.88	0.806	0.855	0.907	0.886
0.9	0.92	0.913	0.923	0.9	0.929	0.9	0.885	0.903	0.871	0.918	0.919

Chehalis Red 30	Bowron 50	Holmes 44	Indianpoint 40	Slim 66	U Fr 521	L Shuswap 185	M Shuswap 167	Eagle 37	Coldwater 54	Nicola 231	Thompson 674
0	0	0	0	0	0	0.027	0	0.054	0	0	0.01
0	0	0	0	0	0	0.03	0	0.027	0	0	0.01
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0.008	0.012	0	0.009	0.026	0.015
0	0.02	0.023	0.025	0.045	0.016	0	0.012	0.041	0.167	0.134	0.065
0	0.07	0.08	0.063	0.091	0.041	0.024	0.033	0.149	0.102	0.18	0.093
0.05	0.19	0.193	0.138	0.167	0.233	0.116	0.207	0.135	0.046	0.058	0.114
0.017	0.04	0.011	0	0.015	0.012	0.084	0.174	0.108	0.065	0.017	0.083
0	0.01	0	0.013	0.008	0.003	0	0.009	0	0	0.011	0.006
0	0	0	0.025	0.015	0.004	0.003	0	0	0	0	0.001
0.033	0.02	0.045	0.013	0	0.01	0.003	0	0	0	0	0.001
0	0	0.011	0.025	0.015	0.005	0.003	0.006	0	0	0.002	0.003
0.017	0.03	0.057	0.087	0.061	0.032	0.027	0.051	0	0.019	0.019	0.028
0.167	0.24	0.205	0.162	0.152	0.12	0.035	0.057	0.041	0.148	0.115	0.077
0.183	0.14	0.091	0.087	0.121	0.079	0.049	0.036	0.041	0.157	0.195	0.104
0.1	0.06	0.023	0.038	0.015	0.051	0.024	0.009	0.027	0.046	0.009	0.017
0.05	0.04	0.011	0.038	0.03	0.03	0.014	0.021	0	0.009	0.015	0.015
0.017	0.04	0	0.038	0.03	0.02	0.022	0.006	0	0.046	0.022	0.019

continued

Table 1 (continued)

***Ots*102 (continued)**

Alleles	Harrison 302	Chilliwack White 180	L Fr 482	Quesnel 174	Stuart 255	Nechako 129	Chilko 114	Bridge 47	Cottonwood 48	Mid Fr 767	Tete Jaune 262	Chilliwack Red 29
236	0.043	0.067	0.052	0.083	0.045	0.085	0.053	0.032	0.021	0.059	0.053	0.034
241	0.065	0.05	0.059	0.055	0.045	0.043	0.022	0.085	0.031	0.045	0.132	0.069
246	0.05	0.047	0.049	0.009	0.024	0.016	0.031	0.053	0	0.02	0.074	0.017
251	0.03	0.042	0.034	0.026	0.022	0.019	0.009	0	0.01	0.018	0.048	0.034
256	0.038	0.022	0.032	0.032	0.012	0.008	0.044	0.011	0.073	0.024	0.122	0.034
261	0.028	0.039	0.032	0.052	0.022	0.023	0.022	0.032	0.063	0.032	0.021	0
267	0.022	0.017	0.02	0	0.029	0.016	0.009	0	0	0.014	0	0.034
273	0.015	0.003	0.01	0.009	0.016	0.008	0.009	0	0	0.01	0	0
279	0.005	0.003	0.004	0.011	0.002	0	0	0	0.021	0.005	0	0
285	0.01	0.014	0.011	0.006	0	0.004	0	0	0	0.002	0	0
291	0.013	0	0.008	0	0	0.004	0.013	0	0	0.003	0	0.034
297	0.01	0.014	0.011	0.009	0	0.004	0	0	0	0.003	0.004	0
303	0.003	0	0.002	0.029	0.004	0.004	0.004	0.021	0	0.01	0.004	0
309	0.002	0.006	0.003	0	0	0	0	0	0	0	0	0
325	0.005	0	0.003	0.003	0	0	0	0	0	0.001	0	0
321	0.002	0	0.001	0	0	0	0	0	0	0	0	0
333	0.002	0	0.001	0	0	0	0	0	0	0	0	0
Hobs	0.714*	0.628*	0.699	0.632*	0.761*	0.713*	0.684*	0.723	0.833	0.714	0.775*	0.862
Hexp	0.927	0.928	0.927	0.931	0.894	0.93	0.842	0.894	0.833	0.906	0.859	0.897

Table 2

Primer sequences, PCR cycle, and gel-running conditions for microsatellite loci *Ots*100, *Ots*101, and *Ots*102.

