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ARTICLE

Genetic monitoring of trout movement after culvert remediation: family matters[1]

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Abstract: We contrasted various genetic analyses to evaluate their utility and constraints for detecting movement of cutthroat trout (*Oncorhynchus clarkii*) through restored culverts in different field settings: population-level metrics of genetic variability (heterozygosity and allelic richness); Bayesian clustering and assignment of individual genotypes from age 1+ fish; and a novel "sib-split" approach, where movement patterns are extracted from the spatial distribution of young-of-year (YOY) full-sibling groups inferred via pedigree reconstruction. Family structure greatly influenced population-level and individual clustering results in our small headwater populations, even though field sampling was implemented to avoid siblings. Sib-split, which **uses** family structure to detect movement, uncovered passage of YOY just weeks after emergence. When retrospectively applied to older individuals, it proved essential in interpreting clustering patterns and captured passage in several families of 1- and 2-year-olds. Where family structuring may negatively affect genetic analyses or, alternatively, be prominent enough to allow application of sib-split is difficult to predict a priori; we discuss benefits and limitations of all approaches under different ecological, spatial, and management scenarios.

Résumé : Nous avons comparé différentes méthodes d'analyse génétique afin d'évaluer leur utilité et leurs limites pour ce qui est de détecter les déplacements de la truite fardée (*Oncorhynchus clarkii*) par des ponceaux remis en état dans différents contextes de terrain. Ces analyses comprennent des mesures de la variabilité génétique (hétérozygotie et richesse allélique) au niveau de la population, le regroupement bayésien et l'affectation de génotypes individuels de poissons de plus de 1 an, ainsi qu'une nouvelle approche dite de « sib-split », dans laquelle les motifs de déplacement sont extraits de la répartition spatiale de groupes de fratrie de jeunes de l'année (YOY) inférés à la lumière de la reconstitution de l'ascendance. La structure familiale influençait considérablement les résultats au niveau de la population et ceux du regroupement des individus dans nos petites populations d'amont, même si l'échantillonnage sur le terrain était conçu pour éviter la fratrie. La méthode sib-split, qui **repose** sur la structure familiale pour détecter les déplacements, a détecté le passage de YOY quelques semaines seulement après l'émergence. Appliquée de manière rétrospective à des individus plus âgés, elle s'est avérée essentielle dans l'interprétation des motifs de regroupement et a relevé les passages dans plusieurs familles d'individus de 1 an et de 2 ans. Il est difficile de prévoir les situations où la structure de la famille pourrait avoir un effet négatif sur les analyses génétiques ou être suffisamment forte pour permettre l'application de l'approche sib-split. Nous abordons les avantages et limites de toutes les approches dans différents scénarios écologiques, spatiaux et de gestion. [Traduit par la Rédaction]

Introduction

An important driver of global biodiversity loss is the isolation of habitats and resulting fragmentation (Manel and Holderegger 2013). Freshwater fish are particularly vulnerable to fragmentation because of the dendritic or branching nature of stream systems (Fagan 2002) where movement is restricted to the linear stream corridor (not the terrestrial matrix; Fausch et al. 2009, 2002). In the United States, hundreds of thousands of dams, diversions, and road culverts present widespread passage barriers to inland salmonids (salmon and trout) and other aquatic organisms (Fausch et al. 2006; GAO 2001; Hendrickson et al. 2008) and are one of the biggest factors leading to their decline. For salmonids, isolation and consequent decreases in habitat size can restrict important localized movements to diverse habitats necessary for basic ecological processes (Dunning et al. 1992; Schlosser and Angermeier 1995) and also precludes among-population dispersal allowing for demographic support and gene flow (Letcher et al. 2007; McElhany et al. 2000). Further, isolation preempts larger-scale migratory movements (Rieman and Dunham 2000) and other episodic movements necessary to find refuge during disturbances such as fires, droughts, or debris flows (Dunham et al. 2003). These aspects of movement are increasingly being recognized as important for maintaining evolutionary potential and population resiliency in salmonids (Waples et al. 2009). Accordingly, the US Forest Service (USFS) and other entities have dedicated a great deal of effort and expense toward removing or restoring barriers — particularly road culverts — to allow passage of salmonids and other aquatic organisms. However, verifying use of remediated passage structures by aquatic organisms may be costly and time-consuming, especially when the monitoring requires intensive field studies involving direct tracking of movement through telemetry or mark–recapture of tagged individuals. Exploration and validation of alternative approaches is needed to provide useful management guidance regarding the most efficient and effective methods for confirming movement and monitoring the effectiveness of passage restoration projects.

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Relative to the extensive efforts required to execute the above individual tracking methods, genetic approaches can be implemented with limited field sampling and often at less expense and increasingly are being used for various aspects of population or individual monitoring (e.g., Schwartz et al. 2007). In many cases, genetic data can provide evidence of movement that is difficult or impossible to capture with the above methods, such as long-distance movement or pulses of movement outside the duration of a seasonal field study (Peacock and Ray 2001). Several studies have demonstrated negative genetic impacts of isolation on trout populations (Neville et al. 2009, 2006*b*; Whiteley et al. 2013; Wofford et al. 2005), but to our knowledge there are no empirical studies evaluating the utility of molecular markers to document passage after culvert removal (other than Whiteley et al. 2014, this issue). Different genetic approaches typically rely on different theoretical assumptions and have different constraints, so the ability to apply any specific analysis to this question may vary depending on factors such as the time since barrier removal, the effective dispersal rate, population stability and size, age and family structure, and the life history stage of interest. Combining information from different unique approaches, however, may help in interpreting patterns from each technique (e.g., Serbezov et al. 2012). Additionally, genetic approaches are uniquely effective for monitoring hybridization with nonnative species (Peacock and Kirchoff 2004; Weigel et al. 2002), an essential need where invasion may be likely after connectivity has been restored (e.g., Fausch et al. 2009; Neville and Dunham 2011; Rahel 2013).

Here, we contrasted a suite of genetic techniques, including traditional population-level metrics of genetic variability, individual clustering and assignment, and a novel "sib-split" method (Whiteley et al. 2014, this issue) based on pedigree reconstruction of young-of-year (YOY) to evaluate movement of westslope cutthroat trout (WCT, *Oncorhynchus clarkii lewisi*) through remediated culverts in Montana and Idaho. We focused on these complementary techniques because each relies on different assumptions, provides different types of information, is relevant at different time scales (Manel and Holderegger 2013), and focuses on different age classes within the target populations. Our goal was to assess the feasibility of these different genetic methods for determining successful passage, not only to demonstrate "biological success" of passage restoration in the study areas, but also to develop the scientific basis for future monitoring and project planning.

Methods

Brief background on genetic approaches

Sampling needs differ for each genetic approach, so here we provide a brief description of each type of analysis to provide context before describing our study system and design below. Population-level metrics, such as estimates of genetic diversity within populations or differentiation among populations, are commonly used to evaluate the conservation status of populations of concern (Kozfkay et al. 2011; Nielsen et al. 2009; Schwartz et al. 2007; Taylor et al. 2011). These population-level descriptors can give unique and important information (i.e., when the long-term **effect** of movement is of interest and (or) when past processes may influence current genetic patterns; Neville et al. 2006*a*; Peacock and Ray 2001). The utility of these metrics for monitoring, however, may be limited by various factors, including the a priori need to delineate "populations" in a landscape or riverscape (Manel et al. 2003), a long time frame for expected responses (Langduth et al. 2010), and theoretical assumptions that are likely not met in many natural populations (e.g., drift-migration equilibrium, equal reproductive opportunities, random mate choice; Bossart and Prowell 1998; Whitlock and McCauley 1999). These attributes may be particularly problematic in continuous and dynamic stream systems (Dunham et al. 2007; Fausch et al. 2002; Waples et al. 2008), which lack obvious habitat features that clearly define populations and where, for instance, drift-migration equilibrium is often not a valid assumption (e.g., Hitt and Roberts 2012; Neville et al. 2006*b*). However, we included several population-level genetic metrics for comparative purposes because (*i*) this type of sampling is what is most frequently carried out by fisheries managers, (*ii*) they target age classes of interest to many restoration situations, and (*iii*) population-level metrics have successfully demonstrated negative impacts of isolation in multiple fishes (Neville et al. 2009; Raeymaekers et al. 2009; Wofford et al. 2005). When interpreted with other genetic approaches and insight from demographic data, these population-level metrics may provide useful complementary metrics (Peacock and Ray 2001) for evaluating genetic recovery after reconnection.

