Genetic variation in westslope cutthroat trout reveals that widespread genetic rescue is warranted

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Abstract

Although human fragmentation of freshwater habitats is ubiquitous, the genetic consequences of isolation and a roadmap to address them are poorly documented for most fishes. This is unfortunate, because translocation for genetic rescue could help mitigate problems. We used genetic data (32 SNPs) from 203 populations of westslope cutthroat trout to (1) document the effect of fragmentation on genetic variation and population structure, (2) identify candidate populations for genetic rescue, and (3) quantify the potential benefits of strategic translocation efforts. Human-isolated populations had substantially lower genetic variation and elevated genetic differentiation, indicating that many populations are strongly influenced by random genetic drift. Based on simple criteria, 23 populations were candidates for genetic rescue, which represented a majority (51%) of suitable populations in one major region (Missouri drainage). Population genetic theory suggests that translocation of a small number of individuals (~5 adults) from nearby populations could dramatically increase heterozygosity by up to 58% (average across populations). This effort provides a clear template for future conservation of westslope cutthroat trout, while simultaneously highlighting the potential need for similar efforts in many freshwater species.

Keywords

Genetic variation, isolation, genetic rescue, fragmentation, genetic differentiation, freshwater fish

Introduction

Freshwater species are unfortunately positioned as the most-imperiled group of biota on earth (Tickner et al. 2020), and populations of freshwater vertebrates are declining at faster rates than either terrestrial or marine species (e.g., Collen et al. 2009). The imperilment of freshwater biota is due to various human stressors, but especially human water consumption (dewatering), pollution, habitat degradation, invasive species, climate change, and widespread habitat fragmentation (Dudgeon et al. 2006, Dudgeon et al. 2019, Reid et al. 2019). Fragmentation is pervasive, and can take many forms, ranging from river-spanning dams, to impassable flow modifications on small streams (e.g., culverts) (Gido et al. 2016). While the ecological consequences of fragmentation are often obvious – species are unable to complete their life-cycle – widespread fragmentation is also dramatically altering evolutionary processes in almost all freshwater aquatic life.

Long-term population declines coupled with habitat fragmentation have resulted in many native freshwater species existing as small, semi- or fully-isolated populations (e.g., Barbarossa et al. 2020), with significant implications for population and species persistence. Importantly, the evolutionary trajectory of small isolated populations becomes dominated by random genetic drift, where the loss of genetic variation due to stochastic processes is not counteracted by gene flow among populations (Allendorf et al. 2013). Isolated populations are therefore at an increased risk of extinction because of inbreeding, and are less able to adapt to (increasingly) stressful conditions because of reduced genetic variation and small genetic effective population size (i.e., natural

selection is less effective when the genetic effective size is small) (e.g., Bijlsma and Loeschke 2012).

Because genetic isolation is clearly problematic and globally ubiquitous, conservation geneticists have argued that fragmentation and resulting isolation represents the greatest genetic threat to biodiversity worldwide (Frankham et al. 2017). To mitigate this problem, there is increasing interest in using intentional translocation of individuals to assist gene flow between fragmented populations, which can restore genetic variation and adaptive potential, alleviate inbreeding depression, and, ultimately, increase population persistence, often called 'genetic rescue' (Tallmon et al. 2004, Whiteley et al. 2015, Bell et al. 2019). Some researchers have argued that a paradigm shift is needed in conservation biology such that translocations for genetic rescue becomes a common and widespread practice (Ralls et al. 2018), as the genetic benefits (heterosis) to small, isolated populations appear to strongly outweigh the potential risks (outbreeding depression) (Frankham 2015). While several famous examples of genetic rescue can be found for various plants and terrestrial animals (see Whiteley et al. 2015), assisted gene flow is still a rare conservation practice, especially for freshwater species (Frankham et al. 2017). The limited implementation of genetic rescue for freshwater species stems from several sources, but perhaps most obviously, the relatively limited availability of genetic data for many freshwater species greatly hinders our ability to identify (1) whether genetic rescue may be appropriate for a given species, and if so (2) which populations may benefit from genetic rescue. In other words, for the vast majority of freshwater species we have a poor understanding of the negative effects of

widespread human-induced fragmentation on genetic variation within and among populations at large geographic scales, and thus, the potential opportunity afforded by genetic rescue.

The ecological consequences of human fragmentation are typified in cutthroat trout (*Oncorhynchus clarkii sp.*), a group of (sub)species native to western North America, all of which experienced dramatic population declines during the course of the last century (Penaluna et al. 2016). Like most species of conservation concern, the underlying drivers of decline are multi-faceted, but the widespread release and subsequent expansion of invasive species have been especially problematic (Bell et al. 2021). In particular, non-native brook trout appear to outcompete cutthroat trout (Peterson et al. 2004), while introduced rainbow trout hybridize with cutthroat trout, ultimately leading to genomic extirpation of local populations (Allendorf and Leary 1988). Over much of their distribution, non-hybridized populations of cutthroat trout are now isolated, oftentimes intentionally with barriers, to headwater streams where they exist as small populations that are genetically and demographically independent from one another (Fausch et al. 2009).

The conservation status of non-hybridized westslope cutthroat trout (*O. c. lewisi*; here forward WCT) differs among populations separated by North America's Continental Divide. West of the Continental Divide in the headwaters of the Columbia River basin, WCT are relatively widespread and often interconnected with one another. East of the Continental Divide in the headwaters of the Missouri and South Saskatchewan River

drainages, WCT are extremely rare and universally limited to small isolated headwater stream fragments, though they were widespread and abundant when Europeans first began inhabiting this region during the nineteenth century (Shepard et al. 2005). Indeed, WCT are currently listed as threatened under Canada's Species at Risk Act (COSEWIC 2016). This creates a template where actively assisting gene flow to promote genetic rescue of WCT may be necessary, especially for remaining populations in the Missouri River drainage, and other portions of the WCT distribution highly fragmented in the past century (e.g., Saskatchewan drainage). Although broad-scale descriptions of genetic variation and differentiation within and among populations of non-hybridized WCT have been conducted (Leary et al. 1985, Drinan et al. 2011, Young et al. 2018), a comprehensive analysis that includes a majority of extant populations has not occurred at large spatial scales.

The overall goal of this project was to document the effects of contemporary habitat fragmentation on the distribution of genetic variation and evolutionary trajectory of WCT. Specifically, we used data from 203 populations of WCT from throughout 26 river basins in Montana, USA to address the following questions: (1) what are patterns of intra-population genetic variation for WCT in the Northern Rocky Mountain region; (2) what is the genetic population structure of non-hybridized WCT; (3) how has isolation and fragmentation influenced genetic variation and differentiation; and (4) how much of an effect would assisted gene flow for genetic rescue have on extant genetic variation within populations? The results from this work directly inform how human actions have

influenced evolutionary dynamics in native species, while also demonstrating the need for future conservation actions like translocation for genetic rescue.