Locus	Primer sequence	PCR cycle denature	Anneal	Extend	No. of cycles	Gel conditions: % acrylamide	Gel conditions: Voltage (V)	Gel conditions: Time (h)
*Ots*100	F 5′TGAACATGAGCTGTGTGAG-3′ R 5′-ACGGACGTGCCAGTGAG-3′.	94°C/20s	57°C/20s	72°C/20s	30	7	60	18
*Ots*101	F 5′ACGTCTGACTTCAATGATGTTT-3′ R 5′TATTAATTATCCTCCAACCCAG-3′	94°C/30s	53°C/30s	72°C/30s	30	8	70	17
*Ots*102	F 5′AGGATCCAATAAGGAGTGATA-3′ R 5′ACTAGGTATCCCCTTAACCA-3′	94°C/20s	50°C/10s	72°C/20s	30	6	60	17

Data analysis

A pedigree analysis was performed on chinook salmon families to document the inheritance of microsatellite alleles at each locus. Chinook salmon families were obtained from domesticated strains originating in Robertson Creek and Big Qualicum River, B.C. For each locus, we examined inheritance in 6 families (12 offspring per family). Population genetic data were analyzed by using GENEPOP version 3.1 (Raymond and Rousset, 1995a) and ARLEQUIN version 1.1 (Schneider et al., 1997). Allele frequencies and heterozygosities were estimated for each population at each locus and conformation to Hardy-Weinberg equilibrium (HWE) was tested with a simulated Fisher's exact test (Guo and Thompson, 1992). A gametic disequilibrium test was also performed. Differences in allele frequencies among different sample years, populations, and regions (populations grouped into regions) were examined by using pairwise tests in ARLEQUIN which is analogous to Fisher's exact test (described in Raymond and Rousset, 1995b). An analysis of molecular variation (AMOVA) was also performed by using ARLEQUIN to measure the distribution of molecular variance at several levels: among individuals, among samples taken in different years for the same population, among all populations (with year classes combined), and among regions. *F*-statistics (Wright, 1951) and their stan-

Table 1 (continued)

Chehalis Red 30	Bowron 50	Holmes 44	Indianpoint 40	Slim 66	U Fr 521	L Shuswap 185	M Shuswap 167	Eagle 37	Coldwater 54	Nicola 231	Thompson 674
0.033	0.02	0	0.125	0.038	0.047	0.016	0.006	0	0.019	0.006	0.01
0.2	0.01	0.136	0.013	0.053	0.102	0.005	0	0	0	0.009	0.004
0.017	0.01	0.034	0.025	0.008	0.046	0.014	0.003	0	0	0.006	0.007
0.083	0	0.034	0.025	0.03	0.039	0.008	0.009	0	0	0.004	0.006
0.017	0.01	0.023	0.025	0.03	0.073	0.016	0.009	0	0	0	0.007
0	0.03	0.023	0	0.008	0.016	0.016	0.006	0.014	0	0.002	0.007
0	0	0	0	0	0.002	0.019	0.003	0	0	0	0.006
0.017	0	0	0	0	0.001	0.003	0.006	0.014	0	0.004	0.004
0	0	0	0	0	0	0.016	0.015	0	0	0	0.008
0	0	0	0	0	0	0.011	0.021	0.081	0.009	0	0.013
0	0	0	0.025	0.023	0.007	0.103	0.066	0.068	0	0	0.048
0	0.01	0	0.013	0.038	0.009	0.116	0.096	0.095	0	0.011	0.065
0	0	0	0	0.008	0.003	0.057	0.093	0.041	0.111	0.089	0.08
0	0.01	0	0	0	0.001	0.041	0.027	0	0.046	0.065	0.044
0	0	0	0	0	0	0.016	0.003	0.027	0	0	0.007
0	0	0	0	0	0	0.038	0.003	0.041	0	0	0.013
0	0	0	0	0	0	0.038	0.003	0	0	0	0.011
0.9	0.8	0.75	0.875	0.829	0.808	0.535*	0.575*	0.297*	0.796	0.68*	0.602
0.9	0.88	0.886	0.925	0.857	0.896	0.941	0.898	0.92	0.889	0.883	0.933

