

Research



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Dispersal and selection mediate hybridization between a native and invasive species

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Hybridization between native and non-native species has serious biological consequences, but our understanding of how dispersal and selection interact to influence invasive hybridization is limited. Here, we document the spread of genetic introgression between a native (*Oncorhynchus clarkii*) and invasive (*Oncorhynchus mykiss*) trout, and identify the mechanisms influencing genetic admixture. In two populations inhabiting contrasting environments, non-native admixture increased rapidly from 1984 to 2007 and was driven by surprisingly consistent processes. Individual admixture was related to two phenotypic traits associated with fitness: size at spawning and age of juvenile emigration. Fish with higher non-native admixture were larger and tended to emigrate at a younger age—relationships that are expected to confer fitness advantages to hybrid individuals. However, strong selection against non-native admixture was evident across streams and cohorts (mean selection coefficient against genotypes with non-native alleles (s) = 0.60; s.e. = 0.10). Nevertheless, hybridization was promoted in both streams by the continuous immigration of individuals with high levels of non-native admixture from other hybrid source populations. Thus, antagonistic relationships between dispersal and selection are mediating invasive hybridization between these fish, emphasizing that data on dispersal and natural selection are needed to fully understand the dynamics of introgression between native and non-native species.

1. Introduction

Species invasions have substantial ecological and evolutionary consequences that ultimately threaten species persistence, ecosystem services and biodiversity [1,2]. Novel species assemblages can result in introgressive hybridization between native and non-native species—a phenomenon that is increasing worldwide owing to species translocations, habitat modifications and global climate change [3–5]. This is concerning for biodiversity because human-mediated introgression can lead to the extinction of native genotypes [6], the disruption of local adaptations and their associated gene complexes that have evolved over thousands of generations [7], and the homogenization of the Earth's biota [5,8].

Human-induced hybridization has contributed to the decline and extinction of numerous plant and animal species [9]. Nevertheless, the ecological and evolutionary mechanisms promoting introgressive hybridization are poorly understood in natural populations, despite the importance of this information for the effective management and conservation of native biodiversity [3,10,11]. Thus, there is a growing need to better characterize how dispersal and natural selection interact to influence the spread of invasive introgression within and among native populations.

Hybridization between native and non-native species is especially common in freshwater fish owing to the widespread translocation of numerous exotic species [12]. Additionally, many fish exhibit external fertilization, behavioural similarities and genomic compatibilities—all traits that promote introgressive hybridization [13]. Rainbow trout (RBT, *Oncorhynchus mykiss*), the most widely distributed invasive salmonid worldwide [14], produce fertile offspring when crossed with cutthroat trout (*O. clarkii*), and introgression often continues until a hybrid swarm forms and native cutthroat trout genomes are lost [6]. Hybridization with RBT threatens all remaining cutthroat trout subspecies in western North America [15]; two subspecies are now extinct, five are listed as threatened under the U.S. Endangered Species Act (ESA), and one is listed under the Canadian Species at Risk Act. Westslope cutthroat trout (WCT; *O. clarkii lewisi*) is the most widely distributed subspecies of cutthroat trout, and hybridization between RBT and WCT is the primary conservation threat to this species [16]. Although hybridization is pervasive in cutthroat trout, the mechanisms promoting the spread of RBT hybridization are poorly understood in nature; this source of uncertainty has been a contentious issue for the conservation of many imperiled taxa [17] and cutthroat trout, in particular [18,19].

Previous research has shown that hybridization between RBT and WCT can spread rapidly across river networks [20,21] despite evidence from one population demonstrating that reproductive success (number of juvenile trout that survive to out-migration) rapidly declines with increasing levels of RBT admixture [22]. This paradox suggests that hybridization may spread owing to sustained dispersal of hybrid individuals with reduced fitness [23,24], or that the strength of outbreeding depression is reduced in certain populations or environments. To address this uncertainty, we expand on previous work [22] and use data from two recently invaded populations to test whether hybridization is driven by positive selection for RBT admixture and/or the continued immigration of RBT and RBT \times WCT hybrids from other source populations.

We use data spanning 24 years in two native WCT populations occupying streams with contrasting environmental characteristics to address three objectives: (i) quantify how individual RBT ancestry is related to phenotypic variation and determine whether phenotypic data accurately predict the direction of selection acting on RBT; (ii) measure direction and strength of selection acting on RBT admixture; and (iii) determine whether dispersal of highly admixed adults from other locations promotes introgression. Together, this work provides a rare and detailed assessment of the mechanisms influencing the spread of invasive genetic introgression in wild animal populations.

