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INFLUENCE OF BARRIERS TO MOVEMENT ON WITHIN-WATERSHED GENETIC VARIATION OF COASTAL CUTTHROAT TROUT

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Abstract. Because human land use activities often result in increased fragmentation of aquatic and terrestrial habitats, a better understanding of the effects of fragmentation on the genetic heterogeneity of animal populations may be useful for effective management. We used eight microsatellites to examine the genetic structure of coastal cutthroat trout (*Oncorhynchus clarki clarki*) in Camp Creek, an isolated headwater stream in western Oregon. Our objectives were to determine if coastal cutthroat trout were genetically structured within streams and to assess the effects of natural and anthropogenic barriers on coastal cutthroat trout genetic variation. Fish sampling occurred at 10 locations, and allele frequencies differed significantly among all sampling sections. Dispersal barriers strongly influenced coastal cutthroat trout genetic structure and were associated with reduced genetic diversity and increased genetic differentiation. Results indicate that Camp Creek coastal cutthroat trout exist as many small, partially independent populations that are strongly affected by genetic drift. In headwater streams, barriers to movement can result in genetic and demographic isolation leading to reduced coastal cutthroat trout genetic diversity, and potentially compromising long-term population persistence. When habitat fragmentation eliminates gene flow among small populations, similar results may occur in other species.

Key words: conservation genetics; dispersal barriers; habitat fragmentation; headwater streams; isolation; Oncorhynchus clarki clarki; *salmonids.*

INTRODUCTION

Habitat fragmentation has been linked to a variety of changes throughout ecological hierarchies, including alterations of individual dispersal behaviors (Stow et al. 2001), shifts in population dynamics (Huhta et al. 2004), reductions in community complexity (Driscoll 2004), and ecosystem-level changes through modifications of trophic cascades (Tallmon et al. 2003). If metapopulations are involved, fragmentation of habitat can destroy critical dispersal pathways, eliminating reestablishment of extirpated populations and resulting in a ''debt of extinction'' (sensu Hanski 1996).

From a genetic perspective, the disruption of migration corridors can result in reduced gene flow, isolating populations and decreasing genetic diversity through the processes of genetic drift and inbreeding (Slatkin 1985). Habitat fragmentation has been implicated in a loss of genetic variation in a multitude of organisms, including roe deer (*Capreolus capreolus*; Wang and Schreiber 2001), winter moths (*Operophtera brumata*; Van Dongen et al. 1997), and a Rhone River percid (*Zingel asper*; Laroche and Durand 2004). In salmonids, researchers have noted decreased genetic diversity in fragmented stream networks where populations are isolated above waterfalls (Carlsson and Nilsson 2001, Castric et al. 2001, Costello et al. 2003, Taylor et al. 2003). Although the link between genetic diversity and fitness is not firmly established (Wang et al. 2002), it is likely that genetic variation is necessary for populations to adapt and persist in the face of environmental change (Allendorf et al. 1987).

The ecological and genetic consequences of large-scale anthropogenic alterations (e.g., forest clear-cutting, large dam construction) are well described through rigorous study by the scientific community. Yet, smaller localized alterations are far more common and widely distributed than these large-scale disturbances and therefore can be equally important provided that these alterations induce analogous changes in genetic and ecological processes. This study addresses the hypothesis that habitat fragmentation can induce genetic differentiation at relatively small spatial scales.

As a result of extensive road building, small stream migration barriers are numerous and widely distributed in the Pacific Northwest. For instance, on U.S. Forest Service lands in Oregon, of ~2750 culverts surveyed, 82%, or 2255 structures failed to meet federal standards for adult and juvenile fish passage (M. Furniss, *personal communication*). This number excludes road

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crossings on private holdings and other federal and state lands, so the true number of anthropogenic fish passage impediments in small streams is probably much higher. Although the relative differences in the effects of anthropogenic barriers and natural barriers on fish populations is unclear, the sheer number of human-related migration obstacles raises important questions concerning the effects of barriers on fish population dynamics, genetics, and persistence.

For example, prior studies have noted that dispersal barriers can affect salmonid population structure (Carlsson and Nilsson 2001, Castric et al. 2001, Neraas and Spruell 2001, Costello et al. 2003, Taylor et al. 2003). These investigations have provided information about evolutionary and contemporary influences on salmonid genetic variation, but despite their strengths, the focus has been on differences among watersheds at relatively large spatial scales. Fausch et al. (2002) argue that stream fish conservation measures have often failed because research is not focused at intermediate spatial scales (10^3–10^5 m) that are pertinent for many stream fishes and that are relevant to human management of watersheds. In an attempt to address this problem, this study investigates salmonid genetic structure within a small watershed, where land use activities, stream fish management, and coastal cutthroat trout (*Oncorhynchus clarki clarki*) occur concomitantly.

