Nested analysis of genetic diversity in northwestern North American char, Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*)

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Abstract: Partitioning within-species genetic diversity is fundamental to conservation of the bioheritage, current viability, and evolutionary potential of individual taxa. We conducted a hierarchical analysis of genetic diversity in Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) involving analysis of hybrid zones between Dolly Varden and bull trout, analysis of phylogenetic structure within species across their native ranges using mitochondrial DNA, and a microsatellite DNA survey of population subdivision of bull trout within single watersheds. Our analyses documented hybridization and some introgression between Dolly Varden and bull trout across a geographically widespread zone of secondary contact between the two species. Both species were subdivided into two major mtDNA lineages, and one lineage in Dolly Varden may have arisen through introgression with bull trout. Bull trout have low levels of microsatellite diversity within populations, but there was substantial interpopulation variation in allele frequencies. Allele frequency distributions suggested that recent, severe bottlenecks occur frequently in some bull trout populations. Our results illustrate partitioning of genetic variation at distinct levels of biological organization (species, phylogeographic lineages, local populations), and we address how such nested variation is fundamental to conservation of biodiversity.

Résumé : La compartimentation de la diversité génétique intraspécifique est une opération essentielle pour la conservation de l'héritage biologique, de la viabilité actuelle et du potentiel évolutif de taxons particuliers. Nous avons procédé à une analyse hiérarchique de la diversité génétique de la Dolly Varden (*Salvelinus malma*) et de l'Omble à tête plate (*Salvelinus confluentus*) qui comprend une étude des zones d'hybrides entre les deux espèces, une analyse basée sur l'ADN mitochondrial de la structure phylogénétique des espèces sur toute leur répartition géographique d'origine et un inventaire des microsatellites de l'ADN dans les subdivisions de populations de l'Omble à tête plate à l'intérieur de bassins hydrographiques particuliers. Nos analyses révèlent l'existence d'hybridation et d'introgression dans une large zone géographique de contact secondaire entre les deux espèces. Chacune des espèces se divise en deux lignées principales d'après son ADNmt; l'une des lignées de la Dolly Varden est peut-être apparue par introgression avec l'Omble à tête plate. L'Omble à tête plate possède une faible diversité des microsatellites à l'intérieur des populations, mais il y a une importante variation des fréquences d'allèles entre les populations. Les distributions de fréquence des allèles laissent croire que d'importants goulots d'étranglement récents se sont produits à plusieurs reprises dans quelques populations d'Omble à tête plate. Notre étude est un exemple de la compartimentation de la variation génétique à plusieurs niveaux distincts de l'organisation biologique (espèce, lignées phylogénétiques, populations locales); nous discutons de la pertinence fondamentale d'une telle variation emboîtée pour la conservation de la biodiversité.

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Introduction

Biological diversity is fundamentally hierarchical in nature, and the binomial nomenclature system developed by Linneaus is the most common application of a formal hierarchical system of biodiversity (Mayr 1982). Two issues related to current taxonomic practices, however, continue to generate controversy: the nature and origin of species and recognition of biodiversity below the species level. First, species concepts and mechanisms of speciation have been a

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continual source of debate in evolutionary biology (Taylor 1999), and these uncertainties and the status of individual taxa as valid species remain lively aspects of evolutionary research (e.g., Howard and Berlocher 1998). Second, the last 20 years have seen a heightened awareness of the presence of evolutionary divergence within species and its importance to conservation of particular species (Utter 1981; Avise 1994). The subspecies category is the most formal recognition of such variability, but other nontaxonomic (yet only slightly less controversial) designations such as "evolutionary significant units" (ESU) (Waples 1995) have gained rapid acceptance in the conservation literature (e.g., Moritz 1994). Molecular assays of biodiversity have been instrumental in documenting the hierarchical structure of intraspecific variation (Avise 1994; Bernatchez 1995). This is particularly true for freshwater fishes where the often dendritic nature of stream geomorphology promotes development of a similar hierarchical pattern at the genetic level (e.g., Vrijenhoek et al. 1985; Meffe and Carroll 1994).

Char, fishes of the genus *Salvelinus*, have provided evolutionary biologists with rich material to investigate the nature and origin of biodiversity at and below the species level (e.g., McPhail 1961; Phillips et al. 1999). This Holarctic genus consists of at least 11 well-recognized species, but the exact composition of the genus and the systematic relationships of species have a long history of uncertainty (e.g., Behnke 1980). For instance, Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) have largely parapatric distributions in northwestern North America. Dolly Varden are coastal in distribution from Washington State to western Alaska and east to the Mackenzie River (Haas and McPhail 1991). Bull trout tend to be more inland in distribution and are found from Nevada to Yukon Territory (see fig. 1 in Baxter et al. 1997). A few areas of sympatry have been identified using morphological and biochemical/molecular analyses and appear to be confined to coastal regions of large rivers (e.g., lower Fraser River) and in and around the Coast/Cascade Mountain divide and the Olympic Peninsula (Cavender 1978; Baxter et al. 1997; Leary and Allendorf 1997). The status of Dolly Varden and bull trout as distinct species, however, has been uncertain, and at one time, bull trout were considered conspecific with Dolly Varden (e.g., McPhail 1961). More recent morphological (Cavender 1978; Haas and McPhail 1991) and molecular phylogenetic data (e.g., Phillips et al. 1995) have supported their status as distinct species. Genetic evidence of the two char maintaining themselves as distinct gene pools in sympatry (Baxter et al. 1997; Leary and Allendorf 1997) has provided the most direct evidence that the two char are distinct biological species. We know little, however, about the extent of contact between Dolly Varden and bull trout and about patterns of hybridization and gene flow on both a large and small geographic scale.

In addition to the species status of individual taxa, documenting the depth and phylogenetic relationships of evolutionary lineages within species is important for phylogeographic inference (Avise 1994). Further, the nature and origins of phylogenetic divisions within species are considered an important step in setting conservation priorities (Moritz 1994; Bernatchez 1995). Deeper (and older) lineages may represent the most important components of a species' "bioheritage" because they may be less replaceable than more recent divergences (Bowen 2000). We have, however, only recently gained some understanding of such diversity in bull trout (Taylor et al. 1999), and what information does exist for Dolly Varden (e.g., Phillips et al. 1999) excludes large portions of its range.

Finally, the level of distinction among local populations within major lineages is also an important issue in understanding both the origin of diversity within individual species and the conservation of such diversity. For instance, there is a rich history of investigations of local population structure in salmonid fishes using biochemical and molecular markers (Ryman and Utter 1987; Allendorf and Waples 1996). Particular attention has been focussed on the use of such data to understand the relationship between heterozygosity and viability of individual populations as well as the level of gene flow and its relationship to demographic independence among local populations. Although some work has been completed on local population structure of bull trout in the United States (Leary et al. 1993; Spruell et al. 1999), there is little known about population structure and its implications for demography for either bull trout or Dolly Varden in Canada, which represents the heart of the range of both species.

In this paper, our primary goals were to further define the contact zones between Dolly Varden and bull trout in northwestern North America, resolve the extent of phylogenetic distinction within Dolly Varden to facilitate comparative analysis with bull trout, and test for population subdivision within phylogenetic lineages of bull trout to help define conservation units on a local basis. Our secondary goal was to illustrate how particular classes of molecular loci and assays are appropriate for specific questions at different levels of biological organization.