dard deviations were calculated for each locus and for all loci combined according to Weir and Cockerham (1984) by using FSTAT (Goudet, 1995). FSTAT also provided pairwise F_{st} values for populations and regions. We used the notations F_{st}, F_{is}, and F_{it} for Weir and Cockerham's (1984) θ, f, and F, respectively. In all analyses, probability values were adjusted to correct for the number of simultaneous tests as discussed by Lessios (1992). Thus, the significance level is taken to be 0.05/number of simultaneous comparisons.

Graphical analysis of genetic relationship between populations was performed. A neighbor-joining (NJ) (Saitou and Nei, 1987) dendrogram was constructed by using PHYLIP 3.5c (Felsenstein[1]). The allele frequency matrix was resampled 1000 times in a bootstrap resampling procedure and Cavalli-Sforza and Edwards (1967) chord distances among populations were estimated for each matrix. A consensus NJ dendrogram of chord distances was generated to determine the stability of nodes within the dendrogram. For presentation, a NJ dendrogram with the original branch lengths was constructed and bootstrap values over 50% were plotted onto the nodes of the dendrogram.

In several populations we suspected a null allele at *Ots*102. These populations had moderate heterozygote deficiencies and a large number of individuals whose other two loci amplified but that failed to amplify at *Ots*102, even after PCR stringency was relaxed. We generated an estimate of the frequency of the null allele by recoding, as null allele homozygotes, two out of every three of the individuals failing to amplify at *Ots*102. Corrected allele fre-

Table 3

Observed precision of allele size determination (bp) for repeated analysis of heterozygous fish at loci *Ots*101, *Ots*100, and *Ots*102. *"n"* is the number of times each fish was analyzed. Standard deviation (SD) was calculated and the range of observed measurements is shown.

Locus	*n*	Mean	SD	Range
*Ots*101	44	221.77	1.08	220–224
	103	199.47	0.96	196–201
	44	167.93	0.79	166–169
	103	153.49	0.94	151–155
*Ots*100	18	367.17	1.86	360–367
	103	322.07	1.73	318–325
	18	281.28	1.23	279–283
	103	251.81	1.13	249–254
*Ots*102	74	270.35	1.34	268–273
	70	225.54	1.10	223–228
	70	188.30	1.08	186–190
	74	181.15	0.86	180–183

[1] Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package), version 3.4. Univ. Washington, Seattle, WA. [Available from author, Department of Genetics, Box 357360, Univ. Washington, Seattle, WA 98195-7360.]

