View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by CiteSeerX

Conserv Genet
DOI 10.1007/s10592-010-0083-y

RESEARCH ARTICLE

Genetic variation and effective population size in isolated populations of coastal cutthroat trout

**Andrew R. Whiteley · Kim Hastings ·
John K. Wenburg · Chris A. Frissell ·
Jamie C. Martin · Fred W. Allendorf**

Received: 6 September 2009 / Accepted: 26 March 2010
© Springer Science+Business Media B.V. 2010

Abstract Following glacial recession in southeast Alaska, waterfalls created by isostatic rebound have isolated numerous replicate populations of coastal cutthroat trout (*Oncorhynchus clarkii clarkii*) in short coastal streams. These replicate isolated populations offer an unusual opportunity to examine factors associated with the maintenance of genetic diversity. We used eight microsatellites to examine genetic variation within and differentiation among 12 population pairs sampled from above and below these natural migration barriers. Geological evidence indicated that the above-barrier populations have been isolated for 8,000–12,500 years. Genetic differentiation among below-barrier populations ($F_{ST} = 0.10$, 95% C.I. 0.08–0.12) was similar to a previous study of more southern populations of this species. Above-barrier populations were highly differentiated from adjacent below-barrier populations (mean pairwise $F_{ST} = 0.28$; SD 0.18) and multiple lines of evidence were consistent with asymmetric downstream gene flow that varied among streams. Each above-barrier population had reduced within-population genetic variation when compared to the adjacent below-barrier population. Within-population genetic diversity was significantly correlated with the amount of available habitat in above-barrier sites. Increased genetic differentiation of above-barrier populations with lower genetic diversity suggests that genetic drift has been the primary cause of genetic divergence. Long-term estimates of N_e based on loss of heterozygosity over the time since isolation were large (3,170; range 1,077–7,606) and established an upper limit for N_e if drift were the only evolutionary process responsible for loss of genetic diversity. However, it is likely that a combination of mutation, selection, and gene flow have also contributed to the genetic diversity of above-barrier populations. Contemporary above-barrier N_e estimates were much smaller than long-term N_e estimates, not correlated with within-population genetic diversity, and not consistent with the amount of genetic variation retained, given the approximate 10,000-year period of isolation. The populations isolated by waterfalls in this study that occur in larger stream networks have retained substantial genetic variation, which suggests that the amount of habitat in headwater streams is an important consideration for maintaining the evolutionary potential of isolated populations.

Keywords Genetic diversity · Population structure · Isolated populations · Salmonid · Effective population size

Electronic supplementary material The online version of this article (doi:10.1007/s10592-010-0083-y) contains supplementary material, which is available to authorized users.

A. R. Whiteley (✉)
Department of Natural Resources Conservation, University of Massachusetts, 160 Holdsworth Way, Amherst, MA 01003, USA
e-mail: awhiteley@nrc.umass.edu; awhiteley@gmail.com

K. Hastings
Resources Management and Science, Yosemite National Park, P.O. Box 700, El Portal, CA 95318, USA

J. K. Wenburg
Conservation Genetics Laboratory, U.S. Fish and Wildlife Service, Alaska Region, 1011 E. Tudor Road, Anchorage, AK 99503, USA

C. A. Frissell
Pacific Rivers Council, PMB 219, 1 Second Avenue East, Suite C, Polson, MT 59860, USA

J. C. Martin · F. W. Allendorf
Division of Biological Sciences, University of Montana, Missoula, MT 58912, USA

Introduction

A primary focus in conservation genetics is to understand factors that influence the persistence of small populations. Isolation of small populations can be caused by natural or anthropogenic barriers to dispersal (Young et al. 1996; Keyghobadi et al. 2005; Trizio et al. 2005; Deiner et al. 2007). Loss of genetic variation and an increased probability of inbreeding depression in small isolated populations may interact with demographic processes to lead to a decreased probability of population persistence (Newman and Pilson 1997; Frankham 1998; Saccheri et al. 1998; Soule and Mills 1998; Crnokrak and Roff 1999; Hedrick and Kalinowski 2000; Nieminen et al. 2001). As the rate of population fragmentation and isolation increases, it becomes increasingly important to examine factors that influence the maintenance of genetic diversity and therefore the likelihood of persistence of small isolated populations.

Populations that have been isolated for long periods of time are best suited for studies that examine fundamental processes associated with maintenance of genetic variation in small populations (White and Searle 2007). Islands have been the primary type of long-isolated habitat analyzed in this regard. For example, several studies find evidence for a correlation between within-population genetic variation and island area (Frankham 1997; Cheylan and Granjon 1998; Hinten et al. 2003; White and Searle 2007; Ortego et al. 2008). While inferences based on island studies provide important insights, novel environmental conditions encountered on islands may affect population persistence, making island populations more prone to extinction than long-isolated non-island populations. Island studies also often lack information on the timing of population isolation. Finally, few island systems are unencumbered by complications due to extensive human alterations of the landscape. Studies of factors that affect population persistence in replicate non-island isolated populations free of human influence would be valuable to determine if island results can be generalized, and may provide additional insights where time since isolation can be reliably inferred.

Linear stream habitats are prone to fragmentation by natural and anthropogenic factors that include dams, road crossings, and waterfalls (Dunham et al. 1997; Warren and Pardew 1998; Taylor et al. 2003; Wofford et al. 2005; Deiner et al. 2007; Fukushima et al. 2007; Rahel 2007). Stream salmonids appear to be particularly prone to impacts from fragmentation. Isolation has been shown to increase population extinction risk in one stream salmonid (Letcher et al. 2007). Other studies have found a positive relationship between population persistence and amount of habitat above barriers for two other salmonid species (Harig and Fausch 2002; Morita and Yamamoto 2002). Isolated populations above stream barriers are similar to those on islands, in that they may be threatened by loss of genetic variation and inbreeding (Wang et al. 2001; Novinger and Rahel 2003). Genetic effects of stream barriers have been observed to include loss of genetic diversity in and increased genetic differentiation of above-barrier populations (Angers et al. 1999; Bouza et al. 1999; Carlsson and Nilsson 1999; Costello et al. 2003; Taylor et al. 2003; Wofford et al. 2005; Neville et al. 2006; Guy et al. 2008). However, important questions remain about fundamental evolutionary processes associated with population isolation in above-barrier populations. For example, gene flow is predicted to be asymmetric and greater in the downstream direction, but occasional upstream gene flow could have large demographic and genetic consequences for the above-barrier populations. In general, few studies have examined factors that influence the maintenance of genetic diversity in fish populations that have been isolated by waterfall barriers for thousands of years.

Replicate isolated fish populations that are well suited for examining the effects of long-term isolation on population persistence occur throughout southeastern Alaska, USA. These populations are isolated because of geological events that have occurred since the last major glacial ice advance. Freshwater habitats in this region were eliminated during the Pleistocene, under the continuous coverage of the Cordilleran ice sheet. Retreat of the ice sheet was largely complete in this region by about 12,500 BP (Mann 1986), leaving many new streams exposed for colonization by fish from saltwater, which can occur very quickly, on the order of years to decades (Milner et al. 2008). High rates of uplift of the earth's outer crust, termed isostatic rebound, subsequently occurred in response to release from the weight of the ice sheet. Where uplift exposed geological discontinuities in streambeds, waterfalls sometimes blocked upstream fish migration and created isolated fish populations upstream of the barrier falls. Historical estimates of the isostatic rebound in this region allow the time since isolation of these populations to be determined. This landscape process has affected several fish species. Here we focus on the coastal cutthroat trout (*Oncorhynchus clarkii clarkii*), one of four major subspecies of this polytypic species (Allendorf and Leary 1988). Anadromous populations can presently be found immediately below waterfall barriers and isolated populations occur immediately above the same falls. Thus, it is possible to sample replicate above- and below-barrier population pairs in many watersheds. Furthermore, human influence is negligible in much of this region, which eliminates confounding factors associated with anthropogenic habitat modifications. In addition, the stream-resident life form of coastal cutthroat trout found above waterfall barriers is too small to have attracted attention as a food source for humans or a candidate for transplantation, therefore most populations remain in a largely natural state.