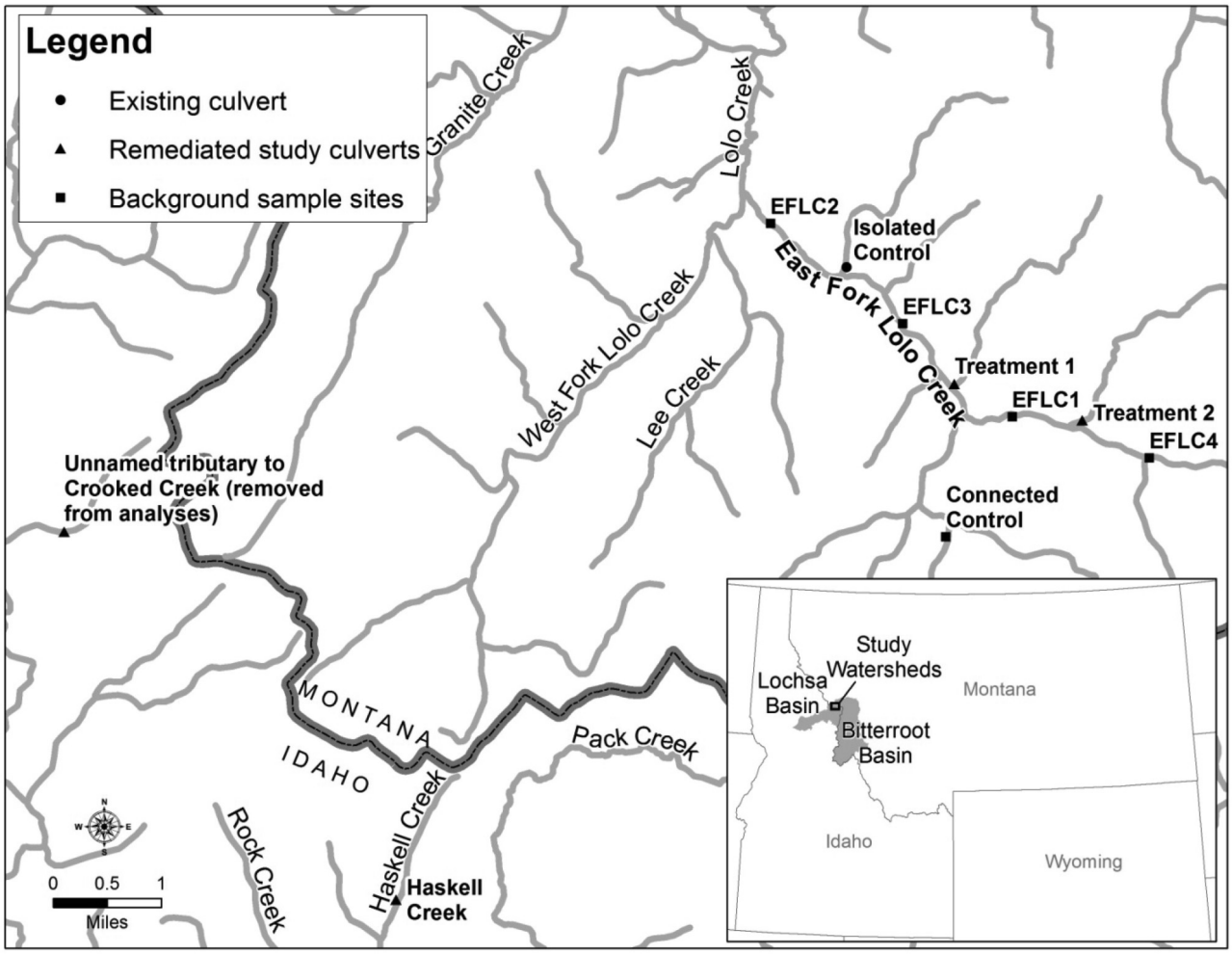
In contrast with traditional population-level measurements, individual-based approaches use information in the individual genotypes to define structure. Importantly, under certain scenarios (see below) these approaches offer the ability to track individual movement directly, in current time, and they circumvent restrictive population theoretical assumptions of drift-migration equilibrium and thus more readily incorporate the dynamics of natural populations (Manel et al. 2005; Paetkau et al. 2004). One approach we used here, a genetic clustering method, defines population boundaries analytically by organizing individual genotypes into clusters that fit theoretical expectations of population structure (Pritchard et al. 2000). The ability to detect movement simultaneously with determination of population structure (i.e., movement is inferred if an individual strongly assigns to a cluster different from where it was captured; Paetkau et al. 2004) has made these approaches attractive for ecological studies (Castric and Bernatchez 2004; Cegelski et al. 2003; Eldridge et al. 2001; Lee et al. 2007). Clustering approaches have proven effective in detecting barrier effects (Safner et al. 2011) and may be particularly useful where the treatment (barrier remediation) is very recent. The power to detect movement depends on the existence of at least moderately strong genetic differentiation among populations of interest (Manel et al. 2005; Whiteley et al. 2014, this issue); cutthroat trout express both migratory and resident life histories (Gresswell 2011; Rieman and Dunham 2000; Schmetterling 2001), but strong natal site fidelity typically leads to substantial genetic structuring (Neville et al. 2006*b*; Taylor et al. 2003), so we expected this requirement to be met in our system. Other limitations are that interpretation of clustering results can be difficult (Gilbert et al. 2012), and results can be influenced by factors such as family structure (Anderson and Dunham 2008; Rodriguez-Ramilo and Wang 2012).

A second individual-based method we employed is based on pedigree reconstruction of full-sibling families and was first used in a fisheries setting by Hudy et al. (2010) to characterize movement of YOY brook trout (*Salvelinus fontinalis*); the authors of this study first realized the potential application of sibship analyses for addressing culvert passage (termed sib-split for this purpose; Whiteley et al. 2014, this issue). The idea behind the approach is that finding full siblings on both sides of a restored culvert indicates movement of YOY through the culvert. A major benefit of sib-split is that only a postremediation sample is needed to confirm current, real-time movement. This is a novel method and there are important differences in the spawning and movement behavior among trout species that could affect the utility of sib-split, so we were particularly interested in evaluating its power and limitations in a natural field setting in a species other than brook trout.

Study design and sites

Study sites were headwater streams in the interior Columbia River basin, USA. We capitalized on an ongoing study of culvert impacts and restoration effectiveness on WCT in the Lolo Creek watershed in western Montana (Fig. 1). Lolo Creek is a tributary to the Bitterroot River in western Montana and was selected for this initial study based on Lolo National Forest plans to remove or replace numerous culverts that were identified as partial or

Fig. 1. Map of the Bitterroot and Lochsa River drainages in Montana and Idaho (lower right inset). The main panel shows the primary study sites in East Fork Lolo Creek (EFLC) as the Isolated and Connected Controls, the four mainstem background sites (EFLC1–EFLC4), and the two treatment streams where culverts were remediated with passage-friendly structures in 2008. Also shown are the remediated culvert sites in Haskell Creek (the third treatment stream) and an unnamed tributary to Crooked Creek (ultimately removed from study). (Note: 1 mile = 1.852 km.)



complete passage barriers for trout. This provided an opportunity to concentrate multiple sample sites within a single geographic area, and thus control for some confounding variables (climate, hydrology, fish community), and facilitated a before–after control–intervention (BACI) analysis (Balkenhol and Waits 2009) for most analytical methods (except for sib-split analyses, see below). Field crews conducted preliminary surveys in 2008 to determine fish occupancy in 12 tributaries in the upper Lolo Creek drainage, primarily in East Fork of Lolo Creek (EFLC) and its tributaries (Fig. 1). These surveys indicated that native WCT and nonnative brook trout were the most common fishes detected at all sites, although nonnative rainbow trout (RBT, *Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) were also present in the system.

From this collection of sites, we selected two "treatment streams" that are unnamed tributaries of EFLC where culverts ranked as "partial barriers" by USFS Region 1 were scheduled to be replaced with passage-friendly culverts in late summer 2008 (Fig. 1). Passage status (total barrier, partial barrier, passable) for structures at stream–road crossings was assessed applying region-specific criteria (Hendrickson et al. 2008; USFS 2003) to an inventory and assessment procedure developed for use across the USA (Clarkin et al. 2005). Two adjacent tributaries were selected as control sites: one with an unremediated culvert barrier (Isolated Control) and another with no passage barriers (Connected Control; Fig. 1). With additional funding in 2011, we established a new study site at a comparable "treatment" stream (Haskell Creek) in the nearby Lochsa River drainage in Idaho, which had undergone similar culvert remediation in 2010 (Fig. 1). Because this culvert had already been replaced, we could not implement comparable BACI analyses here, but could still use individual assignment and sib-split genetic techniques to detect movement through the remediated culvert.

Sampling for population-level genetic inferences

Sampling for population-level metrics occurred in mid- to late summer before emergence of YOY WCT. We balanced our genetic sampling efforts to collect tissues from approximately 50 WCT spread across the entirety of each sample site for accurate representation of population genetic diversity and to avoid sampling siblings (Hansen et al. 1997). In the EFLC drainage, within each treatment stream and in the Isolated Control stream, we selected six to seven sample reaches starting above the culvert and extending to the upper distributional limit of fish in the stream (1200–1700 m upstream depending on the stream), where we used single- or multiple-pass electrofishing to capture age 1+ fish. In the Connected Control stream, we sampled similarly, starting above the conflu-

ence with EFLC, to capture fish representative of the observed spatial distribution and age–size structure. Fish were weighed and measured, and we collected small fin clips from each individual. Tissue samples were dried on gridded chromatography paper (LaHood et al. 2008) for later use in the lab. Population-level samples were collected in EFLC in the summer of 2008 before culverts were remediated and again 3 years after remediation in 2011. In 2011 we added population-level sample sites below each treatment culvert in EFLC as well as above and below the remediated culvert in Haskell Creek.

