doi:10.1046/j.1420-9101.2003.00619.x

Evidence for bimodal hybrid zones between two species of char (Pisces: *Salvelinus*) in northwestern North America

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Keywords:

char;
hybridization;
introgression;
reproductive isolation;
salmonidae;
speciation.

Abstract

Dolly Varden (*Salvelinus malma*, Pisces: Salmonidae) and bull trout (*Salvelinus confluentus*) have widely overlapping, but largely parapatric ranges in watersheds in northwestern North America from Washington State to northern British Columbia. Genetic analysis of natural populations using diagnostic molecular markers revealed widespread local sympatry and hybridization with hybrids comprising 0–25% of the local samples. In a detailed analysis of hybridization using four nuclear DNA markers and mitochondrial DNA within the Thutade Lake watershed, northcentral British Columbia, hybrid genotypes constituted up to 9% of the population of juvenile char. There were significant deviations from Hardy–Weinberg, gametic, and cytonuclear equilibria, and local samples showed bimodal frequency distributions of genotypes. Pure parental and inferred backcross genotypes were most common, and F_1 and F_n hybrids were comparatively rare. Interspecific hybridization was asymmetrical, with most F_1 hybrids (five of six) bearing *S. confluentus* mtDNA. The introgression of nuclear and mitochondrial alleles was asymmetrical, with *S. confluentus* mtDNA and Growth Hormone 2 introgressing into *S. malma* significantly more than either introgression of the three other nuclear loci, or introgression of *S. malma* alleles into *S. confluentus*. Substantial prezygotic isolation between the species likely depends on the large body size difference between them in sympatry: *S. malma* have small bodies and a stream resident life history (12–21 cm adult fork length at maturity), while *S. confluentus* are larger and adfluvial, i.e., they migrate to Thutade Lake where they grow to maturity before returning to tributary streams to spawn (40–90 cm at maturity). These traits may limit interspecific pairings because of size assortative pairing and size-dependent reproductive habitat use.

Introduction

Barriers to natural hybridization fall into two major categories: prezygotic and postzygotic (or pre- and post-mating; Mayr, 1963). Prezygotic barriers, i.e., those that prevent hybridization, typically involve mating behaviours and/or gamete recognition. Mating behaviour can prevent or limit hybridization in several ways, such as assortative mating or the timing or location of mating. Gamete interactions can also play an important role, and may almost completely counter the effects of random mating between species (e.g. Howard *et al.*, 1998; Rieseberg *et al.*, 1998). Post-zygotic barriers take the form of intrinsic genomic incompatibilities (endogenous selection) and/or extrinsic interactions between phenotype and the environment (exogenous selection) that select against hybrids (Arnold, 1997). Understanding the relative roles of pre- and post-zygotic processes in the evolution of reproductive isolation is a fundamental aspect of speciation research (Howard *et al.*, 1998). Hybrid zones, areas where divergent lineages come into

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contact and hybridize, have been important model systems to examine ecological and genetic processes maintaining genetic distinction in the face of gene flow, the distribution of hybrid genotypes, and their relevance to speciation (Barton & Hewitt, 1985; Hewitt, 1989; Butlin, 1998).

The relative importance of pre- and post-zygotic barriers, have influenced much of the debate on the maintenance of hybrid zones over time (Moore, 1977; Barton, 1979; Barton & Hewitt, 1985; Arnold, 1997). More recently, Jiggins & Mallet (2000) reviewed the classification of hybrid zones based on the frequency distribution of genotypes, i.e., unimodal, bimodal, or flat distributions. Within this broad classification, bimodal hybrid zones appeared to be strongly associated with systems where prezygotic isolation was well developed, but Jiggins & Mallet (2000) stressed that more examples of this association from nature were needed to assess the generality of the association between premating isolation and genetic structure. The documentation of the modality of hybrid zones and its association with patterns of reproductive isolation is also important for our understanding of the role of geography in the process of speciation. For instance, Coyne & Orr (1997) highlighted a potential role for reinforcement (Dobzhansky, 1937) to explain the tendency for prezygotic isolation to evolve before post-zygotic isolation in *Drosophila*. In this study we report the occurrence of bimodal hybrid zones, and their apparent association with prezygotic isolation, between two species of char (Pisces: Salmonidae) in northwestern North America.

Dolly Varden char (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) are native to drainages of the North Pacific and have largely parapatric distributions in northwestern North America (Haas & McPhail, 1991). *Salvelinus malma* are coastal in distribution from the western Pacific to Alaska, and south to the Olympic Peninsula, WA. *Salvelinus confluentus* have a more inland distribution from Yukon Territory, Canada, south to northern California and Nevada. The two species also occur sympatrically in areas along the Cascade/Coastal mountain crests in northwestern North America (see Baxter *et al.*, 1997). There has been controversy as to the validity of separate species designations for these taxa (Cavender, 1978; Baxter *et al.*, 1997; Leary & Allendorf, 1997), and at one time *S. confluentus* was regarded as part of the *S. malma* 'species complex' (e.g. McPhail, 1961). More recent morphological and genetic data, however, have established the distinct status of these species (e.g. Cavender, 1978; Haas & McPhail, 1991; Phillips *et al.*, 1995). In fact, molecular phylogenetic data indicate *S. malma* and *S. confluentus* are not sister species (Grewe *et al.*, 1990; Crane *et al.*, 1994; Phillips *et al.*, 1994). Based on morphological analysis, the two species were suspected to hybridize in a few places where they came into contact (e.g. Cavender, 1978), and Baxter *et al.* (1997) reported the first confirmation of hybridization and introgression between species using molecular analyses. Redenbach & Taylor (2002) used comparative phylogeographical analyses to document historical introgression between the two species. These previous studies provided a broad phylogenetic perspective on the interaction between the two species and the historical factors in past and current hybridization. There remains, however, little detailed information on the geographical extent of current hybridization between *S. malma* and *S. confluentus*, or on the structure of their hybrid zones and the processes that influence such structure. Detailed study of these two species where they come into contact in multiple areas also provides an 'acid test' of their status as biological species (Avise, 1994). In addition, zones of secondary contact include regions proposed as terrestrial 'suture zones' in northwestern North America (Remington, 1968). Documentation of contact zones for char provide an opportunity to assess concordance between aquatic and terrestrial suture zones (Remington, 1968). Finally, there is good comparative ecological data for *S. malma* and *S. confluentus* in sympatry (e.g. Hagen & Taylor, 2001) which can be exploited to understand the potential role of ecology in structuring hybrid zones and influencing genetic distinction in the face of gene flow (Jiggins & Mallet, 2000).

Our study had two major goals. First, we wanted to determine the prevalence of interspecific hybridization within the sympatric range of *S. malma* and *S. confluentus* to see if previous incidences of hybridization were isolated spatially and, if not, to determine the geographical extent of hybridization.

Secondly, we wanted to undertake a detailed examination of natural hybridization between *S. malma* and *S. confluentus* in a single area to assess the genetic structure of the hybrid zone and, therefore, provide an independent assessment of hybrid zone structure and its association with patterns of pre/post-zygotic isolation (*sensu* Jiggins & Mallet, 2000). More generally, the area of overlap between *S. malma* and *S. confluentus* encompasses a region of contact between divergent lineages in several other taxa (Redenbach & Taylor, 2002). There has, however, been relatively little documentation of hybridization between animal taxa across a broad geographical scale in coastal areas of northwestern North America (but see Soltis *et al.*, 1997 for plants). This region, however, merits investigation as other portions of northwestern North America have been identified as 'suture zones' of contact and hybridization in comparative analysis of terrestrial biotas (e.g. Remington, 1968).

Materials and methods

Population survey localities

Samples were collected from nine river systems within the sympatric range of *S. malma* and *S. confluentus*, throughout British Columbia, Canada (Table 1, Fig. 1).

Table 1 Parental species and molecular-based hybrid identification in selected watersheds in northwestern North America. Numbers in parentheses following locality names refer to locations in Fig. 1. Unless accompanied by a preceding number, all hybrid genotypes represent single observations.

Locality	Drainage	*Salvelinus malma*	*Salvelinus confluentus*	Hybrids	Hybrid genotypes*	F_{IS} GH	F_{IS} MTB
Southgate R. (1)	BC southcoast	10	11	7	2 H/H, 4 H/v, b/v	0.581	0.859
Toba R. (2)	BC southcoast	9	0	1	H/v	–	–
Omineca R. (3)	upper Peace R.	25	2	0	–	1.0	1.0
Goathorn Cr. (4)	Skeena R.	44	47	0	–	1.0	1.0
Thutade L.† (5)	upper Peace R.	354	580	56	12 b/H, 11 H/H, 2 b/v, 17 H/v, 5 v/H, 9 H/b	0.914	0.934
Iskut R. (6)	Nass R.	33	13	1	H/v	0.949	1.0
Tahltan R. (7)	Stikine R.	0	7	2	2 H/H	–	–
Chutine R. (8)	Stikine R.	15	1	3	b/v, 2 H/v	0.621	1.0

*'H' indicates heterozygosity, 'b' indicates homozygosity for *S. confluentus* alleles, 'v' indicates homozygosity for *S. malma* alleles. Symbol left of the slash indicates GH2 locus, that right of the slash indicates genotype for MTB locus. For instance, an 'H/v' individual is heterozygous at GH2 and homozygous for *S. malma* alleles at MTB.

†Results are for GH2 and MTB only to facilitate comparison with other watersheds where only GH2 and MTB were assayed (see text).