In this paper, we use microsatellites to examine the distribution of genetic variation within, and genetic differentiation among, pairs of coastal cutthroat populations separated by waterfall barriers. We then ask the following questions: Is there evidence of asymmetric downstream gene flow? And, what factors contribute to the maintenance of genetic diversity in the above-barrier populations?

Materials and methods

Study area

The Alexander Archipelago follows the coastline of the southeastern Alaskan panhandle between 54°40′ N and 58°30′ N. The archipelago covers approximately 500 km of latitude and 150 km of longitude, and includes, by one estimate, over 22,000 islands (USFS 1997). These islands contain many freshwater habitats that are separated by saltwater habitat. Compared to continental watersheds elsewhere in North America, the numerous island watersheds in the Alexander Archipelago are quite small. The longest island streams are about 30 km and the majority of streams are <10 km from origin to saltwater.

We selected 12 streams in generally pristine settings in central southeastern Alaska with populations of coastal cutthroat trout above apparent permanent upstream movement barriers (Table 1; Fig. 1). Sample streams were chosen to represent a gradient in amount of above-barrier habitat, ranging from small drainages that were apparently barely sufficient to support an isolated population, to drainages at least an order of magnitude larger in size (Table 1). We generally avoided populations that had access to a lake upstream of the barrier because we wanted to focus on the smallest natural populations we could find. In addition, lakes are more likely than streams to have experienced fish stocking or other forms of anthropogenic supplementation. The amount of linear habitat available to the populations above waterfalls ranged from approximately 1 to 29 km (Table 1). Bankfull width was typically about 5 m. In some cases, coastal cutthroat trout were the only fish found in the above-barrier portion of a stream, while in others, Dolly Varden (*Salvelinus malma*) were also present.

We used geological evidence to estimate the age of each waterfall barrier. These estimates relied on radiocarbon dates for shell fragments from exposed, uplifted glaciomarine sediments, which were used to establish the earliest time that land at different elevations could have emerged from saltwater. From a larger dataset of 65 radiocarbon-dated, exposed glaciomarine sediments, we selected the most recent date found in each 15 m vertical elevation band and regressed it on log-transformed elevation to produce a formula for predicting the time that a given elevation emerged from the sea during Holocene uplift (Table 1; Fig. S1; Hastings 2005). These estimates provide a maximum amount of time that a barrier could have reduced gene flow, since there was likely a time period during which fish passage still occurred as the waterfall formed and since isostatic rebound is a dynamic and nonlinear process.

Table 1 Site names, abbreviation codes, geographic coordinates, and estimates of habitat parameters for each of the study streams

Site name	Site code	*N* (a/b)	Latitude (°N)	Longitude (°W)	Available habitat (m)	Barrier elevation (m ASL)	t_{geo} (YBP)
Duncan HW	DN	30/33	56.77	−133.11	29,700	130	12,000
North Arm	NA	29/29	57.20	−133.26	28,800	100	8,500
West Fools	WF	30/30	56.25	−132.09	14,100	38	11,000
Dry Straits	DS	27/21	56.62	−132.62	7,800	15	8,500
Rugby	RG	29/20	56.21	−132.69	3,900	61	8,000
Whitecap	WC	27/31	56.14	−132.16	1,600	138	11,500
Jenkins	JE	30/29	56.42	−132.18	1,400	58	12,500
McHenry	MH	22/26	55.97	−132.38	900	89	10,500
Hiller	HL	20	56.65	−133.84	20,000	52	10,000
Portage	PO	30	56.91	−133.14	12,700	15	11,500
Mason	MA	29	56.58	−132.92	9,200	81	11,000
Leprechaun	LP	30	56.66	−133.04	2,000	14	12,500

Samples sizes (*N*) are shown for above (a) and adjacent below-barrier (b) population samples. Available habitat represents a measure of the total stream distance above each barrier waterfall in meters (m). Barrier elevation is the height above sea level (ASL) of each waterfall. Geological estimates of time since isolation (t_{geo}) based on barrier elevation is shown in YBP. Populations are arranged by decreasing amount of available above-barrier habitat, except for the final four streams. The below barrier sites for these last four streams were either not sampled (LP) or were removed due to the presence of rainbow trout genes (HL, MA, PO)

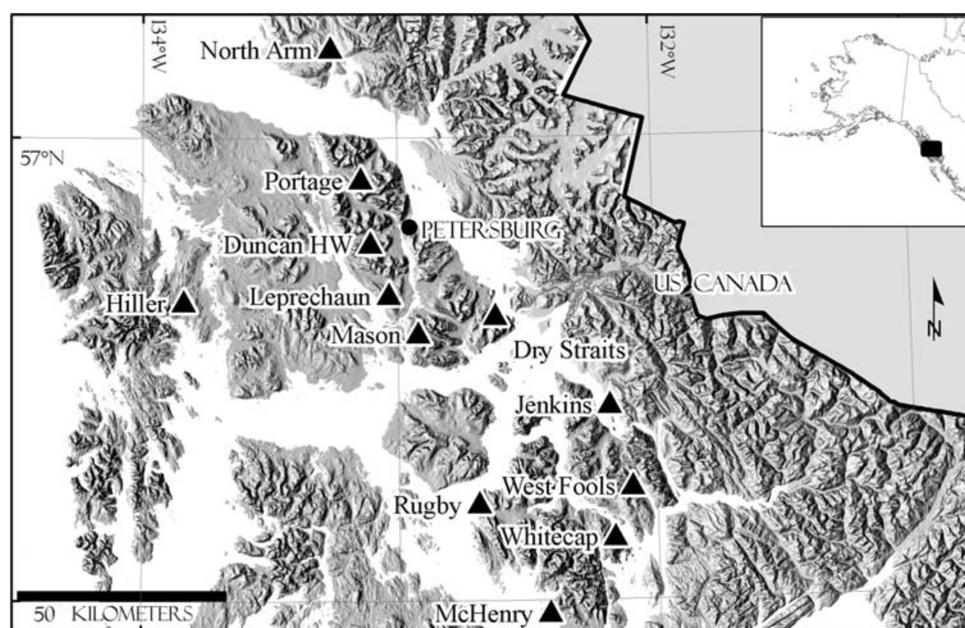

Fig. 1 Map of sample sites. Each *triangle* is located at the mouth of a stream and represents an above/below-barrier population pair

Sample collection

We collected fin clips from approximately 30 coastal cutthroat trout from both above- and below-barrier sites in each of the 12 streams (Table 1; Fig. 1; hereafter abbreviated by a site code followed by -a or -b for above- and below-barrier sites, respectively). Three below-barrier population samples were removed upon discovery of rainbow trout (*Oncorhynchus mykiss*) alleles (see below); a fourth was removed because we were unable to collect enough individuals. Therefore, the final analysis included eight above/below-barrier population pairs and four above-barrier populations without matching below-barrier sites. Fish were captured using minnow traps, electrofishing, or hook-and-line. Fish from below barriers were sampled as close to saltwater as possible to increase the likelihood of sampling anadromous individuals. Above barriers, we sampled fish throughout the available habitat in the smaller drainages, and at centrally situated and apparently representative sites within the larger drainages. Fin clips were stored in 95% ethanol.

