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Hybrid zone structure and the potential role of selection in hybridizing populations of native westslope cutthroat trout (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*)

EMILY M. RUBIDGE* and ERIC B. TAYLOR
Department of Zoology and Native Fish Research Group, University of British Columbia, 6270 University Blvd, Vancouver, BC, Canada, V6T 1Z4

Abstract

Introgressive hybridization is a common feature of many zones of contact between divergent lineages of fishes. This is particularly common when taxa that are normally allopatric come into artificial (human-induced) secondary contact. We examined 18 native populations of westslope cutthroat trout (*Oncorhynchus clarki lewisi*, WCT) to determine the extent of introgressive hybridization with introduced rainbow trout (*O. mykiss*, RBT) and the genetic structure of hybridizing populations in the upper Kootenay River, southeastern British Columbia, Canada. Using four diagnostic nuclear loci we calculated a hybrid index, inbreeding coefficient, F_{IS}, and the linkage disquilibrium correlation coefficient, R_{ij}, for each locality to determine the distribution of genotypes in each population. We also categorized the 142 hybrid individuals found across localities into four hybrid classes based on their genotypes. The majority of localities (11/18) showed a unimodal distribution of genotypes skewed towards genotypes of WCT. Two localities, however (lower Gold Creek and Lodgepole Creek) showed a flat to bimodal distribution and one site (lower Bull River) showed a unimodal distribution skewed towards RBT genotypes. The majority of hybrid individuals were classified genotypically as WCT backcrosses (59%) and post-F_1 individuals (24%). We found a skewed ratio of pure WCT to pure RBT (17 : 1) and only four F_1 hybrids (3%), suggesting that the spread of RBT alleles may be facilitated by hybrids straying to neighbouring populations. We also tested for the action of selection in one population using cohort analyses, but found little evidence of differential selection between pure WCT and hybrid individuals. Pooled across age classes there were significant differences in genotypic frequencies among loci suggesting differential introgression. There was no asymmetry to the hybridization between rainbow trout and westslope cutthroat trout because both species' mitochondrial DNA haplotypes were observed at similar frequencies in the hybrids. Our analyses suggest that hybrid swarms are likely to form in the upper Kootenay River drainage and that certain native WCT populations in British Columbia are at risk of local genomic extinction.

Keywords: conservation, hybridization, hybrid zone structure, introduced species, linkage disequilibrium, selection, westslope cutthroat trout

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Introduction

Hybrid zones result when genetically distinct populations meet and mate, producing offspring of mixed ancestry (i.e. hybrids, Barton & Hewitt 1989). These zones can vary from a few metres to several kilometres in width (Hewitt 1989); they can persist through evolutionary time (e.g.

Correspondence: Emily M. Rubidge. *Present address: Division of Ecosystems Sciences, Department of Environmental Science, Policy & Management, University of California-Berkeley, 151 Hilgard Hall No. 3110, Berkeley, CA 94720-3110, USA. E-mail: erubridge@nature.berkeley.edu

Arnold 1997; Baxter *et al*. 1997), or rapidly result in the collapse of one or both parental species (e.g. Whitmore 1983; Echelle & Connor 1989). Natural hybridization has been reported to occur in a variety of taxa (Arnold 1997) and is often associated with areas where previously isolated populations have come into secondary contact (Hewitt 1989).

Hybrid-zone populations are often classified by the nature of the frequency distributions of genotypes based on a defined hybrid index (where the minimum and maximum values represent the pure parental genotypes). A hybrid swarm, defined by Harrison (1993) as a diverse array of recombinant types, refers to a unimodal hybrid zone distribution where hybrid genotypes predominate. A bimodal hybrid zone consists largely of genotypes that are similar to either parental form, and with few intermediate hybrid genotypes present (i.e. F_1 or early generation post-F_1 genotypes or backcrosses; e.g. Redenbach & Taylor 2003). Jiggins & Mallet (2000) described a continuum of hybrid zones from a unimodal distribution to a bimodal distribution, which may represent different stages in speciation. This continuum has also been shown to happen in reverse when two previously isolated and closely related species come into contact (e.g. Rhymer *et al*. 1994) and merge into a single species.

Documenting the structure of hybrid zones can help in the understanding of the processes that generate and maintain them. For instance, Jiggins & Mallet (2000) suggested that bimodal hybrid zones are characteristic of species that show well-developed (but incomplete) pre-reproductive isolation. In the absence of such isolation, however, bimodal hybrid zones may signal the action of disruptive selection against hybrid genotypes. By contrast, unimodal hybrid zones suggest that pre-reproductive isolation is largely incomplete and/or that intrinsic (environmentally independent) or extrinsic (environmentally dependent) selection against hybrids is absent.

Salmonid fishes (salmon, trout, char, grayling and whitefish) have provided many examples of natural hybridization, and the wealth of knowledge on the ecology and evolutionary history of these fishes provides rich material for the study of hybridization and its evolutionary consequences (Taylor 2004). In addition, the consequences of artificial contact between divergent lineages mediated by human introductions are increasingly important to the conservation of salmonid biodiversity (Allendorf *et al*. 2001). The westslope cutthroat trout (*Oncorhychus clarki lewisi*, WCT) is one of four major subspecies of cutthroat trout (Allendorf & Leary 1988; Behnke 1992). Over the last century, WCT populations have suffered dramatic declines throughout their historic range because of a number of factors including hybridization and introgression with introduced species, particularly non-native rainbow trout (*O. mykiss*, RBT, e.g. Leary *et al*. 1984). Allendorf *et al*. (2004) suggested that WCT are threatened by 'genomic extinction' in the United States, a term defined by Epifanio & Philipp (2001) as a loss of an evolutionary lineage through introgression with another lineage. Although the alleles of the native WCT genome will be present in the introgressed lineage, the novel combinations of alleles and genotypes that have evolved over evolutionary time will be lost (Leary *et al*. 1995; Allendorf *et al*. (2004). Introgressive hybridization has been suggested as the single greatest threat to the remaining WCT populations (Allendorf & Leary 1988), but the processes determining the genetic structure of hybridizing populations in nature (e.g. the relative strengths of prezygotic and postzygotic barriers to gene flow) are not well understood.

WCT and RBT evolved predominantly in allopatry throughout their historic ranges (Behnke 1992). In the few naturally sympatric populations in the drainages of the Clearwater and Salmon rivers (Idaho), prezygotic barriers such as spatial and temporal reproductive segregation appear to limit interspecific mating (Liknes & Graham 1988). The coastal cutthroat trout (CCT, *Oncorhynchus clarki clarki*) is naturally sympatric with native RBT and although hybridization does occur between CCT and RBT, the ability of these two closely related species to coexist and remain distinct in sympatry is usually attributed both to prezygotic and postzygotic isolation (e.g. Campton & Utter 1985; Trotter 1987; Young *et al*. 2001). By contrast, there is little evidence of postzygotic isolation in hybridizing populations of WCT and RBT. Laboratory studies suggest that F_1 WCT × RBT hybrids have reduced fitness compared to the parental types (Leary *et al*. 1995; Allendorf *et al*. 2004), but this contrasts with inferences from wild populations which suggests that RBT hybridization can spread quickly throughout a drainage (e.g. Rubidge *et al*. 2001; Hitt *et al*. 2003). There have, however, been no explicit tests of WCT × RBT hybrid fitness in their natural environments.