2. Material and methods

(a) Study populations

Our study was conducted in Langford and Cyclone creeks, two second-order tributaries of the North Fork Flathead River in Montana, USA. Langford and Cyclone creeks differ in several key abiotic attributes that may be important for hybridization dynamics [25–28]; Cyclone is larger (average stream width = 4.2 m) and relatively warm (mean summer temperature = 13.1°C; max = 18.6°C), while Langford is smaller (average stream width = 2.6 m), thermally stable and relatively cold (mean summer temperature = 9.4°C; max = 10.8°C) [25]. WCT,

RBT and hybrids in Langford and Cyclone creeks exhibit migratory life histories, although a small resident component exists. After emergence, juveniles rear in natal streams for 2 to 3 years before emigrating to the Flathead River or Flathead Lake, where they grow to maturity and return to spawn 2–4 years later in the spring.

Langford and Cyclone creeks are 40.3 km and 59.7 km, respectively, from the putative source of RBT to the North Fork Flathead River system—Abbot Creek [20,21,25]. RBT stocking in the Flathead River system ceased in 1969, but a source of RBT persisted at this location. In both historical and contemporary samples, the overall proportion of RBT admixture in Abbot Creek was more than 90%, and included individuals ranging from highly backcrossed hybrids to pure RBT. In the early 1980s, hybridization was rare or absent in WCT populations located upstream from Abbot Creek, but RBT invasion and subsequent RBT \times WCT hybridization spread rapidly upstream during the subsequent decades (see [29] for a description).

(b) Sampling

From 2003 to 2007, fish weirs were operated near the mouths of Langford and Cyclone creeks to capture adult trout (reproductively mature individuals) during their upstream spawning migration and juveniles during their downstream migration (table 1). Except during extreme spring floods (flood events were rare in Langford Creek but occurred annually in Cyclone Creek), migrant weirs capture the vast majority of juvenile (more than 75 mm total length—age 2 years) and adult trout. For example, 355 adult trout were sampled migrating upstream, but there were only 21 (5.6% of total) adult fish that were captured migrating downstream (post-spawning) that were not previously captured during their upstream migration, indicating that most fish are captured as they migrate past the fish weirs. Weirs were continuously operated during the entire first year of the study in Langford Creek (March 2003–February 2004) and no trout were captured during the winter months (December–March), a finding consistent with other studies showing that juvenile salmonid migrations, including those of cutthroat trout, are driven by water temperature and stream flow in late-spring and early-summer [30]. In subsequent years, weirs in both streams were operated from late-March or early-April until as late as November, but fish were never captured after July. Because of high spring flows in Cyclone Creek, juvenile traps were installed immediately following peak flows in May; adult traps were operated throughout the spawning migration (April–June) except for short periods (several hours up to 1 day) during absolute peak flows. Peak adult migration timing occurred in May, and peak juvenile out-migration was in June.

Although a small number of adult or juvenile fish may have migrated before the weirs were deployed each spring, the effect should be marginal given our exhaustive sampling of the populations throughout their primary migratory period. If sampling bias exists, our estimates of RBT admixture in adult fish would be lower because adult RBT tend to migrate earlier than WCT [31]. Furthermore, juvenile WCT migrate downstream slightly earlier than RBT (C. Muhlfeld 2003–2007, unpublished data), so our estimates of RBT admixture in juvenile samples would be higher. Together, this would reduce our ability to detect selection against RBT (see §2(d)).

Fry traps, which sampled approximately 75% of the total cross-sectional area of the channel, were deployed immediately downstream of the weirs at Langford and Cyclone creeks in 2007 (June–November) because age-0 individuals (less than 75 mm) were too small to be captured in the weir. We did not detect a substantial out-migration of age-0 fish in either stream, which is concordant with previous studies showing poor survival associated with age-0 emigration in salmonid species in the

Table 1. The numbers and proportions (in parentheses) of adult (*A*) and juvenile (*J*) fish with at least 50% proportion RBT (pRBT) admixture. The high admixture category refers to those fish with a minimum of 50% pRBT that are not F_1 hybrids or pure RBT. The total number of fish includes all fish captured for that particular year and life stage.

sample	F_1 s	high admixture	RBT	total number captured
Langford Creek				
2003A	12 (0.40)	12 (0.41)	2 (0.07)	29
2004A	13 (0.30)	9 (0.20)	1 (0.02)	44
2005A	3 (0.06)	19 (0.37)	2 (0.04)	52
2006A	1 (0.02)	23 (0.38)	5 (0.08)	61
2007A	8 (0.18)	14 (0.32)	2 (0.05)	44
2003J	0	4 (0.40)	0	10
2004J	0	21 (0.48)	0	44
2005J	4 (0.01)	108 (0.33)	0	323
2006J	2 (0.01)	21 (0.09)	0	224
2007J	0	18 (0.33)	0	54
Cyclone Creek				
2003A	3 (0.08)	1 (0.03)	0	36
2004A	2 (0.05)	0	0	39
2005A	1 (0.04)	1 (0.04)	0	26
2006A	0	2 (0.08)	0	24
2007A	0	1 (0.05)	0	22
2003J	0	1 (0.1)	0	10
2006J	0	0	0	74
2007J	0	1 (0.02)	0	55

Flathead system [32] and elsewhere [33]. Total length (mm), date and sex (of adults if known) were recorded for each captured fish. Scales were used for ageing of most adult fish and 35% of juvenile fish from Langford Creek (only six juveniles from Cyclone Creek were aged).