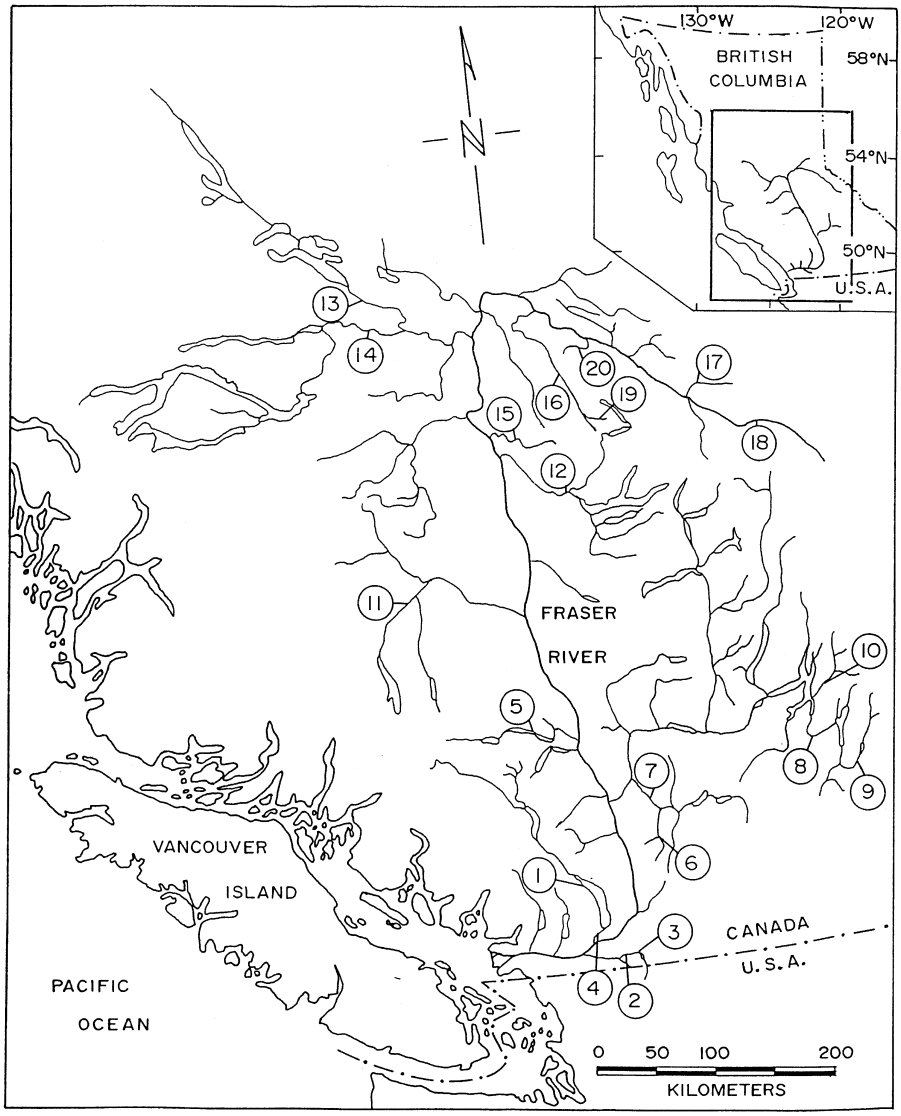

Figure 1

Map of the Fraser River watershed showing locations of chinook salmon populations with inset of the map of British Columbia. Numbers are placed at the collection sites where the populations were obtained: 1 = Harrison, 2 = Chilliwack-white, 3 = Chilliwack-red, 4 = Chehalis-red, 5 = Bridge, 6 = Coldwater, 7 = Nicola, 8 = Lower Shuswap, 9 = Middle Shuswap, 10 = Eagle, 11 = Chilko, 12 = Quesnel, 13 = Stuart, 14 = Nechako, 15 = Cottonwood, 16 = Bowron, 17 = Holmes, 18 = Tete Jaune, 19 = Indianpoint, 20 = Slim.

quencies in the presence of a null allele were generated by using the maximum likelihood method of the utilities option in GENEPOP. The data set with corrected allele frequencies was tested in a NJ dendrogram analysis.

Results

Pedigree analysis and allele assignment

All loci displayed normal Mendelian inheritance; each heterozygous parent (two allele bands) passed each of its two bands to approximately 50% of its offspring, and each homozygous parent (one allele band) passed its single band to 100% of its offspring. Assignment of alleles was based on the empirically determined standard error of band size estimation as reported in Table 3. The size range for each allele was set to allow for 95% confidence of allele assignment.

Heterozygosity and allele frequencies

Heterozygosity was consistently high at *Ots*101 and *Ots*100 (Table 1), ranging from 0.636 to 0.975 at *Ots*101(0.86 average), and from 0.80 to 0.947 (0.88 average) at *Ots*100. Het-

