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EVIDENCE FOR HISTORICAL INTROGRESSION ALONG A CONTACT ZONE BETWEEN TWO SPECIES OF CHAR (PISCES: SALMONIDAE) IN NORTHWESTERN NORTH AMERICA

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Abstract.—Phylogeographic analyses can yield valuable insights into the geographic and historical contexts of contact and hybridization between taxa. Two species of char (Salmonidae), Dolly Varden (*Salvelinus malma*) and bull trout (*S. confluentus*) have largely parapatric distributions in watersheds of northwestern North America. They are, however, sympatric in several localities and hybridization and some introgression occurs across a broad area of contact. We conducted a comparative phylogenetic analysis of Dolly Varden and bull trout to gain a historical perspective of hybridization between these species and to test for footprints of historical introgression. We resolved two major Dolly Varden mitochondrial DNA (mtDNA) clades (with 1.4–2.2% sequence divergence between haplotypes) that had different geographical distributions. Clade N is distributed across most of the range of Dolly Varden, from southern British Columbia through to the Kuril Islands in Asia. Clade S had a much more limited distribution, from Washington state, at the southern limit of the Dolly Varden range, to the middle of Vancouver Island. The distribution and inferred ages of the mtDNA clades suggested that Dolly Varden survived the Wisconsinan glaciation in a previously unsuspected refuge south of the ice sheet, and that Dolly Varden and bull trout were probably in continuous contact over most of the last 100,000 years. When bull trout were included in the phylogenetic analysis, however, the mtDNA of neither species was monophyletic: Clade S Dolly Varden clustered within the bull trout mtDNA clade. This pattern was discordant with two nuclear phylogenies produced (growth hormone 2 and rRNA internal transcribed sequence 1), in which Dolly Varden and bull trout were reciprocally monophyletic. This discordance between mtDNA- and nDNA-based phylogenies indicates that historical introgression of bull trout mtDNA into Dolly Varden occurred. Percent sequence divergence within these introgressed Dolly Varden (clade S) was 0.2–0.6%, implying that the introgression occurred prior to the most recent glaciation. Our analysis and other evidence of contact between divergent lineages in northwestern North America strongly suggests that the area may be the site of previously unsuspected suture zones of aquatic biotas.

Key words.—Glaciation, hybridization, hybrid zones, introns, mitochondrial DNA, *Salvelinus*.

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Phylogeography is the study of the geographic distributions of evolutionary lineages and the geographic processes that influence these distributions with a focus on diversity within and between closely related species (Avise 2000). One factor that can influence phylogeographic inference within species is hybridization and introgression between taxa. Conversely, adopting a phylogeographic perspective can yield important insights into the historical and geographic contexts of hybridization (Butlin 1998; Rieseberg 1998). For example, phylogeographic analyses have demonstrated that taxa have come into contact after variable periods of allopatry (e.g., Dowling et al. 1997; Latta and Mitton 1999; Irwin et al. 2001). In some instances, secondary contact is accompanied by hybridization and introgression that may be ongoing or that may have left an evolutionary footprint on current genetic structure (e.g., Arnold 1997; Soltis et al. 1997; Wilson and Bernatchez 1998). Such interactions are often associated with recently glaciated areas where taxa have come into contact postglacially (e.g., Remington 1968; Barton and Hewitt 1985; Hewitt 1989; Latta and Mitton 1999).

Northwestern North America was heavily glaciated during the most recent (Wisconsinan) glacial period, and there is evidence that many taxa currently distributed in this area persisted in two or more glacial refugia (e.g., McPhail and Lindsey 1970; Taylor et al. 1996, 1999; Soltis et al. 1997; Stone and Cook 2000). However, there have been few investigations of genetic interactions between taxa following probable secondary contact in this region. One notable exception concerns work on plants that indicated secondary contact and current and past hybridization and introgression between taxa (e.g., Soltis et al. 1997; Latta and Mitton 1999). Contact zones in a wider variety of taxa are important to investigate because they can reveal the ecological and genetic processes influencing reproductive isolation (e.g., Irwin et al. 2001), especially if the process is incomplete, as in hybrid zones (Szymura and Barton 1991; Jiggins and Mallet 2000).

Char (*Salvelinus*) are salmonid fishes with a Holarctic distribution that has been heavily influenced by Pleistocene glaciations. Several studies indicate isolation and postglacial dispersal from two or more refugia (e.g., Wilson and Hebert 1998; Taylor et al. 1999; Brunner et al. 2001) as well as secondary contact and hybridization between species (Hammar et al. 1991; Bernatchez et al. 1995; Wilson and Bernatchez 1998). The long history of confusion surrounding *Salvelinus* ''species complexes'' (McPhail 1961; Behnke 1980) and conflicting molecular phylogenies (e.g., Grewe et al. 1990; Phillips et al. 1999) may, in fact, be at least partly due to current hybridization between sympatric species pairs (Arnold 1997).

Dolly Varden char (*S. malma*) and bull trout (*S. confluentus*) are native to drainages of the north Pacific and have largely parapatric distributions in northwestern North America. Dolly Varden are generally coastal in distribution from the western Pacific to Alaska and south to the Olympic Peninsula,

Washington. Bull trout have a more inland distribution from Yukon and Northwest Territories, Canada, to northern California and Nevada. Although largely parapatric, they live sympatrically in areas along the Cascade and coastal mountain crests in northwestern North America (see Baxter et al. 1997). For many years, Dolly Varden and bull trout were considered to be geographic variants within the Arctic char (*S. alpinus*) species complex. McPhail's (1961) morphological analysis, however, described a southern form (Dolly Varden) within this complex, which became known as the ''*S. malma* species complex.'' Subsequently, Cavender (1978) showed that within the species complex in northwestern North America were two forms that were sufficiently divergent in morphology to be designated distinct species: *S. malma* and *S. confluentus*. Judging from comparative molecular sequence analyses, Dolly Varden and bull trout are not sister-species and probably last shared a common ancestor more than 1 million years ago (Grewe et al. 1990; Phillips et al. 1995).

Baxter et al. (1997) presented the first genetic evidence of natural hybridization between bull trout and Dolly Varden. Subsequently, the two species have been shown to hybridize along an extensive area of (presumably) secondary contact in northwestern North America (Taylor et al. 2001). In sympatry, the species remain genetically distinct despite some gene flow between them (Z. Redenbach and E. B. Taylor, unpubl. data). Although the phylogeography of bull trout has been well studied (e.g., Leary et al. 1993; Taylor et al. 1999), the phylogeography of Dolly Varden has received less attention (Phillips et al. 1999). In fact, our knowledge of the distribution of Dolly Varden continues to expand as data accumulate that populations exist in watersheds farther south (Leary and Allendorf 1997) and east (Baxter et al. 1997; Z. Redenbach and E. B. Taylor, unpubl. data) of the currently described range. The majority of southern Dolly Varden populations are present only in recently deglaciated areas. The current distribution and the lack of Dolly Varden in all but the extreme headwaters of interior drainages imply that Dolly Varden recolonized much of the west coast North America from a northern refuge, likely Beringia (McPhail and Lindsey 1970). By contrast, bull trout probably survived Pleistocene glaciations in at least two refugia at the southern edges of the Cordilleran ice sheet: the Chehalis River valley and the lower Columbia River valley (Taylor et al. 1999). The question of whether Dolly Varden survived in a second refuge south of the ice sheets is important because it determines whether Dolly Varden and bull trout have been in contact continuously throughout the Pleistocene glacial events or whether the current sympatry is a zone of secondary contact created only in the last 14,000 years. Because natural selection may strengthen or reinforce reproductive isolation after secondary contact (e.g., Butlin 1989; Rundle and Schluter 1998), areas colonized by allopatric refugial populations could have significantly different levels of current hybridization than areas of historical sympatry and help to reveal the processes involved in the evolution of reproductive isolation, for example, the potential role of reinforcement.

