Microsatellite DNA analysis of coastal populations of bull trout (*Salvelinus confluentus*) in British Columbia: zoogeographic implications and its application to recreational fishery management

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Abstract: Microsatellite DNA variation was assayed among 383 bull trout (*Salvelinus confluentus*) from 20 Pacific coastal localities from the Skeena River (central British Columbia) to the Olympic Peninsula (western Washington State). An average of 1.7 alleles was resolved per population and heterozygosity averaged 0.35. Twenty-six fish were identified as bull trout × Dolly Varden (*Salvelinus malma*) hybrids. Population subdivision was substantial ($\theta = 0.33$), but subdivision was higher ($\theta = 0.46$) when interior populations ($N = 37$) were included, indicating a major genetic distinction between "coastal" and "interior" bull trout. Bull trout populations north of the Squamish River were genetically more similar to interior bull trout than to other more southern coastal populations, suggesting that they had been founded by headwater transfers from interior populations. Individual assignment of bull trout averaged 53.4% correct assignment among populations (range: 12%–95%). Mixture analysis indicated that most fish from the lower Fraser River recreational fishery originated from major nearby tributaries (e.g., Pitt, upper Lillooet, and Chilliwack rivers). Our results substantiate the existence of two major evolutionary lineages of bull trout and highlight the importance of tributary habitats for the persistence of local populations, as well as for those that forage in downstream areas on the lower Fraser River.

Résumé : Nous avons évalué la variation des microsatellites d'ADN chez 383 ombles à tête plate (*Salvelinus confluentus*) provenant de 20 localités de la côte du Pacifique depuis la Skeena (centre de la Colombie-Britannique) jusqu'à la péninsule Olympique (ouest de l'état de Washington). On retrouve en moyenne 1,7 allèle par population et l'hétérozygotie moyenne est de 0,35. Vingt-six poissons ont été identifiés comme des hybrides d'ombles à tête plate et de Dolly Varden (*Salvelinus malma*). La subdivision de la population est considérable ($\theta = 0{,}33$), mais elle est plus importante ($\theta = 0{,}46$) lorsqu'on inclut les populations de l'intérieur ($N = 37$), ce qui indique l'existence d'une forte distinction entre les ombles à tête plate de « la côte » et celles de « l'intérieur ». Les populations d'ombles à tête plate au nord de la Squamish ressemblent plus génétiquement aux populations de l'intérieur qu'aux autres populations côtières plus au sud, ce qui laisse croire qu'elles ont été fondées par des transferts d'amont à partir des populations de l'intérieur. L'attribution des poissons individuels à leur population d'origine a un taux de succès de 53,4 % (étendue : 12–95 %). Une analyse de mélange indique que la plupart des poissons provenant de la pêche sportive sur le Fraser inférieur ont leur origine dans les grands tributaires avoisinants (par exemple, les rivières Pitt, Lillooet supérieure et Chilliwack). Nos résultats appuient l'existence de deux lignées évolutives majeures chez l'omble à tête plate et soulignent l'importance des habitats des tributaires pour la persistance des populations locales et pour celles qui se nourrissent dans les parties aval du Fraser inférieur.

[Traduit par la Rédaction]

Introduction

The bull trout, *Salvelinus confluentus*, is native to western North America from Nevada north to the Northwest Territories west of the continental divide, but extends eastward to western Montana and the headwaters of the South and North Saskatchewan and Athabasca rivers in Alberta (Cavender 1978). In British Columbia (BC), bull trout are native to all aquatic ecoregions (sensu Taylor 2004*a*) except for Vancouver Island and the Queen Charlotte Islands. Across this range, the species is thought to consist of two major evolutionary lineages below the species level: the "coastal" and "interior" bull trout. Coastal and interior lineages have been loosely defined as those sets of populations that occur west and east, respectively, of the Coast–Cascade Mountain ranges (Taylor et al. 1999). This subdivision has been recognized in terms of morphology (e.g., Cavender 1994; Haas and McPhail 2001) and mitochondrial DNA (Taylor et al. 1999) across the species' range, as well as in the United States (US) in terms of allozyme and microsatellite DNA

Received 29 June 2005. Accepted 16 November 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 20 April 2006.
J18766

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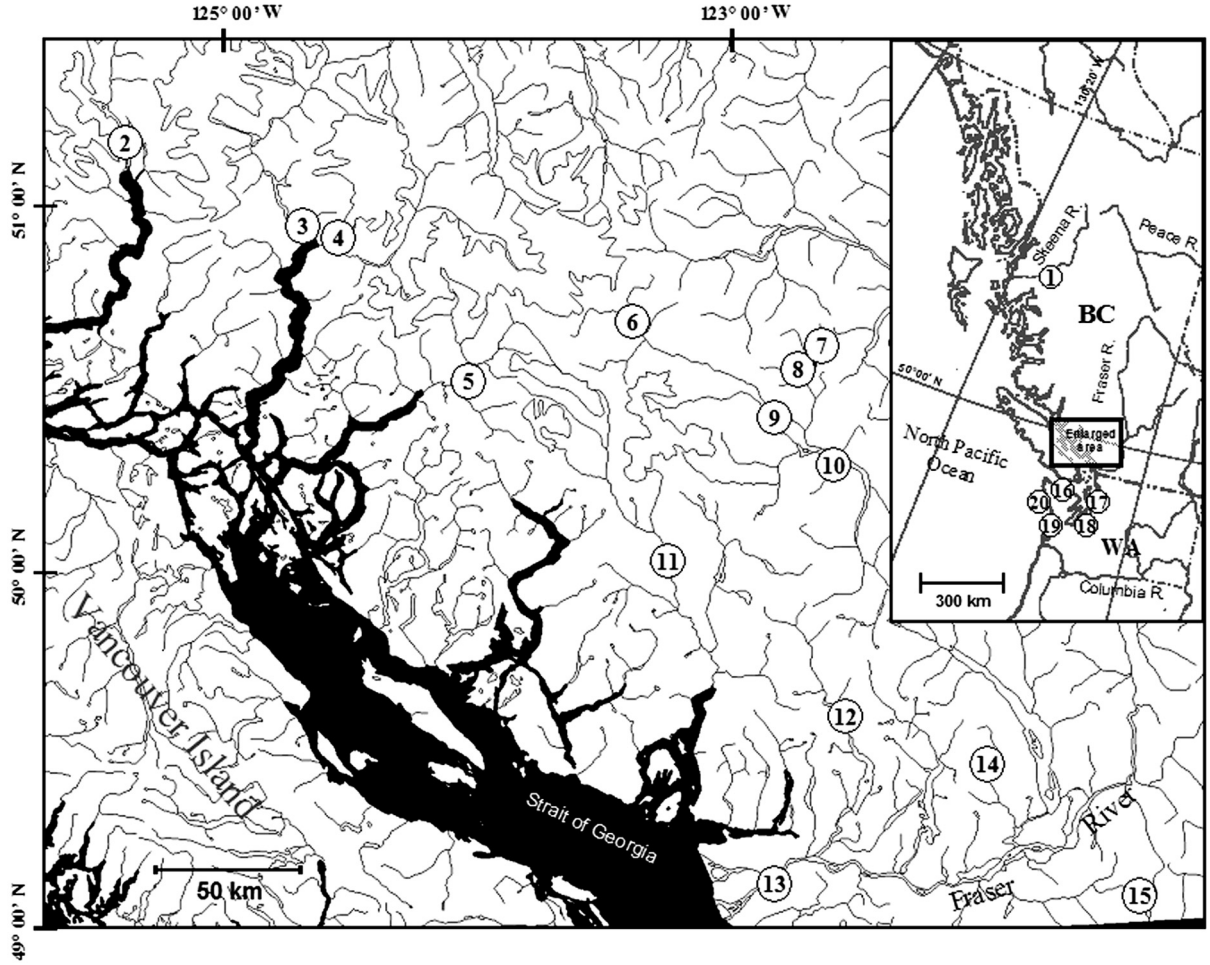
 doi:10.1139/F06-026

Fig. 1. Map of localities assayed for microsatellite DNA variation in bull trout (*Salvelinus confluentus*, populations 1–19) and Dolly Varden (*Salvelinus malma*, population 20). 1, Gitnadoix River; 2, Klinaklini River; 3, Homathko River; 4, Southgate River; 5, Toba River; 6, Ryan River; 7, Phelix Creek; 8, Birkenhead Lake; 9, upper Lillooet River; 10, Ure Creek; 11, Squamish River; 12, Pitt Lake and upper Pitt River; 13, lower Fraser River; 14, Chehalis Lake; 15, Chilliwack Lake; 16, Elwha River; 17, Sauk River; 18, Skokomish River; 19, Queets River; 20, Soleduck River. Inset shows relationship of study area to western North America.