Sampling for individual assignment tests

Effective implementation of clustering and individual assignment requires sampling all or most of the populations that could influence overall structure and provide a source of immigrants (Manel et al. 2005). Therefore, in addition to relying on our population-level samples described above, we collected additional "Background Samples" at several locations in EFLC. We sampled two background sites (EFLC1 and EFLC2) in 2008 and repeated this sampling and also added two more sites (EFLC3 and EFLC4) in 2011 (Fig. 1). Collectively, our population-level and Background Sample site locations were chosen as representative of the area in EFLC occupied by WCT. The culvert remediation locations in Haskell Creek was quite far upstream from other source populations, so we simply used the up- and downstream samples for clustering analysis here, as more extensive sampling was beyond the scope of this study.

2011 sampling to detect movement in YOY using sib-split

Sampling for YOY WCT was conducted in mid- to late fall in each treatment stream (Treatment Streams 1 and 2 in EFLC, as well as Haskell Creek) in 2011. We did not have preremediation samples for this technique, but a strength of sib-split for monitoring is that it can be used to infer movement with only one sampling period. We sampled one reach extending 300 m upstream and another extending 300 m downstream of the culvert, except for in Treatment Stream 1 where the culvert was just 80 m above the confluence with EFLC and which included a small side channel that we also sampled. Beginning at the edge of the culvert, we block-netted three 100 m reaches and used multiple-pass electrofishing within these 100 m reaches to capture all YOY possible. Because we not only wanted to implement sib-split effectively but also wanted to gauge the level of field sampling necessary to capture movement, if it occurred, we stratified our sampling further and recorded individual YOY locations every 20 m. We targeted a sample size of 100 YOY above and below each culvert. A small fin clip was taken from each individual and dried on chromatography paper as outlined above.

Genetic laboratory work

DNA was extracted from samples using a Qiagen DNeasy Tissue Kit (Qiagen Inc., Valencia, California). DNA was diluted to 10 $ng \cdot \mu L^{-1}$ after quantification with fluorometry. Because RBT were found in our system, we evaluated hybridization between WCT and RBT in all individuals using a single PCR multiplex of six markers developed for hybrid detection (Table A1; Ostberg and Rodriguez 2004). Each marker amplifies an allele specific to either RBT or WCT in "pure" individuals, but hybrids should be distinguishable by heterozygous genotypes at one or more markers (see Neville et al. 2009 for further discussion). This set of markers should confer relatively high power for detecting hybridization. For instance, the probability of mistaking a first-generation backcross for a pure individual using five markers is 5%, and with six markers the possibility of making such a mistake is less than 2% (Boecklen and Howard 1997).

Age 1+ fish collected for population and clustering samples were amplified at 13 microsatellite loci isolated from Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*; Peacock et al. 2004; Robinson et al. 2009; Table A1), run in multiplex reactions. To achieve higher power for sib-split, YOY ultimately were genotyped at an additional eight multiplexed microsatellites (see below) isolated from RBT (Palti et al. 2002; Rexroad and Palti 2003; Rexroad et al. 2002; Table A1).

For the Lahontan cutthroat trout and hybrid loci, each reaction contained 6 μL of 2× QIAGEN Multiplex PCR Master Mix (providing a final concentration of 3 $mmol \cdot L^{-1}$ $MgCl_2$), 40 ng of DNA, and 0.2 $\mu mol \cdot L^{-1}$ of each forward and reverse primer and was adjusted to a final volume of 12 μL using molecular grade water. We followed an economic approach for dye-labeling primers (Schuelke 2000), using a modified three-primer nested PCR protocol: reverse primers were "pigtailed" by adding a short repeat (GTTTCTTT) to the 5′ end to reduce stutter, the sequence-specific forward primer was appended with one of three M13 tails (that matched the third primer), and the third dye-specific M13 primer was labeled with one of three fluorescent dyes. Multiplex PCR reactions were based on a touchdown PCR, which allows primers with slightly different optimal annealing temperatures to be run together. The standard protocol included a 15 min hot start at 95 °C, followed by 40 cycles of 95 °C for 30 s; the touchdown of seven cycles at 65 °C, seven cycles at 61 °C, seven cycles at 58 °C, and 20 cycles at 55 °C, in which the first 21 cycles amplified using the locus-specific primer and the final 20 cycles amplified using the labeled M13 tail; and 72 °C for 30 s. For the additional RBT loci run on YOY, multiplex reactions were run as above, but with primer concentrations of 0.05, 0.2, and 0.1 $\mu mol \cdot L^{-1}$ for each forward primer, reverse primer, and primer with the labeled M13 tail, respectively. All PCR fragments were sized with a Perkin Elmer Applied Biosystems 3730 Genetic Analyzer at the Nevada Genomics Center (Reno). Individuals were genotyped with Genemapper version 3.7 (Applied Biosystems).

Analyses of genetic data

Population-level metrics

Our population-level analyses included samples from our treatment streams (above culverts in 2008, above and below culverts in 2011) and control streams in the EFLC system and the 2011 samples above and below culverts in Haskell Creek. We used FSTAT (Goudet 2001) to test for linkage disequilibrium between each pair of loci across all samples, using the program's Bonferroni adjustment of critical significance to account for simultaneous tests. We assessed each sample for Hardy–Weinberg equilibrium at each locus using FSTAT, again adjusting for multiple tests. We also used FSTAT to calculate Nei's (1987) unbiased measure of gene diversity (H_E) and allelic richness (R_S), a rarified estimate of the number of alleles that is independent of the sample size and has been shown to be particularly sensitive to known population bottlenecks or isolation (El Mousadik and Petit 1996; Leberg 2002; Neville et al. 2009; Petit et al. 1998). R_S was calculated for the above samples in a single analysis. Additionally, because R_S corrects for sample size and thus results can vary significantly based on the smallest sample size in a given analysis, differences in R_S were evaluated via pairwise contrasts between only our preculvert and postculvert remediation samples in each study stream; this kept sample size adjustments appropriate to the comparison at hand (e.g., to evaluate changes in R_S in Treatment Stream 2, only the sample sizes for collections in this stream in 2008 and 2011 were used for the adjustment of R_S). Because we did not have above and below comparisons in our treatment streams in 2008 to compare with 2011, for 2008 we evaluated genetic divergence of our isolated populations by calculating pairwise F_{ST} (using FSTAT) between the two above-culvert samples in Treatment Streams 1 and 2. In 2011 we again evaluated pairwise F_{ST} between the two above-culvert samples in Treatment Streams 1 and 2, as well as between above and below sites within each stream. We expected if movement was substantial following culvert remediation, it would be reflected in

Hardy–Weinberg and linkage disequilibrium and possibly in increases in H_E and R_S, with concurrent decreases in genetic differentiation in our above-culvert treatment sites.

Individual clustering and assignment

We implemented the Bayesian clustering algorithm in STRUCTURE version 2.3.3 (Pritchard et al. 2000) assuming an admixture model with correlated allele frequencies. We evaluated all EFLC population and background samples simultaneously in a given analysis, but ran collections from 2008 and 2011 separately. In 2008 we evaluated 1–7 clusters (k) and in 2011 we assessed k = 1–10 because of the additional samples collected in this year. Similar analyses were performed in Haskell Creek alone for 2011 with k = 1–7. For all analyses we used a burn-in length of 100 000 and 100 000 Markov chain Monte Carlo replicates for each of five runs. For each analysis we determined the most likely number of clusters using both the mean log-likelihood of the data (i.e., as recommended by the authors; see STRUCTURE documentation), as well as the Delta k method outlined by Evanno et al. (2005) based on the second-order rate of change of the likelihood function; both statistics were compiled in STRUCTURE HARVESTER version 0.6.8 (Earl et al. 2012). We used the Greedy algorithm with 10 000 random inputs in CLUMPP version 1.1.2 (Jakobsson and Rosenberg 2007) to determine the degree of consistency among runs based on the pairwise similarity statistic (H') and to match clusters that may have been labeled differently across each run.

Family structure can influence interpretation of clustering results (Anderson and Dunham 2008; Rodriguez-Ramilo and Wang 2012; Vaha et al. 2007), and we attempted to minimize this effect by spreading field collections throughout the stream and targeting age 1+ individuals. However, to confirm appropriate sampling, we used the maximum likelihood estimation in program COLONY (Jones and Wang 2010) to evaluate via pedigree analysis any influence of family structure. Because of its ability to reconstruct sibling families based only on genotypes from a single generation (i.e., with no information on parental genotypes), COLONY has been increasingly applied to field studies (Hudy et al. 2010; Kanno et al. 2011; Read et al. 2012). We estimated full-sibling families using the full-likelihood algorithm, updated allele frequencies, an assumption of male polygamy (computational time prohibited assumption of polygyny as well), and no prior for family relationships. Our power based on 13 microsatellites is certainly lower than that for our 21 locus dataset for YOY (see below for more detailed discussion of this issue), but here we were not as focused on the family relationships of each individual per se, but wanted to evaluate any overall issues with family structure in our interpretations of results from STRUCTURE.