Within Canada, the last contiguous range of WCT occurs in the southeast corner of British Columbia in the upper Kootenay River drainage (Fig. 1). Once thought to be a refuge for remaining native WCT gene pools, the upper Kootenay River drainage has recently shown evidence of increased RBT hybridization: 78% (18/23) of the streams examined exhibited evidence of hybridization (Rubidge 2003; Rubidge & Taylor in press). In this study, we had two principal objectives. First, we detailed the genotypic structure of 18 populations found to contain RBT and WCT alleles. We then used these data to determine if hybrid swarms had formed in the upper Kootenay River drainage and thus to make inferences about the associations between hybrid zone structure and the prevalence of pre- or postzygotic processes influencing the structure of these hybrid zones. Second, we examined hybridization in one locality in greater detail, the St Mary River, to determine if there was differential survival occurring between hybrids

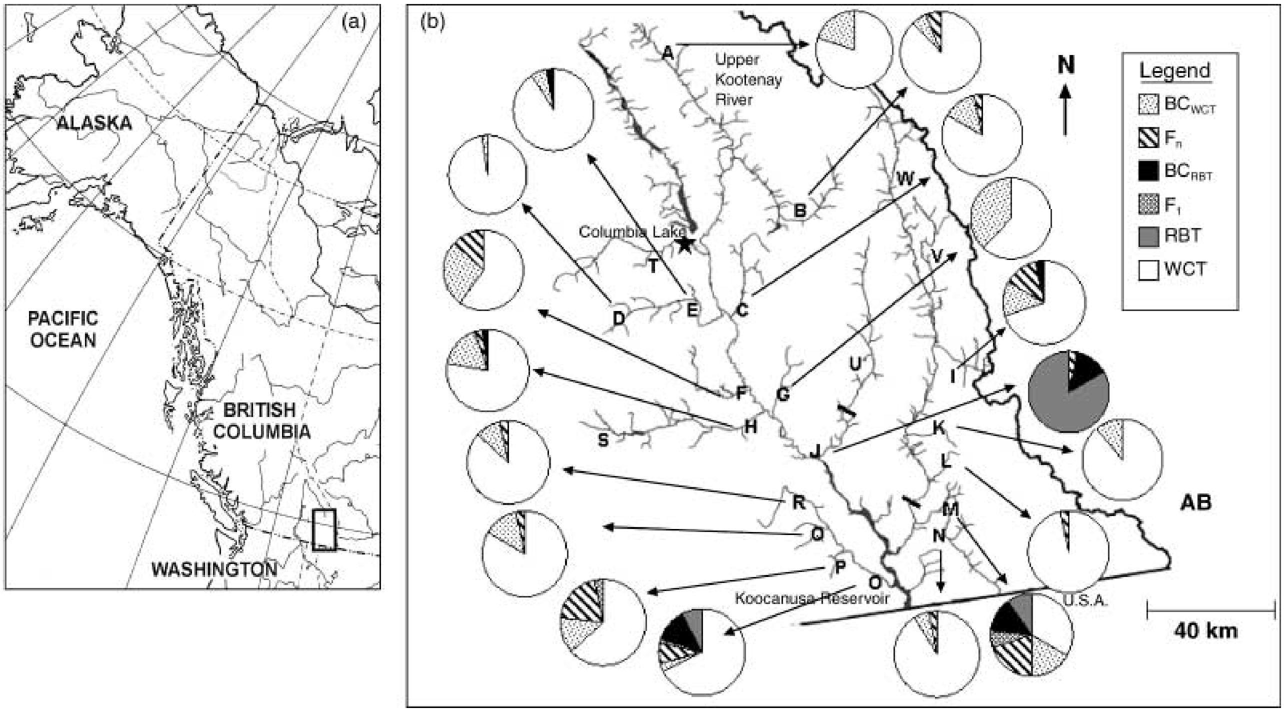

Fig. 1 Sample localities and westslope cutthroat trout (WCT; *Oncorhynchus clarki lewisi*) and rainbow trout (RBT; *O. mykiss*) hybridization in the upper Kootenay River drainage, British Columbia; (a) Inset rectangle shows study area in western North America. (b) Localities sampled for the presence of RBT hybridization: A, upper Kootenay River mainstem; B, White River; C, Lussier River; D, upper Skookumchuk Creek; E, lower Skookumchuk Creek; F, Mather Creek; G, Wild Horse River; H, lower St Mary River; I, Michel Creek; J, lower Bull River; K, Coal Creek; L, Morrissey Creek; M, Lodgepole Creek; N, Wigwam River; O, lower Gold Creek; P, Bloom Creek at Gold Creek; Q, Teepee Creek at Gold Creek; R, upper Gold Creek; S, upper St Mary River; T, Findlay Creek; U, upper Bull River; V, Fording River; W, upper Elk River (BC, British Columbia; AB, Alberta; USA, United States of America). Evidence of RBT hybridization was found at sample localities A to R. Pie charts represent the proportion of hybrid classes and parental types identified. Black bars represent impassable hydroelectric dams and the star represents an impassable canyon. There was no evidence of RBT hybridization at localities S to W, therefore no pie charts are presented.

and pure parental fish by examining the frequency of hybrids across age classes (cohort analyses). We predicted that WCT × RBT hybrids would be less fit than pure WCT in the WCT environment based primarily on the evidence of reduced fitness of hybrids in laboratory studies.

Materials and methods

Study area

The Kootenay River is a major tributary of the Canadian portion of the Columbia River Basin, the third largest drainage basin in British Columbia. This study took place in the upper Kootenay River drainage, which reaches from its source on the west slope of the Rocky Mountains to the first border crossing (Fig. 1). Tissue samples from 981 fish were collected from 23 sample localities in 12 different river systems from 1999 to 2001. Methods used were outlined by Rubidge *et al*. (2001) and included angling, electroshocking and the use of dipnets in isolated pools.

DNA extraction and molecular markers

DNA was extracted from each tissue sample (10–20 mg) using the GENTRA Puregene DNA Extraction Kit following the manufacturer's protocol, diluted to 100 ng/μL, and stored at −20 °C. Parental species and hybrids were identified using four diagnostic nuclear loci: two restriction fragment length polymorphisms (RFLPs) and two species-specific simple-sequence repeats (SSR) dual primer polymerase chain reaction (dpPCR). The RFLP loci were based on sequence variation in the Ikaros (IK) intron and the heat shock protein (Hsc 71) (Baker *et al*. 2002). These intron regions were amplified using the PCR and were incubated with restriction enzymes (*Hin*fI for IK and *Taq*I for Hsc 71) following the enzyme supplier's protocol (New England Biolabs). The two dpPCR loci (OCC16 and OM13, Ostberg & Rodriguez 2002) were amplified using the conditions described in Rubidge (2003) but these did not differ significantly from those used in the original documentation (see Ostberg & Rodriguez 2002). PCR products and restriction fragments were visualized on 1.5–2.5% agarose gels stained

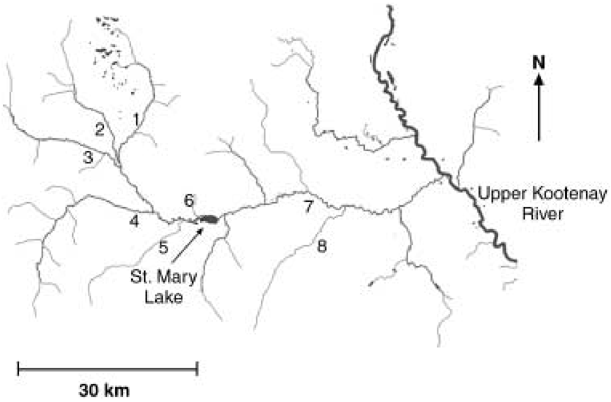

Fig. 2 Sample localities within the St Mary River system, upper Kootenay River drainage used in the cohort analyses. (1) White Creek; (2) Dewar Creek; (3) upper St Mary River mainstem; (4) Redding Creek; (5) Meachen Creek; (6) Alki Creek; (7) lower St Mary River mainstem; (8) Perry Creek. The upper St Mary River samples (localities 1–6) are defined as those areas above St Mary Lake while those below the lake (7, 8) constitute the lower St Mary River samples.

with ethidium bromide. More details of amplification and primer information are available in Rubidge (2003). The results of related analyses identified 18/23 localities as containing alleles from both species (Rubidge & Taylor in press). Only these 18 localities were analysed further in this study to determine the hybrid zone structure of the hybridized populations (Fig. 1).

