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Spatial partitioning and asymmetric hybridization among sympatric coastal steelhead trout (*Oncorhynchus mykiss irideus*), coastal cutthroat trout (*O. clarki clarki*) and interspecific hybrids

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Abstract

Hybridization between sympatric species provides unique opportunities to examine the contrast between mechanisms that promote hybridization and maintain species integrity. We surveyed hybridization between sympatric coastal steelhead (*Oncorhynchus mykiss irideus*) and coastal cutthroat trout (*O. clarki clarki*) from two streams in Washington State, Olsen Creek (256 individuals sampled) and Jansen Creek (431 individuals sampled), over a 3-year period. We applied 11 *O. mykiss*-specific nuclear markers, 11 *O. c. clarki*-specific nuclear markers and a mitochondrial DNA marker to assess spatial partitioning among species and hybrids and determine the directionality of hybridization. F_1 and post-F_1 hybrids, respectively, composed an average of 1.2% and 33.6% of the population sampled in Jansen Creek, and 5.9% and 30.4% of the population sampled in Olsen Creek. A modest level of habitat partitioning among species and hybrids was detected. Mitochondrial DNA analysis indicated that all F_1 hybrids (15 from Olsen Creek and five from Jansen Creek) arose from matings between steelhead females and cutthroat males implicating a sneak spawning behaviour by cutthroat males. First-generation cutthroat backcrosses contained *O. c. clarki* mtDNA more often than expected suggesting natural selection against F_1 hybrids. More hybrids were backcrossed toward cutthroat than steelhead and our results indicate recurrent hybridization within these creeks. Age analysis demonstrated that hybrids were between 1 and 4 years old. These results suggest that within sympatric salmonid hybrid zones, exogenous processes (environmentally dependent factors) help to maintain the distinction between parental types through reduced fitness of hybrids within parental environments while divergent natural selection promotes parental types through distinct adaptive advantages of parental phenotypes.

Keywords: cutthroat trout, ecological segregation, hybridization, species-specific markers, selection, steelhead

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Introduction

Hybridization has been a subject of intense study in evolutionary biology, ranging from speciation models, to natural selection, to reproductive isolating mechanisms and has confounded and challenged numerous species concepts (Harrison 1993; Arnold 1997). Hybrid zones may develop where genetically divergent populations come into contact and reproduce, forming offspring of mixed ancestry (Barton & Hewitt 1985). The distribution of a hybrid zone reflects dispersal, genetic compatibilities, selection, behaviour, habitat preferences, and/or resources (Barton & Hewitt 1989; Rand & Harrison 1989; Harrison 1993; Avise 1994), and the physical structure characterized by a clinal (Szymura & Barton 1991; Hewitt 1993) or mosaic distribution of hybrids (Howard 1986; Rand & Harrison 1989).

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Endogenous and exogenous processes enforce selective constraints on hybrids affecting their fitness (Rice & Hostert 1993; Rundle & Whitlock 2001). Endogenous processes (also referred to as intrinsic processes) impose selection at the developmental and genomic levels because of discordance between the parental species that is independent of the environment. Exogenous processes (also referred to as extrinsic processes) represent environmentally dependent selection on hybrid phenotypes interacting within parental environments (Rice & Hostert 1993; Rundle & Whitlock 2001; Taylor 2004). Hybrid fitness within the parental environment varies compared to the parental species (Arnold & Hodges 1995; Grant & Grant 1996; Hatfield & Schluter 1999), and is reflected by differential survival among distinct classes of hybrid genotypes (Arnold & Hodges 1995).

The introgression of genes between species within hybrid zones creates a complex mixture of genotypes via backcrossing hybrids. Introgression can be characterized by unimodal genotypic distributions representing hybrid swarms, bimodal genotypic distributions representing predominance of parental genotypes, or by intermediate (flat) genotypic distributions (Jiggins & Mallet 2000). Hybrid swarms often develop when allopatric species come into contact through introductions if reproductive isolating mechanisms have not evolved (Forbes & Allendorf 1991; Wild & Echelle 1992; Leary *et al*. 1995). Backcrossing and introgression do not necessarily lead to hybrid swarms and in many instances backcrossed hybrids and parental types coexist within the same environment (Lamb & Avise 1986; Dowling *et al*. 1989; Koppelman 1994).

Hybridization in temperate freshwater fishes is facilitated by external fertilization, disparity in parental population sizes, inadequate pre- or postmating isolating mechanisms, a shortage of suitable spawning habitat, habitat degradation, or introductions (Hubbs 1955; Campton 1987). *Oncorhynchus mykiss irideus* (coastal steelhead and rainbow trout) and *O. clarki clarki* (coastal cutthroat trout) are two naturally sympatric, temperate freshwater fish species that occasionally hybridize under natural conditions (Campton & Utter 1985; Young *et al*. 2001). Both species exhibit chromosomal, genetic and morphological divergence (Gold *et al*. 1977; Thorgaard 1983; Leary *et al*. 1987; Behnke 1992; Ostberg & Thorgaard 1999), suggesting a divergence estimate of approximately 2 million years (Behnke 1992). Hybridization between *O. m. irideus* and *O. c. clarki* is thought to be restricted by ecological, spatial and temporal separation of spawning (Trotter 1989; Behnke 1992), though not completely (Campton & Utter 1985; Johnson *et al*. 1999; Young *et al*. 2001).

Both species have anadromous and non-anadromous life histories (*O. m. irideus* are known as steelhead and rainbow trout, respectively, and *O. c. clarki* are known as sea-run cutthroat trout and resident coastal cutthroat trout). In this study, we refer to steelhead trout specifically as STH, and both sea-run and resident coastal cutthroat trout as CCT.

Both STH and sea-run CCT typically spend 2–3 years in fresh water as juveniles before undergoing the physiological changes (parr–smolt transformation) that enable seawater tolerance (Trotter 1989; Behnke 1992). STH typically spend 1–3 years in the Pacific Ocean (Behnke 1992) and migrate thousands of kilometres before returning to their natal streams as mature adults between 36 and 80 cm in fork length (Shapovalov & Taft 1954; Withler 1966). Sea-run CCT typically over-summer in estuaries and near-shore marine coastal environments, over-winter in fresh water, and mature at 25–45 cm fork length (Behnke 1992). Non-anadromous life histories vary widely for both species, but in general, these fish are smaller at maturity than their anadromous counterparts.

The objective of our study was to use *O. c. clarki* and *O. mykiss* species-specific, nuclear DNA markers (Ostberg & Rodriguez 2002) and mitochondrial DNA (mtDNA) to resolve directional and spatial patterns of hybridization and introgression between naturally sympatric *O. c. clarki* and *O. m. irideus* in two creeks draining into the southwestern Strait of Juan de Fuca, Washington State. Thus, we developed two hypotheses for testing (i) whether hybridization would be reciprocal between the species, and (ii) whether if a habitat partitioning occurred between the two species, then a hybridization gradient would exist. The first hypothesis stems from previous studies that identified reciprocal hybridization in a limited number of STH–CCT hybrids (Hawkins 1997; Young *et al*. 2001). Rejection or acceptance of this hypothesis could then assist in determining if the F_1 hybridization event was the result of random mating events or specific mating behaviours/strategies (such as sneak spawning by males). The second hypothesis stems from spatial habitat partitioning between sympatric STH and CCT. Typically, STH occupy habitat within lower stream reaches while CCT occupy upper stream reaches (Hartman & Gill 1968). Our intention was to determine if the hybrid zone conformed to a clinal distribution pattern between spatially partitioned parental habitats by sampling a series of stream reaches. We also examined fork length variation among the species and hybrids and aged fish by scale analysis to determine if hybrid vigour existed as an observable difference in growth among the species and hybrids. This study provides novel insights into salmonid hybrid zones by detecting clinal hybrid gradients between spatially partitioned parental habitat and fitness differences among hybrid classes.