Methods

Sample collection

To identify remaining non-hybridized WCT throughout the headwaters of the Columbia and Missouri Rivers, genetic samples have been collected from over one-thousand locations (e.g., Muhlfeld et al. 2017). Here, we focus on genetic data from 203 populations largely found in Montana, USA and the headwaters of the Flathead drainage in British Columbia (Canada) that were verified to be non-hybridized, or where we could easily remove a few hybrids that appeared to be recent migrants into an otherwise non-hybridized population, all of which have consistent genotypic data at the same set of SNP loci (see below). Overall, 62 populations were located among 14 watersheds (here defined using HUC-8 watershed codes) east of the Continental Divide in the Missouri River drainage, and 141 populations were located in 13 watersheds west of the Continental Divide in the Columbia River basin (Fig. 1; Table S1 and S2). WCT spawn in first and second order tributaries, and as such, we treated “streams” as populations. When samples were collected from multiple reaches within a stream, we verified that genetic differentiation was non-existent, or trivial (F_{ST} < ~0.03). We limited our analysis to population samples of at least 10 individuals, except one population (South Fork North Fork Divide Creek) where the sample represented all fish that could be captured because of very small population size. The median sample size across

populations was 25 (range 9 to 317). In total, we used genetic data from 7,316 individuals.

Genotyping

Genetic analyses were conducted at the University of Montana (Missoula, Montana USA) Conservation Genetics Laboratory. DNA was extracted from fin tissue with a detergent based cell lysis buffer and ammonium acetate protein precipitation, followed by isopropyl alcohol DNA precipitation. DNA was re-suspended in 100ul TE buffer and diluted to 20-100ng/ul. All fish were genotyped at 95 single nucleotide polymorphs (SNPs) using a Fluidigm EP1 Genotyping System (see Muhlfeld et al. 2016). The majority of loci (61) were species diagnostic between WCT, Yellowstone cutthroat trout (*O. c. bouvieri*) and rainbow trout. The remaining 34 loci are polymorphic within WCT populations throughout the upper Columbia and Missouri River basins.

Polymorphic loci included SNPs found within express-sequence tags (Campbell et al. 2012), and from anonymous sites identified with RADseq (Hohenlohe et al. 2011) that were subsequently developed into targeted assays (as described in Amish et al. 2012). The genomic location of all markers relative to the rainbow trout genome (Pearse et al. 2019) is described in Table S3. Two of the 34 loci demonstrated a consistent excess of heterozygotes across populations, suggesting they may be duplicated, a common occurrence given the ancestral genome-duplication that occurred in salmonid fishes (Allendorf et al. 2015). Those two loci were removed; thus, we focus all analyses herein on 32 polymorphic SNP loci. Importantly, 32 loci spanning at least 17 chromosomes

(Table S3) are sufficient for addressing our primary research goals focused on relative values of population genetic variation and differentiation (Allendorf 2017).

Importantly, WCT colonized the Missouri River basin from populations west of the Continental Divide (likely the upper Middle Fork Flathead River basin) (Young et al. 2018) and harbor a subset of the genetic variation found west of the Continental Divide (Drinan et al. 2011, Young et al. 2018). However, the 32 loci used in this study are variable in populations on both sides of the Continental Divide (Table 1), and thus, ascertainment bias should not influence the results. Indeed, populations from the Missouri and Columbia River basins were used during initial SNP locus discovery (Campbell et al. 2012, Amish et al. 2012), and during development and optimization of the panel.

Data analysis

Patterns of population genetic variation

We used expected heterozygosity (H_e) and the proportion of polymorphic loci (P) as measures of within population genetic variation. As estimates of genetic variation, H_e and P have strengths and weaknesses. The strength of H_e is that it is based on the allele frequencies at the loci, is less sensitive than observed heterozygosity (H_o) to sample size, and is a fundamental parameter in population genetic theory (Allendorf et al. 2013). However, H_e is not particularly sensitive to the loss of low frequency alleles (0.01-0.05). Low frequency alleles have low heterozygosity and, therefore, generally contribute little to the estimate of H_e. In contrast, *P* treats the presence or absence of all

alleles equally regardless of how variable a locus is within the population, and thus, *P* is particularly sensitive to the loss of low frequency alleles through genetic drift and bottlenecks (Allendorf 1986, Luikart et al. 1998). Instead, a major weakness is that *P* is also sensitive to sample size. Since the weakness of H_e as an estimate of amounts of genetic variation is a strength of *P*, and vice versa, we used both statistics to best summarize genetic variation.

We estimated H_e and *P* for each local population using the R package *poppr* (Kamvar et al. 2014). At the watershed level, we calculated *P* for those watersheds where we had genetic data for at least three populations by combining all data from each local population. For the same watersheds, we also calculated the mean expected heterozygosity ($\overline{H_e}$) across populations.

Genetic population structure

The amount of genetic divergence among samples was quantified using F_{ST} (Weir and Cockerham 1984). We considered hierarchical levels within watersheds (W), major rivers (Columbia and Missouri; R), and the total geographic area considered (T) (Fig. 2). We summarized estimates of F_{ST} at the following levels: between populations within watersheds (F_{SW}); among all populations in the Missouri or Columbia River basins (F_{SR}); among watersheds in the Missouri and Columbia basins (F_{WR}); between east and west of the Continental Divide (F_{RT}); and between all populations (global F_{ST}). We also summarized population specific mean pair-wise F_{ST} between all populations in the Missouri or Columbia River basins (population specific F_{ST} with each major river

basin: $\overline{F}_{SR}$). In addition to $\overline{F}_{SR}$ we also calculated Foll and Gaggiotti's (2006) population specific F_{ST} using GESTE, but results were qualitatively very consistent to $\overline{F}_{SR}$, and not presented here.

As with H_e and P, we only calculated F-statistics at the watershed scale for those watersheds with at least three population samples. We computed all *F*-statistics using the package *hierfstat* (Goudet 2004) in Program R (R Core Team 2018). To visualize allele frequency divergence, we also constructed a UPGMA dendrogram based on Cavalli-Sforza and Edwards (1967) Chord distance using *poppr*.