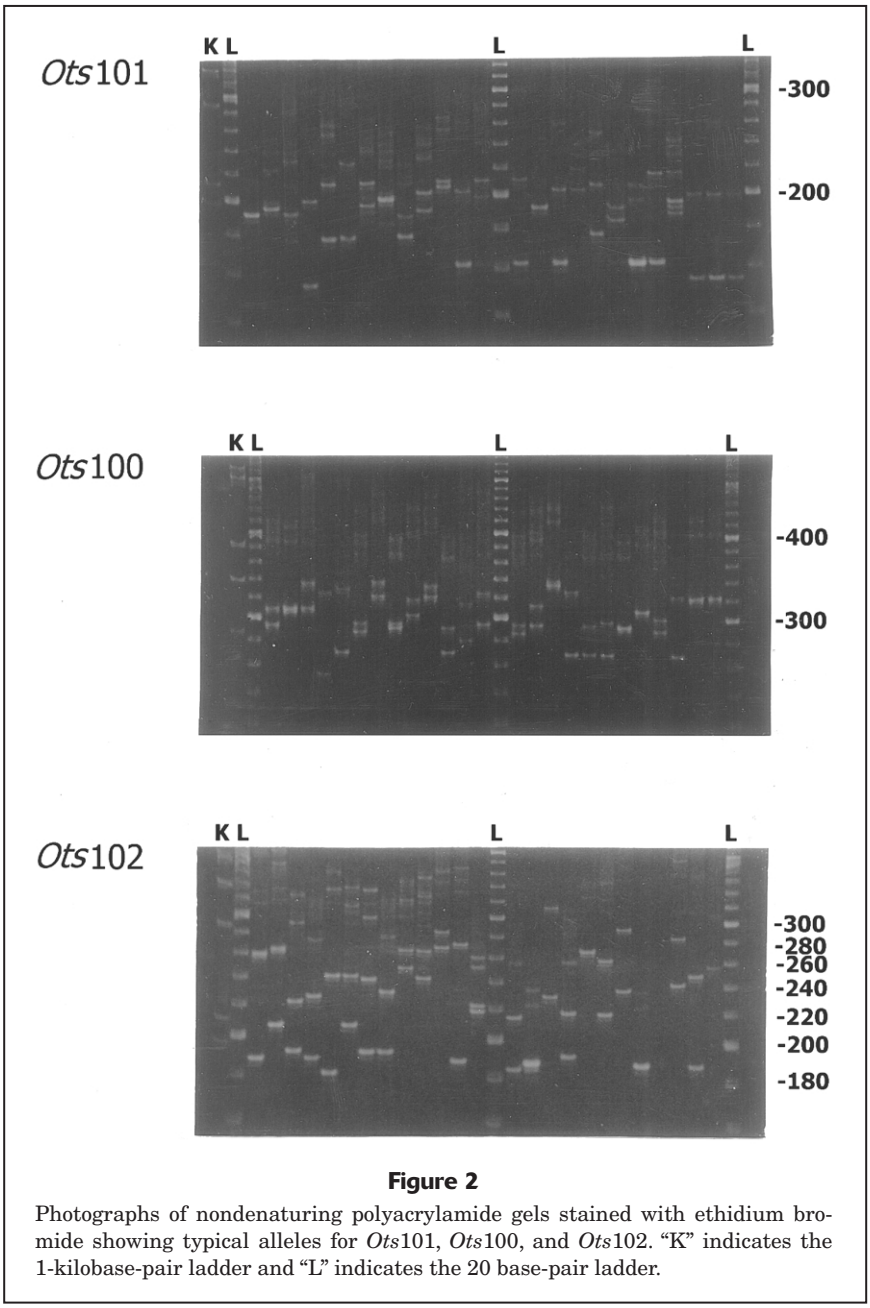

Figure 2

Photographs of nondenaturing polyacrylamide gels stained with ethidium bromide showing typical alleles for *Ots*101, *Ots*100, and *Ots*102. "K" indicates the 1-kilobase-pair ladder and "L" indicates the 20 base-pair ladder.

erozygosity values had a wider range at *Ots*102, ranging from a low of 0.297 in Eagle River to a high of 0.9 in Chehalis-red (average of 0.706). The apparently low heterozygosity found in some populations at *Ots*102 may have been partially due to the presence of a null allele. With the exception of Bridge River, all allele frequencies showed significant variation among the different populations. In pairwise tests, Bridge River was not significantly different (P nondifferentiation=0.00004) from Harrison River, Chilliwack-white, Chilliwack-red, Chehalis-red, Coldwater River, Middle Shuswap River, Eagle River, Stuart River, and Cottonwood River. All other populations were significantly different from each other.

Hardy-Weinberg equilibrium and treatment of the null allele

We tested each of the 20 populations for significant deviations from HWE proportions at each locus. Conformation to HWE was rejected at the 5% level (P<0.05/20) in four populations (Harrison River, Tete Jaune River, Cottonwood River, and Eagle River) at *Ots*101, in one population (Bridge River) at *Ots*100, and in 12 populations at *Ots*102 (see Table 1). All rejections of conformation to HWE were due to a deficiency of heterozygotes. Single locus F_{is} values for *Ots*101, *Ots*100, and *Ots*102 were 0.079, 0.050, and 0.261, respectively, and 0.127 for

all loci combined. All values were significantly greater than zero (P<0.005), indicating that populations tended towards disequilibrium at each locus. Gametic disequilibrium tests indicated that loci were unlinked.