Materials and methods

Sampling

We captured STH, CCT and their hybrids in Olsen and Jansen creeks located on the Olympic Peninsula in

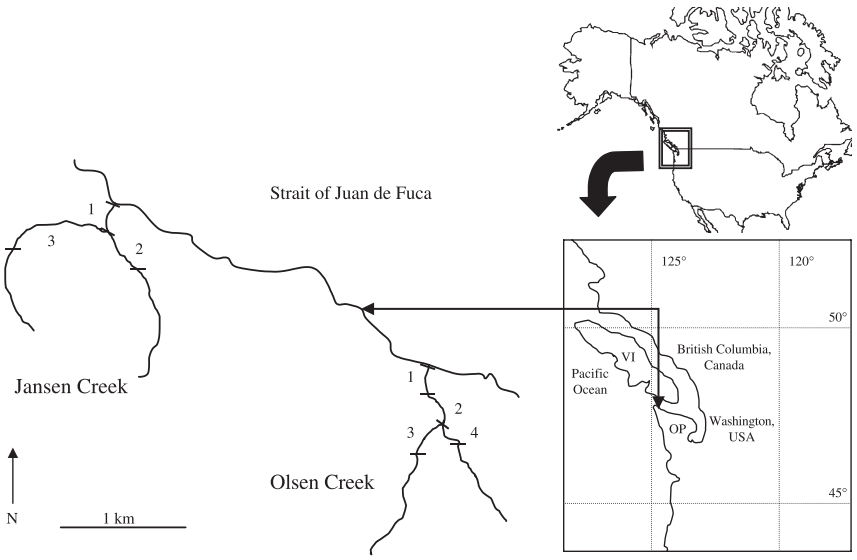

Fig. 1 Map of the sample locations and the partitioned stream reaches within Olsen and Jansen creeks. Numerals refer to stream reach partitions, OP refers to the Olympic Peninsula in Washington State, and VI refers to Vancouver Island in British Columbia.

northwestern Washington State (Fig. 1), using a backpack electrofisher with a single-pass application up the streams between March 2000 and May 2002. Previous investigations indicated a high incidence of hybridization within these creeks. Fin tissues, scale samples, and fork length were collected from 256 and 431 individuals captured from Olsen and Jansen creeks, respectively. Both creeks are second-order streams draining into the Strait of Juan de Fuca. Stream order describes stream size and drainage (the lower orders represent smaller streams) and is determined by tributary number (Gallagher 1999). The Jansen Creek watershed contains 520.4 ha with an average gradient of 3.8%, while the Olsen Creek watershed contains 299.9 ha with an average gradient of 2.7%.

Each creek was partitioned into stream reaches based on distance to test for habitat partitioning and a hybridization gradient (Fig. 1). Olsen Creek was partitioned into four reaches, each of approximately 250 m. Jansen Creek was partitioned into three reaches, reach 1 was approximately 250 m in length, reach 2 was approximately 500 m and reach 3 was approximately 750 m. Sampling intensities varied among years because of the weather.

Nuclear DNA extraction and analysis

Fin tissues were placed into individual tubes containing a mixture of T1 lysis buffer and Proteinase K. DNA extractions were performed using NucleoSpin Multi-96 Tissue Kits (Clontech).

We used 20 species-specific primers to amplify a total of 11 *Oncorhynchus mykiss* and 11 *O. c. clarki* species-specific, nuclear markers (Table 1) (Ostberg & Rodriguez 2002). The *O. mykiss* primers appear to be diagnostic for the *O. m. irideus*, *O. m. gairdneri* and *O. m. stonei* subspecies combined. All 10 *O. c. clarki* primers and nine of the 10 *O. mykiss* primers amplified 21 species-specific products that were either fixed or had frequency differences greater than 0.990 between *O. c. clarki* and *O. mykiss* reference populations. The other *O. mykiss* primer (OM-34) amplified a species-specific product with a frequency difference greater than 0.982 between reference populations (Ostberg & Rodriguez 2002).

Since 17/20 primers amplify a single, specific dominant product in either *O. mykiss* or *O. c. clarki* only, we developed a diplex system [(amplifying one dominant species-specific product from each species in a single polymerase chain reaction (PCR)] to control for failed PCRs (Table 1). Eight different *O. mykiss* and nine different *O. c. clarki* primers were amplified under diplexed conditions. Two of the *O. mykiss*-specific primers (OM-13 and OM-27) were used in two different diplex combinations. The primer OM-4 was diplexed with a previously undescribed primer OT-75 (5′-AGGTGACGTGAGTAGAATGG-3′, 5′-GGGAACTCTTGGCTGTTAAG-3′, GenBank accession number AY296062) because *O. c. clarki* primers with similar annealing temperatures to OM-4 either amplified products of comparable size to the OM-4 product, and thus products were difficult to discern, or poor amplification resulted. The primer OT-75 is monomorphic in both *O. mykiss* and *O. c. clarki*, amplifying a series of 400–550 base pair (bp) products.

Three primers, OM-11, OM-15 and OCC-16, amplify species-specific, size-differentiated products in both *O. mykiss* and *O. c. clarki*. PCR conditions for OM-11, OM-15 and OCC-16 followed the methods of Ostberg & Rodriguez (2002). Although the *O. c. clarki* product amplified by OM-11 occurs at a frequency of 0.918 in reference populations (Ostberg & Rodriguez 2002) it was not used in the final

Table 1 Diplexed and non-diplexed primers (μM concentrations in parentheses), product sizes and annealing temperatures for primers amplifying species-specific products (Ostberg & Rodriguez 2002)

Primer set 1 (STH specific)	Primer set 2 (CCT specific)	Approximate product size (bp)		Annealing temp (°C)
		Primer set 1 STH product	Primer set 2 CCT product	
Diplexed primers				
OM-9 (0.5)	OCC-12 (0.042)	375	150	60†
OM-27 (0.25)	OCC-19 (0.5)	200	300	60
OM-34 (0.5)	OCC-11 (0.025)	1050	350	66
OM-13 (0.042)	OCC-6 (0.5)	175	300	52
OM-4 (0.5)	OT-75 (0.025)	290	—*	54†
OM-27 (0.25)	OCC-1 (0.05)	200	300	60
OM-1 (0.25)	OCC-7 (0.125)	300	475	64
OM-31 (0.025)	OCC-14 (0.25)	325	150	56
OM-13 (0.125)	OCC-3 (0.5)	175	290	54
OM-26 (0.5)	OCC-8 (0.05)	350	100	64
Non-diplexed primers				
OM-11 (0.5)		200	250	62
OM-15 (0.5)		225	1000	64
OCC-16 (0.5)		280	380	50

Non-diplexed primers yield PCR products where the fragment size is species-specific while the individual primers used for diplexed reactions only amplify a single product in one species or the other.
*A CCT-specific product is not amplified with this primer combination.
†Primers using 40 PCR cycles, all other primers used 35 PCR cycles.

hybridization analysis. Thus, three *O. mykiss* and two *O. c. clarki* species-specific products used in the hybrid analysis were derived from these primers. Amplified products were visualized on 2.0% agarose gels stained with ethidium bromide, and sizes were estimated according to a 100-bp ladder standard.

Species and hybrid classifications

Individuals were designated as CCT (composed of all 11 *O. c. clarki* markers and no *O. mykiss* markers), STH (composed of all 11 *O. mykiss* markers and no *O. c. clarki* markers), F_1 hybrid (composed of all 11 *O. mykiss* and *O. c. clarki* markers), or post-F_1 hybrid (all others marker combinations). Post-F_1 hybrids refer to individuals hybridized beyond F_1, and are classified as a single group unless otherwise stated. The post-F_1 classification probably contains a mixture of various backcross generations.

Mitochondrial DNA analysis

The maternal lineages for 430 and 255 individuals from Jansen and Olsen creeks, respectively, were identified by PCR amplification of the mitochondrial ND-2 region followed by digestion with the restriction enzyme *Csp*6I. We were unable to amplify one individual from each creek, thus the mtDNA samples sizes differ from the nuclear DNA sample size by one for each creek. To verify the utility of the procedure a total of 44 known *O. mykiss* were tested from the following locations with the sample number in parentheses: Dworshak National Fish Hatchery, ID (n = 8); Skookumchuck River, WA (n = 8); Chilliwack River, BC (n = 8); Kenai River, AK (n = 8); Crystal Lake Hatchery, CA (n = 8); and Sacramento River, CA (n = 4). A total of 32 known *O. c. clarki* were also tested from the following locations: Cowlitz Trout Hatchery, WA (n = 8); Gines Creek, AK (n = 8); San Josef River, BC (n = 6); Cummins Creek, OR (n = 6); and May Creek, CA (n = 4). Amplification of the ND-2 region was performed in 20-μL reaction volumes consisting of 50 ng total DNA, 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2% Triton X-100, dNTPs at 200 μM each, 0.5 units *Taq* DNA polymerase, and 0.5 μM primers. The primer sequences were 5′-GGCTCAGGCACCAAATACTAA-3′ and 5′-TAAGCTATCGGGCCCATACC-3′. PCRs were amplified for 40 cycles, beginning with a 93 °C dwell for 2 min, followed by 93 °C denaturing for 15 s, 56 °C annealling for 1 min, and 72 °C extension for 1 min and 30 s. After amplification the ND-2 product was digested with the restriction enzyme *Csp*6I (MBI Fermentas) following the manufacturer's specifications. The results yielded a specific digestion pattern of approximately 650, 375 and 180 bp for *O. c. clarki* and 575, 500 and 300 bp for *O. mykiss*.