Materials and methods

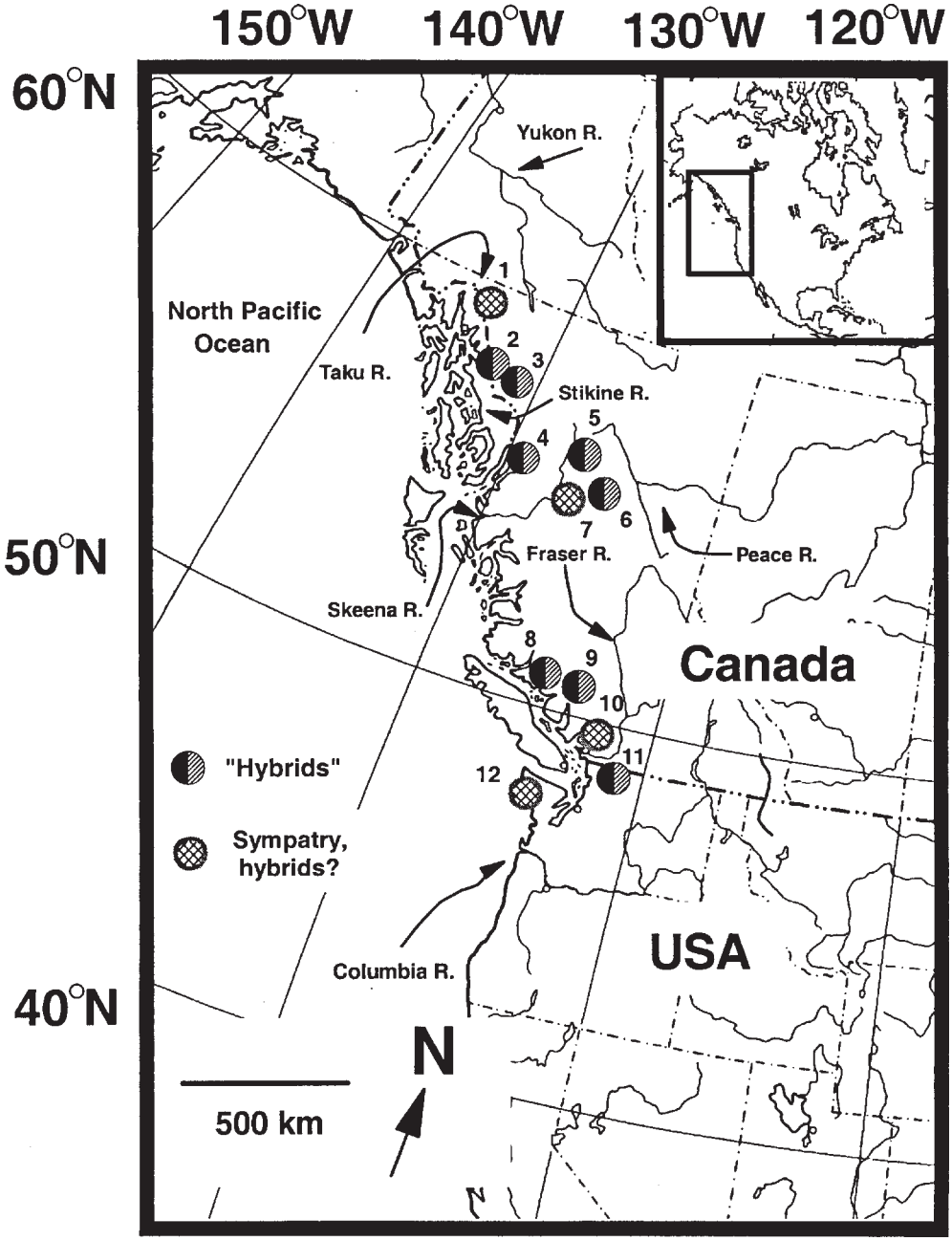
General laboratory methods

Our analyses consisted of various polymerase chain reaction (PCR) based assays of regions of DNA that either were diagnostic for Dolly Varden or bull trout or resolved polymorphisms within either species. For these analyses, genomic DNA was extracted from fin clip samples that were stored in 95% ethanol. DNA was extracted using standard proteinase K – pronase tissue digestion and phenol–chloroform- or salt-based purification.

Hybridization between Dolly Varden and bull trout

We surveyed geographic areas for evidence of sympatric populations and for the presence of interspecific hybrids using samples that where obtained opportunistically or as part of a detailed study of hybridization within a single watershed (Fig. 1). Sample localities were tested for the presence of either or both species and for hybrid individuals by examining introns or intergeneric spacers at three independently segregating loci (Table 1). Two of the three markers resolved size-based differences between the species that required no further analysis. The third locus, ITS-1, was subjected to restriction enzyme analysis as outlined in Baxter et al. (1997). All markers have been assayed on a number of individuals (N = 30–100) for each locus from throughout the geographic range of each species to confirm their diagnostic nature (Baxter et al. 1997; Redenbach 2000). PCR assays were run in either a Stratagene "Robocycler" or an MJ Research PCJ 100 thermal cycler using be-

Fig. 1. Distribution of Dolly Varden, bull trout, and hybrids between these species based on molecular analyses at three nuclear loci. Also indicated are regions of sympatry established by molecular analyses but at which no hybrids have yet been detected. Sample codes are given in Table 5. Samples 11 and 12 represent the Skagit River and the East Fork Quinnalt River assayed by McPhail and Taylor (1995) and Leary and Allendorf (1997), respectively. Inset shows sample area in relation to North America.



tween 0.200 and 0.800 nM dNTPs and 0.5–1.0 unit of *Taq* polymerase per reaction.

We identified hybrids as fish of mixed ancestry. Using the diagnostic markers, we recognized hybrid genotypes as those individuals that were (*i*) heterozygous for species-specific fragments at all loci (putative F1 hybrids), (*ii*) heterozygous at one locus but homozygous for species-specific alleles at the other loci (putative backcrosses), or (*iii*) homozygous at all loci but for opposite specific-specific alleles at at least one of those loci (F1 × F1 or later-generation hybrids or backcrosses). Our analysis is conservative in that with three diagnostic markers, some fish classified as "pure" parental genotypes could actually be advanced-generation hybrids or backcrosses (see Avise 1994). At sites where parental and hybrid genotypes were detected ($N = 5$), we tested for differences in the percentages of hybrids using chi-square randomization tests (Roff and Bentzen 1989).

Phylogenetic groupings within species

For phylogenetic inference within both Dolly Varden and bull trout, we assayed sequence variation in the mtDNA NADH subunit 1 gene as outlined by Taylor et al. (1999). Sequences obtained from an extensive survey of variation in bull trout (Taylor et al. 1999) were combined with data collected from Dolly Varden from throughout their geographic range (Table 2). We also used ND1 sequence data collected from lake trout (*Salvelinus namaycush*) to serve as an outgroup taxon in our analyses. For Dolly Varden, our preliminary analyses indicated the presence of two divergent ND1

Table 1. Primers, PCR conditions, species-specific diagnoses, and sources of molecular markers used in DNA analyses of Dolly Varden and bull trout.

Primer	Sequence	Cycles/annealing temperature (°C)	$MgCl_2$ (mM)	Allele size (basepairs)	Source
GH7	CTT ATG CAT GTC CTT CTT GAA			DV: 600	McKay et al. 1996
GH51	GTC AAG CTG ATA CAA CTC	40/50	1.5	BT: 540	
MTBF2	ATG CAC CAG TTG TAA GAA AG			DV: 520	J. Baker[b]
MTBR2	GTC GGA AGG ACA GCA GGG	40/56	1.5	BT: 700	Unpublished data
MD-1	CTT GAC TAT CTA GAG GAA GT			DV: 700	Phillips et al. 1995
ITS-R	AGC TTG CTG CGT TCT TCA TCG A	40/55	1.5	BT: 490,210[c]	
N1F	GGT ATG GGC CCG AAA GCT TA				Neilsen et al. 1998
N1R	GCC TCG CCT GTT TAC CAA AAA CAT	35/54	2	Clade A:340/200	
ND1C[a]	TGC AGC CGC TAT TAA GGG TTC G	na	na	Clade B:500/250[d]	Redenbach and Taylor 1999

Note: Loci are growth hormone (GH), metallothionein (MTB), internal transcribed spacer 1 (rDNA, ITS-1), and the mtDNA NADH-1 subunit (ND1). All sequences are written 5′–3′. na, not applicable.

[a]Used as sequencing primer.

[b]Fisheries Research Institute, University of Washington, P.O. Box 357908, Seattle, WA 98195-7980, U.S.A.

[c]RFLP difference resolved with *Sma* I.