The objectives of our research were twofold. First, we wished to test the hypothesis that Dolly Varden are subdivided into two or more evolutionary lineages stemming from isolation in distinct glacial refugia. Strong phylogeographic patterns have been demonstrated for other northwestern North American species (e.g., Taylor et al. 1996, 1999; Soltis et al. 1997; Stone and Cook 2000) and phylogeographic assessment of Dolly Varden may help clarify the role of secondary contact versus range expansion from a common refuge in generating hybrid zones (Rieseberg 1998). Second, given current hybridization and introgression between Dolly Varden and bull trout, as well as the dynamic geological setting for char hybrid zones in northwestern North America, we wished to test for a historical component to hybridization in this area. To these ends, we conducted a comparative phylogeographic survey of DNA sequence variation at mitochondrial (mtDNA) and nuclear (nDNA) loci in Dolly Varden and bull trout to assess the level of genetic and geographic concordance in lineage distinctions. Discordance in cytoplasm- and nuclear-based phylogenies has been used in a variety of taxa to implicate historical introgression (Arnold 1997; Rieseberg 1998). Relatively few of these analyses, however, have been conducted for animal taxa, particularly in western North America, where the potential for such interactions is high owing to repeated opportunities for isolation and secondary contact following Pleistocene glacial events. Investigation of such contact zones is important for a number of reasons. First, they can help to understand the biogeographic and evolutionary history of particular lineages and their interactions, such as those in northwestern North America. In addition, analysis of contact zones in different areas contributes to a comparative database that can help determine whether evolutionary processes, such as hybridization and reinforcement, are general features of contact zones or particular to specific taxa or areas (e.g., Hewitt 2001).

Materials and Methods

Sample Collection

Dolly Varden samples were obtained from throughout their range, from Washington state, British Columbia, Alaska, and the Kuril Islands in the western Pacific (Table 1). Bull trout sequences for this study were taken from Taylor et al. (1999) and were chosen to represent the two major mtDNA clades present throughout their range (Table 1). Other *Salvelinus* species were collected opportunistically or their sequences were obtained from the literature (Table 1; see Results).

Molecular Methods

The DNA was extracted from 20 mg of adipose or caudal fin tissue by overnight digestion at 37°C in a 0.5% sarcosyl/0.2 M EDTA solution with Pronase (Roche Diagnostics, Laval, Canada), followed by a single phenol/chloroform extraction (as described in Taylor et al. 1996). DNA was precipitated in isopropanol, washed in 70% EtOH, and the DNA pellet was either dried in a vacuum-condenser for 5 min or left overnight to air dry. The pellet was resuspended in TE buffer (pH 8.0; amount depending on the relative size of the DNA pellet) and stored at −20°C.

Three loci were sequenced for this study (Table 2). Growth hormone 2 (GH2) is a coding gene, and the GH2 primers amplify intron C (McKay et al. 1996). The ribosomal DNA

TABLE 1. Sample localities, sample sizes, numbers of northern (clade N) and southern (clade S) haplotypes, and names of Dolly Varden mitochondrial (mtDNA) haplotypes resolved in phylogeographic analyses. Sample sizes indicate the number of mtDNA haplotypes sequenced/those assayed by restriction fragment length polymorphism analyses. Also shown are the names of Dolly Varden mtDNA haplotypes that were assayed for growth hormone (GH) and ribosomal DNA ITS (rDNA) sequence variation, and the locations of lake trout (mtDNA, GH, rDNA) and Arctic char (GH) that were sequenced. Numbers following sample location names are sites shown in Figure 2. NA, not applicable; UD, undetermined mtDNA sequence haplotype; only diagnostic Clade N or S nucleotide sites were resolved. Sequences marked with an asterisk are incomplete.

Sample	Drainage	*N*	Clade N	Clade S	mtDNA	GH	rDNA
Dolly Varden							
Udobnaya River (1)	Kuril Islands, Russia	1/4	5		DV2	DV2	DV2
Porozhistaya River (2)	Kuril Islands, Russia	1/3	4		DV2		
Little Togiak Lake (3)	Bristol Bay, AK	1/5	6		DV1		
Iliamna Lake (4)	Bristol Bay, AK	2/5	7		DV1, DV3		
Klutina River (5)	Copper River, AK	1/5	6		DV1		
Bonnet Plume River (6)	Peel River, Yukon	/10	10				
Blackstone River (7)	Peel River, Yukon	/5	5				
Tulsequah River (8)	Taku River, BC	3/4	7		DV1, 6, 10	DV10	DV10
Iskut River (9)	Stikine River, BC	1/2	3		DV1		
Tahltan River (10)	Stikine River, BC	1/	1		DV6		
Chutine River (11)	Stikine River, BC	1/	1		DV9		
Zolzap River (12)	Nass River, BC	/10	9	1			
Omineca River (13)	Upper Peace River, BC	1*/		1	UD		
Ogden Channel (14)	Central coast, BC	1/	1		DV11		
Noyes Sound (15)	Central coast, BC	1/	1		DV8		
Ecstall River (16)	Skeena River, BC	1*/2	3		UD		
Ayton Creek (17)	Skeena River, BC	1/	1		DV6	DV6	DV6
Brent Creek (18)	Queen Charlotte Islands, BC	1/	1		DV1		
Aero River (19)	Queen Charlotte Islands, BC	1/	1		DV1		
Feather Creek (20)	Queen Charlotte Islands, BC	/2	2				
Honna River (21)	Queen Charlotte Islands, BC	1/	1		DV5		
Three Mile Creek (22)	Queen Charlotte Islands, BC	/1	1				
Ian River (23)	Queen Charlotte Islands, BC	/5	5				
Ain River (24)	Queen Charlotte Islands, BC	/5	5				
Kumealon Creek (25)	Midcoast, BC	1/	1		DV1		
Noosneck River (26)	Midcoast, BC	1/5	6		DV4		
Ocean Falls (27)	Midcoast, BC	/11	11				
Wakeman River (28)	South coast, BC	/2		2			
Dallery Creek (29)	Midcoast, BC	1/5	2	4	DV1		
Southgate River (30)	South coast, BC	1/		1	DVB	DVB	DVB
Upper Deserted River (31)	South coast, BC	/4	4				
O'Connell Lake (32)	Vancouver Island, BC	1/8	9		DV1		
Claninick River (33)	Vancouver Island, BC	1/2	3		DV1		
Keogh River (34)	Vancouver Island, BC	/10	5	5			
Eve River (35)	Vancouver Island, BC	/5		5			
Misty Lake (36)	Vancouver Island, BC	1/	1		DV1		
Zeballos River (37)	Vancouver Island, BC	1/3	4		DVE		
Jessie Lake (38)	Vancouver Island, BC	/1		1			
Thelwood Creek (39)	Vancouver Island, BC	1/5	2	4	DVC		
Phillips River (40)	Vancouver Island, BC	1/5	1	5	DV7		
Cowichan Lake (41)	Vancouver Island, BC	1/4	5		DV1	DV1	DV1
Mamquam River (42)	South coast, BC	1/		1	DVB		
Mill Creek (43)	South coast, BC	1/3		4	DVD	DVD	DVD
Capilano River (44)	South coast, BC	1*/2		3	UD		
Seymour River (45)	South coast, BC	1*/2		2	UD		
Loon Lake (46)	South coast, BC	/8		8			
Silverhope Creek (47)	South coast, BC	1/		1	DVB		
Quinault River (48)	Olympic Peninsula, WA	1/8		9	DVA		
Dungeness River (49)	Olympic Peninsula, WA	/5		5			
South Fork Nooksack River (50)	Puget Sound, WA	/5		5			
Bull trout							
Silverhope Creek (51)[1]	South coast, BC				BTCa		
Squamish River (52)[2]	South coast, BC				BTCb		
Metiolus River (53)	Lower Columbia River, OR				BTCc		
Bear Creek (54)	Snake River, OR				BTIa		
Upper Anderson Creek (55)	Upper Fraser River, BC				BTIc		
Hotel Creek (56)	Upper Liard River, BC				BTIb		
Miscellaneous char							
DV × BT F_1 hybrid	Upper Peace River, BC	1/			BTIa		
Lake trout	Nation River (Upper Peace River, BC)	1/	NA	NA	NA	NA	NA
Arctic char	Norway	NA	NA	NA	NA	NA	NA

[1] Overlaps location of Dolly Varden sample 46–47.
[2] Overlaps location of Dolly Varden sample 42–43.

TABLE 2. Primers, polymerase chain reaction conditions, and sources of molecular markers used in DNA sequence analyses of Dolly Varden and bull trout. Loci are growth hormone (GH), internal transcribed spacer 1 (rDNA, ITS-1), and the mitochondrial NADH-1 subunit (ND1). All sequences are written 5′–3′.