Because the genetic effects of population isolation can be similar across species, results from this study should prove useful to a variety of ecosystem managers. Whether populations are isolated as a result of anthropogenic activities or ''natural'' events, a better understanding of how migration barriers influence genetic processes will improve the science of ecosystem management and restoration. In an attempt to study the effects of habitat fragmentation on genetic structure, we used eight microsatellites to provide an assessment of genetic differentiation in coastal cutthroat trout from an isolated watershed. Our objectives were to assess coastal cutthroat trout population structure in a small stream network and to evaluate the effects of fish passage barriers on coastal cutthroat trout genetic variation.

Methods

Study site and sampling procedures

Camp Creek, a third-order stream in the Umpqua River basin of western Oregon, was chosen for this study (Fig. 1). The Camp Creek watershed is primarily composed of sedimentary rock with a drainage area of 2200 ha. Although extensive logging has occurred on some tributaries and ridge tops, old-growth Douglas-fir (*Pseudotsuga menziesii*) and red cedar (*Thuja plicata*) are present throughout the riparian corridor. The study sections of Camp Creek are isolated from anadromous fish by a 15-m waterfall (barrier 1, Fig. 1). Coastal cutthroat trout, reticulate sculpin (*Cottus perplexus*), and longnosed dace (*Rhinichthys cataractae*) are the only fish species present, and there are no records of fish stocking in the Camp Creek basin. Although we have no data on recreational fishing in the basin, because of small fish size and the relatively remote location, it is likely that fishing is rare.

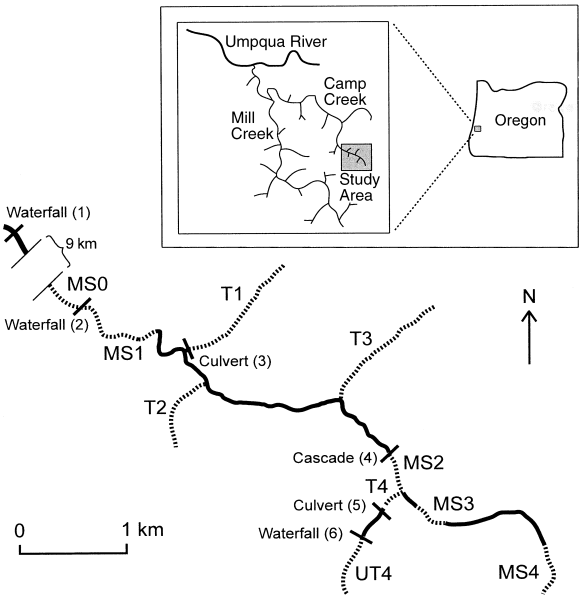

FIG. 1. Map of coastal cutthroat trout distribution and sampling sections in the Camp Creek study area, western Oregon, USA. Sampling sections are identified by dashed lines. Solid bars indicate barriers to fish passage. Captions associated with bars specify barrier types, and numbers in parentheses identify barriers. Key to abbreviations: MS, mainstem; T, tributary; UT, upper tributary.

During the summer of 2002, the watershed was surveyed in order to identify barriers to fish passage. Professional fishery biologists used visual assessments to identify two culvert barriers and four geomorphic barriers (Fig. 1). The geomorphic barriers included a 15-m bedrock falls (barrier 1), a 5-m bedrock falls (barrier 2), a 30-m long bedrock cascade with a 15% slope (barrier 4), and a 2-m boulder falls (barrier 6). Culverts (barriers 3 and 5) were tested and verified as fish passage barriers using FishXing v. 2.2 software (*available online*).[5] An examination of historical aerial photographs indicated that the two culverts were installed in the mid-to late-1950s. We were unable to date geomorphic barriers or empirically verify them as fish passage barriers.

All genetic sampling locations were determined by tributary junctions or fish-passage barriers, excluding sites MS3 and MS4 (Fig. 1). Sites MS3 and MS4 were selected because it was important to sample the entire watershed, and no tributaries or passage barriers occurred in the relatively extensive upper portions of

[5] ⟨http://stream.fs.fed.us/fishxing/⟩

Camp Creek. In all, genetic sampling occurred at 10 sites in the Camp Creek watershed.

In August 2002, electrofishing crews began sampling at barrier 2 (Fig. 1) and proceeded upstream, sampling every pool and cascade in the fish bearing portions of the watershed. Prior to release, trout were measured (nearest millimeter fork length) and weighed (nearest 0.1 g), and a small portion of caudal fin tissue (1.5 mm^2) was collected. In sections MS1, T1, T2, and T3, up to 10 fin clips were taken from trout (>50 mm in length) in each 10-mm size class until 100 samples were collected or until sampling crews reached the end of trout distribution. At site MS0 and at all sites above barrier 3, fin clips were obtained from every captured trout. Fin tissue was stored in 2-mL vials with a calcium sulfate desiccant (Indicating Drierite, W. A. Hammond Drierite, Xenia, Ohio, USA). During May 2003, we returned to the basin and using a hook and line, additional samples were collected at site MS1, and site MS0 was sampled for the first time. Thus, nine sites were sampled solely in 2002, one site in 2003 (MS0), and one site in both years (MS1). Length–frequency histograms were used to identify trout age groups.