(c) Genetic analyses

To estimate individual ancestry, all sampled fish from 2003 to 2007 were genotyped using eight species-diagnostic microsatellite loci [22]. The proportion RBT admixture (pRBT = total number of RBT alleles/total number of alleles genotyped) was calculated for each sample year and each individual. First-generation hybrids between WCT and RBT have a pRBT of 0.5 and are heterozygous for alleles from each parental taxon at each diagnostic locus. Fish with a pRBT of 0.5 that were not heterozygous for alleles from the parental taxa at all loci are post- F_1 hybrids. To compare admixture values in samples collected from 2003 to 2007 with historical levels of admixture, we used data from samples collected in 1984 and 1998. Those samples were genotyped with six allozyme loci [34] and four PINE loci (paired interspersed nuclear elements [20]), respectively, that were diagnostic between RBT and WCT.

(d) Data analyses

(i) Relationship between proportion of rainbow trout and traits associated with reproductive success

Generalized linear models were used to quantify relationships between individual RBT ancestry and length at spawning and age at juvenile out-migration. Body length is positively related to reproductive success in salmonid fishes, including *Oncorhynchus* spp. [22], because larger individuals are more fecund and can better

defend optimal spawning locations [35–37]. The timing of key life-history transitions, including the age of out-migration, can also influence reproductive success (as measured from spawning adults to subsequent juvenile out-migrants). Fish that spend 3 years in their natal streams risk additional mortality compared to those that out-migrate at age two (they may, however, have improved survival post-migration) [38]. Thus, if hybridization is related to age at out-migration, it could substantially influence our measure of fitness (i.e. from adult to juvenile—described below).

We used normal linear models to test for relationships between individual pRBT and the total length (mm) of all adult fish migrating into Cyclone and Langford creeks. In addition to testing for an effect of pRBT, we also tested whether sex and stream were related to variation in adult length. A logit link function was used to model how individual pRBT was related to the proportion of individuals out-migrating at age 2, as more than 99% of out-migrating juvenile fish emigrated at age 2 or 3 (i.e. a binomial response). Ultimately, we were interested in whether relationships between phenotypic variation and pRBT were predictive of the observed direction of selection occurring on RBT hybridization.

(ii) Selection on rainbow trout admixture

To measure the direction of selection on RBT hybridization in each stream, we tested whether pRBT significantly differed between samples of adult fish and their juvenile progeny [39]. In a randomly mating population without natural selection, the pRBT in spawning adults should be equal to pRBT in the offspring, with some variation owing to drift and sampling. Alternatively, higher or lower pRBT in offspring relative to adults is evidence for positive or negative selection against RBT hybridization, respectively. Thus, our estimates of selection

span the period of adult spawning, embryonic rearing and juvenile survival prior to out-migration. Although not representative of total lifetime fitness, these are critical periods for population dynamics and life-history evolution in salmonid fishes [40] that encompass major components of overall fertility and viability.

We tested for differences between pRBT in adults and juveniles by using all samples combined across years, and by testing for differences between the appropriately paired parental and offspring generations. Ninety-two per cent of juvenile individuals out-migrated at age 2, so we tested for differences in pRBT between adults in year t (e.g. spring 2004) and juveniles in year $t + 2$ (e.g. spring 2006). Because an excess of parental types were captured in all samples (i.e. evidence for non-random population structure or mating), we used non-parametric bootstrap resampling over individuals (1000 replicates) to construct standard errors for the pRBT in each sample, and to construct confidence intervals around our test statistic (pRBT in adult fish – pRBT in juvenile fish). Non-random population structure in RBT \times WCT hybrid zones is relatively common [21,41] and probably reflects the combined effects of dispersal and selection ([39] and see §3), and some degree of assortative mating owing to slight differences in reproductive timing [31]. We used a conservative $\alpha = 0.01$ as our significance threshold because the bootstrap method accounts only for sampling error, but error owing to genetic drift occurring between the adult and juvenile generations [42,43] may also be occurring. We used a randomization (i.e. permutation) approach to calculate the p -value that the observed difference in pRBT between the juvenile and adult sample could have occurred by chance from 5000 random samples of the data.