variation (Leary et al. 1993; Spruell et al. 2003). In the US, bull trout are listed as threatened under the Endangered Species Act (USFWS 1999), while in BC the species is blue-listed as a species of "special concern". Across at least the southern portion of the range in BC and the US, bull trout are threatened in many areas by a combination of overexploitation, habitat degradation, and interactions with exotic species. To try to understand important gaps in the basic biology and evolutionary history of bull trout, there has been a great deal of recent research conducted on the species. One aspect of this increased activity has been intensive genetic surveys of population subdivision to try to understand the level of genetic and demographic independence among populations and the critical habitat features that influence this structure. For instance, Costello et al. (2003) surveyed variation at seven microsatellite DNA loci in bull trout from 37 samples in the Pine (Peace River) and upper Kootenay River systems of BC. These authors found substantial differentiation among populations and documented the importance of instream migration barriers (waterfalls, cascades) in structuring genetic differentiation among populations. While the authors found evidence of migration of bull trout between some large and interconnected habitats (e.g., mainstem tributaries to the upper Kootenay River), other tributaries showed strong genetic and demographic isolation from one another. By contrast, there has been no published work on the level of genetic subdivision or migration among populations for the coastal group of bull trout. Coastal bull trout are of particular interest from the standpoint of exchange among populations because they are reported to exhibit amphidromous behaviour (i.e., making short forays to sea; Cavender 1978; Kraemer 2003), a behaviour recently confirmed in part of their range (Brenkman and Corbett 2005). Salmonid fishes that make migrations to sea are typically less genetically subdivided than those that are restricted to fresh water (Ward et al. 1994). Consequently, for more informed management of bull trout in coastal regions of western North America, it would be prudent to collect genetic data to assess population subdivision for coastal populations rather than rely on inferences based on similar data for interior populations.

Information on population structure would be important for biodiversity management of coastal bull trout for two other reasons as well. First, a locally important recreational fishery for bull trout occurs in the lower Fraser River (especially between New Westminster and Vancouver). Bull trout

Table 1. Sample localites, watersheds, collection year, and sample sizes of all bull trout (*Salvelinus confluentus*) collected for microsatellite analysis.

Population	Watershed	Year collected	Sample size
Gitnadoix River	Skeena River	2003	22
Homathko River	Central coast	2002–2003	30
Klinaklini River	Central coast	1998–2004	10
Toba River	Central coast	1999	10
Southgate River	Central coast	1999	25
Squamish River	South coast	1999–2004	20
Lillooet River[a]	Lower Fraser River	2001	20
Birkenhead Lake	Lillooet River	2000	20
Phelix Creek	Birkenhead Lake	2001	30
Ryan River	Lillooet River	2001	15
Ure Creek	Lillooet Lake	2001	16
Chehalis Lake	Lower Fraser River	1997	15
Chilliwack Lake	Lower Fraser River	1998–2000	15
Pitt River	Lower Fraser River	2000	29
Lower Fraser River[b]	Fraser River	2000–2003	30
Elwha River	Olympic Peninsula (north)	2000	10
Queets River	Olympic Peninsula (west)	1998–1999	10
Sauk River	Puget Sound (Skagit River)	1998–2004	31
Skokomish River	Puget Sound (south)	1998–1999	5
Soleduck River[c]	Olympic Peninsula (west)	1998	10

[a]Mainstem.
[b]Lower 20 km of Fraser River between New Westminster and Richmond, B.C.
[c]Dolly Varden, *Salvelinus malma*.

are directly targeted or are caught incidentally in fisheries for salmon. Several tributaries to the lower Fraser River or adjacent areas contain large populations of bull trout (e.g., Chilliwack–Vedder system, Pitt Lake–River system, Squamish River) and, given the sea-going behaviour of bull trout, could contribute fish to this fishery. Understanding which systems contribute to the lower Fraser River fishery would help to prioritize populations and their habitats for protection. In addition, several of these tributaries have locally important recreational fisheries of their own (e.g., Chilliwack Lake, Squamish River, Pitt Lake, and upper Pitt River), and knowledge of the differentiation among populations could contribute to understanding their uniqueness.

Second, bull trout are found in coastal areas in BC from Vancouver north to streams on the north coast (e.g., lower Skeena River, Taku River). It is, however, unknown to what extent these northern, coastally distributed populations represent fish derived from coastal lineage or from descendants that colonized coastal areas from the interior (e.g., by headwater transfers). The Coast Mountains is an area where the headwaters of interior draining and coastal draining river systems interdigitate and where faunal exchanges are strongly suspected to have occurred, including those involving char (*Salvelinus* spp.) (McPhail and Lindsey 1986; Baxter et al. 1997). It appears that up to at least the Squamish River, coastally distributed bull trout are indeed part of the coastal evolutionary lineage (e.g., Taylor et al. 1999), but the status of populations further north is unknown.

In this study, we used polymerase chain reaction (PCR)-based analysis of microsatellite DNA loci in coastal bull trout to determine (*i*) the level of genetic distinctiveness among populations of coastally distributed bull trout, particularly relative to similar data collected for bull trout from the interior of BC, (*ii*) the origins of north coastal populations of bull trout (i.e., do they represent sea-based dispersal from the coastal lineage or headwater transfers via fresh water from interior areas?), and (*iii*) the relative contributions of tributary populations of bull trout angled in the lower Fraser River recreational fishery. Answers to these questions are important to understand the evolutionary history and origin of coastal populations because genetic differentiation is typically associated with demographic independence among populations. In nature, low migration between populations is typically associated with high levels of molecular genetic differentiation (reviewed by Bohonak 1999). Genetic analysis of coastal populations can, therefore, contribute to conservation of bull trout biodiversity both at deeper evolutionary and contemporary time scales.

Materials and methods

Sample watersheds

Bull trout tissue samples consisted of fin clips stored in 95% ethanol and were collected from 19 localities ranging from Puget Sound and the Olympic Peninsula north to the lower Skeena River (Table 1, Fig. 1). Samples were collected by electrofishing, fish fences, and by angling using a variety of methods between 1997 and 2004. Samples from the lower Fraser River were collected by recreational anglers from areas downstream of New Wesminster and to Richmond, BC, between 2001 and 2004.

Microsatellite analysis

After screening many microsatellite loci, seven were chosen for inclusion based on clarity of resolution and degree of polymorphism: *Omy*77, *Sfo*18, *Ssa*197, *Sco*19, *Sco*23,

*Ssa*456, and *Ssa*311 (see Costello et al. 2003 for details). PCRs were carried out with ^{32}P-labelled primers in 10 μL volumes of 10 mmol·L^{-1} Tris–HCl (pH 8.3), 1.5 mmol·L^{-1} $MgCl_2$, 0.8 mmol·L^{-1} dNTP, and 0.1 units of *Taq* polymerase in MJ PTC 100 and 200 thermocyclers using cycling parameters outlined in Costello et al. (2003). PCR products were electrophoresed through 6% Long Ranger™ polyacrylamide gels and visualized on Kodak Biomax™ MS film. Alleles were scored by eye with reference to standardized individuals run on every gel and to an M13 sequencing ladder.

Dolly Varden (*Salvelinus malma*) are found sympatrically with many of the coastal bull trout populations sampled. Because the two species are similar morphologically and easily confused and because they often hybridize in sympatry (Redenbach and Taylor 2002, 2003), we wanted to eliminate any possible occurrences of Dolly Varden in our samples. From suspected areas of sympatry, samples were screened for variation at two nuclear loci that can identify bull trout, Dolly Varden, and their hybrids, as described by Redenbach and Taylor (2002, 2003). In addition, a sample of Dolly Varden from the Soleduck River in Washington State was screened for the same set of seven microsatellite loci. We then used the known Dolly Varden as well as the extensive data set of Costello et al. (2003) and applied multilocus assignment tests to classify all samples as either Dolly Varden or bull trout. We calculated a hybrid index to quantify the level of genetic identity of each sample using the following equation:

$$I_H = 1 - \{\log (p_X)/[(\log(p_X) + \log(p_Y)]\}$$

where p_X denotes the likelihood of being a Dolly Varden and p_Y is the likelihood of being a bull trout (Hansen et al. 2001). The hybrid index ranges from 0 to 1, and in our case, as values approach 0 this denotes increasing similarity to Dolly Varden; as they approach 1 they denote increasing similarity to bull trout. The program GENECLASS (Version 2.0, Piry et al. 2004) was used to calculate the likelihood values for identity as Dolly Varden or bull trout. Hybridization between Dolly Varden and bull trout, however, is a natural phenomenon in the study area (Baxter et al. 1997; Redenbach and Taylor 2002, 2003) and contributes to genetic diversity in char. Consequently, we only eliminated individuals that had a hybrid score of 0.50 or lower (i.e., those fish whose genome consists of <50% bull trout). This procedure was not intended as a rigorous analysis of hybrid zone structure, but simply to eliminate obvious Dolly Varden or advanced generation backcrosses with Dolly Varden from the subsequent analyses.

