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Parallel introgression and selection on introduced alleles in a native species

Rachael A. Bay[1] | Eric B. Taylor[2] | Dolph Schluter[2]

[1]Department of Evolution and Ecology, University of California, Davis, Davis, California

[2]Department of Zoology and Biodiversity Research Centre, University of British Columbia, Vancouver, British Columbia, Canada

Correspondence
Rachael A. Bay, Department of Evolution and Ecology, University of California Davis, Davis, CA.
Email: rbay@ucdavis.edu

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Abstract

As humans cause the redistribution of species ranges, hybridization between previously allopatric species is on the rise. Such hybridization can have complex effects on overall fitness of native species as new allelic combinations are tested. Widespread species introductions provide a unique opportunity to study selection on introgressed alleles in independent, replicated populations. We examined selection on alleles that repeatedly introgressed from introduced rainbow trout (*Oncorhynchus mykiss*) into native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) populations in western Canada. We found that the degree of introgression of individual single nucleotide polymorphisms from the invasive species into the native is correlated between independent watersheds. A number of rainbow trout alleles have repeatedly swept to high frequency in native populations, suggesting parallel adaptive advantages. Using simulations, we estimated large selection coefficients up to 0.05 favoring several rainbow trout alleles in the native background. Although previous studies have found reduced hybrid fitness and genome-wide resistance to introgression in westslope cutthroat trout, our results suggest that some introduced genomic regions are strongly favored by selection. Our study demonstrates the utility of replicated introductions as case studies for understanding parallel adaptation and the interactions between selection and introgression across the genome. We suggest that understanding this variation, including consideration of beneficial alleles, can inform management strategies for hybridizing species.

KEYWORDS
adaptation, contemporary evolution, ecological genetics, genomics/proteomics, hybridization

1 | INTRODUCTION

Hybridization and introgression play an undeniable role in evolution. Although traditionally thought of as the breakdown of reproductive isolation (Mayr, 1942), we now know that hybridization is common in natural populations, potentially occurring in >10% of animal species (Mallet, 2005). Hybridization can lead to reinforcement through the evolution of prezygotic isolation (Kronforst, Young, & Gilbert, 2007). On the other hand, hybridization can also lead to new, potentially adaptive combinations of genotypes (Grant & Grant, 2016; Grant, Grant, Markert, Keller, & Petren, 2004). This access to an extended gene pool from which to produce novel phenotypes could be particularly advantageous under rapidly changing environmental conditions (Hamilton & Miller, 2016).

Humans have increased the frequency of hybridization in the wild, largely by bringing formerly allopatric species into contact. This is common in species that are stocked for game or have accidental introductions from domestic populations. Hybridization through

introduction has occurred across much of the tree of life, from mammals (Goodman, Barton, Swanson, Abernethy, & Pemberton, 1999) to birds (Barilani et al., 2005; Blanco Aguiar et al., 2008) to plants (Bleeker & Hurka, 2001). Occasionally, humans can facilitate hybridization by altering the ecological landscape in ways that either erode signals used for mate selection (Seehausen, Van Alphen, & Witte, 1997) or form new habitat that supports species coexistence (Bleeker & Hurka, 2001). For example, in two Brassicaceae species in the genus *Rorippa* in Scotland, the creation of drainage ditches provided a novel environment allowing persistence of a hybrid zone between otherwise ecologically segregated species (Bleeker & Hurka, 2001). Even in cases of introductions, human alteration of the environment can increase hybridization levels (Harbicht, Alshamlih, Wilson, & Fraser, 2014). In the case of the endangered California Salamander (*Ambystoma californiense*), for example, populations inhabiting artificial ponds have higher levels of hybridization with introduced congeners than those in natural vernal pools (Riley, Bradley Shaffer, Randal Voss, & Fitzpatrick, 2003).

The outcome of anthropogenic hybridization is complex (Arnold & Hodges, 1995), depending on the interactions of each genome of the parental species with the environment, as well as interactions between the genomes themselves. If hybrids are much lower in fitness than either parental species, hybridization could result in the decline or extinction of one species. This could happen quite rapidly; simulations based on annual plants show that extinction due to hybridization is possible in fewer than five generations (Wolf, Takebayashi, & Rieseberg, 2001). However, hybrids can often be more fit than parental species (Clayton & Price, 1994; Qian, Liu, & Meng, 2003). Some of this complexity arises because if hybridization persists into advanced generations recombination will decouple different portions of the genome and natural selection will result in varying levels of introgression (Schumer et al., 2018; Toews et al., 2016; Turissini & Matute, 2017). In California Tiger Salamanders, for example, while the majority of alleles introgressed from Barred Tiger Salamanders (*Ambystoma tigrinum mavortium*) have not spread outside the introduced range, a few "super invasive" alleles have spread much more quickly, likely facilitated by selection on these genomic regions (Fitzpatrick et al., 2010).

There are several established cases where introgression between species has introduced variants with major adaptive advantages. For example, an allele associated with warfarin resistance in house mice (*Mus musculus domesticus*) was originally acquired through hybridization with Algerian mice (*Mus spretus*) (Song et al., 2011). Similarly, a chromosomal segment involved in insecticide resistance has been transferred between African *Anopheles* species and quickly rose in frequency as insecticides became more common in the region (Norris et al., 2015). In part because of conflicting selective forces, conservation of hybridizing species has been a topic of much debate (Allendorf, Leary, Spruell, & Wenburg, 2001; Hamilton & Miller, 2016). In fact, some argue for a "gene-centric" rather than "species-centric" view of introgression precisely because different introduced genes will have different overall impacts on fitness (Crispo, Moore, Lee-Yaw, Gray, & Haller, 2011).