Primer	Sequence	Cycles/annealing temp (°C)	$MgCl_2$ (mM)	Source
GH7[1]	CTT ATG CAT GTC CTT CTT GAA			McKay et al. (1996)
GH51	GTC AAG CTG ATA CAA CTC	40/50	1.5	
MD-1	CTT GAC TAT CTA GAG GAA GT			
ITS-R	AGC TTG CTG CGT TCT TCA TCG A	40/52–55	1.5	Phillips et al. (1995)
KP-2[2]	AAA AAG CTT CCG TAG GTG AAC CTG C			
ND1F	GGT ATG GGC CCG AAA GCT TA			Neilsen et al. (1988)
ND1R	GCC TCG CCT GTT TAC CAA AAA CAT	35/54	2.0	
ND1C[1]	TGC AGC CGC TAT TAA GGG TTC G	NA	NA	Redenbach and Taylor (1999)

[1] Used as sequencing primer.
[2] Used as sequencing primer for rDNA.

(rDNA) primers (ITS-1) amplify the first internally transcribed region of spacer DNA between rDNA genes and have been widely used in char molecular systematics (e.g., Phillips et al. 1995). Finally, the mtDNA primers amplified an approximately 2-kb fragment consisting of several mitochondrial genes: a portion of the $tRNA^{Gln}$ gene, the entire $tRNA^{Ile}$, NADH dehydrogenase subunit 1, and $tRNA^{Leu\text{-}1}$ genes, and a portion of the 16S rRNA gene. We sequenced approximately 570 bp of this fragment including the Gln, Ile tRNAs, and the 5′ end of the NADH-1 gene (Appendix 1).

Polymerase chain reactions (PCRs) were run with varying conditions for the three markers (Table 2). A typical reaction consisted of a total volume of 75 μl, with 800 mM of total dNTPs (200 mM for GH2), 0.6 mM of each primer (0.25 mM for GH2), 1.5 mM $MgCl_2$ (2.0 mM for ND1), 1× *Taq* DNA polymerase buffer (Gibco, Invitrogen, Burlington, Canada), 3.75 units of *Taq* DNA polymerase, and 1 μl of whole DNA. Full details of PCR conditions for each locus are given in Taylor et al. (2001). Sequencing reactions for mtDNA, GH, and rDNA were performed as outlined in Taylor et al. (2001) and Redenbach and Taylor (1999).

The two mtDNA sequence clades resolved (see below) could be diagnosed by incubating the 2.0 kb amplicon with *Hae* III or *Hin*f I to resolve clade-specific restriction fragment length polymorphisms (RFLPs). These diagnostic RFLPs consisted of 550-, 490-, 300-, 200-, 190-, and 100-bp fragments in one clade and 550-, 380-, 240-, 200-, 190-, and 100-bp fragments in the other clade (plus several smaller fragments) using *Hae* III. We used the *Hae* III diagnostic RFLPs to further define the geographic distribution of each clade in larger samples (up to $N = 10$) from throughout the range of Dolly Varden.

Phylogenetic Analysis

We analyzed all three sequenced molecular markers using the PHYLIP computer analysis package (Felsenstein 1995). We calculated sequence divergences using DNADIST in PHYLIP applying the Kimura two-parameter distances model of nucleotide substitution (Kimura 1980). Data were subjected to bootstrapping (1000 replicates, SEQBOOT), and analyzed by a distance tree-building program (NEIGHBOR, using neighbor-joining method and randomizing input order). The 1000 resulting NEIGHBOR trees were combined using majority-rule consensus tree analyses (CONSENSE), with specified outgroups, as described in the Results. Bootstrap values greater than 70% were considered to be strong support, as they generally correspond to a greater than 95% probability that a clade is consistently resolved (Hillis and Bull 1993). 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. We calculated haplotype diversity (Nei 1987), nucleotide diversity, and nucleotide divergences (Nei and Tajima 1981) within and between mtDNA haplotype clades and char species using the DA program of REAP (McElroy et al. 1992).

RESULTS

Mitochondrial DNA Phylogeography

Thirty-seven Dolly Varden sampled from Washington to the Kuril Islands were sequenced for 570 bp of the mitochondrial tRNA/NADH-1 genes (Table 1). We resolved 16 haplotypes (labeled DV-1 to DV-11 and DV-A to DV-E; Table 1, Appendix 1). Most haplotypes were present in only one individual, with the exception of DV-1, DV-2, DV-6, and DV-B, which were found in 13, two, three, and three individuals, respectively. Phylogenetic analysis of the mtDNA haplotypes revealed that Dolly Varden and bull trout are paraphyletic. Dolly Varden haplotypes fell into two clades, clade N (for northern, see below) and clade S (for southern), both of which were found in more than 90% of the bootstrap replicates (Fig. 1). Diagnostic point mutations identified by sequencing also supported each of the major clade distinctions in the tree (Appendix 1). Clade N was monophyletic, contained 11 haplotypes obtained from 28 individuals, and had 92% bootstrap support. The Dolly Varden clade S, however, clustered with all coastal bull trout mtDNA haplotypes (BT-Ca-c; Fig. 1) rather than with Dolly Varden clade N haplotypes. Dolly Varden haplotype DV-B was, in fact, identical in sequence to BT-Ca, one of the three coastal bull trout haplotypes (Taylor et al. 1999). Bootstrap support for the clade encompassing coastal bull trout and clade S was 63% and included a diagnostic point mutation (a T-C transition).

There was no derived mutation shared by all clade S Dolly Varden to distinguish them from coastal bull trout; they had only the lack of a diagnostic mutation to distinguish them

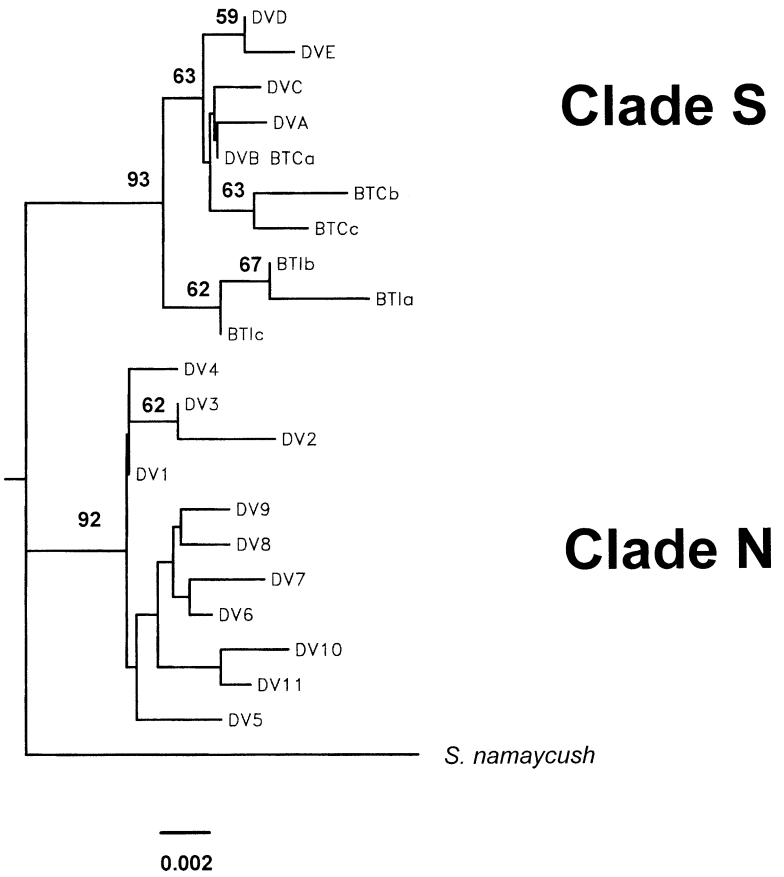

FIG. 1. Phylogenetic tree of *Salvelinus* mitochondrial DNA rooted with *S. namaycush* based on neighbor-joining analysis of Kimura two-parameter distance inferred from 570 bp of the tRNA-Gln (36 bp), tRNA-Ile (73 bp), and NADH-1 (453 bp) genes. Bootstrap support levels from 1000 resamplings are reported (where more than 50%) at branch points. Haplotypes are defined in Table 1 and Appendix 1. *Salvelinus confluentus* (BT-I, interior, and BT-C, coastal) haplotypes were obtained from Taylor et al. (1999). Scale bar represents units of genetic distance.