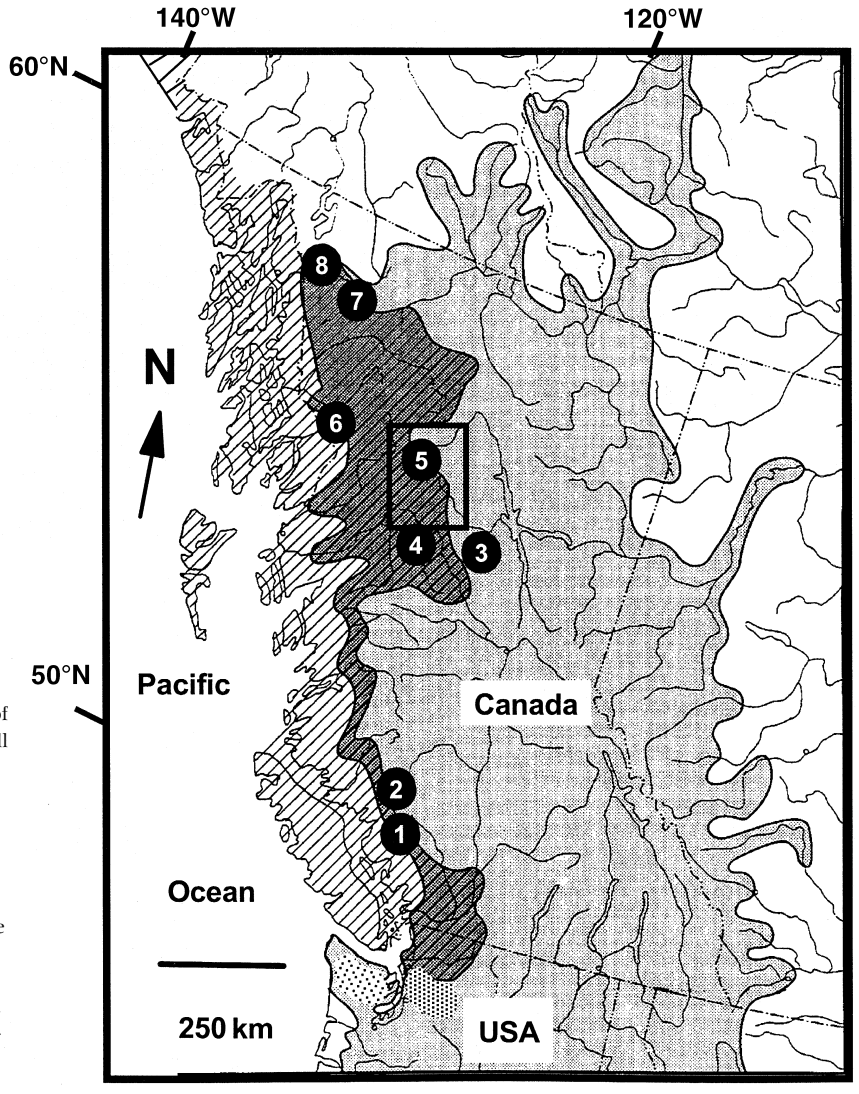

Fig. 1 The parapatric species distributions of Dolly Varden (*Salvelinus malma*, stipled), bull trout (*S. confluentus*, shaded), and their overlap (stipled-shaded) in northwestern North America and the geographical distribution of sample localities. 1 = Southgate River, 2 = Little Toba River, 3 = Omineca River (upper Peace River), 4 = Goathorn River (Skeena River drainage), 5 = Thudate Lake watershed (upper Peace River drainage), 6 = Iskut River (Nass River drainage), 7 = Chutine River (Stikine River drainage), and 8 = Tahltan River (Stikine River drainage). The location of the Thutade Lake watershed is indicated by the boxed area.

These samples ranged in size from nine to 990 individuals and consisted of fin clips stored in 95% ethanol. Samples from tributary streams were collected by electroshocking or with baited minnow traps. Field identifications were based on morphological differences between species; *S. conluentus* have longer upper jaws and more branchiostegal rays than *S. malma* (see Haas & McPhail, 1991 for details). Consequently, any ambiguous identifications were tentatively classified as hybrids.

Thutade Lake watershed collections

The Thutade Lake watershed is located in north-central British Columbia (Fig. 1) and forms part of the headwaters of the Mackenzie River (Arctic) drainage to which it connects via the Finlay and Peace rivers. The lake is pristine, 40 km long and fed by several tributary rivers and streams that represent areas of sympatry between *S. malma* and *S. confluentus* that probably stem from postglacial watershed exchange between the Peace (interior drainage) and Skeena (Pacific drainage) rivers (Fig. 2; McPhail & Lindsey, 1986). Sampling within the watershed was conducted on two geographical scales. First, sample sites were spread throughout the watershed, in six tributaries of the lake (Fig. 2). On a finer scale, 17 separate sites spread over 16 km of Kemess Creek and its tributaries were sampled during August 1997 and 1998 to examine species and hybrid distributions within a single drainage (e.g. Bustard, 2000). Juvenile samples were collected by electroshocking and grouped into age classes based on age-size class distributions (J. Hagen, personal communication, fork length in mm): 0+ (i.e. newly emerged fry to 1 year of age) ≤ 55 mm, 1+ (i.e. 1–2 years of age) = 55–80 mm, 2+ = 81–110 mm, 3+ = 111–140 mm, and >3+ > 140 mm. Owing to small sample sizes, we pooled the 2+, 3+ and >3+ age classes into a single age class, >1+, for analysis. The creek was divided into three equal length sections each with a similar number of sample sites ($n = 6$, 5, and 6) and the distribution of genotypes among sections was examined

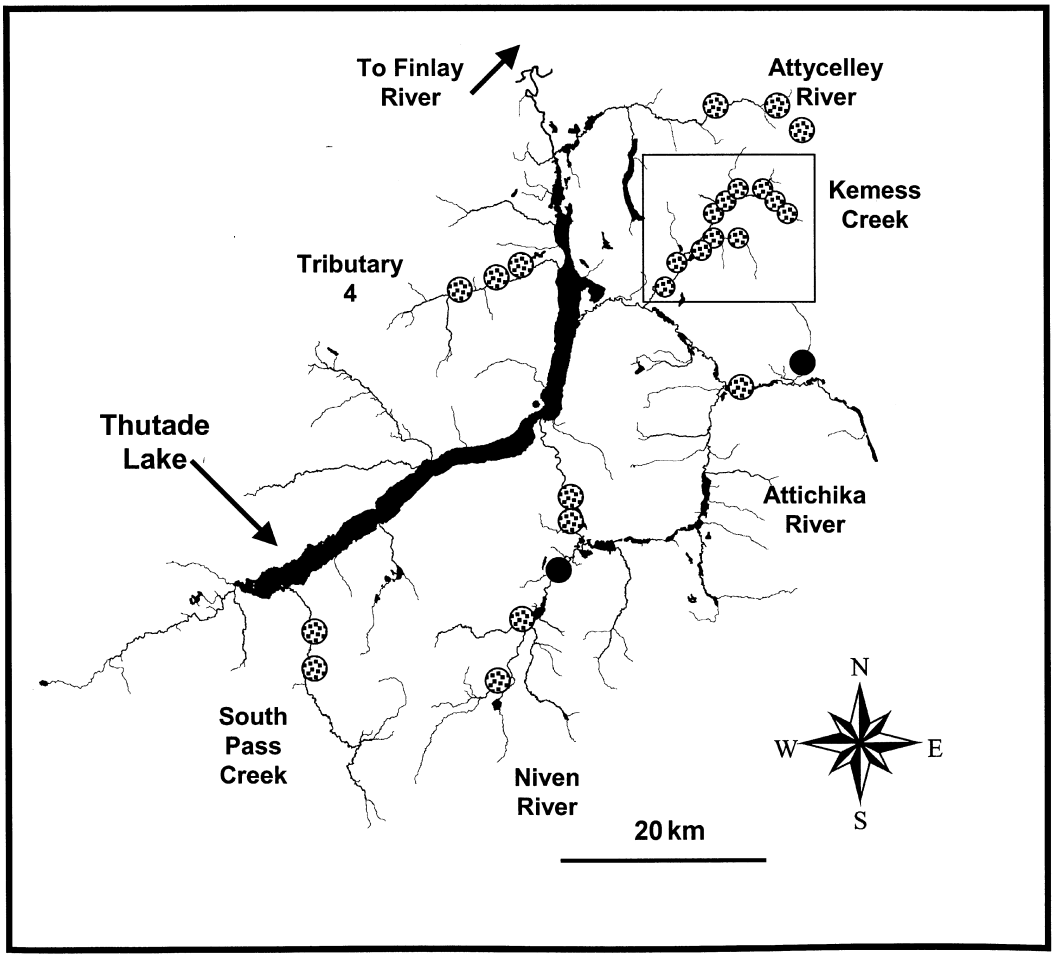

Fig. 2 Thutade Lake watershed. Sample sites are marked by black circles and the those where hybrids were found are stipple-filled circles. The boxed area shows Kemess Creek and the range of sample sites ($n = 17$ in total).

using chi-square randomization tests (Roff & Bentzen, 1989). Adult *S. confluentus* were angled from holding pools in a tributary of Kemess Creek (the Attichika River, $n = 29$) or were collected using gill nets from Thutade Lake ($n = 15$). Standard length and, for adults, sex were recorded. For all samples, adipose fin clips, and occasionally whole fry, were taken and stored in 95% ethanol.

Molecular assays

DNA was extracted from about 20 mg of adipose or caudal fin tissue by overnight digestion at 37 °C in a 0.5% Sarcosyl/0.2 M ethylenediaminetetraacetic acid solution with Pronase, followed by a single phenol/chloroform extraction (as described in Taylor *et al.*, 1996) or by using the Purgene DNA extraction kit (Gentra Systems, Inc., Minneapolis, MN, USA). DNA was precipitated in isopropanol, washed in 70% EtOH, and the DNA pellet was dried and then resuspended in TE buffer (pH 8.0) and stored at −20 °C.