Species introduced at multiple locations provide a specific opportunity to test whether alleles are adaptive across independent populations. The general question of evolutionary repeatability has been a longstanding question in evolutionary biology (Bolnick, Barrett, Oke, Rennison, & Stuart, 2018). Well studied examples like divergence of *Anolis* ecomorphs across Caribbean islands (Losos, 1998) or repeated divergence of sympatric stickleback species (Boughman, Rundle, & Schluter, 2005; Schluter, Clifford, Nemethy, & McKinnon, 2004) suggest that similar ecological contexts promote parallel phenotypic evolution. Laboratory experiments show mixed results, as replicate populations in identical conditions do not always accumulate the same mutations (Woods, Schneider, Winkworth, Riley, & Lenski, 2006). Still, we do see examples in wild populations in which the same genetic variants are repeatedly used during parallel phenotypic divergence, such as the *Eda* allele associated with low plate number in stickleback that have colonized freshwater environments (Colosimo, 2005). Introgression between introduced and native species provides a natural repeated experiment with which to test whether putatively adaptive alleles have repeated effects across multiple populations.

Hybridization between introduced rainbow trout (*Oncorhynchus mykiss*) and native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) has been studied extensively as a system in which introgression can have dramatic conservation implications. Rainbow trout and westslope cutthroat trout are largely allopatric (Penaluna et al., 2016), but rainbow trout have been stocked in lakes and streams across much of the range of westslope cutthroat trout since the 19th century. Since then the species have undergone such extensive hybridization that managers are concerned about loss of the native westslope cutthroat trout due to "genomic extinction" (Allendorf & Leary, 1988). Indeed, native westslope cutthroat trout numbers have experienced declines over the last century (COSEWIC, 2016; Mayhood & Taylor, 2011; Weigel, Peterson, & Spruell, 2003). Although hybrids have lower fitness in the wild than the parental species (Muhlfeld et al., 2009), levels of hybridization vary with environmental factors such as water temperature, elevation, and stream size (Weigel et al., 2003; Yau & Taylor, 2013), potentially suggesting interactions between the introgressed genomes and the abiotic environment. Additionally, increases in hybridization in recent years have been linked to climate change-driven environmental shifts (Muhlfeld et al., 2014). For these reasons, it is important to understand the potential for different genomic regions and individual loci to impact hybrid fitness. Here, we use genome-wide markers to test for rainbow trout alleles under positive selection in hybridizing native populations of westslope cutthroat trout. We focus on identifying alleles that show repeated signals of adaptation across multiple independent watersheds, using two newly sequenced populations from British Columbia (BC) and Alberta (AB), Canada, along with six populations from a previous study. The goal of the study is to help resolve the genomic consequences of hybridization, more specifically: (a) to examine whether levels of introgression at the same loci are repeated across independent introductions; (b) to identify introduced alleles that consistently show signals of adaptation; and (c) to estimate levels of selection that explain observed patterns of introgression. Our results, paired with knowledge from previous studies, can help inform management of populations experiencing human-induced hybridization.

TABLE 1 Samples used for introgression analysis

Name	Ancestry	Province	Year	*N*	Reference
Lower Bull River	RBT	BC	2000	13	Rubidge and Taylor (2005)
Gold Creek	Admixed	BC	2000	15	Rubidge and Taylor (2005)
Upper Bull River	WCT	BC	1999	15	Rubidge and Taylor (2005)
Mount Lassen	RBT	AB	2000	15	Taylor et al. (2007)
Sullivan Creek	Admixed	AB	2007	15	Yau and Taylor (2013)
J-H7a	WCT	AB	2007	15	Yau and Taylor (2013)

Note: Ancestry was inferred as rainbow trout (RBT), westslope cutthroat trout (WCT) or admixed based on previous microsatellite studies. Sample size (*N*) represents the number of samples remaining after removing those with low sequencing coverage.

2 | MATERIALS AND METHODS

2.1 | Study populations

We used DNA extracted for previous studies (Table 1; Figure 1) (Rubidge & Taylor, 2005; Taylor, Tamkee, Sterling, & Hughson, 2007; Yau & Taylor, 2013). All DNA was extracted from tissue using standard protocols, but extraction methods did vary by study; Yau and Taylor (2013) used phenol-chloroform extraction and Rubidge and Taylor (2005) used PUREGENE extraction kits. We used DNA for 90 samples from six locations (15 randomly