Microsatellite typing

Genomic DNA was extracted from small portions of tissue (0.5 mm^2) in 200 μL of 5% Chelex 100 (Bio-rad Laboratories, Hercules, California, USA) in 96-well PCR trays (0.2 μL) using a PT-100 thermocycler (MJ Research, Waltham, Massachusetts, USA). Tissue extracts were heated at 65°C for 3 h, boiled at 103°C for 10 min, and stored at 4°C. Eight microsatellite loci were used to characterize coastal cutthroat trout genetic variability in the Camp Creek watershed. All forward primers were labeled with fluorescent phosphoamidite (HEX, TET, or FAM). We developed two multiplex sets: set A (*Ots-209* and *Ots-212* [Greig et al. 2003]) and set B (*One-102, One-103,* and *One-108* [Olsen et al. 2000] Appendix A). *Ots-9,-10* (Banks et al. 1999), and *Omy-1046* (Rexroad et al. 2002) were amplified individually in separate PCR reactions (Appendix A). DNA fragments were fractioned by size on a 5% acrylamide gel and visualized using an MJ Research BaseStation DNA fragment analyzer. Gels were manually scored using MJ Bioworks Cartographer version 1.2.3sg software (MJ Research, Waltham, Massachusetts, USA). In order to maximize sample sizes, we made second attempts at PCR reactions that failed to produce scoreable products during initial processing.

Genetic and statistical analysis

Allele frequencies, number of alleles per locus, allelic richness, and estimates of genetic distance (F_{st}; Wright 1951), were calculated using FSTAT software (Goudet 1995). The F_{st} statistic provides a general measure of genetic differentiation that is comparable across species. Significance of F_{st} values was evaluated by permutation procedures as implemented in FSTAT. Measures of allelic richness are sensitive to the number of individuals sampled, so allelic richness values were standardized by sample sizes (El Mousadik and Petit 1996, Petit et al. 1998). In some situations, the genetic distance between populations can be related to the geographic distance separating those populations. To test for this phenomenon, we compared genetic (F_{st}) and geographic distance matrices using a Mantel test as implemented by FSTAT (2000 randomizations).

As another measure of genetic differentiation, genotype distributions between all locus-population combinations were compared using exact tests and Markov chain methods as implemented in GENEPOP version 3.3 (Raymond and Rousset 1995). Parameters for all Markov chain iterations included: dememorization number of 1000, 200 batches, and 1000 iterations. The GENEPOP software was also used to calculate observed heterozygosities and gene diversities (expected heterozygosity) and to assess deviations from Hardy-Weinberg expectations and genotypic linkage equilibrium between loci. When appropriate, Bonferroni adjusted P values were used for evaluating statistical significance (Rice 1989). Adjustments included corrections for multiple comparisons of populations ($n = 11$), microsatellite loci ($n = 8$), or loci combinations within populations ($n = 28$).

Additional tests for population differentiation were performed using procedures implemented by STRUCTURE (Pritchard et al. 2000) and using the assignment test of WHICHRUN (Banks and Eichert 2000). The STRUCTURE program is a Bayesian genetic clustering procedure that assumes complete linkage and Hardy-Weinberg equilibrium within populations and estimates the population of origin of individuals (Z) and the allele frequencies of all populations (P). Prior probability distributions for Z and P are estimated using observed genotype data. In the STRUCTURE program, an anonymous routine iterates through alternate assignment of individual genotypes into k groups to maximize linkage equilibrium and Hardy-Weinberg equilibrium within groups with no regard for where the samples were collected. We evaluated ln likelihoods for $k = 1$–15 by averaging results from 20 iterations (burn-in 50 000 replications, 100 000 MCMC replicates). Sample locations were then assigned to clusters that contained the majority of individuals captured in the respective location. The WHICHRUN software is an individual-based population assignment program that uses genotypic data to allocate individuals to their most likely source populations. Ten replicate data sets ($N = 10\,000$) were simulated to assess statistical power for correct population assignment using WHICHLOCI (Banks et al. 2003). In order to visualize genetic structure in Camp Creek, phylograms were generated using SEQBOOT, GENEDIST, NEIGHBOR, and CONSENSE computer programs, as implemented in the PHYLIP software package (Felsenstein 1991). Trees were edited using TREEVIEW (Page 1996).

