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LETTER

Genetic variation and hybridization determine the outcomes of conservation reintroductions

Colter A. Feuerstein[1] | **Ryan P. Kovach**[2] | **Carter G. Kruse**[3] | **Matt E. Jaeger**[4] | **Donovan A. Bell**[1,2] | **Zachary L. Robinson**[1] | **Andrew R. Whiteley**[1]

[1]Wildlife Biology Program, W.A. Franke College of Forestry and Conservation, University of Montana, Missoula, Montana, USA

[2]Montana Fish, Wildlife & Parks, Missoula, Montana, USA

[3]Turner Enterprises Inc., Bozeman, Montana, USA

[4]Montana Fish, Wildlife & Parks, Dillon, Montana, USA

Correspondence
Colter A. Feuerstein, Wildlife Biology Program, W.A. Franke College of Forestry and Conservation, University of Montana, Biological Sciences HS104, 32 Campus Drive, Missoula, MT 59812, USA.
Email: colter.af96@gmail.com

Present addresses:
Zachary L. Robinson, Columbia River Inter-Tribal Fish Commission–Hagerman Genetics Laboratory, Hagerman, ID, USA, and Colter A. Feuerstein, Montana Fish, Wildlife & Parks, Billings, MT, USA.

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Abstract

The preservation of genetic variation is fundamental in biodiversity conservation, yet its importance for population viability remains contentious. Mixed-source reintroductions, where individuals are translocated into a single vacant habitat from multiple genetically divergent and often depauperate populations, provide an opportunity to evaluate how genetic variation and hybridization influence individual and relative population fitness. Population genetic theory predicts that individuals with higher genetic variation and hybrids among populations should have higher fitness. We tested these two hypotheses by analyzing individual and population-scale data for westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) in four mixed-source reintroductions. We observed more hybrid and fewer nonhybrid offspring than expected across four independent mixed-source reintroductions. We also found clear evidence that heterozygosity influenced individual reproductive and relative population fitness. Overall, we found a strong, positive relationship between genetic variation, hybridization, and transplant fitness, emphasizing the importance of genetic variation and population mixing in conservation.

KEYWORDS

conservation, fitness, genetic variation, heterosis, hybridization, reintroduction, translocation

1 | INTRODUCTION

Amid unprecedented declines in biodiversity, imperiled species commonly persist in small, isolated populations, fragmented across a once connected landscape. Remnant populations of conservation concern often have low genetic variation (i.e., heterozygosity), due to increased inbreeding and genetic drift, which in turn can influence population viability (Allendorf et al., 2022; Bozzuto et al., 2019; Frankham et al., 2017; Saccheri et al., 1998). Although genetic variation is fundamental to population persistence (Burger & Lynch, 1995; Lande & Barrowclough, 1987;

Ralls et al., 2018; Reed & Frankham, 2003), its role in conservation is frequently debated due to a lack of empirical data connecting genetic variation to population viability and conservation success in natural populations (Kardos et al., 2021; Teixeira & Huber, 2021). Thus, understanding how genetic variation influences conservation outcomes is crucial, especially as more species are pushed toward extinction.

Reintroductions are a critical conservation tool that are increasingly used to refound populations following extirpation (Seddon & Armstrong, 2016; Soorae, 2018). Mixed-source reintroductions that use populations with varying genetic variation provide an excellent opportunity to address questions regarding the importance of genetic variation in conservation practice. Increasing evidence suggests that hybridization (mixing) between genetically depauperate populations can improve fitness by alleviating inbreeding depression (i.e., heterosis) (Edmands, 2007; Whiteley et al., 2015; Johnson et al., 2017; Robinson et al., 2017; Bell et al., 2019; Bozzuto et al., 2019; Pregler et al., 2023), and that individuals with higher heterozygosity may have increased fitness (Lacy, 1997; Charpentier et al., 2005; Bozzuto et al., 2019; Scott et al., 2020). Therefore, mixing source populations via reintroduction may serve as a critical method for increasing both population viability and individual fitness by mitigating inbreeding effects and increasing genetic variation (Hoffmann et al., 2021). Despite this potential, few studies have addressed the relationships between individual heterozygosity, hybridization, and conservation success in nature. In turn, it remains unclear whether sourcing individuals for reintroductions from one or multiple populations is optimal, and whether population genetic variation should be considered when selecting source populations (Jamieson & Lacy, 2012; Taylor et al., 2017; Biebach et al., 2019).