Sib-split analyses

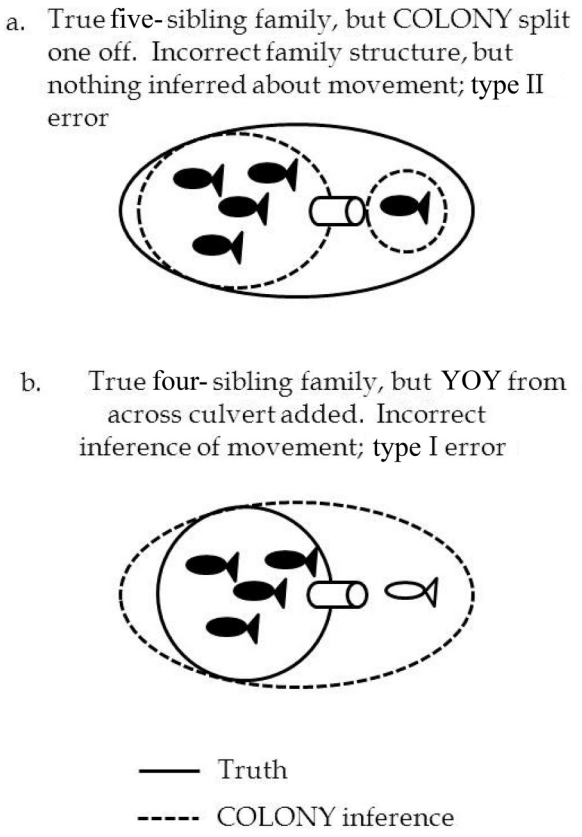
Program COLONY was also used to estimate full-sibling families in our 2011 YOY collections. Under certain scenarios, the program has a tendency to split larger sibling groups (Almudevar and Anderson 2012) and may not accurately estimate very small full-sibling families (Hudy et al. 2010; Kanno et al. 2011; Wang and Santure 2009), so we undertook a multistep simulation process (Fig. A1) to evaluate the behavior of COLONY based on genetic variation observed in microsatellites markers in our sample populations. We present those methods and results here, because this process determined our final locus set for subsequent analyses. We first simulated genotypes of a cohort of YOY fish using the program PEDAGOG (Coombs et al. 2010*a*). PEDAGOG models the evolutionary and ecological dynamics of populations using individual-based simulations while recording genotypic and pedigree information for all individuals in each generation. We initiated the program with the 13-locus genotypic data from our 2011 YOY collections in Treatment Stream 2 (we retained all loci for COLONY runs, as COLONY is relatively robust to linkage disequilibrium; Wang and Santure 2009). We assumed male polygamy, set population growth parameters such that we were able to simulate 10 generations with realistic family sizes, simulated genotypic errors (miscall rate = 0.001), and incorporated missing genotypes at a level similar to missing genotypes in our own data (proportion of loci genotyped = 0.9, SD = 0.2). From this effort we obtained simulated genotypes of a new 10th-generation YOY cohort at least as large as our field collection, with a known pedigree. We then imported these simulated YOY genotypes into COLONY (Fig. A1) and estimated full-sibling families using three runs of the full likelihood with medium precision, updated allele frequencies, male polygamy, and no prior information on family structure. The full-sibling relationships estimated by COLONY were then aligned with the known pedigree (generated in PEDAGOG) using the program PedAgree (Coombs et al. 2010*b*; Fig. A1). PedAgree presents summary statistics that are helpful in evaluating the overall performance of COLONY, but these do not fully capture some details of pedigree (in)accuracy, such as the splitting of families. For instance, if a family is erroneously split into two families by COLONY, sibling assignment accuracy will still be estimated by PedAgree to be 100% for families above a threshold size if the individuals assigned to each subfamily are true siblings, but this accuracy does not reflect that all siblings were not correctly assigned to one family. Therefore, we used PedAgree for alignment purposes but tallied similarities and inconsistencies related to splitting and grouping independent of the program statistics.

PEDAGOG generated 367 YOY, with 92% of individuals genotyped at 11 or more loci (only two individuals were genotyped at the minimum eight loci). Based on initial simulation results and because COLONY typically does not perform as well with small families (Hudy et al. 2010; Wang and Santure 2009), we excluded families with fewer than four siblings from our interpretations. The true pedigree from PEDAGOG consisted of 54 full-sibling families with four or more siblings. Sixteen families had seven or more siblings, and all but one of these was reconstructed correctly by COLONY. In the remaining families, there were 14 observed cases of splitting, where COLONY separated siblings — usually by splitting one individual off as a singlet from a larger group. In our interest of determining passage through culverts, this result could lead to type II errors, where we may not infer movement when it actually occurred. For instance, if an individual(s) from a true sibling group was found on the other side of the culvert, but was also falsely split as a separate family by COLONY, we would have incorrect information about family structure but would not infer movement because we would not know the individual(s) was related (Fig. 2*a*). There were five cases where COLONY incorrectly added a sibling to a cohort to which it was unrelated. This scenario could lead to type I errors and cause us to infer movement incorrectly, if the falsely added sibling was found on the opposite side of the culvert from the other (truly related) siblings (Fig. 2*b*).

As we wanted to minimize both sources of error, we genotyped all of the YOY from field collections at a total of 21 loci to improve power (Wang and Santure 2009). We repeated the above process, this time initiating PEDAGOG with observed data at 21 loci, generating new YOY with a known pedigree and comparing this pedigree to the estimates of full-sibling families from COLONY using the same parameters as above. This time PEDAGOG generated 360 YOY spread across 46 families with four or more siblings. Seventy-eight percent of these individuals were genotyped at 19–21 loci, and 5% were genotyped at the lowest observed number, 17 loci. With this larger marker set, COLONY assigned all 24 of the families with six or more siblings correctly. There were still 12 cases of splitting that could possibly cause us to miss movement (type II error; Fig. 2*a*), which were observed in the 22 families with four to five siblings. Importantly, there were no instances where COLONY incorrectly assigned unrelated individuals to a family (i.e., no type I error; Fig. 2*b*).

After we determined that COLONY could estimate full-sibling families accurately — and minimize the likelihood we would infer

Fig. 2. Schematic of the two different types of error based on improper pedigree reconstruction. (*a*) The true family contains five members (outlined with a solid circle), while COLONY split one individual off (with the four-member and one-member families in dashed circles). Here, we would erroneously conclude the family has four members and would miss a movement event that led to siblings being found on either side of the culvert, leading to a type II error. (*b*) The true family has four members (outlined with a solid circle), but COLONY incorrectly added an individual to the family (white individual included in the dashed circle). If this individual was located on the other side of the culvert, we would incorrectly infer movement, leading to a type I error.



movement where it did not occur — we used sib-split to evaluate movement of YOY through the remediated culverts in our study sites. We used COLONY as above to estimate full-sibling family groups from our 21-locus YOY genotypes collected in the field. In EFLC, we combined YOY from both treatment streams into one analysis to account for any (unlikely) movement of siblings between streams, but performed a separate analysis in Haskell Creek.

Results

In 2011 we collected only four fish from our Isolated Control stream and therefore decided to drop the Isolated Control stream from our 2011 analyses but include it in the 2008 STRUCTURE analyses.

Hybrid individuals

Hybridization was common in our EFLC collections. In 2008 we found 35 age 1+ rainbow–WCT hybrids in our treatment and control collections in EFLC (Table A2). Twenty-two were in our upper Treatment Stream 1 (52% of sampled individuals); five were in upper Treatment Stream 2 (12% of sampled individuals); eight were in the Isolated Control stream (17% of sampled individuals, dropped in 2011), and ironically none were found in the Connected Control. We identified 32 age 1+ hybrids in our 2011 EFLC study streams comprising 0%–43% of our samples (Table A2). Again, no hybrids were found in the Connected Control. Treatment Stream 1 housed 10 hybrids, with four found throughout the stream below the culvert and six collected above the culvert distributed to the upper-most sampling area (+1400 m). Twenty-two hybrids were found in Treatment Stream 2; two of these were found in our site immediately above the culvert (0 m) and the rest were distributed below the culvert. One hybrid was found in Haskell Creek 600 m above the culvert. In 2011, within our 300 m sampling reaches above and below each treatment culvert, 31 hybrid YOY were collected throughout the downstream section of Treatment Stream 1, while 17 were found above the culvert, exclusively in the first 40 m save for one hybrid YOY collected 240 m above the culvert. Interestingly, no hybrid YOY were found in Treatment Stream 2 or in Haskell Creek. All hybrids from all samples (including Background Samples, not shown in Table A2 for simplicity) were removed from further analyses.