Because of a suspected null allele at *Ots*102, most populations showed a deficiency of heterozygotes at this locus; this locus also had a high and significant F_{is} value. When the dendrogram analysis was redone with the corrected data set, the regional structure was improved, in that all the middle Fraser River populations grouped together and bootstrap values were higher for regional nodes. Thus the major conclusion of this study was unaffected by the suspected null allele. However because the null allele was not observed in the pedigree analysis, its presence remains speculative and we have kept the original data set for the analysis presented here. Observed homozygote excesses are likely not due to our inability to resolve alleles because we observed a large number of individuals that amplified at the other two loci of the study but failed to amplify at *Ots*102, suggesting the presence of a null homozygote. Observed heterozygote deficiency could also be due to a partial disequilibrium; however no single population showed disequilibrium at all three loci.

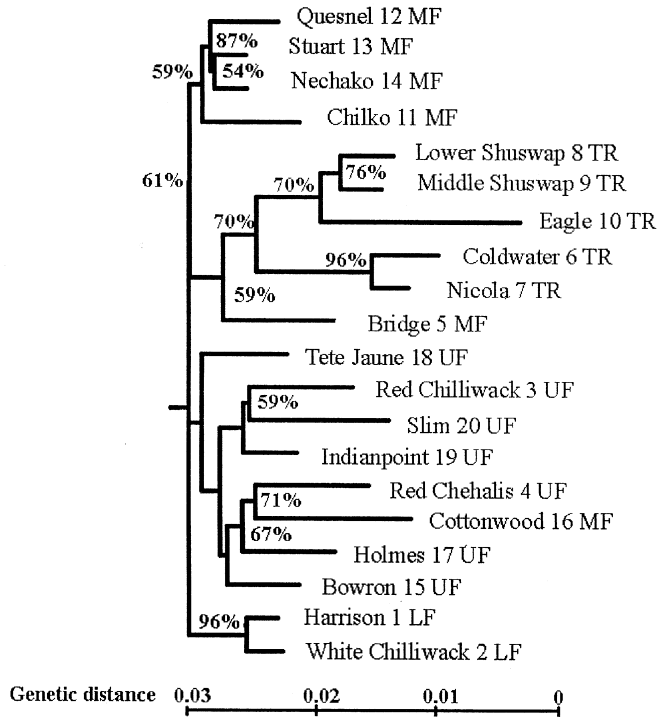

Figure 3

Neighbor-joining dendrogram of 20 chinook salmon populations from the Fraser River watershed. The dendrogram shows branch lengths, and the bootstrap values at the nodes were the percentage of dendrograms in which the populations beyond the node were grouped together out of 1000 dendrograms. The numbers following the place names correspond to map numbers in Figure 1. "MF", "TR, "UF" and "LF" indicate middle Fraser, Thompson River, upper Fraser and lower Fraser River respectively.

Temporal stability of allele frequencies, and population and regional variability

Comparisons of samples from different years for the same population suggested that there is temporal stability in allele frequencies within populations. With the exception of a single comparison within the Harrison River samples (the 1988 samples differed from the 1992 samples [P nondifferentiation=0.0003]), sample sets within populations were indistinguishable. Analysis of molecular variance showed that 97.57% (P<0.01, 5719 df) was within populations and that 1.80% (P<0.01, 16 df) of the total genetic variance was among populations. A small but significant 0.63%, (P<0.01, 3 df) of the genetic variance was apportioned between regions. Because population year classes were not significantly different from each other and variability among populations exceeded variability among year classes, year classes were combined into single populations for the rest of the analysis. All regions were significantly different from each other in pairwise tests (P<0.008).

Dendrogram

To determine if there was a pattern to allele frequency differences, we constructed a NJ dendrogram (Fig. 3) of pairwise genetic distances. This analysis suggested that geography at least partially underlies genetic relationships among chinook salmon populations in the Fraser River drainage. The NJ dendrogram consisted of four major branches, and are congruent with regional groupings (Fig. 3). The lower Fraser River branch includes Harrison River and Chilliwack and is supported in 96% of the dendrograms. The Thompson River branch is supported at 70% and includes Bridge River (from the middle Fraser River) in 59% of the dendrograms. The middle Fraser River branch is supported in 59% of the dendrograms. The largest branch, supported in 32% of the dendrograms, includes all the upper Fraser River populations, as well as the Chilliwack-red and Chehalis-red hatchery populations, and Cottonwood River from the middle Fraser River region.