Freshwater organisms are frequently threatened by population fragmentation and isolation (Brauer & Beheregaray, 2020; Su et al., 2021). The westslope cutthroat trout (*Oncorhynchus clarkii lewisi*), a freshwater salmonid native to western North America, has substantially declined throughout its historical range due to the introduction of nonnative species (Shepard et al., 2005; Bell et al., 2021). Many remaining westslope cutthroat trout populations are small and isolated with extremely low genetic variation (Kovach et al., 2022). To reduce the negative genetic effects of isolation, preserve remaining genetic variation, and increase range-wide abundance and distribution, several conservation efforts have eliminated nonnative species from historically occupied westslope cutthroat trout streams and reintroduced multiple source populations into newly vacated habitat. Here, we used genetic data from four mixed-source westslope cutthroat trout reintroductions in the upper Missouri River basin of Montana to describe how genetic variation and among-population hybridization influence conservation outcomes at individual and population scales (Figure 1a).

2 | METHODS

2.1 | Study design and sample collection

To restore westslope cutthroat trout to historically occupied habitat, nonnative salmonid species above artificial or natural stream barriers were removed from the North and South Forks of Greenhorn (NF Greenhorn and SF Greenhorn), Peet, and Ruby Creeks. Following nonnative salmonid removal, source individuals were captured from nine geographically and genetically separated populations and translocated to NF Greenhorn, SF Greenhorn, Peet, and Ruby Creeks from 2015 to 2018 (Figure 1a,b). Two source populations were used to refound Peet, Ruby, and SF Greenhorn, whereas three sources were used in NF Greenhorn. Source individuals were released near or in the same stream sections each year. Genetic samples were collected from all source individuals used to refound NF Greenhorn and SF Greenhorn but not from source individuals used to refound Peet and Ruby. Yearly translocations of source individuals from the five populations used to refound NF Greenhorn and SF Greenhorn were mostly equal, whereas source translocations into Peet and Ruby were variable by population and year (Table S1).

Reproduction between source individuals began in 2016 in Ruby and 2017 in NF Greenhorn, SF Greenhorn, and Peet. We sampled reintroduction streams in 2019 and 2020 to capture offspring, the vast majority of which (>98%) were first-generation (F_1) progeny of adult transplants (Supporting Information). To capture offspring, we sampled 200–1000 stream m at release sites, then systematically sampled additional 200–1,000-m reaches, spaced semi-evenly throughout SF Greenhorn, Peet, and Ruby reintroduction streams. In NF Greenhorn, we followed a similar sampling design, but approximately doubled sampling efforts at each site (1000–1500 m) to increase sample size. These methods allowed us to subsample the entire occupied habitat of each stream, minimize capturing offspring family groups (i.e., capturing offspring representative of the entire population), and maximize sampling size and distance. Offspring were captured using a backpack electrofisher, genetic samples were collected from fins, and individual length was measured to the nearest millimeter.