[d]RFLP differences resolved with *Hae* III.

clades. The two clades could be distinguished by restriction site differences resolved with a number of enzymes (*Hae* III, *Hin*f I). We employed *Hae* III to resolve the presence of either or both clades in larger samples (up to $N = 10$) from throughout the range of Dolly Varden. As we were interested in the phylogeny of Dolly Varden mtDNA across the range of the species, we focussed our sampling on obtaining sequences from as wide an area as possible but did not sample extensively within populations. Phylogenetic analysis consisted of sequence divergence estimates (Kimura two-parameter) and maximum parsimony analyses. These analyses incorporated bootstrap resampling of sequence matrices ($N = 1000$) to assess the robustness of derived groupings. We also used maximum-likelihood analyses (Kishino and Hasegawa 1989) to statistically test for monophyletic groupings in combined analyses of Dolly Varden and bull trout sequences. All phylogenetic analyses were conduced using programs from the Phylip software package (Felsenstein 1993).

Population structure of bull trout

Samples were obtained during the summer of 1997 from Harrison Lake (Red Deer River, Banff National Park, Alta.) and Ice River (tributary of Kicking Horse River, upper Columbia River near Golden, B.C., in Yoho National Park). Bull trout were also sampled from two interior British Columbia watersheds: the upper Kootenay River watershed and a small portion of the lower Peace River, the Pine River watershed. The Kootenay River samples ranged from Ram Creek (Wigwam River) system near Elko, B.C., north to Skookumchuck Creek near Canal Flats, B.C. (about 200 km distant). The Pine River samples concentrated on two watersheds: the Sukunka and Murray rivers. Samples were collected in both 1997 (Kootenay and Sukunka rivers) and 1998 (Murray River) (Table 3).

We assayed microsatellite DNA variation using loci that had been isolated from other salmonid species (three loci) and also used two loci isolated from a bull trout genomic library (E.B. Taylor, unpublished data). PCR conditions followed standard procedures (Table 4), and all reactions were completed using 32-P end-labelled primers and PCR products were scored from autoradiographic films after polyacrlyamide electrophoresis (typically 6% Long Ranger gels).

Allelic variation within population samples was tested for departures from Hardy–Weinberg equilibrium as well as for departures from linkage equilibrium between loci. Pairwise tests of allele frequency differentiation between populations within watersheds were accomplished using Monte-Carlo resampling statistics. A variety of mutation-based and drift-based genetic distance algorithms are available for the calculation of population subdivision and genetic distances among samples. We considered drift-based methods of genetic subdivision to be the most appropriate. First, the post-glacial origin of the freshwater populations of bull trout in our study areas sets their maximum age at about 12 000 years (Taylor et al. 1999). Over such short time frames, particularly when population histories may have involved large changes in population sizes, demographic processes probably overwhelm any post-colonization mutation-based differentiation patterns. Second, over such short evolutionary time periods, drift-based metrics alleles models tend to outperform alternatives based on mutation models (Paetkau et al. 1997), particularly in the range of the numbers of sample and loci employed in our study (Gaggiotti et al. 1999).

Consequently, we used methods based on the variance in allele frequencies (F_{st}) without regard to molecular size differences among alleles as a drift-based measure of genetic subdivision. Similar approaches have been adopted in other investigations of post-glacial fish population genetics (e.g., Douglas et al. 1999). At each locus, we also calculated F_{st} (as estimated by θ) and its statistical significance based on allelic frequency differences using Genepop (Raymond and Rousset 1995) and Fstat (Goudet 1995). We also examined the level of genetic subdivision when samples were arranged hierarchically, i.e., genetic variation between watersheds (Peace versus Kootenay), variation among populations within watersheds, and variation within populations. This analysis was completed using the analysis of variance like design of Arlequin (Schneider et al. 1997), and the statistical significance of variance components was tested using randomization procedures in the Arlequin analysis.

In all statistical tests, the sequential Bonferroni procedure (Rice 1989) was followed to adjust the rates of Type I error over simultaneous tests to 0.05. Finally, we also utilized the microsatellite allele frequencies to test for evidence of recent bottlenecks in bull trout using the "mode-shift" indicator as implemented in Bottleneck (Piry et al. 1999). Populations that have undergone recent bottlenecks are expected to show a reduction in the proportion of low-frequency alleles relative to alleles of moderate abundance. Recent bottlenecks are those that have occurred within 40–80 generations, and the mode-shift test has resolved differences in empirical allele frequency distributions between bottlenecked and non-bottlenecked populations across a number of species (Luikart et al. 1998). The detection of recent bottlenecks in bull trout may be especially important, as populations so affected may not have had time to adapt to potential problems imposed by small population

Table 2. Sample localities and sample sizes for Dolly Varden phylogeographic analyses.

Sample	Drainage	*N*	Northern clade	Southern clade
Udobnaya R.	Kuril Is., Russia	1/4	5	
Porozhistaya R.	Kuril Is., Russia	1/3	4	
Togiak L. (1)	Bristol Bay, Alaska	1/5	6	
Iliamna L. (2)	Bristol Bay, Alaska	1/5	6	
Klutina R. (3)	Copper R., Alaska	1/5	6	
Bonnet Plume R. (4)	Peel R., Yukon	/10	10	
Blackstone R. (5)	Peel R. Yukon	/5	5	
Tulsequah R. (6)	Taku R.	3/4	7	
Iskut R. (7)	Stikine R.	3/	3	
Zolzap R. (8)	Nass R.	/10	9	1
Ogden Channel (9)	Central coast B.C.	1/	1	
Noyes Sound (10)	Central coast B.C.	1/	1	
Ecstall R. (11)	Skeena R.	1/2	3	
Brent Cr. (12)	Queen Charlotte Is.	1/	1	
Aero R. (13)	Queen Charlotte Is.	1/	1	
Feather Cr. (14)	Queen Charlotte Is.	/2	2	
Honna R. (15)	Queen Charlotte Is.	1/	1	
Three Mile Cr. (16)	Queen Charlotte Is.	/1	1	
Ian R. (17)	Queen Charlotte Is.	/5	5	
Ain R. (18)	Queen Charlotte Is.	/5	5	
Kumealon Cr. (19)	Midcoast B.C.	1/	1	
Noosneck R. (20)	Midcoast B.C.	1/5	6	
Ocean Falls (21)	Midcoast B.C.	/11	11	
Dallery Cr. (22)	Midcoast B.C.	1/5	2	4
Wakeman R. (23)	South coast B.C.	/2		2
Southgate R. (24)	South coast B.C.	1/		1
Upper Deserted R. (25)	Midcoast B.C.	/4	4	
O'Connell L. (26)	Vancouver Is.	1/8	9	
Claninick R. (27)	Vancouver Is.	1/2	3	
Keogh R. (28)	Vancouver Is.	/10	5	5
Misty L. (29)	Vancouver Is.	1/	1	
Eve R. (30)	Vancouver Is.	/5		5
Jessie L. (31)	Vancouver Is.	/1		1
Thelwood Cr. (32)	Vancouver Is.	1/5	2	4
Phillips R. (33)	Vancouver Is.	1/5	1	5
Zeballos R. (34)	Vancouver Is.	2/2		4
Cowichan L. (35)	Vancouver Is.	1/4	5	
Mamquam R. (36)	South coast B.C.	1/		1
Mill Cr. (37)	South coast B.C.	1/3		4
Capilano R. (38)	South coast B.C.	/2		2
Seymour R. (39)	South coast B.C.	/2		2
Loon L. (40)	South coast B.C.	/8		8
Quinault R. (41)	Olympic Pen., Wash.	1/8		9
Dungeness R. (42)	Olympic Pen., Wash.	/5		5
South Fork Nooksack R. (43)	Puget Sound, Wash.	/5		5
Lake trout	Nation R. (upper Peace R., B.C.)	1/		

Note: Sample sizes separated by a solidus indicate the number sequenced left of the solidus and those assayed by restriction fragment length polymorphism analyses right of the solidus. Number codes following river and lake names denote sample localities given in Fig. 3.

sizes and may signal populations at risk of losing heteozygosity or variation at quantitative loci affecting fitness over the longer term (Luikart et al. 1998).