from bull trout haplotypes (a T-C transition present in BT-Cb and BT-Cc at position 359, Appendix 1). The paraphyly of Dolly Varden mtDNA was also supported by maximum-likelihood and parsimony analyses (phenograms not shown) and constraining trees to monophyly of clade N and clade S monophyly was significantly worse (ln likelihood = −1034.9) than the most likely tree (ln likelihood = −996.6, $P < 0.05$). Bull trout haplotypes (from Taylor et al. 1999) are paraphyletic as well, with the interior clade (BT-I) being monophyletic (62% support) and the coastal clade (BT-C) paraphyletic with clade S Dolly Varden, as described above. The interior clade of bull trout also included a diagnostic C-T mutation at position 291. A Dolly Varden/bull trout hybrid with an F_1 genotype, as determined by heterozygosity at each of four nuclear loci (Redenbach 2000), had a mtDNA sequence haplotype identical to BT-Ia. Restriction site differences between clades S and N were resolved with *Hae* III to further define their geographic distributions, and haplotypes from clade S occurred over a smaller area than those of clade N (Fig. 2). Clade S was generally found only in the south of British Columbia and in Washington. An exception was a newly discovered population of Dolly Varden in the upper Omineca River headwaters of the Peace River (Fig. 2). Clade N was distributed from the southern tip of Vancouver Island to the Kuril Islands in the western Pacific, encompassing almost the entire range of Dolly Varden (the island of Hokkaido was not sampled). Twelve individuals with the northern haplotype, DV-1, were distributed from Vancouver Island to Bristol Bay, Alaska (Fig. 2, Table 1).

There was about the same amount of sequence divergence between bull trout haplotypes (0.2–1.6%) as between clade N Dolly Varden haplotypes (0.2–1.4%). Only six haplotypes, however, were found in 22 bull trout individuals collected throughout the bull trout range (Taylor et al. 1999), whereas 11 clade N haplotypes were found in 24 Dolly Varden individuals (if clade S is included, 16 Dolly Varden haplotypes were found in 37 individuals with an increased maximum sequence divergence of 2.2%). Haplotype diversity was lower in bull trout (0.58 ± 0.080; Taylor et al. 1999) than Dolly Varden (0.73 ± 0.064). Even clade S Dolly Varden mtDNA, which falls within the coastal bull trout clade phylogenetically, has higher haplotype diversity than bull trout (0.79 ± 0.09). Sequence divergence and nucleotide diversity within clade N (0.2–1.4%, 0.38%) were greater than those within clade S (0.2–0.6%, 0.26%). The haplotype diversities within each of the two clades, however, were very similar (0.73 ± 0.064 and 0.79 ± 0.086).

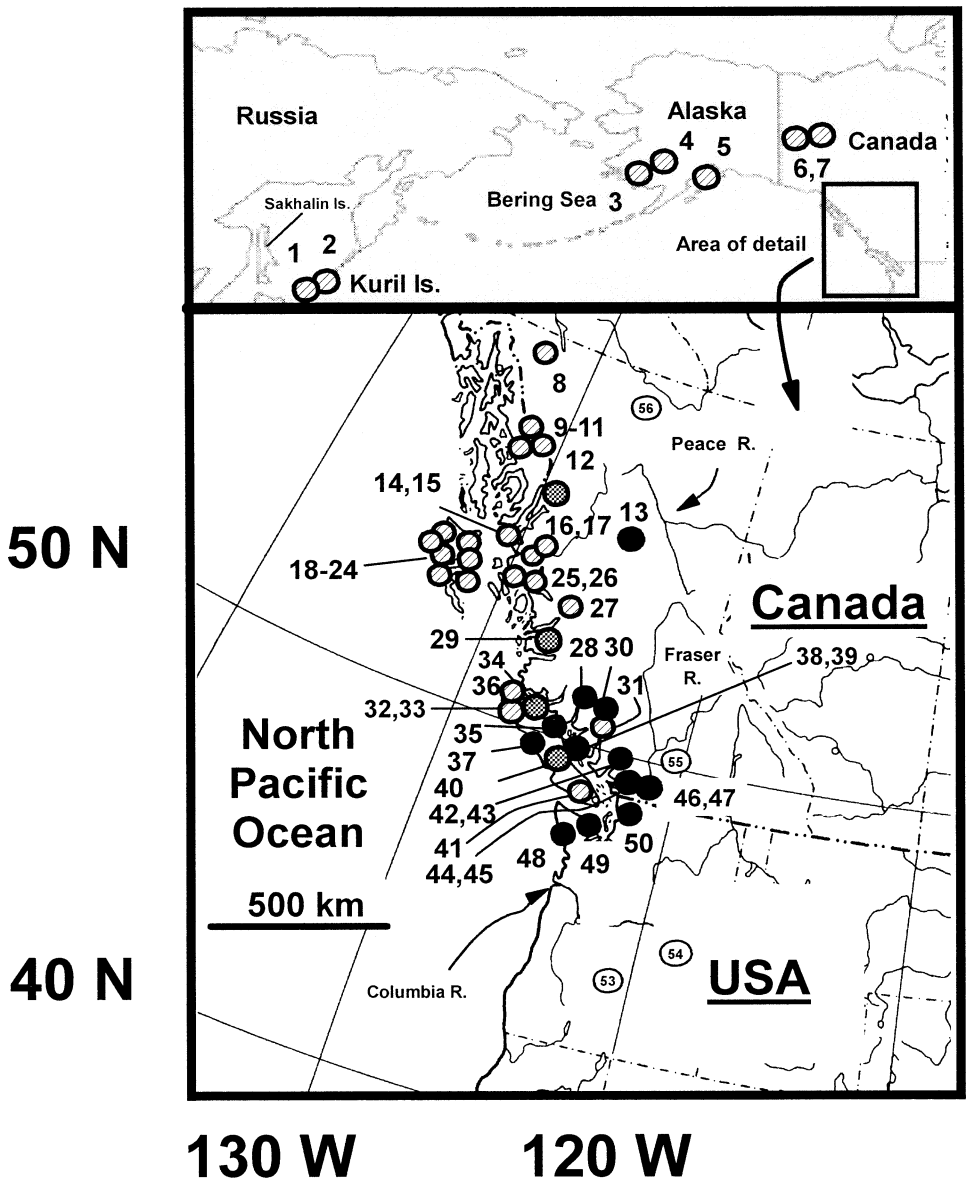

FIG. 2. Geographic distribution of Dolly Varden mitochondrial DNA clades. Black symbols represent clade S, hatched symbols clade N, and cross-hatched symbols are mixed sites. Sample localities by numbers are defined in Table 1.

Ribosomal DNA ITS-1 and GH2 Phylogenies

The individuals sequenced at GH2 and ITS-1 were specifically chosen for their mtDNA haplotype and collection location, so that the two Dolly Varden and two bull trout mtDNA clades would be well represented (Table 1). Phylogenetic analysis of 484 base pairs of GH2 showed Dolly Varden and bull trout to be reciprocally monophyletic, with significant bootstrap support (Fig. 3a). Bootstrap support for the bull trout clade, grouping coastal and interior bull trout, was 99% in the GH2 tree. Bootstrap support for the Dolly Varden clade was 99%, and consisted of individuals of the mtDNA clades N and S from southern British Columbia to the Kuril Islands. In fact, Dolly Varden individuals with four different mtDNA haplotypes (DV-1, DV-10, DV-B, and DV-D) sampled from British Columbia yielded identical GH2 sequences (Appendix 2). Overall sequence divergence within the Dolly Varden clade ranged from 0.00% to 0.42%. Within bull trout sequence divergence was similarly small (0.21%), whereas sequence divergence between Dolly Varden and bull trout ranged from 4.31% to 5.00%. Lake trout GH sequence, used as the outgroup, was less divergent in sequence from Dolly Varden (1.47–1.90%) than from bull trout (4.56–4.79%).