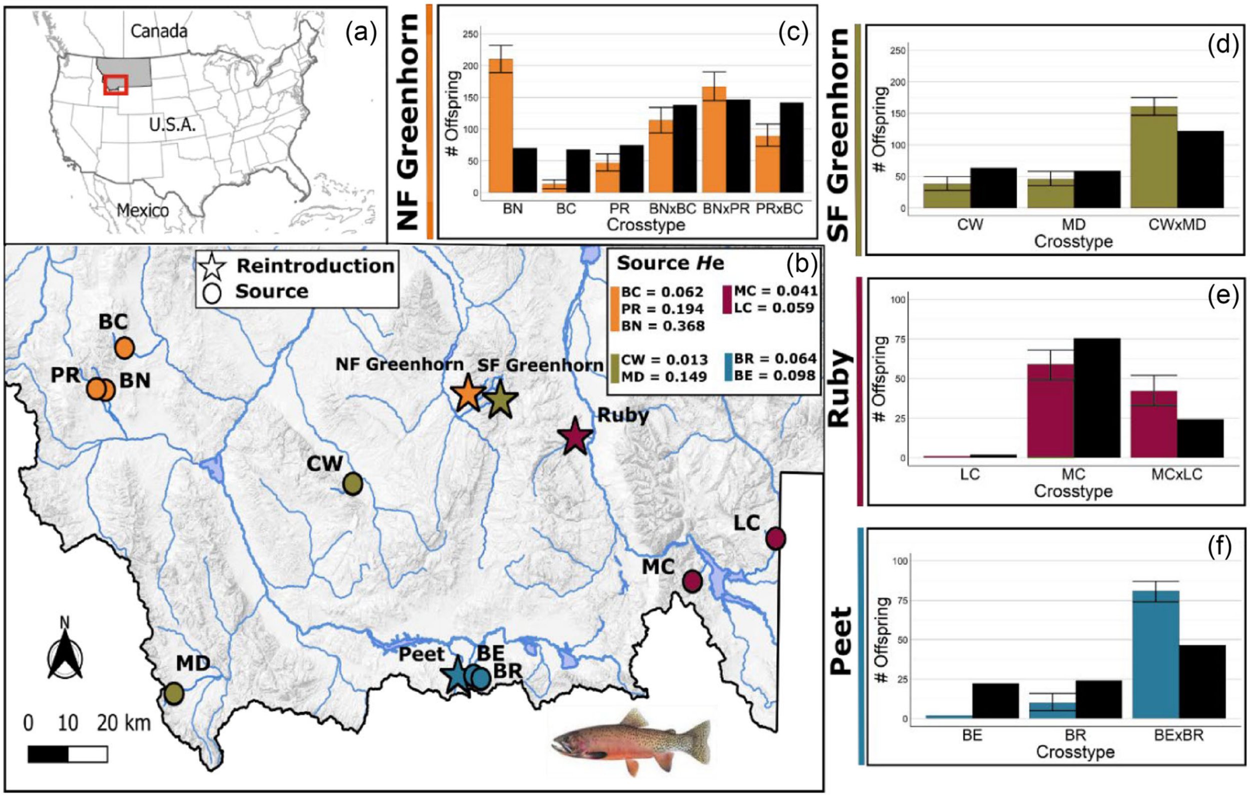

FIGURE 1 Newly founded, mixed-source westslope cutthroat trout populations dominated by genetically variable source populations and hybrid crosstypes. Map of Montana, USA (a) showing source populations (acronyms) and their corresponding, color-coded reintroduction locations (full names); see text for naming explanation of source and reintroduction streams. The expected heterozygosity of each source population (b) and number of observed (colored bars) versus expected (black bars) offspring assigned to crosstype following reintroduction efforts into NF Greenhorn (c), SF Greenhorn (d), Ruby (e) and Peet (f) Creeks. Note that 95% confidence intervals are shown for observed offspring.

2.2 | Genetic data and analysis

We used tissue samples from every translocated individual ($n = 686$) and sampled offspring ($n = 1257$) for population and parentage assignment in NF Greenhorn and SF Greenhorn. For Peet and Ruby reintroductions, we used tissue samples from source populations BE ($n = 24$), BR ($n = 25$), LC ($n = 29$), and MC ($n = 26$), and offspring from Ruby ($n = 102$) and Peet ($n = 93$) for population assignment. To distinguish between source and reintroduction populations throughout this manuscript, we used acronyms for source population names (e.g., BE, BR, LC, and MC) and kept reintroduction names the same (e.g., Peet and Ruby). In total, 2242 genetic samples from founder individuals and subsequent progeny were genotyped using GT-seq (Campbell et al., 2015). We used an existing GT-seq panel of 373 westslope cutthroat trout single nucleotide polymorphisms (SNPs) (one of which was a sex ID marker) developed by the Idaho Fish & Game Eagle Fish Genetics Lab. After removing loci that consistently deviated from Hardy–Weinberg proportions across populations, we proceeded with parentage and population assignment testing using 251 (NF Greenhorn and SF Greenhorn), 81 (Peet), and 76 (Ruby) variable locus datasets. Additionally, we genotyped SF Greenhorn source individuals and progeny using a RAD Capture panel that targeted 10,000 RAD tags (Strait et al., 2021) to increase parentage assignment accuracy. We similarly removed loci that consistently deviated from Hardy–Weinberg proportions and proceeded with approximately 4800 SNPs in SF Greenhorn (Supporting Information).