Results

Species distributions and hybridization

The geographic distribution of Dolly Varden and bull trout has traditionally been inferred from morphological analyses, and in several cases, sympatry was documented and hybridization suspected (e.g., Cavender 1978; Haas and McPhail 1991). We used diagnostic molecular markers to examine 12 localities from six drainage areas for the presence of both species and of interspecific hybrids, 10 of which had not been suspected as contact zones previously (Fig. 1; Table 5). Seven of the 12 sites contained both Dolly Varden and bull

Table 3. Sample sizes (N), numbers of observed alleles (N_a), and expected (H_e) and observed (H_o) heterozygosities per locus and per bull trout population assayed.

Locus	HL	IR	SK	RC	CC	NBR	UBC	BR	Mean
Omy 77									
N	43	30	36	45	17	21	22	15	28.6
N_a	1	2	2	2	1	1	2	2	1.6
H_e	0	0.42	0.44	0.49	0	0.46	0.40	0.18	0.30
H_o	0	0.40	0.30	0.58	0	0.52	0.36	0.06	0.28
Sfo 18									
N	43	30	36	45	17	21	22	18	2.9
N_a	1	1	2	2	2	2	2	2	1.7
H_e	0	0	0.26	0.10	0.50	0.50	0.40	0.48	0.28
H_o	0	0	0.25	0.11	0.52	0.71	0.40	0.31	0.29
Ssa 197									
N	43	30	36	46	16	21	24	18	29.2
N_a	1	2	2	2	2	2	2	2	1.8
H_e	0	0.49	0.31	0.46	0.11	0.50	0.08	6.48	0.30
H_o	0	0.50	0.33	0.46	0.13	0.80	0.08	0.56	0.36
Sco 19									
N	43	30	35	45	17	21	23	18	29
N_a	1	5	6	4	2	3	4	3	3.5
H_e	0	0.46	0.41	0.27	0.39	0.09	0.16	0.24	0.25
H_o	0	0.33*	0.43	0.27	0.53	0.09	0.17	0.17	0.25
Sco 23									
N	43	30	35	46	17	21	22	18	29
N_a	1	3	2	1	2	2	2	2	1.8
H_e	0	0.49	0.37	0	0.38	0.52	0.46	0.42	0.33
H_o	0	0.60	0.43	0	0.29	0.52	0.58	0.61	0.39

Note: Populations: HL, Harrison Lake (Red Deer River drainage, Alberta); IR, Ice River (upper Columbia River, B.C.); SK, Skookumchuck Creek (upper Kootenay River, B.C.); RC, Ram Creek (upper Kootenay River, B.C.); CC, Chamberlain Creek (lower Peace River, B.C.); NBR, North Burnt River (lower Peace River, B.C.); UBC, Upper Brazion Creek (lower Peace River, B.C.); BR, Burnt River (lower Peace River, B.C.).

*$P < 0.006$ (Bonferroni-corrected α for eight within-locus simultaneous tests of Hardy–Weinberg equilibrium).

Table 4. Locus, source species, reference, total alleles observed (N_a), sample size (N), molecular size range, and total expected heterozygosity (H_e) for five microsatellite loci assayed in bull trout.

Locus	Source species	Reference	Annealing temperature (°C)	N_a	N	Size range (basepairs)	H_e
Omy 77	Rainbow trout	Morris et al. 1996	56	3	228	279–283	0.45
Sfo 18	Brook trout	Angers et al. 1995	65	2	233	150–156	0.36
Ssa 197	Atlantic salmon	O'Reilly et al. 1996	58	2	225	119–123	0.48
Sco 19[a]	Bull trout	F CTT GAA ATT AGT TAA ACA GC R* CCA AAC TAC CCA ATA ATC	53	11	231	158–216	0.27
Sco 23[a]	Bull trout	F TGT GAA AAG GGG AGA CAG AG 3′ R GGA GAC AGG GGA TGG AAA AG	61	3	236	185–193	0.39

Note: All reactions used the forward primer as the labelled primer unless indicated by an asterisk. All sequences are written 5′–3′.

[a]E.B. Taylor, unpublished data.

trout (i.e., they were homozygous for species-specific alleles at all three nuclear loci), and seven sites contained at least one hybrid individual (i.e., each such individual was heterozygous for at least one locus or was homozygous for alternative species-specific alleles at two or more loci). Two of the zones of sympatry represent confirmations of range extentions for Dolly Varden. Dolly Varden were found in two tributaries of the upper Peace River (Mackenzie River drainage): Thutade Lake (cf. Baxter et al. 1997) and the Omineca River (Fig. 1; Table 5). The extent of hybridization varied among the localities; in samples with at least 15 individuals assayed, the percentage of hybrid-class individuals varied from a high of 25% in the Southgate River to 2% in the Iskut River ($\chi^2 = 16.1$, 4 df, $P = 0.007$) (Table 5).

Phylogenetic groupings within species

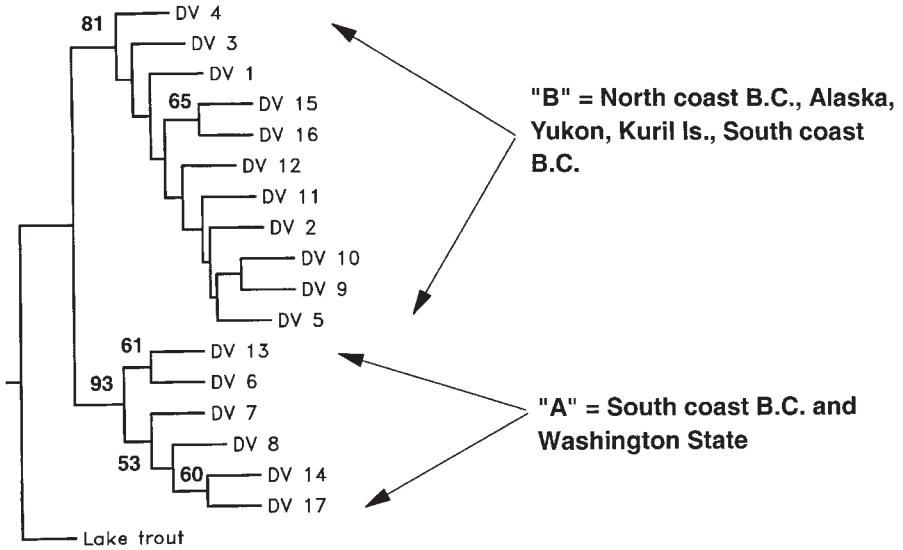
Taylor et al. (1999) resolved two phylogenetic groupings of bull trout using restriction site and sequence analysis of five regions of the mtDNA genome. "Coastal" bull trout were found west of the Coastal and Cascade Mountain crests and "interior" bull trout were found east of these mountains. Our current sequence analysis of the ND1 region in Dolly Varden (Appendix, Table A1) also resolved two groupings that differed from one another by about 1.1% in sequence

Table 5. Parental species and hybrid identification in selected watersheds.

Sample	Drainage	Dolly Varden	Bull trout	Hybrids
Tulsequah R. (1a)	Taku R.	7		
Nakina R. (1b)	Taku R.		10	
Tatsatua R. (1c)	Taku R.		5	
Chutine R. (2)	Stikine R.	15	1	3
Tahltan R. (3)	Stikine R.			1
Iskut R. (4)	Nass R.	33	13	1
Thutade Lake (5)	Upper Peace R.	49	127	9
Omineca R. (6)	Upper Peace R.	24	2	1
Goathorn Cr. (7)	Skeena R.	15	10	
Toba R. (8)	South coast B.C.	9		1
Southgate R. (9)	South coast B.C.	10	11	7
Squamish R. (10)	South coast B.C.	20	10	

Note: Number codes following river and lake names refer to sites given in Fig. 1.

Fig. 2. Phylogenetic tree of Dolly Varden mtDNA based on parsimony analysis of 503 basepairs of the ND1 gene. Numbers at branch points represent bootstrap percentages based on 1000 resamplings of the sequence matrix. The lake trout ND1 sequence was used as the outgroup. Sample identifications for all sequences are given in the Appendix (Table A2).