Codominant diagnostic nuclear markers were used to identify hybrid individuals. Growth Hormone 2 (GH2) is a coding gene, but the GH2 primers amplify intron C (McKay *et al.*, 1996). Metallothionine (MTB) also is a coding gene and as with GH2 the primers used amplify an intron (Baker *et al.*, 2002). In both markers, an indel mutation(s) appears in the intron, producing polymerase chain reaction (PCR) amplified fragments with visibly different lengths for the two species (Taylor *et al.*, 2001). The ribosomal DNA primers amplify the first internal transcribed sequence (ITS-1) of the rDNA. The ITS-1 locus has a diagnostic *Sma*I restriction site present only in *S. confluentus* (Phillips *et al.*, 1995; Baxter *et al.*, 1997). A short interspersed repetitive element (SINE) from the *Fok* family (FOK 223) of SINE's (retroposons originating from tRNA, with random reincorporation into the genome via a cDNA intermediate) has an inserted element present only in *S. malma* and Arctic char (*S. alpinus*, Hamada *et al.*, 1998). This insertion produces a diagnostic difference in PCR amplified fragment lengths between *S. malma* (360 base pairs) and *S. confluentus* (180 base pairs). A mitochondrial DNA restriction fragment length polymorphism was used to identify the mitochondrial cytotype of individuals (McPhail & Taylor, 1995; Baxter *et al.*, 1997). The mitochondrial NADH5/6 dehydrogenase coding gene (mtDNA) has a *Hin*d III restriction site present only in *S. malma* in this area (Redenbach & Taylor, 2002). The diagnostic nature of these markers (i.e. *S. malma* and *S. confluentus* were fixed for alternate alleles at all loci), was validated by analysis of samples from throughout the allopatric ranges of both species ($n = 25$–100 per species, per locus, at various localities, Baxter *et al.*, 1997; Taylor *et al.*, 2001; Redenbach & Taylor, 2002).

The PCR reactions were run using primers and conditions for GH, MTB, ITS-1, and mtDNA as outlined in Taylor *et al.* (2001) and Redenbach & Taylor (2002). The primers used to amplify the FOK fragments were FOK223F (5′ cgc agg atc gag tgt cag tc 3′) and FOK223R (5′ tag ctc caa ccc ata tct ga 3′, Hamada *et al.*, 1998). A typical reaction for each locus consisted of a total volume of 25 μL with 800 mM of total dNTPs (200 and 320 mM for GH2 and MTB), 0.6 mM of each primer (0.25 and 0.08 mM for GH2 and MTB), 1.5 mM $MgCl_2$ (2.5 mM for ND5/6), 1× GibcoBRL Taq DNA polymerase buffer, 1.25 units of Taq DNA polymerase, and 1 μL of genomic DNA. DNA was denatured at 95 °C for one cycle, and at 94 or 92 °C for the remaining steps, extension was at 72 °C, and annealing temperatures and number of cycles varied (see Taylor *et al.*, 2001, 23 cycles and 55 °C for FOK223). PCR was terminated with a single 5-min extension at 72 °C, after which samples were stored at 4 °C. Restriction digests were performed as per manufacturer's instructions (New England Biolabs, Beverly, MA, USA), overnight, using 6 μL of PCR product in a total volume of 20 μL. The PCR amplified and restriction length differences were visualized using 1.5% agarose gels stained with ethidium bromide.

Identification of hybrid classes

Char from most localities were assayed at two nuclear loci, GH2 and MTB, to obtain an overview of hybridization across a broad geographical range and to resolve the presence both of F_1 and later generation hybrids. All individuals collected from the Thutade Lake watershed in 1997 and 1998 ($n = 1034$) were analysed using the four nuclear markers (GH2, ITS-1, MTB, and FOK) and mitochondrial DNA (ND5/6) to obtain a more precise estimate of hybridization and to detect any asymmetrical hybridization.

Individuals were classified as either *S. malma*, *S. confluentus*, or hybrids based on their composite nuclear and mtDNA genotypes. Individuals were considered to be 'pure' *S. malma* or *S. confluentus* if they contained only alleles diagnostic for the parental species at all loci, nuclear and mitochondrial. Individuals were considered to be hybrids if they contained any mix of alleles from the two parental species.

The hybrids were subdivided into F_1, F_n, backcross (BC) or mitochondrially introgressed (IG) genotype classes. F_1 genotype individuals were defined as fish that were heterozygous at all nuclear loci. The female parent for F_1 genotypes was inferred from the mtDNA haplotype. F_n genotype individuals (or reverse BCs) were those that were homozygous for alternate species alleles for at least two nuclear loci, with any genotype for remaining loci. BC genotypes were heterozygous at one to three nuclear loci, with the remaining nuclear loci homozygous for one parental species. In *S. confluentus* BCs (BCBT), the remaining nuclear loci where homozygous for *S. confluentus* alleles, and in *S. malma* BCs, they were homozygous for *S. malma* alleles (BCDV). Mitochondrially IG individuals had a 'pure' nuclear genotype across all four loci, with the other species' mitochondrial DNA (i.e. there was no nuclear evidence of hybridization).

These four hybrid classes were intended to describe the genotype of individuals, but do not necessarily reflect their parentage. For example, an individual classified as a F_1 genotype could actually be the result of a cross between a true F_1 and a parental individual, which would be expected to produce offspring heterozygous at all four nuclear loci 6.25% of the time. Similarly, an individual classified as a BC genotype could be anything from a first generation BC to an *n*th generation BC. Hereafter, the term 'hybrid' refers to any/all of these genotypic classes, while more specific terms refer to specific genotypic classes.

Because only four nuclear markers were used, this type of identification could result in overestimates of numbers of pure parental, F_1, and mtDNA-IG fish, as well as underestimates of the number of BC individuals. Individuals identified as F_1 using four nuclear loci could actually be later generation hybrids or BCs. As later generation BCs would be expected to have fewer heterozygous loci, an analysis of four nuclear alleles would also be expected to missclassify some individuals with later-generation hybrid genomes as parental genotypes, thereby underestimating the total percentage of hybrid individuals in the population. Scoring additional nuclear markers would reduce this source of error (for example the percentage of expected first generation BCs heterozygous at all loci is 6.25, 3.12, 1.56, and 0.78% for 4, 5, 6, and 7 nuclear loci), but the use of four markers is sufficient for coarse classification of genotypes (cf. Boecklen & Howard, 1997).

Population genetic analyses

To test for nonrandom mating within samples, we calculated Weir & Cockerham's (1984) estimate of the inbreeding coefficient (F_{IS}) as a measure of heterozygote deficit (Jiggins & Mallet, 2000) using GENEPOP Version 3.1d (Raymond & Rousset, 1995). F_{IS} is equivalent to one minus the observed frequency of heterozygotes divided by the expected frequency of heterozygotes. F_{IS} values indicate heterozygote deficiencies (positive values), heterozygote excess (negative values) or Hardy–Weinberg expectations (a value of zero). We tested the statistical significance of the F_{IS} using an exact Hardy–Weinberg test (Weir, 1990) under the null hypothesis of random mating. This test was performed for each locus in all tributaries, using the complete enumeration method (Louis & Dempster, 1987) in GENEPOP (Raymond & Rousset, 1995).

We calculated nuclear gametic disequilibria (D′) for all six permutations of nuclear loci pairs as per Hartl & Clark (1997), with the exception that we ignored 'double heterozygotes' in calculating D because we could not determine their gametic phase, and because they were such a small proportion of the population their effect would not be great (Avise & Van den Avyle, 1984; Campton, 1987). D′ is equal to D, the observed disequilibrium, divided by D_m, the theoretical maximum disequilibrium. D′ can vary from −1 to +1, with positive values indicating an association between intraspecific alleles at the two loci being compared. We tested the statistical significance of D′ values by assessing deviations between observed proportions of genotypic classes and the expected proportions under a model of no linkage (physical or otherwise) between genotypes at the two loci (i.e. random mating in the char population with respect to these loci and/or no selection against particular genotypic classes). This was tested using GENEPOP (Raymond & Rousset, 1995) via a contingency table for the probability test (or Fisher's exact test) and a Markov Chain (dememorization 2000, 200 batches, 1500 iterations).

We calculated cytonuclear disequilibria (cD) as per Asmussen *et al.* (1987) for all four possible pairs of nuclear loci and mtDNA. The four cD values calculated are labeled cD_1, cD_2, cD_3, and cD. cD_1 through cD_3 refer to the difference between observed individuals in genotypic classes versus those expected given observed nuclear and mitochondrial allele frequencies. A positive cD_1 indicates that *S. confluentus* mtDNA was associated with *S. confluentus* nuclear homozygotes more than expected given random association, a positive cD_2 indicates that *S. confluentus* mtDNA was associated with nuclear heterozygotes than expected, and a negative cD_3 indicates that more *S. malma* mtDNA was associated with *S. malma* nuclear homozygotes more frequently than expected (Asmussen *et al.*, 1987). cD measures allelic disequilibria, and a positive cD indicates that more *S. confluentus* nuclear alleles were associated with *S. confluentus* mtDNA alleles than expected given random association.