In the analysis of the 410-bp rDNA ITS-1 sequence, Dolly Varden and bull trout were again reciprocally monophyletic with significant bootstrap support (Fig. 3b). Every individual sequenced was different for at least one base pair. The Dolly Varden clade (99% support) contained two major subclades, which corresponded to both subspecific and geographic delineations, but did not correspond to distinctions between

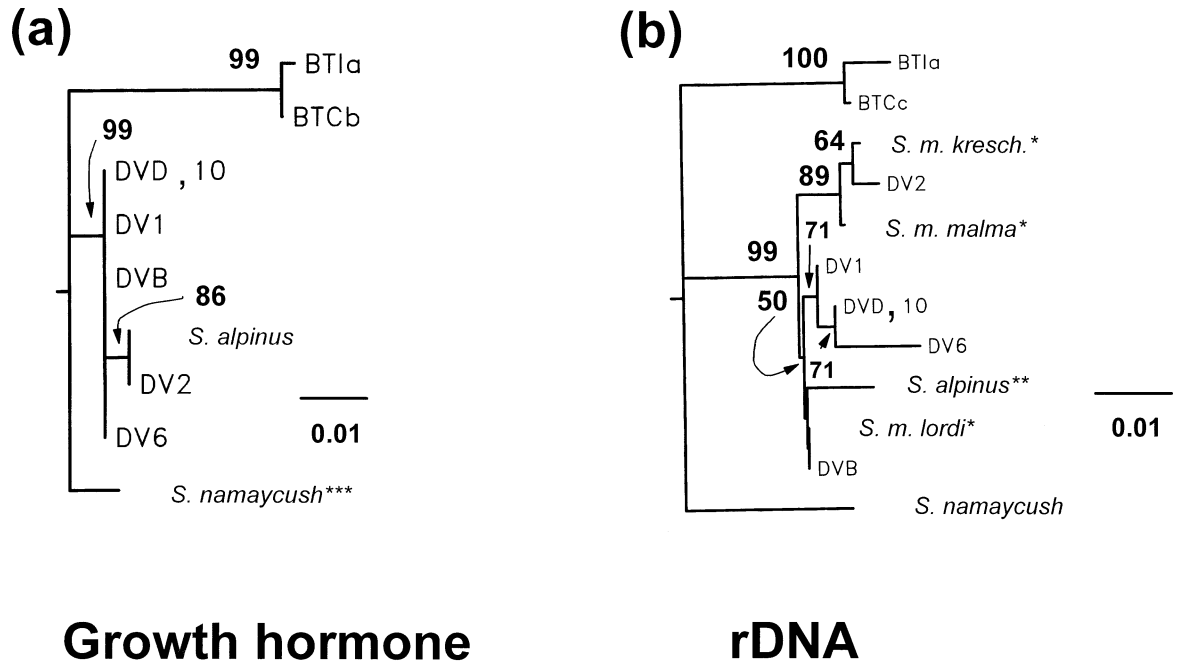

FIG. 3. Neighbor-joining tree of Kimura two-parameter distances for (a) 484 bp of GH2 intron C and (b) 410 bp of ribosomal DNA internal transcribed spacer region (rDNA ITS-1) sequences for *Salvelinus* species. Haplotypes are defined in Table 1 and Appendix 2 and 3, respectively. Trees were rooted using sequences from lake trout *Salvelinus namaycush*. Bootstrap support levels (where more than 50%) from 1000 resamplings are marked at branch points. Sequences marked with an asterisk are from Phillips et al. (1999), those with two asterisks from Pleyte et al. (1992), and with three asterisks from McKay et al. (1996). Scale bars represent units of genetic distance.

mtDNA clades S and N. For instance, *S. m. kraschenninnikovi* and *S. m. malma*, collected from Sakhalin Island, Russia (Phillips et al. 1999), grouped with DV-2, collected in the Kuril Islands, with 89% support. *Salvelinus m. lordi*, collected from southeastern Alaska, grouped with British Columbian individuals with 50% bootstrap support (DV-1, DV-10, DV-B, and DV-D). The percentage sequence divergence of ITS-1 within the Dolly Varden clade ranged from 0.0% to 1.5%. Coastal and interior bull trout grouped together and separately from all Dolly Varden ITS-1 sequences with 100% support. Finally, there was a lower percentage sequence divergence between Dolly Varden and bull trout in mtDNA (1.2–2.6%) than exhibited with either GH2 (4.31–5.0%) or ITS-1 (4.12–6.08%). In both GH2 and ITS-1, the percentage sequence divergence between species was five to 10 times greater than that within species, whereas the range of mtDNA percentage sequence divergences between species overlapped that within species.

DISCUSSION

Comparative Phylogeography and Contact Zones in Northwestern North America

Contact zones are geographic areas that house one or more divergent evolutionary lineages whose ranges are otherwise allopatric. Such regions may stem from secondary contact between allopatrically derived lineages or by primary intergradation, processes that are difficult to distinguish (Endler 1977). When such contact is associated with interbreeding, hybrid zones are produced. Many such zones have been described and they are often associated with recently deglaciated areas (reviewed by Hewitt 1989). A variety of phylogeographic studies of taxa in northwestern North America have reported contact zones between divergent lineages (e.g., fish, O'Reilly et al. 1993; Bernatchez and Wilson 1998; Taylor et al. 1999; mammals, Stone and Cook 2000; plants, Soltis et al. 1997). Remington (1968) described ''suture zones'' as broad areas of contact and hybridization between formally isolated species that are roughly coincident across a broad range of taxa. Two areas (northern Rocky Mountain and northern Cascade areas) were described as suture zones in northwestern North America based on the distribution and interaction of terrestrial floral and fauna, and aquatic faunas were suggested to display suture zones that would be independent of those for terrestrial biotas (Remington 1968). The increasing documentation of contact zones between divergent lineages, coupled with our data on contact and hybridization between Dolly Varden and bull trout, suggests that much of northwestern North America represents sites of recontact of biotas once isolated in separate Wisconsinan refugia. Consequently, Pleistocene glacial events in this area have been a major factor in the origin of aquatic suture zones.

For Dolly Varden and bull trout, and other species that shared the same glacial refugia, our data suggest that the contact zone between these char species stems from dispersal of Dolly Varden both from Beringia and from a Chehalis Refuge and dispersal of bull trout from Chehalis Refuge as well as from the Pacific Refuge (Fig. 4). For instance, clade N Dolly Varden mtDNA is distributed from the Kuril Islands in the western Pacific through Alaska to southern British Columbia. Clade N, therefore, was almost certainly present in the Bering Refuge, which consisted of unglaciated portions