Genetic data analysis

The following tests were performed using GENEPOP (Version 3.3, Raymond and Rousset 2001) for all collections with sample sizes of at least 15. Tests for deviations from Hardy–Weinberg equilibrium were performed for each locus–population combination using an exact test in which p values were estimated using a Markov chain method. Tests for genotypic linkage disequilibrium for all combinations of locus pairs within a population were also made using a Markov chain method with GENEPOP default values. Tests for population differentiation between all possible pairs of populations were performed for each locus and over all loci combined using log-likelihood (G)-based exact tests with default values. Basic descriptive statistics of sample size (N), number of alleles (N_a), allelic richness (N_r), and observed (H_o) and expected (H_e) heterozygosity were compiled using FSTAT (version 2.9, Goudet 2001). F_{ST} values were estimated using θ (Weir and Cockerham 1984) and calculated in ARLEQUIN (version 2.0, Schneider et al. 2000) with significance based on a permutation process.

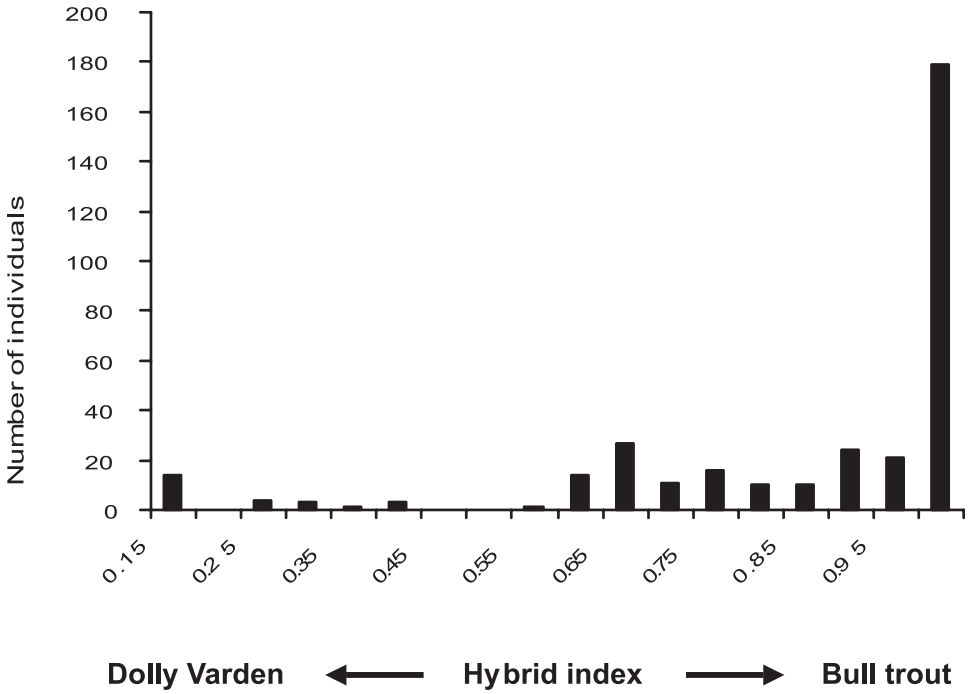
We also applied the analysis variance approach of Excoffier et al. (1992) to partition microsatellite variation into its various components (among populations within coastal and interior regions versus between coastal and interior regions) using ARLEQUIN. Where appropriate, all statistical tests were conducted at table-wide significance levels of $\alpha = 0.05$ using the sequential Bonferroni adjustment (Rice 1989). Overall genetic differentiation among populations was summarized by inferring neighbour-joining (Saitou and Nei 1987) and unweighted pair-group method with arithmetic averages (UPGMA, Sneath and Sokal 1973) trees based on pairwise measures of Cavalli-Sforza and Edwards (1967) genetic distances. Both tree reconstruction methods produced the same general topology, and we present overall similarities among populations using the UPGMA method for visual clarity. In general, we based our inferences on allele frequency variation among populations assuming no mutation-based differentiation, because estimates based on the former appear to be more appropriate for recently diverged populations (e.g., Goldstein et al. 1995; Gaggiotti et al. 1999).

To determine the relative contributions of populations that contribute fish to those caught by angling in the lower Fraser River sports fishery, we used the Bayesian approach to mixed-stock analysis as implemented in the program GMA (genetic mixture analysis, Kalinowski 2003). GMA uses the method of Rannala and Mountain (1997) to estimate the probability of observing a genotype in a population. After the probability of observing each fish has been calculated using this method, maximum likelihood estimates of the mixture proportions can be made following standard methods (e.g., Millar 1987). Essentially, the contribution estimations for each population in the baseline are calculated as those that maximize the probability of obtaining the observed mixture sample of genotypes. The GMA analysis differs from other mixture algorithms (e.g., Debevec et al. 2000) in terms of calculating the probability of sampling alleles from the baseline population. GMA uses a Bayesian esimator and is better at accomodating situations where alleles found in the mixture sample are not in the baseline sample (Winans et al. 2004).

We included simulation analyses as well as a method to assess the robustness of mixture estimates. Replicate mixtures and baselines are simulated from the actual baseline by drawing individuals randomly from the baseline file. We simulated mixtures of 50 individuals (i.e., multilocus genotypes) by random sampling with replacement and estimated mixture proportions for 10 000 replicate analyses. The variability about the mixture proportions across all replicates gives an indication of how well baseline data can estimate mixture proportions (Kalinowski 2003).

We also used maximum likelihood-based assignment procedures. These analyses make use of multilocus genetic profiles of individual fish to assign them to a priori groups (i.e.,

Fig. 2. Distribution of hybrid indices based on variation at seven microsatellite loci for all char (*Salvelinus* spp.) collected in the study. A score of 0 indicates Dolly Varden (*Salvelinus malma*) and a score of 1.0 indicates bull trout (*Salvelinus confluentus*). First-generation hybrids between the species would have scores of 0.5.



potential sample populations of origin) that had been characterized at the same loci (reviewed by Hansen et al. 2001) using GENECLASS. Individuals were assigned to the population in which they had the highest log-likelihood of occurring. We also used GENECLASS to test more explicitly for migration between populations by calculating the likelihoods of individuals being first-generation migrants. Using variation across the seven loci, the likelihood score was calculated as L_h, where L_h is the likelihood of drawing a particular individual's genotype from the population residing in the locality from which it was sampled given the observed set of allele frequencies (Paetkau et al. 2004). We chose to calculate L_h because we probably did not sample all potential source populations (Paetkau et al. 2004). Individuals were assigned to the population with the highest log-likelihood score, and potential immigrants were identified as individuals assigned to a population other than the one from which they were sampled. To test the null hypothesis that an individual was actually born in the population from which it was sampled, we used the genotype resampling procedure recommended by Paetkau et al. (2004). An individual fish was rejected as a member of the population from which it was sampled if the observed likelihood of population membership was represented by 1% or less of the simulated, randomly generated values for the population in question. In all assignment analyses, samples from the Elwha and Queets rivers and from the Sauk and Skokomish rivers were pooled into an Olympic Peninsula group and a Puget Sound group, respectively, owing to limited sample sizes for each component population (i.e., most *N*s were ≤10).

Mixed stock analysis and assignment of individuals are distinct in that the former estimates mixture contributions based on the genotypes of individuals in proportion to the probability that each genotype originates from each of the possible source populations without regard to the probable origin of individual genotypes per se. By contrast, the assignment procedure classifies each individual to a single population only and is more informative in terms of potential movement of individual fish among localities.

Results

Presence of Dolly Varden in bull trout samples

After screening all samples at the microsatellite loci, we identified a total of 26 fish that had hybrid indices of 0.5 or lower (Fig. 2). Nine of these animals were from the Gitnadoix River system, two from the Homathko River, 13 from the Southgate River, and two from the Toba River. Analysis of these data using factorial correspondence analysis clearly separated the known Dolly Varden from bull trout, but several individual char were clearly intermediate between Dolly Varden and the majority of bull trout (data not shown). All of these intermediate individuals represented the hybrid and introgressed individuals from the Toba, Southgate, Homathko, and Gitnatdoix rivers identified previously using the hybrid indices; these intermediates also contained one or more Dolly Varden alleles at two single copy nuclear DNA loci (E.B. Taylor, unpublished data). These char of hybrid origin were removed from further analysis of bull trout microsatellite variation.

Microsatellite polymorphism within populations

Across all 383 char, all but one of the loci (*Ssa*311) were polymorphic, with the number of alleles observed ranging from two at *Ssa*197 and *Sco*23 to a high of 11 at *Sco*19. Expected heterozygosity ranged from 0.0 at *Ssa*311 to 0.327 at *Omy*77 and averaged 0.167. Within populations, the mean number of alleles averaged across loci ranged from 1.4 in

Table 2. Summary of variation at microsatellite loci within each of the sample populations.