As with D′, we tested the statistical significance of cD_i values by assessing deviations between observed proportions of genotypic classes and the expected proportions under a model of no association between nuclear and mitochondrial alleles (i.e. random mating in the char population with respect to these loci and/or no selection against particular genotypic classes). These tests were performed following the same method as for D′, but using the RXC computer program (available at http://www.public.asu.edu/~mmille8/rxc.htm) to calculate a metropolis algorithm to obtain an unbiased estimate of the exact probability value. For all of these statistical tests, we used the sequential Bonferroni adjustment to compensate for tests across multiple loci and multiple tributaries, to ensure a maximum Type I error rate of alpha = 0.05 (Rice, 1989).

Results

Macrogeographical distribution of hybridization

Within the ranges of *S. malma* and *S. confluentus* sampled (Fig. 1), six of eight localities were 'locally sympatric', i.e., they had 'pure' individuals of both species present (Table 1). If, however, sympatry is measured at the level of the allele, all eight sites contained alleles from both

species. Hybridization, defined as the presence both of *S. malma* and *S. confluentus* nuclear alleles within a single individual, was observed in four of the six locally sympatric sites throughout BC, from the Toba River on the southcoast of BC to the Stikine River in northern BC (Table 1, Fig. 1). Evidence of hybridization was also found at two localities where one of the parental species was absent from our sample (only *S. confluentus* in the Tahltan River and only *S. malma* in the Toba River; Table 1). Hybridization was not observed at all localities, and recorded levels of hybridization varied greatly between sites, from a low of 2.1% in the Iskut River to a high of 25% in Southgate River (Table 1, $P < 0.001$).

In the Thutade Lake watershed, *S. malma* and *S. confluentus* were locally sympatric in five of the six tributaries to Thutade Lake (Table 2). All six tributaries, however, contained hybrids, which formed from 3.4 to 18.4% of the tributary population samples (Table 2). These hybrids, 89 of 990 stream resident individuals assayed, fell into five genotypic classes and formed a bimodal distribution of genotypes in the watershed (Fig. 3). Of the 44 adult putative *S. confluentus* sampled (identified by life history and morphology), all 29 from the Attichika River and 14 of 15 from Thutade Lake were classified as *S. confluentus* across all four nuclear loci and mtDNA, while a single adult (sampled from the lake) had an F_1 genotype and *S. confluentus* mtDNA. The proportions

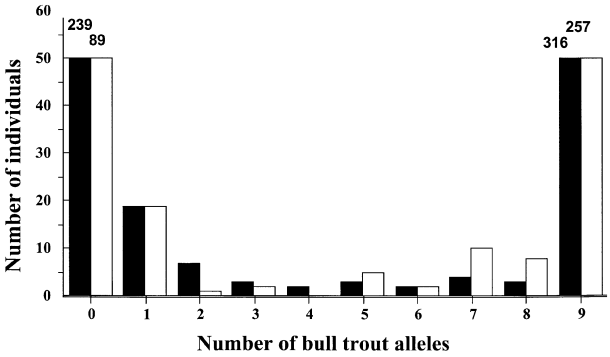

Fig. 3 Bimodal hybrid zone between *Salvelinus malma* and *S. confluentus* in Kemess Creek (black bars) and from five other tributaries of the Thutade Lake watershed combined (white bars), British Columbia. Shown is the frequency distribution of *S. confluentus* alleles per individual juvenile char ($n = 990$ fish) ranging from 0 (equivalent to homozygous for *S. malma* alleles at all for nuclear loci and exhibiting *S. malma* mtDNA) to nine (equivalent to homozygous for *S. confluentus* alleles and exhibiting *S. confluentus* mtDNA). Because of small numbers of hybrid individuals, the height of bars at 0 and 9 have been set to a maximum of 50 and actual numbers of individuals fixed for *S. malma* or *S. confluentus*, respectively, alleles/haplotype are given above each bar.

Table 2 Summary of total and percentage of hybrids found in each tributary of the Thudate Lake watershed and the across all tributaries (Thutade total). Genotypes were inferred from four nuclear and one mtDNA marker, and do not necessarily reflect true ancestry.

Locality	Total	BT	BCBT	F1	Fn	BCDV	IGDV	DV	Percent hybrids
Kemess Cr.	598	316	9	2	2/1	9/16	2	239	6.7/6.5
South Pass Cr.	64	47	0	0	0	/2	5	10	10.9/3.1
Tributary 4	87	61	5	0	1	/1	9	10	18.0/8.0
Attichika R.	88	74	4	3	0	0	0	7	8.0/8.0
Niven R.	94	75	10	0	1	1	2	5	14.9/12.8
Attycelley R.	59	0	0	0	0	1/1	0	57	3.4/3.4
Thutdate total	990	573	28	5	4/1	11/20	20	328	9.0/8.0

BT, homozygous for *Salvelinus confluentus* alleles at all four loci with *S. confluentus* mtDNA; BCBT, *S. confluentus* backcross (BC), heterozygous for at least one locus, homozygous at remaining; F_1, heterozygous at all four loci; F_n, post-F_1 hybrid, homozygous for alternative species alleles at two or more loci; BCDV, *S. malma* BC, heterozygous for at least one locus, homozygous at remaining; IGDV, introgressed *S. malma*, homozygous at all four nuclear loci for *S. malma* alleles, but with *S. confluentus* mtDNA; DV, homozygous for *S. malma* alleles at all four loci with *S. malma* mtDNA. For each hybrid class, values left of slash represent numbers with *S. confluentus* mtDNA, those right of the slash represent numbers with *S. malma* mtDNA. When no slash is present, all hybrids had *S. confluentus* mtDNA. Percentages of hybrids was calculated for all sample sizes of at least 20, and values left of slash represent percentages of all hybrid genotypes, those right of the slash represent percentage of hybrids not including introgressed *S. malma*.

of the two parental species in a sample varied between tributaries to Thutade Lake and across the other British Columbia localities, but there was no significant association between the relative frequencies of parental species and the percentage of hybrids in the population ($P = 0.47$, Z. Redenbach & E.B. Taylor, unpublished data).

Hardy–Weinberg, gametic, and cD

In locally sympatric populations fewer heterozygotes were observed than expected, yielding positive F_{IS} values ranging from 0.581 to 1.0 (Table 1). Deviations from expected values were significant at all localities and loci but one, the Chutine River at MTB, using the sequential Bonferroni correction (Table 1).

The same pattern was observed in the Thutade lake watershed; genotype frequencies deviated significantly from expectations at all four nuclear loci ($P < 0.0001$) for all five sympatric tributaries. In every case, heterozygote deficiencies produced positive F_{IS} values ranging from 0.621 to 1.0 (Table 3).

The Thutade Lake data clearly indicated nonrandom mating within the various samples. Contingency tests showed significant gametic disequilibria, for all six nuclear locus pairs in the five sympatric tributaries to Thutade Lake (Table 3), under the null hypothesis of independence of genotypes between loci, for all locus pairs in all tributaries ($P < 0.0001$). Contingency testing revealed significant cD under the null hypothesis of genotypes at each nuclear locus are independent from cytotype, for all locus pairs in Kemess Creek and in the

Table 3 Inbreeding coefficients (F_{IS}), gametic disequilibria (D′), and cytonuclear disequilibria (cD_i) for five tributaries of Thutade Lake.

Tributary	F_{IS}	D′	cD_1	cD_2	cD_3	cD
Kemess Creek	0.925/0.963	0.972/0.986	0.228/0.231	−0.004/0.008	−0.236/−0.223	0.229/0.232
South Pass Creek	0.918/1.0	0.989/1.0	0.138/0.138	−0.025/0.0	−0.138/−0.112	0.125/0.138
Tributary 4	0.847/0.968	0.907/0.991	0.092/0.096	−0.004/0.003	−0.097/−0.087	0.089/0.097
Attichika River	0.625/0.807	0.801/0.866	0.068/0.072	0.002/0.006	−0.074/−0.074	0.071/0.073
Niven River	0.621/0.778	0.733/0.870	0.044/0.047	0.002/0.005	−0.049/−0.049	0.046/0.048
Thutade total	0.753/0.888	0.858/0.932	0.085/0.086	−0.002/−0.001	−0.086/−0.084	0.085/0.086

The ranges, separated by slashes, given indicate those statistics obtained for the four nuclear loci and mtDNA (i.e. yielding four F_{IS}, six (pairwise) D′ and four of each cD_i). For cD_i statistics, only Kemess Creek has sufficient sample size for statistical analyses. In this case, all other tributaries were pooled and are represented by the 'Thutade total' sample. All values are statistically significant at $P < 0.001$ (see text for details).

sample consisting of all other tributaries pooled ($P < 0.001$). cD varied much more between tributaries than did either F_{IS} or gametic disequilibria (Table 3). All three genetic statistics, however, showed similar results between loci within a tributary. In general, positive cD, and cD_1 (across loci and tributaries) indicated nonrandom mating between species and an association between *S. confluentus* mtDNA and homozygotes for *S. confluentus* nuclear alleles. By contrast, negative cD_3 indicated a strong association between homozygous *S. malma* nuclear genotypes and *S. malma* mtDNA. Finally, positive and negative cD_2 (Table 3) indicated shifting associations between nuclear heterozygotes and *S. confluentus* (or *S. malma*) mtDNA among loci. This variation in the sign of cD_2 resulted from cD_2 being positive (indicating an association between heterozygotes and *S. confluentus* mtDNA) for all loci except for GH2. In three of the five samples, and for the pooled sample, negative cD_2 indicated an association between GH2 heterozygotes and *S. malma* mtDNA (Table 3), a result that may stem from biased introgression of *S. confluentus* GH2 alleles into *S. malma* (see below).