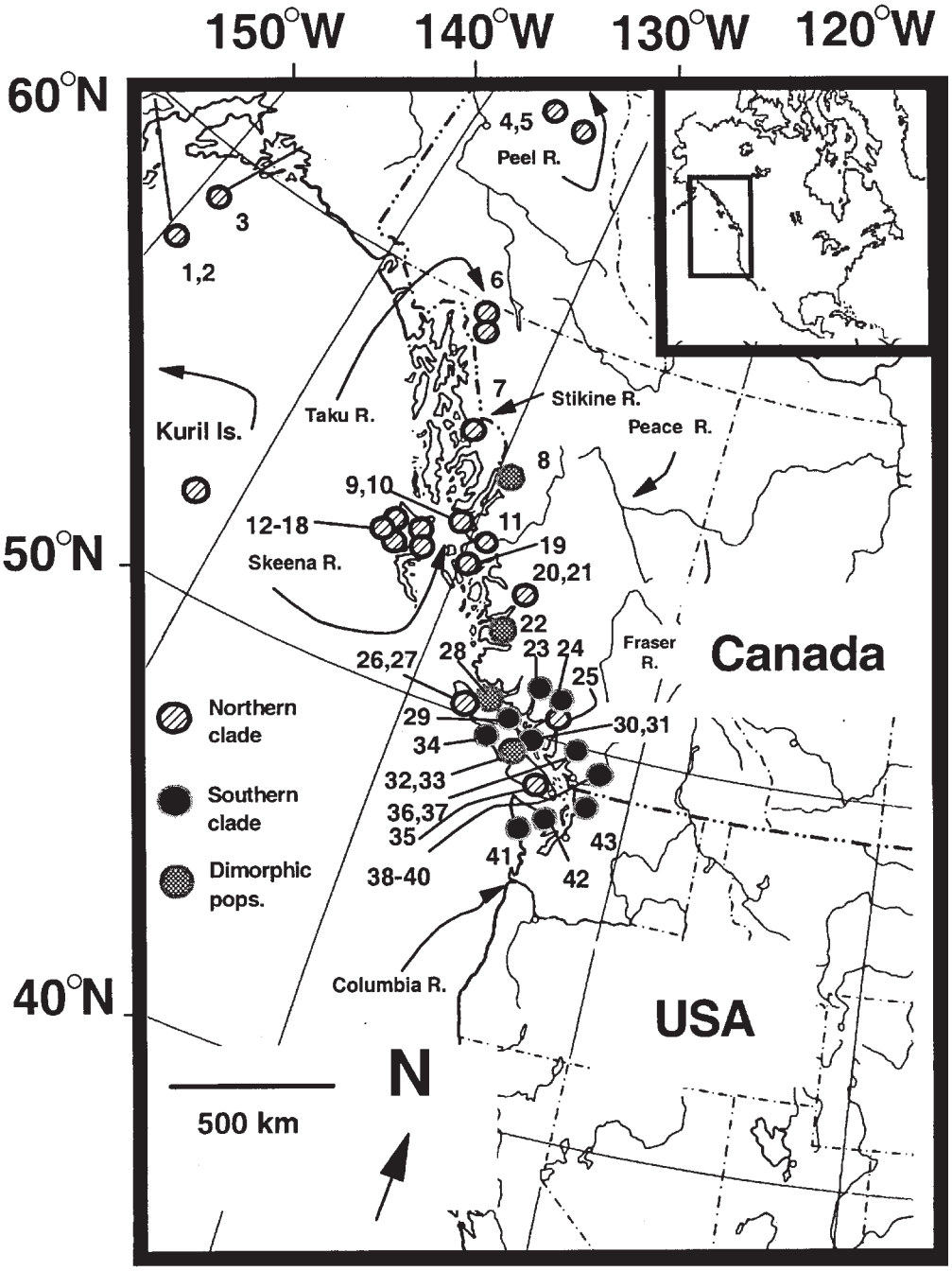
(Fig. 2). These two clades of Dolly Varden mtDNA were well supported in the parsimony analysis accompanied by bootstrap analysis (minimum of 81% support). Several restriction sites were diagnostic in the samples that we examined, and we used *Hae* III to resolve the presence of one or both clades in further samples (Table 2; Fig. 3). One clade (A) was found on the Olympic Peninsula, Vancouver Island, and south coastal British Columbia and north to the headwaters of the Nass River. The other clade (B) corresponded to samples from the northwest Pacific Ocean (Kuril Islands), western Alaska, north coastal British Columbia, the Queen Charlotte Islands, and the Peel River (a northwestern Yukon tributary of the Mackenzie River) and extended down to southern Vancouver Island (Figs. 2 and 3).

Combining the 17 Dolly Varden haplotypes with the six bull trout ND1 sequence haplotypes suggested that Dolly Varden mtDNA is paraphyletic; members of all Dolly Varden clade A haplotypes are more closely related to bull trout mtDNA than they are to Dolly Varden clade B haplotypes (61% bootstrap support) (Fig. 4). In fact, bull trout mtDNA is also paraphyetic, as interior bull trout mtDNA clusters separately from a lineage composed of coastal bull trout and group A Dolly Varden mtDNA (63% bootstrap support) (Fig. 4). Enforcing bull trout monophyly and Dolly Varden monophyly resulted in phylogenetic trees that were significantly worse (log likelihood $D = -40.2$, $P < 0.05$) than the maximum-likelihood tree that had the same topology as the parsimony tree (phenogram not shown). The six Dolly Varden ND1 haplotypes that were part of the mtDNA clade with the six bull trout haplotypes, however, were found in individuals that all possessed the rDNA ITS-1, MTB, and GH alleles that are diagnostic for Dolly Varden and vice versa (Redenbach 2000).

Microsatellite polymorphism within populations

In general, microsatellite polymorphism in bull trout across all loci was relatively low (Appendix, Table A2). Most populations had only two alleles at all loci (except for *Sco* 19), and one population (Harrison Lake) was fixed for a single allele at all five loci. There was only one significant departure from Hardy–Weinberg equilibrium involving a reduced number of heterozygotes from expected (*Sco* 19 in Ice

Fig. 3. Geographic distribution of two clades of Dolly Varden mtDNA based on sequence and restriction fragment polymorphism analysis (with *Hae* III) of the ND1 gene. Pies represent the presence of either or both clades but not their relative frequencies. Inset shows sample area in relation to North America. Sample codes are given in Table 2.



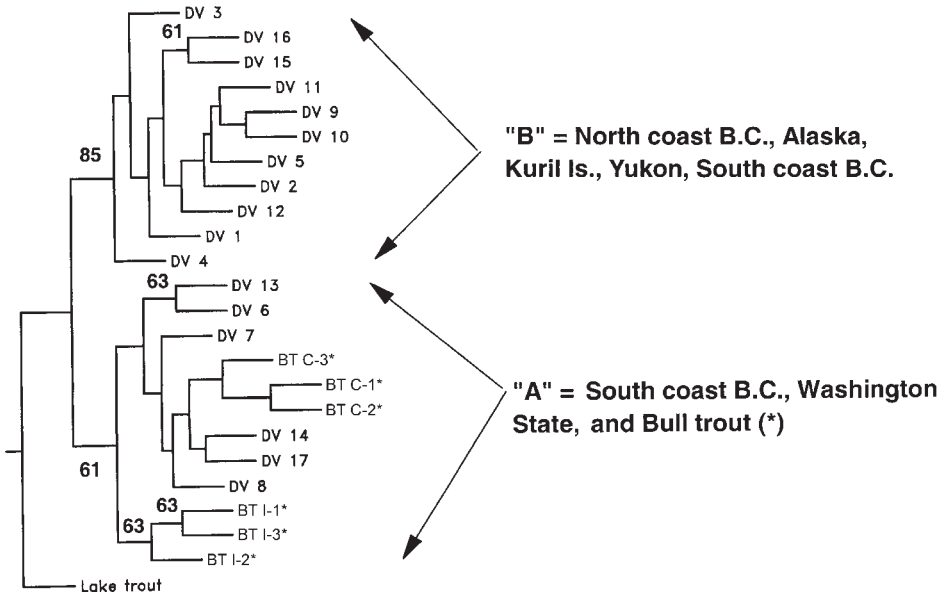
River) (Table 3). All loci appeared to be in linkage equilibrium as well (minimum $P = 0.06$ within populations and 0.32 across populations). The populations tended to have low and variable average gene diversities (expected heterozygosities), typically ranging from 0.29 (Ram Creek) to 0.47 (Burnt River). A notable exception was Harrison Lake with an average gene diversity of 0.009 and an observed heterozygosity of 0.0.