To quantify the strength of selection acting on RBT admixture, we used the observed changes in allele frequencies at each diagnostic locus to calculate the relative fitness (w_i) of genotypes with RBT alleles [44]. For this analysis, we focused only on the paired parental and offspring samples (adults in year t versus juveniles in year $t + 2$). For simplicity, we ignored allelic identity (there were multiple RBT and WCT alleles at each microsatellite locus) and focused only on allelic state (i.e. RBT or WCT allele) for all calculations. In other words, we treated an individual with two RBT alleles as a 'RBT homozygote' even if the two RBT alleles differed in repeat motif. Thus, we used a simple two-allele model and separately calculated the relative fitness of genotypes containing RBT alleles using a model assuming directional selection with additive effects (i.e. intermediate effects), and a model assuming dominance for the advantageous allele at each locus. Specifically, we solved for the relative fitness values necessary to produce the observed change that occurred between the adult and juvenile sample (eqn 8.4 in [42]). For each paired sample comparison, we took the mean of the selection coefficients ($1 - w_i$) across loci to obtain an average estimate of the strength of selection acting on genotypes with RBT alleles.

Because of issues of linkage disequilibrium in hybrid zones [42], genotypes within an individual suffer from some degree of non-independence depending on individual ancestry, where non-independence is maximized in an F_1 hybrid and decays with subsequent backcrossing. As such, our estimates of the variance surrounding the mean selection coefficients across loci are likely to be biased slightly low [45]. Additionally, the accuracy of the estimated selection coefficients themselves likely suffer from several sources of error, including the fact our data do not come from perfectly discrete generations and that the strength and direction of selection probably vary as a result of individual genetic background (i.e. parental type versus F_1 , versus F_2 versus backcross; [46]). Nevertheless, the estimated selection coefficients provide an approximation of the strength of selection occurring in this system.

(iii) Rainbow trout and hybrid dispersal

Observed counts and total proportions of spawning adults and out-migrating juveniles with predominantly RBT ancestry (at

least 50% pRBT) were used to infer the relative roles of RBT and hybrid dispersal in promoting hybridization between WCT and RBT. An excess of adult fish with high pRBT compared with the previous juvenile out-migrants indicates that some adults with high pRBT must be immigrants from downstream source populations of RBT (sites with high pRBT are located only downstream of our study streams; [21,25]). In each stream, year and life stage (adult versus juvenile), we organized fish into three classifications of RBT ancestry: F_1 hybrid, pure RBT and more than 50% pRBT but not F_1 or pure RBT. We then compared the numbers and proportions of juvenile out-migrants to the numbers of spawning adults for each category of RBT ancestry in each stream. Comparisons were made across all years (e.g. we compared the total number of pure out-migrating RBT juveniles and RBT adults in Langford Creek), and for paired samples that roughly corresponded to the same generation. For example, if zero F_1 or pure RBT migrated out of Langford or Cyclone creeks in year t , but numerous F_1 and pure RBT arrived to spawn in years $t + 2$ or $t + 3$ (the average number of years spent rearing in downstream habitats), then the majority of these fish must have been migrants since they did not originate (i.e. out-migrate) from that stream. In each stream, we also tested for significant differences in the overall proportions of adult and juvenile fish with high pRBT (more than 50% pRBT) using binomial t -tests. All statistical analyses were conducted in Program R [47]. All data are deposited at Dryad (doi:10.5061/dryad.qk746).

3. Results

(a) Spread of hybridization over two decades

The proportion of RBT admixture (pRBT) increased markedly in Cyclone and Langford creeks from 1984 to 2003, but admixture values during the period of intensive field sampling (2003–2007) were relatively stable (figure 1a,b). In 1984, hybridization was not detected in Cyclone Creek ($n = 15$; the probability of detecting 5% pRBT was greater than 0.99) and was almost non-detectable in Langford Creek ($n = 12$); one trout captured at Langford had three RBT alleles out of a possible 12. By 1998, RBT introgression was detected in 40% and 75% of fish sampled in Cyclone ($n = 25$) and Langford creeks ($n = 20$), respectively. From 2003 to 2007, RBT alleles were detected in 58% of adults ($n = 147$) and 40% of juveniles ($n = 139$) in Cyclone Creek and in 87% of adults ($n = 232$) and 82% of juveniles ($n = 635$) sampled in Langford Creek.

(b) Relationships between proportion of rainbow trout and traits associated with reproductive success

There were significant relationships between individual pRBT and both traits related to reproductive success in salmonid fishes: adult size and juvenile age at out-migration. For adult fish, length was positively related to pRBT ($b_1 = 3.89$, s.e. = 0.99; $p < 0.001$) (figure 2a). On average, a pure RBT adult is approximately 62 mm longer than a pure WCT adult, an increase of 20–30% depending on sex. Length at spawning was also strongly related to sex (females were larger; $p < 0.001$), but length did not differ between the two populations ($p = 0.676$).