A subset of CCT from Olsen and Jansen creeks (approximately 10%) produced the fragment pattern diagnostic for *O. mykiss*. We sequenced the 500-bp *O. mykiss* fragment

from two known, non-hybridized *O. mykiss* from Dworshak National Fish Hatchery, ID, and two CCT from Jansen Creek displaying the diagnostic *O. mykiss* fragments to verify that some individuals identified as CCT contained *O. mykiss* mtDNA.

Statistical analysis

A χ^2 contingency table was used to test for temporal variation of STH, CCT, post-F_1 and F_1 hybrids among sample years. The three sample years were then combined and angular transformations were performed on the species and hybrid frequencies to homogenize the variance (Ott 1988). An analysis of variance (ANOVA) was applied to test for differences within and among stream reaches within each creek and Fisher's least significant difference determined *post hoc* differences among groups. Spatial distributions between F_1 and post-F_1 hybrids within reaches were tested for significance using *t*-tests.

χ_2 tests and Yates correction for two categories of data were used to compare marker distributions in post-F_1 hybrids between Jansen and Olsen creeks for each species-specific marker. The mean for each marker between creeks was considered to be the expected distribution, assuming each marker was inherited randomly in post-F_1 hybrids.

To determine if post-F_1 hybrids represented a hybrid swarm or recent and recurring hybridization events, we used a binomial distribution and the model from Boecklen & Howard (1997) to generate expected marker frequency distributions for first- (bc_1), second- (bc_2), and third-generation (bc_3) backcross hybrids . Backcrosses were expected to contain all markers from the backcrossing species whereas the inheritance of markers from the non-backcrossing species was expected to follow a normal distribution around a mean number of markers, assuming markers were inherited independently, random mating and no selection. Thus, STH backcrosses (STHbc) contain all *O. mykiss*-specific markers and a variable number of *O. c. clarki*-specific markers. This analysis incorporated only post-F_1 hybrid genotypes consisting of 11 *O. c. clarki* markers and *x O. mykiss* markers (CCT backcrosses, CCTbc) and *x O. c. clarki* markers and 11 *O. mykiss* markers (STHbc), where $x = 1$–10 markers.

The binomial test (Ott 1988) was used to determine if the distribution of mtDNA within first-generation CCT backcrosses ($CCTbc_1$) deviated from the expected distribution. The test assumed (i) F_1 hybrids contained *O. mykiss* mtDNA exclusively (see Results, all F_1 hybrids contained only *O. mykiss* mtDNA), (ii) equal mating between F_1 hybrids and species, (iii) random mating, and (iv) equal fitness. Following these assumptions the expected proportion of *O. mykiss* mtDNA to *O. c. clarki* mtDNA in $CCTbc_1$ would be 1 : 1. Since a high percentage of the expected bc_2 and bc_3 frequency distributions overlap with the expected bc_1 frequency distributions (Fig. 4), we focused our analysis on the upper half of the $CCTbc_1$ distribution (individuals containing six to nine *O. mykiss*). This allowed for less than a 3.5% overlap of the expected bc_2 frequency distribution while analysing nearly 50% of the bc_1 frequency distribution, assuming that the upper and lower halves of the $CCTbc_1$ frequency distributions were similar within Olsen and Jansen creeks. We generated a confidence value for correctly classifying individuals as $CCTbc_1$ by calculating the proportion of $CCTbc_2/CCTbc_1$ using the expected and observed frequency modes for $CCTbc_2$ and $CCTbc_1$ from Fig. 4(a). The proportion of $CCTbc_2/CCTbc_1$ (3.03) was multiplied by the expected frequency of $CCTbc_2$ containing six to nine *O. mykiss* markers (0.034) adjusting the expected frequency of $CCTbc_2$ (0.103). Finally, an adjusted proportion of $CCTbc_2/CCTbc_1$ was calculated (17%), yielding an 83% confidence value that the observed distribution of $CCTbc_1$ did not contain $CCTbc_2$ individuals. The confidence value probably represented a conservative calculation because the observed mode corresponding to the expected $CCTbc_2$ mode probably contained individuals hybridized beyond bc_2, initially inflating the $CCTbc_2/CCTbc_1$ proportion.

Fork length variation between creeks and among pooled creeks was compared using the non-parametric Wilcoxon signed-rank test because variances were not homogeneous. The Tukey–Kramer procedure for unequal sample sizes determined *post hoc* fork length differences among years for species, and among species and hybrids.

Scale analysis

Scale analysis followed the methods of Chugunova (1963). Briefly, scale samples were scraped from storage envelopes into watch glasses and sorted under a dissecting scope. Those without tears or regeneration were covered with a 10% KOH solution for approximately 2–5 min and subsequently transferred to a watch glass filled with deionized water for further cleaning. Once thoroughly cleaned, the scales were left to dry and then mounted between two glass slides per individual. Each slide was viewed on a microfiche screen and growth patterns were analysed to determine age. We restricted the scale analysis of STH and CCT to coincide with the fork length distribution overlap between age 1 and age 2 STH and CCT. A subset of scales from STH ($N = 87$) and CCT ($N = 100$) 61–130 mm fork length were aged, as well as a subset of post-F_1 hybrids ($N = 41$) 64–205 mm fork length, and F_1 hybrids ($N = 16$) 110–177 mm. Four F_1 hybrids could not be aged because of poor scale quality. Fork lengths were binned into 10 mm groups for age analysis. F_1 brood years were back calculated by subtracting the age of the individual from the sample year.

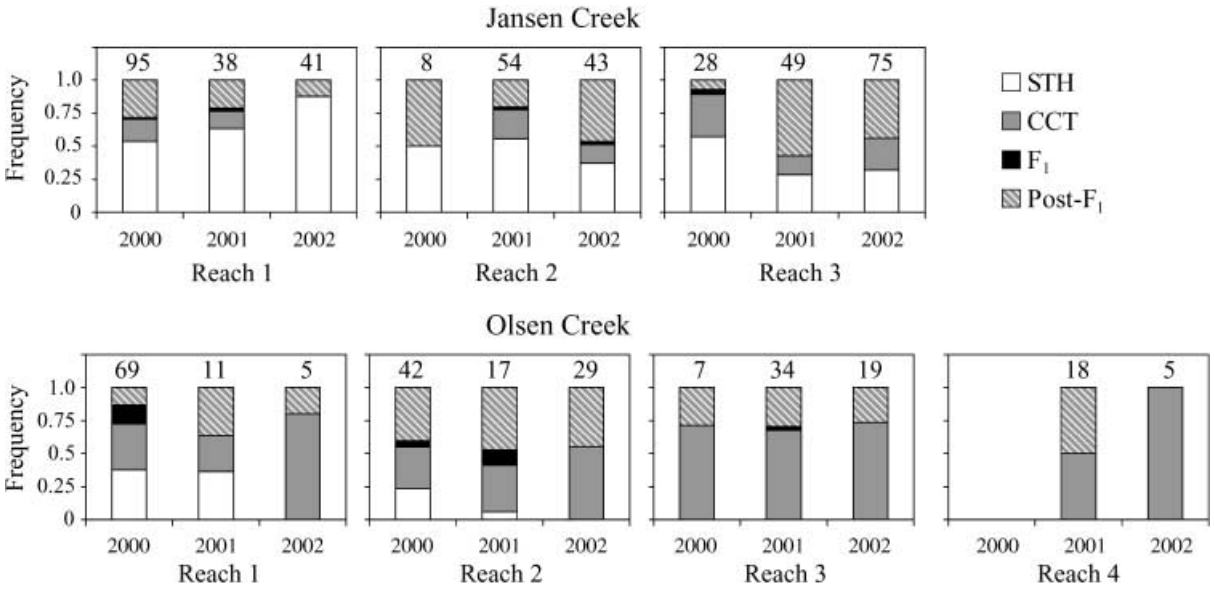

Fig. 2 Temporal frequency distributions for STH, CCT, F_1 and post-F_1 hybrids among years for each sample reach within Jansen Creek and Olsen Creek. The number of individuals represented by each data set is presented above each bar.

Results

Nuclear DNA analysis

The distribution of STH, CCT and hybrids differed among sample years, among reaches, and between creeks (Fig. 2). F_1 and post-F_1 hybrids constituted a total of 1.2% and 33.6% of the population within Jansen Creek and 5.9% and 30.4% within Olsen Creek, respectively. The presence of hybrids throughout all reaches in both creeks suggested that hybridization was persistent and spatially extensive although in-stream migrations could also explain these data (Moring *et al*. 1986; Henderson *et al*. 2000).