When large numbers of juveniles were present in a sample, or when sampling occurred over consecutive years, we tested for nonrandom sampling of family groups (Hansen et al. 1997) and temporal stability of allele frequencies by examining genotypic distributions, deviations from Hardy-Weinberg expectations, and genotypic linkage disequilibrium between age groups/sample years within sampling locations. In genetically ''ideal'' populations (populations of infinite size, random mating, no migration, etc.; Hartl and Clark 1997), allele frequencies should be stable over time. Therefore, when multilocus genotypic differences were encountered across age groups/sample years at a sample location, the respective subsamples were considered to be separate populations. Within age-0 samples, deviations from Hardy-Weinberg expectations or multiple failures of tests for genotypic linkage equilibrium (>10% failures per population [Banks et al. 2000]) were viewed as evidence of family sampling.

Results

Loci diagnostics

Sample sizes for individual populations ranged from 31 (*One-102* in T2) to 118 (*Omy-1046* in MS3) and the mean was 68 (Appendix B). All eight of the microsatellite loci analyzed were polymorphic in Camp Creek coastal cutthroat trout. Across all populations, the number of alleles per locus ranged from 3 (*Ots-209*) to 11 (*One-102*) with an average of 8.0 alleles per locus; however, upstream of barrier 2, the average number of alleles per locus dropped to 4.1. A total of 20 private alleles (rare alleles observed in a single population) were documented, and at least one private allele (mean frequency = 0.038) occurred at each locus. Seventeen of these private alleles were found in the MS0 population; upstream of barrier 2, three private alleles (average frequency 0.013) occurred in two loci (*One-102* and *One-108*) and three populations (T3, T4, and MS3) each contained one private allele.

Deviations from Hardy-Weinberg equilibrium were observed in 9 of 82 (10.9%) possible tests within loci, across populations ($\alpha = 0.05/11 = 0.0045$), and in 10 of 82 (12.2%) possible tests across loci, within populations ($\alpha = 0.05/8 = 0.00625$). Heterozygote deficits were spread among six populations and five loci with no more than three deficits at any one locus or population. A total of 15 of 267 (5.6%) possible loci combinations failed tests for genotypic linkage equilibrium ($\alpha = 0.05/28 = 0.0018$). These failures occurred across 10 loci combinations with no more than three at any one loci pair.

Temporal stability and family sampling

We were able to test for temporal stability of allele frequencies and sampling of related individuals because of two sampling events: (1) The MS1 sample location was sampled during consecutive years, and (2) length–frequency histograms indicated that several samples contained enough age-0 trout for reasonable subsampling (>10 age-0 trout at sites MS2, T4, UT4, MS3, and MS4). Detailed results are described in the next four paragraphs, but in summary, allele frequencies were temporally stable in most samples (with the exclusion of T4), and relatively few highly related individuals were including in each sample (with the exclusion of MS4).

Tests for genotypic differentiation between sample years in the MS1 location revealed no significant temporal changes in allele frequencies ($\alpha = 0.05/8 = 0.0063$). Therefore, the MS1 samples from 2002 and 2003 were pooled into one sample. Comparisons across age groups at other sample locations indicated that three populations (MS2, MS4, and T4) contained genotypic differences between age-0 and age-1+ trout. In the MS2 and MS4 populations, age groups differed at a single locus (*Omy-1046* and *Ots-10,* respectively), but in the T4 location, age groups differed at five of seven possible loci comparisons (*Ots-212, Omy-1046, Ots-10, One-102, One-103*; $\alpha = 0.05/8 = 0.0063$). Because of the significant multilocus differences between age groups, samples from the T4 location were split into two samples (one containing only age-0 trout and the other composed of age-1+ trout).

Testing for differences in allelic frequencies in temporal samples from small populations can result in statistical differences due to genetic drift (Waples 1990). Although the outcome from the temporal analyses in the T4 location may be a result of this phenomenon, this was the only location exhibiting temporal instability, and the sample was ''split'' into two age groups as a conservative measure.

Tests for genotypic linkage disequilibrium and Hardy-Weinberg expectations within age groups indicated potential sampling of many highly related individuals in the MS4 age-0 population. In the MS4 age-0 samples, 5 of 21 (24%) possible loci comparisons failed tests for genotypic equilibrium ($\alpha = 0.05/28 = 0.0018$). In addition, three of seven polymorphic loci in the MS4 age-0 population differed significantly from Hardy-Weinberg expectations ($\alpha = 0.05/8 = 0.00625$). Poor discriminatory power among loci precluded the correction of the age-0 MS4 sample for relatedness (Banks et al. 2000); therefore, all age-0 fish were removed from the MS4 sample before further analysis. None of the other age-class subsamples revealed genotypic linkage disequilibrium at >10% of possible comparisons, and only one population showed evidence for deviation from Hardy-Weinberg expectations. This deviation occurred at a single locus (*Omy-1046*) in the UT4 age1+ population ($\alpha = 0.05/8 = 0.00625$).