In Langford Creek, the proportion of juvenile trout out-migrating at age 2 (as opposed to age 3) increased with increasing values of individual pRBT ($b_1 = 0.30$, s.e. = 0.09; $p < 0.001$) (figure 2b). Nearly all of the juvenile trout with pRBT values

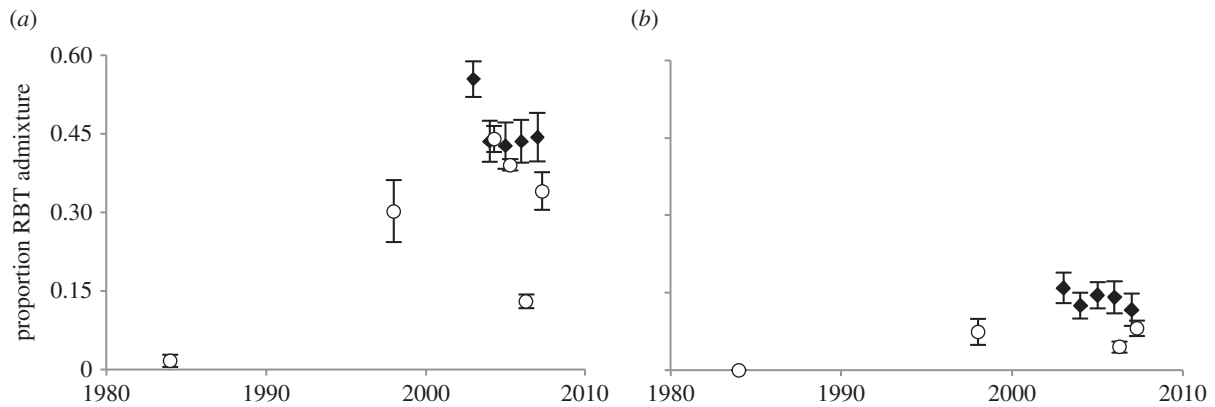


Figure 1. Rainbow trout admixture proportions (pRBT) across time in Langford (a) and Cyclone (b) creeks. The open circles are samples of juvenile fish and the dark diamonds are adult fish. The error bars around each estimate are bootstrap standard errors.

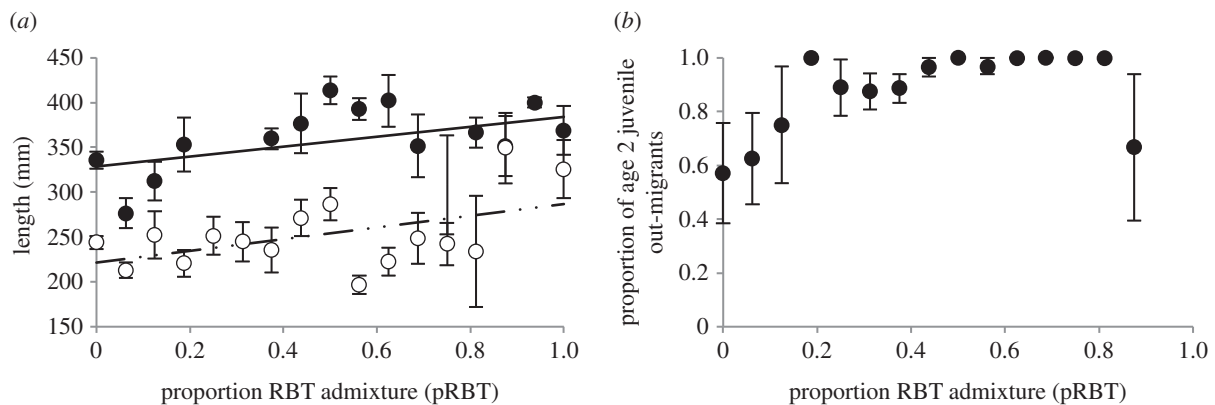


Figure 2. Relationships between individual pRBT and adult length (a) and the proportion of juvenile trout out-migrating at age 2 as opposed to age 3 (b). In panel (a), the open circles are for males while black dots are for females. Error bars are standard errors.

greater than 0.5 out-migrated at age 2 (98%, s.e. = 0.02). However, the proportion of trout out-migrating at age 3 was 37% (s.e. = 0.11) for fish with pRBT less than 0.15. Thus, RBT and their hybrids generally migrate downstream at a younger age than many pure or nearly pure WCT.