Temporal variation of STH, CCT, F_1 and post-F_1 hybrids sampled among years within Jansen Creek was not significant (χ^2, $P = 0.492$, 6 d.f.), but was significant within Olsen Creek (χ^2, $P < 0.0001$, 6 d.f.). The variation within Olsen Creek may have been the result of limitations of our sampling effort (different months were sampled with different intensities among years, numbers of fish sampled differed each year, and sampling intensities differed each year) and/or ecological factors (poor recruitment and year-to-year survival differences among fish). Within each stream, the proportion of STH to CCT was greatest in the lower reaches and progressively declined upstream (Figs 2 and 3). STH were not observed within the upper two reaches of Olsen Creek.

Within-reach comparisons among species and hybrids (pooled F_1 and post-F_1 hybrids) indicated that within reaches 1 and 2 Jansen Creek, STH were more abundant than CCT, and also more abundant than hybrids within

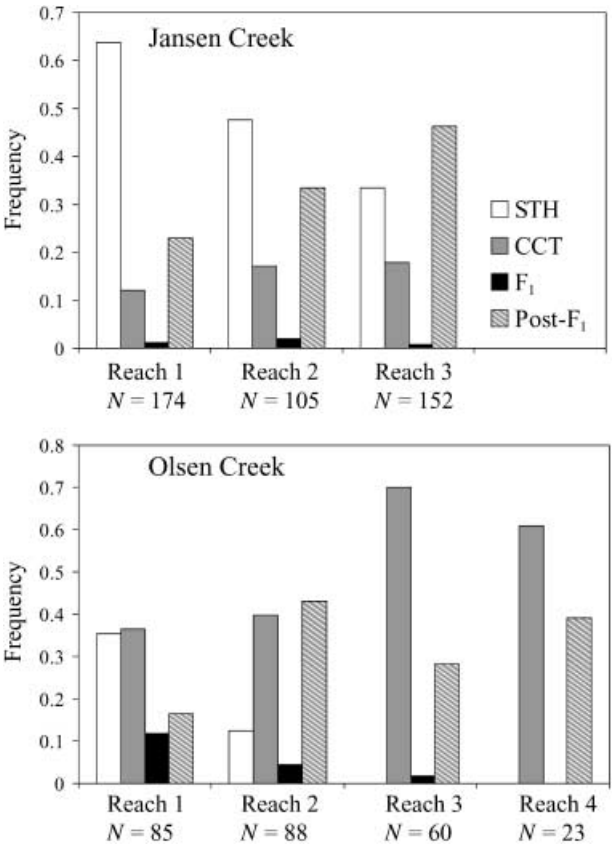

Fig. 3 The total frequency distributions for STH, CCT, F_1 hybrids and post-F_1 hybrids within each sample reach for Olsen Creek and Jansen Creek. The number of individuals (N) is represented below each sample reach.

Table 2 Significant within-reach differences detected among species and hybrids using ANOVA of arcsine transformed frequencies and Fisher's LSD (confidence coefficient = 0.95)

Test	ANOVA	*P*	Significant differences based on Fisher's LSD
Jansen Creek within Reach 1	$F_{2,6} = 13.090$	0.006	STH distinct from CCT and hybrids
Jansen Creek within Reach 2	$F_{2,6} = 6.875$	0.028	STH distinct from CCT
Jansen Creek among CCT, STH, hybrids	$F_{2,6} = 39.872$	> 0.001	STH and hybrids distinct from CCT
Olsen Creek within Reach 2	$F_{2,6} = 9.260$	0.015	CCT and hybrids distinct from STH
Olsen Creek within Reach 3	$F_{2,6} = 320.529$	> 0.001	CCT distinct from hybrids and STH
Olsen Creek hybrids among reaches	$F_{2,6} = 9.084$	0.015	reach 2 hybrids distinct from reaches 1 and 3
Olsen Creek among CCT, STH, hybrids	$F_{2,6} = 5.878$	0.039	CCT distinct from STH

reach 1 (Table 2) suggesting that STH dominate CCT in the downstream habitat. In Olsen Creek, CCT and hybrids were more abundant than STH in reach 2, and CCT were more abundant than hybrids in reach 3. Hybrids within Olsen Creek were more abundant in reach 2 than in reaches 1 and 3. Temporal analysis indicated that STH and hybrids were more abundant than CCT in Jansen Creek, and CCT were more abundant than STH in Olsen Creek. Spatial distributions between F_1 and post-F_1 hybrids indicated that post-F_1 hybrids were more abundant within reaches 1 ($P = 0.012$) and 2 ($P = 0.037$) in Jansen Creek and within reaches 2 ($P = 0.023$) and 3 ($P > 0.001$) in Olsen Creek. Spatial distributions between CCT, STH and hybrids were not significant among reaches.

The ability to distinguish among backcross generations is limited because large numbers of markers are needed and the expected marker frequency distributions for two or more different backcross generations can overlap (Boecklen & Howard 1997). Rather than assign genotypes to backcross generations we used the expected frequency modes to characterize the hybrid zones. Post-F_1 hybrids from both creeks contained a variety of marker combinations indicating the existence of a variety of hybrid classes. The observed frequency distributions of *Oncorhynchus mykiss* markers in CCTbc corresponded to the expected frequency modes for the first two backcross generations, expressing modality around the expected means for bc_1 and bc_2 and suggesting that some backcrossing in post-F_1 hybrids was represented by recent and recurring hybridization events (Fig. 4a). The observed frequency distributions of *O. c. clarki* markers in STHbc did not correspond to any of the expected frequency modes (Fig. 4b).

Both Jansen and Olsen creeks represented bimodal hybrid zones, indicated by a predominance of individuals genetically similar to parental genotypes (Fig. 5). The bimodal nature of parental genotypes suggests that the populations within Jansen and Olsen creeks do not represent a hybrid swarm. Also, the genotypic distributions reveal that the majority of hybrids contain few *O. mykiss* markers, suggesting that backcrossing to CCT is more common than backcrossing to STH.

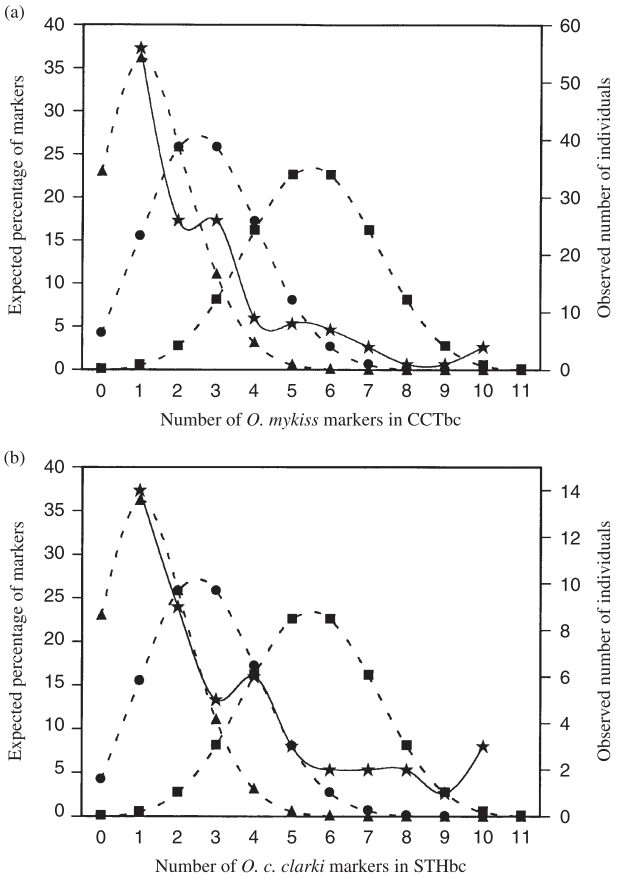

Fig. 4 Relative percentages of the expected marker distributions (dashed lines) in bc_1 (■), bc_2 (●), and bc_3 (▲) using 11 species-specific markers and (a) the observed number of CCTbc individuals with respective *Oncorhynchus mykiss* markers (stars), and (b) the observed number of STHbc individuals with respective *O. c. clarki* markers (stars). The data from Jansen and Olsen creeks are combined.