Population	*Omy*77	*Sfo*18	*Ssa*197	*Sco*19	*Sco*23	*Ssa*456	*Ssa*311
Gitnadoix River							
H_e	0.646	0.621	0.507	0.542	0.426	0.631	0.000
H_o	0.636	0.227	0.182	0.591	0.046	0.227	0.000
N_a	3	2	2	3	1	2	1
N_r	2.99	1.99	1.99	2.53	1	1.99	1
Homathko River							
H_e	0.364	0.377	0.127	0.461	0.459	0.474	0.000
H_o	0.400	0.333	0.067	0.267	0.400	0.400	0.000
N_a	2	2	1	4	2	2	1
N_r	1.99	1.98	1	2.76	1.99	1.99	1
Klinaklini River							
H_e	0.479	0.000	0.000	0.563	0.000	0.337	0.000
H_o	0.500	0.000	0.000	0.700	0.000	0.400	0.000
N_a	2	1	1	3	1	2	1
N_r	2	1	1	2.80	1	2	1
Toba River							
H_e	0.337	0.626	0.505	0.100	0.336	0.484	0.000
H_o	0.400	0.300	0.400	0.100	0.000	0.200	0.000
N_a	2	2	2	2	1	2	1
N_r	2.0	2.0	2.0	2.0	1.0	2.0	1.0
Southgate River							
H_e	0.381	0.606	0.507	0.561	0.607	0.620	0.000
H_o	0.320	0.280	0.040	0.120	0.120	0.120	0.000
N_a	3	2	2	2	2	2	1
N_r	2.77	2.0	1.67	1.97	2.0	2.0	1.0
Squamish River							
H_e	0.053	0.199	0.053	0.542	0.000	0.000	0.000
H_o	0.053	.105	0.053	0.526	0.000	0.000	0.000
N_a	2	3	3	5	1	1	1
N_r	1.42	2.34	1.42	3.51	1.0	1.0	1.0
Birkenhead River							
H_e	0.435	0.186	0.000	0.186	0.000	0.287	0.000
H_o	0.467	0.067	0.000	0.200	0.000	0.333	0.000
N_a	2	2	1	2	1	2	1
N_r	2.0	1.91	1.0	1.91	1.0	1.97	1.0
Chehalis River							
H_e	0.291	0.000	0.000	0.273	0.000	0.053	0.000
H_o	0.333	0.000	0.000	0.316	0.000	0.053	0.000
N_a	2	1	1	2	1	2	1
N_r	2.0	1.0	1.0	1.97	1.0	1.42	1.0
Chilliwack River							
H_e	0.453	0.000	0.000	0.441	0.000	0.497	0.000
H_o	0.267	0.000	0.267	0.316	0.000	0.267	0.000
N_a	3	1	1	3	1	3	1
N_r	2.53	1.0	1.0	2.93	1.0	2.79	1.0
Lillooet River							
H_e	0.296	0.262	0.000	0.097	0.000	0.309	0.000
H_o	0.350	0.300	0.000	0.100	0.000	0.050	0.000
N_a	2	2	1	2	1	3	1
N_r	1.98	1.97	1.0	1.65	1.0	2.58	1.0
Ryan River							
H_e	0.067	0.129	0.000	0.186	0.000	0.000	0.000
H_o	0.067	0.133	0.000	0.200	0.000	0.000	0.000
N_a	2	2	1	2	1	1	1
N_r	1.53	1.79	1.0	1.91	1.0	1.0	1.0
Phelix Creek							
H_e	0.259	0.065	0.000	0.000	0.000	0.033	0.000
H_o	0.167	0.067	0.000	0.000	0.000	0.033	0.000
N_a	2	2	1	1	1	2	1
N_r	1.95	1.47	1.0	1.0	1.0	1.27	1.0

Table 2 (*concluded*).

Population	*Omy*77	*Sfo*18	*Ssa*197	*Sco*19	*Sco*23	*Ssa*456	*Ssa*311
Ure Creek							
H_e	0.417	0.466	0.000	0.387	0.000	0.063	0.000
H_o	0.313	0.438	0.000	0.375	0.000	0.063	0.000
N_a	2	2	1	2	1	2	1
N_r	2.0	2.0	1.0	1.99	1.0	1.5	1.0
Upper Pitt River							
H_e	0.192	0.000	0.000	0.101	0.000	0.448	0.000
H_o	0.207	0.000	0.000	0.103	0.000	0.517	0.000
N_a	3	1	1	3	1	2	1
N_r	2.09	1.0	1.0	1.76	1.0	2.0	1.0
Lower Fraser River							
H_e	0.127	0.000	0.000	0.161	0.000	0.427	0.000
H_o	0.133	0.000	0.000	0.100	0.000	0.400	0.000
N_a	2	1	1	5	1	2	1
N_r	1.72	1.0	1.0	2.27	1.0	1.99	1.0
Olympic Peninsula – Puget Sound[a]							
H_e	0.220	0.000	0.000	0.030	0.000	0.451	0.000
H_o	0.181	0.000	0.000	0.030	0.000	0.060	0.000
N_a	2	1	1	5	1	2	1
N_r	1.72	1.0	1.0	2.27	1.0	1.99	1.0
Soleduck River[b]							
H_e	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H_o	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N_a	1	1	1	1	1	1	1
N_r	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Coastal bull trout (overall)							
H_e	0.327	0.181	0.056	0.260	0.052	0.274	0.00
H_o	0.313	0.164	0.052	0.250	0.039	0.207	0.00
N_a	2.56	1.75	1.31	3.25	1.19	2.13	1.0
N_r	2.12	1.60	1.21	2.22	1.19	1.85	1.0
Interior bull trout							
H_e	0.248	0.229	0.276	0.324	0.207	0.098	0.158
H_o	0.255	0.243	0.300	0.313	0.226	0.103	0.150
N_a	1.74	1.71	1.84	3.89	1.63	1.45	1.42
N_r	1.62	1.64	1.81	2.69	1.55	1.35	1.39

Note: H_e, expected heterozygosity; H_o, observed heterozygosity; N_a, number of alleles; N_r, allele richness. The values for Interior bull trout represent mean values across 37 populations (see Costello et al. 2003).

[a]Consists of a pooled sample of fish from the Sauk (Skagit River drainage), Skokomish, Elwha, and Queets rivers.

[b]Dolly Varden, *Salvelinus malma*.

the Chehalis and Ryan rivers and Phelix Creek to a high of 2.0 in the Squamish and Southgate rivers (Table 2). Allele richness varied from an average of 1.2 per locus in Phelix Creek to a high of 1.93 in the Gitnadoix River system and 4.4 across all loci and populations. Similarly, gene diversity (the probability that any two randomly chosen alleles would be different averaged across all loci, Nei 1987) ranged from a low of 0.05 in Phelix Creek and the Ryan River to a high of 0.32 in the Gitnadoix River.

Across 52 tests of deviations from Hardy–Weinberg equilibrium, two tests were significant. Both tests involved *Ssa*456: one in the Lillooet River sample and the other in the Olympic Peninsula sample (both $P < 0.0001$). Tests for linkage disequilibrium between loci within each population showed that none were significant ($P > 0.05$).

Microsatellite differentiation among populations

We summarized genetic differentiation among populations as F_{ST} (θ) or the proportion of the total variation in microsatellite allele frequencies that is attributable to differences among populations. For the samples of bull trout in this study, θ was substantial at 0.46 and was significantly greater than 0 ($P < 0.0001$). For specific pairwise comparsions, however, θ varied widely from a low of 0.0 (e.g., between Homathko and Southgate rivers) to a high of 0.78 between Phelix Creek and the Klinaklini River (Table 3). Some small-scale differentiation was observed. For instance, bull trout sampled from a spawning tributary of Birkenhead Lake (Phelix Creek) were significantly distinct ($\theta = 0.07$, $P < 0.005$) from fish sampled from the lake proper (Table 3). Similarly, bull trout from Chehalis Lake were significantly distinct from bull trout originating from the nearby Pitt River watershed ($\theta = 0.45$, Table 3). By contrast, fish from the Pitt River were not distinct from fish sampled from Chilliwack Lake ($\theta = 0.07$, Table 3).