In an interspecific mating, the female parent can be identified by the mtDNA of the F_1 hybrids and five of the six F_1 genotype hybrids (five juveniles and one adult) contained *S. confluentus* mtDNA. The single exception was heteroplasmic, containing both *S. malma* and *S. confluentus* mtDNA. A binomial test on the distribution of mtDNAs in the five homoplasmic indicated that interspecific hybridization was significantly asymmetrical ($n = 5$, $P = 0.031$), and biased in the direction of *S. malma* male by *S. confluentus* female matings. This unidirectionality is supported by the mtDNA in the other genotypic classes of hybrid (see below) and the significantly positive cD_2 across most loci (Table 3).

Introgression

Of the 89 juvenile hybrids from the Thutade Lake watershed, there were only five F_1 genotypes and six F_n genotypes limiting the size of any potential hybrid swarm (i.e. pool of interbreeding hybrid individuals; Table 2, Fig. 3). BC genotypes ($n = 59$) and mitochondrial introgressions ($n = 20$) were the most common hybrid classes (Fig. 3), and indicated that introgression has occurred.

In the six tributaries, introgression of *S. malma* nuclear alleles into *S. confluentus* ranged from 0 to 2.65%, while introgression of *S. confluentus* nuclear alleles into *S. malma* ranged from 0 to 3.13% (Table 4). Tributaries varied in the predominant directions of introgression, but the directionality did not correspond to the prevalence of the two parental species at the site, which may reflect sampling error, or perhaps local differences in mating preference. The watershed average was 1.45% introgression of *S. confluentus* nuclear alleles into *S. malma* and 1.00% introgression of *S. malma* nuclear alleles into *S. confluentus* (Table 4). If, however, GH2 is removed from he analysis the overall nuclear introgression into *S. malma* (1.02%) is very similar to that of nuclear introgression into *S. confluentus* (1.00%).

Locus-by-locus scrutiny, however, revealed that *S. confluentus* alleles for the four nuclear loci introgress into *S. malma* to different degrees (based on the number of foreign alleles, and under the null hypothesis of equal introgression, $\chi^2 = 12.4$, $P < 0.01$). Introgression of *S. confluentus* GH2 into *S. malma* occurred at 2.7 the average extent observed at the other three loci (21 of 758 alleles scored), each of which introgressed by about the same degree (7, 9, and 7 alleles for ITS-1, MTB, and FOK) (Fig. 4). In *S. confluentus*, however, *S. malma* alleles at the four loci introgressed at similar levels (observed introgression of 11, 10, 12, and 15 of 1202 scored alleles for GH2, ITS-1, MTB, and FOK, $\chi^2 = 1.2$, ns) (Fig. 4).

A similar pattern of increased heterozygosity of GH2 over another locus (MTB) was noted outside the Thutade Lake watershed (Table 1). Across all localities, including Thutade Lake, 49 individuals were heterozygous for GH2, but only 32 were heterozygous for MTB, and this was consistent across five of the six localities containing hybrids when each was considered separately (GH2/

Table 4 Introgression of nuclear and mtDNA alleles between *Salvelinus malma* and *S. confluentus* in the Thudate Lake watershed.

Tributary	Species	*n*	No. heterospecific nuclear alleles	Percent introgression	No. heterospecific mtDNA alleles	Percent mtDNA introgression
Kemess Cr.	*S. confluentus*	325	17	0.65	0	0
	S. malma	268	35	1.63	13	4.9
South Pass Cr.	*S. confluentus*	47	0	0	0	0
	S. malma	17	4	2.94	5	29.4
Tributary 4	*S. confluentus*	66	7	1.33	0	0
	S. malma	20	1	0.62	9	45.0
Attichika R.	*S. confluentus*	78	6	0.96	0	0
	S. malma	7	0	0	0	0
Niven R.	*S. confluentus*	85	18	2.65	0	0
	S. malma	8	2	3.13	3	37.5
Attycelley R.	*S. confluentus*	0	0	0	0	0
	S. malma	59	2	0.42	1	1.7
Total	*S. confluentus*	601	48	1.0	0	0
	S. malma	379	44	1.45	31	8.2

All individuals that were identified as pure *S. confluentus* or backcrosses (BCs) to *S. confluentus* have been pooled into '*S. confluentus*' while all those identified as pure *S. malma*, BCs to *S. malma*, or mtDNA-introgressed *S. malma* have been pooled into '*S. malma*.' All putative F_1 and F_n individuals ($n = 6$ and 5 fish, respectively) were excluded from the analysis. Four nuclear loci were examined and percent introgression was, therefore, calculated as the fraction of heterospecific alleles relative to the total number of alleles (8).

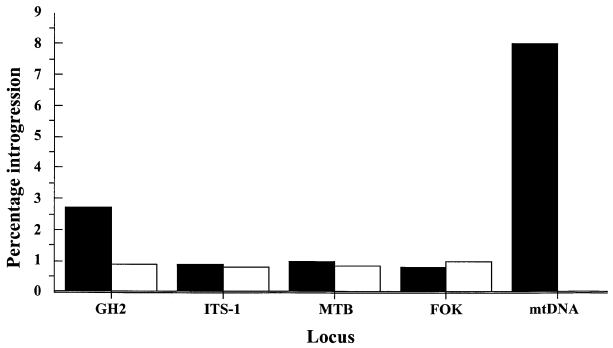

Fig. 4 Introgression of species-specific alleles in *Salvelinus malma* and *S. confluentus* at four nuclear loci and for mitochondrial DNA. Percent introgression refers to the number of heterospecific alleles present divided by the total number of alleles (i.e. $2n$ for nuclear, n for mitochondrial). Black bars represent percentage introgression of *S. confluentus* alleles into *S. malma*, white bars represent percentage introgression of *S. malma* alleles into *S. confluentus*.

MTB: 6/2, 1/0, 37/28, 1/0, 2/2, 2/0; order as in Table 1). This difference between GH2 and MTB was, however, not significant (pooled $\chi^2 = 3.16$, ns), and the Tahltan River was an exception with equal heterozygosities for the two loci (2/2; Table 1).

Mitochondrial introgression appeared to be unidirectional because we observed no introgression of *S. malma* mtDNA into *S. confluentus* (Table 4, Fig. 4). Introgression of *S. confluentus* mtDNA into *S. malma* ranged from 0 to 45% in the six Thudate Lake tributaries. Of 89 hybrids, 67 had *S. confluentus* mtDNA, 21 had *S. malma* mtDNA, and one was heteroplasmic. Five of six F_1 genotypes contained *S. confluentus* mtDNA, as did all *S. confluentus* BC genotypes, all mitochondrially IG *S. malma* individuals, most (four of five) F_n genotypes, and many (11 of 31) *S. malma* BC genotypes (Table 2). The only hybrid individuals with *S. malma* mtDNA were *S. malma* BC genotypes (20 of the 31 BCDV), a single F_n genotype, and the heteroplasmic F_1 (Table 2).

Microgeography of hybridization

The distribution of fry (0+) and juveniles (1+ and >1+ classes) in Kemess Creek differed between the two *Salvelinus* species ($P < 0.001$ for comparisons between species for both age classes, Table 5). *Salvelinus malma* fry occurred near sites of groundwater upwelling in both 1997 and 1998 that were located in the headwaters of Kemess Creek (across six sites, $n = 61$ fry), and in a

Table 5 Distribution of *Salvelinus malma*, *S. confluentus*, and hybrid char among three sections of Kemess Creek. Genotypes were inferred from variation at four diagnostic nuclear loci. Localities correspond to three, approximately 5 km long sections of Kemess Creek. Hybrids include F_1, post-F_1, and backcross genotypes.

Genotype	Lower Kemess Cr.	Middle Kemess Cr.	Upper Kemess Cr.
S. malma – fry	57	1	61
S. malma – juveniles	38	20	64
Hybrids – fry	17	2	5
Hybrids – juveniles	4	5	6
S. confluentus – fry	144	61	43
S. confluentus – juveniles	40	32	12
Total	300	121	190

small tributary stream in the lower region of Kemess Creek (across five sites, $n = 57$), both of which are also preferred spawning sites for *S. malma* (Bustard, 1998, 2000). Juvenile *S. malma*, however, were distributed more evenly throughout Kemess Creek, but were still relatively more concentrated in the upper and lower most sections than *S. confluentus* ($P < 0.001$ between life stages of *S. malma*, Table 5). By contrast, *S. confluentus* fry and juveniles were similarly and more widely distributed in Kemess Creek as were the spawning locations of adults ($P = 0.07$ for difference between *S. confluentus* age classes, Table 5; Bustard, 1998, 2000). Although hybrids were distributed throughout Kemess Creek, hybrid fry tended to be more concentrated in the lower portions of Kemess Creek than juvenile hybrids ($P = 0.043$, Table 5). Both F_1 genotypes, however, were juveniles (86 and 143 mm in length) and were found within the headwaters near concentrations of the *S. malma* and *S. confluentus* spawning areas. Similarly, the F_n genotypes (two fry and one juvenile), which were likely the offspring of a mating between two *S. malma* BCs, were all found in the headwaters, while *S. malma* BC genotypes were also concentrated near *S. malma* spawning areas as fry ($n = 15$), but throughout the system as juveniles ($n = 10$, Table 5). By contrast, *S. confluentus* BC genotypes (seven fry and two juveniles) were all found in more downstream areas of Kemess Creek.