Final data adjustments, therefore, included pooling the 2002 and 2003 samples from MS1, splitting T4 into two samples of age-0 and age-1+ trout (resulting in sample T4-0 and T4-1), and removing all age-0 fish from the MS4 sample (resulting in sample MS4-1).

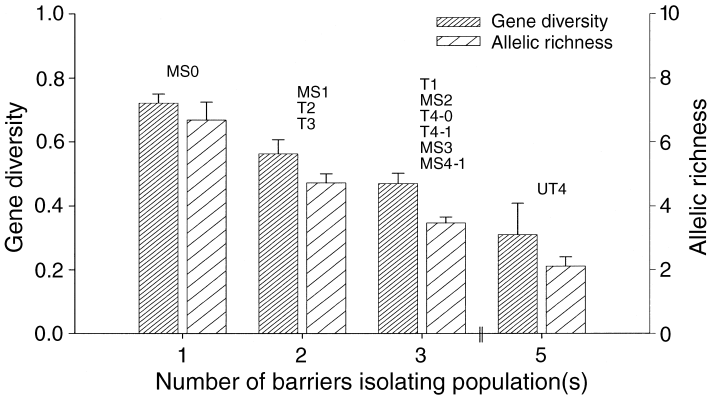

FIG. 2. Gene diversity and allelic richness (mean + 1 SE) of 11 Camp Creek populations in relation to the number of barriers (anthropogenic and geomorphic) located downstream of the respective population. None of the populations in Camp Creek is isolated by four barriers.

Following these data adjustments, heterozygote deficits decreased within loci, across populations (from 10.9% to 6.1%) and within populations, across loci (from 12.2% to 7.3%), and total genotypic linkage disequilibrium decreased from 5.6% to 4.9%.

Gene diversity and genetic differentiation

Mean within-population gene diversity was 0.50, and mean allelic richness was 3.96. In general, gene diversity and allelic richness decreased with increasing distance upstream and the associated increase in genetic isolation (Fig. 2, Appendix B). Tributaries that were connected with mainstem habitats tended to have relatively high levels of allelic richness and gene diversity, and low values were associated with samples upstream of barriers to gene flow.

When comparing each population pair across loci, significant genotypic differences were detected in 369 of 433 (85.2%) possible tests ($\alpha = 0.05/8 = 0.00625$). Genotype distributions differed between population pairs at a majority of loci (mean = 6.7 loci; range = 2–8 loci; Table 1). Genetic differences between populations were also evident from pairwise F_{st} estimates (mean = 0.124; range = 0.014–0.393; Table 1). The largest F_{st} values were associated with the two fully isolated populations (T1, UT4). The mean pairwise F_{st} value for populations that were not separated by barriers (0.062) was significantly different from the mean F_{st} between barrier-separated populations (0.144; Mann Whitney, $P < 0.01$); however, this result was related to the effects of the tributaries that were completely isolated from the remainder of the stream network (sites T1 and UT4). Excluding these two populations from the analysis, differences in mean pairwise F_{st} values between separated (0.085) and connected (0.062) populations were not significant (two-sample t test, $P > 0.05$). No significant relationship was observed between genetic distance and geographic distance.

A neighbor-joining phylogram of Cavalli-Sforza and Edwards chord distance (Cavalli-Sforza and Edwards 1967) illustrates the influence of fish-passage barriers on coastal cutthroat trout genetic structure in Camp Creek (Fig. 3). Phylogram organization is related to the spatial location of mainstem barriers, dividing the phylogram into the lower (MS0), middle (MS1, T1, T2, T3), and upper watershed (MS2, MS3, T4-0, T4-1, UT4, MS4). In addition, in the middle and upper wa-

TABLE 1. Population structure and genotypic differentiation of coastal cutthroat trout in Camp Creek, western Oregon, USA.

Sample section	MS0	MS1	T1	T2	T3	MS2	T4-0	T4-1	UT4	MS3	MS4-1
MS0		0.04	0.19	0.06	0.03	0.07	0.10	0.10	0.19	0.06	0.09
MS1	8		0.22	0.03	0.01	0.04	0.08	0.08	0.19	0.05	0.09
T1	8	8		0.25	0.24	0.26	0.37	0.33	0.39	0.23	0.32
T2	8	6	7		0.04	0.08	0.11	0.10	0.21	0.04	0.10
T3	6	2	7	4		0.05	0.09	0.09	0.21	0.04	0.06
MS2	8	5	8	7	5		0.05	0.03	0.15	0.03	0.06
T4-0	8	8	8	7	7	4		0.12	0.20	0.09	0.15
T4-1	7	6	8	8	6	5	5		0.20	0.07	0.06
UT4	8	8	6	8	8	8	7	7		0.12	0.17
MS3	8	7	6	8	4	5	7	7	8		0.06
MS4-1	8	7	8	8	7	5	4	4	7	7	

Notes: Values above the diagonal represent pairwise F_{st} values, and numbers below the diagonal represent the number of loci (out of 8) that revealed significant genotypic differentiation between populations ($\alpha = 0.05/8 = 0.00625$). Following Bonferroni adjustments, all F_{st} values were significant at $P < 0.001$, excluding the T3/MS1 comparison (significant at $P < 0.01$).