A mitochondrial DNA (mtDNA) restriction site polymorphism was used to identify the mitochondrial haplotype of a subset of hybrid individuals. The primer combination GluDG/12Sar (Palumbi 1996) amplifies an approximately 3.0 kilobase pair fragment of the mtDNA spanning the cytochrome *b* gene, the control region, and a portion of the 12S rRNA gene. Species-diagnostic haplotypes are revealed when this fragment is cut with the restriction enzyme, *Ava*II (see Rubidge *et al*. 2001). The diagnostic nature of each of the nuclear loci and mtDNA was validated and discussed by Rubidge *et al*. (2001) and Ostberg & Rodriguez (2002).

Cohort collection

We also collected samples from one locality for different age classes of fish both in 2000 and 2001. The headwaters of the St Mary River are in the Purcell Mountains and the river flows east to drain into the upper Kootenay River about 2 km north of Fort Steele near Cranbrook, British Columbia. The upper and lower portions of the river are separated by St Mary Lake (Fig. 2).

In the St Mary River, approximately 100 tissue samples were collected from each of the four age classes in 2000 (0 +, 1 +, 2 + , 3 +) above and below St Mary Lake. Age classification was based on size, location of sampling and retention of juvenile characteristics such as parr marks (dark oval bands on the lateral surface of subadult fish). The criteria used to define age classes were as follows: 0 +, fry or young of the year, < 55 mm in length; 1 +, year old fish, approximately 60–130 mm; 2 +, fish larger than 130 mm that retained parr marks; and > 3 +, adults, fish larger than 180 mm that have no retention of juvenile characteristics. In the following summer, 2001, each age class was re-sampled to conduct a cohort analysis of hybrid survival (see below).

Hybrid classification

At each locus, an individual was classified as WCT if it was homozygous for the WCT allele, RBT if it was homozygous for the RBT allele, or a 'hybrid' if it was heterozygous and contained the alleles for both species. Across all four loci then, an individual was classified as being a 'hybrid' if it possessed at least one (of a possible eight) alleles that were heterospecific. Hybrid individuals were further subdivided into three different hybrid classes; F_1, F_n, or backcross genotype classes. The goal of this classification was to group the hybrid genotypes into two broad classes, those that are the result of crosses between parental types (F_1 hybrids, identified as being heterozygous at each locus) and later generation genotypes (individuals that are homozygous at one or more loci). These later generation hybrids were further classified according to whether they were homozygous for at least one locus from each parental type (labelled F_n genotypes) or from only one parental type (labelled BC or backcross genotypes). In the latter case, the homozygous loci are diagnostic for WCT in a WCT backcross genotype (BC_{WCT}) or RBT in an RBT backcross genotype (BC_{RBT}).

Hybrid zone structure

To characterize the genetic structure of hybrid zones, Jiggins & Mallet (2000) recommended that future studies provide individual data or some means of visualizing the distribution of genotypes. We calculated the hybrid index used by Szymura & Barton (1991) as the frequency distribution of the number of individuals in the population with zero, one, two, … eight RBT alleles. Because we used four loci, the maximum hybrid index value possible was eight (i.e. all eight possible alleles were RBT alleles). An individual with no RBT alleles present at any loci would have a hybrid index of zero (or be considered a 'pure' WCT). A problem with this index is that it does not distinguish heterozygotes from homozygotes with alternative alleles. For example, an F_1 individual is heterozygous at all four loci giving a hybrid index value of four; but there are other genotypes that also give a hybrid index value of four, such as an F_n that is homozygous for WCT at two loci and homozygous for RBT at the other two loci. The possible number of F_1 hybrids present, however, will be estimated from the results of the hybrid class analysis.

Population genetic analysis

If all individuals collected at a locality were produced by random mating then allele frequencies should conform to Hardy–Weinberg proportions. If, however, immigration of RBT alleles (via pure RBT or hybrids) is recent, or individuals were mating assortatively, or if selection was acting against/for hybrids (heterozygotes in this case) then one would expect deviations from Hardy–Weinberg proportions. We calculated Weir & Cockerham's (1984) inbreeding coefficient as a measure of heterozygote deficit (Jiggins & Mallet 2000) in populations that deviated significantly from Hardy–Weinberg equilibrium using GENEPOP Version 3.1d (Raymond & Rousset 1995). The inbreeding coefficient (F_{IS}) is equal to $1 - (P_{Aa}/2p_Ap_a)$ where P_{Aa} is the observed frequency and $2p_Ap_a$ is the expected frequency of heterozygotes. F_{IS} values range from –1 to +1. Positive values indicate a heterozygote deficit, negative values a heterozygote excess, and 0 indicates what is expected under random union of gametes.

Linkage disequilibrium (i.e. nonrandom gametic association between alleles of different genes) between pairs of loci is helpful when describing the distribution of hybrid genotypes and when estimating the 'age' of the hybridized population (Forbes & Allendorf 1991). Strongly bimodal populations will have high linkage disequilibrium and near maximum heterozygote deficiencies, whereas hybrid swarms or unimodal populations will not reveal any genetic associations among parental alleles if they are randomly mating (Jiggins & Mallet 2000; Allendorf *et al*. 2001; Redenbach & Taylor 2003). When previously isolated and genetically distinct populations (i.e. WCT and RBT) come into secondary contact, gametic disequilibria will be high initially. Eventually it will erode over time if there is some level of mixing between gene pools, there is no selection, and there is no immigration of parental types continually into the zone of contact. Recent immigration of parental types, positive assortative mating, or selection against hybrids will, however, slow or prevent the disappearance of disequilibria. Given that hybrid swarms have been documented between WCT and RBT in other drainages (i.e. randomly mating populations; e.g. Leary *et al*. 1984), the presence of linkage disequilibrium in hybridized populations of these species suggests that random mating has not yet been established and hybridization is recent. The absence of linkage disequilibrium in hybridized populations, however, does not exclude the possibility of recent hybridization; in populations where there is recent hybridization of post-F_2 or backcrossed individuals linkage disequilibrium may not be present. We calculated linkage disequilibrium between all six possible pairs of loci using GENETIX (Belkhir *et al*. 2001). The correlation coefficient (R_{ij}), described by Weir (1979), is determined from the composite linkage disequilibrium coefficient, Δ_{ij}, which takes into account associations between alleles whether they are in coupling or repulsion (Black & Krafsur 1985). A sequential Bonferroni correction for multiple tests was used to prevent artificial inflation of alpha values in both the Hardy–Weinberg equilibrium test and the linkage disequilbrium tests. To compare the mean F_{IS} values and the mean R_{ij} values between years (1999 and 2000) in sample localities that were sampled in both years (lower Gold Creek and lower St Mary River) we used an Independent-Samples *t*-test implemented in SPSS 11.0.

Detecting selection using cohort analysis

We used both 'static' and 'dynamic' cohort analyses to test if we could detect differential survival between hybrids and parental individuals (e.g. Dowling & Moore 1985; Bert & Arnold 1995). A static cohort analysis involves sampling different age classes during the same year. The fish were collected in August 2000 and assigned to an age cohort based on size and retention of juvenile characteristics (see collection methods for more details). During August 2001, we attempted to collect tissue samples from each of the age classes again, re-sampling each cohort from the previous year to conduct a dynamic cohort analysis. The dynamic analysis followed each age class over 1 year, to determine if the proportion of hybrids changed during the course of that year. In all cases, fish were collected in the same habitats across cohorts to minimize any spatial variability in selective regimes.