Effects of isolation and fragmentation on variation and differentiation

To quantify how anthropogenic fragmentation influences genetic variation and differentiation in WCT, we categorized each population based on its connectivity with other populations. We created three specific categories for isolation: (1) *human-isolated* populations are those populations that have been isolated by contemporary human activities; (2) *naturally-isolated* populations are those populations that were historically isolated by geologic or hydrologic features (e.g., waterfalls and stream-drying); and (3) *connected* populations are those populations that have perennial or seasonal stream connectivity with at least one other population. Forms of human isolation included dams, water quality fragmentation (due to mining wastewater), impassable culverts, irrigation water withdrawal or infrastructure, and demographic isolation. Complete demographic isolation is fairly common in some watersheds (e.g., Big Hole River); in short, stream systems are still physically connected with one another, but WCT only

exist in very small relict populations within an inhospitable matrix of habitat that is saturated by competitively superior invasive species. Isolated populations needed to be restricted to a singular habitat patch (first or second order stream). Larger streams (second or third order) that were fragmented near their terminus (downstream confluence), but contained multiple first or second order streams containing WCT were considered *connected*. We surveyed local fish biologists (Montana Fish, Wildlife & Parks) to confirm the isolation status of each local population (Table S1 and S2).

We then compared patterns of population genetic variation and genetic differentiation among the different categories. We used an ANOVA and Tukey's HSD to compare the mean genetic variation (H_e and P) and mean differentiation ($\overline{F}_{SR}$) values among the different categories. In the Columbia River basin, this included human-isolated, naturally isolated, and connected populations, but in the Missouri River drainage this only included human and naturally isolated populations. We further used Tukey's HSD in an ANOVA framework to compare naturally and human-isolated populations in the Missouri Drainage to populations in all three categories in the Columbia drainage. For these analyses, we only present the relevant Tukey HSD results, as the overarching ANOVA model is partly redundant with the previous analysis.

To further describe the effects of fragmentation on genetic differentiation in the Columbia River basin, we quantified the correlation (Pearson's) between the proportion of isolated populations in a watershed (both natural and human-induced) and F_{WT}. Last,

we calculated F-statistics at the various hierarchical levels after excluding isolated populations (i.e., only using connected populations).

Potential effect of assisted translocation on genetic variation

We used a simple series of genetic criteria to identify populations that are suitable candidates for genetic rescue efforts. We focused specifically on those populations where contemporary evolutionary dynamics are likely to be strongly determined by stochastic genetic drift and are potentially at high-risk of demographic impacts from inbreeding depression. As such, we identified those populations that were isolated by human causes (i.e., isolated within the last ~100 years), have very little genetic variation (H_e and P in the lowest quartile for all WCT populations), and have extremely high genetic differentiation from other populations either east or west of the Continental Divide ($\overline{F}_{SR} > 0.40$). We refer to those populations as "target" populations from here forward.

We used the existing genetic data and population genetic theory to quantify how assisted gene-flow could influence genetic variation in each target population. Specifically, we first estimated the expected change in allele frequencies at each locus after one generation and after a single gene flow event with migration rate of 0.1 using a simple continent-island model (e.g., Equation 9.10 in Allendorf et al. 2013). Specifically, for each locus we used $q'_A = (1-m)q_A + mq_B$ to predict allele frequencies in the recipient (target) population (q'_A) following gene flow, where m is the migration rate from the donor to the recipient population, q_A is the original allele frequency in the recipient

(target) population, and q_B is the allele frequency in the donor population. We then used predicted allele frequencies to calculate expected heterozygosity. To standardize results across populations, we quantified the percent change in mean expected heterozygosity post assisted translocation and subsequent gene flow.

For many of the target populations of WCT, the migration rate (0.1) is roughly equivalent to translocating 5 reproductively successful individuals into some of the smallest populations (genetic effective populations size (N_e) ~ 50), or approximately 10 individuals into larger populations (N_e = 100). These N_e values are likely reasonable for isolated WCT populations (Carim et al. 2016), and the migration rate represents a compromise between enhancing genetic variation while retaining genetic distinctiveness, and is similar to empirical genetic rescue attempts in other salmonid populations (Robinson et al. 2017) and guidelines developed for other conservation situations (Hedrick 1995). For each target population, we calculated the percent change in expected heterozygosity based on gene flow from each local population of WCT found in the same watershed. Using donor populations from the same watershed should reduce the risk of outbreeding depression (Hedrick & Fredrickson 2010), which is often a concern for salmonid fish (e.g., Rollinson et al. 2014). For those populations in watersheds with fewer than three populations, we also included all donor populations from watersheds that were spatially contiguous with the watershed of interest (Table S4). For each target population, we summarized results by averaging the expected percent change in heterozygosity across all potential donor populations.

Results

Population genetic variation

Genetic variation in WCT populations ranged widely. Some populations were variable at all 32 loci, while other populations were polymorphic at few if any loci (Table S1 and S2). Similarly, heterozygosity ranged from 0.0 to 0.399. As expected given differences in conservation status, patterns of population genetic variation in the Missouri River drainage were very different from those in the Columbia River basin.

Local populations of WCT in the Missouri River basin generally had very low genetic variation (Fig. 3a, c). Across all populations, the proportion of polymorphic loci (*P*) was 0.339. The vast majority of populations (82.7%) were variable at fewer than 50% of SNP loci, and 33.3% of populations were variable at fewer than 25% of the SNP loci analyzed (Table S1). Only four populations (6.5% of the total) were variable at 75% or more of the SNP loci. As such, average expected heterozygosity (H_e) was low (median = 0.090), ranging from 0.004 to 0.283 (Table S1).

At the watershed scale in the Missouri River basin, *P* ranged from 0.406 (Ruby) to 1.000 (Big Hole) (Table 1). The number of populations within a watershed was strongly and positively associated (Pearson's r = 0.720) with *P*. Thus, genetic variation was highest in the Big Hole, Beaverhead, and Red Rock watersheds. Paradoxically, those same watersheds had the lowest average H_e, suggesting there was considerable genetic variation among populations within watersheds, but not within local populations themselves.

In contrast, in the Columbia River basin the mean *P* among all local populations was 0.781, or more than twice the average value in the Missouri River basin (Fig. 3b). Only 12.7% of populations were variable at fewer than 50% of SNP loci, while 71.8% of populations were variable at 75% or more of the loci analyzed, over ten-times the percentage in the Missouri basin. The median H_e among populations was 0.299, which was over three times as high as median H_e in the Missouri drainage, and ranged from 0 to 0.397 (Table S2, Fig. 3d).

In the Columbia basin, estimates of *P* at the watershed scale ranged from 0.906 to 1.00 (Table 1). Like genetic variation in the Missouri River basin, *P* at the watershed scale was strongly correlated with the number of replicate populations within a watershed (Pearson's r = 0.627), but even with a minimum of three populations within a watershed, more than 90% of loci were variable (see Middle Kootenai and Stillwater).

Population genetic structure

Population genetic structure in WCT varied widely by population and region. Across watersheds, genetic differentiation ranged from fairly low (F_{SW} = 0.055) to extremely high (F_{SW} = 0.672; Table 1), and at a global level, genetic differentiation was high among populations (F_{ST} = 0.415). Like population genetic variation, patterns of genetic differentiation varied between the Missouri River basin and the Columbia River basin.