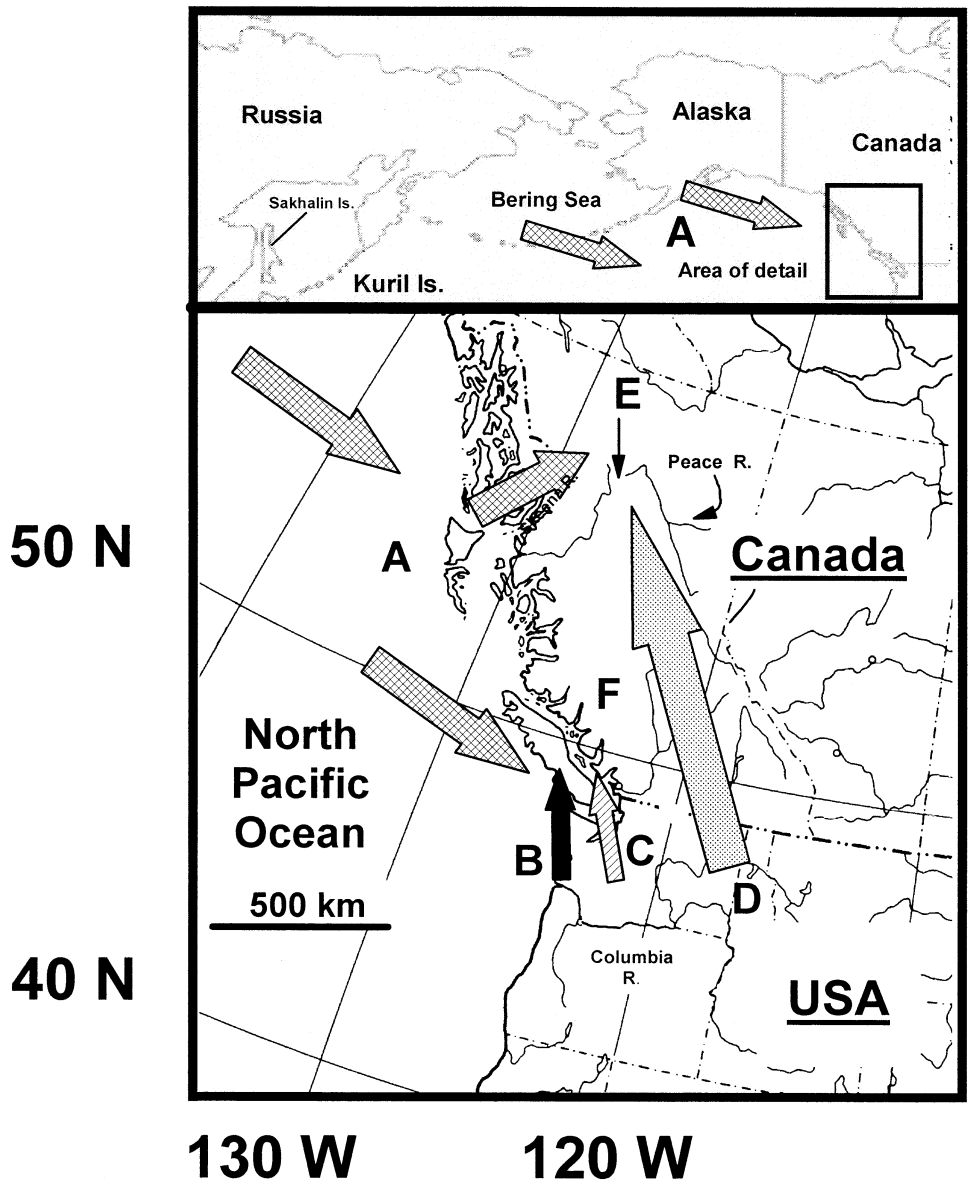

FIG. 4. Hypothesized postglacial dispersal routes and zones of secondary contact between Dolly Varden and bull trout. Shaded arrows and accompanying letters represent dispersal from putative Wisconsinan glacial refugia. A and crosshatched arrows, dispersal of Dolly Varden from Beringia; B and solid arrows, dispersal of Dolly Varden from Chehalis Refuge; C and hatched arrow, dispersal of bull trout from Chehalis Refuge; D and stippled arrows, dispersal of bull trout from Pacific Refuge; E, region of secondary contact between Dolly Varden resulting from watershed exchanges (e.g., in low-lying areas between the upper Skeena River, colonized from the Pacific Ocean by Dolly Varden, and the upper Peace River, colonized by bull trout dispersing from the Columbia Refuge); and F, region of sympatry between Dolly Varden and bull trout resulting from codispersal from Chehalis Refuge.

of the Yukon River valley, adjacent parts of Kamchatka, and exposed portions of the Bering Sea. Because our data suggest that clade N haplotypes are the only mtDNA types present through most of the species' range, it is also likely that the Bering Refuge provided much of the mtDNA diversity in Dolly Varden that recolonized southern areas. By contrast, clade S has a more limited geographic distribution in southwestern British Columbia and Washington state, with scattered occurrences in more northern areas. This narrow distribution implies that there was a second Dolly Varden refuge located south of the Cordilleran ice sheet. Because Dolly Varden is a coastal species, with a primarily anadromous life history, this refuge almost certainly was coastal. Given the concentration of clade S in areas around Olympic Peninsula and the data indicating that the Chehalis River valley was a glacial refuge for fish (McPhail 1967; McPhail and Taylor 1999; Taylor et al. 1999), Dolly Varden also likely persisted here during the Wisconsinan.

Bull trout also have been hypothesized to have survived the Wisconsinan glaciation in two refugia, the Chehalis and the Pacific (Columbia River valley south of the ice sheet) refugia, both south of the ice sheets (Taylor et al. 1999). If true, bull trout and Dolly Varden may not have been completely isolated during the Wisconsinan. Although Beringian

Dolly Varden and Pacific Refuge bull trout may have been allopatric, the two species were probably historically sympatric in the Chehalis Refuge. This suggests that the current sympatric range in northwestern North America consists of two semidistinct areas: a zone of secondary contact between formally allopatric species (in northern through mid–British Columbia) and a possible zone of range expansion for two continuously sympatric species populations (southern British Columbia and adjacent portions of Washington state, areas E and F, respectively, Fig. 4). Differing durations of contact between species may result in differing levels of reproductive isolation (Hewitt 1989). Preliminary data on comparative population hybridization surveys, however, revealed no significant differences in levels of hybridization between areas of secondary contact and range expansion (Z. Redenbach and E. B. Taylor, unpubl. data).

Dating of divergence events based on mtDNA sequence divergence is inherently uncertain (e.g., see discussion in Avise 2000), but an average rate of 1% per million years has been suggested for salmonids based on calibration of whole-molecule divergences and fossils (Smith 1992). Both Redenbach and Taylor (1999) and Taylor et al. (1999) found comparable levels of sequence divergence between ND1 sequences and RFLP surveys of multiple fragments in two species of salmonids, and our ND1 divergences among *Salvelinus* are only slightly lower than whole-molecule estimates (Grewe et al. 1990). In addition, McKay et al. (1996) estimated a substitution rate of 0.83% per million years from comparative sequence analysis of ND3 in several salmonid species. Consequently, a substitution rate of 1% per million years for *Salvelinus* ND1 sequences is a reasonable one. The within-clade sequence divergences of 0.2–0.6% found in clade S, therefore, indicates that it originated prior to the end of the Wisconsinan glaciation, about 15,000 years ago, but clade N may be the original Dolly Varden mtDNA type because it contains lineages that are more divergent from one another. The presence of a few individuals bearing clade S haplotypes in midcoastal regions (Nass River, Skeena River, and Omineca River), however, suggests that clade S may have also persisted in the Bering Refuge. In addition, further sampling could indicate additional clade S haplotypes north of their current recorded range. Alternatively, the presence of clade N in the Chehalis Refuge during the Wisconsinan cannot be discounted completely. Clade N is distributed to the southern tip of Vancouver Island and southwestern British Columbia (e.g., Skagit River), and Dolly Varden may make extensive long-distance movements through the ocean (e.g., DeCicco 1992). Of much greater surprise than Bering Refuge Dolly Varden reaching southern British Columbia is the observation that Chehalis Refuge Dolly Varden have apparently colonized only midway up Vancouver Island and along the adjacent mainland coast. If the population sizes of Chehalis Refuge Dolly Varden population were very small, it might explain this apparent lack of colonization success. Two further hypotheses, however, may explain the apparently limited northward postglacial dispersal of Chehalis Dolly Varden. First, throughout most of their range Dolly Varden are anadromous, but in sympatric populations, Dolly Varden tend to assume a resident life history, whereas bull trout maintain a generally adfluvial or anadromous migratory life history (McPhail and Taylor 1995; Baxter et al. 1997; Hagen and Taylor 2001). If Wisconsinan Chehalis Dolly Varden were also largely stream-resident, then as the ice sheets began to retreat they would have been less likely to disperse through marine waters in a stepping-stone fashion than anadromous Dolly Varden from the Bering Refuge, thus limiting their northward spread. A second hypothesis for the limited northward spread of clade S is the idea that selection may favor clade S and clade N mtDNA in southern and northern populations of Dolly Varden, respectively (cf. Glemet et al. 1998; Wilson and Bernatchez 1998).