Table 1. Details of estimates of genetic diversity in East Fork Lolo Creek, Montana, for the Connected Control and both above-culvert sites in the treatment streams, as well as estimates for the above-culvert site in Haskell Creek, Idaho, in 2011.

Sample site	Year	*N*	H_E	R_S	Pair R_S	YOY *N*
Connected Control	2008	33	0.77	7.02	7.46	—
	2011	29	0.72	6.02	6.44	—
Treatment 1 above	2008	21	0.73	6.15	6.15	—
	2011	43	0.72	6.72	6.62	242
Treatment 2 above	2008	38	0.70	5.43	6.08	—
	2011	51	0.72	5.94	6.60	205
Haskell Creek above	2011	73	0.83	9.90	12.32	176

Note: Details for each site include the collection year; the number of age 1+ individuals collected (*N*) for population-level and STRUCTURE analyses after hybrid removal; Nei's genetic diversity (H_E); allelic richness estimated across all sites in one analysis for each year (R_S); and allelic richness estimated as a within-site comparison including both sample years and for Haskell Creek in 2011 (Pair R_S). Also given are the total YOY analyzed in each stream for sib-split after hybrid removal (distributed both above and below the culvert).

Population-level metrics

After table-wide adjustment for multiple tests (adjusted $P = 0.00083$), there was only one instance of significant heterozygosity excess and one of heterozygosity deficit in 2008, both at different loci and in different populations. In 2011 there remained nine instances of significant Hardy–Weinberg disequilibrium (eight were heterozygosity deficits, one was a heterozygosity excess; table-wide adjusted $P = 0.00035$, due to more samples); the most frequent occurrence was for locus 30 in 5 of 11 samples (two Background Samples, the Isolated Control, and the above-culvert samples in both EFLC treatment streams), with the remaining four instances spread across samples and loci. In 2008 (six samples), there were 27 locus pairs × sample combinations showing significant linkage disequilibrium (LD) after table-wide adjustment ($P = 0.0001$). OCH14 and OCH20 showed significant LD in every sample. Sixteen cases of LD were in the Isolated Control stream (dropped from 2011 analyses), while other instances were spread across locus pairs and samples. In 2011 (11 samples), there were 69 locus pair × sample combinations showing significant LD using a table-wide $P = 0.00006$. Again, OCH14 and OCH20 showed LD in every sample, and thus OCH14 was dropped from further analyses (except pedigree analyses, see above). Samples collected above the restored culverts (Treatment Streams 1, 2, and Haskell Creek) had the majority of remaining significant LD tests (12, 22, and 10, respectively), while the rest were spread across locus pair – sample combinations. Further analysis (see below) suggested these deviations were likely caused by family structure.

In 2008, H_E in the EFLC above-culvert treatment and control sites ranged from 0.61 (Isolated Control 2008, not shown in Table 1) to 0.77 (Connected Control, Table 1), while in 2011 H_E was 0.72 in all relevant samples (Connected Control and upper Treat-

ment Streams 1 and 2). There were no differences in H_E within sites across years (two-tailed t test, $P > 0.05$); H_E in Haskell Creek was 0.83 in 2011. R_S evaluated across all sites ranged from 5.43 in the above-culvert sample in Treatment Stream 2 to 7.02 in the Connected Control in 2008 (Haskell Creek was not sampled in 2008). In 2011, values ranged from 5.94 (Treatment Stream 2) to 9.90 in Haskell Creek. R_S values compared only between each above-culvert (or Connected Control) sample across the 2 years are shown in Table 1 (pair R_S) and did not vary significantly across years in any site. In 2008 pairwise F_{ST} between the two above-culvert sites in Treatment Streams 1 and 2 was 0.08, whereas in 2011 this estimate was 0.03; in 2011 the above-culvert versus below-culvert comparison within each treatment stream was 0.00 for Treatment Stream 1 while in Treatment Stream 2 this estimate was 0.04.

Individual clustering and assignment

For the 2008 EFLC samples (which included the Isolated Control dropped in 2011), both the mean log-likelihood and the Delta k method of Evanno et al. (2005) suggested $k3$ as the most appropriate level of structuring (Fig. A2*a*), whereas in 2011, without the Isolated Control, $k2$ was most supported (Fig. A2*b*). The H_ statistic from CLUMPP was 0.99 for both (across runs for 2008 $k3$ and across runs for 2011 $k2$), suggesting a high degree of pairwise similarity among replicates and little discrepancy in calculated proportional ancestry among runs within each analysis. In 2008, most individuals in the Isolated Control were represented by a cluster (light grey areas, Fig. 3*a*) not detected elsewhere in the system, while some were assigned to a cluster (dark grey areas, Fig. 3*a*) characteristic of the two EFLC background sites, the Connected Control, and Treatment Stream 1 above the culvert. Most individuals in Treatment Stream 2 were assigned to a third cluster (white areas, Fig. 3*a*), with a few assigned to the dark grey cluster. COLONY demonstrated marked family structure in our 2008 EFLC age 1+ samples and identified 10 full-sibling families that had 4–16 members. Two of these families were found in Treatment Stream 1 (above the culvert only for this year, one 4- and one 9-member family) and four were in Treatment Stream 2 (above the culvert only for this year, two 5-, one 6-, and one 16-member family). Only one EFLC family suggested evidence of movement over a culvert (i.e., via sib-split), with one individual captured in the EFLC1 Background Sample being grouped with four siblings above the culvert in Treatment Stream 2 (denoted by stars in Fig. 3*a*).

In 2011, the Connected Control and Background Samples were characterized similarly to 2008, with most individuals assigning to the dark grey cluster (Fig. 3*b*). The new below-culvert sample from both treatment streams, however, had many individuals assigning to the white cluster (Fig. 3*b*), and there was a substantial change in individual assignments in the above-culvert sample from Treatment Stream 1, where many individuals here also strongly assigned to the white cluster (Fig. 3*b*). Without other information, this could be interpreted as evidence of substantial movement through the remediated culvert into upper Treatment Stream 1 (either from Treatment Stream 2 or possibly from the below-culvert portion of Treatment Stream 1, which we did not sample in 2008), but COLONY results demonstrated this change in assignments was almost entirely due to family structure. COLONY identified 11 families across our sample sites this year with four or more full siblings, with one family containing 33 members. **All** of the white individuals below and above the culvert in Treatment Stream 1 were estimated to be from one full-sibling family. Similarly, the white individuals found above and below the culvert in Treatment Stream 2 were also estimated to be from a separate family of full siblings. Though this family structure changes our interpretation of the process of movement, it still provides evidence of movement, as full siblings from these families **were** found on opposite sides of the culvert; in total, four of the 11 age 1+ families showed evidence of movement (identified by different symbols in Fig. 3*b*). Individual sizes indicated these were families of 1-year-olds (54–80 mm total length (TL) sampled in August), although one family was likely to be 2, possibly 3, years old (137–166 mm TL, sampled 8 September).

Despite sampling only above and below the culvert in Haskell Creek, both the mean log-likelihood and the Delta k method suggested three genetic clusters were most appropriate (Fig. A2*c*); here the analysis clearly had a more difficult time determining consistent proportional ancestry for individuals among runs (reflected in an H_ statistic of 0.55), and individual assignments were again difficult to interpret without other information. COLONY results clarified that patterns of individual ancestry in STRUCTURE were again influenced by family structure and uncovered seven full-sibling families ranging from 4 to 13 individuals. Six of these families showed evidence of movement, with siblings found on either side of the culvert (each family shown by different symbols in Fig. 3*c*). Sibling sizes again indicated these families were 1 and 2 and possibly even 3 years old.

Sib-split analyses

Identification of full-sibling families by COLONY from YOY collected in 2011 gave clear evidence of movement across the remediated culvert for this age class in all treatment sites. In Treatment Stream 1, there were 24 families with four or more full siblings, with family sizes ranging from 4 to 41 individuals (left column, Fig. 4*a*). Individual families generally were well-distributed throughout our sampling sites, and in 21 of these 24 families, full siblings were found on both sides of the culvert. Treatment Stream 2 had 10 families with four or more full siblings, with sizes ranging from 4 to 57 members, whose locations were spread throughout sampling reaches above and below the culvert (Fig. 4*b*). Haskell Creek had 13 families with four or more members, ranging from 4 to 40 full siblings (Fig. 4*c*). In Haskell Creek, movement was uncovered in 5 of the 13 families, with full siblings collected on both sides of the culvert.