Discussion

Genetic distinction in sympatry

Over a broad geographical range of contact, and in a detailed examination in the Thutade Lake watershed, *S. malma* and *S. confluentus* formed two incompletely isolated, nonrandomly intermating populations. There was significant heterozygote deficiency (F_{IS}) and significant nuclear and cytonuclear disequilibria (D' and cD_i), although hybrids constituted up to 25% of all char sampled in some areas, and 9% of those in the Thutade Lake study. The genetic distinction between sympatric *S. malma* and *S. confluentus* is consistent with differences between species in life history, reproductive ecology, and zoogeography (Baxter *et al*., 1997; Hagen & Taylor, 2001; Redenbach & Taylor, 2002). Such differentiation supports their designation as distinct species under a variety of definitions despite remaining hesitation in some jurisdictions (e.g. Templeton, 1989; Mallet, 1995; Mayr, 1996; cf. Leary & Allendorf, 1997). Extant sympatric populations of *S. malma* and *S. confluentus* have developed through processes of dispersal and watershed exchanges since the retreat of the Wisconsinan glaciers beginning about 15 000 years ago (McPhail & Lindsey, 1986; Redenbach & Taylor, 2002). Presumably these species have been hybridizing and exchanging genes at least for that length of time, yet they persist as genetically distinct in sympatry.

Maintenance of genetic distinction

Prezygotic isolation may play an important role in structuring hybrid zones with bimodal genotype distributions (Jiggins & Mallet, 2000). Aspects of the reproductive ecology of sympatric *S. malma* and *S. confluentus* are consistent with this idea. There are no morphological impediments to hybridization between *S. malma* and *S. confluentus* because, as with most fish, fertilization is external. Assortative mating, however, probably plays a large part in prezygotic isolation, because only a maximum of 0.5% of juvenile char in the Thutade Lake watershed had an F_1 genotype. We suggest that processes of prezygotic isolation play a more prominent role in accounting for the low incidence of F_1 hybrids, rather than post-zygotic selection against hybrids at early life history stages, for four reasons. First, intrinsic selection against juvenile F_1 hybrids apparently is limited; they suffer no survival disadvantage relative to parental genotypes under laboratory conditions (Haas & McPhail, 1991). Secondly, analysis of habitat use and diet in sympatric juvenile char suggest that differences between *S. malma* and *S. confluentus* in streams of the Thutade Lake watershed are slight (Hagen & Taylor, 2001). Consequently, there is no obvious basis for selection against phenotypically intermediate juvenile hybrid char in terms of habitat or trophic ecology as suggested for other sympatric fish species where parental species are more strongly specialized to alternative niches (e.g. reviewed in Schluter, 1998; Taylor, 1999). Thirdly, although they were rare, F_1 hybrids were found throughout the age classes sampled including two 1997 fry, one 1998 fry, two 1998 >1+ age class juveniles, and one adult. This dispersion of F_1 genotypes indicates at least some survival across years for hybrids. Finally, the many BC hybrids and, to some extent, advanced generation hybrids indicates that hybrids survive, are fertile, and can mate successfully.

By contrast, several processes might contribute to prezygotic isolation, particularly reproductive ecology. First, *S. confluentus* typically mature and spawn earlier in the year than do *S. malma* (Bustard, 1998; Hagen & Taylor, 2001). Secondly, *S. malma* and *S. confluentus*, and salmonid fishes in general typically exhibit size-assortative mating (Leggett, 1980; Foote & Larkin, 1988; Sigurjonsdottir & Gunnarsson, 1989; Maekawa *et al*., 1993, 1994; Sexauer, 1994; Baxter, 1997). Consequently, the large body size-at maturity difference should promote a high degree of reproductive isolation between species (12–21 cm for *S. malma* and 40–90 cm for *S. confluentus*; Hagen & Taylor, 2001). Thirdly, *S. malma* and *S. confluentus* also have distinct spawning site preferences that are largely a function of interspecific differences in size-at-maturity, which probably contribute to spatial isolation between species during reproduction (Bustard, 1998; Hagen & Taylor, 2001). These observations strongly suggest that the high degree of reproductive

isolation between *S. malma* and *S. confluentus* in sympatry is promoted by prezygotic processes, and support the hypothesis of Jiggins & Mallet (2000) that bimodal hybrid zones, in which there are few F_1 hybrids relative to BCs and parental genotypes, result under such conditions.

Post-zygotic selection against hybrids may also contribute to the maintenance of a biomodal hybrid zone in char. For instance, *S. confluentus* have 78 chromosomes while *S. malma* are polymorphic, but have 82 chromosomes in populations most proximal to the geographical range of *S. confluentus* (Cavender & Kimura, 1989; Phillips *et al.*, 1999). Consequently, intrinsic genomic incompatibilities are possible particularly for post-F_1 hybrids. In addition, although there is no obvious basis for extrinsic selection against stream-resident juvenile hybrids in the Thutade Lake watershed, selection may act against hybrids at other life history stages such as during lakeward migration or residence, or during reproduction (Hagen & Taylor, 2001). Life history differences between hybridizing taxa have been suggested to account for the absence of hybrids at adult life history stages in other salmonids and deserve study in *Salvelinus* (e.g. Campton & Utter, 1985).

Asymmetry of hybridization

We observed differential introgression across the four nuclear loci which could be the result of variable selection. In particular, nuclear loci introgress to different degrees in the two parental species. All four loci in *S. confluentus* and three of the four loci in *S. malma* introgress to the same extent (i.e. they have the same proportion of foreign to parental alleles). *Salvelinus confluentus* GH2, however, introgressed into *S. malma* 2.7 times more than the other nuclear loci (Fig. 4). Such apparently differential introgression may result from positive selection for, heterospecific GH2 alleles in *S. malma*. Similar observations of interlocus variation in introgression have been suggested to be the result of differential selection in other taxa (e.g. Shoemaker *et al.*, 1996; Poteaux *et al.*, 1998; Hey, 2001; Martinsen *et al.*, 2001), and because the same pattern was repeated in populations outside the Thutade Lake, differential introgression of GH2 appears to be a general phenomenon. The well known phenotypic effects of differential expression of Growth Hormone loci in salmonid fish (reviewed by Bjornsson, 1997) may make it susceptible to selection.

Our data also suggest differential introgression of *S. confluentus* mitochondrial DNA into *S. malma* because all but one of the putative F_1 genotype hybrids ($n = 6$) contained exclusively *S. confluentus* mtDNA which, given the maternal inheritance of mtDNA, indicates that the interspecific mating is *S. malma* male by *S. confluentus* female. This unidirectionality is supported by the mtDNA in the other genotypic classes of hybrids; *S. malma* mtDNA was found only in *S. malma* BC genotypes, while all other hybrid genotype classes exhibited *S. confluentus* mtDNA. Unidirectionality was also observed in *S. malma*/*S. confluentus* hybridization in the Skagit River, in southwestern British Columbia (McPhail & Taylor, 1995), and is common in interspecific hybridization (e.g. Lamb & Avise, 1986; Kitano *et al.*, 1994). Although further sampling is required to confirm the introgression bias and to see if it is geographically widespread, knowledge of the mating system and biology of sympatric char suggests that biased introgression of *S. confluentus* mtDNA is probably common. Further, because there is no evidence of biased mortality of hybrids from reciprocal crosses between *S. malma* and *S. confluentus* (Haas & McPhail, 1991), and because we observed *S. confluentus* mtDNA in F_1 and other hybrid classes, post-zygotic causes of strongly asymmetrical hybridization are unlikely (e.g. Rand & Harrison, 1989; Wirtz, 1999). By contrast, two of the 11 prezygotic processes described by Wirtz (1999) likely explain the predominance of *S. confluentus* mtDNA in hybrid char: reproductive behavioural differences between species, and unidirectional 'sneak' fertilizations.

First, *S. confluentus* mature earlier in the season than *S. malma* in the Thutade Lake watershed and any overlap in spawning times occurs at the end of the *S. confluentus* spawning period (Hagen & Taylor, 2001). In addition, salmonid males generally ripen earlier than females, and females remain ripe later (e.g. Kitano, 1996). It is, therefore, more likely that a *S. malma* male would become ripe in time to encounter a *S. confluentus* female (i.e. this scenario requires less spawning time overlap) than that a *S. confluentus* male would remain ripe long enough to encounter a ripe *S. malma* female. Secondly, 'sneaking' is a common parasitic mating behaviour widespread in salmonid fishes, including char, and occurs when a smaller male rushes into the nest of a larger mating pair of the same species and attempts to fertilize the eggs of the female (Svedang, 1992; Maekawa *et al.*, 1993, 1994; Taborsky, 1998). In allopatry, sneaking has been observed both in *S. confluentus* and in *S. malma* (Maekawa *et al.*, 1993; Baxter, 1997). In the Thutade Lake watershed, as in most sympatric sites, *S. malma* is the smaller of the two species (Hagen & Taylor, 2001). A hybrid mating, therefore, would be expected to occur when a *S. malma* male sneaks on a *S. confluentus* spawning pair. In fact, sneaking has been proposed as an explanation for interspecific hybridization in other char and salmonids (e.g. McGowan & Davidson, 1992; Kitano *et al.*, 1994). We suggest, therefore, that although body size differences between *S. malma* and *S. confluentus* in sympatry may limit interspecific pairings, they may, in turn, promote some interspecific matings by sneaking.

If the avenue for hybridization is limited to the sneaking of male *S. malma* on *S. confluentus* spawning pairs, then complete prezygotic reproductive isolation may be difficult to establish, because *S. malma* are employing an intraspecific parasitic reproductive strategy that, presumably, is successful in allopatric populations of

S. malma (e.g. Gross, 1985; Maekawa *et al.*, 1993, 1994). In addition, any selective penalty for interspecific sneaking by *S. malma* males (e.g. reduced interaction with conspecifics) is reduced because sneaking takes place before the main *S. malma* spawning activity. Jiggins & Mallet (2000) suggested that reinforcement of premating isolation should be more likely in bimodal hybrid zones, such as we have described for *S. malma* and *S. confluentus*. The hypothesis that we propose for the asymmetrical hybridization via sneak fertilizations, however, may represent a scenario where the potential for reinforcement to enhance reproductive isolation is constrained.