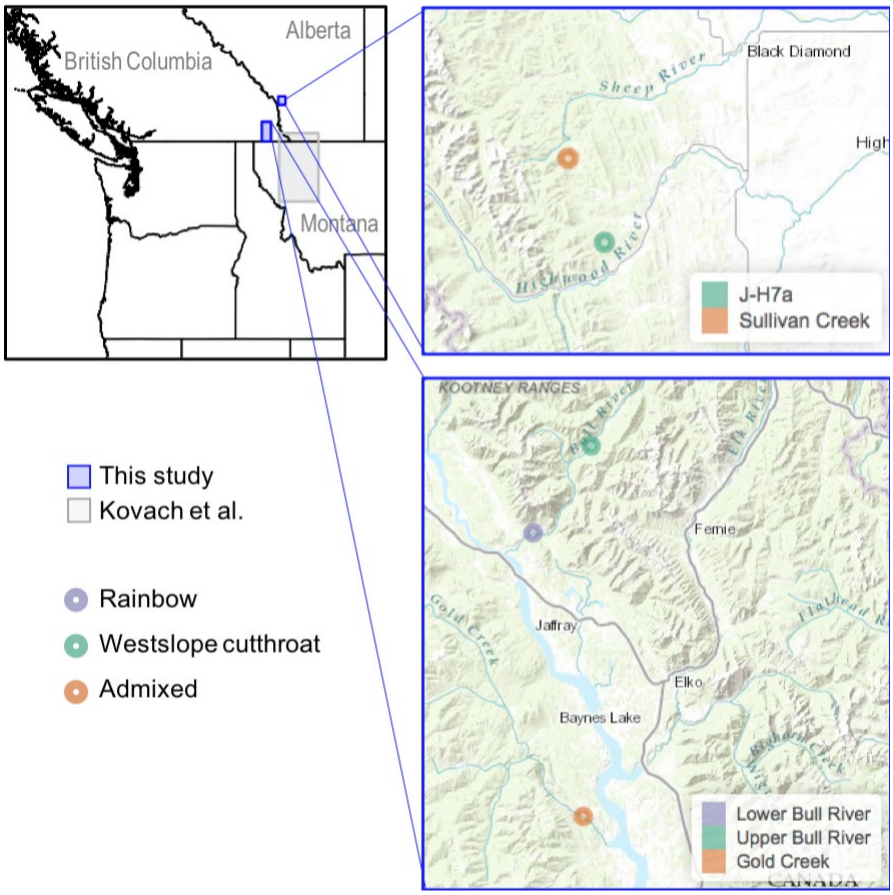

FIGURE 1 Locations of populations from western Canada and the USA used in this study. Top left shows study regions, with blue rectangles indicating populations from Alberta (upper) and British Columbia (lower), Canada, sequenced in this study and gray rectangle indicating the study region covered by Kovach et al. (2016). Expanded maps show specific locations of sampling sites. Individuals from the Mount Lassen stock were also sequenced, although not represented in this figure

selected samples from each location): two admixed, two westslope cutthroat trout, and two rainbow trout. Two locations (Gold Creek, BC and Sullivan Creek, AB) were chosen because microsatellite studies found them to have high levels of admixture. Two locations near the admixed populations were chosen for their low levels (<1%) of rainbow trout ancestry (upper Bull River, BC and J-H7a, AB). For rainbow trout samples, we selected a location in BC (lower Bull River) found to have a naturalized population (rainbow trout ancestry = 97.1%) and samples from the Mt. Lassen stocking populations commonly used in AB. Gold Creek and the Bull River are tributaries of the upper Kootenay River in BC and upper and lower Bull River are separated from each other by a natural waterfall that is now the site of a run-of-the-river hydroelectric plant. The Kootenay River eventually drains to the Pacific Ocean via the Columbia River. Sullivan Creek is a tributary of the Highwood River, Bow River drainage in southwestern Alberta, which flows east of the Continental Divide as part of the Hudson Bay drainage. The sample "JH7a" is from an unnamed creek tributary to Flat Creek, also a tributary of the Highwood River.

2.2 | GBS sequencing

DNA was cleaned with the Zymo Genomic DNA Clean and Concentrator Kit. GBS libraries were constructed following the protocol of Elshire et al. (2011) with the restriction enzyme *PstI*. All individuals were barcoded separately and pooled into a single library. Libraries were sequenced at Genome Québec in a single 125 bp paired-end lane on an Illumina HiSeq v4 platform.

Quality filtering and demultiplexing of raw sequencing reads was conducted using the process_radtags.pl script, part of the STACKS pipeline (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). Adapter sequences were removed and the sliding window method implemented in STACKS was used to discard sequences with low quality (-s 20). Only read pairs in which both reads passed the quality filters were retained for downstream analysis. Filtered reads from each sample were mapped to the Atlantic salmon (*Salmo salar*) reference genome (ICSASG_v2) (Davidson et al., 2010) using stampy (Lunter & Goodson, 2011). In addition to the Atlantic salmon genome being a higher quality reference, the similarity in divergence of both trout species from the Atlantic salmon reference (Kitano, Matsuoka, & Saitou, 1997) could potentially reduce mapping bias that could occur using the rainbow trout genome. Single nucleotide polymorphisms (SNPs) were identified using ANGSD (Korneliussen, Albrechtsen, & Nielsen, 2014) with initial parameters: minor allele frequency >0.01, SNP *p*-value <1e-6, base quality *Q* >30, minimum individuals >18. This individual coverage filter was used only for initial SNP identification and more stringent per population requirements were used for analysis of introgression (see below).

2.3 | Population structure

We conducted principal components analysis of SNP genotypes using PCAngsd (Meisner & Albrechtsen, 2018), and used covariance matrices to calculate eigenvectors and generate PCA plots in R version 3.5.0 (R Core Team, 2014). Additionally, we used NGSadmix (Skotte, Korneliussen, & Albrechtsen, 2013) to estimate ancestry proportions for each individual. We ran a range of *K* values (1–6) with 20 replicate runs each and determined the optimal value using the Evanno method as implemented in CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015). We also used CLUMPAK to combine runs and identify alternative solutions for each K value.

We next identified SNPs fixed between westslope cutthroat and rainbow trout species, first removing individuals from "pure" populations that had admixed ancestry in our admixture analysis. After removing these individuals, we used genotype likelihoods to estimate allele frequencies for each admixed population (Sullivan Creek, AB and Gold Creek, BC) as well as each parental species using ANGSD. Measuring allele frequencies directly from genotype likelihoods has recently been shown to lead to more accurate inference than first estimating individual genotypes (Warmuth & Ellegren, 2019). From these allele frequency estimates, we identified SNPs that were fixed between species (estimated alternate allele frequency <0.0001) and had data for at least 10 individuals from each admixed population and at least 10 individuals from each "pure" species.

We then investigated the distributions of allele frequencies of these diagnostic SNPs within the two admixed populations. To examine the degree of parallel introgression between these two populations, we first tested correlation of the frequencies of rainbow trout alleles using a standard linear model in R. To determine whether this correlation was driven by alleles of high or low introgression, we binned SNPs from each admixed population into five even bins (20% of SNPs each) based on quantile scores. We then tested for overlap between the bins among admixed populations and compared with a null expectation based on randomizing the data 1,000 times.