(c) Selection against rainbow trout admixture

In both Cyclone and Langford creeks, the pRBT in spawning adults was substantially higher than the pRBT in out-migrating juveniles, indicating strong selection against fish with RBT admixture (figures 1a,b and 3a,b). This pattern was consistent across streams, years and various methods of combining or splitting data; in all comparisons, pRBT was higher in adults than juveniles. Across all samples in both streams, the pRBT in out-migrating juveniles was significantly lower than pRBT in spawning adults (figure 3a). For all paired tests between parental and progeny generations, the pRBT was greater in adult samples than in the corresponding juvenile out-migrants (figure 3a). The decreases in pRBT were significant ($p < 0.01$) for three of the five independent, paired tests, and the Fisher's combined probability for all five tests (significance values obtained from randomization) was highly significant ($p < 0.0001$).

For each paired comparison between parents and offspring, the observed changes in RBT allele frequencies at each diagnostic locus ($n = 8$) were used to estimate selection coefficients acting on genotypes with RBT alleles. There was consistent evidence that genotypes with RBT alleles had

reduced relative fitness (from adult spawning to juvenile out-migration) compared with genotypes with only native WCT alleles. The observed changes in RBT allele frequencies between samples from the parental and subsequent progeny generation were sufficiently large that an intermediate model for directional selection (additive effects of each RBT allele) was unable to produce the observed genetic change that occurred at 18 of the 40 comparisons among loci and paired samples (five paired comparisons with eight loci). Therefore, we do not present results for the intermediate model.

Under a model for dominant directional selection (i.e. the relative fitness (w) of a genotype with one RBT allele is equal to w for the genotype with two RBT alleles), the average estimated selection coefficients ($s = 1 - w$) across loci for samples from Langford Creek ranged from $s = 0.32$ (s.e. = 0.11; adults 2005 and juveniles 2007) to $s = 0.88$ (s.e. = 0.10; adults 2004 and juveniles 2006; figure 3b). Similarly, the average estimates of s acting against RBT genotypes in Cyclone Creek were 0.73 (s.e. = 0.09; adults 2004 and juveniles 2006) and 0.44 (s.e. = 0.12; adults 2005 and juveniles 2006). Overall, the mean of the average estimates of s across streams and paired comparisons was 0.60 (s.e. = 0.10). The estimates of selection coefficients were similar among the eight diagnostic loci, where the range in the mean s per locus was 0.46–0.77 over all paired comparisons. These estimates of s reflect a general genomic effect rather than selection at each individual locus. That is, individuals who have an RBT allele at one of the diagnostic loci are more likely to have RBT genes over their entire genome than individuals that do not have an RBT allele.