In the Missouri River drainage, genetic differentiation among local populations was extremely high (F_{SR} = 0.450). Over 94% of pair-wise estimates of F_{ST} among populations of WCT in the Missouri drainage were greater than 0.2, and 40.1% were greater than 0.5 (Table S5). At a smaller scale, genetic differentiation was also extremely high among populations within watersheds (global F_{SW} = 0.364). Genetic differentiation was lowest among populations in the Red Rock River basin (F_{SW} = 0.269) and highest within the Ruby River basin (F_{SW} = 0.672; Table 1). At a more course hierarchical level, global F_{ST} among watersheds in the Missouri drainage (F_{WR}) was 0.150. Thus, genetic differentiation among local populations within watersheds was greater than twice as large as the observed genetic differentiation among watersheds.

Across all hierarchical levels, genetic differentiation was lower in the Columbia River basin than in the Missouri River basin. Nevertheless, genetic differentiation among all local populations was still high (F_{SR} = 0.303). However, many estimates were relatively small at least compared to differentiation in the Missouri River basin; 7.6% of estimates were less than 0.1, and 33.4% were less than 0.2 (Table S6). Genetic differentiation among populations of WCT within watersheds (global F_{SW}) was 0.204, which is roughly half of the value in the Missouri River basin. F_{SW} ranged widely, from 0.055 in the Bitterroot to 0.570 in the Middle Kootenai drainage. The genetic differentiation among watersheds (F_{WR}) in the Columbia River drainage was 0.126. Overall genetic differentiation between WCT east and west of the continental divide (F_{RT}) was high (0.225), but smaller than differentiation between many local, neighboring populations.

A dendrogram based on Cavalli-Sforza and Edward's chord distance generally confirms the results of the hierarchical F-statistics (Fig. S1). Populations of WCT in the Missouri River drainage all grouped with one another, but subsequent grouping by watershed was less cohesive. In the Columbia basin, populations from the same watershed or proximate watersheds typically grouped with one another with the exception of isolated populations with low to moderate genetic variation (see also Table S2).

Effects of fragmentation

Results from ANOVA tests suggest that P ($F_{(2, 138)}$ = 69.39), H_e ($F_{(2, 138)}$ = 80.46) and $\overline{F}_{SR}$ ($F_{(2, 138)}$ = 100.00) were significantly different (P < 0.001) among naturally isolated, human isolated, and connected populations in the Columbia River basin (Fig. 3). Tukey's HSD showed that all three categorical levels were significantly different from one another for all three response variables (P < 0.003), where naturally isolated populations had the lowest genetic variation and highest differentiation on average, and human-isolated populations had lower genetic variation and higher differentiation than interconnected populations. In contrast, in the Missouri River basin P, H_e, and $\overline{F}_{SR}$ were not significantly different (P > 0.894) between human-isolated and naturally-isolated populations (Fig. 3). Subsequent results (Tukey's HSD) further showed that human-isolated and naturally-isolated populations in the Missouri River drainage have significantly P and H_e, and higher $\overline{F}_{SR}$ than interconnected and human-isolated populations in the Columbia basin (P < 0.006), but had similar P, H_e, and $\overline{F}_{SR}$ as naturally isolated populations in the Columbia drainage (P > 0.121). In other words, the genetic variation and differentiation found in populations throughout the Missouri

drainage is equivalent to that found in naturally isolated populations in the Columbia River drainage.

In the Columbia basin, the extent of genetic differentiation within a watershed (F_{SW}) was strongly correlated with the proportion of isolated populations within a watershed (Pearson's R = 0.641, n = 11, P = 0.042; Fig. 4). Relative to the hierarchical F-statistics from the entire data set for the Columbia Basin, removing isolated populations (both natural and anthropogenic) reduced genetic differentiation among all populations by 23.8% (F_{SR} = 0.231), and among populations within watersheds by 42.2% percent (global F_{SW} = 0.118). The F_{SW} estimate for interconnected populations in the Columbia River basin was over three times smaller than the global estimate of F_{SW} in the Missouri Drainage (0.364).

Potential effects of assisted translocation for genetic rescue

We identified 23 target populations that appear to be immediate candidates for genetic rescue efforts based on their isolation status (human isolated), standing genetic variation (lowest quartile of observed genetic variation), and genetic divergence ($\overline{F}_{SR}$ > 0.40). Twenty (87%) of those populations were found in the Missouri River drainage (Table S1), and three (13%) were found in the Columbia drainage (Fig. 1; Table S2). The majority (51.3%) of human-isolated populations (20 of 39) in the Missouri River basin were candidates for genetic rescue.

We failed to detect any genetic variation in one of those populations (Autumn Creek), and thus, the expected percent change in genetic variation following assisted gene flow for that population is infinite. Therefore, we focused on the expected change in genetic variation for the remaining 22 target populations (Table 2). Genetic variation was predicted to strongly increase in all populations, with the average expected increase in genetic variation per population ranging from 9.6 to 582%. The median expected increase in genetic variation across all populations was 33.1%. Importantly, those average values are based on expected outcomes of translocations from each population in the same watershed, or contiguous watersheds, often from populations with very low genetic variation. In all cases, some population combinations resulted in much larger predicted changes in genetic variation; the maximum predicted increase in genetic variation per population ranged from 27.9 to 705.5%, with a median value of 58.4%. Population specific results can be found in Supplementary Tables S7-S16.

Discussion

Human-isolation appears to have strongly influenced evolutionary dynamics in numerous populations of WCT. Genetic variation was substantially lower and genetic differentiation was significantly elevated in human-isolated populations, especially those found in the Missouri River drainage. The consequences of human-isolation for genetic variation and differentiation are approaching (Columbia drainage) or equivalent to (Missouri drainage) that observed in naturally-isolated populations. This is remarkable given that the period of isolation is dramatically different – approximately 100 years for human-isolated populations vs. 1000s of years for naturally isolated populations. The

widespread and presumably rapid loss of genetic variation in human-isolated populations of WCT demonstrates that new conservation strategies are needed to bolster genetic variation and ameliorate potential inbreeding depression. Fortunately, strategic translocations of small numbers of fish (<10 adults) could strongly increase genetic variation.

Genetic variation and population structure

Broadly speaking, the patterns of genetic variation and genetic differentiation herein are consistent with previous research on WCT. A number of studies have documented relatively high genetic differentiation among local populations, but sampling was often relatively sparse and conducted over multiple major watersheds or the species entire range (Leary et al. 1985, Taylor et al. 2003, Drinan et al. 2011). Similarly, studies have also noted that isolated populations of WCT have lower genetic variation and higher genetic differentiation, but focused on naturally isolated populations (Taylor et al. 2003) or was limited to one part of the WCT range (Carim et al. 2016). Our results include sufficient sampling of interconnected and isolated populations to tease apart the relative effect of human-isolation, even compared to naturally-isolated populations.