The SNPs in which rainbow trout allele frequency was in the top 5% tail for both admixed populations were considered candidates for positive selection on rainbow trout alleles. Based on the Atlantic salmon reference genome, we identified genes within 25 kb of these candidate SNPs. We used the R package topGO (Alexa & Rahnenfuhrer, 2010) to test for enrichment of particular gene ontology categories. Specifically, we used Fisher's exact test to identify biological process categories in which more genes were in our candidates list than expected based on a background list of all genes near SNPs in our data set, using a false discovery rate cutoff of <0.01 and a minimum category size of five genes.

2.4 | Simulations

To estimate levels of introgression and selection pressure compatible with observed allele frequencies, we used simulations based on a Wright-Fisher model. The simulation was based on a single population of westslope cutthroat trout and selection coefficient for the rainbow trout allele (*s*) which was introduced at a given level of introgression (*i*), by increasing the allele frequency at the end of each generation. Initially, we ran a neutral model (*s* = 0) varying population

size from 100 to 10,000 and the level of introgression from 0% to 1% of the population size per generation, running 1,000 replicates for each parameter set for 50 generations each (~2 years per generation for the last century). To estimate *i* for each admixed population, we compared the empirical mean rainbow trout allele frequency to the means of simulated neutral distributions under different levels of introgression. Once we estimated *i* for each population, we ran additional replicates (100,000) for *N* = 100, 500, and 1,000 to provide higher resolution on the upper tail of the distribution for comparison with the empirical distribution.

Because we found that at *N* > 1,000 population size did not impact allele frequency distribution (Figure S1) we used a population size of *N* = 1,000 in the remaining simulations. Next, we varied both selection (*s* = 0–0.2) and introgression (i= 0%–1%), again running 1,000 replicates per condition and 50 generations. These simulations were intended to determine the level of selection necessary to produce the highest frequency rainbow trout alleles observed in our admixed populations—these are candidates for adaptive introgression. We employed the conservative assumption that highly introgressed alleles were within the upper tail of rainbow trout allele frequencies under a given simulated selection regime. We therefore estimated the 95th quantile as well as maximum rainbow trout allele frequency in each simulated condition and compared that to the maximum rainbow trout allele frequency in the empirical data.

2.5 | Comparison with previous genomic data

Kovach et al. (2016) examined introgression in populations across several watersheds in Montana and BC (Figure 1). To examine the generality of our findings, we asked whether we observed parallel signatures of introgression across the 21 populations in this data set. We downloaded genotypes at 9,380 species diagnostic SNPs for 339 individuals from 21 populations from DRYAD (Kovach et al., 2016). For each population, we calculated the frequency of the rainbow trout allele at each SNP. Because we are interested in understanding selection in admixed populations, we kept only six populations with moderate levels of admixture (mean rainbow trout allele frequency 5%–95%). For all pairwise combinations of the remaining populations, we calculated the number of shared "highly introgressed SNPs", or those in the top 5% tail in both populations. To generate a null distribution, we also randomized SNPs 100 times per pairwise comparison.

3 | RESULTS

3.1 | Distributions of introduced alleles

We generated 544,232,344 pairs of 125 bp reads, 88% of which were retained after filtering for barcodes, adapters, and quality. After discarding two individuals, both from the lower Bull River population, we identified 93,488 SNPs which could be used for population structure and admixture analysis. Principal components analysis revealed structuring by both species and geography (Figure 2). The first principal components axis (PC1 – 30.8% of variance) clearly separated westslope cutthroat from rainbow trout, with hybrids at intermediate values. PC2 (8.0% of variance) distinguished the Mount Lassen rainbow trout population used for stocking in AB from the naturalized rainbow trout population at lower Bull River in BC. PC3 (4.9% of variance) separated BC populations, both pure westslope cutthroat trout and admixed, from AB populations. In our Bayesian hierarchical clustering analysis using NGSadmix, these data were best described by two groups (*K* = 2; Table S1) separating "pure" westslope cutthroat from rainbow trout, with admixed populations showing ancestry from both groups. Both naturalized and stocking

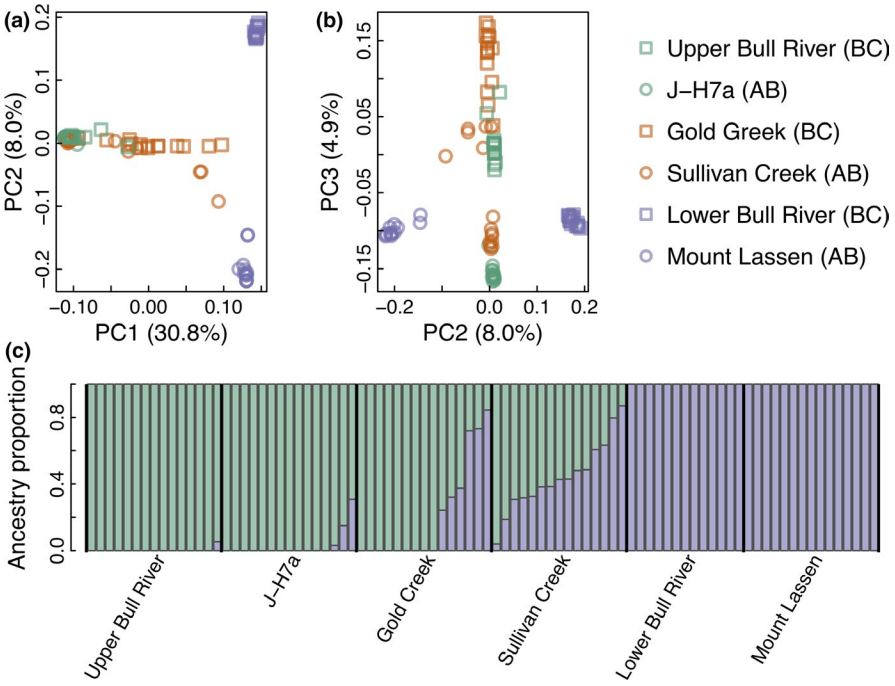

FIGURE 2 Population structure in rainbow and westslope cutthroat trout samples from British Columbia (BC) and Alberta (AB). Top panel (a, b) shows principal components analysis, with squares representing samples from BC and circles representing samples from AB. Purple represents rainbow trout in both principal components (PC) and lower ancestry plots (c) and green represents westslope cutthroat trout. In the PC plot, admixed populations are shown in orange