We arranged all populations into two groups: those found in, and tributary to, the lower Fraser River ($N = 9$ populations) and those draining by independent means to the coast ($N = 8$). When this hierarchy was analyzed by analysis of molecular variance (AMOVA), 13.4% ($P = 0.023$) of the

variation was attributable to differences between these groups, 36.9% ($P < 0.001$) to differences among populations within these groups, and the remaining 49.7% ($P < 0.001$) was due to differences among individuals within populations. Upon inspection of allele frequencies, however, it became evident that certain more northerly distributed coastal populations (i.e., north of the Squamish River) contained alleles not found in more southern populations, but present in interior populations studied by Costello et al. (2003). For instance, the *Sfo*18*150 allele is common in the interior of BC and adjacent portions of the US interior and is present in more northern, coastal populations, such as the Gitnadoix, Klinaklini, Southgate, Toba, and Homathko rivers (Fig. 3*a*). This allele, however, is very rare in populations from the Squamish River southward. Similarly, the *Omy*77*283 allele is absent from coastal rivers from the Squamish River southwards, but reaches a mean frequency of 0.14 in the middle–north coast rivers and 0.78 in interior bull trout (Fig. 3*b*; see supplementary data Table S1 for allele frequency data).[2] Consequently, we grouped these middle–north coastal and interior populations into an "interior" group and all others into a "coastal" group. Analysis of this hierarchy indicated that 46.1% of the variation was attributable to between group differences, only 15.9% of the variation was attributable to differences among populations within these two groups, and 37.9% was due to differences among individuals within populations (all $P < 0.001$). We next combined our data with data on 37 populations from the interior upper Kootenay River and Pine River drainages examined by Costello et al. (2003). Pooling all the current samples into our coastal group to compare against the 37 interior populations resulted in 39.6% of the variation being due to differences between coastal and interior groups, 24.2% to differences among populations within these groups, and 36.2% to differences among individuals within populations (all $P < 0.001$). We also placed the Gitnadoix to Toba rivers' north coastal samples into the Pine – upper Kootenay interior group. Under this hierarchy, the coastal versus interior comparison resulted in an increase to 46.1% of the variation between these groups, 21.3% was due to differences among populations with coastal and interior groups, and 32.7% was due to differences among individuals within populations (all $P < 0.001$). By contrast, when samples were placed into northern and southern population groups (i.e., north and south of the latitudinal midpoint of all samples), only 3.1% of the total variation was due to differences between these groups, which was not significant ($P = 0.06$). By contrast, 48.6% and 48.2% of the variation was attributable to differences among populations within northern and southern groups and within populations, respectively (both $P < 0.001$). In summary, differentiation between groups of bull trout was maximized when coastally distributed bull trout from north of the Squamish River were included in an interior genetic grouping and not in a genetic grouping consisting of coastal populations from the Squamish River, the lower Fraser River and its tributaries, and the Olympic Peninsula – Puget Sound. Population subdivision among just the coastal genetic group of bull trout (e.g., not including samples from the Gitnadoix to Toba rivers) was estimated as $\theta = 0.33$ ($P < 0.001$). The affinity of northern coastal populations with interior bull trout was also resolved with high levels of bootstrap support in a clustering of populations based on Cavalli-Sforza and Edwards' (1967) measure of genetic distance among populations (Fig. 4).

Assignment of individual bull trout and mixture analysis

The "learning" samples of bull trout (i.e., fish collected from known localities and that represented potential source populations for fish angled from the lower Fraser River) consisted of 353 fish from 16 localities. Self-assignment of these fish resulted in low overall assignment success; only 53.4% of these learning sample fish were correctly assigned to their collection localities. Success, however, varied considerably among populations. For instance, assignment success was as high as 83%, 92%, and 95% in Phelix Creek, Gitnadoix River, and Chehalis Lake, respectively. By contrast, only 12% of the Birkenhead Lake fish were correctly self-assigned (although that increased to 60% if its inlet, Phelix Creek, was included as a potential source population), and assignment success was only 52% for the Squamish River (see supplementary data Table S2).[2] In most cases, low assignment success was attributable to misassignment to nearby systems. For instance, the upper mainstem Lillooet River had only 35% assignment success, but most misassignments of these fluvial flish were to smaller tributaries of this river (e.g., Ryan River and Ure Creek accounted for 70% of the misassignments). In addition, central coast rivers (Southgate, Toba, Klinaklini rivers) accounted for 85% of the 13 Homathko River central coast bull trout that were not self-assigned.

Our analyses identified 11 putative first or early generation migrants between sample populations. Each of these fish had less than a 1% probabilility of originating from the locality from which it was sampled, and most were inferred to be members of populations that were geographically most proximate to the sample locality (Table 4). For instance, within the Lillooet River drainage, five of the six inferred immigrants came from one or more component rivers within the drainage (Ryan River, Ure Creek, Chehalis River, mainstem Lillooet River; Table 4). Similarly, among the four central coast populations (Homathko, Klinakini, Toba, and Southgate rivers), three of five inferred immigrants were assigned to one or more of the other central coast populations (Table 4). By contrast, a few fish were inferred to have originated as dispersers, or their recent descendants, from more distant localities (Table 4).

The 30 bull trout angled from the lower Fraser River were individually assigned to 6 of the 16 putative source localities. Most fish (13) were assigned to the Ryan River (upper Lillooet River system), while the second most were assigned to the Pitt River system (7). Only 1 of the remaining 10 fish was assigned to a tributary (the Squamish River) that did not drain to the lower Fraser River. Including exclusion probabilities in the assignment tests, however, indicated that very

[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5021. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

Table 3. Matrix of pairwise F_{ST} (θ) values between populations.

	Brkh	Chel	Chwk	LFr	Lill	Phel	Pitt	Ryan	Ure	Opps	Squam	Gitn	Homa	Klin	Shgt
Chel	0.50012														
Chwk	0.22017	0.37907													
LFr	0.32942	0.48280	0.07147												
Lill	0.26918	0.50375	0.08771	0.06361											
Phel	0.06886	0.71081	0.47849	0.56291	0.51155										
Pitt	0.30867	0.45834	0.07089	–0.01281	0.06766	0.54753									
Ryan	0.37815	0.65079	0.15510	0.12313	0.05149	0.64650	0.14314								
Ure	0.16254	0.52913	0.14570	0.21648	0.09788	0.40174	0.21559	0.13266							
Opps	0.34473	0.46772	0.08703	0.00205	0.07585	0.56986	–0.00558	0.16352	0.24733						
Squam	0.35518	0.60069	0.13432	0.17284	0.10918	0.59383	0.19081	0.05324	0.10454	0.21585					
Gitn	0.48800	0.59923	0.45863	0.58192	0.51662	0.65338	0.57645	0.56335	0.45385	0.59550	0.52439				
Homa	0.40025	0.58978	0.48603	0.57288	0.49696	0.54121	0.56362	0.55750	0.38428	0.58278	0.54483	0.31899			
Klin	0.62549	0.74666	0.65256	0.76150	0.69242	0.78554	0.75463	0.77951	0.58766	0.76748	0.72581	0.28038	0.13251		
Shgt	0.35517	0.54508	0.42513	0.55122	0.46215	0.56210	0.53756	0.55318	0.34229	0.56078	0.52560	0.26879	–0.00429	0.16900	
Toba	0.59199	0.72770	0.59977	0.73016	0.67116	0.76682	0.72432	0.75007	0.57549	0.74182	0.67378	0.28829	0.37027	0.44596	0.34652

Note: Only those comparisons where populations had sample sizes of at least 15 were included. Underlined values are not significantly greater than 0 (at a table-wide $P > 0.05$). θ represents the proportion of the total variance in allele frequencies that is a result of differences between populations (relative to variability within individuals and populations). Brkh, Birkenhead Lake; Chel, Chehalis Lake; Chwk, Chilliwack Lake; LFr, lower Fraser River; Lill, Lillooet River; Phel, Phelix Creek; Pitt, upper Pitt River; Ryan, Ryan River; Ure, Ure Creek; Opps, Olympic Peninsula and Puget Sound; Squam, Squamish River; Gitn, Gitnadoix River; Homa, Homathko River; Klin, Klinaklini River; Shgt, Southgate River; Toba, Toba River.

few of the putative source localities could be excluded as sources of individual bull trout at a type I error rate of 5% (i.e., an error would be made 5% of the time by excluding a given population; Fig. 5). All of the lower Fraser River fish, however, were excluded as originating from the Gitnadoix, Homathko, Toba, and Klinaklini rivers and Phelix Creek with at least 90% confidence. Exclusion analysis indicated that the Chilliwack, Pitt, and Lillooet rivers had the highest average probability of assignment followed closely by the Chehalis and Squamish rivers and the pooled Olympic Peninsula and Puget Sound samples (Fig. 5).