In the Columbia River drainage, genetic variation in human-isolated populations has decreased, on average, by more than 30% relative to interconnected populations (Fig 2). Furthermore, estimates of genetic differentiation were strongly influenced by the inclusion of isolated populations, demonstrating that the genetic structure of this species

is now strongly influenced by human fragmentation. It is important to note that habitat patch size may be a confounding variable in these analyses, as isolated streams will have smaller upstream habitat patches, almost by definition, and smaller habitat patch sizes are typically associated with lower genetic variation in stream salmonids (Whiteley et al. 2013, Kovach et al. 2015). Indeed, it seems quite likely that isolation and small habitat patch size are almost certainly working together to reduce genetic variation and increase differentiation in WCT. Thus, it is important to note that the observed patterns are not due to isolation alone, but pragmatically the underlying driver (isolation vs. small population size) is of less concern than the actual biological outcome: low genetic variation and very extreme genetic differentiation in many human-isolated populations.

The genetic consequences of isolation were more acute in the Missouri River drainage, where genetic variation and differentiation in human-isolated populations are now equivalent to naturally isolated populations that have been fragmented from other WCT populations for thousands of years. Relatively small amounts of genetic variation and extreme differentiation of WCT populations in the Missouri River drainage reflects the complete loss of a migratory life history throughout the entire river basin, coupled with a legacy of extensive human impacts, especially invasive species and land-use, that generally exceeds human impacts in the Columbia River drainage (Shepard et al. 1997, Shepard et al. 2005). Populations declines have been far more dramatic in the Missouri drainage (Shepard et al. 1997).

Thus, anthropogenic isolation, coupled with habitat degradation and expansion of invasive species, appears to have markedly influenced the evolutionary dynamics of WCT. Unfortunately, because of the ongoing threat of hybridization and competition with other invasive species, fragmentation is now critical for native trout conservation in many regions (Faush et al. 2009) and a growing number of trout populations are being intentionally isolated. Collectively, previous studies (e.g., (Fumagalli et al. 2002, Wofford et al. 2005, Neville et al. 2006, Whiteley et al. 2013) and our data emphasize that isolation comes with genetic costs, as expected by conservation genetic theory (Allendorf et al. 2017).

Assuming that genetic variation and differentiation among interconnected populations in the upper Columbia represents a template for microevolutionary dynamics (i.e., gene flow, genetic drift, and selection) elsewhere, these data suggest that genetic drift is likely dominant in many populations, which in turn suggests that N_e is often small. This is consistent with very small estimates of N_e in isolated WCT populations – often less than 50 – reported in Carim et al. (2016). Thus, evolutionary dynamics in many human-isolated populations of WCT may be largely driven by stochastic random processes, which greatly reduces the efficacy of natural selection (Allendorf et al. 2013). Even where natural selection may be strong enough to overwhelm drift in small populations, reduced genetic variation in isolated populations, like those herein, limits evolutionary potential, as genetic variation is necessary for adaptive evolution (Willi et al. 2006, Hoffman et al. 2017).

Moreover, small effective populations sizes coupled with rapid loss of genetic variation greatly increase the risk that populations are suffering from inbreeding depression (Reed et al. 2003). Fundamentally, when populations become isolated and rapidly decline in size (true for WCT and many other fish species), they become exposed to more deleterious genetic variation that has increased in frequency due to small effective population size and close-inbreeding (Kardos et al. 2021). These theoretical expectations have been confirmed in experimental (e.g., Markert et al. 2010, Ørested et al. 2019) and wild populations (e.g., Bozzuto et al. 2019, Grossen et al. 2020, von Seth et al. 2021) that have low fitness and high genetic load. Furthermore, an increasing number of studies have documented a positive relationship between individual heterozygosity and fitness (Scott et al. 2020, Hasselgren et al. 2021, Stoffel et al. 2021). An implicit assumption herein, and in most of conservation genetic work, is that estimates of genetic variation scale negatively with risk of inbreeding depression and loss of evolutionary potential, and this is supported by the broader literature. While exceptions certainly exist, and the relationship is imperfect, estimates of genetic variation and differentiation remain the best means for pragmatic conservation aimed at addressing the genetic consequences of population isolation (Frankham et al. 2017, Kardos et al. 2021).

Genetic rescue

Genetic rescue offers a management tool that can address the genetic consequences of fragmentation in situations where it is largely impossible to re-connect human-isolated populations (Tallmon et al. 2004, Whiteley et al. 2015). We used a

simple set of genetic and ecological criteria to prioritize which populations are potentially most in need of genetic rescue, the vast majority of which were found in the Missouri River drainage. Various other criteria could be used in place of or in addition to our prioritization template (e.g., see Frankham et al. 2017), however, results based on population genetic expectations suggest our schema was appropriate. All populations were expected to experience substantial increases in genetic variation following a small gene flow event of approximately 5 to 10 individuals (Table 2).

The strong positive effect of translocation on genetic variation in target populations, even in the Missouri River drainage where population genetic variation is generally low, arises because more than 36% the genetic variation in WCT is found among populations (based on F_{SW} and F_{SR}). Each isolated population appears to have undergone random genetic drift resulting in substantial allele frequency differences among populations, and low genetic variation within populations. Indeed, most SNP loci were polymorphic within a watershed but not within a population (Table 1). This strong differentiation, a function of isolation itself, lends itself particularly well to increasing genetic variation with strategic translocation for genetic rescue.

On average, randomly selecting another donor population from within the same watershed results in an expected increase in genetic variation of 36%, and strategic translocation (based on observed allele frequency differences or differences in genetic variation) could result in an increase of genetic variation in focal target populations exceeding 50% on average. This is likely an underestimate because increased fitness

of intraspecific hybrids following translocations increases genetic variation more than if the fitness effects were neutral (Robinson 2020), as was assumed in our simple population genetic predictions. Further, the results provide a clear template by which to exclude population combinations that provide a negligible increase in genetic variation in the focal population, which is particularly important given that translocation comes at a demographic cost to the donor population.

Importantly, genetic rescue also offers an opportunity to restore natural processes (gene flow) and thus, the evolutionary legacy of WCT. Multiple analyses demonstrated that genetic differentiation within watersheds was fairly low when populations are interconnected (Fig. 3f, Fig. 4), which argues that genetic rescue, in addition to bolstering genetic variation, will also help restore natural connectivity and gene flow. Indeed, WCT were a highly migratory species prior to isolation, and still complete long-distance migrations in select (interconnected) river basins throughout the Columbia River basin (e.g., Schmetterling 2003).