This graphic analysis of genetic relationships shows regional groupings consisting of lower, middle, and upper Fraser River population groups, and a well-defined Thompson River group. These groupings are modestly supported because most of the genetic variance is within populations and only a small amount of the variance is among regions.

Population and regional differences

Wright's F statistics (Wright, 1951) were calculated to determine the degree of structuring between and within the regional population groupings. When all the populations were kept separate, the F_{st} values indicated diver-

gence among populations with single locus values of 0.011 (SD=0.002) for *Ots*101, 0.021(SD=0.004) for *Ots*100 and 0.038 (SD=0.007) for *Ots*102. The multiple locus value was 0.023 (SD=0.008). To determine the patterns of genetic relationship between sample groups within the individual regional groupings, pairwise F_{st} values were calculated for each pair of samples (Table 4). The highest pairwise F_{st} values were observed between populations of different regional groups. In order to estimate the degree of genetic isolation between the regional groupings suggested by dendrogram analysis, populations were combined by geographic region and pairwise F_{st} values were calculated (Table 5). The greatest value observed was between the Thompson River and upper Fraser River regions (0.0161) and the lowest value was between the upper and middle Fraser regions (0.006). F_{st} values were significant at each locus: *Ots*101 0.006 (SD=0.002), *Ots*100 0.008 (SD=0.00)1, and *Ots*102 0.022 (SD=0.008), for combined loci 0.012 (SD=0.005) (all $P<0.005$).

Discussion

Our results show significant genetic diversity within and between chinook salmon populations spawning in the tributaries of the Fraser River. The genetic relationship between the populations from the different tributaries suggest that there are four regional assemblages: upper Fraser River, middle Fraser River, lower Fraser River, and the Thompson River. These regional assemblages are concordant with the interpretation of the population structure seen by Teel et al. (2000) based on allozyme analysis. This concordance was observed with two very different marker sets. We employed three markers with average observed heterozygosities of 86%, 88%, and 71%, whereas out of the 25 polymorphic enzymes used by Teel et al., the highest heterozygosity was 0.441. The concordance of these two marker types with geography suggests that the genetic differences observed are not due simply to genetic drift but

Table 4

Pairwise F_{st} values among populations of the Fraser River watershed. Vertical lines indicate regional groupings. Abbrevations are as follows: Haris. = Harrison, Chil.w = Chilliwack-white, Che.-r = Chehalis-red, Ipoint = Indianpoint, Chil.-r = Chilliwack-red, TJ = Tete Jaune, Coldwt. = Coldwater, L.Shu. = Lower Shuswap, Cotton = Cottonwood, Ques. = Quesnel.

	Haris.	Chil.-w	Che.-r	Holmes	Bowron	Ipoint.	Chil.-r	Slim	TJ	Nicola	Coldwt.	L. Shu.	M. Shu.	Eagle	Cotton	Bridge	Ques.	Stuart	Nechako	Chilko
Haris.																				
Chil.-w	0.003																			
Che.-r	0.016	0.018																		
Holmes	0.019	0.020	0.010																	
Bowron	0.021	0.019	0.013	0.003																
Ipoint.	0.016	0.011	0.014	0.008	0.002															
Chil.-r	0.019	0.019	0.019	0.012	0.011	0.001														
Slim	0.028	0.023	0.018	0.018	0.007	0.009	0.010													
TJ	0.023	0.019	0.025	0.012	0.020	0.015	0.012	0.024												
Nicola	0.026	0.028	0.040	0.036	0.031	0.027	0.026	0.034	0.045											
Coldwt.	0.027	0.025	0.038	0.035	0.032	0.030	0.031	0.039	0.047	0.004										
L. Shu.	0.022	0.023	0.032	0.023	0.021	0.018	0.020	0.027	0.029	0.039	0.043									
M. Shu.	0.029	0.031	0.039	0.027	0.024	0.024	0.024	0.034	0.030	0.044	0.049	0.006								
Eagle	0.034	0.033	0.044	0.023	0.028	0.032	0.029	0.041	0.037	0.038	0.035	0.023	0.027							
Cotton	0.024	0.027	0.023	0.020	0.018	0.025	0.030	0.037	0.029	0.038	0.036	0.037	0.037	0.047						
Bridge	0.021	0.018	0.019	0.010	0.009	0.011	0.017	0.022	0.019	0.023	0.018	0.028	0.024	0.032	0.029					
Ques.	0.017	0.015	0.025	0.013	0.009	0.005	0.008	0.021	0.017	0.032	0.032	0.019	0.022	0.032	0.025	0.017				
Stuart	0.018	0.014	0.026	0.015	0.014	0.009	0.008	0.022	0.013	0.032	0.030	0.024	0.026	0.032	0.025	0.018	0.009			
Nechako	0.011	0.009	0.018	0.017	0.013	0.007	0.007	0.021	0.016	0.022	0.020	0.023	0.026	0.030	0.020	0.011	0.007	0.005		
Chilko	0.026	0.022	0.043	0.023	0.021	0.018	0.017	0.037	0.018	0.045	0.046	0.037	0.034	0.041	0.024	0.026	0.017	0.011	0.018	