Genetic parentage analysis (Cockburn et al., 2021) was used to assign NF Greenhorn and SF Greenhorn offspring to candidate parents and population crosses (crosstypes) (Table S2). Stringent parentage assignment rules resulted in successfully assigning 640 NF Greenhorn offspring and 179 SF Greenhorn offspring to parents (Supporting Information; Table S2). We used *STRUCTURE* (Hubisz et al., 2009) to assign offspring to crosstypes in Ruby and Peet. Simulations revealed high accuracy in assigning offspring to source populations (100%) and NF Greenhorn and SF Greenhorn parents (>94%; Supporting Information).

2.3 | Statistical analyses—Crosstype success and relative population fitness

To describe relative source population fitness as a function of population mixing and genetic variation, we estimated expected heterozygosity (H_e; the proportion of heterozygous genotypes expected under Hardy–Weinberg proportions) using R package *hierfstat* (Goudet & Jombart, 2021) for all source populations (Figure 1b) and compared observed to expected offspring crosstype proportions in NF Greenhorn and SF Greenhorn. Expected crosstype proportions were estimated using the initial reintroduction proportions of each source population assuming random mating and equal survival and reproduction from 2016 to 2020. We performed a chi-square test between observed and expected crosstype proportions and adjusted significance values for multiple testing using sequential Bonferroni procedures (Rice, 1989).

We then summarized relationships between genetic variation, hybridization, and crosstype success among all reintroduction streams. Specifically, we calculated observed crosstype heterozygosity ($n = 15$) and z-score standardized estimates for each reintroduction stream separately. We then calculated crosstype success as the difference between observed and expected offspring counts, which was z-score standardized for each stream separately. We used nonlinear regression to model crosstype success and assumed an exponential relationship between crosstype success and observed crosstype heterozygosity (Supporting Information).

2.4 | Statistical analyses—Individual fitness

Our sampling design (namely that we sampled all source parents and a larger proportion of the F1 generation) allowed us to conduct additional tests of crosstype success in NF and SF Greenhorn. We estimated full-sibling, age-1 family size (i.e., family size of the first cohort produced by source populations) and the number of offspring per parent (individual reproductive success). Crosstype effects on family size were evaluated using a generalized linear model (GLM) with a Poisson distribution and log link. Since family size is potentially influenced by parental body size (Koch & Narum, 2021) and density (Nicola et al., 2008), we included maternal length and cohort year as covariates (Supporting Information). Although family size is influenced by multiple factors, including the number of eggs produced by a female and fertilization rate, crosstype should be most closely related to survival from fertilization to age-1, and we thus consider the influence of crosstype on family size to be a proxy for the influence of crosstype on juvenile survival (a life-stage that cannot be monitored using other methods). We compared family size estimates between hybrid crosstypes and their parental crosstype counterparts as an additional heterosis test.

We examined individual reproductive success by modeling two separate processes in NF Greenhorn and SF Greenhorn: (i) whether a source individual survived and reproduced (i.e., survival and reproduction) as a function of individual heterozygosity, sex, length, translocation year, and population of origin, and, given that an individual reproduced (ii) reproductive success as a function of the same covariates. A Bernoulli GLM with a logit link function was used to model the first process (logistic regression model), while a zero-truncated Negative Binomial GLM with a log link function was applied for the second process (count model). This model, commonly known as a hurdle model, has been used for similar analyses (Huisman et al., 2016; Samuelson et al., 2018). We then multiplied the predicted fitness from the two processes to obtain an aggregate fitness estimate to further evaluate fitness effects. All GLMs were analyzed in a Bayesian framework. We report statistical support using the probability of direction (*pd*), which is the proportion of estimates from the posterior distribution that are in the same direction as the median and analogous to a p-value.

Although population of origin was included as a covariate in these hurdle models to account for among population variation and distinguish between population- and individual-level effects, we further examined survival and reproduction, and reproductive success separately for individuals from each NF Greenhorn source population (e.g., modeling individual reproductive success separately for BN, BC, and PR). Such analyses were necessary to describe individual fitness within (not among) each source population and further determine whether a population-level effect was responsible for individual-level results.