Discussion

Our study contrasted multiple genetic analytical approaches to evaluate their utility for detecting movement of WCT through restored culverts in a natural field setting. We chose several different metrics — some more likely to be effective than others — that incorporated different sampling schemes, spanned multiple response times, and used genetic information from different age classes of stream resident trout. Our results suggest that, despite the broadly accepted **potential** for such genetic evaluation and monitoring to be efficient and effective compared with more traditional ecological approaches (e.g., Schwartz et al. 2007), application of genetic techniques in natural trout populations can be complex and context-dependent. We found that even where sampling of age 1+ fish was conscientiously implemented to avoid siblings (e.g., Hansen et al. 1997), results were greatly dependent on family structure in a given population at hand, thus biasing population-level analyses and making information on family structure essential for correct interpretation of individual-based clustering analyses. The pervasive presence of siblings, as well as hybrid individuals, made application of genetic tools difficult for monitoring movement in the native trout species of interest in our study (and prioritized for culvert remediation in the region). One exception was sib-split (Whiteley et al. 2014, this issue), which **uses** information about family structure to detect movement and was shown to have high power to do so across several age classes in our study system. However, this method also has important caveats, which we present later in the Discussion.

Invasion by nonnative species and the potential for hybridization can create difficult trade-offs for biologists weighing fish passage decisions (e.g., Fausch et al. 2009), and hybridization complicated our efforts to detect WCT movement in our study system. Genetic markers are essential for evaluating hybrid patterns and monitoring

Fig. 3. Results from STRUCTURE simulations in East Fork Lolo Creek (EFLC), Montana, for 2008 (*k*3, panel *a*) and 2011 (*k*2, panel *b*) and Haskell Creek, Idaho, in 2011 (*k*3, panel *c*). Each vertical bar represents an individual fish, with the different shades representing the clusters identified by STRUCTURE, such that each fish is assigned a proportional ancestry in the different clusters. Fish are organized by sample collections across the *x* axis (separated by black bars), although location was not included in the analysis. Individuals identified by COLONY to be from the same full-sibling family (with four or more members) are denoted by symbols (stars, ovals, etc.); note that **only** those families demonstrating cross-culvert movement are shown for clarity, and symbols for individuals in some of these families (e.g., ovals in Fig. 3*b*) could not be shown because of space limitations.

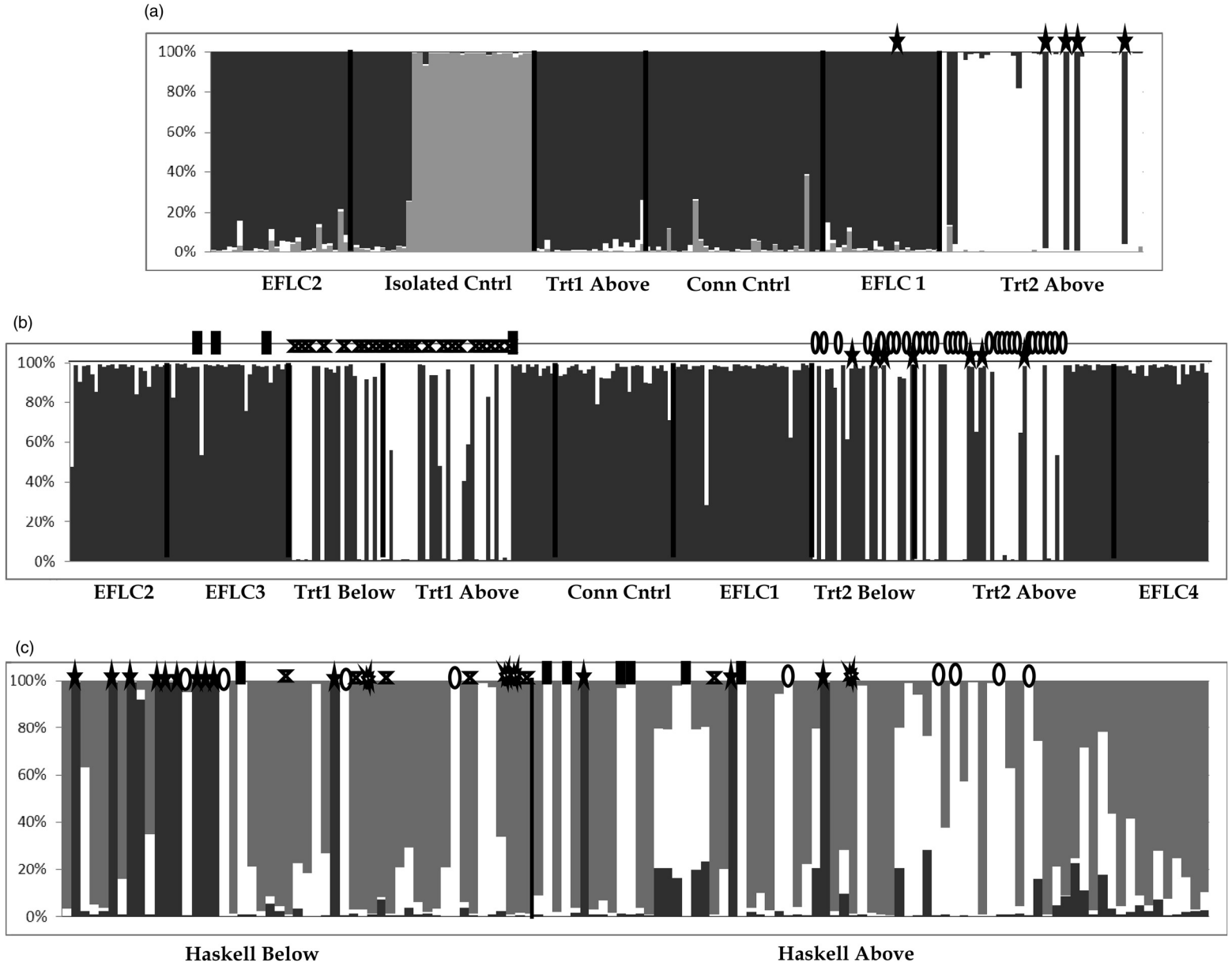


Fig. 4. Results of the reconstruction of full-sibling family groups by program COLONY from YOY collected in the field. Only families with four or more individuals were included and used for inference. East Fork Lolo Creek Treatment Stream 1, Treatment Stream 2, and Haskell Creek are shown in panels *a*, *b*, and *c*, respectively. Each row represents a family, with the full-sibling family size shown in the first column and the distribution of individuals in each of our 20 m sampling strata across columns, beginning with the lowest downstream site and moving upstream to the most upstream site. The culvert is indicated by a dark grey column. For instance, for Treatment Stream 1, the first family had 40 full siblings, with 12 siblings found in different 20 m stretches below the culvert and the remaining 28 spread from 0 to 280 m (top of +260 site) above the culvert.

(a)

Family size ↓ downstream ← upstream

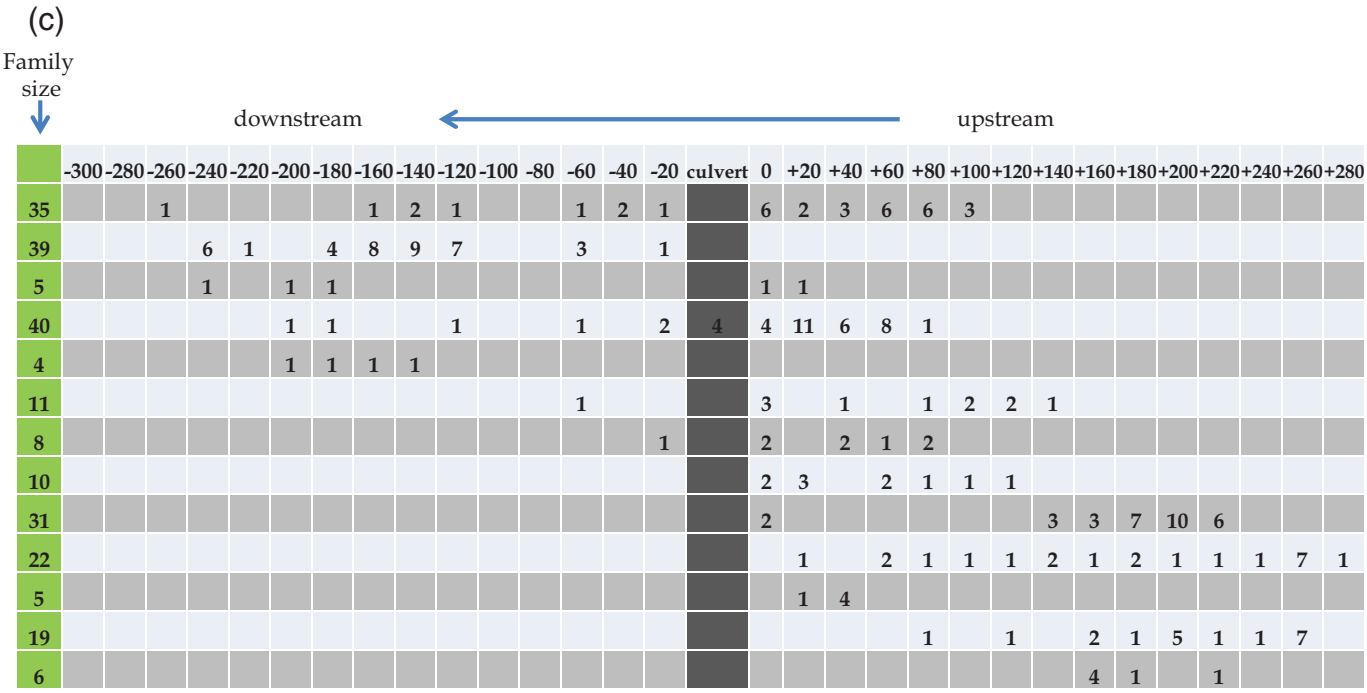
	S-20	-80	-60	-40	-20	culvert	0	+20	+40	+60	+80	+100	+120	+140	+160	+180	+200	+220	+240	+260
40	2	4	4	2			1	2	5	1	3	3	4	3	1		2		2	1
30	3		3	2				2	1	1	2	1		2	3	1	3	1	2	3
16	2	2	2		4									2	3	1				
17	2	1	1	3					1			1	1	3			2	1		1
4	1														1		1			1
8	1	1	1	2	1				1	1										
27	1	6	8	5	1		2		4											
5		1	2														2			
41		2	7		1			2	2	2	2	2	3	11	1	1	3			2
14		1	2	2	4			1	3	1										
24		3	5	1	8				1	1	2	2	1							
14		1	2	1	5		4	1												
4		1											1	1			1			
13		1	1	1								2	2	2	2		1			1
26		1	3	1			1	2	1	2	1	3	3	1	1	1	2	3		
6		3	1		1				1											
5		1									1	1	2							
11		1	1										2	1			1	2	1	1
7			3	1	1		2													
6			1										2		1					
8				1						1				1	1		2			
22										1	1	1	2	2	3	4	2	2	1	3
4											1				2					1
4																1		1		1