populations of rainbow trout showed 100% ancestry from a single cluster. Populations representing pure westslope cutthroat trout had very little rainbow trout ancestry (0.3% and 3.2%). The contribution of rainbow trout was entirely driven by four individuals having mixed ancestry. These individuals were removed from downstream analysis. Populations previously identified as admixed showed mixed ancestry in NGSadmix analysis, with individuals ranging from 0% to 84% rainbow trout ancestry (population means: Gold Creek [BC] = 21.5%, Sullivan Creek [AB] = 44.5%).

We found significant parallelism in patterns of SNP introgression by replicate invasions of rainbow trout, especially in those SNPs at the frequency extremes. We identified 3,552 high quality SNPs with fixed differences between the two parental species, combining populations from BC and AB. In the Gold Creek (BC) admixed population, mean rainbow trout allele frequency was 0.23, whereas Sullivan Creek (AB) had even higher levels of rainbow trout ancestry (mean allele frequency = 0.44). Overall frequencies of individual rainbow trout alleles were correlated between the two admixed westslope trout populations (r = 0.21; p < 0.001; Figure 3a). This was driven by higher than expected sharing of high and low frequency rainbow trout alleles in the two populations (Figure 3b): 282 rainbow trout SNPs occurred at low frequency (0%–20%) in both admixed populations compared to 98–164 SNPs expected by chance (mean = 128.4). Another 263 SNPs occurred at high frequency in both populations (80%–100%), compared to 114–172 SNPs expected by chance (mean = 142.7).

The same 59 SNPs were extremely introgressed (top 5%) in both admixed populations from AB and BC, indicating a steep parallel rise to high frequency of these rainbow trout alleles. These SNPs were spread across the genome, located on 23 different chromosomes. Analysis of genes within 25kb of these SNPs showed enrichment for several GO categories related to response and transport of foreign compounds, including potential toxins (Table 2). Many of these categories were driven by candidate SNPs near genes encoding five different ATP binding cassette (ABC) transporter proteins. These proteins transport compounds across membranes and have been shown to be involved in xenobiotic resistance and, in humans, drug resistance (Sarkadi, Özvegy-Laczka, Német, & Váradi, 2004; Sharom, 2008; Sturm & Segner, 2005). All genes near SNPs that are highly introgressed in both populations are listed in Table S2.

To test the generality of these patterns, we also reanalyzed the data from a previously published set of rainbow trout diagnostic SNPs across six admixed populations of with moderate levels of introgression (5%–95% rainbow trout ancestry based on mean rainbow trout allele frequency across 9,380 diagnostic SNPs): Belmont Creek, Blanchard Creek, Cyclone Creek, Dutch Creek, Elk Creek, and Finley Creek (Kovach et al., 2016). We focused exclusively on the parallel signatures, because they indicate putatively adaptive introgression. In this data set, we found that population pairs shared more high frequency rainbow trout alleles than expected based on a null distribution generated by randomizations: 22 of 30 pairwise comparisons (73.3%) had more shared SNPs with high rainbow trout allele frequency (top 5%) than 95% of randomized comparisons (Figure 4). This pattern held for comparisons both within and between watersheds; 14/22 comparisons between watersheds and 8/8 comparisons within the same watershed showed higher than expected concordance. In addition to these pairwise comparisons, we identified multiple SNPs that were highly introgressed in more than two populations. We see parallel signatures of highly introgressed rainbow trout alleles across three populations at 193 SNPs,