Although we do not provide direct evidence for genetic rescue, which requires data of fitness and population growth (Whiteley et al. 2015; Bell et al. 2019; Robinson et al. 2020), increases in within-population genetic variation will reduce genome-wide homozygosity, and thus, genetic rescue seems probable in these populations. Further, the large increases in genetic variation predicted from assisted gene flow should translate to higher adaptive potential and lower vulnerability to changing environmental conditions. For example, adaptive capacity in the face of climate change depends, at

least in part, on standing genetic variation (e.g., Bay and Palumbi 2015). Nevertheless, experimental tests of translocation for genetic rescue are needed to validate theory. Preliminary results from a genetic rescue experiment in brook trout (*Salvelinus fontinalis*) suggests that this general approach was initially (through the first generation) successful (Robinson et al. 2017), both in terms of bolstering genetic variation and also improving individual and population fitness. Similar experimental tests are underway for WCT (Bell and Whiteley, *unpublished data*).

While the basic concept of genetic rescue is simple, attempting genetic rescue is not trivial and is certainly not without risk (Tallmon et al. 2004, Bell et al. 2019). Evidence for local adaptation in salmonid fishes (Taylor 1991) increases the risk that outbreeding depression could potentially occur as a result of translocations. Because of that, empirical attempts at genetic rescue have been rare in salmonid fishes, and in fishes more broadly (Wells et al. 2019). Our prioritization scheme for identifying target populations attempts to minimize the risk of outbreeding depression by focusing only on the most genetically depauperate populations where heterosis should be maximized (Frankham 2015). Similarly, using donor populations from within the same watershed decreases the likelihood of outbreeding depression, while simultaneously restoring historical patterns of connectivity and gene flow.

Furthermore, we focused only on those populations that were isolated due to human fragmentation and are suffering from contemporary loss of genetic variation. Populations that have been naturally isolated for thousands of years might suffer from

fixed genetic load, but are also more likely to have purged deleterious genetic variation that may result in inbreeding depression (Garcia-Dorado 2015, Hedrick and Garcia-Dorado 2016). Instead, the rapid loss of genetic variation within a population is particularly concerning, as deleterious recessive genetic variation of both moderate and large effect can be exposed at relatively high frequency (e.g., Grossen et al. 2020), which greatly increases the risk of inbreeding depression.

Last, we used a small migration rate (m=0.1) that minimized the potential for genetic swamping if admixed individuals are favored (as expected), but is small enough that any outbreeding depression, if it were to occur, would be limited to small portion of the population. Additionally, it is critical to note that genetic rescue efforts for species like WCT (threatened with non-native hybridization with rainbow trout and Yellowstone cutthroat trout) must insure that translocations do not inadvertently move interspecific hybrids; fortunately, genomic methods can identify late-generation hybrids with small amounts of non-native ancestry (Kovach et al. 2016), and similar methods can now be easily developed in other species (McFarlane and Pemberton 2019). Ultimately, low effective population size and high inbreeding are consequences of poor or limited habitat, and if these underlying problems aren't resolved, the genetic issues will eventually arise again. We recommend translocations for purposes of genetic rescue in three cases: 1) To promote persistence when no other options are available, 2) to buy time until other strategies are implemented (e.g., habitat improvement, invasive suppression, dam removal, etc.), and 3), ideally as part of a broader conservation strategy that includes other actions.

Conclusions

Overall, these results are directly relevant to innumerable freshwater fish species worldwide. Human fragmentation of freshwater ecosystems is ubiquitous, which greatly increases the likelihood that the results presented herein are also pervasive; indeed, widespread connectivity in riverine habitats is now the rarity, not vice versa. Furthermore, the vast majority of native fish species are declining in abundance, not increasing (e.g., Jelks et al. 2008). This strongly suggests that evolutionary dynamics in many populations of conservation concern are increasingly driven by random (non-adaptive) genetic drift. Similar empirical analyses remain rare, but are emerging in the literature with similar conclusions (e.g., Coleman et al. 2013, Pavlova et al. 2017). Thus, attempting genetic rescue is likely warranted for populations of many fish species – it is certainly a relevant conservation tool that merits discussion for similar salmonid fishes, many of which have populations that are declining in abundance, increasingly isolated, and strongly effected by genetic drift (Fumagalli et al. 2002, Wofford et al. 2005, Neville et al. 2006, Whiteley et al. 2013 Camak et al. 2021).

Although translocation for genetic rescue is likely a viable tool for freshwater fish conservation, it must be grounded in strong empirical data both prior to action and post-translocation (i.e., monitoring outcomes). It is critical that genetic rescue efforts do not contribute to the massive problem of unmonitored translocation and release of fishes that still continues today (Laikre et al. 2010). This analysis benefits greatly from an exceptional decades long genetic monitoring program (beginning in 1979) that first

identified remaining non-hybridized populations, and now, can be used to plan and prioritize genetic rescue efforts. Although robust genetic inventories have been conducted for some species, especially salmonids, this type of template is not available for many freshwater fish species. This emphasizes the immense value that large-scale genetic monitoring can provide for immediate and long-term conservation efforts – indeed, the need to expand genetic monitoring has never been greater as fish are increasingly subject to escalating human stressors. We strongly encourage fish biologists and managers to begin describing extant genetic variation and structure in many native species so that similar analyses can identify genetically depauperate populations and strategize future conservation efforts. Those efforts will be most effective when they are strategic and directly integrated with local conservation actions, including prioritization of genetic rescue efforts.

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Tables

Table 1. Population genetic variation and differentiation within watersheds in the Missouri and Columbia River basins. 'N' is the number of local populations with data within each watershed. Here, $\overline{H_e}$ is the average expected heterozygosity (across populations) and *P* is the total proportion of polymorphic loci. F_{SW} is the global estimate of the fixation index (i.e., F_{ST}) among populations within each watershed.

Watershed	Basin	N	$\overline{H_e}$	*P*	F_{SW}
Beaverhead	Missouri	7	0.082	0.688	0.464
Belt	Missouri	5	0.128	0.656	0.381
Big Hole	Missouri	16	0.086	1.000	0.415
Red Rock	Missouri	10	0.110	0.938	0.269
Ruby	Missouri	5	0.058	0.406	0.672
Upper Missouri	Missouri	7	0.080	0.813	0.499
Bitterroot	Columbia	22	0.353	1.000	0.060
Blackfoot	Columbia	13	0.339	0.969	0.139
Flint-Rock	Columbia	19	0.334	0.969	0.127
Lower Clark Fork	Columbia	20	0.242	0.969	0.216
Middle Fork Flathead	Columbia	18	0.201	1.000	0.405
Middle Kootenai	Columbia	3	0.178	0.938	0.570
North Fork Flathead	Columbia	14	0.266	0.969	0.201
South Fork Flathead	Columbia	10	0.249	0.969	0.191
Stillwater	Columbia	3	0.180	0.906	0.194
Swan	Columbia	8	0.209	1.000	0.336
Upper Clark Fork	Columbia	5	0.247	0.938	0.229

Table 2. Expected change in genetic variation in candidate populations for genetic rescue. 'Change' is the average percent change in genetic variation across all population combinations. 'Max' is the maximum percent change in genetic variation. Summary statistics describe extant genetic variation (H_e and P) and differentiation ($\bar{F}_{SR}$).