The observed distribution of markers in post-F_1 hybrids did not deviate from expectations for *O. c. clarki*-specific markers, but did deviate for two *O. mykiss*-specific markers (the OM-1 product and the OM-34 product). The OM-1 product occurred more often than expected (χ^2, $P < 0.025$),

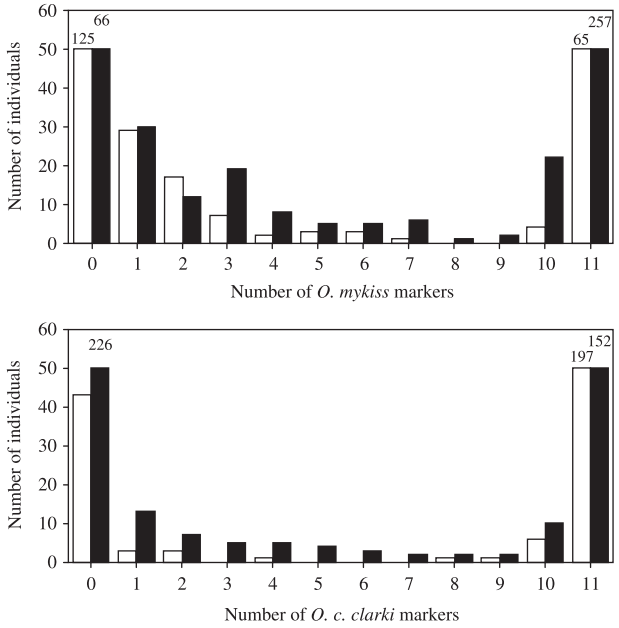

Fig. 5 Bimodal frequency distributions of the number of individuals with a particular number of *Oncorhynchus c. clarki*- and *O. mykiss*-specific markers in Olsen Creek (white bars) and Jansen Creek (black bars).

and the OM-34 product less often than expected (χ^2, $P < 0.025$). Twenty-one individuals expressed 10 *O. mykiss*-specific markers each, and of these 62% lacked the OM-34 product. Within CCTbc expressing only one *O. mykiss* marker, deviations from expected values were observed for the OM-1 product (χ^2, $P < 0.001$), OM-9 product (χ^2, $P < 0.025$), OM-27 product (χ^2, $P < 0.025$) and OCC-16 *O. mykiss* product (χ^2, $P < 0.005$). Deviations may represent non-random mating between hybrids and CCT, genetic drift, natural selection, or polymorphisms within CCT. An analysis of 46 resident CCT, isolated from STH, above an anadromous migratory barrier in an adjacent creek (Bullman Creek) revealed no *O. mykiss* markers, suggesting that polymorphisms were not the cause (data not shown). The OCC-1 product and OM-15 *O. c. clarki* product always occurred together, the OCC-8 product always occurred with the OCC-11 product but the OCC-11 product occurred with the OCC-8 product at a frequency of 0.903, and the OM-11 *O. mykiss* product and OM-15 *O. mykiss* product co-occurred at a frequency of 0.967 in all post-F_1 hybrids. Sequence analyses of these co-occurring markers indicated no apparent homology between sequences or primer binding sites.

Mitochondrial DNA analysis

All F_1 hybrids (five from Jansen Creek and 15 from Olsen Creek) contained *O. mykiss* mtDNA and thus were the progeny of female STH and male CCT matings. Individuals backcrossed toward STH exclusively contained *O. mykiss* mtDNA, whereas individuals backcrossed toward CCT contained either *O. c. clarki* or *O. mykiss* mtDNA (Fig. 6). The majority of post-F_1 hybrids from both creeks were composed of either 11 *O. c. clarki*-specific markers and a low number *O. mykiss*-specific markers (≤ 4) or vice versa. This indicates that backcrossing was more common

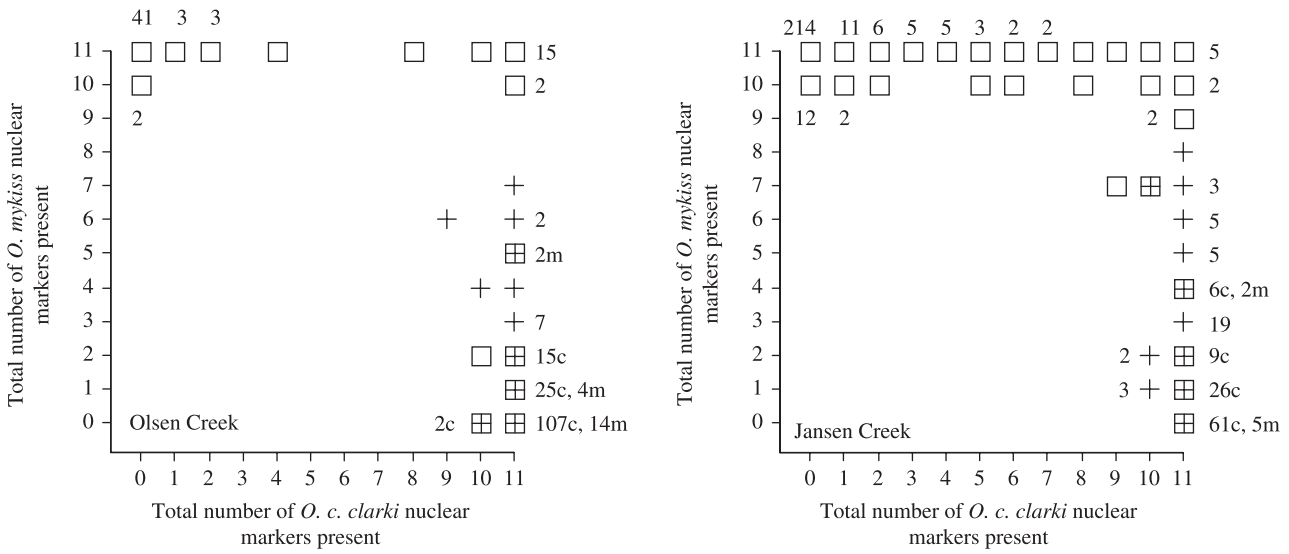

Fig. 6 Distribution of *Oncorhynchus mykiss* and *O. c. clarki* species-specific markers and mtDNA for each individual within Olsen and Jansen creeks: *Oncorhynchus mykiss* mtDNA (□), *O. c. clarki* mtDNA (+). The number of individuals with each *O. mykiss* and *O. c. clarki* marker combination is indicated, unless only one individual is represented. Where both mitotypes are present for one marker combination, c represents the number of CCT and m the number of STH.

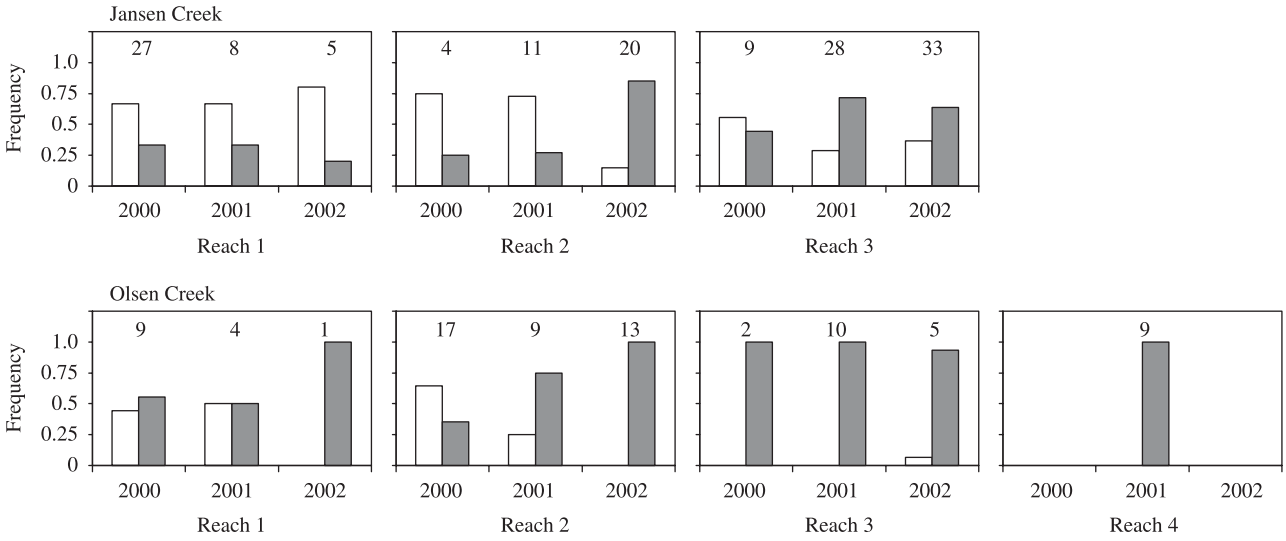

Fig. 7 Temporal frequency distributions for *Oncorhynchus mykiss* mtDNA (white bars) and *O. c. clarki* (shaded bars) in post-F_1 hybrids for each sample reach within Jansen Creek and Olsen Creek. The number of individuals represented within the data set for each reach and year is presented above each year.