The mixture analysis was broadly consistent with the individual assignment analysis and suggested that three populations contributed to the mixture. The Pitt River was estimated to contribute the most (90.1%) of the angled bull trout to the mixture followed by two other lower Fraser River tributaries: the Chilliwack River (5.3%) and Ryan River (upper Lillooet River, 4.6%). Simulation procedures indicated that the predominant contribution of Pitt River bull trout to the lower Fraser River mixture was stable across replicates (Fig. 6). There was, however, some uncertainty concerning the ability of our data to accurately resolve the relative contribution of other populations, particularly those outside the lower Fraser River. When populations were pooled into "Fraser River, "South Coast" (Squamish River, Olympic Peninsula, and Puget Sound), and "Central Coast" (all coastal rivers north of the Squamish River), the mean (± standard deviation) mixture proportions across 10 000 simulated mixtures were 0.86 (0.14), 0.14 (0.13), and 0.0 (0.0), respectively. This suggests that most of the uncertainy in assigning mixture proportions centres on the relative contributions of non-Fraser River south coast populations.

Discussion

Presence and hybridization with Dolly Varden

Previous genetic analyses have identified sympatric populations of bull trout and Dolly Varden in several areas along the Coast–Cascade mountain crest (e.g., Baxter et al. 1997; Leary and Allendorf 1997; Redenbach and Taylor 2002). In most of these areas, hybrids between the species have also been recorded (e.g., Redenbach and Taylor 2002, 2003). Our current analysis corroborates these findings and extends them to yet another watershed (the Gitnadoix River) using independent genetic loci and points to the need to screen populations of "bull trout" for possible inclusion of Dolly Varden or alleles from Dolly Varden in studies involving coastal char. It is interesting to note that hybridization between the species in central and north coast populations appears to be higher than in areas of sympatry on the south coast. For instance, despite being at least broadly sympatric in the Squamish River (E.B. Taylor, unpublished data) and in several areas on the Olympic Peninsula (e.g., Leary and Allendorf 1997) and Puget Sound (McPhail and Taylor 1995), hybridization has only been detected in the Skagit River. A possible explanation for this apparent regional difference in the extent of hybridization is that south coastal bull trout and Dolly Varden may have had a longer period of co-evolutionary history than those in the central and north coast. The latter may have only come into contact postglacially during headwater exchanges (Baxter et al.

1997). Longer periods of co-evolutionary history may have strengthened reproductive isolation between species through reinforcement and resulted in lower hybridization on the south coast. A similar situation has been proposed to explain the general trend for lower hybridization between naturally sympatric populations of coastal cutthroat trout (*Oncorhynchus clarkii clarkii*) and rainbow–steelhead trout (*Oncorhynchus mykiss*) than between westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and introduced rainbow trout in southeastern BC (reviewed in Taylor 2004*b*). The combined use of nonlethal, microsatellite-based assays and hybrid indices or assignment tests provides a convenient method to study hybridization between sympatric salmonid species.

Variation within coastal bull trout samples

Consistent with earlier results for interior bull trout (Costello et al. 2003), bull trout collected from a range of localities in coastal BC exhibited relatively low amounts of microsatellite variation within populations. The average (across loci and populations) number of alleles of 1.7 (range 1.4–2.1) was very similar to that reported for interior bull trout (1.9). Similarly, average expected heterozygosity was relatively low (0.35), but higher than that for interior bull trout (0.24). Bull trout both from coastal and interior areas, therefore, display considerably lower within-population variation than has been recorded for a number of other freshwater salmonds (means of 8.0 alleles per locus and heterozygosity of 0.62; see Costello et al. 2003). The relatively low within-population variation in bull trout at microsatellite loci is consistent with earlier results based on allozymes (e.g., Leary et al. 1993), mitochondrial DNA (Taylor et al. 1999), and microsatellites in populations from the US (e.g., Spruell et al. 1999). Costello et al. (2003) argued that the relatively low variation in bull trout resulted from a combination of historical (e.g., propagule-type recolonization from glacial refugia and founder events) and possibly contemporary demographic factors (e.g., small sizes of many spawning populations; Baxter and McPhail 1996).

By contrast, higher levels of microsatellite DNA variation have been reported for tetranucleotide loci, at least for populations in the southern portion of the range of *S. confluentus* (W. Ardren, US Fish and Wildlife Service, Abernathy Fish Technology Center, Longview, WA 98632, USA, personal communication). In addition, the apparently relatively low microsatellite DNA variation within populations does not necessarily imply they are genetically depauperate in genetically based morphological, behavioural, or life-history traits (cf. Pfrender et al. 2000), but this does have implications for individual classification in mixed-population analyses (see below).

Variation among samples

Nothwithstanding relatively low variation within populations, genetic differentiation among populations of coastal bull trout was substantial, and in fact, the θ value observed (0.46) exceeded that (0.39) recorded for bull trout from the interior-draining Pine and upper Kootenay rivers (Costello et al. 2003). The higher level of subdivision observed in the current study probably results from the inclusion of both coastal and interior genetic lineages of bull trout in our analysis. When only members of the coastal lineage were examined, θ dropped to 0.33. These data indicate that coastally distributed populations of bull trout are highly distinct at microsatellite loci. Such high levels of genetic distinction probably also stem from our sampling of watersheds that are highly distinct spatially. For instance, the upper Lillooet River system is separated from the upper Squamish River by about 200 km of freshwater and near-shore coastal habitat. By contrast, geographically more proximate populations (e.g., the upper Pitt and Chilliwack rivers; Southgate and Homathko rivers) showed no major genetic divergence at the loci examined. Interior bull trout showed a strong pattern of isolation-by-distance that probably also applies for coastal bull trout (i.e., dispersal among populations is constrained by geographic distance and perhaps by intrinsic barriers; for example, behavioural or physiological avoidance of marine waters by some populations). Within-system differentiation such as that observed in the Birkenhead Lake – Phelix Creek

Fig. 3. Mean (+ standard deviation) frequency of the (*a*) *Sfo*18*150 allele and (*b*) the *Omy*77*283 allele in bull trout (*Salvelinus confluentus*) collected from south coast (N = 14), north coast (N = 5), and interior (N = 37) localities.