(b)

Family size ↓ downstream ← upstream

	-300	-280	-260	-240	-220	-200	-180	-160	-140	-120	-100	-80	-60	-40	-20	culvert	0	+20	+40	+60	+80	+100	+120	+140	+160	+180	+200	+220	+240	+260	+280
19	2		1	2		1		1			2	1			2				2			3	1			1				
24	1	2			1	1		1	1	1	1						5				2	3		1	1		1			2	
54	2	2	1				2	2	2		4		5	4	12		4	9	5												
29	2			1		1		1						1	1		1	4		1	3	3	1			1	3	3	1	1	
15	1						1	1	3								1			1	1	4							1		1
43	1				1	2	2	1	2			1	1	1	1		1	2	1	3	3	4	3	2	3	3	3	2			
40	1	1		2		2		1			3				3		4	2		2	2	4	1		2		3		1	3	3
57									1		1		1	1	2			3	2	4	3	3	1	2	4	2	2	3	6	8	8
14									1		1						1	3							2	3		1	2		
4																			1			1				1			1		

Fig. 4 (*concluded*).



changes over time (Bennett and Kershner 2009; Kozfkay et al. 2011; Neville and Dunham 2011; Rubidge et al. 2001). Here, diagnostic markers revealed the presence of hybrids in our study system, but the spatial and temporal patterns of hybridization were surprising. First, we observed no hybrids in our Connected Control sample in either sample period, but observed hybrids in both treatment streams above the culverts. We expected the opposite pattern based on the (partial) culvert barriers in both treatment streams and the absence of any obvious physical barrier between the Connected Control and EFLC (where RBT and hybrids were detected). Second, hybridization appeared to **decrease** after the culvert remediation in the upstream sites of both treatment streams (Table A2), partly because several large, assumedly "pure", WCT families dominated our 2011 population-level (age 1+) samples in both treatment streams. Third, in Treatment Stream 2 we sampled no hybrid YOY despite the presence of hybrid adults in both time periods. These patterns could indicate a number of different processes, but determining which is most important would require additional study. First, trout could have passed the culverts in the treatment streams before remediation, as there is some evidence that the fish passage criteria are risk-averse and tend to overestimate passage restriction in some cases (e.g., Burford et al. 2009). Second, if remediation facilitated successful reproduction by larger-bodied (and more fecund) migratory WCT that were also genetically pure, then the frequency of hybrids in the sample population might decrease. Third, there may have been changing patterns of survival or reproductive success of hybrids versus native fish in the focal stream reaches, although this is a particularly complicated issue that would require detailed individual- or population-level study in a field or laboratory setting (e.g., Fraser et al. 2008; Muhlfeld et al. 2009). Regardless, an important practical concern was that hybridization complicated our use of genetic tools and greatly increased the expense of our study because of the need to genotype all individuals to remove hybrids from our final samples (but see below for discussion of this issue related to sib-split).

Part of our analyses focused on using population-level genetic metrics to evaluate fish passage using a BACI experimental approach. We found these metrics to display few clear response patterns, which was not surprising given the violations of theoretical assumptions and confounding influences that are typical for systems like those we studied. We observed no differences in heterozygosity and allelic richness in our treatment (or control) samples across the two sampling periods. We did observe that all three above-culvert sample populations maintained significant linkage disequilibrium at multiple loci after culvert remediation, as would be expected if recent movement into these sites occurred (Waples and England 2011). However, these patterns would also be expected to arise from the family structure observed in our study (Whiteley et al. 2013), emphasizing that even when we observed a population-level response that conformed to our expectations, we could not definitively attribute it to postremediation movement because of potential interactions with other factors. Similarly, differentiation (F_{ST}) decreased between the two above-culvert samples after the culverts were remediated. We did not have 2008 below-culvert samples to enable within-stream comparisons (i.e., above–below pairwise F_{ST}) across time, but pairwise F_{ST} values for samples above and below each culvert in 2011 were low ($F_{ST} = 0$ in Treatment Stream 1 and 0.04 in Treatment Stream 2) and consistent with expectations of gene flow between these sites. However, the 2011 cross-culvert distribution of the two large families in each treatment stream, as well as their assignment to the same genetic cluster, suggests family structure may have reduced observed relevant F_{ST} values in our study. That F_{ST} may have been downward-biased by family structure is corroborated by theoretical expectations for a longer time lag for decreased differentiation following stream reconnections (e.g., Langduth et al. 2010; Raeymaekers et al. 2009).

Conceptually, the clustering and assignment approach we implemented should have reasonable power to identify age 1+ migrants across the remediated culverts in our treatment streams; our populations showed the requisite level of (moderate–high) divergence when isolated (Manel et al. 2005; Saenz-Agudelo et al. 2009), and this approach has performed well at identifying barrier effects in simulations (Safner et al. 2011; but see Whiteley et al. 2014, this issue). In particular, STRUCTURE did indicate marked changes after culvert remediation in the genetic characteristics of individuals found above the restored culvert in Treatment Stream 1, which in 2011 shifted ancestry towards the white cluster (Fig. 3*b*). However, pedigree analyses determined that the identified "movers" into upper Treatment

Stream 1 (fish captured in upper Treatment Stream 1 in 2011 that assigned to the white cluster) were actually part of one large full-sibling family collected both above and below the culvert in Treatment Stream 1. The distribution of these siblings could be used post hoc to infer movement through the culvert, (e.g., via sib-split), but using STRUCTURE alone we might have incorrectly interpreted the nature of this movement. From STRUCTURE results alone, we could have inferred that each age 1+ individual moved purposefully through the culvert into upper Treatment Stream 1, possibly even immigrating from Treatment Stream 2, whereas the most parsimonious interpretation from pedigree analysis suggests that movement through the culvert arose via the dispersion of siblings born in the stream (though perhaps the progeny of immigrant parents). It is also possible, although perhaps less likely, that the same parents spawned on both sides of the culverts to generate two separate full-sibling families. Family structure has been recognized to influence clustering results (Anderson and Dunham 2008; Rodriguez-Ramilo and Wang 2012), and its effect can become more apparent with the increased resolution of a larger molecular marker set (see Whiteley et al. 2014, this issue; Vaha et al. 2007), but we were surprised at the high frequency of age 1, and even ages 2 or 3, siblings in our samples and their consequent strong effect on patterns and interpretation of individual clustering and assignment.

Removal of siblings is prudent to meet expectations of all of the above analyses (e.g., Anderson and Dunham 2008; Whiteley et al. 2013). In our current study, this would require removing at least half of the age 1+ individuals from each of our Treatment Streams 1 and 2 above-culvert samples, greatly reducing our final sample size and thus **still** compromising estimates (e.g., Antao et al. 2011). Evaluation of these needs comes at great expense because family structure is difficult to predict and siblings can only be identified after genotypes are obtained. This tradeoff — the potential necessity of genotyping many individuals beyond the targeted sample size so that family members (or hybrids) can be removed — reduces the general appeal of genetic data specifically for monitoring movement, at least soon after culvert removals in small stream populations such as those we studied.