We calculated the following statistics for each age group: (i) percentage of hybrid individuals, (ii) the Weir & Cockerham (1984) estimate of the inbreeding coefficient as a measure of heterozygote deficit (F_{IS}), and (iii) the linkage disequilibrium correlation coefficient R_{ij} (Weir 1979). Calculating the mean number of heterospecific alleles for each cohort from genotypes of individual fish yielded similar results to estimating the percentage of hybrid individuals for each age class. For simplicity, we report results based on the percentage of hybrid individuals in each cohort. We calculated the mean value for F_{IS} and R_{ij} across all loci for each age class. Under the null hypothesis of no differential selection between hybrid and parental genotypes we expected the percentage of hybrids, F_{IS}, and R_{ij} to remain relatively constant among age classes. By contrast, selection against (for) hybrids would be signalled by an increase (decrease) both in F_{IS} and R_{ij}. We compared the means of these statistics between age classes using a one-way analysis of variance (ANOVA) if all assumptions were met, and a nonparametric Kruskal–Wallis test if ANOVA assumptions (i.e. homogeneity of variances) were violated.

The number of hybrid individuals across age classes was compared using χ^2 contingency tests and Fisher's exact tests. Both SPSS 11.0 and JMPIN 4.0 statistical software were used. We also tested for differential introgression among loci by comparing genotypic frequencies of homozygotes (WCT), heterozygotes (hybrids), and homozygotes (RBT), across cohorts for each locus individually using Monte Carlo statistics on $R \times C$ matrices (http://itb.biologie.hu-berlin.de/~nils/stat/). One thousand random matrices were generated with the same row and column totals as in the empirical matrix. Methods of the randomization and generation of the test statistics are described in Blüthgen *et al*. (2000).

Results

Hybrid categories

The majority of fish at all localities (with the exception of two: lower Bull River and Lodgepole Creek) were classified as WCT (Table 1). Westslope cutthroat trout backcrosses (BC$_{\mathrm{WCT}}$) were the most common hybrid genotype identified in the upper Kootenay River drainage across all 18 localities; in 1999, 50% and in 2000, 60% of the hybrid fish were classified as BC$_{\mathrm{WCT}}$. The next most common class of hybrids was post-F$_1$ generation hybrids (F$_n$) at 29% in 1999 and 23% in 2000. Rainbow trout backcrosses (BC$_{\mathrm{RBT}}$) were more common in 1999 (21%) than in 2000 (12%). In both years, first-generation hybrids (F$_1$) were absent or very rare in the upper Kootenay River; only four potential F$_1$ hybrids were observed across all 142 hybrids examined. All F$_1$ individuals were collected in 2000 from three localities; Lodgepole Creek on the Wigwam River system, Bloom Creek (a tributary of Gold Creek) and lower Gold Creek (Fig. 1b). Fish classified as WCT were found at every locality sampled except for lower Bull River where all but five fish were identified as RBT, the remaining five fish were of hybrid descent. By contrast, of the 30 fish sampled from Lodgepole Creek, only a third were classified as WCT. Lodgepole Creek and lower Gold Creek were the only localities that contained every hybrid class and both WCT and RBT parental genotypes (Fig. 1b). At four localities (upper Kootenay River, upper Skookumchuk Creek, Coal Creek, Wild Horse River) we found only WCT and BC$_{\mathrm{WCT}}$. Although pure RBT were only found at three locations, BC$_{\mathrm{RBT}}$ were found at an additional three locations (lower Skookumchuk Creek, lower St Mary River and Michel Creek) suggesting that RBT are in these systems or at least in nearby streams.

Table 1 Percentages of individuals classified as westslope cutthroat trout (WCT), rainbow trout (RBT), and their hybrids from the upper Kootenay River systems based on genotypes across four diagnostic nuclear loci

Locality (N)	WCT (%)	Hybrids (%)	RBT (%)
A. upper Kootenay River (15)	80.0	20.0	0
B. White River (33)	87.9	12.1	0
C. Lussier River (30)	83.3	16.7	0
D. upper Skookumchuk Creek (40)	97.5	2.5%	0
E. lower Skookumchuk Creek (33)	90.9	9.1	0
F. Mather Creek (30)	60.0	40.0	0
G. Wild Horse River (45)	62.2	37.8	0
H. lower St. Mary River 1999 (31)	87.1	12.9	0
lower St. Mary River 2000 (104)	83.7	16.3	0
I. Michel Creek (30)	70.0	30.0	0
J. lower Bull River (30)	0	16.7	83.3
K. Coal Creek (36)	88.9	11.1	0
L. Morrissey Creek (30)	96.7	3.3	0
M. Lodgepole Creek (30)	33.3	56.7	10.0
N. Wigwam River (34)	91.2	8.8	0
O. lower Gold Creek 1999 (36)	69.4	25.0	5.6
O. lower Gold Creek 2000 (30)	66.7	23.3	10.0
P. Bloom Creek (30)	63.3	36.7	0
Q. Teepee Creek (30)	83.3	16.7	0
R. upper Gold Creek (30)	86.7	13.3	0

Sample sizes are given in parentheses.

Hybrid zone structure

The frequency distribution of genotypes within sample localities was classified as follows: unimodal, left-skewed; flat; bimodal; and unimodal, right-skewed. The genotype distribution for most of the populations was unimodal, left-skewed towards 'pure' WCT (e.g. Wild Horse River, Fig. 3a) with no RBT parental types detected. These populations did not significantly deviate from Hardy–Weinberg proportions and did not show significant linkage disequilibrium. Ten additional localities [upper Kootenay River, White River, Morrissey Creek, Wigwam River, upper Skookumchuk River, Teepee Creek, upper Gold Creek, Coal Creek, lower St Mary River (2000) and Mather Creek] showed very similar genotypic distributions to the Wild Horse River. Lodgepole Creek had a flat distribution of genotypes (Fig. 3b), lower Gold Creek approached a bimodal distribution (Fig. 3c), and the lower Bull River genotypic distribution was right-skewed towards RBT, with no WCT parental types present (Fig. 3d).

Westslope cutthroat trout allele frequencies (averaged over four loci) varied with sample location from 0.03 in the lower Bull River to 0.63–0.99 across the other 17 localities containing both species' alleles. Four localities deviated significantly from Hardy–Weinberg proportions, and seven showed significant linkage disequilibrium. Lower Gold Creek (both 1999 and 2000 samples), lower St Mary River (1999 only), Lodgepole Creek and Michel Creek all deviated significantly from Hardy–Weinberg proportions and had significant linkage disequilibrium (Table 2). Three localities, Lussier River, Bloom Creek and lower Skookumchuk