Population	Watershed	H_e	P	$\bar{F}_{SR}$	Change	Max
Brays Creek	Beaverhead	0.062	0.281	0.424	18	31.2
Jake Canyon Creek	Beaverhead	0.064	0.156	0.513	30.2	50
Bender Creek	Big Hole	0.034	0.125	0.583	56.1	98.2
Little American Creek	Big Hole	0.056	0.258	0.459	27.1	57.1
Blind Canyon Creek	Big Hole	0.054	0.125	0.423	26.5	59.6
Rabbia Creek	Big Hole	0.087	0.344	0.416	14.2	35.9
S. Fork N. Fork Divide Creek	Big Hole	0.045	0.097	0.439	36	66.8
Doolittle Creek	Big Hole	0.057	0.156	0.435	26	56
Squaw Lake	Big Hole	0.076	0.250	0.501	22.7	50.9
Twelvemile Creek	Big Hole	0.104	0.281	0.408	9.6	27.9
Wild Horse Creek	Gallatin	0.038	0.125	0.449	42.8	95.5
Bean Creek	Red Rock	0.063	0.188	0.438	19.4	55.3
Bear Creek	Red Rock	0.020	0.063	0.541	91.6	207
Craver Creek	Red Rock	0.066	0.188	0.416	17.1	49.6
Dark Hollow Creek	Ruby	0.055	0.344	0.548	18.3	25.8
Ramshorn Creek	Ruby	0.004	0.031	0.776	582	705.5
Lone Willow Creek	Smith	0.034	0.219	0.609	93.3	129.8
Hall Creek	Upper Missouri	0.060	0.188	0.556	37.6	57.2
S. Fork Quartz Creek	Upper Missouri	0.027	0.125	0.638	101.4	142.1
Staubach Creek	Upper Missouri	0.021	0.094	0.593	102.6	149.7
Autumn Creek	Middle Fork Flathead	0.000	0.000	0.607	∞	∞
Munson Creek	Lower Clark Fork	0.060	0.161	0.560	92.3	115.8
Slowey Gulch	Middle Clark Fork	0.069	0.219	0.603	106.4	151.8

Figures

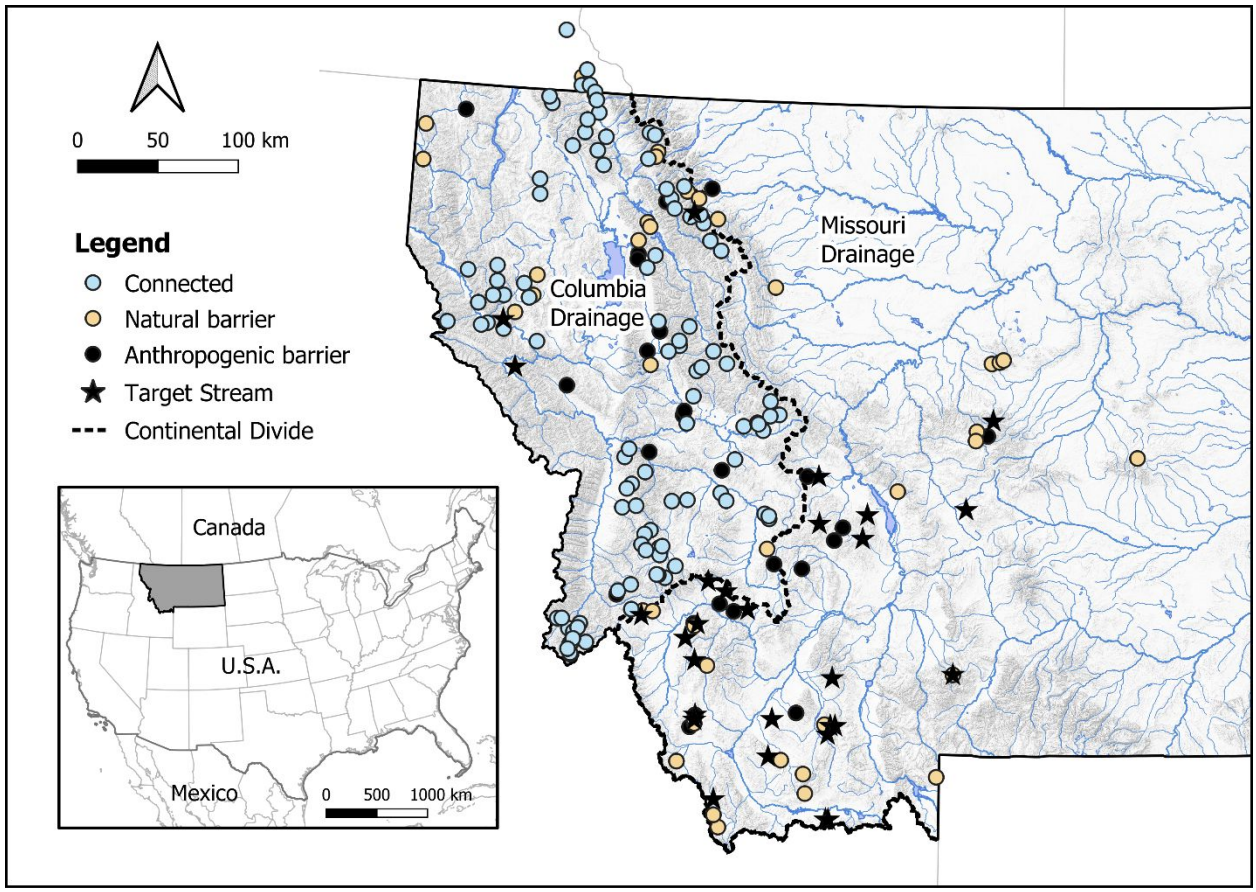

Figure 1. Map of study area. Population samples (dots) are colored based on their isolation status (see legend). The Continental Divide that separates the Columbia River basin (on left) from the Missouri River basin (on right) is depicted by the black dashed line. Candidate populations for genetic rescue are highlighted with a star (Target Stream in Legend). Map layers were accessed from the Montana State Library (https://geoinfo.msl.mt.gov).