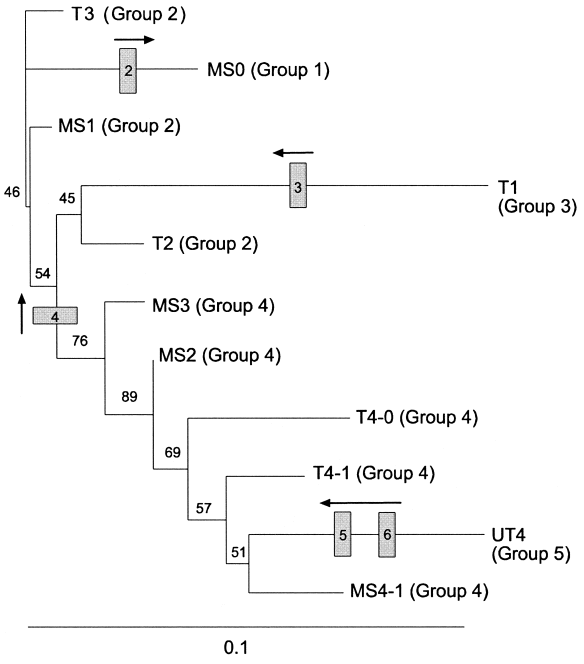

FIG. 3. Neighbor-joining phylogram of 11 Camp Creek coastal cutthroat trout populations using Cavalli-Sforza and Edwards chord distance at eight microsatellite loci. Numbers at nodes represent the percentage of bootstrap simulations that support the associated groups (1000 replicates). Gray bars represent fish passage barriers, numbers in bars identify barriers (as depicted in Fig. 1), and arrows indicate potential downstream directional gene flow. Group numbers indicate Bayesian cluster assignments.

tersheds, the large divergence of tributary populations T1 and UT4 is also associated with passage barriers.

Bayesian posterior probabilities were highest for $k = 5$, providing evidence that five clusters captured most of the genetic structure found in Camp Creek. These five groups were similar to the structure observed in the phylogram and were strongly associated with barriers to movement: Group 1 (MS0), Group 2 (MS1, T2, T3), Group 3 (T1), Group 4 (MS2, T4-0, T4-1, MS3, MS4-1), and Group 5 (UT4; Fig. 3). Because Groups 2 and 4 contained multiple sampling locations, we pooled individuals from these sampling locations into the groups indicated by STRUCTURE and evaluated these groups for linkage disequilibrium and Hardy-Weinberg equilibrium. When pooled, these groups displayed linkage disequilibrium at 14% (Group 2) and 25% (Group 4) of the loci combinations and failed tests for Hardy-Weinberg equilibrium at 22% of the comparisons in both groups.

Individuals were not randomly reassigned among populations, nor were all individuals reassigned to their sampled populations as sampled population reassignment ranged from 37% (T3) to 98% (UT4; Fig. 4). Simulations revealed that WHICHRUN could be expected to correctly assign 74.8 ± 5.3% of the individuals, and assuming that fish barriers were correctly identified, some highly unlikely reassignments did occur (Fig. 4). Although it is possible that some structures were incorrectly classified as barriers, we are highly confident in our visual assessments of fish passage barriers. In addition, a year-long mark–recapture study of Camp Creek coastal cutthroat trout never identified an individual moving upstream across the barriers denoted in this study (Hendricks 2002). Limited power for assignment tests likely occurred as a result of low allelic variation and relatively low differentiation in allele frequencies among several populations.

DISCUSSION

Results from this study suggest that headwater coastal cutthroat trout persist as partially independent populations and that fish-passage barriers can dramatically and rapidly influence coastal cutthroat trout genetic variation. Where dispersal was possible, gene flow was adequate for preserving allelic richness and genetic diversity. However, despite open dispersal pathways in some locations, levels of gene flow were not sufficient to maintain uniform allele frequencies among populations.