3 | RESULTS

3.1 | Relative population and crosstype fitness

Observed crosstype proportions were different from random expectation in all four reintroduction sites (Figure 1; Table S4). In NF Greenhorn, offspring from BN, the most genetically variable population ($H_e = 0.368$), were three times more abundant than expected, while offspring from BC, the least genetically variable population ($H_e = 0.062$), were five times less abundant than expected (Figure 1c; Table S4). Hybrid offspring from crosstypes with BC

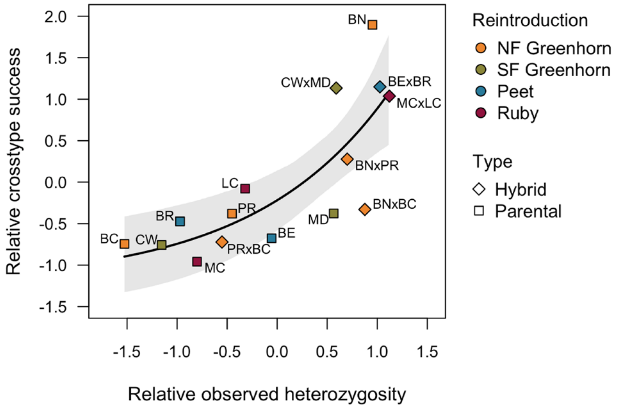

FIGURE 2 Crosstypes with greater relative heterozygosity had increased relative success. Colors describe reintroduction stream and shapes show crosstype type: parental or hybrid. See text for naming explanation of source and reintroduction streams. Relative observed heterozygosity values are described by low (negative) and high (positive) heterozygosity values. Values along both axes were standardized within reintroduction streams to account for competitive dynamics and to emphasize the importance of relative fitness within a site. The black line describes an exponential relationship between crosstype success and heterozygosity. The gray band represents the 95% credible interval.

ancestry were underrepresented, while offspring from PRxBN hybrids were more abundant than expected (Figure 1c; Table S4). In SF Greenhorn, offspring from the least genetically variable parental crosstype (CW, $H_e = 0.013$) were less abundant than expected, whereas offspring from the hybrid crosstype (CWxMD) were more abundant than expected (Figure 1d; Table S4). In Ruby and Peet Creeks, hybrids (BExBR and MCxLC) were nearly two times more abundant than expected (Figure 1e,f; Table S4).

To summarize these findings, we compared relative crosstype heterozygosity to relative crosstype success (the difference between observed and expected offspring) (Table S4). We found a strong positive relationship between crosstype success and crosstype genetic variation ($pd > 0.999$; Figure 2). Relative success increased by 110% for each one standard deviation increase in relative observed heterozygosity (Figure 2; Tables S4 and S9).

3.2 | Individual fitness

In NF Greenhorn, family size was elevated for parental crosstypes with higher genetic variation (BN and PR) compared to the parental crosstype with the lowest genetic variation (BC) (Figure 3a; Table S5). Family size in the most genetically variable population was 283% higher than the population with the lowest variation ($pd = 0.999$). Four of six comparisons between parental and hybrid crosses sup-