Microsatellite amplification and allele scoring

We extracted DNA with the Pure Gene® kit (Gentra Systems) following the manufacturer's protocol. Target sequences were PCR amplified for eight microsatellite loci: *SFO8*, *OMY77*, *ONEμ11*, *OGO4*, *OGO8*, *OCL1*, *OCL2*, and *OCL4* (Table S1). We visualized fluorescently labeled PCR products on acrylamide gels with a Hitachi FMBIO-II fluorescent imager. Product sizes were determined using MapMarkerLOW™ size standards (Bio Ventures Inc.). Each gel included previously amplified individuals to ensure consistent scoring across all gels. Allele frequency data are available from the authors upon request.

Hybridization with rainbow trout

We screened all coastal cutthroat trout samples from below-barrier populations for evidence of hybridization with sympatric rainbow trout because these species are known to hybridize (Allendorf et al. 2004). We also randomly selected 10 fish from each above-barrier population to screen for evidence of hybridization, which we considered unlikely as no rainbow trout were observed above the barrier in any stream. We used genetic markers derived from paired interspersed nuclear elements (PINEs, Spruell et al. 2001). Conditions for PINE PCR, electrophoresis details, and methods for scoring amplification products followed those described by Spruell et al. (2001). We identified hybrids using four previously developed PINE markers shown to be diagnostic between rainbow trout and coastal cutthroat trout (Kanda et al. 2002; Hitt et al. 2003). We detected rainbow trout alleles in 14, 22, and 11 individuals in HL-b, MA-b, and PO-b, respectively and excluded these sites from further analyses. We did not detect any evidence of hybridization with rainbow trout in any of the other below-barrier populations or in any of the above-barrier populations.

Genetic data analysis

Allele frequencies, deviations from Hardy–Weinberg expectations, gametic disequilibrium, observed (H_O) and expected (H_E) heterozygosity, per locus and population,

mean within-population expected heterozygosity (H_S), and mean allelic richness per population (AR; mean number of alleles scaled to the smallest sample size; $N = 15$) were calculated with GENEPOP ver. 3.4 (Raymond and Rousset 1995) and FSTAT ver. 2.9.3.2 (Goudet 2001). We calculated loss of heterozygosity and allelic richness for the above-barrier populations with the assumption that below-barrier populations are suitable representations of the amount of genetic variation contained within the above-barrier populations upon isolation.

Pairwise exact tests for genic differentiation, F-statistics, and pairwise F_{ST} values were calculated with GENEPOP ver. 3.4 (Raymond and Rousset 1995) and FSTAT ver. 2.9.3.2 (Goudet 2001). We used θ analogues (Weir and Cockerham 1984) for estimates of F_{ST}. We estimated: (1) the divergence among below-barrier populations, which are believed to be capable of exchanging migrants; (2) the divergence among the isolated, above-barrier populations; and (3) the average divergence between paired above- and below-barrier samples in each stream. To estimate above-below barrier differentiation for each of the four unpaired above-barrier samples, we used the average pairwise F_{ST} value between each of these above-barrier sites and all eight of the below-barrier samples. Randomization tests were used to test whether F-statistics were significantly greater than zero. We used F_{ST} instead of R_{ST} because F_{ST} estimates are more conservative when relatively few microsatellite loci are used (<20) and populations have diverged recently (Gaggiotti et al. 1999). We also calculated F_{ST}' according to Hedrick (2005). F_{ST}' is a standardized measure of genetic divergence that is related to the maximum possible value F_{ST} can attain for the genetic diversity present in the sample. We used F_{ST}' to examine the relationship between genetic divergence between above and below-barrier population pairs and the within-population genetic diversity in above-barrier populations because F_{ST}' is less influenced than F_{ST} by the amount of genetic diversity within samples (Hedrick 2005; Heller and Siegismund 2009; Ryman and Leimar 2009). We adjusted the results from multiple tests for conformation to Hardy–Weinberg expectations and gametic disequilibrium with the sequential Bonferroni procedure (Rice 1989).

We used JMP ver. 7 (SAS Institute, Inc.) to perform a principal components analysis (PCA) of allele frequencies, using the covariance matrix. The largest allele at each locus was omitted to account for the non-independence of allele frequencies within a locus (Cavalli-Sforza et al. 1993).

Asymmetric gene flow

We used STRUCTURE ver. 2.1 (Pritchard et al. 2000; Falush et al. 2003) to test for migration between above- and below-barrier population pairs. Each population pair was examined separately without prior population information. We used 100,000 replicates and 20,000 burn-in cycles under an admixture model where we applied the 'infer α' option (α is the Dirichlet parameter for degree of admixture) with a separate α for each population under the F model. We used the correlated allele frequencies model under the $\lambda = 1$ option, where λ parameterizes the allele frequency prior and is based on the Dirichlet distribution of allele frequencies. We allowed F to assume a different value for each population, which allows for different rates of drift among populations. We performed five runs for $K = 1$ and 2 for each population pair.

We also used BAYESASS ver. 1.3 (Wilson and Rannala 2003) to obtain a more contemporary estimate of gene flow between above and below-barrier population pairs. BAYESASS implements a Bayesian approach for the estimation of population-specific inbreeding coefficients and asymmetric migration rates among populations. The model assumes linkage equilibrium but allows for deviations from Hardy–Weinberg expectations. It also assumes migration rates are constant for two generations prior to sampling. We used MCMC runs of 20×10^6 iterations with a burn-in of 2×10^6 and a thinning interval of 2,000 iterations. We used delta values of 0.10 for the allele frequency and F parameters, and 0.05 for the migration rate parameter because these values resulted in acceptance rates between 40 and 60%. We performed 5–10 runs with a different starting point for each population pair. We calculated the Bayesian deviance for all MCMC runs and report the run with the lowest deviance value. We also eliminated any runs that had migration rates for both populations very close to the prior of 0.33 (Faubet et al. 2007). We used a modified version of the program to obtain raw MCMC trace files, from which we calculated distribution means and 95% highest posterior density (HPD) intervals (the boundary of the shortest span that includes 95% of the probability density of a parameter).

Above-barrier populations

We used Pearson correlations to test the relationship between within-population estimates of genetic variation (H_S and AR), estimates of effective population size (N_e, see below), and available habitat. One-tailed significance values were used because predictions were directional.

The paired nature of our sampling scheme and the geographic isolation of the above-barrier populations allowed us to estimate long-term N_e analytically based on loss of heterozygosity over time for each isolated population. We calculated long-term N_e with the following formula:

$$N_e = \frac{1}{2(1 - e^a)}, \text{ where } a = \frac{\ln\left(\frac{H_t}{H_0}\right)}{t} \tag{1}$$

(Crow and Kimura 1970).

In this equation, H_t is mean expected heterozygosity at generation t. For this parameter we used estimates of mean expected heterozygosity (H_S) for each above-barrier population. For H_0, the presumed heterozygosity of the founding population t generations in the past, we used estimates of H_S from paired contemporary below-barrier populations. This assumes that genetic diversity in the below-barrier populations is representative of the founding population of fish originally trapped above the waterfall barriers in each stream. For each of the four above-barrier populations that lacked an adjacent below-barrier paired population, we used mean values of H_S for the eight other below-barrier populations for the parameter H_0.