However, partly as a consequence of this tight family structure, sib-split focused on the YOY age class proved the most effective genetic method for detecting movement by WCT in our study streams. This was a somewhat surprising result, as we were concerned that life history characteristics of the target species here — WCT, which spawn in late spring – early summer, resulting in YOY emergence late in the growing season — might preclude application of sib-split because the only viable sampling opportunity is within weeks of emergence. In contrast, brook trout spawn in the fall and YOY emerge in the spring, thus allowing YOY brook trout approximately 4 months to move before late summer sampling (e.g., Hudy et al. 2010; Whiteley et al. 2014, this issue). We initially assumed YOY dispersal distances would be constrained in our study, affecting the utility of sib-split to detect culvert passage, but the method clearly detected movement across all three of the remediated culverts, even just shortly after emergence. Ultimately, our sampling so soon after emergence may have been advantageous in allowing us to collect relatively large families before substantial mortality or out-of-site movement may have occurred. In fact, in all three sites the sampling could have been restricted to just 60 m above and below the culvert (Fig. 4), thus reducing the costs of genetic analyses. Minimally sufficient sampling schemes will obviously depend on the distribution of YOY in a given study area, but our results (see also Whiteley et al. 2014, this issue) suggest a pilot study or a staged approach to genotyping (i.e., collecting more broadly above and below a barrier but running initial analyses only on a subset of individuals) may help avoid unnecessary genotyping and reduce costs. Finally, where simply detecting movement is the goal, an advantage of sib-split is that it should still be effective even where hybrids occur. Here we removed hybrids, as our focus was on monitoring movement and its impacts in a native trout, but where this is not of concern, hybridization may actually increase the power of sib-split given the increased allelic diversity from two parental species.

Results from this current study and insight from other parts of our geographic region (the western US) do, however, suggest some constraints on sib-split to consider for future monitoring planning. First, there are some streams in the west and elsewhere where sib-split based on YOY collections will not likely be feasible. For instance, our original study design included a fourth treatment stream (a tributary to Crooked Creek, Idaho, shown in Fig. 1 but removed from analyses), but total recruitment failure in 2011 precluded the use of sib-split targeting YOY in this stream. Predictable application of sib-split may be difficult for populations at the edge of environmental tolerance (see, e.g., Coleman and Fausch 2007*a*, 2007*b*; Harig and Fausch 2002) or where recruitment is highly variable for any reason. Additionally, in many regions with high topographic relief (e.g., in the northern Rockies of Idaho; Neville et al. 2009), roads are often located in valley bottoms, and the associated culverts are found at the confluence with larger mainstem rivers; sampling YOY in such rivers (which would be the below-culvert habitat) is difficult, and there may be a lower likelihood that YOY would remain in close proximity to each other to allow for family reconstruction, given the larger habitat and higher flows. In other, more arid regions such as in the Great Basin Desert, cutthroat trout are typically constrained to the very headwaters of streams, while downstream habitats — where culverts are often located — often dry up shortly after spring flows. In this latter case, the distribution of late summer-emerging YOY would be irrelevant for evaluating movement over culverts, and sib-split would be feasible only for older age classes that would have had an opportunity to move through culverts during higher spring flows (H.M. Neville, unpublished data). In our present study, we were able to utilize information about family structure in older age groups of WCT to successfully apply sib-split post hoc to infer movement. The same may not be true in systems where older age classes of fish are more dispersed or have suffered substantial mortality.

Finally, other authors have proposed a "majority rule" to determine the directionality of movement from sib-split, whereby movement is inferred in the direction with the fewest siblings under the assumption that the majority of siblings would still be located closest to the natal redd (Hudy et al. 2010; Whiteley et al. 2014, this issue). We did not feel comfortable quantifying the actual number of movers based on inferring directionality from sibling distributions in our study. For instance, in Treatment Stream 1, based on the majority rule we would infer upstream movement of YOY in 9 of 24 families where more individuals were captured below the culvert than above (Fig. 4*a*). We caution that results such as this could be biased by the established splitting behavior of COLONY (Almudevar and Anderson 2012); that is, it is possible in some of these instances that a larger upstream family may have been split into two or more groups, as we demonstrated by simulation. Similarly, in instances where downstream movement is inferred (more individuals found above the culvert, assumed to be the redd location), it may well be that other siblings in the downstream family simply dispersed out of our sample site (into the mainstem EFLC, in this case). Differential movement and survival in upstream versus downstream habitats, because of different physical conditions or fish assemblages, for instance, may influence observed sibling dispersion patterns (Bujold et al. 2004; Crisp and Hurley 1991; Westley et al. 2008). Thus, we recommend caution when inferring movement based on sibship without other corroborating information (or sampling in all suitable habitat, which would be prohibitive in most situations). Similarly, we recommend careful analysis of the behavior of COLONY relative to the locus set used and the research question being addressed to evaluate the likelihood of type I and II errors that could occur (see also Whiteley et al. 2014, this issue). With our YOY collections and microsatellite marker set, we feel confident that these errors were minimized and enabled accurate reconstruction of the siblings

within each family identified, despite some potential family splitting. Each source of error likely increased with our analysis of age 1+ individuals, which were analyzed with a subset of the loci evaluated for YOY, leading us to focus more on general patterns of movement than specific assignments of individuals for these samples. In general, each type of error may be more or less acceptable for any given question, but one should fully understand and explore the impact of these possible errors on interpretations of movement or the ecological–evolutionary question of interest. The careful matching of question and method is a fundamental consideration (Manel et al. 2005). While sib-split focusing on YOY successfully detected movement, additional information — most likely at the population level and including hybridization patterns — would be needed to establish whether such movement improved the status or viability of our target population(s) in the long term.

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Appendix A

Appendix tables and figures appear on the following pages.

Table A1. Loci used in this study, with the species in which they were discovered or the hybrid loci identified, the samples for which they were used, and the literature reference.

Locus	Species	Samples	Reference
OCH5, 6, 13, 14, 16, 17, 18, 20, 22, 24, 29, 30, 35	Lahontan cutthroat trout	Age 1+ and YOY	Peacock et al. 2004; Robinson et al. 2009
OCH21	Lahontan cutthroat trout	YOY	Robinson et al. 2009
OMM1329, 1323, 1220, 1325, 1037, 1272, 1018	Rainbow trout	YOY	Palti et al. 2002; Rexroad et al. 2002; Rexroad and Palti 2003
OCC34, 35, 36, 37, 38; OM55	Hybrid	All	Ostberg and Rodriguez 2004

Fig. A1. Schematic of the process for determining the accuracy of COLONY in reconstructing pedigrees from individuals with known pedigrees, which were simulated in program PEDAGOG based on observed levels of genetic diversity in our field collections.

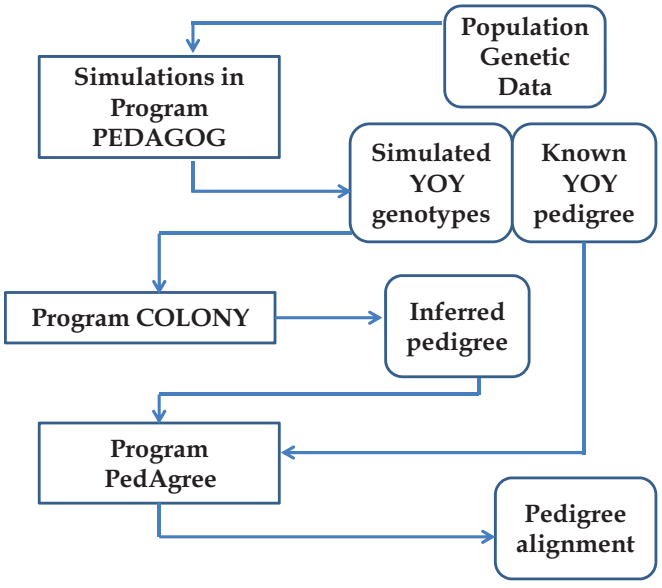

Table A2. For each sample site in the East Fork Lolo Creek, Montana (Isolated and Connected Controls, and above and below the culvert in both treatment streams), and Haskell Creek, Idaho, the number of westslope cutthroat–rainbow trout hybrids and percentage of the total sample containing hybrids are given for age 1+ and YOY collections, respectively.

	2008 age 1+		2011 age 1+		2011 YOY	
Sample site	No. of hybrids	% Sample	No. of hybrids	% Sample	No. of hybrids	% Sample
Control						
Isolated	8	17%	NA	NA	NA	NA
Connected	0	0%	0	0%	NA	NA
Treatment 1						
Above	22	52%	6	12%	17	6%
Below	NA	NA	4	14%	31	17%
Treatment 2						
Above	5	12%	2	4%	0	0%
Below	NA	NA	20	43%	0	0%
Haskell Creek						
Above	NA	NA	1	1%	0	0%
Below	NA	NA	0	0%	0	0%

Note: All hybrids were removed from further analysis. NA, not applicable.

Fig. A2. For panels *a*, *b* and *c*, the left panels show the mean log-likelihood from STRUCTURE, while the right panels show Delta *k* following Evanno et al. (2005) for different numbers of clusters *k* (*x* axis) simulated in STRUCTURE. Results for EFLC 2008 are in panel *a*, where *k*3 was inferred, while *k*2 was the most appropriate level of structuring for 2011 in panel *b*. Panel *c* shows results for Haskell Creek, for which *k*3 was inferred.