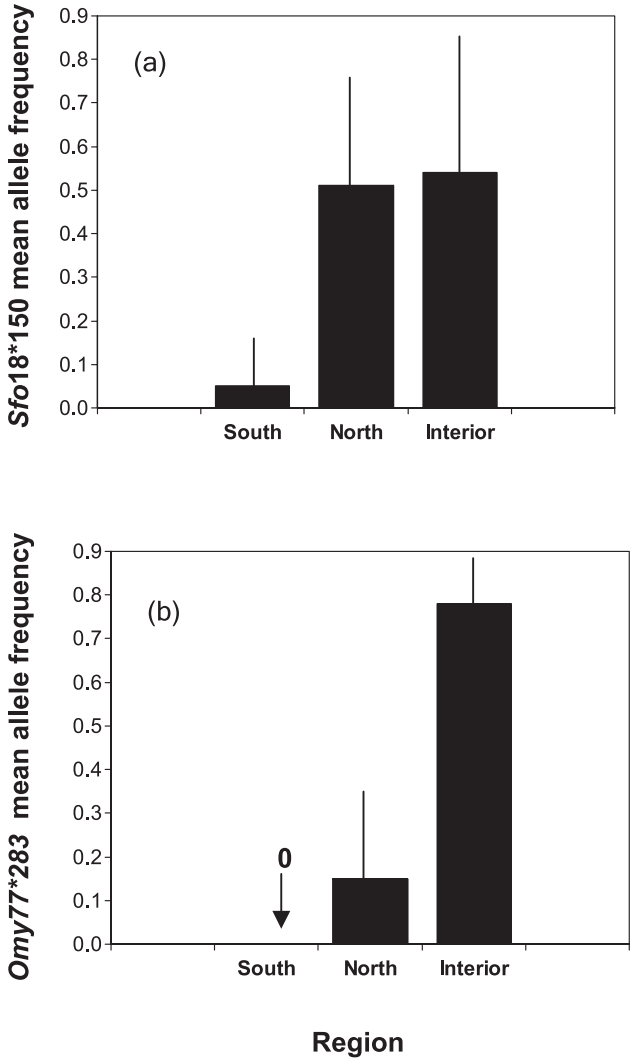
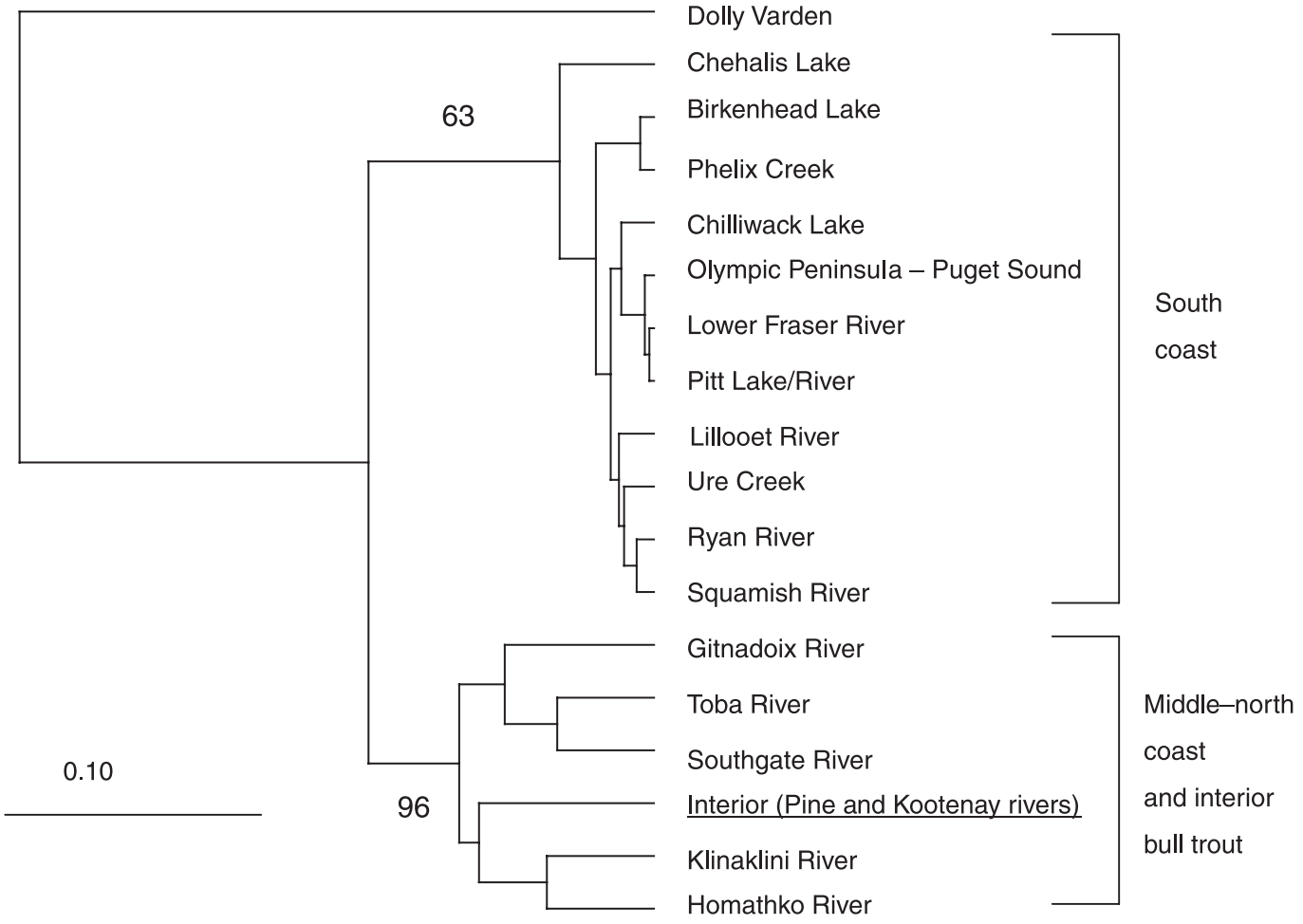


Fig. 4. Dendrogram of genetic similarily among samples of bull trout (*Salvelinus confluentus*) estimated from variation across seven microsatellite loci and clustering of Cavelli-Sforza and Edwards' (1967) genetic distances between samples using the unweighted pair-group method with arithmetic averages (UPGMA). Underlined samples represent those from interior populations studied by Costello et al. (2003). Numbers along branches represent bootstrap scores from 1000 pseudoreplicate analyses.



samples illustrates microspatial differentiation that may be present in some systems. Costello et al. (2003) also reported such fine-scale differentiation in interior populations of bull trout. In some cases, this differentiation may represent divergence between adfluvial and fluvial populations or (as in the case between Chehalis Lake and upper Pitt River populations) differentiation between lake resident and amphidromous populations. The lack of differentiation between upper Pitt River and Chilliwack Lake populations suggests that both of these populations may be amphidromous and mix to a large degree in common feeding areas such as the lower Fraser River. By contrast, owing to their distinction from more migratory populations from the upper Pitt River and Chilliwack Lake, our data suggest that samples from some lakes (e.g., Chehalis Lake, Birkenhead Lake) may move less between lakes and the lower Fraser River.

Regional differentiation and postglacial colonization

Perhaps the most biologically striking aspect of population subdivision in *S. confluentus* is the pronounced difference between coastal and interior populations. That bull trout appear to exhibit a major distinction at the intraspecific level between coastal and interior populations has been observed in previous genetic (e.g., Leary et al. 1993; Taylor et al. 1999; Spruell et al. 2003) and morphological analyses (Cavender 1994; Haas and McPhail 2001). This was most clearly apparent from our data; 46% of the variation was attributable to differences between coastal and interior population groups — a much higher percentage than other hierarchies tested (e.g., north or south of the latitudinal midpoint of localities). Our data, therefore, strongly support the idea that bull trout are composed of at least two major genetic lineages. (cf. Taylor et al. 1999). This coastal–inland separation is a common one in fish and other taxa and likely stems both from historical isolation in distinct glacial refugia as well as from postglacial climatological changes (reviewed by Brunsfeld et al. 2001).

We also found that coastally distributed populations north of the Squamish River (e.g., Klinaklini, Gitnadoix, Toba, Southgate rivers) were genetically more similar to interior populations than to coastal populations from the Squamish River and southward. The genetic similarity of middle–north coast bull trout populations to those from the interior was first noted by Taylor et al. (1999) in a survey of mitochon-

Table 4. Identification of potential immigrants between populations of bull trout (*Salvelinus confluentus*) as determined by assignment tests and variation across seven microsatellite loci.

Sample locality	Locality of inferred immigrant(s)	Midpoint distance from sample locality (km)
Gitnadoix River	Klinaklini River	700
Homathko River	Southgate River[a]	100
Klinaklini River	Homathko River	300
Southgate River	Ure River	560
Squamish River	Ure Creek	330
Phelix Creek	Ryan River[a] (2)	75
Chehalis River	Pitt River–Lake	130
Lillooet River	Chehalis River[a] (2)	150
Ure Creek	Lillooet River[a]	60

Note: The source localities of inferred immigrants are shown, as are the approximate waterway distances between localities, and each inferred immigrant represents a single occurrence unless indicated otherwise in parentheses. Fish were inferred to be immigrants if they had less than a 1% probability of belonging to the locality from which they were sampled. Inferred source populations for immigrants had probabilities of assignment that were at least 10 times that of the locality from which the individual was sampled.

[a]The locations are from the same watershed as the sample location or enter the ocean within 5 km of each other.

drial DNA (mtDNA) variation. In particular, the Klinaklini River shared mtDNA haplotypes with many interior populations. Bull trout from coastal areas south of of these middle–north coastal regions (e.g., Squamish River, lower Fraser River) do not exhibit mtDNA haplotypes or microsatellite alleles (e.g., *Omy*77*283 allele) that are very common in interior areas. Further, the headwaters of these middle–north coastal rivers interdigitate with interior watersheds (e.g., between the Klinaklini River and the interior-draining Chilko River). Consequently, faunal exchange between headwaters of coastal and interior drainages is the most likely explanation for some coastal populations being genetically more similar to interior populations than to other coastal populations. Such faunal transfers along the Coast Mountains crest have also been suggested to explain the distributions of several freshwater fishes in BC (e.g., McPhail and Lindsey 1986; Baxter et al. 1997) and are not uncommon in other mountainous areas of the world (Waters and Wallace 2000; Froufe et al. 2003; Mesquita et al. 2005). It would be interesting to sample more densely within transitional rivers that contain both lineages such as the Fraser River to determine if and at what spatial scale the two lineages may come into contact. The mtDNA work of Taylor et al. (1999) suggests that the transition between lineages may occur over as few as a few tens of kilometres in the Fraser River canyon area.

Bull trout in coastal watersheds, therefore, appear to have originated from two independent invasions: one resulting from postglacial dispersal northward along coastal routes from the Chehalis or Pacific refugia and a second "overland" route from the interior drainages, whose bull trout dispersed postglacially from a middle–upper Columbia River refuge (cf. Taylor et al. 1999; Costello et al. 2003). Our analysis of bull trout genetic variation is a further example of how the dynamic nature of the geological history of western North America has provided opportunities for genetic divergence within species and for the subsequent distribution of such lineages in contemporary landscapes (cf. Taylor and McPhail 2000; Jacobs et al. 2004).