For the parameter t for each above-barrier population, we used geological estimates of waterfall ages. We used a mean generation interval of 4 years to convert t from years to generations. Stream-resident cutthroat trout from above-barrier populations mature early at a smaller size. Previous work indicates that fish in these isolated populations spawn from age 2 to 4, with a mean spawning age of 3 (Wyatt 1959; Nicholas 1978; June 1981; Rehe 2007). Fish from below-barrier populations are anadromous and mature later at larger size (Trotter 1989). Spawning for this life-history form occurs between ages 4 and 6, with a mean of 5 years (Rehe 2007). We used the mean of both life history forms (4 years) as the generation interval.

We estimated contemporary N_e for each above barrier population with ONeSAMP (Tallmon et al. 2008) and LDNE (Waples and Do 2008). ONeSAMP calculates eight summary statistics and uses Approximate Bayesian Computation (ABC) to estimate N_e from a single population sample. LDNE uses a linkage disequilibrium method to calculate N_e estimates and incorporates the bias correction from Waples (2006). Both programs make the following assumptions: selectively neutral and unlinked loci, closed populations, and discrete generations. ONeSAMP and LDNE provide a contemporary estimate of inbreeding N_e. Because cutthroat trout are iteroparous and have overlapping generations, estimate are intermediate to the number of breeders (N_B) and N_e of the generation prior to that sampled (Waples 2005; Palstra and Ruzzante 2008). For priors on N_e, in ONeSAMP we used 2.0 as the low value and both $0.5 \times N_c$ (demographic estimates of population size) and N_c as the upper value. N_c was estimated based on intensive sampling of one stream (LP-a). We used a three-pass removal sampling design (Bryant 2002) to sample 50 m reaches and program CAPTURE (White et al. 1978; Rexstad and Burnham 1991; Burnham and Anderson 1998) to generate an abundance estimate for the entire stream. We used the density estimate from LPA to estimate N_c for the other above-barrier populations. These N_c estimates are not reported here because we did not estimate N_c independently for each of the populations. The use of a range of priors allowed us to assess sensitivity of N_e estimates to those priors. If estimated N_c was greater than 10,000, the upper limit for priors with this program, we used this upper limit as the prior. Monomorphic loci were excluded from the analysis. For LDNE, the lowest allele frequency used was 0.01 (a larger threshold allele frequency provided similar results).

Recent population bottlenecks could be responsible for small contemporary N_e estimates in our above-barrier populations (see below). To test for evidence of bottlenecks since population isolation, each above-barrier population was tested for heterozygosity excess compared to that expected at mutation-drift equilibrium. We used the program BOTTLENECK (Cornuet and Luikart 1996; Piry et al. 1999) with the two-phase mutation model of microsatellite evolution (DiRienzo et al. 1994) with 10% of the infinite allele model and 90% of the stepwise mutation model (White and Searle 2007). A one-tailed Wilcoxon test for heterozygosity excess was used as the test for a bottleneck.

We used EASYPOP ver. 2.0.1 (Balloux 2001) to examine the influence of mutation as a source of new genetic diversity in above-barrier populations. We performed single population simulations assuming that each above-barrier population has been isolated for 10,000 years (2,500 generations). We used a maximum of 10 alleles (mean for our data set) and a step-wise mutation model. We assumed no gametic disequilibrium and an equal number of males and females. We ran ten replicate simulations for three mutation rates (1×10^{-3}, 1×10^{-4}, 1×10^{-5}) and N_e values of 50, 100, 250, 500, and 1,000, 5,000, and 10,000 to fully encompass the empirical long-term estimates of N_e. Mutation rate estimates for microsatellites have a large degree of uncertainty (Ellegren 2004) but the typical range observed by most studies to date has been 1×10^{-3}–1×10^{-5} (Jones et al. 1999; Shimoda et al. 1999; MacKiewicz et al. 2002; Steinberg et al. 2002; Ellegren 2004; Yue et al. 2007). Simulations were started with minimal variation and we recorded the final H_S after 2,500 generations.

Results

Tests for deviation from Hardy–Weinberg proportions were significant in 10% of the cases (13 of 130 tests; $P < 0.05$), where 6.5 were expected by chance at $\alpha = 0.05$. Significant tests were distributed across seven of eight loci and nine of 20 populations. After sequential Bonferroni correction, either for approximately eight tests within each population sample or for approximately 20 tests per locus, four comparisons remained significant (one in DN-a and

LP-a, two in RG-a; one for *OCL1* and *OCL2*, two for *OCL4*). Significant gametic disequilibrium was detected for 12% of the cases (51 of 417 tests; $P < 0.05$). Upon sequential Bonferroni correction for approximately 28 locus pairs in each population, 13 tests remained significant, 12 of which occurred in below-barrier populations. Eight of the 13 significant tests occurred in RG-b.

Each of the above-barrier populations had lower estimated within-population genetic variation than any below-barrier population (Table 2). For the below-barrier populations, mean expected heterozygosity (H_S) was 0.62 (range 0.55–0.66) and mean allelic richness (*AR*) was 4.8 (range 3.9–5.5; Table 2). For above-barrier populations, mean H_S was 0.32 (range 0–0.50) and mean *AR* was 2.3 (range 1–3.6; Table 2). If we assume that current estimates of genetic diversity within the below-barrier populations are representative of the genetic diversity of the above-barrier populations at the time of population isolation, then above-barrier populations had a mean reduction in H_S of 48% and *AR* of 65% (Table 2).

We observed substantial variation in allele frequencies among sample sites (Table S2). Allele frequency differences were significant among all pairwise comparisons for the 20 population samples at each locus individually, and over all eight loci ($P < 0.0001$). Overall F_{ST} was 0.39 (95% C.I. 0.36–0.42). We observed a dramatic difference in the estimate of F_{ST} that included only below-barrier populations ($F_{ST} = 0.10$, 95% C.I. 0.08–0.12), compared to the estimate that included only above-barrier populations ($F_{ST} = 0.57$, 95% C.I. 0.53–0.61). Mean pairwise F_{ST} between immediately adjacent above- and below-barrier populations was 0.28 (SD 0.18). Standardized estimates of F_{ST}' following Hedrick (2005) were greater than unstandardized F_{ST} estimates. Mean overall F_{ST}' was 0.69. The estimate of F_{ST}' that included only below-barrier populations was 0.26 and the estimate than included only above-barrier populations was 0.84. Mean pairwise F_{ST}' between immediately adjacent above- and below-barrier populations was 0.48 (SD 0.24). Within-stream pairwise F_{ST}' estimates were less than the mean pairwise F_{ST}' estimated for the above-barrier population in a pair and all below-barrier populations from other streams in seven of eight cases (Wilcoxon paired sign-rank test = 16, $P = 0.023$). Jost's *D* (Jost 2008), which is mathematically independent from H_S (Jost 2009), showed highly similar results to F_{ST}' (data not shown) and thus observed patterns of genetic differentiation are unlikely to be an artifact of the measure of genetic differentiation that we used.