A role for ecological speciation?

The role of ecology in speciation has received renewed attention (Orr & Smith, 1998; Schluter, 1998). The term 'ecological speciation' describes the evolution of reproductive isolation as a consequence of specialization to alternative ecological niches (e.g. Schluter, 1996). Hybrid zones between *S. malma* and *S. confluentus* may contribute to understanding the potential importance of ecological speciation at a level of divergence greater than that usually associated with speciation in postglacial fishes (Schluter, 1996, 1998). For instance, molecular phylogenetic analysis of *S. malma* and *S. confluentus* indicates that they are not sister species and last shared a common ancestor several million years ago (Grewe *et al.*, 1990; Pleyte *et al.*, 1992; Crane *et al.*, 1994). The two species, however, are capable of exchanging genes and intrinsic genetic incompatibilities and feeding ecology of juvenile hybrids appear to make limited contributions to reproductive isolation. By contrast, although direct evidence is lacking, migratory ecology of juvenile fish, and feeding and reproductive ecology of adult char may still play a role in extrinsic selection against hybrids.

Ecological speciation is a fairly specific mechanism whereby divergent selection promotes the evolution of specializations to alternative niches which may lead to post-zygotic selection against phenotypically intermediate hybrids (Schluter, 1996). The likelihood of reproductive isolation is increased if the ecological specializations also, directly or indirectly, decrease the likelihood of heteromorphic mating, i.e., they promote the evolution of prezygotic isolation (Rice & Hostert, 1993). Size-assortative mating in sympatric char, resulting from ecological specialization to migratory or stream-resident life history, therefore, may indirectly promote the evolution of reproductive isolation in a manner similar to that proposed for more recent (<15 000 years) divergences in other north temperate fishes (Schluter & Nagel, 1995; Wood & Foote, 1996). Whether or not such selection for divergent traits initiates the evolution of reproductive isolation directly, is a consequence of post-speciation divergence, or acts in reinforcement are major unknowns in speciation research. Interestingly, char diagnosed as *S. malma* and *S. confluentus* using morphological and biochemical/molecular characters also have widespread distributions in northwestern North America as allopatric populations where they appear to overlap in size-at-maturity and general life history to a greater extent than when sympatric (e.g. Maekawa *et al.*, 1993; McPhail & Baxter, 1996; Underwood *et al.*, 1996). These observations suggest that character displacement in life history, and its morphological and behaviour correlates, may be an important process in the evolution of *S. malma* and *S. confluentus* and, more generally, that ecological processes contribute to speciation in the face of gene flow (Rice & Hostert, 1993).

Acknowledgments

Our research could not have been completed without the assistance and experience of D. Bustard and J. Hagen. We thank A. Altman for laboratory assistance, J. Baker for sharing MTB primer sequences with us, G. Bell, J. Hagen, S. Karl, J.D. McPhail, M. Whitlock, and two reviewers for helpful discussion, comments, and advice. This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) by grants to EBT, and an NSERC Postgraduate Scholarship awarded to Z.R. We appreciate the logistical support provided by Kemess Mines, Inc. and the British Columbia Ministry of Water, Land and Air Protection (Fisheries Branch).

References

Arnold, M.L. 1997. *Natural Hybridization and Evolution*. Oxford University Press, New York.

Asmussen, M.A., Arnold, J. & Avise, J.C. 1987. Definition and properties of disequilibrium statistics for associations between nuclear and cytoplasmic genotypes. *Genetics* **115**: 755–768.

Avise, J.C. 1994. *Molecular Markers, Natural History and Evolution*. Chapman & Hall, New York.

Avise, J.C. & Van den Avyle, M.J. 1984. Genetic analysis of reproduction of hybrid white bass X striped bass in the Savanna River. *Trans. Am. Fish. Soc.* **113**: 563–570.

Baker, J., Bentzen, P. & Moran, P. 2002. Molecular markers distinguish coastal cutthroat trout from coastal rainbow trout/steelhead and their hybrids. *Trans. Am. Fish. Soc.* **131**: 404–417.

Barton, D.M. 1979. The dynamics of hybrid zones. *Heredity* **43**: 341–359.

Barton, N.H. & Hewitt, G.M. 1985. Analysis of hybrid zones. *Annu. Rev. Ecol. Syst.* **16**: 113–148.

Baxter, J.S. 1997. *Aspects of the Reproductive Ecology of Bull Trout (Salvelinus confluentus) in the Chowade River, British Columbia*, MSc thesis. University of British Columbia, Vancouver.

Baxter, J.S., Taylor, E.B., Devlin, R.H., Hagen, J. & McPhail, J.D. 1997. Evidence for natural hybridization between Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) in a northcentral British Columbia watershed. *Can. J. Fish. Aquat. Sci.* **54**: 421–429.

Bjornsson, B.T. 1997. The biology of salmon growth hormone: from daylight to dominance. *Fish Physiol. Biochem.* **17**: 9–24.

Boecklen, W.J. & Howard, D.J. 1997. Genetic analysis of hybrid zones: numbers of markers and power of resolution. *Ecology* **78**: 2611–2616.

Bustard, D. 1998. *Kemess South Project: 1998 Fish Monitoring Studies*. Consult. Rept. prep. by D. Bustard and Assoc., Smithers, B.C. for Kemess Mines, Inc., Vancouver, Canada.

Bustard, D. 2000. *Kemess South Project: 2000 Fish Monitoring Studies*. Consult. Rept. prep. by D. Bustard and Assoc., Smithers, B.C. for Kemess Mines, Inc., Vancouver, Canada.

Butlin, R.K. 1998. What do hybrid zones in general, and the *Chorthippus parallelus* zone in particular, tell us about speciation? In: *Endless Forms. Species and Speciation* (D. J. Howard & S. H. Berlocher, eds), pp. 367–378. Oxford University Press, New York.

Campton, D.E. 1987. Natural hybridization and introgression in fishes: methods of detection and genetic interpretations. In: *Population Genetics and Fishery Management* (N. Ryman & F. Utter, eds), pp. 161–192. University of Washington Press, Seattle, WA, USA.

Campton, D.E. & Utter, F.M. 1985. Natural hybridization between steelhead trout (*Salmo gairdneri*) and coastal cutthroat trout (*S. clarki*) in two Puget Sound streams. *Can. J. Fish. Aquat. Sci.* **42**: 110–119.

Cavender, T.M. 1978. Taxonomy and distribution of the bull trout, *Salvelinus confluentus* (Suckley), from the American Northwest. *Calif. Fish Game* **64**: 139–174.

Cavender, T.M. & Kimura, S. 1989. Cytotaxonomy and interrelationships of Pacific basin *Salvelinus*. *Physiol. Ecol. Japan Spec.* **1**: 49–68.

Coyne, J.A. & Orr, H.A. 1997. 'Patterns of speciation in *Drosophila*' revisited. *Evolution* **51**: 295–303.

Crane, P.A., Seeb, L.W. & Seeb, J.E. 1994. Genetic relationships among *Salvelinus* species inferred from allozyme data. *Can. J. Fish. Aquat. Sci.* **51** (Suppl. 1): 182–197.

Dobzhansky, T.H. 1937. *Genetics and the Origin of Species*, 1st edn. Columbia University Press, New York.

Foote, C.J. & Larkin, P.A. 1988. The role of male choice in the assortative mating of sockeye salmon and kokanee, the anadromous and non-anadromous forms of *Oncorhynchus nerka*. *Behaviour* **106**: 43–62.

Grewe, P.M., Billington, N. & Hebert, P.D.N. 1990. Phylogenetic relationships among members of *Salvelinus* inferred from mitochondrial DNA divergence. *Can. J. Fish. Aquat. Sci.* **47**: 984–991.

Gross, M.R. 1985. Disruptive selection for alternative life histories in salmon. *Nature* **313**: 47–48.

Haas, G.R. & McPhail, J.D. 1991. Systematics and distribution of Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) in North America. *Can. J. Fish. Aquat. Sci.* **48**: 2191–2211.

Hagen, J. & Taylor, E.B. 2001. Habitat partitioning as a factor limiting gene flow in hybridizing populations of Dolly Varden char (*Salvelinus malma*) and bull trout (*S. confluentus*). *Can. J. Fish. Aquat. Sci.* **58**: 2037–2047.

Hamada, M., Takasaki, N., Reist, J.D., DeCicco, A.L., Goto, A. & Okada, N. 1998. Detection of the ongoing sorting of ancestrally polymorphic SINEs toward fixation or loss in populations of two species of charr during speciation. *Genetics* **150**: 301–311.

Hartl, D.L. & Clark, A.G. 1997. *Principles of Population Genetics*, 3rd edn. Sinauer Associates, Sunderland, MA, USA.

Hewitt, G.M. 1989. The subdivision of species by hybrid zones. In: *Speciation and its Consequences* (D.J. Otte and J.E. Endler, eds), pp. 85–110. Sinauer Associates, Inc., Sunderland, MA, USA.

Hey, J. 2001. *Genes, Categories, and Species. The Evolutionary and Cognitive Causes of the Species Problem*. Oxford University Press, Oxford.

Howard, D.J., Gregory, P.G., Chu, J. & Cain, M.L. 1998. Conspecific sperm precedence is an effective barrier to closely related species. *Evolution* **52**: 511–516.

Jiggins, C.D. & Mallet, J. 2000. Bimodal hybrid zones and speciation. *Trends. Ecol. Evol.* **15**: 250–255.

Kitano, S. 1996. Size-related factors causing individual variation in seasonal reproductive success of fluvial male Dolly Varden (*Salvelinus malma*). *Ecol. Freshw. Fish* **5**: 59–67.