Historical Introgression and Reticulate Evolution in Char

Unlike the mtDNA phylogeny, GH2 and ITS-1 phylogenies suggested that Dolly Varden and bull trout are distinct monophyletic lineages. An entire clade of geographically localized Dolly Varden mtDNA (clade S), however, is more closely related to bull trout mtDNA than to all other Dolly Varden (clade N). Coastal bull trout even shared a haplotype with clade S Dolly Varden. This mtDNA paraphyly could be explained by incomplete lineage sorting, convergent mutation, or current and historical mtDNA introgression. We favor the introgression explanation for the following reasons. First, Dolly Varden and bull trout can and do hybridize in nature (McPhail and Taylor 1995; Baxter et al. 1997; Redenbach 2000; Taylor et al. 2001). This hybridization appears to be typically unidirectional as revealed by the asymmetric introgression of mtDNA (Redenbach 2000). We have hypothesized (Baxter et al. 1997; Z. Redenbach and E. B. Taylor, unpubl. data) that the unidirectional hybridization results from the common salmonid parasitic reproductive strategy known as sneaking. During such spawnings, small-bodied males, in this case Dolly Varden (Hagen and Taylor 2001), sneak fertilizations during pairing of larger-bodied male and female bull trout. Furthermore, it is known that mtDNA can introgress permanently across a species barrier (e.g., Ferris et al. 1983; Spolsky and Uzzell 1984), and that this introgression is often the best explanation for discordancies between mitochondrial and nuclear phylogenetic trees (Arnold 1997).

Second, it is unlikely that either retention of ancestral polymorphism or convergent evolution of sequence variants could explain the geography of paraphyletic relationships between mtDNAs of bull trout and Dolly Varden. The shared sequence variants are not distributed throughout the range of both species, as expected 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 localities. In addition, the paraphyletic Dolly Varden and bull trout mtDNAs share six mutations, necessitating six convergent substitutions to explain their similarity. This number of homoplasic events is likely less parsimonious than similarity through introgression (cf. Bernatchez et al. 1995). The Dolly Varden mtDNA paraphyly is best explained, therefore, by introgression of coastal bull trout mtDNA into Dolly Varden at the southern portions of its North American range. Similar events have been observed within several pairs of char species (Bernatchez et al. 1995; Wilson and Bernatchez 1998). In these cases, however, the introgressed populations were cur-

rently allopatric. Introgression, therefore, was clearly shown to have occurred historically, presumably during temporary bouts of sympatry in recolonized deglaciated areas. By contrast, the introgressed Dolly Varden are often sympatric with bull trout and geographically interspersed with clade N Dolly Varden.

Several points, however, indicate the introgression producing clade S was historical and not exclusively contemporaneous. First, even though clade S haplotypes share a more recent common ancestor with bull trout haplotypes with which they are currently broadly sympatric (e.g., haplotypes BTCa-c), clade S Dolly Varden have diverged from coastal bull trout haplotypes (Fig. 1). If mtDNA introgression were due exclusively to current hybridization, all clade S haplotypes would be expected to be identical to the coastal bull trout haplotypes (i.e., BT-Cb and BT-Cc). Second, the level of sequence divergence within clade S suggests that if a single introgression event occurred, it was prior to the Wisconsinan. Again, assuming a constant molecular clock of about 1% per million years (see above) yields estimated minimum divergence times of 100,000 years for some haplotypes within clade S. Because these estimates suggest that the introgression of clade S predates the Wisconsinan glaciation, historical introgression is again supported over current hybridization as sole explanation for mtDNA paraphyly.

Third, if clade S had originated from current introgression, its geographic distribution should closely match that of coastal bull trout and be concentrated on the southwestern mainland of British Columbia and adjacent portions of Washington, Oregon, and northern California (Taylor et al. 1999). Clade S Dolly Varden, however, are more widely distributed than coastal bull trout. For instance, clade S Dolly Varden were found at localities such as the Omineca, Nass, and Southgate Rivers in central and northern British Columbia (Fig. 2), all considerably north of the current distribution of coastal bull trout (Taylor et al. 1999). If introgression of bull trout mtDNA into Dolly Varden results only from current hybridization, mtDNA haplotypes in Dolly Varden at these sites should be closely related to those of the local bull trout (i.e., interior bull trout in the Nass, Omineca, and Southgate Rivers). More substantial geographical evidence favoring a role for historical introgression is that clade S Dolly Varden occur on Vancouver Island, where bull trout are currently absent (McPhail and Lindsey 1986; cf. Wilson and Bernatchez 1998; Shimizu and Ueshima 2000).

Finally, the sequence divergence between bull trout and Dolly Varden was much greater for nuclear markers (GH2: 4.31–5.00%, ITS-1: 4.12–6.08%) than for mtDNA (1.2–2.6% between clade N and bull trout) sequences. It is possible that a hybridization event between Dolly Varden and bull trout prior to divergence among clade S mtDNA haplotypes resulted in this decreased level of mitochondrial relative to nuclear sequence divergence between the species.

Soltis et al. (1997) described a dichotomy of northern and southern chloroplast DNA (cpDNA) clade distinctions in five angiosperms and one fern in northwestern North America that is similar in geographic distribution to our results, particularly for Dolly Varden. These authors also suggested that these clades are the result of 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. An even more striking congruence in phylogeographic pattern between these plants and char was the observation that the herb *Tellima grandifora* contained a southern group of populations that appears to have obtained its chloroplast genome via ancient hybridization and cpDNA capture from a species of *Mitella* (reviewed by Soltis et al. 1997). The congruence in pattern between char and plants suggests that historical processes have strongly impacted not only the phylogeographic distribution of intraspecific lineages in diverse taxa, but also evolutionary interactions between species such as introgression. In particular, these analogous situations highlight the potential role of the dynamic geological history of northwestern North America in initiating vicariance and influencing dispersal patterns and subsequent evolutionary interactions between species (see also Latta and Mitton 1999; Golden and Bain 2000).

Phillips et al. (1999) showed (with rDNA sequences) that Dolly Varden were paraphyletic with respect to Arctic char. The authors suggested that such paraphyly may have its origin in hybridization and introgression between the species in the northern portions of the geographic range of Dolly Varden. Although Savvaitova (1980) discounted the role of hybridization in the evolutionary history of Salvelinus (cf. Behnke 1980), our data and those of Phillips et al. (1999) indicate that Dolly Varden have been subject to introgression from congeners at both extremes of their North American geographic range: Arctic char in the north and bull trout in the south. Such historical interactions may explain much of the morphological variability and resulting taxonomic confusion in the ''*alpinus-malma*'' complex (McPhail 1961; Savvaitova 1995; Phillips et al. 1999). In fact, historical hybridization and introgression may have been a persistent theme in char and have contributed to ''evolutionary novelties'' (sensu Arnold 1997) in *Salvelinus*, particularly in Pacific lineages that show extensive morphological and life-history variability (Behnke 1980; Savvaitova 1980). More generally, our analysis is a further indication of the potential importance of historical processes (isolation and subsequent dispersal from distinct glacial refugia) as factors in the development of mosaic-like hybrid zones (Hewitt 1996). Dowling et al. (1997) showed a strong phylogeographic pattern to mtDNA introgression in a contact zone between two species of cyprinid fishes (genus *Luxilus*); the predominant mtDNA lineage undergoing introgression was strongly influenced by the geographic distribution and the direction of postglacial dispersal of mtDNA clades from Pleistocene refugia. Our data suggest a similar pattern in *S. malma* and *S. confluentus*; local contingencies resulting from glaciation and shifting watercourses have likely played an important role in determining zones of sympatry (Baxter et al. 1997) resulting in contact zones of different ages and a mosaic pattern of semi-isolated hybrid zones (e.g., old and recent hybrid interactions). Our data indicate that current areas of contact between Dolly Varden and bull trout stem from two processes (Fig. 4): (1) secondary contact between Dolly Varden and bull trout dispersing postglacially from the Bering and Columbia (Pacific) Refugia, respectively; and (2) range expansion from a common refuge in the Chehalis River valley. As Dowling et al. (1997) point out, such mosaic hybrid zones varying in the

extent and pattern of hybridization between lineages may be exploited in char and other taxa to understand the evolution of reproductive isolation itself.

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APPENDIX 1

ND1 mtDNA sequences. Haplotype DV-1 is given in full. Base pairs 1–36 represent a partial sequence for the tRNA-Gln gene, base pairs 39–111 represent the tRNA-Ile gene, and base pairs 119–570 represent the beginning of the NADH-1 gene (975 bp in full length). For the other haplotypes, only variant sites are listed. Sample locations for given haplotypes are listed in Table 1. BT-Ix, BT-Cx, and *Salvelinus fontinalis* sequences are from Taylor et al. (1999); asterisks indicate location of *Hae* III restriction site in DV1 full sequence.