Principal components analysis based on allele frequencies revealed a central cluster of below-barrier populations and a scattered distribution of above-barrier populations (Fig. 2). PC axes one through four explained 19, 15, 13,

Table 2 Genetic diversity parameters, proportion of genetic diversity lost in above-barrier populations, and estimates of N_e

Population pair	H_S (a/b)	*AR* (a/b)	H_S lost (%)	*AR* lost (%)	Contemporary N_e–ONeSAMP	Contemporary N_e–LDNE	Long-term N_e
DN	0.500/0.609	3.38/4.69	18	36	79.6 (46.5–371.0)	63.2 (16.8–∞)	7,606
NA	0.503/0.604	3.62/4.74	17	30	18.4 (11.1–47.2)	22.1 (11.3–61.0)	5,806
WF	0.206/0.625	1.84/5.18	67	80	13 (8.3–49.7)	6.4 (1.2–122.3)	1,239
DS	0.454/0.598	2.83/4.90	24	53	35.2 (28.2–60.8)	–	3,857
RG	0.264/0.668	1.74/4.54	60	79	19.3 (12.8–46.4)	–	1,077
WC	0/0.611	1.00/4.88	100	100	–	–	–
JE	0.396/0.658	2.37/5.54	40	70	61.5 (39.6–216.4)	110 (10.6–∞)	3,077
MH	0.169/0.555	1.46/3.94	70	84	9.8 (5.65–25.8)	25.5 (0.6–∞)	1,104
HL[a]	0.405/0.616	3.06/4.80	34	46	80.7 (38.6–418.5)	53 (9.8–∞)	2,980
PO[a]	0.345/0.616	2.59/4.80	44	58	47.9 (26.4–219.7)	79 (14.4–∞)	4,280
MA[a]	0.264/0.616	2.03/4.80	57	73	27.5 (20.7–64.8)	32 (2.9–∞)	1,623
LP[a]	0.305/0.616	1.96/4.80	50	75	18.0 (11.7–57.0)	8 (1.5–298.9)	2,223

H_S represents the mean expected heterozygosity and *AR* represents the mean allelic richness for (a) above and (b) below-barrier sites. H_S and *AR* lost show the percentage reduction of H_S or *AR* in each above-barrier population relative to the adjacent below-barrier population. Contemporary N_e estimates were calculated for the above-barrier sites with ONeSAMP (95% credible limits in parentheses) or LDNE (95% confidence intervals in parentheses). Long-term N_e was calculated based on loss of heterozygosity from the assumed ancestral below-barrier population over the geologically estimated time since isolation. N_e could not be calculated for the genetically monomorphic WC population

[a] Populations for which mean H_S or *AR* from the eight below-barrier populations retained in the analysis were used for the below-barrier estimate of H_S or *AR*

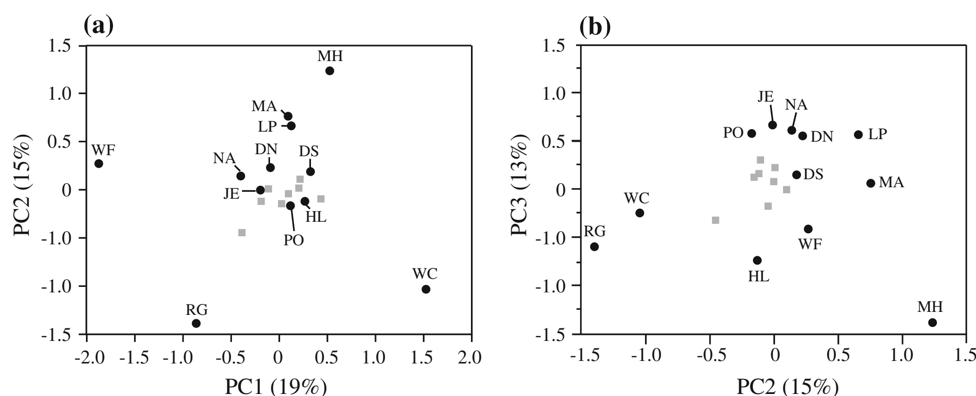

Fig. 2 Principal components analysis of allele frequency variation. *Black dots* represent above-barrier populations and *grey squares* represent below-barrier populations. Proportion of variation attributable to PC axes 1 and 2 (**a**) and PC axes 2 and 3 (**b**) is shown. Population labels are shown only for above-barrier populations

and 10% of the allele frequency variation, respectively. The central cluster of below-barrier populations is consistent with the hypothesis that gene flow has prevented differentiation of these sites and could also reflect the greater genetic diversity of these sites. There was a wide distribution of above-barrier populations around the central cluster, with little apparent geographic pattern (Fig. 2). For example, WF-a and WC-a were geographically proximate but appeared in different extremes of PC space (Fig. 2a). The wide scatter of above-barrier sites in the PCA analysis reflects the increased genetic differentiation and reduced genetic diversity of the above-barrier populations.

Asymmetric gene flow

We used the program STRUCTURE to test for asymmetric gene flow. There was little evidence of gene flow in the upstream direction, with the exception of one individual in NA whose entire genome assigned to the below-barrier population (Fig. 3b) and one individual in DN, for which

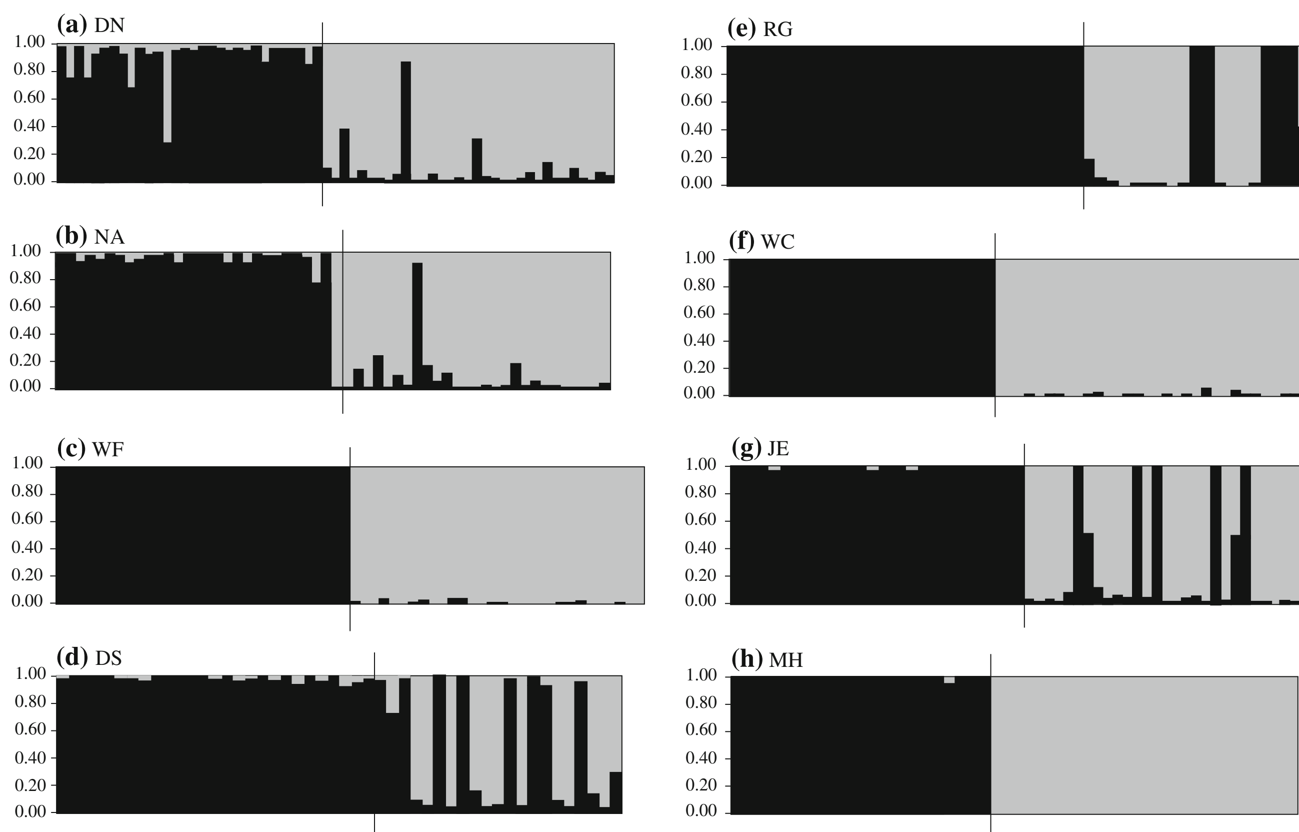

Fig. 3 Proportion of the genome of each individual assigned by STRUCTURE to either the above-barrier population (*black*) or below barrier population (*grey*) for each of the eight above/below-barrier population pairs (**a**–**h**). Each column corresponds to an individual. Individuals from above- and below-barrier sample sites are to the left (*black*) and right (*grey*) of the vertical line in each panel, respectively

approximately 75% of the genome assigned to the below-barrier population (Fig. 3a). The mean proportion of the genome of above-barrier sampled individuals that belonged to the adjacent below-barrier cluster was 0.03 (range 0.004–0.09). There was evidence of greater gene flow in the downstream direction for some of the population pairs (Fig. 3). The mean proportion of the genome of below-barrier sampled individuals that belonged to the adjacent above-barrier cluster was 0.15 (range 0.004–0.46).