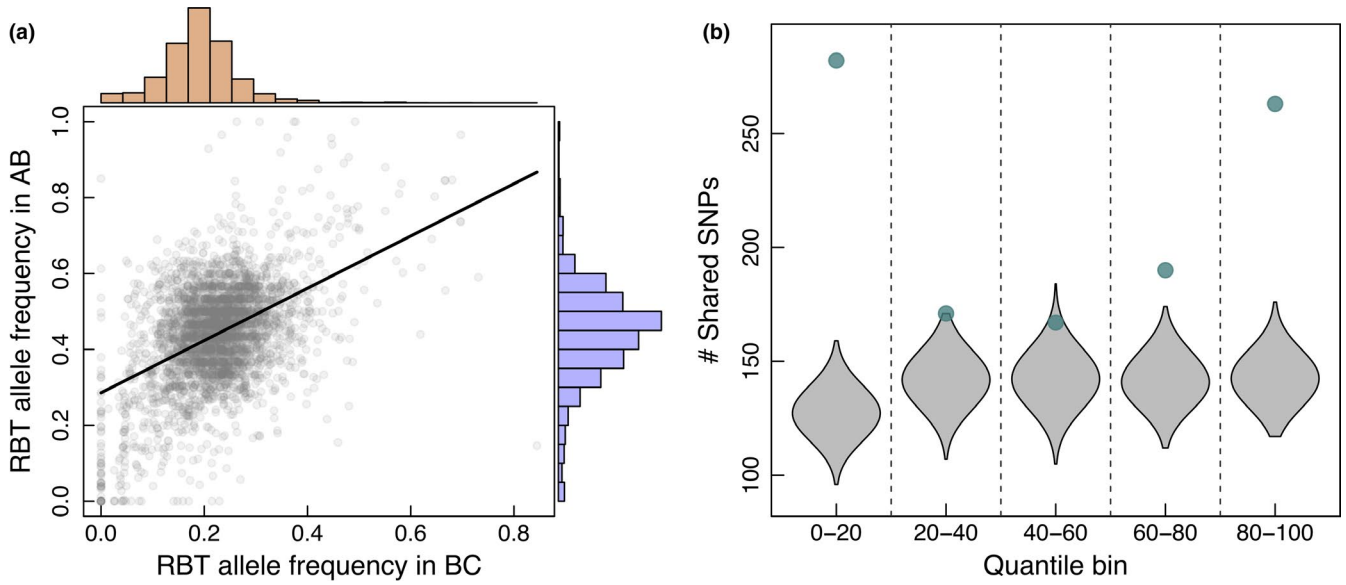

FIGURE 3 Correlation of allele frequencies in two admixed populations for 3,552 single nucleotide polymorphisms (SNPs) that were fixed between "pure" rainbow (RBT) and westslope cutthroat trout populations. (a) Linear association across all SNPs. Histograms show the distribution of allele frequencies for the admixed populations from British Columbia (BC: top) and Alberta (AB: right). (b) Shared SNP occurrence within rainbow trout allele frequency quantile bins between BC and AB admixed populations. Violin plots show null distribution created using 1,000 randomizations and points are actual values [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Biological process Gene Ontology (GO) terms enriched ($p < 0.01$) for single nucleotide polymorphisms in which rainbow trout allele frequency is high (>95th quantile) in both admixed populations

GO.ID	Term	Annotated	Significant	*p*-value
GO:0046685	Response to arsenic-containing substance	6	3	0.00016
GO:0001885	Endothelial cell development	7	3	0.00028
GO:0046618	Drug export	2	2	0.00043
GO:0009713	Catechol-containing compound biosynthetic process	8	3	0.00044
GO:0042423	Catecholamine biosynthetic process	8	3	0.00044
GO:0033059	Cellular pigmentation	10	3	0.00092
GO:0046189	Phenol-containing compound biosynthetic process	10	3	0.00092
GO:0008345	Larval locomotory behaviour	3	2	0.00126
GO:0072089	Stem cell proliferation	42	5	0.00153
GO:0015893	Drug transport	46	5	0.00232
GO:0006950	Response to stress	768	26	0.00253
GO:0006584	Catecholamine metabolic process	14	3	0.00264
GO:0007595	Lactation	14	3	0.00264
GO:0009712	Catechol-containing compound metabolic process	14	3	0.00264
GO:0030537	Larval behaviour	5	2	0.00409
GO:0042421	Norepinephrine biosynthetic process	5	2	0.00409
GO:0055085	Transmembrane transport	252	12	0.00442
GO:0006706	Steroid catabolic process	6	2	0.00606
GO:0042415	Norepinephrine metabolic process	6	2	0.00606
GO:0042493	Response to drug	229	11	0.00615
GO:0016042	Lipid catabolic process	38	4	0.00733
GO:0048149	Behavioural response to ethanol	7	2	0.00837
GO:0071158	Positive regulation of cell cycle arrest	22	3	0.00994

across four populations at 55 SNPs, and across five populations at seven SNPs. Although we did not find SNPs in which the rainbow trout allele was highly introgressed across all six populations, in the seven SNPs where we saw parallel signatures across five populations, the same population was counter to the pattern in all seven cases. Of these seven SNPs, five are on chromosome 4 (although three are on unlocalized scaffolds within that chromosome) of the rainbow trout genome assembly and the remaining two are on unplaced scaffolds.

3.2 | Levels of introgression and selection

We used simulations to estimate strength of selection on adaptive alleles by comparing them to the background of neutral introgression rates for each admixed population separately. For the Gold Creek population, a simulated neutral introgression rate of $i = 0.0045$ best fit the distribution of rainbow trout allele frequencies, with a mean frequency of 0.222 (empirical mean = 0.224). The width of this distribution, however, was narrower than the empirical distribution, depending on the population size used in simulations (Figure 5). For Sullivan Creek, we estimated an introgression rate of $i = 0.0089$ (simulated mean = 0.440; empirical mean = 0.440). Although we simulated population size ranging from 100 to 10,000 individuals, the simulated population size did not impact the mean rainbow trout allele frequency and therefore inferred stocking rate (Figure S1).

With a simulated population size of $N = 1{,}000$ in Gold Creek, rainbow trout alleles at nine SNPs had frequencies higher than expected under neutral simulations. At $N = 500$, there was one such SNP. Under these simulations, the maximum allele frequency for a neutral rainbow trout allele was 0.66 ($N = 1{,}000$) or 0.76 ($N = 500$). The maximum allele frequency observed in this population was 0.84. In Sullivan Creek, we observed 22 SNPs having higher rainbow trout allele frequency than expected from the