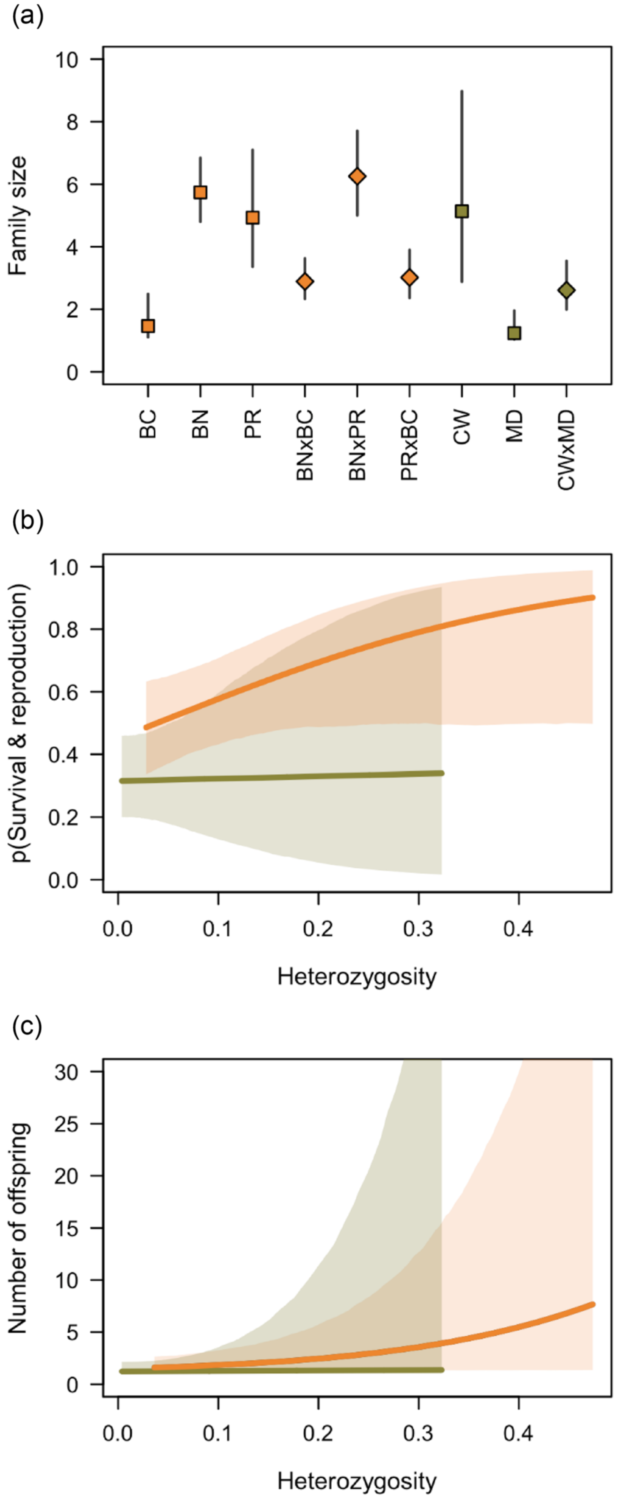

FIGURE 3 Crosstype had a strong effect on family size, while individual heterozygosity positively influences the probability of survival and reproduction, and reproductive success. Family size (a) was predicted as a function of crosstype in NF Greenhorn (orange) and SF Greenhorn (green), where hybrid crosstypes are represented by diamonds and parental crosstypes are shown by squares. See text for naming explanation of source and reintroduction streams. The probability of survival and reproduction (b) and reproductive success given survival and reproduction (c) were predicted as a

(Continues)

FIGURE 3 (Continued)
function of individual heterozygosity in NF Greenhorn (orange) and SF Greenhorn (green). Confidence bars (a) and bands (b and c) are 95% credible intervals. The full 95% CIs are not fully shown in C to better show the mean effects.

ported heterosis, with hybrids having greater family sizes than the corresponding source populations in all cases except when hybrid crosses had BC ancestry (e.g., BNxPR crosstypes had larger family sizes relative to both PR and BN crosstypes) (Figure 3a; Table S5).

In SF Greenhorn, parental crosstype genetic variation was not associated with family size (Figure 3a; Table S5). However, SF Greenhorn family size results were strongly influenced by one highly successful family from the genetically depauperate CW population; CWxMD family size was more than two times larger than CW family size when this outlier was removed.

Individuals in NF Greenhorn with higher genetic variation were more likely to survive and reproduce ($pd = 0.972$) and had more offspring given successful reproduction ($pd = 0.967$; Figure 3b; Table S6). A one standard deviation increase in heterozygosity from the mean was predicted to increase the probability of survival and reproduction by 16.3% and the number of offspring produced given reproduction by 68.0%. Notably, the relationship between individual fitness and heterozygosity was further strengthened in NF Greenhorn when evaluating aggregate fitness (i.e., the combined effect of heterozygosity on survival and reproduction, and number of offspring given reproduction) with a one standard deviation increase in heterozygosity from the mean predicted to increase aggregate fitness by 95.4% ($pd = 0.994$; Figure S3).

We sampled less intensively in SF Greenhorn compared to NF Greenhorn and thus performed a weaker test of the relationship between individual genetic variation and fitness. Survival and reproduction ($pd = 0.53$) and reproductive success ($pd = 0.642$) in SF Greenhorn did not have significant relation with heterozygosity, but both estimates were positive (Figure 3c; Table S7). We evaluated individual fitness within source populations in NF Greenhorn and found mixed evidence for the influence of heterozygosity on the number of offspring produced within source populations (Figure S4). However, we found that higher individual heterozygosity increased survival and reproduction within all source populations (Figure S4; Table S8), which provided additional support for a heterozygosity effect at the individual-level.