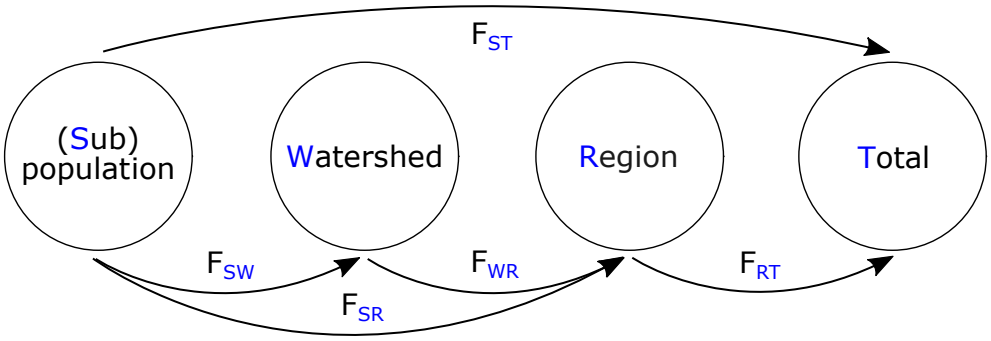

Figure 2. A diagram depicting the various F-statistics that were computed across four-hierarchical levels: the local Subpopulation, Watershed (HUC 8), Region (Missouri or Columbia basin), and Total.

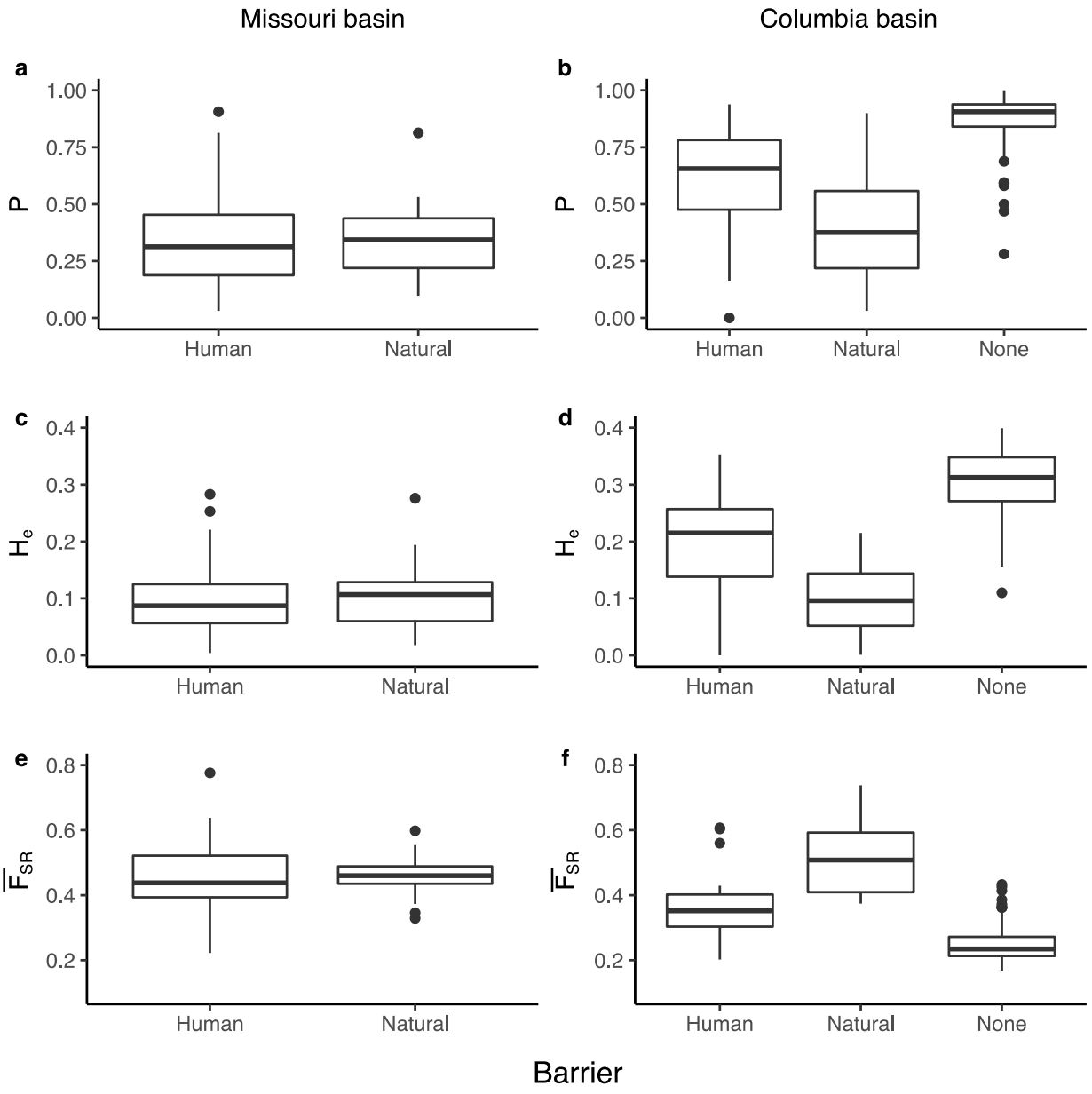

Figure 3. Box and whisker plots depicting the distribution of population genetic variation (*P* and H_e) and mean population differentiation ($\overline{F}_{SR}$). Population data are grouped by Barrier and thus isolation status – isolated by "Human" barriers, "Natural" barriers, or interconnected ("None"). Results for westslope populations in the Missouri drainage are left (panels a, c, e), while results for Columbia drainage populations are right (panels b, d, f).

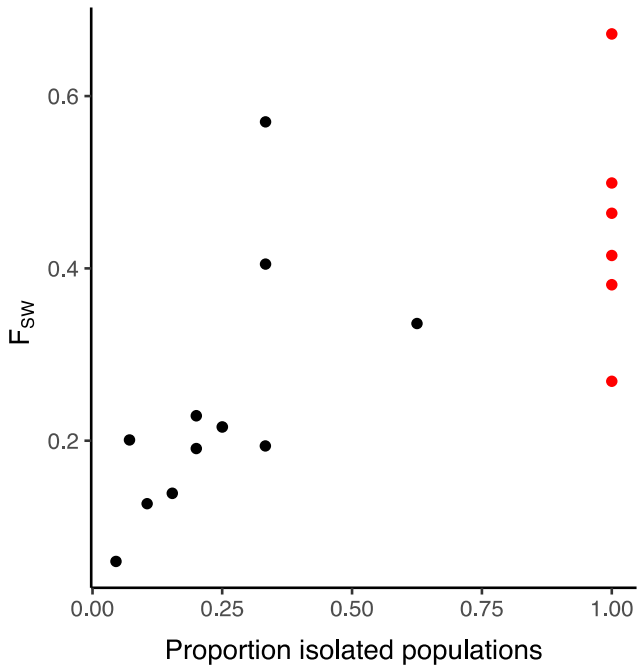

Figure 4. Genetic differentiation among populations within watersheds (F_{SW}) relative to the proportion of isolated populations within a watershed. The black points are for watersheds in the Columbia River basin, while the red points (far right) are for watersheds in the Missouri River basin.