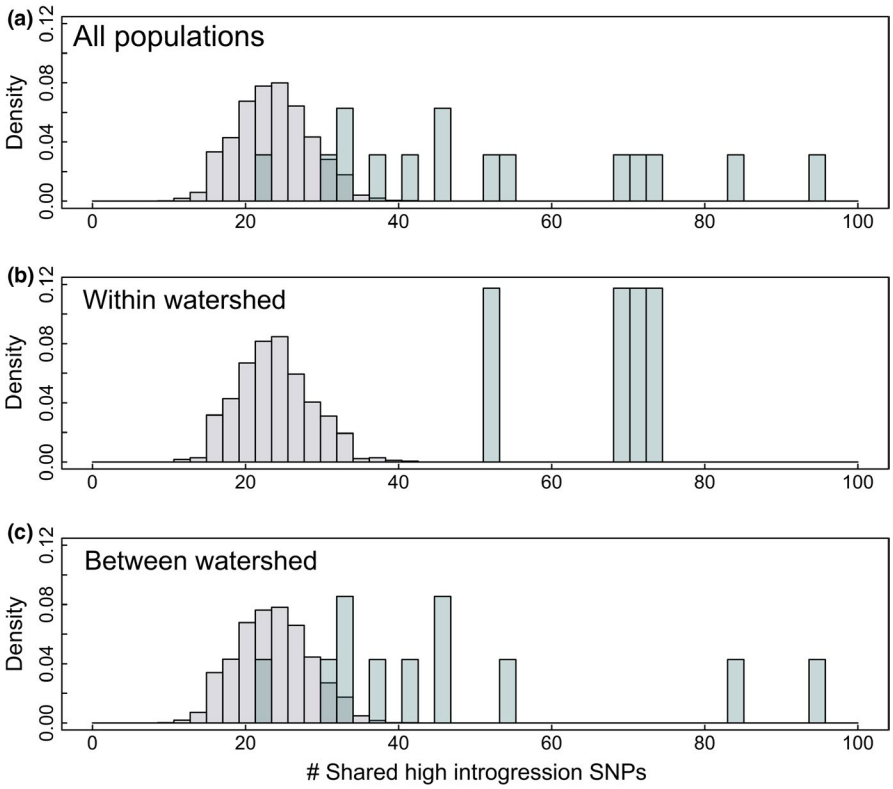

FIGURE 4 Pairwise comparison of highly introgressing alleles in six admixed populations genotyped by Kovach et al. (2016). Histograms show the number of overlapping high introgression Single nucleotide polymorphisms (SNPs; within the top 5% for each population) for each combination of two populations. Green bars are actual pairwise comparisons and grey bars represent a null distribution created by randomizing SNPs 100 times per pairwise comparison. Here we show overlap in (a) all pairwise comparisons (n = 30) as well as (b) comparisons within the same watershed (n = 8) and (c) comparisons between populations in different watersheds (n = 22)

simulated N = 1,000 distribution, and five SNPs with excess frequencies compared with the N = 500 distribution. We found fixed rainbow trout alleles at five SNPs, but the maximum simulated neutral allele frequencies were considerable higher than in Gold Creek (0.832 under N = 1,000 and 0.976 under N = 500). Under N = 100, rainbow trout allele frequencies span the full range of possible values under neutrality (0–1), but the neutral allele frequency distributions do not provide a good fit to the observed distributions (Figure 5).

What levels of selection are required to explain introgression patterns in admixed populations? We used simulations to vary the level of selection on rainbow trout alleles. A conservative approach to estimating the selection coefficient is to compare allele frequencies for introgression outliers to the upper tail of the distribution simulated under a selection model rather than to the mean. We therefore compare both the 95th quantile and maximum rainbow trout allele frequency at different levels of simulated selection with the observed maximum rainbow trout allele frequencies in our two admixed populations. For each location, we therefore calculate two selection coefficients based on whether we compare maximum observed frequencies to the 95th quantile or the simulated maximum (Figure S2). At the level of introgression estimated in Gold Creek, a selection coefficient of s = 0.05 (95th quantile) or s = 0.01 (max) is required to produce the maximum rainbow trout allele frequencies observed in that population. In Sullivan Creek, we estimate a selection coefficient of s = 0.03 (95th quantile) or 0.006 (max) to produce fixed rainbow trout alleles.

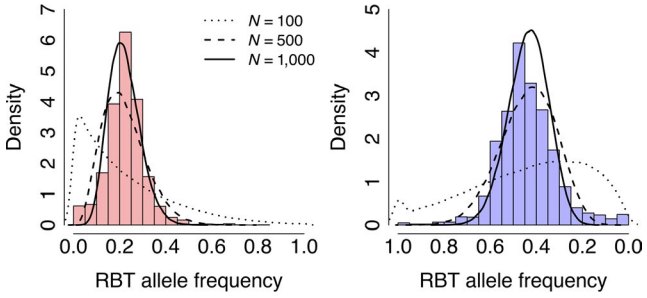

FIGURE 5 Distribution of observed rainbow trout (RBT) allele frequencies in British Columbia (left) and Alberta (right) admixed populations compared to allele frequencies simulated under a neutral model

4 | DISCUSSION

Introgression between closely related species offers the opportunity for evolutionary "experiments" with new allelic combinations. In natural hybrid zones, there are examples in which introgression can be deleterious or adaptive, depending on the genomic region and the environmental conditions (Harbicht et al., 2014; Mallet, 2005). Human activity can lead to new instances of hybridization and introgression, either by altering the environment to facilitate range expansion, by disrupting sexual signals, or through introduction of non-native species (Allendorf et al., 2001; Bleeker & Hurka, 2001; Vilà, Weber, & Antonio, 2000). In an example of human-induced introgression, we found evidence for parallel, potentially adaptive introgression between native westslope cutthroat trout

and introduced rainbow trout. Specifically, the same alleles from rainbow trout repeatedly rose to high frequency in independent admixed westslope cutthroat trout populations. This pattern was apparent in both our data set of two highly admixed populations in BC and AB, as well as a previously published data set from multiple watersheds across Montana and BC.