Table 3 The total number (*N*), frequency of *O. mykiss* and *O. c. clarki* mtDNA, and the species-specific proportion of mtDNA frequency to nuclear DNA (nucDNA) frequency in post-F_1 hybrid individuals within each reach of Olsen Creek and Jansen Creek

	N	*O. mykiss* mtDNA	*O. c. clarki* mtDNA	*O. mykiss* mtDNA/ nucDNA	*O. c. clarki* mtDNA/ nucDNA
Olsen Creek					
Reach 1	14	0.429	0.571	1.381	0.829
Reach 2	38	0.342	0.658	1.018	0.991
Reach 3	17	0.059	0.941	0.482	1.072
Reach 4	9	0.222	0.778	1.084	0.978
Total	78	0.291	0.709	1.043	0.983
Jansen Creek					
Reach 1	40	0.700	0.300	1.172	0.745
Reach 2	35	0.400	0.600	0.907	1.073
Reach 3	70	0.357	0.643	1.028	0.985
Total	145	0.462	0.538	1.053	0.958

than matings between F_1 hybrids or between F_1 hybrids and backcrosses. Backcrossing toward CCT appeared more common than backcrossing toward STH.

Temporal variation between *O. mykiss* and *O. c. clarki* mtDNA in post-F_1 hybrids was detected within Jansen Creek (χ^2, $P = 0.007$, 2 d.f.) and Olsen Creek (χ^2, $P < 0.001$, 2 d.f.). *Oncorhynchus mykiss* mtDNA occurred at the highest frequency in post-F_1 hybrids within the lowest reach of Jansen and Olsen creeks and declined in correspondingly higher reaches (Fig. 7). The proportion of mtDNA to nuclear DNA species markers indicates differential introgression within post-F_1 hybrids based on location (Table 3). Within reach 1 in both Jansen and Olsen creeks the proportion of *O. mykiss* mtDNA to *O. mykiss* nuclear DNA was greater than 1.0 indicating that post-F_1 hybrids had higher frequencies of *O. mykiss* mtDNA than *O. mykiss* nuclear DNA, whereas in Olsen Creek the proportion within reach 3 suggests a deficit in *O. mykiss* mtDNA. These results may reflect partitioning of spawning habitat and differences in habitat utilization between female STH and CCT. Interestingly, the total proportion between creeks was similar for *O. mykiss* (1.043 and 1.053) and *O. c. clarki* (0.983 and 0.958). The distribution of *O. c. clarki* mtDNA was spatially significant between reach 1 and 3 in Jansen Creek ($P = 0.044$) and in *O. mykiss* mtDNA between reach 1 and 3 in Olsen Creek ($P = 0.013$).

Nineteen CCT individuals (10.2%) from both creeks contained *O. mykiss* mtDNA (Fig. 6). Although CCT are highly heterogeneous between streams (Wenburg *et al*. 1998) and mtDNA is a rapidly evolving molecule (Brown *et al*. 1979; Avise 1994) the ND-2 region reliably differentiates maternal lineages of STH, CCT and their hybrids (Young *et al*. 2001; our confirmation in the Materials and Methods). To verify that this represented past hybridization events, a 500-bp fragment diagnostic for *O. mykiss* was sequenced from two known, non-hybridized *O. mykiss* and two individuals classified as CCT from Jansen Creek exhibiting the *O. mykiss* fragment pattern. Sequence alignments revealed complete homology, indicating that some individuals within our study appeared to be CCT based on nuclear markers but were probably later generation hybrids, and more properly referred to as 'CCT-like' individuals.

The distribution of *O. c. clarki* mtDNA in $CCTbc_1$ containing six to nine *O. mykiss* markers was greater than expected

(a single $CCTbc_1$ contained *O. mykiss* mtDNA and 12 contained *O. c. clarki* mtDNA, $P = 0.0016$, 83% confidence value). The 83% confidence value was probably conservative (see methods) and represents two individuals of the 13 $CCTbc_1$ containing six to nine *O. mykiss* markers that could actually be $CCTbc_2$. After removing any two individuals containing *O. c. clarki* mtDNA from the sample (potential $CCTbc_2$), the distribution of *O. c. clarki* mtDNA in $CCTbc_1$ was still highly significant ($P = 0.005$). The same test could not be applied to first-generation STH backcrosses ($STHbc_1$) because $STHbc_1$ cannot have a shared maternal lineage when all F_1 hybrids contain *O. mykiss* mtDNA. Furthermore, between F_1 and $CCTbc_1$ containing six to nine *O. mykiss* markers there was an estimated 84.6% reduction in *O. mykiss* mtDNA (100% of F_1 contained *O. mykiss* mtDNA and 15.4% $CCTbc_1$ contained *O. mykiss* mtDNA), while the distribution of *O. mykiss* mtDNA appeared to be relatively consistent between $CCTbc_1$ and 'CCT-like' individuals containing only *O. c. clarki* nuclear markers (10.2%). These data suggest that hybrids (F_1 and post-F_1) did not have equal fitness or non-random mating occurred.

Fish size

Length frequency distributions for each species, F_1 and post-F_1 hybrids indicated that CCT, STH and post-F_1 hybrids were distributed throughout the size categories, whereas F_1 hybrids were distributed in the upper two-thirds of the size categories (Fig. 8). No young-of-the-year fish (age 0) were observed during our sampling, based on fork length. Temporal variation in fork length was significant only within Jansen Creek among STH (Wilcoxon signed-rank test, $P < 0.0005$, d.f. = 2) and post-F_1 hybrids (Wilcoxon signed-rank test, $P < 0.0001$, d.f. = 2). Pairwise comparisons indicated that the mean STH fork length for the sample year 2001 was significantly larger than the sample year 2000, and the mean post-F_1 hybrid fork length for 2001 was significantly larger than 2000 and 2002 (Tukey–Kramer procedure). These differences may reflect sampling inconsistencies, year-to-year survival, or recruitment. Mean fork length for each species, F_1, and post-F_1 hybrids did not differ significantly between creeks and both creeks and all sample years were pooled for further fork length analysis. Mean fork lengths for pooled creeks and years, followed by one standard deviation in parentheses, were: CCT = 107.4 mm (28.1), STH = 91.5 mm (18.5), post-F_1 = 104.1 mm (27.5), and F_1 = 132.0 mm (19.8). Mean fork length varied significantly among species and hybrid classes (Wilcoxon signed-rank test, $P < 0.0001$, d.f. = 3). The Tukey–Kramer procedure for pairwise comparisons indicated that F_1 hybrids were significantly larger than all other groups, STH were significantly smaller than all other groups, and post-F_1 hybrids and CCT were not significantly different in mean fork length.

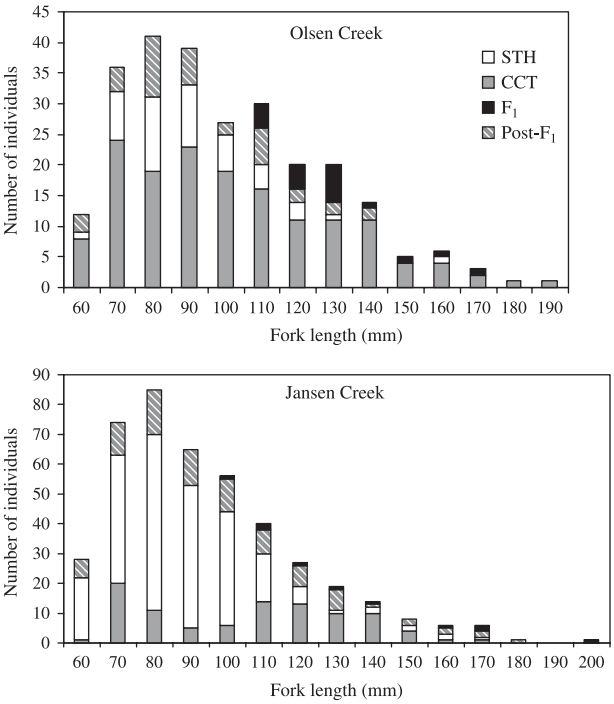

Fig. 8 Length frequency distributions for CCT, STH, F_1 and post-F_1 hybrids. All individuals are grouped into 10-mm increments and the single adult STH (460 mm) is not included.

Table 4 The number of age 1 and age 2 STH ($N = 87$) and CCT ($N = 100$) determined for each corresponding binned fork length range

Fork length range (mm)	STH age 1	STH age 2	CCT age 1	CCT age 2
61–70	3	—	3	—
71–80	5	—	6	—
81–90	22	1	27	1
91–100	21	1	22	2
101–110	14	11	9	15
111–120	1	3	1	10
121–130	—	5	—	4

An absence of individuals is indicated by a dash (—).