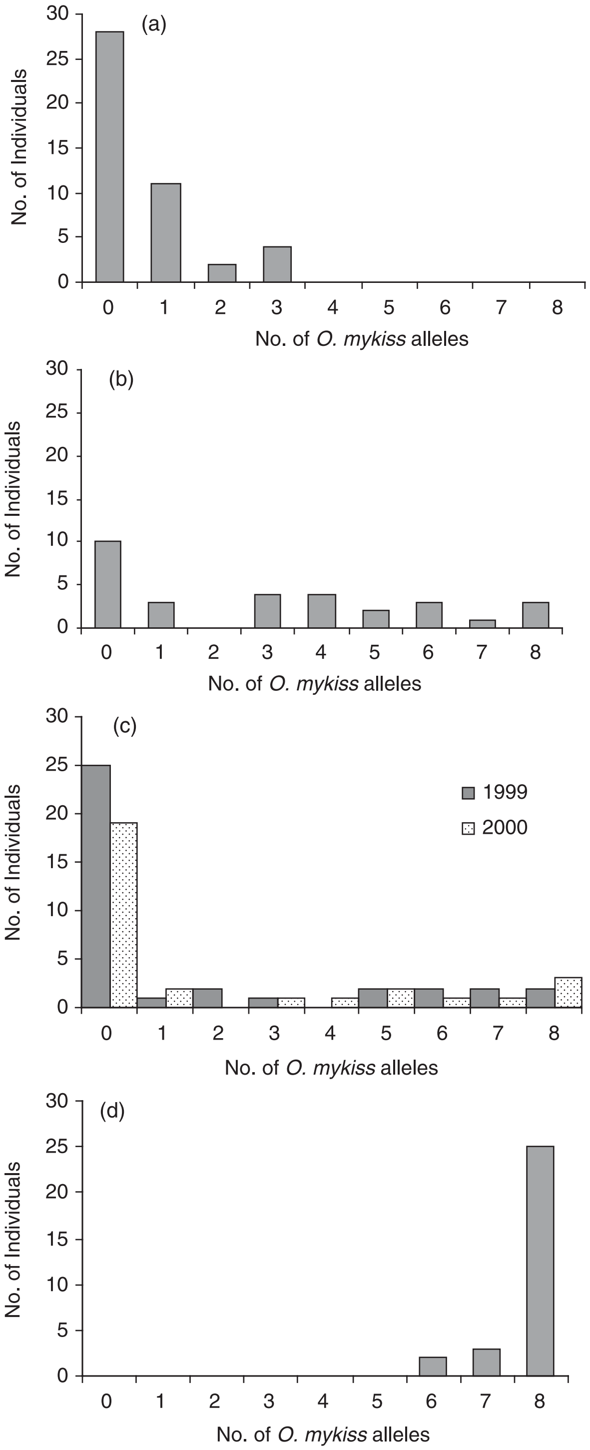

Fig. 3 Hybrid zone structure of westslope cutthroat trout and rainbow trout in four populations from the upper Kootenay River drainage. Values on the x-axis represent the number of RBT alleles present ranging from zero ('pure' WCT) to eight ('pure' RBT). (a) Wild Horse River; (b) Lodgepole Creek; (c) lower Gold Creek; (d) lower Bull River.

Table 2 Inbreeding coefficient (F_{IS}; Weir & Cockerham 1984, a measure of heterozygote deficit), for four nuclear loci in three populations that significantly deviated from Hardy–Weinberg proportions (in all cases $P \leq 0.0002$)

	F_{IS}			
Location	Ikaros	Hsc 71	Occ 16	Om13
Lower St Mary's River 1999	−0.034	1*	1*	1*
Lower Gold Creek 1999	−0.029	0.862*	0.316	0.645*
Lower Gold Creek 2000	0.521*	0.601*	0.637*	0.594*
Lodgepole Creek	0.367	0.657*	0.356	0.440
Michel Creek	0.354	0.766*	—	0.529*

F_{IS} values range from −1 to +1, with positive values indicating heterozygote deficit, negative values indicating heterozygote excess, and 0 equal to expected under random mating.
*indicates F_{IS} values that are statistically different from 0 at an alpha level of 0.05, corrected for multiple tests with a sequential Bonferroni (0.05/20 = 0.0025).

Creek conformed to Hardy–Weinberg equilibrium (average $F_{IS} = 0.139$, 0.119, and −0.009, respectively), but they exhibited significant linkage disequilbria between pairs of loci. Hardy–Weinberg equilibrium can be restored after one generation of random mating, whereas linkage relationships decay more slowly, depending on the rate of recombination and the mating regime. The samples collected from lower St Mary River in 1999 deviated from Hardy–Weinberg equilibrium (mean $F_{IS} = 0.794$), but the 2000 samples from the same population did not (mean $F_{IS} = 0.142$) suggesting that WCT × RBT hybridization is relatively recent at this locality. In the four populations that deviated from Hardy–Weinberg equilibrium all loci had significant positive mean F_{IS} values, indicating a heterozygote deficiency, but only lower Gold Creek (in 2000) had a significant heterozygote deficiency at all four loci (F_{IS} ranged from 0.521 to 0.637). In Michel Creek, Occ16 was fixed for the WCT allele therefore F_{IS} was not calculated in this case.

Gametic disequilibria were calculated for all six nuclear locus pairs for all 18 localities that showed evidence of hybridization (Table 3). Contingency tests revealed significant genetic associations (H_O = genotypes at one locus are independent from genotypes at another locus) between at least two of the possible six locus pairs in 7/18 admixed localities. The values of R_{ij} ranged widely from 0.046 to 0.999. The Occ16 locus was fixed for the WCT allele in the Lussier River and therefore R_{ij} could not be calculated for pairs involving Occ16 at this locality. All calculated R_{ij} values were positive, indicating an association between alleles from the same parental species between loci. Four out of the six pairs of loci in the lower Gold Creek 1999 samples showed significant linkage disequilibrium; the 2000 samples from lower Gold Creek showed all six pairs in significant

Table 3 Linkage disequilibrium correlation coefficient, R_{ij}, between pairs of nuclear loci (Weir 1979), in populations that indicated significant disequlibrium between pairs of loci

	R_{ij}					
Location	Ikaros/Hsc	Ikaros/Occ16	Ikaros/Om13	Hsc/Occ16	Hsc/Om13	Occ16/Om13
Lower Skookumchuk Creek	0.480	0.046	0.482	0.719*	0.999*	0.718*
Lower Gold Creek 1999	0.515*	0.216	0.103	0.793*	0.570*	0.648*
Lower St Mary River 1999	0.370	0.576*	0.576*	0.718*	0.718*	0.999*
Lower Gold Creek 2000	0.937*	0.999*	0.934*	0.960*	0.856*	0.914*
Lussier River	0.850*	Fixed	0.959*	Fixed	0.973*	Fixed
Bloom Creek	0.472	0.268	0.541*	0.329	0.598*	0.541
Lodgepole Creek	0.739*	0.643*	0.737*	0.445*	0.631*	0.684*
Michel Creek	0.754*	0.557	0.948*	0.457	0.821*	0.492

R_{ij} values range from −1 to +1 and $R_{ij} = 0$ indicates that loci are in linkage equilibrium.
*indicates R_{ij} values that are statistically different from 0 at an alpha level of 0.05, corrected for multiple tests with a sequential Bonferroni (0.05/120 = 0.0004).

Table 4 The number of westslope cutthroat trout (WCT), rainbow trout (RBT), four classes of hybrid genotypes, and the percentage of hybrids found from among individual cohorts in the lower St Mary River in 2000 and 2001

Age class (N)	WCT	F_1	F_n	BC_{WCT}	BC_{RBT}	RBT	% hybrids	Mean F_{IS} (SD)	Mean R_{ij} (SD)
Year 2000									
> 3 + (104)	87	0	3	14	0	0	16.3	0.144 (0.189)	0.309 (0.285)*
2 + (93)	70	1	3	19	0	0	24.7	0.032 (0.257)	0.313 (0.285)*
1 + (112)	89	0	3	19	1	0	20.5	0.213 (0.150)*	0.422 (0.145)*
0 + (100)†	82	0	5	13	0	0	18.0	0.136 (0.362)*	0.240 (0.103)*
Total (409)	328	1	14	65	1	0	19.8		
Year 2001									
> 3 + (0)	0	0	0	0	0	0	n/a	n/a	n/a
2 + (5)	2	0	0	2	0	1	40.0	n/a	n/a
^{a}1 + (49)	28	0	3	16	2	0	43.0	0.064 (0.099)	0.438 (0.154)*
0 + (46)	40	0	1	5	0	0	13.0	−0.006 (0.007)	0.035 (0.014)
Total (100)	70	0	4	23	2	1	29.0		

The mean F_{IS} value averaged across four loci and the mean R_{ij} value averaged across six loci pairs are also given. Standard deviations (SD) of these means are given in parentheses.
*Significance at a Bonferroni corrected alpha of 0.05/4 = 0.0125 for F_{IS} values and 0.05/24 = 0.002 for R_{ij} value.
†Cohort born in 2000 and re-sampled in 2001.

linkage disequilibrium. The average R_{ij} value in 1999 was 0.474 whereas the average R_{ij} value in 2000 was significantly higher at 0.933 ($t = -4.2$, d.f. = 10, $P = 0.002$). By contrast, in the two years in which the lower St Mary river was sampled, the opposite was true. The average R_{ij} for 1999 was 0.659, but for 2000 it was significantly lower at 0.309 ($t = 3.683$, d.f. = 10, $P = 0.004$), indicating that linkage disequilibrium had decayed over the year.