The extent of fine-scale, within-watershed genetic structure observed in this study was equal to large-scale, among-watershed differentiation noted in anadromous coastal cutthroat trout (Wenburg et al. 1998, Wenburg and Bentzen 2001). However, much of the genetic heterogeneity observed at large scales was attributed to biological factors that limit gene flow, such as natal homing; in Camp Creek, most of the genetic structure occurred as a result of physical migration barriers and genetic drift. Population structure in Camp Creek consists of reduced gene diversity and dramatic divergence in allele frequencies in barrier isolated pop-

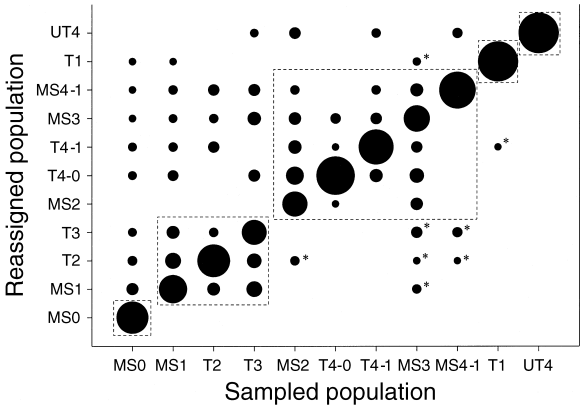

FIG. 4. Individual reassignment of Camp Creek coastal cutthroat trout. Circles represent the percentage of individuals assigned to a particular population. Dashed lines enclose reassignments between populations not separated by barriers. Asterisks highlight unlikely reassignments associated with individuals moving upstream across barriers. Only reassignments >2% are shown.

ulations, as well as minor genetic differentiation within continuous stream sections.

The effects of barriers and genetic drift are demonstrated by high F_{st} values, reduced heterozygosity, and the rapid loss of alleles associated with population isolation. In addition, Bayesian analysis clustered samples based on the presence or absence of open dispersal pathways between populations. Nevertheless, a variety of results indicate that minor genetic structure also exists among populations where dispersal is possible. Significant F_{st} values between all sampling locations indicate that allele frequencies differed throughout the basin, regardless of the presence or absence of barriers. In addition, a lack of random individual reassignments among populations supports the observation that fine-scale genetic structure is present. Also, the observed increase in deviations from linkage and Hardy-Weinberg equilibrium when samples were pooled by the absence of barriers provides additional evidence for genetic structure among ''connected'' populations. This fine-scale structure exists despite the fact that very little migration is required to maintain homogenous allele frequencies among populations (Hartl and Clark 1997).

Migration of coastal cutthroat trout in Camp Creek was somewhat difficult to assess because assignment tests were hampered by low power. Although most fish were reassigned to their source populations, data suggest that some migration does occur. Using mark–recapture techniques, Hendricks (2002) reported that some Camp Creek coastal cutthroat trout moved from 100 to 1000 m, distances greater than those separating many populations in this study. Although it is impossible to determine the potential genetic contribution of these fish, Hendricks (2002) noted that the most extensive trout movement occurred during spawning season, suggesting that movement was reproductively motivated. Results from assignment tests and mark–recapture data, in addition to the small spatial scales involved in this study, suggest that some migration occurs among populations in Camp Creek. Because genetic drift strongly impacts small populations (Allendorf 1986), and headwater streams frequently support low trout densities (Connolly and Hall 1999, Novinger and Rahel 2003), it is likely that genetic drift maintains the genetic heterogeneity in Camp Creek despite some gene flow between populations.

However, there are additional processes other than genetic drift that can contribute to fine-scale genetic structure. Ecological factors, such as habitat suitability (Gowan and Fausch 1996) or predation (Fraser and Gilliam 1995), and physical filters that limit, but do not eliminate dispersal (e.g., high gradient reaches, log jams, beaver ponds; Kocik and Ferreri 1998), could alter migration rates and influence trout genetic structure. In addition, because genetic variation is affected by past demographic events, disturbances such as debris flows or droughts could result in population bottlenecks that can cause reduced genetic variation and increased genetic differentiation. Indeed, recent debris flows (1996) in the UT4 and MS4 sampling locations may have influenced the genetic structure observed in Camp Creek. In headwater streams, high spatial and temporal variability in a variety of factors, including habitat quality and quantity, the presence and persistence of migration impediments, and the occurrence of stochastic events, is likely reflected in large temporal and spatial variation in salmonid demographic and genetic organization.

If taken out of context, the genetic effects of natural and anthropogenic barriers appear to be similar. Even so, it is likely that in small watersheds, the spatial distribution and permanence of anthropogenic barriers may differ from geomorphic passage obstructions, and these differences could dramatically alter the resulting effects on trout population structure. Field observations of over 50 watersheds in western Oregon indicate that natural barriers are rarely found at tributary junctions (R. E. Gresswell, *unpublished data*). Tributaries provide a major function as sediment delivery systems, and the deposition of alluvium at tributary junctions likely reduces the probability of barrier formation as well as the likelihood that a barrier could persist over time. In contrast, road construction along the narrow terraces of headwater streams is frequently associated with culvert installations that may act as long-term impediments to fish movement. Regardless of the difference between natural and anthropogenic fish passage impediments, it is highly likely that extensive road building has substantially increased the frequency of small stream barriers. Our data suggest that this additional stream fragmentation could result in reduced fine-scale genetic diversity of coastal cutthroat trout.