Estimates of m from BAYESASS, which provide a more contemporary estimate of gene flow, were highly concordant with those from STRUCTURE and revealed further evidence of gene flow asymmetry. Posterior distributions of upstream m were close to zero for all eight populations (Fig. 4). Posterior distributions were consistent with the occurrence of downstream contemporary gene flow in NA, DS, RG, and JE but not DN, WF, WC, or MH (Fig. 4).

Above-barrier populations

For the above-barrier populations, the amount of available habitat was significantly correlated with both measures of within-population genetic variation, H_S ($r = 0.65$; $P = 0.01$) and AR ($r = 0.83$, $P = 0.0004$). This pattern was highly evident in calculations of loss of genetic diversity. The three sites with the largest above-barrier stream networks (mean habitat = 26,167 m) lost an average of 23% of their heterozygosity and 37% of their allelic richness. The three smallest streams (mean habitat = 1,300 m) lost an average of 70% of their heterozygosity and 84% of their allelic richness.

We observed a significant negative relationship between within-stream (above relative to below-barrier) genetic differentiation and estimates of within-population genetic diversity of the above-barrier population in each pair. The correlation between above/below pairwise F_{ST}' and above-barrier population H_S was -0.82 ($P = 0.01$; Fig. 5) and between pairwise F_{ST}' and AR was -0.73 ($P = 0.04$; data not shown). This correlation supports the hypothesis that genetic drift is the predominant evolutionary process driving differentiation of the above-barrier sites at the loci examined.

Long-term estimates of N_e were based on the loss of heterozygosity over the interval of time since the above-barrier populations have been isolated according to our geological estimates. These estimates represent N_e over the many generations since isolation. The mean of estimates of long-term N_e was 3,170 (range 1,077–7,606; Table 2). Amount of available habitat was significantly correlated with long-term N_e ($r = 0.78$, $P = 0.002$). Long-term N_e estimates were significantly correlated with H_S ($r = 0.89$, $P = 0.0002$) and AR ($r = 0.89$, $P = 0.0003$).

We estimated contemporary N_e for above-barrier populations with ONeSAMP (N_e–ONeSAMP), a summary

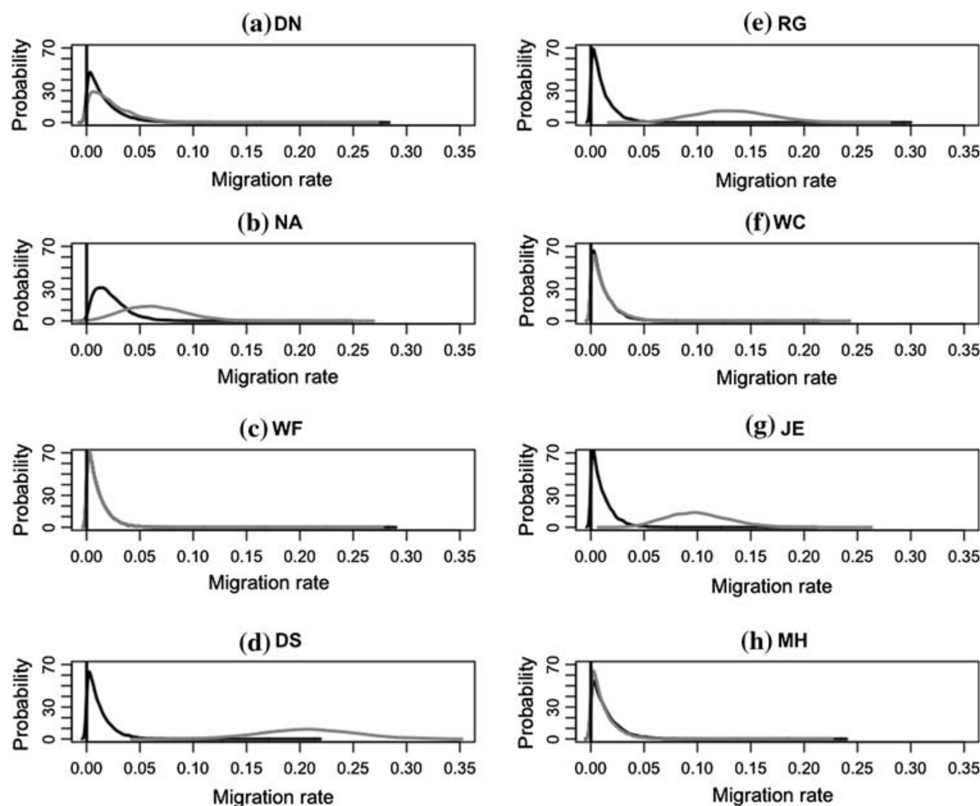

Fig. 4 Posterior distributions of contemporary migration rates from BAYESASS between above/below-barrier population pairs. **a–h** Correspond to each of the eight population pairs. *Black lines* correspond to upstream gene flow. *Grey lines* correspond to downstream gene flow

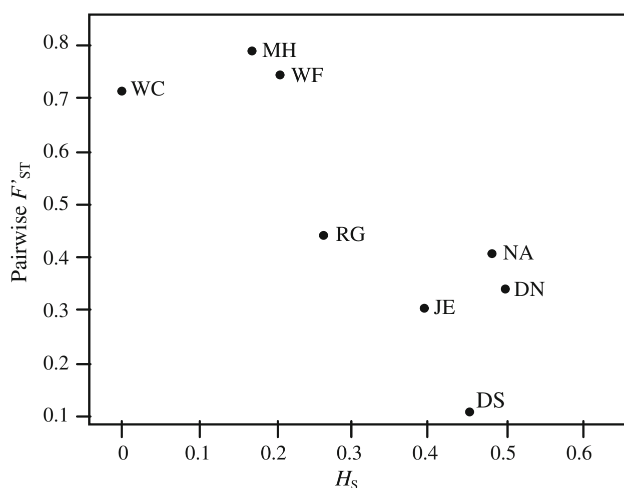

Fig. 5 Relationship between genetic differentiation of within-stream population pairs and within-population genetic variation of the above-barrier population ($r = -0.82$, $P = 0.01$). Pairwise F_{ST}' values were calculated for each above- and below-barrier population pair. H_S corresponds to mean within-population heterozygosity of the above-barrier populations in each pair