Mitochondrial DNA analysis

Fish from two localities that contained individuals from all hybrid classes, lower Gold Creek and Lodgepole Creek, were assayed ($N = 24$) to determine their mtDNA haplotypes. There appeared to be no asymmetry to the hybridization between RBT and WCT because both species' haplotypes were observed in the hybrid individuals tested; 13 had the RBT haplotype and 11 had the WCT haplotype (Fisher's exact test $P = 0.36$). The three F_1 hybrids that were successfully amplified for mtDNA also showed evidence of bidirectional hybridization.

Cohort analysis in St Mary River

Between August 7 and August 14, 2000, 409 fish were sampled from the lower St Mary River and 405 fish were sampled from the upper river (above St Mary Lake). In the lower river, 19.8% were identified as hybrid individuals (Table 4).

No hybrids were detected in the upper river above St Mary Lake. Pure RBT were not identified anywhere in this system in 2000. A total of 100 fish were sampled between August 16 andAugust 22, 2001 from the lower river, 29% of these fish were identified as hybrid individuals and one pure RBT juvenile was identified. We did not resample the upper St Mary River in 2001 because no hybrids were detected in 2000. Our results suggest that hybrids and RBT are extremely rare or absent in the upper river considering that over 400 fish were sampled and not one fish was identified as such. The majority of the hybrids identified in all age classes in both years were classified as BC_{WCT}. One F_1 hybrid was found in the 1 + cohort in 2000.

The percentage of hybrids in the static analysis (different age classes sampled in 2000) ranged from 16.3% in the > 3 + cohort to 24.7% in the 2 + cohort. There was no significant difference in the proportion of hybrid individuals identified between each age class in 2000 ($\chi^2 = 2.41$, $P = 0.49$). All four cohorts showed significant positive linkage disequilibrium and the R_{ij} values ranged from 0.026 to 0.627 (Table 4). A Kruskal–Wallis test comparing the mean R_{ij} values indicated that there was no significant difference in this value among cohorts. Similarly, the mean F_{IS} value, did not differ significantly among cohorts ($F = 0.357$, d.f. = 3, $P = 0.79$), indicating that there was no detectable change in the heterozygote deficit with age.

There were only five age 2 + and no > 3 + trout sampled in 2001. Owing to these sampling difficulties in the older age classes in 2001, the static and dynamic cohort analyses were limited to comparisons between the 0 + and 1 + cohorts. Although sample sizes were smaller in 2001, a significant difference in the prevalence of hybrids was detected between cohorts sampled in 2001 ($\chi^2 = 11.68$, $P = 0.003$); 13% of the 2001 0 + cohort were hybrids whereas 43% of the 1 + samples were identified as hybrids (Table 4).

In the dynamic cohort analysis (Table 4 and Discussion) that compared the fry (0 + in 2000) and fingerling (1 + in 2001) stages, the proportion of hybrids observed in our sample more than doubled over the year ($\chi^2 = 10.1$, $P = 0.0015$). In addition, the 0 + cohort deviated from Hardy–Weinberg proportions, indicating a heterozygote deficiency (mean $F_{IS} = 0.136$). When this cohort was resampled in 2001 as 1 + fish, however, this deficiency was no longer significantly different from zero (mean $F_{IS} = 0.064$). Although there was significant linkage disequilibrium within both cohorts, there was no significant difference in the mean R_{ij} value when fish were sampled as 0 + and again as 1 + individuals ($F = 4.7$, d.f. = 1, $P = 0.06$).

Differential introgression among loci

There was no detectable difference in the frequencies of homozygous WCT genotypes, heterozygous hybrid genotypes and homozygous RBT genotypes in the 2000 samples at any of the four loci across all four age classes treated individually ($P = 0.112$–0.472). Pooled across age classes, however, there were significant differences in genotypic frequencies among loci ($P < 0.0001$, Table 5). The Hsc 71 locus identified more homozygous RBT genotypes than the other three loci. Upon removal of the Hsc 71 frequencies from the contingency test, significant differences were still detected ($P = 0.006$), suggesting that the Occ16 locus identified fewer heterozygous hybrid genotypes than the other two loci. Upon removal of both Hsc 71 and Occ16, the result of the contingency test was nonsignificant ($P = 0.48$).

Table 5 Pooled genotypic frequencies of: homozygous westslope cutthroat trout genotypes (ww), hybrid genotypes (wr), and homozygous rainbow trout genotypes (rr) from lower St Mary River (2000 sample) by locus

		Genotypic frequency			
Locus	*N*	ww	wr	rr	T_{obs}, *P*-value
Ikaros	408	361	46	1	
Hsc 71	398	352	32	14	
Occ16	405	386	18	1	
Om13	403	374	27	2	9155.20, $P < 0.0001$

Monte-Carlo randomizations (1000 runs) were used on $R \times C$ contingency tables to determine if the genotypic frequency differed among loci and the observed test statistic (T_{obs}) and associated P-value are shown.

Discussion

Estimates of hybrid prevalence

Hybridization is widespread in the upper Kootenay River drainage, but the number of hybrids identified in our study is probably a conservative estimate of the actual number of hybrids present. For instance, the intent of our hybrid class analyses was to describe the genotype of the individuals, but they do not necessarily describe the parentage of an individual because it is not possible to be sure of a hybrid's exact ancestry in most cases. The use of four diagnostic nuclear loci to distinguish between a first-generation hybrid and a backcrossed individual is fairly accurate (6.25% chance of error), but distinguishing between a later generation backcross and a pure parental species is much more difficult. Using four loci, the chance of misidentifying a later generation backcross (BC_{WCT-3} and higher) as a pure WCT is over 50% (Boecklen & Howard 1997). Even if the number of diagnostic loci were doubled (eight loci) there is still a 34% chance of misidentifying a third-generation BC_{WCT} as a genetically pure WCT (Boecklen & Howard 1997). Consequently, using only four nuclear loci for this type of classification results in an overestimate of pure parental and F_1 individuals and an underestimate of backcross individuals. Our analysis therefore is a conservative estimate of the full range of possible hybrid genotypes present and our estimates of the number of 'pure' WCT present in the upper Kootenay River system are almost certainly inflated.

Hybrid zone structure and demographic inferences

The majority of the 142 fish identified as hybrids were classified as backcrosses to WCT (59%). This suggests that not only are hybrids viable and fertile, but that RBT alleles are being spread through extensive backcrossing with pure WCT (introgressive hybridization). Less than 3% of hybrids were classified as first generation hybrids (F_1). This lack of F_1 hybrids could be a result of the skewed ratio of WCT to RBT (27 : 1) found across all 23 localities that we sampled in the upper Kootenay River drainage. The ratio is typically even more extreme than this average value because 76% of the pure RBT were found at a single locality, the lower Bull River. Although RBT are not currently distributed evenly among localities and appear to be absent from most hybridized localities (15 out of 18), their alleles have successfully spread through the upper Kootenay River system. Possible explanations of how RBT alleles have spread can be inferred from the structure of the genotypic distribution of the populations at each locality.