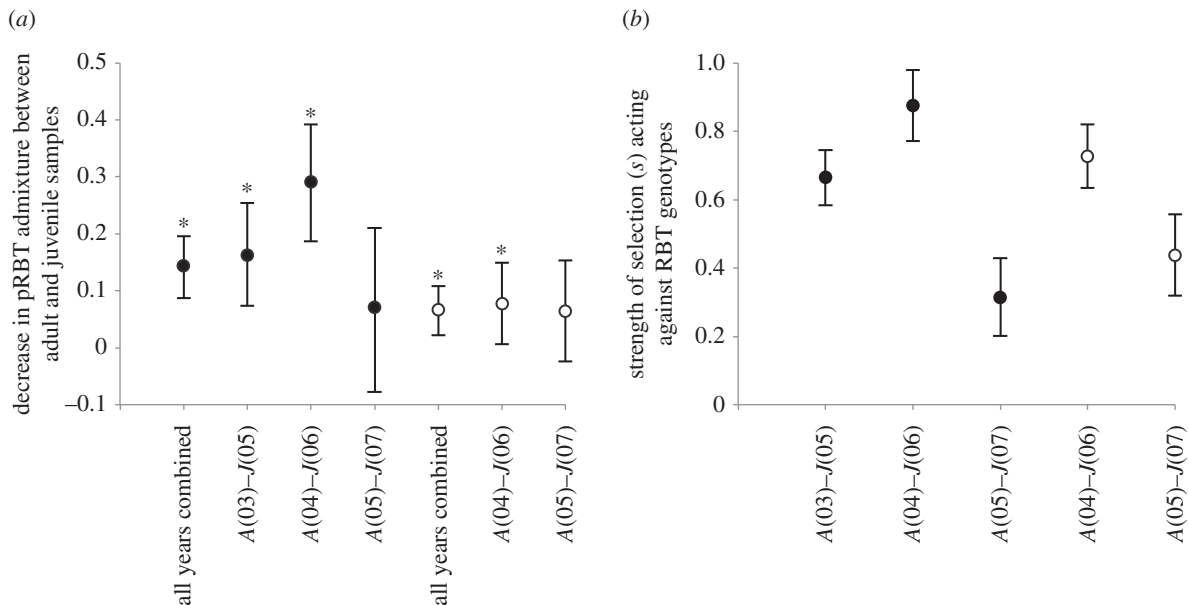


Figure 3. Differences in pRBT between adult and juvenile fish from Langford and Cyclone creeks (a), and the estimated strength of selection acting against genotypes with RBT alleles (b). The comparisons on the x-axis refer to estimates based on data from adult and juvenile trout combined across years (all years combined), or to estimates based on data from parental (e.g. adults in 2003 = A(03)) and offspring (e.g. juveniles in 2005 = J(05)) generations. The black filled symbols are for Langford Creek, while the open symbols are for Cyclone Creek. In (a), estimates that were significantly different from zero ($p < 0.01$) are highlighted with an asterisk (*) symbol. In (b), a selection coefficient (s) of 0.0 means that genotypes with or without RBT alleles have identical fitness, while an s value of 1.0 means that genotypes with RBT alleles essentially had no reproductive success. The error bars in (a) are bootstrap 99% CIs, while the error bars in (b) are standard errors.

(d) Rainbow trout and hybrid dispersal

The number of spawning adults with predominantly RBT ancestry (at least 50% pRBT) greatly exceeded the number of out-migrating juveniles with at least 50% pRBT (table 1). These data strongly indicate that immigration (i.e. dispersal) of highly admixed fish from other RBT source populations perpetuates introgression in these streams. In particular, there was a substantial disparity in the number of out-migrating F_1 hybrids and pure RBT compared to the number of in-migrating adult F_1 hybrids and pure RBT. For example, 12 pure RBT adults were captured migrating into Langford from 2003 to 2007 (5.2% of adults), but no pure RBT juveniles were ever captured migrating out of the creek, despite substantial sample sizes (overall juvenile $n = 655$). In other words, there is no evidence that pure RBT were born and out-migrated from Langford Creek, but pure RBT spawning adults were observed migrating into Langford Creek every year for which we had data. Thus, most, if not all, of the pure RBT must be from other locations. Similarly, there were 37 F_1 hybrid adults captured migrating into Langford Creek (total $n = 230$; 16%), but only six F_1 out-migrating juveniles were ever captured leaving Langford Creek ($N = 655$; 1%), which provides further support that the vast majority of F_1 hybrids arriving to spawn in Langford Creek were from other locations. In Cyclone Creek, we captured six F_1 in-migrating adults, but no F_1 out-migrating juvenile fish were captured. Pure RBT were never captured in Cyclone Creek.

The observation of more highly admixed spawning fish than highly admixed juvenile out-migrants is also evident when using paired samples consisting of collections from the same cohort at the juvenile (i.e. out-migrant) and adult life stages. From 2003 to 2005, four F_1 hybrid and no pure RBT juveniles were captured out-migrating from Langford Creek. Of the in-migrating adults captured in 2006 and 2007 (corresponding to juveniles that would have out-migrated from

2003 to 2005), nine were F_1 s and seven were pure RBT. The overall proportion of in-migrating adult fish with at least 50% pRBT (includes F_1 s, backcrosses, and putatively pure RBT) was significantly greater than the overall proportion of out-migrating juvenile fish with at least 50% pRBT in both Langford ($p < 0.001$) and Cyclone ($p = 0.028$) creeks.

4. Discussion

Despite strong selection against non-native genetic admixture, invasive hybridization increased rapidly in two native trout populations inhabiting streams with contrasting abiotic and biotic characteristics. The continuous immigration of adult trout with high proportions of RBT genes from outside 'source' populations appeared to be the primary mechanism promoting hybridization in both populations. These results emphasize that genomic extinction can occur even when native populations (individuals) have substantially higher relative fitness than individuals with non-native genes [23,24]. This phenomenon was clearly highlighted in our study; at the end of the study period, only 17% of the fish in Langford Creek were pure WCT despite the fact that selection coefficients acting against RBT introgression were as strong as 0.88.

(a) Effects of hybridization on phenotypic traits

Admixture proportions in individual trout were related to phenotypic traits associated with fitness in salmonid fishes. For both traits of interest—length at spawning and age at out-migration—selection should favour RBT and their hybrids, at least over the period on which we focused (adult to juvenile). There was a strong positive relationship between pRBT and length for adult fish. Because introgressed fish were larger, they likely had greater egg production, larger eggs (see also [48]) and were better able to defend spawning sites. Indeed,

individual length is often directly related to reproductive success for a variety of salmonid species [22,35,49,50]. Additionally, fish with higher levels of pRBT admixture were more likely to out-migrate at an earlier age (age 2 as opposed to age 3). This result implies that we should have captured more out-migrating juveniles that were admixed because fish with low or no pRBT reside in the streams longer, during which time additional mortality occurs.