Microsatellite polymorphism among populations

There was marked among-population variation in allele frequencies (Appendix, Table A2). Fixation indices (θ) across all eight sample sites ranged from 0.07 (*Sco* 19) to 0.42 (*Sfo* 18 and *Ssa* 197). The overall level of subdivision was high ($\theta = 0.33$, 95% CI = 0.2164–0.3986), which was significantly greater than 0 ($P < 0.005$). Values of θ within watersheds were slightly lower; mean θ within the Columbia/Kootenay and Peace watersheds was 0.143 and 0.189, respectively (both $P < 0.05$).

In pairwise analyses within watersheds, all but one of the population pairs (North Burnt and Burnt rivers, $P > 0.05$) were significantly different from one another in allele fre-

Fig. 4. Phylogenetic tree of Dolly Varden and bull trout mtDNA based on parsimony analysis of 503 basepairs of the ND1 gene. Bull trout sequences (asterisks) are from Taylor et al. (1999). Numbers at branch points represent bootstrap percentages based on 1000 resamplings of the sequence matrix. The lake trout ND1 sequence was used as the outgroup. The annotations "C" and "I" accompanying the bull trout haplotypes designate samples from coastal and interior regions, respectively (see Taylor et al. 1999). Haplotype identities for Dolly Varden are as in Fig. 2 and those for bull trout are given in Taylor et al. (1999).



quencies pooled across loci (maximum $P < 0.001$), but some loci produced more differences than others. Within the upper Kootenay/Columbia system, *Omy* 77 and *Sco* 19 showed all pairwise tests as significant (maximum $P = 0.002$), while in the Peace, *Omy* 77, *Sco* 19, and *Sco* 23 tended to show the greatest number of significant differences (maximum $P = 0.02$). Between watersheds, the greatest differences in allele frequencies were found at *Sfo* 18 and *Sco* 19 (Appendix, Table A2).

Using the theoretical relationship between F_{st} and number of migrants (N_em), $F_{st} \approx 1/(4N_e m + 1)$, estimates of exchange among populations within watersheds (Harrison Lake excluded) ranged from two (Ice River and Ram Creek) to three (Skookumchuck Creek and Ram Creek) individuals per generation in the upper Kootenay/Columbia. In the Peace watershed, estimates ranged from one (Upper Brazion Creek and Burnt River) to three (North Burnt and Burnt rivers) individuals per generation.

Hierarchical analysis of microsatellite diversity based on allele frequencies (F_{st}) indicated that most variation resided within populations ($F_{st} = 0.32$) but with significant variation among populations within watersheds ($F_{sc} = 0.28$) and low, but significant variation between the Peace and upper Kootenay/Columbia watersheds ($F_{ct} = 0.06$) (all $P < 0.01$).

Evidence for population bottlenecks

The microsatellite data was subject to the mode-shift indicator for recent population bottlenecks described by Luikart et al. (1998), and three populations showed an excess of medium-frequency alleles suggestive of recent bottlenecks: Skookumchuck Creek, Chamberlain Creek, and North Burnt River. In addition, The Harrison Lake population exhibited a lack of detectable variation across five loci (mean observed heterozygosity of 0.0), suggesting that this population has been subject to strong founder events or population bottlenecks during or following colonization of the lake.

Discussion

Sympatry and hybridization between Dolly Varden and bull trout

Our data confirm and extend suggestions of sympatry and hybridization between Dolly Varden and bull trout that were based on morphological analyses. Cavender (1978) concluded that the species coexisted in the McCloud River (California), Puget Sound, and Skeena and Taku rivers, with two fish identified as hybrids in the upper Skeena River. Haas and McPhail (1991) reported Dolly Varden and bull trout coexisting in the Nass and Stikine rivers as well. Our data confirmed the presence of both species in these watersheds and identified three further areas of sympatry along the southwestern coast of British Columbia (Toba, Southgate, and Squamish rivers). These data, coupled with earlier molecular-based identifications of both species in the upper Peace River (Baxter et al. 1997), the Olympic Peninsula (Leary and Allendorf 1997), and the Skagit River (a tributary of northern Puget Sound; McPhail and Taylor 1995), indicate that Dolly Varden and bull trout come into contact along most of their largely parapatric distributions in northwestern North America.

Bull trout are thought to have originated in the Columbia River basin (Behnke 1980), and dispersal via inland watershed connections occurred throughout the interior of British Columbia and north to the Yukon and east of the Rocky Mountains to the North and South Saskatchewan River systems. Dispersal to the Olympic Peninsula, Puget Sound, and south coastal British Columbia watersheds via the marine environment has also been suggested (Cavender 1978; Behnke 1980; Haas and McPhail 1991). Dolly Varden proba-

bly colonized most of their southern range via marine dispersal, given their largely coastal distribution and their well-established anadromous behaviour (e.g., DeCicco 1992). Sympatric populations of Dolly Varden and bull trout therefore probably originiated from two processes: double invasions of emerging coastal habitats via marine dispersal and headwater exchanges between inland tributaries of large coastal and interior drainages (e.g., between the Skeena and upper Peace rivers; Baxter et al. 1997). Over their range of contact, of particular curiosity is the absence of bull trout from Vancouver Island (where Dolly Varden are common), which is less than 50 km from sympatric populations on the Olympic Peninsula and the south coast of British Columbia (see Redenbach 2000).

Our data indicate that Dolly Varden and bull trout hybridize over an extensive area of contact. McPhail and Taylor (1995) and Baxter et al. (1997) reported hybridization in two independent watersheds separated by over 1000 km, and therefore, natural hybridization between the two species is clearly a widespread phenomenon. Our surveys were not exhausive in many areas, and sampling was largely opportunistic, but our data suggest that the rate of hybridization is variable among watersheds. For instance, in the Southgate watershed, about 25% of all char examined were classified as hybrids whereas only about 2% were hybrids in the Iskut River sample. Where the ecology of sympatric populations has been studied in detail (McPhail and Taylor 1995; Hagen 2000), size-dependent differences in spawning behaviour and habitat choice appear to play an important role in reproductive isolation between Dolly Varden and bull trout. Perhaps the opportunities for interspecific matings vary among watersheds owing to variability in habitat or life history characteristics of their char populations, and this may contribute to variation in the rate of hybridization (Arnold 1997). An alternative hypothesis is that postmating processes are major determinants of the extent of hybridization and gene flow and that the distinct watersheds that we have sampled vary in the degree to which they select against hybrids.