Kitano, S., Maekawa, K., Nakano, S. & Fausch, K.D. 1994. Spawning behaviour of bull trout in the Upper Flathead Drainage, Montana, with special reference to hybridization with brook trout. *Trans. Am. Fish. Soc.* **123**: 988–992.

Lamb, T. & Avise, J.C. 1986. Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: the influence of mating behavior. *Proc. Nat. Acad. Sci. USA* **83**: 2526–2530.

Leary, R.F. & Allendorf, F.W. 1997. Genetic confirmation of sympatric bull trout and Dolly Varden in western Washington. *Trans. Am. Fish. Soc.* **126**: 715–720.

Leggett, J.W. 1980. Reproductive ecology and behaviour of Dolly Varden charr in British Columbia. In: *Charrs: Salmonid Fishes of the Genus Salvelinus* (E. K. Balon, ed.), pp. 721–737. Dr. W. Junk Publs., The Hague, The Netherlands.

Louis, E.J. & Dempster, E.R. 1987. An exact test for Hardy–Weinberg and multiple alleles. *Biometrics* **43**: 800–802.

McGowan, C. & Davidson, W.S. 1992. Unidirectional hybridization between brown trout (*Salmo trutta*) and Atlantic salmon (*S. salar*) in Newfoundland. *Can. J. Fish. Aquat. Sci.* **49**: 1953–1958.

McKay, S.J., Devlin, R.H. & Smith, M.J. 1996. The phylogeny of Pacific salmon and trout based on growth hormone type-2 (GH2) and mitochondrial NADH dehydrogenase subunit 3 (ND3) DNA sequences. *Can. J. Fish. Aquat. Sci.* **53**: 1165–1174.

McPhail, J.D. 1961. A systematic study of the *Salvelinus alpinus* complex in North America. *Can. J. Fish. Aquat. Sci.* **18**: 793–816.

McPhail, J.D. & Baxter, J.S. 1996. A review of bull trout (*Salvelinus confluentus*) life-history and habitat use in relation to compensation and improvement opportunities. *B.C. Ministry of Environment, Lands and Parks, Fisheries Branch, Fish. Mgmt. Rpt.* **104**: 1–31.

McPhail, J.D. & Lindsey, C.C. 1986. Zoogeography of the freshwater fishes of Cascadia (the Columbia River and rivers north to the Stikine). In: *The Zoogeography of North American Freshwater Fishes* (C. H. Hocutt & E. O. Wiley, eds), pp. 615–637. John Wiley and Sons, New York.

McPhail, J.D. & Taylor, E.B. 1995. *The Skagit Char Project*. Final Report to Skagit Environmental Endowment Commission (project 94-1). Seattle, WA, USA.

Maekawa, K., Hino, T., Nakano, S. & Smoker, W.W. 1993. Mate preference in anadromous and landlocked Dolly Varden (*Salvelinus malma*) females in two Alaskan streams. *Can. J. Fish. Aquat. Sci.* **50**: 2375–2379.

Maekawa, K., Shigeru, N. & Yamamoto, S. 1994. Spawning behaviour and size-assortative mating of Japanese charr in an artificial lake-inlet stream system. *Environ. Biol. Fishes* **39**: 109–117.

Mallet, J. 1995. A species definition for the modern synthesis. *Trends Ecol. Evol.* **10**: 294–299.

Martinsen, G.D., Whitman, T.G., Turek, R.J. & Keim, P. 2001. Hybrid populations selectively filter gene introgression between species. *Evolution* **55**: 1325–1335.

Mayr, E. 1963. *Animal Species and Evolution*. The Belknap Press, Harvard University Press, Cambridge, MA, USA.

Mayr, E. 1996. What is a species, and what is not? *Phil. Sci.* **63**: 262–277.

Moore, W.S. 1977. An evaluation of narrow hybrid zones in vertebrates. *Quart. Rev. Biol.* **52**: 263–277.

Orr, M.R. & Smith, T.B. 1998. Ecology and speciation. *Trends Ecol. Evol.* **13**: 502–506.

Phillips, R.B., Manley, S.A. & Daniels, T.J. 1994. Systematics of the salmonid genus *Salvelinus* inferred from ribosomal DNA sequences. *Can. J. Fish. Aquat. Sci.* **51** (Suppl. 1): 198–204.

Phillips, R.B., Sajdak, S.L. & Domanico, M.J. 1995. Relationships among charrs based on DNA sequences. *Nord. J. Freshw. Res.* **71**: 378–391.

Phillips, R.B., Gidex, L.I., Westrich, K.M. & DeCicco, A.L. 1999. Combined phylogenetic analysis of ribosomal ITS1 sequences and new chromosome data supports three subgroups of Dolly Varden char (*Salvelinus malma*). *Can. J. Fish. Aquat. Sci.* **56**: 1504–1511.

Pleyte, K.A., Duncan, S.D. & Phillips, R.B. 1992. Evolutionary relationships of the salmonid fish genus *Salvelinus* inferred from DNA sequences of the first internal transcribed spacer (ITS 1) of ribosomal DNA. *Mol. Phylog. Evol.* **1**: 223–230.

Poteaux, C., Bonhomme, F. & Berrebi, P. 1998. Differences between nuclear and mitochondrial introgressions of brown trout populations from a restocked main river and its unrestocked tributary. *J. Fish Biol.* **63**: 379–392.

Rand, D.M. & Harrison, R.G. 1989. Ecological genetics of a mosaic hybrid zone:mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. *Evolution* **43**: 432–449.

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

Redenbach, Z. & Taylor, E.B. 2002. Evidence for historical introgression along a contact zone between two species of char (Pisces: Salmonidae) in northwestern North America. *Evolution* **56**: 1021–1035.

Remington, C.L. 1968. Suture-zones of hybrid interaction between recently joined biotas. *Evol. Biol.* **2**: 321–428.

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

Rice, W.R. & Hostert, E.E. 1993. Laboratory experiments on speciation: what have we learned in 40 years? *Evolution* **47**: 1637–1653.

Rieseberg, L.H., Baird, S.J.E. & Desrochers, A.M. 1998. Patterns of mating in wild sunflower hybrid zones. *Evolution* **52**: 713–726.

Roff, D.A. & Bentzen, P. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. *Mol. Phylog. Evol.* **6**: 539–545.

Schluter, D. 1996. Ecological speciation in postglacial fishes. *Phil. Trans. R. Soc. Lond. B Biol. Sci.* **351**: 807–814.

Schluter, D. 1998. Ecological causes of speciation. In: *Endless Forms: Species and Speciation* (D. J. Howard & S. H. Berlocher, eds), pp. 114–129. Oxford University Press, New York.

Schluter, D. & Nagel, L.M. 1995. Parallel speciation by natural selection. *Am. Nat.* **146**: 292–301.

Sexauer, H.A. 1994. *Life History Aspects of Bull Trout, Salvelinus confluentus, in the Eastern Cascades, Washington*, MSc Thesis. Central Washington University, Ellensburg, WA, USA.

Shoemaker, D.D., Ross, K.G. & Arnold, M.L. 1996. Genetic structure and evolution of a fire any hybrid zone. *Evolution* **50**: 1958–1970.

Sigurjonsdottir, H. & Gunnarsson, K. 1989. Alternative mating tactics of Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. *Environ. Biol. Fishes* **26**: 159–176.

Soltis, D.E., Gitzendanner, M.A., Strenge, D.D. & Soltis, P.A. 1997. Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. *Plant Syst. Evol.* **206**: 353–373.

Svedang, H. 1992. Observations on interbreeding between dwarf and normal arctic charr, *Salvelinus alpinus*, from Stora Rösjön, central Sweden. *Environ. Biol. Fishes* **33**: 293–298.

Taborsky, M. 1998. Sperm competition in fish: ''bourgeois'' males and parasitic spawning. *Trends Ecol. Evol.* **13**: 222–227.

Taylor, E.B. 1999. Species pairs in north temperate freshwater fishes: evolution, taxonomy, and conservation. *Rev. Fish Biol. Fish.* **9**: 299–324.

Taylor, E.B., Foote, C.J. & Wood, C.C. 1996. Molecular genetic evidence for parallel life history evolution within a Pacific salmon (sockeye salmon and kokanee, *Oncorhynchus nerka*). *Evolution* **50**: 401–416.

Taylor, E.B., Redenbach, Z., Costello, A.B., Pollard, S.M. & Pacas, C.J. 2001. Nested analysis of genetic diversity in northwestern North American char, Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*). *Can. J. Fish. Aquat. Sci.* **58**: 406–420.

Templeton, A.R. 1989. The meaning of species and speciation: a genetic perspective. In: *Speciation and its Consequences* (D. Otte & J. A. Endler, eds), pp. 3–27. Sinauer Associates, Sunderland, MA, USA.

Underwood, T.J., Millard, M.J. & Thorpe, L.A. 1996. Relative abundance, length frequency, age, and maturity of Dolly Varden in nearshore waters of the Arctic National Wildlife Refuge, Alaska. *Trans. Am. Fish. Soc.* **125**: 719–728.

Weir, B.S. 1990. *Genetic Data Analysis*. Sinauer Publ., Sunderland, MA, USA.

Weir, B.S. & Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.

Wirtz, P. 1999. Mother species–father species: unidirectional hybridization in animals with female choice. *Anim. Behav.* **58**: 1–12.

Wood, C.C. & Foote, C.J. 1996. Genetic differentiation of sympatric anadromous and non-anadromous morphs of sockeye salmon (*Oncorhynchus nerka*). *Evolution* **50**: 1265–1279.

Received 7 January 2003; revised 18 July 2003; accepted 18 July 2003