Ten populations that exhibited a genotypic distribution skewed towards WCT (Fig. 3a) conform to Hardy–Weinberg proportions and show no significant linkage disequilibrium. These populations have levels of RBT introgression that are less than 10%. There are two possible explanations for this genotypic distribution. One is that there was an influx of RBT into the system several generations ago and the RBT alleles now present are a signal of this past hybridization. The second scenario is recent immigration and subsequent hybridization of post-F_2 individuals from neighbouring hybridized localities. The presence of linkage disequilibrium can be a result of recent hybridization, but the absence of it does not exclude the possibility of recent hybridization, i.e. linkage disequilibrium may not be present in a situation where there is recent introgression involving post-F_2 individuals.

Rainbow trout introductions began as early as 1915 in the upper Kootenay River drainage, and for about 40 years (from the 1920s to 1964) the Cranbrook (British Columbia) Hatchery sporadically introduced 'Cranbrook trout' which were produced by intentionally hybridizing RBT and WCT. These hybrid fish were introduced into many streams and lakes in both British Columbia and Alberta (British Columbia stocking records, unpublished data, Ward 1974). It is therefore possible that the genetic structure observed in many populations (the left-skew distribution towards WCT) is a result of this past stocking of hybrids and subsequent backcrossing with WCT. If, however, this were true one would expect that the last genetic study on hybridization in this drainage would have detected it. The last genetic study of WCT × RBT hybridization in the upper Kootenay River was in 1986 (Leary *et al*. 1987). Of the seven localities in common between our study and that of Leary *et al*. (1987), the latter authors found evidence of RBT introgression at only one locality (the White River) and we found RBT introgression here as well as at three other localities (Rubidge 2003; Rubidge & Taylor in press). Although different genetic loci were used, both studies had similar power to detect introgression. Consequently, if the genotypic distribution observed in the present study was a result of an influx of RBT alleles several generations ago, then one would expect that Leary *et al*. (1987) would have detected introgression at more than just the one locality. The absence of RBT alleles in 1986 and the presence of them in 1999 suggest that hybridization (about three or four generations later) is recent in the three streams where new hybridization was detected. In addition, the presence of post-F_2 (F_n) and backcrossed individuals in the populations displaying the left-skewed (towards WCT) unimodal genotypic structure coupled with the rarity of observed F_1 hybrids, suggests that these populations are not closed to immigration by hybrids from other systems.

The fish sampled from Bloom Creek, lower St Mary River, lower Skookumchuk Creek and Lussier River all had a left-skewed genotypic distribution in which WCT were the most common genotype, and conformed to Hardy–Weinberg equilibrium, but they had significant linkage disequilbrium, suggesting that hybridization is recent at these localities. The Michel Creek samples also had a left-skewed genotypic distribution but deviated from Hardy–Weinberg equilibrium and had significant linkage disequilibrium, indicating that hybridization or immigration of RBT is very recent. All of these populations are particularly interesting because although no pure RBT were found, backcrosses to RBT (BC_{RBT}) were found at lower St Mary River, lower Skookumchuk Creek and at Michel Creek, and an F_1 was found at Bloom Creek. So either RBT are present at these populations in very low numbers, or these hybrid individuals have immigrated from a nearby population.

Lower Gold Creek had a distribution of genotypes that was closest to a bimodal distribution. Bimodality is achieved in populations with extremely strong heterozygote deficits and near maximum linkage disequilibrium (Jiggins & Mallet 2000; Redenbach & Taylor 2003). Jiggins & Mallet (2000) suggested that bimodality is indicative of strong assortative mating, but there is little evidence of assortative mating between WCT and RBT because hybrid swarms have formed in other drainages (reviewed in

Shepard *et al*. 2002). The bimodal distribution in lower Gold Creek is most likely the result of recent and continuous immigration of RBT into the area and of subsequent hybridization. Gold Creek flows into Koocanusa Reservoir and the sample location on lower Gold Creek is only about 5 km up from where Gold Creek enters the reservoir. As the reservoir has been extensively stocked with RBT, it could provide a nearby and relatively constant source of RBT that migrate up Gold Creek to spawn (Rubidge & Taylor in press). Consequently, in the absence of selection against hybrids, the Gold Creek population is perhaps at the greatest risk of forming a hybrid swarm.

The last pattern of hybrid zone structure observed in the upper Kootenay River WCT populations was a flat distribution observed at Lodgepole Creek. A flat genotypic distribution consists of a more even mixture of parental and hybrid genotypes (Jiggins & Mallet 2000). The deviations from Hardy–Weinberg proportions and linkage disequilibrium values were modest at Lodegpole Creek and only one-third of the population consisted of fish classified as pure WCT. Lodgepole Creek is a tributary of the Wigwam River and we sampled fish from about 6 km upstream from the Lodgepole/Wigwam confluence. In contrast to the high degree of hybridization in Lodgepole Creek, we detected only 8% hybrids introgression in the mainstem Wigwam River. Given that there is no barrier to migration between these locations, the higher level of introgression in Lodgepole Creek suggests that Wigwam River upstream is at great risk of further hybridization. Both Hitt *et al*. (2003) and Rubidge & Taylor in press) demonstrated a general tendency for hybridization between RBT and WCT to spread in upstream directions.

Cohort analysis and the role of selection

Our results provide little evidence of selection acting against WCT × RBT hybrids in the lower St Mary River. For instance, in the static cohort analysis there was no significant difference in the number of hybrids observed, the degree of heterozygote deficiency, or linkage disequilibrium with increasing age. The static cohort analysis assumes that each cohort not only has the same initial hybridization rate (same number of hybrid offspring produced each year), but also that the environmental selective conditions are constant over the four cohorts sampled in our study (0 +, 1 +, 2 +, 3 + ages classes). Although this environmental constancy is unlikely, the nonsignificant results between the proportion of hybrids across age classes suggests that even in the face of natural environmental fluctuation, there does not appear to be strong selection against hybrid genotypes in the lower St Mary River. A *post hoc* power analysis using standard recursion on the two-sample model (classic one gene with two alleles viability model, Hartl & Clark 1997) indicated that selection would have to be very strong ($s \geq 0.5$) to have at least an 80% chance of detecting a difference when sampling 100 individuals in each of the four cohorts. Therefore, it is likely that if weak to moderate selection is occurring we were unable to detect it because of the relatively small numbers of fish and cohorts sampled.

By contrast, the dynamic analysis sampled the same cohort after 1 year and is consistent with the possibility that hybrids are actually favoured by selection; the proportion of hybrids increased from 18% in the fry stage (0 +) to 43% in the fingerling stage (1 +). In addition, the heterozygote deficiency disappeared over the year, providing evidence that selection may act in favour of hybrids, although linkage disequilibrium did not differ significantly between cohorts. Unfortunately, and despite considerable effort, older age class fish were very difficult to catch in 2001 to extend the dynamic analysis to more age classes. The difficulty in catching older age classes in the summer of 2001 may have been related to warmer water temperatures and lower water levels than the year before. There was below normal precipitation in the winter of 2001 and above normal temperatures in the summer of 2001 (Environment Canada http://www.climate.weatheroffice.ec.gc.ca/climateData/canada_e.html). Most laboratory experiments indicate that trout reduce and eventually cease feeding as water temperatures rise above 22 °C (Dickson & Kramer 1971). At the time of sampling the WCT had most likely moved from feeding areas into deeper cooler pools, making them difficult to sample via angling.