Age analysis

To determine if the significant difference in F_1 hybrid fork length was age dependent or represented a possible case of hybrid vigour for growth, we aged a subset of STH and CCT and determined the fork length transition that corresponded to the age 1 and age 2-year class transition. The age structure for STH and CCT indicated that the size transition between ages 1 and 2 STH and CCT was in the range of 101–110 mm fork length (Table 4). The 16 F_1 hybrids were between ages 2 and 4. Lack of age 1 F_1 hybrids may

explain the significant size difference between F_1 hybrids and STH, CCT and post-F_1 hybrids. Brood year calculations indicate the most recent F_1 hybrid event occurred during 2000 and occurred during each preceding year dating back to 1996. More F_1 hybrids were produced in Olsen Creek during the 1998 brood year than any other year (at least 46.7% of F_1 hybrids aged). The subset of post-F_1 hybrids indicated that they were composed of individuals between ages 1 and 4.

Discussion

Asymmetric hybridization

Contrary to our first hypothesis of reciprocal hybridization F_1 hybrids were produced by matings between female STH and male CCT exclusively. Asymmetric gene flow among hybridizing species may result from fitness differences, population structure, or mating behaviour (Barton & Hewitt 1985; Lamb & Avise 1986; Dowling *et al*. 1989). Within salmonids, asymmetric hybridization has been observed within the genera *Salmo* (McGowan & Davidson 1992; Matthews *et al*. 2000), *Salvelinus* (Baxter *et al*. 1997; Redenbach & Taylor 2003) and *Oncorhynchus* (Dowling & Childs 1992; Rosenfield *et al*. 2000). Wirtz (1999) proposed several hypotheses promoting asymmetric hybridization; two of which, sneak spawning and sexual selection, seem especially relevant. Sneak spawning is an alternative mating strategy used by jacks (small, sexually precocious males) that reduces agnostic encounters by allowing jacks to gain refuge in shallow water or submerged cover while remaining in close proximity to a spawning pair. Alternative mating strategies have been implicated as driving hybridization in numerous species (Gross 1984; Lamb & Avise 1986; Konkle & Philipp 1992; McGowan & Davidson 1992; Kitano *et al*. 1994; Wood & Foote 1996). The size differences between sexually mature STH and CCT may promote hybridization through sneak spawning (Taylor 2004). Indeed, sneak spawning has been implicated as promoting asymmetrical hybridization between species that differ in size at sexual maturity such as Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) (McGowan & Davidson 1992) and between bull trout (*Salvelinus confluentus*) and Dolly Varden (*S. malma*) (Baxter *et al*. 1997; Redenbach & Taylor 2003). Our data indicate a deficit of STH nuclear DNA and an abundance of CCT nuclear DNA in post-F_1 hybrids in reach 1 of both creeks, although STH were significantly more abundant than CCT within reach 1 in Jansen Creek. These results indicate an inconsistent genetic contribution by male CCT to post-F_1 hybrids within STH dominated habitat, suggestive of a sneaking strategy.

The sexual selection hypothesis suggests that as females of a rare species wait upon the spawning grounds for intraspecific mates they become less discriminating and may eventually mate with males of a more common species (Wirtz 1999). Thus, there is a tendency for locally rare species to provide the female parent in interspecific matings (Rakocinski 1980; Avise & Saunders 1984; Avise *et al*. 1997). Sexual selection by female STH against male CCT may be weakened in the absence of STH males or by an inability of STH males to locate STH females. The latter could possibly occur in small coastal streams if habitat restricts within-stream movement of STH, such as low water flows, but still allows small CCT males to seek female STH.

Spatial partitioning

Our second hypothesis was, if both species partitioned habitat then a hybrid gradient would exist. The fact that significant spatial distributions occurred between STH and CCT suggests a modest level of habitat partitioning, which agrees with previous studies (Hartman & Gill 1968). Furthermore, the clinal distribution of mtDNA in post-F_1 hybrids and the proportion of species-specific mtDNA markers to nuclear DNA markers in post-F_1 hybrids suggests that females segregate spawning habitat between species. Lastly, a hybrid gradient was observed within Olsen Creek. These data indicate that partial habitat partitioning and a clinal hybrid gradient existed supporting our hypothesis.

The partitioning of habitat between STH and CCT may be a function of stream or substrate size. The frequency of STH was highest in the lowest reach of each creek, corresponding to the largest volume of water within each creek, while the frequency of CCT was highest in the reaches above the forks in each creek, corresponding to a lesser volume of water within each creek. Furthermore, post-F_1 females appeared to segregate habitat based on their mitotype, which may also be related to stream size or spawning substrate size. The basis of the clinal hybrid gradient may be a distribution overlap between parental types or intermediate parental habitat. Although our interpretations are speculative, a physical habitat analysis would be the next progression for uncovering the basis for habitat partitioning and the clinal hybrid gradient.

Spatial partitioning of habitat segregates sympatric species into ecological niches (Hartman & Gill 1968; Hagen & Taylor 2001) and may create clinal hybrid zones that represent peripheries of overlapping habitat between species (Freeman *et al*. 1991; Johannesson *et al*. 1995; Martinsen *et al*. 2001). Mosaic partitioning represents ecological segregation within a space (Rand & Harrison 1989) and in the case of salmonids may represent spawning segregation based on substrate size, water depth and water velocity. Our data indicate that habitat partitioning between salmonids along small spatial scales may promote clinal hybrid gradients. To the contrary, Taylor (2004) suggested that on small scales, habitat partitioning and local habitat preferences

between sympatric salmonid species probably promote mosaic salmonid hybrid zones. Salmonid hybrid zone structures are probably dictated by ecological requirements of each species in relation to environmental factors. For example, a mosaic hybrid zone may develop when a smaller species (CCT) spawns among patches of smaller substrate in shallow and slower water while a larger species (STH) spawns among patches of larger substrate in deeper and swifter water within the same given stream space. A clinal hybrid gradient may develop when spawning substrate and water depth and velocity specific for each species is segregated in a longitudinal space with some overlapping habitat. Moreover, within a river system where habitat partitioning occurs, hybrid zones may be clinal between the distributions of species at the local level, but appear mosaic in structure at the river-scape level. A large-scale river study may determine if salmonid hybrid zones consist of a series of mosaic clines.

Hybrid fitness

Ideally, an analysis of hybrid fitness should examine an array of recombinant parental genotypes representing distinct hybrid classes rather than lumping all hybrids into a single class, because hybrid fitness has been demonstrated to vary among hybrid classes (Arnold & Hodges 1995). Thus, we examined the hybrid fitness of F_1 and $CCTbc_1$ hybrids. The major obstacle for introgression within these creeks appeared to be obtaining the bc_1 stage, after which exogenous selection may relax. For example, the reduction of *O. mykiss* mtDNA from F_1 to $CCTbc_1$ was fivefold higher than from $CCTbc_1$ to 'CCT-like' individuals. Campton & Utter (1985) and Young *et al*. (2001) suggested that exogenous selection acts upon CCT–STH hybrids during the marine migration stage of the anadromous life history. Exogenous selection against hybrids may be more limited within resident life histories than within anadromous life histories (Utter 2001). Lee & Power (1976) and Jonsson *et al*. (2001) observed that females had a higher propensity for anadromy than their male counterparts, thus female F_1 hybrids may be under harsher selective constraints in the marine environment than males that remain and mature within the stream. Indeed, introduced, non-native resident rainbow trout readily hybridize and form hybrid swarms with native trout (Busack & Gall 1981; Gyllensten *et al*. 1985; Forbes & Allendorf 1991; Charmichael *et al*. 1993) suggesting that selective constraints may be relaxed within the freshwater environment.

Morphological adaptations attributed to parental species provide hybrid phenotypes with fitness advantages in certain environments (Hatfield & Schluter 1999; Rundle 2002). Diverse marine migratory behaviours between STH and CCT necessitate different morphological adaptations, and STH may be more suited for long-distance migrations whereas CCT may be more suited for shorter migrations (Bisson *et al*. 1988; Hawkins & Quinn 1996). Selection against F_1 hybrids would occur in the marine environment if hybrid fitness was dependent upon the interaction and survival of phenotypically intermediate individuals within the parental environment. Wood & Foote (1996) observed that sockeye salmon (*O. nerka*) grew faster than kokanee (the non-anadromous form of sockeye salmon) suggesting that the larger growth in sockeye may enhance their survival during marine migrations compared to kokanee, and Taylor & Foote (1991) suggested that sockeye might also have an advantage over kokanee for swimming performance during marine migrations. If F_1 hybrids were intermediate to parents, then F_1 hybrids would be smaller than STH and may suffer high rates of mortality during the extensive marine migrations. Such differential survival of F_1 hybrids may explain the reduced incidence of *O. mykiss* mtDNA between F_1 and $CCTbc_1$.