With no potential immigration (from upstream or downstream), coastal cutthroat trout in isolated tributaries T1 and UT4 exhibited the lowest gene diversity, lowest allelic richness, and the highest degree of genetic divergence. Indeed, the T1 population, which has only been isolated for 45 years (as of 2003), had roughly 50% fewer alleles than nearby tributaries with mainstem connections. Through losses attributable to the elimination of gene flow, barriers at tributary mouths restrict the spatial distribution of alleles, reduce the ''spreading of risk'' of genetic variation, and eliminate potential genetic refuges from larger scale stochastic events.

The existence of more than 250 coastal cutthroat trout populations above natural barriers in western Oregon and the widespread presence of natural barriers within headwater streams (Gresswell et al. 2003) suggest that coastal cutthroat trout are at least partially adapted to fragmented stream habitats. In the absence of extirpations, fragmented populations can actually retain higher genetic diversity than a single population of the same total size (Kimura and Crow 1963). Thus, at range-wide spatial scales, this fragmentation poten-

tially contributes to coastal cutthroat trout genetic diversity, and it is not recommended that natural barriers be modified for fish passage. However, at small spatial scales, where extirpation risks are high, fragmentation will likely have long-term negative consequences on the genetic variation of individual assemblages of coastal cutthroat trout.

Despite this evidence, the widespread presence of introduced salmonids has created a situation where intentional isolation is increasingly viewed as an appropriate measure for conservation (Kruse et al. 2001, Novinger and Rahel 2003). Evidence suggests that managers should consider intentional isolation only when other conservation strategies have been unsuccessful, and it is important to evaluate trout population sizes, local disturbance regimes, and habitat connectivity in conjunction with population genetic characteristics when determining the potential effects of isolation (Hilderbrand and Kruse 2000, Novinger and Rahel 2003).

Because independent management of genetically distinct populations is predicated on the assumption that genetic structure represents demographic independence, it is important to determine the factors influencing population structure (Carvalho 1993, Moritz et al. 1995). Other studies of fine-scale salmonid genetic structure cite reproductive isolation, due to precise natal homing or barriers to fish movement, as the cause of this fine-scale genetic heterogeneity (Carlsson et al. 1999, Spruell et al. 1999, Carlsson and Nilsson 2000, Herbert et al. 2000, Carlsson and Nilsson 2001, Neraas and Spruell 2001). Although barriers do affect the population structure in Camp Creek, some of the observed fine-scale genetic heterogeneity is likely derived from the effects of genetic drift and is not a result of natural selection and reproductive isolation. Even though salmonids can develop local adaptations at small spatial scales (Olsen and Vollestad 2001, 2003, Koskinen et al. 2002), the apparent gene flow among populations in Camp Creek suggests that management should potentially be focused at spatial scales that are larger than those represented by populations in this study. For proper conservation of salmonids, managers may need to focus not only upon individual populations and critical habitat areas, but also, and perhaps more importantly, on reestablishing linkages among tributary and mainstem populations, linkages that provide headwater salmonids with the demographic and genetic benefits of population connectivity.

Although many populations in Camp Creek have relatively low genetic diversity, these data do not directly address the probability of future population persistence, nor do they suggest that coastal cutthroat trout are resilient to the negative effects of genetic homogeneity. The fact that many isolated watersheds (>500 ha) in western Oregon do not support coastal cutthroat trout R. E. Gresswell, *unpublished data*) suggests that at some temporal scale, isolation leads to extirpation, and this may be related to the process of genetic degradation. Furthermore, in fragmented habitats, demographic and environmental stochasticity alone can lead to population extirpations (Morita and Yokota 2002).

As has been observed in other species, these data suggest that habitat fragmentation can result in a loss of genetic variation. When dispersal pathways are disrupted, gene flow is reduced or eliminated. The ensuing population isolation can exacerbate the effects of genetic drift and can result in reduced genetic diversity. In the past, anthropogenic alterations have resulted in landscape changes that have likely increased the number of fine-scale migration barriers in aquatic and terrestrial environments. It is important that in future activities, managers acknowledge the significance of habitat connectivity and recognize the potential effects of barriers to movement on animal genetic structure. The genetic consequences associated with population isolation may be relevant to a variety of organisms, and ecologists might expect similar results in other species that persist as small populations in fragmented habitats.

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APPENDIX A

A table showing thermocycler profiles, PCR reagents, and multiplex combinations for Camp Creek coastal cutthroat trout DNA amplification is available in ESA's Electronic Data Archive: *Ecological Archives* A015-016-A1.

APPENDIX B

A table showing a microsatellite locus summary for Camp Creek coastal cutthroat trout populations (including subsamples from age groups and sample years) is available in ESA's Electronic Data Archive: *Ecological Archives* A015-016-A2.