The lack of a strong signal of selection in our study is consistent with data from experimental studies in laboratory settings suggesting that endogenous (environmentally independent) selection against hybrids between WCT and RBT, or between subspecies of cutthroat trout, is relatively weak (Ferguson *et al*. 1985; Forbes & Allendorf 1991). Leary *et al*. (1995) showed that experimentally produced WCT × RBT hybrids had equal or higher survival until hatching but experienced slower growth and survival to 112 days postfertilization. Similarly, Ferguson *et al*. (1985) concluded that the relatively high developmental success of hybrids between WCT and RBT suggests an absence of strong postmating isolation. Indeed, a review of fitness of hybrids in nature across a diverse array of taxa found many cases of hybrids being equal or superior to parental taxa in various measures of fitness (Arnold & Hodges 1995). In addition, even if hybrid genotypes are considerably less fit ($w = 0.1$) than parental types, it has been shown that introgressive hybridization may still occur (Epifanio & Philipp 2001).

Interestingly, Young *et al*. (2001) suggested that exogenous selection may limit introgression between hybridizing populations of anadromous RBT (steelhead) and CCT (see also Campton & Utter 1985). This inference was based on their observation of a high number of F_1 hybrids relative to backrosses or later generation hybrids sampled in Puget Sound and the Olympic Peninsula area of Washington State. Young *et al*. (2001) suggested that the greater differences in life history between steelhead trout (involving oceanic migrations) and CCT (involving only short-distance forays to the sea) may impose stronger selection against intermediate hybrid individuals (see also Utter 2000). Consistent with this idea is the experimental observation of intermediate swimming performance of hybrids between the two species that have clear relevance to differences in migration distance (Hawkins & Quinn 1996). This situation contrasts with that in interior RBT and WCT whose migratory life histories are more similar to one another (both make relatively short migrations within tributaries and between tributaries and mainstem rivers). In addition, we observed more inferred backcross and post-F_1 hybrids than we did F_1 hybrids, suggesting that introgression is not as limited as in the case of the coastal trout. Finally, there are hints of reduced performance of hybrids under some conditions (Leary *et al*. 1995), and the direction and strength of selection probably varies depending on the genetics of particular populations as well as with the ecological (e.g. lake vs. stream environments), environmental (e.g. interannual differences in thermal regimes) and associated life-history differences (riverine vs. river–lake migratory populations). For example, both the extent and phenotypic influences of hybridization between Galapagos finches (*Geospiza* spp.) can fluctuate interannually depending on environmental conditions (Grant & Grant 2002). The body of work completed to date on hybridization between WCT and RBT has focused largely on genetic surveys or laboratory studies. Much more work examining the ecological distribution and performance of genotypes in trout hybrid zones in nature is needed to improve our understanding of the factors influencing the interactions between these species. At this point, however, it appears that selection acting to constrain introgression between native WCT and exotic RBT may be very weak (and in fact may favour introgression) in the upper Kootenay River system. The ultimate fate of native WCT gene pools in terms of extent of introgresson with RBT is therefore probably more a function of ecological and demographic factors such as the number and locations of introductions and, barriers to, and rates of, dispersal of introduced RBT.

Asymmetrical introgression

Although the static cohort analysis did not reveal any selection against hybrids, we found evidence of differential introgression between loci that may result from selection at the locus level. Homozygous RBT genotypes were approximately 17 times more frequent at the heat shock (Hsc 71) locus than at the other three loci. By contrast, heterozygote genotypes were approximately 2.5 times less frequent at the Occ16 locus than at the other three loci. These results suggest that selection is acting differentially on these loci. Differential introgression between nuclear loci has been detected in other salmonids. Redenbach & Taylor (2003) reported that bull trout (*Salevlinus confluentus*) growth hormone 2 introgressed significantly more into Dolly Varden (*Salvelinus malma*) than the other three loci used to detect hybrids in a number of areas where the two species meet and hybridize. Similar observations of interlocus variation in introgression have been suggested to be the result of differential selection in other taxa (e.g. Dowling & Moore 1985; Shoemaker *et al*. 1996; Poteaux *et al*. 1998; Martinsen *et al*. 2001). The mechanism(s) of differential introgression, however, is typically unknown and deserves further study.

Interestingly, we found no evidence of the biased mtDNA introgression that has been reported in several other salmonid hybrid systems (see Taylor 2004). The apparently unbiased mtDNA introgression between WCT and RBT is consistent with their similar sizes at maturity and the idea that biased mtDNA introgression is often driven by interspecific differences in body size and its effects on spawning behaviour and size-assortative mating (see Wirtz 1999; Taylor 2004).

Conservation implications

Although RBT have been introduced in various lakes in the upper Kootenay River drainage for at least 80 years (British Columbia stocking records, unpublished data) it appears that continued and expanded introductions in recent years have greatly enhanced WCT × RBT hybridization in this drainage. Hybridization appears to be spreading upstream from Koocanusa Reservoir (Rubidge *et al*. 2001; Rubidge & Taylor in press), and our results suggest that populations in close proximity to the reservoir (Lodgepole Creek and lower Gold Creek) in the absence of selection against hybrids are at greatest risk of genomic extinction. Although the RBT stocking programme into Koocanusa Reservoir in British Columbia was cancelled because of the hybridization issue, the threat of hybridization remains because RBT stocking continues into the reservoir in the USA (B. Westover MWLAP, personal communication 2003). For instance, there is strong evidence that introduced RBT have established a feral population in the lower Bull River (Rubidge, Baxter & Taylor unpublished data), which can serve as a source of pure RBT into surrounding tributaries. In addition, evidence suggests that the spread of RBT is facilitated through hybrid straying (Hitt *et al*. 2003; Rubidge & Taylor in press), which further complicates the protection of pure WCT populations.

There is little that can be done to remove the RBT alleles from the populations in this study that have experienced less than 10% introgression and that do not deviate from the measured population genetic parameters that suggest mating is random. By contrast, in situations where a hybrid swarm has not formed and pure parental types are still present, a species removal programme may be effective in removing RBT alleles before they can introgress into the WCT genome. For example, in lower Gold Creek where the genotypic structure is bimodal, and the heterozygote deficiency and linkage disequilibria are relatively high, removing pure RBT and hybrid individuals may be successful in slowing or preventing further hybridization. It will also aid in preventing hybrids produced at this locality from straying to neighbouring sites (e.g. upstream to Bloom Creek). Consequently, our results suggest that in the absence of management intervention, hybrid swarm formation and local extinctions of pure WCT populations are likely in at least two tributaries of the upper Kootenay River (lower Gold Creek and Lodgepole Creek) and possibly more (Bloom Creek and Michel Creek). The remaining populations with less than 10% RBT introgression are at threat of accumulating more RBT alleles via hybrids straying from nearby hybridized populations and from continued RBT stocking in high-elevation 'landlocked' lakes in this drainage.

Conclusions

Our study has documented the frequency distribution of genotypes in hybridizing populations of introduced RBT and native WCT. The general lack of bimodality in these frequency distributions is consistent with predictions about the relative importance of pre- and postmating processes in hybrid zone structure (Jiggins & Mallet 2000) as well as with our general understanding of the ecology and genetics of interactions between these two species of western trout. Our data have also provided an independent assessment of the strength of selection and we found no evidence of strong selection against hybrids in this system. Our data also provide some general guidelines concerning the sampling regime in terms of sample numbers and length of study time (both need to be increased) when using cohort analysis to detect what is probably weak selection acting on RBT × WCT hybrid genotypes. Variability among localities in the form of the genotypic frequency distributions also provided insight into the dynamic nature of hybrid zones in riverine environments. The proximity to populations that are the source of non-native alleles, the time course of invasions of non-native alleles, and local environmental conditions probably have considerable influence on the form and fate of hybrid zones in different localities even within a relatively small geographical area.

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This research was part of Emily Rubidge's MSc thesis work at the University of British Columbia. Emily is interested in investigating the effects of human activities on the genetic structure of natural populations. Eric Taylor has strong interests in conservation of native fishes and employs molecular and ecological methods in studies of the origins and persistence of biodiversity.