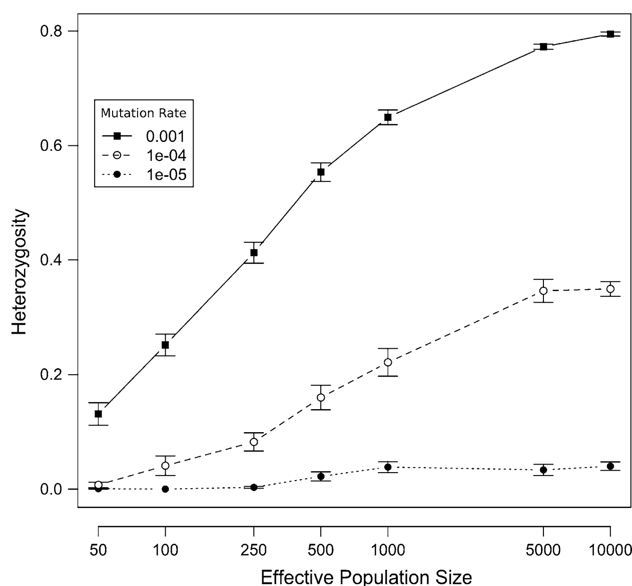

Fig. 6 Simulations to test the influence of mutation on genetic diversity in above-barrier populations. Heterozygosity is shown as a function of effective population size and mutation rate (μ). Heterozygosity is mean H_S across eight simulated loci and across ten replicate simulations after 2,500 generations. Error bars show ±1 SE and the log scale was used for the *x*-axis

statistic based one sample N_e estimator, and LDNE (N_e–LDNE), a one sample estimator based on linkage disequilibrium. Mean N_e–ONeSAMP was 37 (range 9.8–80.7; Table 2) and point estimates were generally not sensitive to priors. Mean N_e–LDNE was 44 (range 6.4–110; Table 2). Point estimates for each estimator were significantly correlated ($r = 0.73$, $P = 0.02$). ONeSAMP provided smaller credible limits than those from LDNE (Table 2). Amount of available habitat was not significantly correlated with N_e–ONeSAMP ($r = 0.42$, $P = 0.10$) or N_e–LDNE ($r = -0.04$, $P = 0.46$). Point estimates for N_e–ONeSAMP were significantly correlated with H_S ($r = 0.61$, $P = 0.02$) and *AR* ($r = 0.60$, $P = 0.03$). Point estimates for N_e–LDNE were not significantly correlated with H_S ($r = 0.43$, $P = 0.12$) or *AR* ($r = 0.30$, $P = 0.44$). Based on the expression used to calculate long-term N_e, estimated mean number of years for the observed loss of H_S for these small estimates of contemporary N_e (mean of point estimates from ONeSAMP and LDNE) was 158.5 (range 29–347; Table 2), which was approximately two orders of magnitude less than the mean geological estimate of isolation time (Table 1).

Recent population bottlenecks could be responsible for small estimates of contemporary N_e. BOTTLENECK analyses revealed evidence for bottlenecks in DS-a, JE-a, and LP-a (Table 1). Each of these populations had an excess of heterozygosity when compared to the expectation at mutation-drift equilibrium (Wilcoxon test, $P < 0.05$). However, after sequential Bonferroni correction based on 12 tests, none of these results remained significant.

High mutation rates at microsatellite markers could be a source of new genetic variation in the above-barrier populations. We used EASYPOP simulations to evaluate the likely contribution of mutation to genetic diversity of above-barrier populations over the time period we consider (2,500 generations) and over a range of effective population sizes. We started simulations with no genetic variation to isolate the effect of mutation on the accumulation of genetic variation. A mutation rate of 1×10^{-3} had a large influence on the simulated amount of heterozygosity (H_S) retained over this time period and across the range of N_e values examined (Fig. 6). A more moderate and probably more representative mutation rate (1×10^{-4}) contributed more than 10% to H_S only at larger population sizes ($N_e > 500$). The lowest mutation rate that we considered (1×10^{-5}) had little effect on genetic diversity across this range of population sizes (Fig. 6). Accounting for increased genetic variation in above-barrier populations due to mutation would lead to smaller estimates of long-term N_e. Contributions to heterozygosity of 10, 25, and 50% by mutation would lead to reduced mean long-term N_e estimates for all above-barrier populations by 25, 45, and 65%, respectively. Values for mean long-term N_e estimates given these mutational contributions were 2,268 (range 967–4,958), 1,646 (range 822–3,094), and 1,058 (range 617–1,685), respectively.

Discussion

Following retreat of continental ice sheets in southeast Alaska, newly formed streams were colonized by coastal

cutthroat trout. We examined populations above and below waterfalls that are approximately 10,000 years old. Genetic differentiation among the below-barrier populations in our study was similar to that observed by Wenburg et al. (1998) for anadromous coastal cutthroat trout populations in western Washington, USA. These results reveal that fine-scale genetic structure has developed in this region in a similar manner as anadromous populations from more southern portions of the species' range. This observation highlights the influence of gene flow caused by the anadromous life history on genetic structure as well as the dramatic effect waterfall barriers have had on genetic differentiation of above-barrier populations in this study.

The consistent pattern of reduced genetic variation within the above-barrier populations and marked genetic differentiation between above- and below-barrier population pairs confirms that the above-barrier populations are isolated. Previous studies have also observed reduced within-population genetic variation and increased among-population genetic divergence of salmonid populations in isolated headwater sites (Angers et al. 1999; Bouza et al. 1999; Carlsson and Nilsson 1999; Costello et al. 2003; Taylor et al. 2003; Wofford et al. 2005; Neville et al. 2006). However, these previous studies have not had the opportunity to examine replicate isolated populations in pristine conditions where the influence of various evolutionary processes on the maintenance of genetic diversity can be tested in the absence of anthropogenic manipulations.

The significant negative relationship between genetic differentiation of the above-barrier populations and their within-population genetic diversity supports the hypothesis that drift has predominantly caused genetic differentiation of the above-barrier sites. The significant correlation between amount of habitat and retention of genetic diversity in the above-barrier populations further supports the role of drift in mediating loss of genetic diversity. Further, this correlation is consistent with studies of animals on islands (Frankham 1997; Cheylan and Granjon 1998; Hinten et al. 2003; White and Searle 2007; Ortego et al. 2008) and demonstrates the island-like conditions of the above-barrier habitat in this study.

The above-barrier populations, particularly those in streams with the most above-barrier habitat, retained surprisingly high genetic diversity in the absence of gene flow from downstream sources. Given the long period of isolation in the absence of gene flow, we expected more populations to have lost all of their genetic diversity. However, only one population (WC-a) lost all of its genetic variation and other populations retained up to 83% of their heterozygosity and 70% of their allelic richness (Table 2; Fig. 2). The extant populations are a subset of all original populations because coastal cutthroat trout are now absent from many of the smaller streams in this region. It is possible that most populations that lost most or all of their genetic variation no longer persist.

Estimates of loss of genetic diversity, along with estimates of long-term N_e, assume that paired below-barrier populations are representative of the ancestral above-barrier population at the time of isolation. It is possible that patterns of genetic structure of the below-barrier anadromous populations have changed over this time period, however, the key parameter of interest is within-population heterozygosity (H_S). Below-barrier populations had highly similar estimates of within-population genetic diversity (Table 2). It appears that enough gene flow has occurred among these populations to maintain similar amounts of within-population heterozygosity. Further, lower genetic differentiation of each above-barrier population from the below-barrier population from the same stream relative to below-barrier populations from other streams provides additional support for our assumption. Greater gene flow among below-barrier populations would have obscured this pattern, homogenized allele frequencies, and led to divergence from the ancestral state in terms of within-population genetic diversity. Thus, we expect below-barrier H_S to have changed minimally over time and we expect any biases associated with our assumption about past genetic diversity to be minimal.