Table 5

Table of pairwise F_{st} values among major regions in the Fraser River drainage.

	Lower Fraser	Mid Fraser	Upper Fraser
Lower Fraser	0.0000		
Mid Fraser	0.0115	0.0000	
Upper Fraser	0.0147	0.0062	0.0000
Thompson	0.0146	0.0125	0.0161

rather reflect patterns of historical colonization and present day gene flow (or lack thereof). Concordance of both allozyme and microsatellite data indicates that the simple method used in our study to designate alleles does not greatly bias or skew the results.

During the retreat of the Wisconsin glacial ice sheet, the headwaters of the Fraser and Thompson Rivers were ice free before the lower Fraser River channel was ice free, and therefore drained through the Columbia River. McPhail and Lindsey (1986) suggested that freshwater fish colonized the upper Fraser River and the Thompson River by means of the Columbia River during this time. Allozyme analysis of chinook salmon show that the Thompson River populations are distinct from the population of the other Fraser River tributaries (Utter et al., 1989; Teel et al., 2000). Similarly, coho salmon from the Thompson River are genetically distinct from coho salmon in the lower Fraser River (Small et al., 1998a). In our analysis, the Thompson River populations formed a distinct group, consistent with the hypothesis that the Thompson River watershed may have been colonized by a different founder group than other regions of the Fraser River. In addition, strong genetic substructuring *within* the Thompson River watershed was observed. This structuring, also observed by Teel et al. (2000), suggests that there may be sufficient genetic isolation within the Thompson River watershed to allow for the persistence of locally adapted populations.

If the upper Fraser and Thompson Rivers were both colonized by means of the Columbia River, then tests of the genetic relationship between these groups might show the upper Fraser and Thompson Rivers more closely related to each other than to other regions. However, based upon our study, they are the most distantly related, suggesting that either the upper Fraser was not colonized by the same population that founded the Thompson River populations, or that migration may have obscured the origins of fish inhabiting this region. This hypothesis is currently being tested by analyzing chinook salmon populations from the Skeena and Nass Rivers (possible source populations) and by increasing the resolution of the genetic structure of coho salmon in the Fraser River watershed by analyzing more microsatellite loci.

The high bootstrap support for the lower Fraser group suggests that this region was colonized by a single founder population. This hypothesis seems likely because the river mouths are separated by approximately 15 kilometers. The close genetic association between the Harrison and Chilliwack Rivers (F_{st}=0.003) indicates either that straying occurs routinely between them or that colonization was so recent that the populations have not diverged.

The red-flesh Chehalis and Chilliwack populations were introduced in the 1980s from broodstocks originating in the upper and middle Fraser River. Sources of the brood stocks included the Bowron, Chilko, and Quesnel Rivers and Slim Creek[2] These populations are artificially maintained by selecting red-flesh fish for broodstock. Our analysis places these populations with the upper Fraser River populations, reflecting their origins.

The regional groupings and patterns of genetic relationships within each region provide a starting point for discussion of the events that led to the repopulating of these regions by chinook salmon and the degree of isolation of different populations. Although only in the early stages, this information forms a base upon which to begin assigning management and fishery enhancement priorities such that genetic diversity present in wild populations is preserved. This information, along with life history and ecological data, will also be useful for the determination of whether a regional grouping of populations can be considered an evolutionarily significant unit (Waples, 1991).

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