Intraspecific phylogeny and reticulate evolution in char

Our analysis of ND1 mtDNA sequences in Dolly Varden over its native range demonstrated the presence of two phylogenetic groupings. Taylor et al. (1999) reported similar findings for the same region of mtDNA in bull trout and suggested that the diversification within bull trout was driven by historical isolation and postglacial dispersal from two glacial refugia: the Chehalis and lower Columbia River Valley. Combined analysis of Dolly Varden and bull trout for ND1, however, indicated a a paraphyletic relationship of mtDNA for both species, i.e., some Dolly Varden haplotypes were more closely related to bull trout than to other Dolly Varden and vice versa. More detailed analyses of nuclear ITS-1 and GH nuclear sequences of Dolly Varden and bull trout with paraphyletic ND1 haplotypes indicate that, unlike the mtDNA results, Dolly Varden and bull trout are reciprocally monophyletic (minimum 98% bootstrap support) at these nuclear loci (Redenbach 2000). Our demonstration of current hybridization between the species over a geographic area that includes sites with paraphyletic mtDNA and the clear distinction between the species in terms of nuclear sequences at two loci strongly hint that the presence of two divergent mtDNA clades in Dolly Varden is attributable to introgression of bull trout mtDNA into Dolly Varden. The pattern of mtDNA paraphyly in the presence of nuclear genome distinction is a classic signature of introgression of cytoplasmically inherited genomes documented in a variety of plants and animals (see Arnold (1997) for a review) including char (e.g., Bernatchez et al. 1995). Two observations suggest that that introgression of bull trout mtDNA into Dolly Varden includes ancient hybridization events as well ongoing hybridization. First, the bull-trout-like Dolly Varden mtDNA is found in areas that currently have no bull trout populations (Vancouver Island), and therefore, mtDNA introgression must have occurred in the past (cf. Wilson and Bernatchez 1998; Shimizu and Ueshima 2000). Second, the paraphyletic Dolly Varden and bull trout ND1 sequences are not identical to each other, which suggests that the mtDNA introgression must have occurred long enough ago for sequence polymorphism to acrue within the paraphyletic Dolly Varden sequences.

It is unlikely that either retention of ancestral polymorphism or convergent evolution of sequence variants could explain the paraphyletic relationships between mtDNAs of bull trout and Dolly Varden. First, the shared sequence variants are not distributed throughout the range of both species, the expected pattern for shared ancestral polymorphisms, but appear to be restricted to areas of current sympatry or to areas that are relatively short dispersal distances from sympatric sites. Second, the paraphyletic Dolly Varden and bull trout mtDNAs share six sequence variants, which necessitates six convergent substitutions to explain their similarity. This number of homoplasic events is clearly less parsimonious than similarity through introgression (cf. Bernatchez et al. 1995).

The distinction of Dolly Varden mtDNA into "northern" and "southern" groupings, with the southern grouping probably derived from mtDNA introgression from bull trout, is remarkably similar to variation in cpDNA between several species of plants in northwestern North America. Soltis et al. (1997) described northern and southern clade distinctions in five angiosperms and one fern and suggested that these clades probably stem from isolation in distinct refugia at the southern margins of the Pleistocene ice sheets and further north on the Olympic Peninsula, Queen Charlotte Islands, and (or) islands in the Gulf of Alaska. One herb species, *Tellima grandifora*, however, was unique because the southern group of populations appears to have aquired its chloroplast genome via ancient hybridization and cpDNA capture from a species of *Mitella* (reviewed by Soltis et al. 1997). This cpDNA capture and its possible analogous situation for mtDNA in Dolly Varden may have been facilitated by sympatry of char (and the plants) in southern glacial refugia or by interactions upon secondary contact.

Reciprocal laboratory crosses between Dolly Varden and bull trout apparently show no bias in viability of F1 offspring (Haas and McPhail 1991), which suggests that paraphyly of bull trout and Dolly Varden mtDNAs could result from bidirectional introgression. It appears, however, that a bias in introgression likely exists in the direction of bull trout introgression of mtDNA into the genome of Dolly Varden. First, the geographic distribution of Dolly Varden

mtDNA clades is not as tightly linked to geography as are those of bull trout (e.g., there is more overlap between clades in Dolly Varden). This suggests that vicariant events may not have been as important in structuring Dolly Varden mtDNA phylogenetic variation and that some other factor may best explain the geographic distribution of mtDNA variants. Second, of the six ND1 sequence sites that diagnose the paraphyletic Dolly Varden and bull trout clade, five are shared with the largely allopatric interior bull trout clade and none are shared with the largely allopatric "northwestern" Dolly Varden clade. Finally, where the two species are currently sympatric, Dolly Varden are typically much smaller at maturity than bull trout (e.g., McPhail and Taylor 1995; Baxter et al. 1997). Baxter et al. (1997) outlined a hypothesis that hybridization is asymmetrical, with smaller-bodied Dolly Varden males acting as "sneakers" during spawning between larger-bodied bull trout male–female pairs. Because mtDNA is, with few exceptions, maternally inherited (Avise 1994), such biased hybridization could provide a mechanism for introgression of bull trout mtDNA into Dolly Varden from backcrossing of F1 hybrids to Dolly Varden.

Population subdivision within bull trout

Bull trout exhibit relatively low levels of within-population variation at microsatellite loci; the number of alleles in each population tended to be low (one to three at all but *Sco* 19), and average heterozygosities were generally less than 50%. In anadromous Arctic char (*Salvelinus alpinus*) from Newfoundland and Labrador, the number of alleles at six loci across seven populations ranged from nine to 48 and expected heterozygosities from 0.19 to 0.97 (Bernatchez et al. 1998). Similarily high heterozygosities were observed at heterologous loci in Arctic char from alpine lakes in Europe (Brunner et al. 1998). Lower heterozygosities were observed in freshwater populations of brook trout (*Salvelinus fontinalis*) in lakes from south-central Quebec (Angers et al. 1995; Angers and Bernatchez 1998), but variability was still generally higher than that observed in bull trout. Low heterozygosity values were also observed in bull trout from the upper Columbia and Klamath rivers at four heterologous loci (P. Spruell, Division of Biological Sciences, University of Montana, Missoula, MT 59812, U.S.A., unpublished data) and in populations sampled from the Lake Pend d'Oreille system (average expected heterozygosities across loci ranged from 0.0 to 0.40; Spruell et al. 1999). These microsatellite data are consistent with earlier studies of allozymes (Leary et al. 1993) and mtDNA (Taylor et al. 1999) that reported relatively low levels of within-population variation at these independent loci. Low heterozygosity values at microsatellite loci in bull trout could result from inherently low mutation rates at these loci for this species, as microsatellites are not necessarily always "hypervariable" (e.g., see Schug et al. 1997). Alternatively, low variability may stem from demographic processes that have reduced effective population sizes in bull trout historically during and following postglacial dispersal (e.g., Hewitt 1996). Reduced population sizes through bottlenecks or founder events would have eliminated considerable allelic variation which has not yet recovered via mutation (cf. Schug et al. 1997).

Notwithstanding low allelic variation at microsatellite loci in bull trout, there is substantial variation among populations in the frequency of alleles that do exist. Average F_{st} values were typically greater than 0.3 and within each region ranged from 0.09 to 0.22. These values are typical of those reported for many other salmonids (e.g., see Wenburg et al. 1998). Our overall value of $F_{st} = 0.33$ is very similar to the value of 0.37 reported by Angers and Bernatchez (1998) for 26 lake populations of brook trout in Quebec. An estimate of effective population size (N_e) can be obtained using the expression $\hat{N}_e \approx H/(1 - H)/4\mu$, where H is heterozygosity and μ is the mutation rate (see Waples 1998). We used the average expected heterozygosity across loci for all populations (0.33) excluding the Harrison Lake population, which was monomorphic at all loci, and a mutation rate of 10^{-4}, which is typical for many microsatellite loci (e.g., reviewed in Feldman et al. 1999). This expression yields an estimated N_e of about 1200 fish among the tributaries that we sampled within each of the Columbia/Kootenay and Pine watersheds. Our estimates of migration among populations under an island model of population structure were typically less than five individuals within each of the Pine and Kootenay watersheds. These data, although subject to a number of assumptions (see Waples 1998; Whitlock and McCauley 1999), strongly suggest that bull trout populations are highly isolated from each other demographically and genetically (cf. Spruell et al. 1999).

Our data suggest that genetic subdivision was greater within the Peace region than in the Columbia/Kootenay, which was surprising given the greater geographic distance among the latter samples. Greater differentiation in the Peace may stem from the inclusion of samples from above impassable (apparently) waterfalls in Chamberlain Creek and Upper Brazion Creek. By contrast, at least between Ram Creek and Skookumchuck Creek on the Kootenay River, fewer habitat structure induced impediments to bidirectional gene flow appear to exist. This hypothesis is being tested by assaying more populations while also characterizing sample sites in terms of geomorphological features that might aid in understanding what landscape features might organize variation within and between populations of bull trout (cf. Angers et al. 1999).