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DV-1

     ___tRNA Gln_____________________  ___tRNA Ile______
  1  TTTTGATCTCTTGAGGATGGGTTCGAATCCCTTCTCTCTAGAATTGAGGG   50
     ____________________________________________________
 51  GACTTGAACCCCTATCAGCCACGCTATCAAGGTGGTCCTTAAGCATTCAG  100
     ___________        _________ NADH-1 _______________
101  GCACAATTCCTTGGAATTAAAGTTGGGGAGGGAGGCCGGCTAGTGCAATA  150
     ____________________________________________________
151  GGAAGCGCGAGGTGTCATAGGACAAGTGCTAGGGTTAATGGAAGGAAGCT  200
     ____________________________________________________
201  TTTTCAAACTAAATGCATAAGTTGGTCGTATCGAAATCGTGGGTAGGAAG  250
     ____________________________________________________
251  CTCGTACTCACAAAAACACAACGGAGAGTAGGGCAGCTTTCGTTATCAGA  300
     ____________________________________________________
301  TTTACGGCTGTTAATTCAGGGAAAGCGGGGATGTGGGATGCGCCTAGAAA  350
     ____________________________________________________
351  TAGGACGGCTGAAAGTGTATTTATTAGAAGAATATTAGCGTATTCAGCCA  400
     ____________________________________________________
401  GGAAGAAGAGGGCGAATGGTCCTCCAGCGTATTCTACGTTGAATCCTGAA  450
     ____________________________________________________
451  ACTAATTCTGATTCTCCTTCTGTGAGGTCAAAAGTGCACGGTTTGTTTCG  500
     ____________________________________________________*
501  GCCAAAGTAGAAATGTATCATATGGCGGCAAGGGTTCAGGCTGGTACTAA  550
     ***
     ____________________
551  TAATCAGATGCTTTCTTGAG                                570

ND1 Variant Sites

                  11111111222222222222333333333344444444555
          11222468124455671123466777889000124555674455666670000
        759235560001316832681017029581035462469671405235810 13

DV-1     TGGTTGTCACATGGCTCACGCGCCACTATCATCACGGCCTGGTAATTTTTGGC
DV-2     ..A.A.........................C.....................
DV-3     ..A.....................................................
DV-4     ........................T.....................................
DV-5     .....CC.................................................
DV-6     .C...A..................................................
DV-7     .C...T..G...............................................
DV-8     CC......................................................
DV-9     .C.G....................................................
DV-10    .C...A.T............................A...................
DV-11    .......T............................A...................
DV-A     ............................TG.......A....C..CA.T
DV-B     ............................TG.......A.......CA.T
DV-C     ...G........................TG.......A.......CA.T
DV-D     .....A......................TG.......A.......CA.T
DV-E     .....A........T.............TG.......A.......CA.T
BT-Ca    ............................TG.......A.......CA.T
BT-Cc    ............A..............TG....T..A.......CA.T
BT-Ia    ..............C............T..TG.......A........A.T
BT-Ib    ............T..C............T..TG.......A........ATT
BT-Ic    ........................T..TG.......A........A.T
S.namay. .........T.C.....GT.C.......C.G.T.....T...........A..
                                                          ---
Diagnostic sites:                          ~  **          *  #* *
```

* Distinguishes Dolly Varden clade N from bull trout and Dolly Varden clade S.
~ Distinguishes interior bull trout from remainder.
\# Distinguishes Dolly Varden clade S and coastal bull trout from remainder.
\- Indicates location of diagnostic *Hae* III (GC^CC) restriction sites.

APPENDIX 2

Growth hormone 2 intron C sequences. The full sequence is given for DV-1, which is identical to those in DV-10, DV-B, DV-D. For the other haplotypes, only variant sites are listed. *Salvelinus namaycush* sequence was obtained from McKay et al. (1996).

DV-1		
1	CCTGACGTTGCCGTCGCCCCCCAGATTCTGATAGTAGTTCCCGTAGGGGG	50
51	GCAGATGCTGAGAGTCATTGTCATCCAGGCTCAGTACGCCGTCCTGGCTC	100
101	CCCTGTGGGAGACAGAGAGAGATGCATGGTGGGGTGGATGTGTGCATATC	150
151	AATATTTGTTTATTAGGCCATTCTAAAAGCACATATAGCAAAAGACACAT	200
201	GCGTAAATGTCCTTGAATGGGCCAATATTCAGTCATTGAATGGGGTGTTA	250
251	CTGATATAGTCACTATTAGTGATGATCATCACAATGTAGCAGACGTTTAG	300
301	AAAGCATAGTTGTATTTCACTTTCATGTCGGTGGAAGATAATAACCACAC	350
351	TGTAAACCCCAATGTGCTGTCATTACTCAAAACTTTTGAGGAAACCGTTG	400
401	CACTTAATATACAGAATCTGACTACAGTTACTTAAAGTGATTTTTATAGT	450
451	CATTACTCAAACCTATAGAGTCACCCTGTAGAGG	484
Variant Sites		
	1111122222222222222233333344	
	1136883566877777888899999904459948	
	3291258455754567836891256896476253 3	
DV-1*	TCCAGTGGTAGAGATCAAGAGAGGTTAAAAAACTG	
DV-2	..G.....C.....	
DV-6	..G.....C.....	
S. alpinus	..G.K.....C.....	
BT-I	G..GKCATCGAGATA-GTT--TA--AG..G....A	
BT-C	...G.CATCGAGATA-GTT--TA--AG..G....A	
S. namaycush	.T.....G.....-AG.-.-GAG.	

* Includes DV-10, DV-B, DV-D.

APPENDIX 3

Ribosomal DNA first internal transcribed spacer region (rDNA ITS-1) sequences. The sequence of DV-1 is given in full. For the other haplotypes, only variant sites are listed. *Salvelinus malma krascheninnikovi* (Smk), *S. m. malma* (Smm), and *S. m. lordi* (Sml) sequences were obtained from Phillips et al. (1999); *S. alpinus* (Sa) and *S. namaycush* (Sn) sequences were obtained from Pleyte et al. (1992).

```
DV-1                                                    Variant Sites

  1 GGTTGCCAGCCGCCGGCATGGGGCTGTGCTCCGAAAACCAAAGTCTGCTG  50                  11111111111222222222222222222222222333333333333333333333444444
 51 TGGGGTGGGTAGGGTATGGGGGCTCACCCCCCCCGCCTCGCCCATCTCTC 100          44557889012355568900122223335567788888889999900002233335666688999000000
101 GGCGCGGGTGTCCTCGGTCTTAGCCCGG-TTCCCCGCTATTCCTTTTGCC 150          13158342669523810746315692386901712345692345901274801288567989189134 56
151 TAAGGGTTGCACCCGACCGGCTCCATCCCTTTTCCCCGTTAGCCACGGCC 200  DV-1    AGTGCCCCGG-CAATATGTGTGAGGCGCGGGCGCGTAATCTTATCGGCGCCGCAAGCGCGCCCCAKCGCG
201 ACATGGCGCACCTATGGGCAGGTGAGTCGGCCGCTACCGAAGGGGACTGG 250  DV-2    .......T.............S........T....A.............C.S...R.....GA...
251 GGGTGTCCGGTGAACCGGGACTTCCCGAAACGTAATCCCATTTATAAGCG 300  DV-10   .................C.......G.........................G......K.....G....
301 GCTTGAGTATCGCCCAGTATCCTCGCTCGGCACCGGGAACCCAGTCAACC 350  DV-B    ....G.......................SC.................................G....
351 GCTCTGCGCCCCGGCGCAGGCGGGGGTTTAATGTCTCCCCCAGCCCTCAC 400  DV-D    ............................YS.....................G.............G...K
401 KGC--GCTTC                                       410  Smk     ....G..T.........................T....A.............C...........G....
                                                         Sml     ....G.....................................................................G....
                                                         Smm     ....G..T.........................T....A.........................G....
                                                         BT-I    .CCT.....SG..-AG..C....C.A.M...T...GTC.A.GTG.RTGR..R....S..R.....G....
                                                         BT-C    .CCTG.....G..-AG..C.............T...GTC.A.GTG..TG.................G....
                                                         Sa      ....G...........C............................T............CG......G....
                                                         Sn      .C.TG--.A..T--.GC..A.C...TC......TSGTC..G.T..................T-.CGA...
```