4 | DISCUSSION

We show the benefits of increased genetic variation among four separate conservation reintroductions under natural conditions. Overall, our results highlight that (i) source populations with lower relative genetic variation were generally less successful, (ii) individuals with lower genetic variation typically had reduced mating success and fewer offspring, and (iii) hybridization mostly increased crosstype success throughout the four study streams. These results provide much-needed empirical support for the importance of genetic variation as a primary metric in conservation genetics and contribute to the increasing literature describing the fitness benefits of intraspecific gene flow under natural conditions.

The higher relative fitness of hybrid crosstypes and crosstypes with increased genetic variation (Figures 1 and 2) suggests that individual heterozygosity and masking of negative inbreeding effects could be responsible for observed fitness effects. Although the underlying genetic mechanism responsible for this pattern (i.e., disassortative mating, inbreeding avoidance, increased genetic variation, or heterosis) remains unclear, heterosis is likely given the extremely low genetic variation and strong genetic divergence of many westslope cutthroat trout populations (Kovach et al., 2022) and evidence of genetic rescue in other populations of this species. That is, the masking of recessive deleterious alleles and the ensuing fitness benefit acting through key vital rates (increased embryonic and juvenile survival) provides the most plausible explanation. While it is important to consider the risks of outbreeding depression beyond the first generation (Edmands, 2007; Huff David et al., 2011), future outbreeding depression in our system is unlikely to overwhelm observed apparent heterosis and population-specific fitness effects because none of the translocated source populations were locally adapted to the reintroduction sites, and we do not expect substantial structural genomic variation among source populations because they were only recently isolated (<200 years) (Frankham et al., 2017).

We found that increased individual heterozygosity provides fitness benefits under natural conditions. This relationship was most apparent in our evaluation of individual probability of survival and reproduction in NF Greenhorn, which may suggest that elevated genetic variation across the genome improves survival and the ability to successfully reproduce in novel habitat (consistent with adaptive potential). However, it is important to note that a population-level effect of heterozygosity (i.e., BN with the highest average heterozygosity, PR with intermediate, and BC with the lowest) was partly responsible for the individual-level fitness signal (Figure S4). Population-level effects may hint at the potential influence and complexity of other abiotic and biotic dynamics involved in mixed-source reintroductions, such as habitat matching (Ewen et al., 2012; Jachowski et al., 2016) (Supporting Information). Overall, our findings consistently align with fitness benefits associated with higher genetic variation at both individual and population scales.

In the face of climate change and continued anthropogenic effects, reintroductions are likely to be increasingly common, and our results confirm that genetic variation is a central component to conservation reintroductions. Our data indicate that genetic variation can strongly influence fitness and conservation outcomes, and as a result, support theoretical (Kardos et al., 2021) and recent empirical evidence (Scott et al., 2020) that suggests genetic variation is a fundamental element of conservation biology and conservation practice. As such, genetic concerns are important not just in broad policy, where they are all too frequently neglected (Hoban et al., 2020), but also for planning key conservation actions such as species reintroductions. Our results strongly suggest that genetic considerations are warranted when selecting individuals or populations for species translocations and expansions. Specifically, genetically variable source stocks appear more likely to succeed in conservation reintroductions. When genetically variable individuals or source populations are unavailable, using multiple genetically depauperate (and genetically divergent) source populations can lead to increased fitness in offspring, a finding that is consistent with the growing body of literature on genetic rescue (e.g., Bell et al., 2019). The latter strategy may be particularly relevant for many species that are strongly fragmented by human activities, especially when isolated populations harbor unique, and potentially important, subsets of genetic variation. Therefore, mixed-source reintroductions are warranted in many conservation applications, especially when source populations have low genetic variation, a common situation in conservation management. Overall, these data clearly support recent calls to incorporate conservation genetic tools when managing fragmented populations (e.g., Ralls et al., 2018). While increasing genetic variation is by no means a panacea, these results clearly demonstrate that it is a critical component to conserving and managing species amid unprecedented worldwide extinctions (Frankham et al., 2017; Johnson et al., 2017; Allendorf et al., 2022).

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DATA AVAILABILITY STATEMENT

Data will be available at data dryad DOI: https://doi.org/10.5061/dryad.sxksn0380.

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SUPPORTING INFORMATION

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

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