Mixture analysis, individual assignments, and conservation implications

Although our recreational fishery sample is a small one, it was obtained over a number of years and from diverse locations in the lower 20 km of the Fraser River. The microsatellite-based classification of bull trout sampled from the lower Fraser River recreational fishery produced two clear results. First, our data indicate that certain river systems apparently do not contribute fish to this fishery (e.g., most middle–north coast rivers, Phelix Creek). Second, although a diversity of populations probably contributes to the recreational fishery for bull trout, the catch appears to be dominated by fish from the Pitt River drainage. As might be expected owing to their general proximity to the lower Fraser River, tributaries such as Pitt Lake and upper Pitt River and the Chilliwack River contributed most to fish caught in the fishery. It is perhaps not surprizing that the Pitt River system is a major contributor because it is the first major bull trout-supporting watershed upstream of the sample locations in the lower Fraser River. In addition, extensive mark–recapture data ($N > 200$ fish) revealed considerable movement of bull trout between the upper Pitt River and many areas in the lower Fraser River (A. Stobbart, Fisheries and Oceans Canada, 38620 Bell Road, Dewdeny, BC V0M 1H0, unpublished data). Perhaps less expected, however, were the apparent contributions of some fish from more distant localities in the Fraser River drainage (e.g., upper Lillooet River and its tributaries) and outside the Fraser River (e.g., Squamish River). Certainly, bull trout have been reported from nearshore marine areas (Suckley 1861; Haas and McPhail 1991; Goetz et al. 2004) and may disperse among watershed using coastal routes, as has been reported for amphidromous brook trout (e.g., Castric and Bernatchez 2003).

Consequently, our analyses suggest that it is important to protect habitats in tributaries such as the Pitt, Chilliwack, and Lillooet rivers to sustain their bull trout populations and fisheries (e.g., see http://www.pittriverlodge.com). In addi-

Fig. 5. Probabilities of assignment of 30 bull trout (*Salvelinus confluentus*) angled from the lower Fraser River to each of 16 putative source localities. Each bar represents the probability of belonging to each putative source averaged across each of the 30 angled bull trout. Assignment probabilities were based on allelic variation across seven microsatellite loci. Underlined names indicate tributaries that drain to the lower Fraser River.

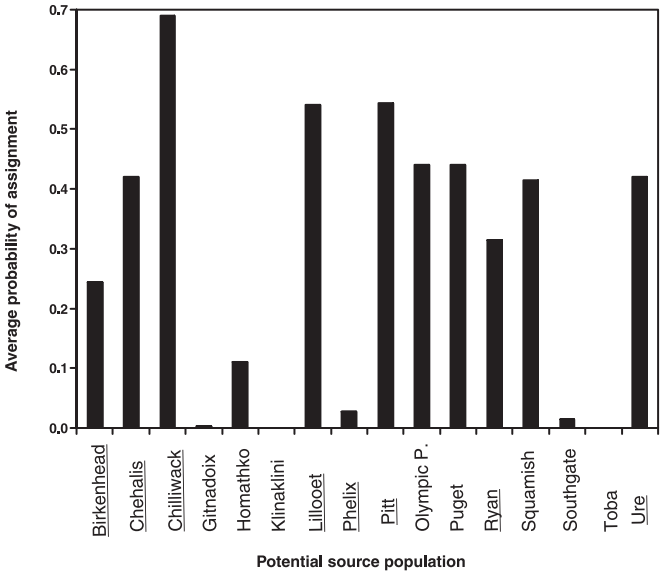
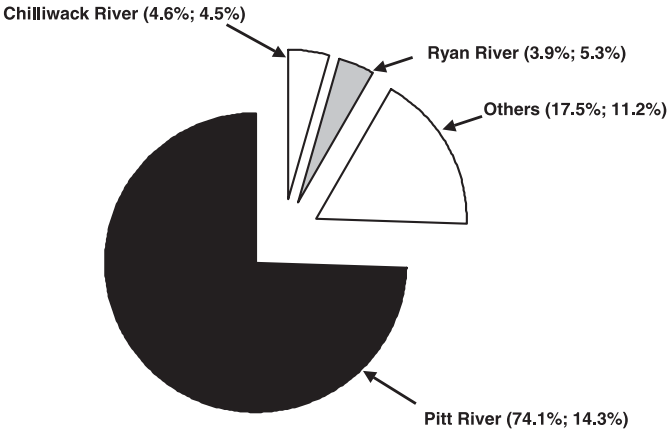


Fig. 6. Estimated percent contributions of source populations of bull trout (*Salvelinus confluentus*) to fish angled from the lower Fraser River between 2000 and 2004. The values represent the mean estimated percent contributions (±1 standard deviation) from 10 000 simulated mixtures from the baseline data (N = 50 fish each) and represent variability about the empirical estimated percentages of 90.1%, 5.3%, 4.6%, and 0.0% for the Pitt, Ryan, Chilliwack rivers, and all other localities pooled, respectively.



tion, our results demonstrate the importance of tributary populations to habitats that receive migrants from these populations as they move downstream for feeding or on their way to the sea. Finally, our recreational fishery sample was from a relatively small area of the lower Fraser River (which extends 150 km upstream to Hope, BC), and there are other large bull trout-producing systems across this area (Chilliwack Lake, Harrison Lake, Coquihalla River). It is probable, therefore, that populations other than the Pitt River contribute substantial numbers of bull trout to recreational fisheries in other portions of the lower Fraser River valley.

Our microsatellite assignment data, however, showed that a number of populations had very similar assignment probabilities for individual fish, which has implications for inferences of movements between river systems (e.g., the upper Pitt and Lillooet rivers). In some instances, individual fish had only marginally different probabilities of being assigned to particular populations. In addition, simulated mixtures showed some variability about the empirical estimates. The accuracy of assignment and mixture analyses depends, principally, on three factors: the numbers of loci assayed, the level of variability of the genetic loci, and the extent of divergence among potential source populations (e.g., Cornuet et al. 1999; Winans et al. 2004). In most cases, θ estimates of divergence between populations were sufficiently high (e.g., 0.60 between the Chehalis and Squamish rivers) to generate high differential assignment success between particular rivers with our small number of loci. The greatest limitation to assignment in the case of bull trout is the relatively low heterozygosity (and low numbers of alleles) within populations at the loci assayed. This means that there are relatively few alleles of distinctive enough frequencies among populations across the seven loci to result in high assignment success. Nerass and Spruell (2001) also reported relatively poor self-assignment success for bull trout sampled from portions of the Lake Pend Oreille – lower Clark Fork River (Idaho–Montana) drainage. A priority for more confident assignment-based conservation issues is the application of three–five more genetic loci that are more variable within populations. For instance, although *Ssa*311 is variable within interior bull trout and is useful for distinguising between coastal and interior bull trout, it was monomorphic within the coastal lineage. Koskinen et al. (2001) obtained virtually 100% assignment success for European grayling (*Thymallus thymallus*) among three tributary populations sampled from Lake Saima in Finland, but heterozygosities and number of alleles were much higher in their study, averaging 0.41 and 7.3, respectively. Testing of a further 20, more variable loci derived from bull trout is currently underway in a collaborative effort (W.R. Ardren, S. Young, P. Spruell, C. Cegelski, and E.B. Taylor, unpublished data).

Our study has demonstrated substantial genetic differentiation among populations of coastal bull trout in central-west and southwestern BC. Most populations in relatively close proximity are genetically distinct from each other, which implies some degree of demographic independence among populations (cf. Costello et al. 2003). Consequently, there is good evidence that populations should be managed as at least semi-independent biological units. In addition, our data corroborate and extend the evidence for two major genetic lineages of bull trout: a coastal and interior lineage. These lineages have been identified using a diverse and independent set of traits (morphology, molecular genetics, geographic distribution, and behaviour) and, consequently, qualify as evolutionary significant units within *Salvelinus confluentus* under a variery of definitions (e.g., Crandall et al. 2000). These data, therefore, strongly suggest that coastal and interior lineages should be considered separately in the upcom-

ing Committee for the Status of Endangered Wildlife in Canada (COSEWIC) status review of bull trout.

Finally, our study illustrates the power and versatility of microsatellite DNA-based assays in biology and conservation. From the same DNA samples and microsatellite assays, we were able to confidently document species identifications and hybridization, resolve population structure and historical signatures of zoogeographic processes, as well as provide independently supported (from mark–recapture studies) estimates of contributions to recreational fisheries. These results will contribute to conservation of the historical legacy of bull trout biodiversity as well as to management of habitat and populations important to recreational fisheries.

Acknowledgements

We appreciate the efforts of many individuals for contributing samples to our study, especially R. Hsu, D. Jesson (BC Ministry of the Environment), P. Spruell (University of Montana), Norm Switzler (Washington Department of Fish and Wildlife, WDFW), and Sewall Young (WDFW). Funding for this project was provided by "seed funding" grant from the Habitat Conservation Trust Fund (No. 04-02-322) and from the Natural Sciences and Enigineering Research Council of Canada. Technical assistance in the laboratory was ably provided by P. Tamkee. We also appreciate the comments of reviewers of this manuscript.

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