Conservation implications

Our analyses demonstrate the widespread sympatry of Dolly Varden and bull trout over much of their largely parapatric geographic ranges. The two species hybridize in areas of contact and some gene flow (introgression) occurs between them (Baxter et al. 1997; Redenbach 2000) and presumably has occurred at least since postglacial times (i.e., over the last 10 000 – 15 000 years). Despite such gene flow, Dolly Varden and bull trout are clearly distinct gene pools and diagnosable over their ranges and, in particular, in areas of sympatry. Distinction in sympatry and in the face of gene flow is the "acid test" of the biological species concept (Avise 1994), and consequently, the recognition of Dolly Varden and bull trout as distinct species is clearly warranted. Nevertheless, it is also clear that the two species' evolutionary histories have not been completely independent. Rather, our phylogeographic analyses suggest that their evolutionary genetic interactions have been frequent and continue to the present day. It is possible that such interactions may have

contributed novel genotypes to both species and to their respective evolutionary trajectories and that such processes may act as creative forces in the current biodiversity of both taxa (Arnold 1997). Our analyses do caution, however, that similar patterns of mtDNA diversification (e.g., two divergent lineages within both Dolly Varden and bull trout) can stem from different causes (introgression versus vicariance, respectively). Different evolutionary causes of lineage diversification within species deserve recognition, particularly with respect to mtDNA, which is often used as a basis for designation of conservation units below the species level (e.g., Moritz 1994).

On a much finer scale of geographic variation, microsatellite variation in bull trout demonstrates that significant amounts of genetic subdivision exist among populations within relatively small areas (e.g., within the Pine River drainage) as well as across larger areas (between the Peace and Kootenay drainages) within a single phylogenetic lineage (i.e., the interior bull trout clade of Taylor et al. 1999). These data imply a high degree of demographic independence among populations within watersheds, an inference that should be considered during management plans for these watersheds. For instance, our data suggest that populations undergoing large reductions in population size will not be "rescued" from extinction by migration from other populations, at least in the short term (also see Spruell et al. 1999). Further, the low levels of molecular variation in bull trout (also seen in other areas for microsatellites, in mtDNA, and allozymes) strongly hint that bull trout have been subjected to large and repeated reductions in effective population size. This may reflect their relatively recent postglacial dispersal into the upper Columbia/Kootenay and Peace River watersheds from refugia outside British Columbia (see Taylor et al. 1999), with bottlenecks and founder events during colonization reducing variation (Hewitt 1996). Reduced variation, in addition to the evidence for recent bottlenecks from our analysis, may indicate that bull trout are vulnerable to potentially deleterious effects of reduced population size, i.e., the molecular data suggest periodic reductives in population sizes that may foster inbreeding (Saccheri et al. 1998). Consequently, bull trout may be a species that is especially sensitive to human-induced reductions in population size that exceed the apparently natural historical reductions inferred from our data.

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Appendix

Table A1. Polymorphic sites in 503 basepairs of aligned ND1 sequences for char sampled in the study.

					11	11	11	222	22	23	33	33	34	44	45	55
	11	22	24	68	14	45	56	113	78	90	00	15	54	56	70	00
	759	23	55	60	01	31	68	261	27	10	35	44	91	03	10	13
DV 1	TGG	TT	GT	CA	CT	GG	CT	ACT	CT	CA	TC	AG	CG	AT	TG	GC
DV 2	.C.	G.	..	..	..	..	..	...	..	..	..	..	..	..	..	..
DV 3	...	..	CC	..	..	..	..	...	..	..	..	..	..	..	..	..
DV 4	...	..	..	..	..	..	..	...	T.	..	..	..	..	..	..	..
DV 5	.C.	..	T.	.G	..	..	..	...	..	..	.T	..	T.	..	..	..
DV 6	...	..	A.	..	..	..	..	...	..	..	.T	G.	.A	..	CA	.T
DV 7	...	G.	..	..	..	..	..	...	..	..	.T	G.	.A	..	CA	.T
DV 8	...	..	..	..	..	..	..	...	..	..	..	..	.A	..	CA	.T
DV 9	.C.	..	A.	..	..	..	..	...	..	..	..	.A	..	..	..	..
DV 10	.C.	..	A.	T.	..	..	..	...	..	..	..	..	..	..	..	..
DV 11	CC.	..	..	..	..	..	..	...	..	..	..	.A	..	..	..	..
DV 12	...	..	..	T.	..	..	..	...	..	..	.T	G.	..	..	..	..
DV 13	...	..	A.	..	..	..	T.	...	..	..	.T	G.	TA	..	CA	.T
DV 14	...	..	..	..	..	..	..	...	..	..	..	..	.A	..	CA	.T
DV 15	..A	..	..	..	..	..	..	...	..	..	C.	..	..	..	..	..
DV 16	..A	.A	..	..	..	..	..	...	..	..	.T	G.	..	..	..	..
DV 17	...	..	..	..	..	..	..	...	..	..	.T	G.	TA	.C	CA	.T
BT C-1	...	..	..	T.	..	..	..	...	..	..	.T	G.	.A	G.	CA	.T
BT C-2	...	..	..	T.	..	..	..	...	..	..	.T	G.	.A	G.	CA	.T
BT C-3	...	..	..	..	..	.A	..	...	..	..	.T	G.	TA	..	CA	.T
BT I-1	...	..	..	..	..	..	.C	...	..	T.	.T	G.	.A	..	.A	.T
BT I-2	...	..	..	..	..	..	..	...	..	T.	.T	G.	.A	..	.A	.T
BT I-3	...	..	..	..	..	T.	.C	...	..	T.	.T	G.	.A	..	.A	TT
Lake Trout	.	.	.	.	T	C	.	.	.	G	T	C	.	C	.	G	.	T	G	.	.	.	.	.	A	.	.

Note: The region sequenced includes a portion of the 16S rRNA gene (basepairs 1–199), Leu-tRNA (basepairs 200–275), and the NADH-1 gene (basepairs 276–503). Haplotype names refer to those in Fig. 2.

Table A2. Allele frequencies at five microsatellite loci in eight bull trout populations.

	Locus and alleles (basepairs)																
	Omy 77				*Sfo* 18			*Ssa* 197			*Sco* 19				*Sco* 23			
Population	*N*	279	283	287	*N*	150	156	*N*	119	123	*N*	174	200	RA	*N*	185	191	193
HL	43	0.000	1.000	0.000	43	1.000	0.000	43	0.000	1.000	42	0.000	1.000	0.000	43	0.000	1.000	0.000
IR	30	0.300	0.700	0.000	30	1.000	0.000	30	0.483	0.517	30	0.000	0.717	0.283	30	0.033	0.350	0.617
SC	36	0.000	0.681	0.319	36	0.847	0.153	36	0.806	0.194	35	0.100	0.757	0.143	35	0.000	0.243	0.757
RC	45	0.000	0.422	0.578	45	0.944	0.056	46	0.641	0.359	45	0.000	0.844	0.156	46	0.000	0.000	1.000
CC	16	0.000	1.000	0.000	17	0.500	0.500	16	0.063	0.938	17	0.265	0.735	0.000	17	0.000	0.265	0.735
NBR	21	0.000	0.643	0.357	21	0.500	0.500	21	0.500	0.500	21	0.024	0.952	0.024	21	0.000	0.519	0.481
UBC	22	0.000	0.273	0.727	25	0.280	0.720	24	0.042	0.958	22	0.022	0.913	0.065	26	0.000	0.519	0.481
BR	15	0.000	0.900	0.100	16	0.406	0.594	15	0.389	0.611	36	0.056	0.861	0.083	18	0.000	0.694	0.306

Note: Sample sites: HL, Harrison Lake; IR, Ice River; SC, Skookumchuck Creek; RC, Ram Creek; CC, Chamberlain Creek; NBR, North Burnt River; UBC, Upper Brazion Creek; BR, Burnt River. To conserve space, nine alleles at *Sco* 19 (158, 190, 192, 198, 202, 204, 206, 210 and 216 basepairs) have been pooled under the designation "RA" for rare alleles.