(b) Antagonisms between selection and dispersal

Based on the observed phenotypic relationships, hybrid fish and RBT were predicted to have higher relative fitness. However, we found consistent evidence of strong selection against RBT admixture in both populations. These results are consistent with a previous study [22] that used parentage analyses with some of the same data to show that adult pRBT was negatively related to the number of out-migrating juveniles produced by adult spawning fish in Langford Creek. We expand on this work by quantifying the strength of selection acting against RBT genotypes and by demonstrating a similar pattern of selection against RBT admixture in another population with different environmental conditions. Our results have three major implications: (i) phenotypic traits may poorly predict direction of selection; (ii) our estimates of the strength of selection acting against RBT, though surprisingly large, may actually be conservative because we did not account for phenotypic biases favouring RBT; and (iii) selection against RBT \times WCT hybridization appears to be occurring in diverse environments.

Across riverscapes, RBT \times WCT hybridization is often prevalent in low elevation streams with warmer water temperatures, whereas non-hybridized populations occur in colder, headwater streams [25,26]. This led to the hypothesis that cold temperatures in headwater streams impart a competitive advantage to native WCT [27,28]. We found evidence for selection against RBT admixture in two streams with contrasting thermal characteristics; Cyclone Creek is considerably warmer than Langford Creek but has a much lower pRBT. Thus, selection against RBT may be present across a wider gradient of environments and habitat features than previously thought. Furthermore, because WCT and RBT have similar optimum growth temperatures in the laboratory (cutthroat: 13.7°C, rainbow: 13.2°C; [51], but also see [52]), temperature alone may not prevent the spread of hybridization. Greater longitudinal and spatial sampling is still needed to identify whether certain conditions alleviate or eliminate selection against RBT hybridization. Indeed, climate-induced shifts in spring precipitation were associated with the rapid expansion of hybridization in the North Fork Flathead River [29], suggesting that episodic changes in climatic conditions might influence selective pressures acting on RBT and/or hybrid dispersal. More work is needed to pinpoint the selective mechanisms and/or life stages during which selection against RBT is occurring and to what degree selective pressures may change in the future.

Our results, combined with several previous studies in this region [21,31], demonstrate that dispersal of hybrid individuals from downstream source populations is a significant factor, and probably the primary mechanism contributing to the spread of hybridization between WCT and RBT. We found substantially more highly introgressed fish migrating into each creek to spawn than highly introgressed juvenile

fish out-migrating from each creek, indicating that many hybrids migrating into Cyclone and Langford creeks are from other hybrid source populations. These data corroborate spatial analyses indicating that hybridization spreads via long distance movements of individuals with high amounts of RBT admixture and stepping-stone invasion at small scales by later-generation backcrosses [21,31].

Within salmonid fishes, outbreeding depression between native and non-native populations has been observed in other contexts [53,54], however, the finding that hybridization can persist or increase because of dispersal is quite noteworthy, with major implications for conservation and management or imperiled plant and animal species. Because the progeny of all hybrids are hybrids [23,24], WCT are clearly threatened with genomic extinction despite selection against hybrid genotypes in diverse stream environments. Though introgression levels were relatively stable in later years, this is probably owing to RBT suppression efforts lower in the river network [55], as opposed to an equilibrium scenario between dispersal and selection. To conserve remaining cutthroat trout populations in connected stream systems, it is clear that the active management or elimination of RBT and hybrid source populations is needed.

While RBT dispersal appears to play a significant role in promoting the spread of hybridization, other factors probably contribute to hybridization dynamics. For example, F_1 hybrids were shown to have reproductive success nearly equivalent to or potentially greater than pure WCT (i.e. heterosis) [22], a common feature of hybrid zones that acts to promote introgressive hybridization [7,56], even when later-generation hybrids have substantially reduced fitness. Moreover, genomic data suggest that there may be positive selection for several RBT alleles across native WCT populations in the North Fork Flathead River [57]. How these individual loci interact with dispersal and genome-wide selection to influence introgression is currently unknown. Nevertheless, our data strongly indicate that at the population level, selection and dispersal play antagonistic roles in mediating hybridization between these species.

(c) Implications

Studies examining how natural selection influences invasive hybridization in the wild are exceedingly rare, limiting general insight on the underlying mechanisms and consequences of human-mediated hybridization. Major implications of this work include: (i) phenotypic traits associated with fitness in other contexts can poorly predict the direction of selection acting in admixed populations; (ii) focusing on selection alone may provide an incomplete picture of the dynamics influencing invasive introgression; and (iii) dispersal can be a major driver in maintaining and spreading hybridization but is frequently ignored because it is difficult to measure [58]. Thus, our study shows that combining data on fitness and dispersal is necessary to fully understand the mechanisms driving invasive hybridization and other eco-evolutionary dynamics [59]; the paucity of such data in wild animal populations makes this a novel step forward in our empirical understanding of how invasive introgression can spread in natural populations. Ultimately, the fact that dispersal pressure from invasive species can overwhelm selection against introgressive hybridization has consequential implications for genetic diversity and global biodiversity.

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