Campton & Utter (1985) suggested that hybrids might have a competitive disadvantage during the critical over-wintering period because they observed only age 0 CCT–STH hybrids in their study. Body morphology may place STH at a competitive advantage for riffles and CCT for pools (Bisson *et al*. 1988) whereas hybrids may be at a competitive disadvantage (Hawkins & Quinn 1996). Within the same population Campton & Utter (1985) also observed age 0 and 1 CCT and age 0 STH only. In our study, both CCT and STH ages 1 and 2, and hybrids (both F_1 and post-F_1) ages 1–4 occurred together, and there did not appear to be a disproportionate reduction of hybrids among fork lengths. Furthermore, hybrids made a considerable contribution to the overall population demographic (approximately 35% in both creeks). Although we cannot infer a competitive disadvantage for age 0 hybrids during their first over-wintering period, our data suggest that hybrids age 1 and older may not be severely disadvantaged in the ensuing over-wintering periods. The lack of both age 1 STH and hybrids observed by Campton & Utter (1985) does not necessarily indicate a competitive disadvantage but may also reflect poor recruitment, temporal variation, or partial habitat partitioning. The assumption of reduced marine survival and co-occurrence of hybrids and parental types in freshwater leads us to conclude that the marine environment plays a role in the survival of F_1 hybrids.

Differential introgression

Differential introgression between nuclear and mitochondrial DNA gene flow may reflect the use of parental environments, resource competition, and lower fitness of some cytonuclear combinations (Lu *et al*. 2001; Babik *et al*. 2003). We found that the introgression of mtDNA was more pronounced and had a greater variance than nuclear DNA in post-F_1 hybrids. Other studies have demonstrated

similar results (Forbes & Allendorf 1991; Poteaux *et al*. 1998; Babik *et al*. 2003). Differential introgression between reaches within both creeks was observed as a frequency-dependent gradient in post-F_1 hybrids, with a higher proportion of *O. mykiss* mtDNA than *O. mykiss* nuclear DNA in the lowest reach of both creeks. Lu *et al*. (2001) observed differential introgression between two distinct morphological lineages of lake whitefish (*Coregonus clupeaformis*) between several lakes leading them to conclude that variable introgression might reflect divergent natural selection as a result of resource competition and/or exploitation of different habitats. The differential introgression between reaches does not represent a random distribution, but rather assemblages with an affinity for the maternal spawning habitat. Although speculative, the differential introgression between reaches may reflect the habitat partitioning observed between parental types with respect to female nest building, and possibly the parental life history that post-F_1 hybrids follow.

Maintenance of species integrity

Genotypic frequency distributions provide a useful means of describing the structure of hybrid zones. Hybrid zones have been characterized as bimodal, representing a predominance of parental genotypes, unimodal representing hybrid swarms, and flat representing a predominance of intermediate genotypes (Harrison & Bogdanowicz 1997). Assortative mating and strong prezygotic isolating mechanisms influence the structure of bimodal hybrid zones, whereas assortative mating is reduced within unimodal hybrid zones (Jiggins & Mallet 2000). Positive size assortative mating is common among salmonids (Gross 1984; Foote & Larkin 1988) and serves a principal role in maintaining the divergence between sympatric sockeye and kokanee salmon (Wood & Foote 1996). The genotypic frequency distributions for both populations within the study were bimodal, indicating a general genetic similarity to parental genotypes and as such, neither population represents a hybrid swarm. The bimodal distribution of genotypes within our study suggests divergent selection against hybrid phenotypes promoting parental phenotypes, or strong premating isolation and weak selection (Jiggins & Mallet 2000).

Within parental environments, divergent selection reduces the fitness of phenotypically intermediate individuals in favour of parental phenotypes (Hatfield & Schluter 1999; Cruz *et al*. 2001; Rundle 2002). In fishes, studies have indicated that divergent natural selection maintains phenotypic discreteness in sympatric populations acting on swimming performance, resource competition, and reproductive behaviours (Schluter 1996; Wood & Foote 1996; Rogers *et al*. 2002; Schluter 2003). Moreover, despite considerable gene flow, species can maintain important phenotypic differences in the presence of robust, divergent natural selection (Johannesson *et al*. 1995; Wood & Foote 1996; Babik *et al*. 2003). Hybrid fishes have reduced developmental stability (Graham & Felley 1985; Leary *et al*. 1985), which may adversely affect morphological adaptations, ultimately reducing hybrid fitness within parental environments. Divergent phenotypic adaptations and critical swimming velocities of STH and CCT may provide parentals with adaptations for specific life histories and hybrids with a disadvantage (Hawkins & Quinn 1996; Bisson *et al*. 1998). Furthermore, swimming behaviours have been demonstrated to be under genetic control (Rogers *et al*. 2002) and as such, STH–CCT hybrids may exhibit intermediate swimming behaviours in the marine environment and lack the size, stamina, and morphological adaptations to endure the extensive STH migrations. Resource competition may occur among parental and hybrid juveniles in freshwater streams where morphological adaptations may provide parental types with an advantage over hybrids for feeding and over-wintering habitat. Lastly, assortative mating has been implicated as a mechanism promoting divergent natural selection (Johannesson *et al*. 1995; Schluter 1996; Wood & Foote 1996). Among salmonids, positive size assortative mating is common (Gross 1984; Foote & Larkin 1988) and could enforce distinction between CCT and STH phenotypes when hybridization occurs. Thus, hybrid intermediacy may be maladaptive within parental environments, enforcing divergent natural selection pressures that act to reinforce the integrity of STH and CCT as species.

Conclusions

Hybrid zones provide unique opportunities to examine mechanisms of speciation and examine hybridization as an evolutionary process (Barton & Hewitt 1985; Arnold 1992; Jiggins & Mallet 2000). Our results suggest that within hybrid zones, exogenous processes help to reinforce parental niches by maintaining genetic divergence between parental types because of reduced fitness of F_1 hybrids within parental environments. Controlled field studies comparing the fitness of F_1 hybrids and parental types within parental environments have yielded similar results implicating exogenous dependent processes as promoting divergence between parental morphs (Hatfield & Schluter 1999). Reinforcement of parental morphs through exogenous dependent factors may be an integral process of speciation, assuming that similar processes maintaining genetically distinct parental types are also important in speciation (Barton & Hewitt 1985; Jiggins & Mallet 2000). Our results also indicated unequal fitness between F_1 and first-generation hybrid backcrosses. Arnold & Hodges (1995) summarized variable fitness differences between hybrid classes and parental genotypes and concluded that hybrid fitness varied compared to that of parental types. Although we were unable to test for fitness differences

between parental types and hybrids, our results indicate that F_1 hybrids had significantly reduced fitness compared to first-generation hybrids, implying that individuals that were phenotypically more similar to parental types had higher fitness than phenotypically intermediate individuals.

Spatial, temporal and ecological partitioning of habitat, and size-assortative mating is believed to minimize interspecific hybridization among sympatric salmonids (Trotter 1989; Behnke 1992; Taylor 2004). However, partial breakdown of one or more of these factors can promote hybridization (Taylor 2004). Our results indicate that within sympatric salmonids, reproductive behaviours and exogenous factors contribute to the structure of salmonid hybrid zones while divergent natural selection acts as a wedge upon hybrids by enforcing the adaptive advantage of parental phenotypes. The sneaking behaviour of males and overlapping spawning habitat may act to promote hybridization while reduced fitness for F_1 hybrids suggests a disadvantage for phenotypically intermediate hybrids within the parental environment. The fact that the majority of individuals represented parental phenotypes combined with reduced fitness for F_1 hybrids suggests exogenous processes acted against intermediate phenotypes while maintaining the distinction between parental types through divergent natural selection. Our results also indicate that salmonid hybrid zones may be clinal in structure.

Further studies assessing the fitness among hybrid generations and genotypic classes compared to parental types during the anadromous life history are warranted to test the hypothesis that hybrids are selected against in the marine environment. Also, fitness, survival and competition studies among juvenile hybrids and parental types could determine if hybrids were disadvantaged during the over-wintering period or other critical freshwater rearing times. Lastly, analyses of hybridization and the physical habitat components within large-scale rivers would be useful for determining habitat parameters that affect hybridization, the basis for partitioning of parental habitat, and whether the structure of salmonid hybrid zones are clinal, mosaic, or a combination of both.

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