Previously, Hohenloe et al. (2013) identified three outlier loci which they termed "super-invasive" alleles, where rainbow trout alleles had unexpectedly high frequencies in admixed populations. In a separate study, Kovach at al. (2016) used cline analysis to identify rainbow trout alleles under either positive or negative selection in admixed populations, finding a broad signal of resistance to introgression. Our study adds to these previous findings in several ways. First, we specifically focus on loci under positive selection, or those that may ultimately increase fitness in advanced generation hybrids. Second, while most admixed cutthroat trout populations have relatively low proportions of rainbow trout ancestry, we examined two populations with higher overall admixture, which are most likely to have advanced generation hybrids, allowing for recombination between rainbow trout and westslope cutthroat trout loci. Finally, while previous studies employed outlier analyses to identify loci under selection, we take a less conservative approach by identifying rainbow trout alleles that are simply at high allele frequency in more than one population, capitalizing on the independent nature of different watersheds in this system.

Kovach et al. (2016) documented widespread genomic "resistance" to introgression; the majority of rainbow trout alleles with signals for selection in their analysis were selected against in the westslope cutthroat admixed populations. This is consistent with previous findings that hybrids have overall lower fitness in nature (Muhlfeld et al., 2009). Our data too show that many rainbow trout alleles stay at low frequency in both admixed cutthroat populations (Figure 3). However, with hybridization persisting over a century or longer, there is the opportunity for recombination in advanced generation hybrids. Different genomic regions can therefore act somewhat independently so that even if most hybrids have lower fitness because most rainbow trout alleles are slightly deleterious on a westslope cutthroat trout background, a smaller number of rainbow trout alleles might in fact improve fitness. Because population structure is high in westslope cutthroat trout populations (Allendorf & Leary, 1988; Taylor, Stamford, & Baxter, 2003), independent observations of the same rainbow trout allele rising to high frequency strongly suggest that those alleles are adaptive in the admixed populations. In our data set, we found 59 SNPs distributed broadly across the genome that showed evidence of parallel introgression. These SNPs were found disproportionately often in genes involved in response to foreign compounds (Table 2). For example, five genes with high frequencies of rainbow trout alleles were annotated as ABC transporter proteins, which have known function in disease and toxin resistance (Sarkadi et al., 2004; Sturm & Segner, 2005). In humans, different polymorphisms in this family of proteins are associated with differential responses to drug therapy and disease susceptibility (Sharom, 2008). ABC transporter proteins are known to be expressed in many tissue types in rainbow trout (Fischer et al., 2011), although the effects of different polymorphisms are not known. Within the data set from Kovach et al. (2016) all of the most highly shared signals for positive selection were on chromosome 4, perhaps suggesting either a larger rainbow trout haplotype under selection, or selected SNPs residing in a region of low recombination.

We also used population genetic simulations to estimate levels of introgression and selection in admixed populations. Our estimates of selection coefficients range from quite low to high compared with other wild populations (0.006–0.05). These estimates are nevertheless contingent on a number of assumptions. First, we simulate a single population with constant levels of introgression through stocking. Gene flow of advanced generation hybrids could preferentially introduce rainbow trout alleles already at high frequencies in neighbouring populations. In addition, stocking rate is unlikely to be completely constant and more likely occurred in spurts. These larger pulses of introgression could lead to different evolutionary dynamics. Another assumption is that the mean rainbow trout allele frequency within an admixed population represents neutrality. If the majority of rainbow trout alleles are deleterious, this would cause us to underestimate the rate of introgression and bias our estimates of selection on highly introgressed alleles upward. Our estimates of selection are thus best thought of as the difference in selection coefficient at highly introgressed adaptive alleles compared to genome-wide average selection. Finally, when estimating selection coefficients, we used a population size of 1,000 individuals. In our neutral simulations, we see very little effect of population size on either mean or upper tail of the distribution of allele frequencies, except at very low populations sizes (Figure S1). It is likely that some westslope cutthroat trout populations are very small. For our two admixed populations, however, the shape of the distribution of rainbow trout allele frequencies looks much more similar to that observed by simulating N = 1,000 than N = 100. Population estimates of similarly sized water bodies in the regions are consistent with this assumption. For example, an 11 km section of upper Bull River was estimated to host 538 westslope cutthroat trout in 2006 (Baxter, 2006) and Gorge Creek, a system similar to Sullivan Creek in Alberta, had an estimated 1,197 westslope cutthroat trout (COSEWIC, 2016). If, however, these populations did have much lower population sizes, our selection coefficients would be overestimates of the actual selection pressure. Additionally, we cannot rule out the potential effect of sampling bias on estimation of allele frequencies from admixed populations.

The risk of genomic extinction through hybridization has long been a central focus of management of westslope cutthroat trout populations. Indeed, the observation of lower fitness in hybrid individuals (Muhlfeld et al., 2009), selection against rainbow trout alleles (Kovach et al., 2016), and declining populations sizes (Mayhood & Taylor, 2011; Weigel et al., 2003) highlights the need to consider the deleterious effects of hybridization when designing management practices. Our work along with previous studies, however, suggests that introgression is more complex, involving loci that are maladaptive within admixed populations, but also providing access to an

extended gene pool that contains adaptive alleles not present in westslope cutthroat trout. Especially under the current reality that many admixed populations contain advanced generation hybrids where recombination has broken up different segments of the genome, it is important to understand which rainbow trout alleles are most harmful to the native populations and which might indeed help with population persistence. This understanding could be especially beneficial under the current regime of rapid environmental change, as some evidence shows that rainbow trout and westslope cutthroat trout may have different thermal niches (Bear, McMahon, & Zale, 2011; Yau & Taylor, 2014) and hybridization has generally been increasing in concert with climate change (Muhlfeld et al., 2014). A more thorough understanding of why certain rainbow trout alleles seem to be adaptive and under what conditions we might expect them to be adaptive, as well as the overall fitness consequences of these alleles in advanced generation hybrids is needed to formulate more accurate predictions of how ongoing introgression will impact native populations now and in the future.

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DATA AVAILABILITY

Aligned reads for this study are available through NCBI SRA under BioProject ID PRJNA525868.

ORCID

Rachael A. Bay https://orcid.org/0000-0002-9516-5881

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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