Retention of relatively high levels of genetic diversity for the estimated period of isolation translated into large long-term estimates of N_e in many of the above-barrier populations. The long-term N_e estimates reflect the effective size that would limit drift over thousands of years, if drift were the only evolutionary process responsible for loss of genetic diversity. These long-term estimates establish an upper limit for N_e, but the values themselves should be interpreted with caution because they are likely to be biased high for a number of reasons. If our estimates of isolation time are biased high, generation interval is biased low, or initial heterozygosity estimates are biased low, our estimates of long-term N_e will be biased high. We have already addressed our assumption about initial heterozygosity and we chose a conservative generation interval of 4 years where three might be justified. There is some uncertainty associated with geological estimates of time since isolation, but the geological evidence strongly suggests that waterfalls have been in place for at least thousands of years and it is highly likely that fish have been physically present above these barriers since their formation. The geological estimates of barrier ages were based on the age of marine-deposited evidence at the elevation of the top of the barrier and we used the youngest-aged marine sediment in each elevation stratum. Mann and Hamilton (1995) indicate that modern sea level in southeastern Alaska was reached by 9,000 ^{14}C years before present (YBP) and in the time since, shore lines have varied by only a few meters (Riddihough 1982; Clague 1989).

Mutation, selection, and gene flow could each have contributed to the maintenance of genetic diversity in the isolated above-barrier populations. Any contribution from these three evolutionary processes would lead to an upward bias in long-term estimates of N_e. The influence of each process could also scale with the amount of available habitat. The mutation rate of microsatellites is high enough (Ellegren 2004) that this process is likely to have created new allelic diversity in the amount of time that above-barrier populations have been isolated. Our simulations suggested that mutation could have influenced genetic diversity at moderate mutation rates if effective population size were greater than 500 and incorporation of a modest mutation rate (1×10^{-4}) led to reduced long-term N_e estimates by as much as 45%. The simulations also indicated that larger populations would receive greater input of genetic diversity from mutation and thus contribution from mutation is consistent with the observed positive relationship between genetic diversity and available above-barrier habitat.

Selection against individuals homozygous for deleterious recessive alleles could have indirectly served to maintain genetic diversity in the above-barrier populations. Bensch et al. (2006) demonstrated that selection may favor more heterozygous individuals in a highly inbred population of Scandinavian wolves (*Canis lupus*), though a follow up study with more markers but without a pedigree failed to replicate these results (Hagenblad et al. 2009). The influence of selection would depend on levels of inbreeding in the above-barrier populations (Bensch et al. 2006), which is currently unknown. Selection would also be more effective at removing deleterious recessive alleles from larger populations. Therefore, selection might indirectly increase heterozygosity more effectively in the streams that contain larger populations. It is also possible that selection could increase genetic diversity through frequency-dependent fitness effects that do not depend on inbreeding.

Gene flow into the isolated populations would also serve to maintain genetic diversity (Jorde and Ryman 1996; Laikre et al. 1998). Upstream gene flow, which appears to be highly infrequent over contemporary time scales, could have occurred occasionally in the long-term. In addition, we cannot exclude the possibility that multiple populations exist above the waterfalls in some of the study streams. Fish habitat extended from 0.9 to 28 km upstream from the waterfalls in the streams we studied. Our samples were collected throughout the available habitat in the shorter drainages but in a central location in longer drainages. Thus, enough habitat might occur for population subdivision to have developed in the larger streams. Gene flow among multiple genetically differentiated populations in these streams with larger above-barrier habitat could maintain genetic diversity within each of the smaller above-barrier subpopulations (Jorde and Ryman 1996; Laikre et al. 1998).

Contemporary N_e estimates stood in stark contrast to the long-term N_e estimates. Contemporary N_e was small and not significantly correlated with amount of available habitat (Fig. 2). Effective sizes this small in the above-barrier populations would be expected to lose the observed amount of heterozygosity in a mean of 159 years (range 29–347), based on the same equation we used to calculate long-term N_e and the mean contemporary point estimates of N_e from ONeSAMP and LDNE. Thus, contemporary N_e estimates are inconsistent with the amount of genetic diversity retained by above-barrier populations over the time interval of isolation. Recent bottlenecks could be responsible for small contemporary N_e estimates, though analyses with the program BOTTLENECK provided little evidence in support of this hypothesis. The correlation between genetic diversity and amount of above-barrier habitat also suggests that bottlenecks have not had a large influence on genetic variation in these populations because events that influence population size strongly enough to leave the signature of a bottleneck are likely stochastic in nature and would be expected to influence within-population genetic diversity independently of habitat size.

Our contemporary N_e estimates could be biased low because, when applied to iteroparous organisms with overlapping generations, one-sample N_e estimators provide estimates that correspond to a value intermediate to N_B (number of breeders) and N_e of previous generations (Waples 2005). In addition, if the analysis by Palstra and Ruzzante (2008) for temporal N_e estimators applies to one-sample estimators, our samples sizes of approximately 30 may have low power to detect large N_e. The large discrepancy between contemporary and long-term N_e estimates in our study suggests that, while one-sample N_e estimates are likely to be useful under a variety of circumstances, in this example, the one-sample estimates were not directly relevant to understanding the maintenance of genetic diversity in long-isolated populations. This inference is only possible due to the geological circumstances of the studied populations and the ability to compare contemporary N_e to estimates of long-term N_e obtained by alternative means.

Asymmetric gene flow

The observation that gene flow was strongly biased in the downstream direction over waterfalls is consistent with another study that has examined the direction of gene flow in relation to stream barriers (Crispo et al. 2006). Several lines of evidence support the interpretation that gene flow occurs in the downstream but rarely in the upstream direction in our study streams. Immigration of divergent upstream genomes into downstream populations is the most likely cause of the deviations from Hardy–Weinberg proportions and gametic disequilibrium observed in the below-barrier sites in

general, and the RG sample specifically. The STRUCTURE (Fig. 3) and BAYESASS (Fig. 4) results provided little evidence of upstream gene flow, but strong evidence for downstream gene flow in several streams. The overall pattern is one of heavily biased unidirectional downstream gene flow in some but not all of the streams.

One-way gene flow in headwater stream systems has important evolutionary and conservation implications (Novinger and Rahel 2003; Allendorf et al. 2004). Highly divergent genomes enter the below-barrier populations from the above-barrier populations, potentially providing increased genetic diversity. However, fish from above-barrier populations differ in life-history and have likely experienced different selective regimes. Downstream gene flow could reduce fitness through outbreeding depression in the below-barrier populations. In addition, upstream gene flow from larger below-barrier populations is eliminated as a source of genetic diversity for the above-barrier populations. Recent work has provided evidence for asymmetric gene flow from larger to smaller salmonid populations (Fraser et al. 2004; Hansen et al. 2007), but waterfalls appear to preclude this possibility for above-barrier populations in this study. In addition, the variation we observed among populations in frequency of downstream gene flow suggests that not all populations lose the tendency to migrate upon isolation by barriers to fish movement.

Conclusions

This study offered an unusual opportunity to examine factors that influence the maintenance of genetic diversity in replicate isolated populations. Multiple lines of evidence indicated that above-barrier populations were isolated and gene flow has been asymmetrically in the downstream direction. Thus, presumably larger below-barrier populations are prevented from acting as sources of genetic diversity for the above-barrier populations. Yet, these above-barrier populations have retained substantial diversity that scaled significantly with the amount of available habitat above the waterfalls. Genetic drift appears to predominantly influence the maintenance of genetic diversity within and amount of genetic divergence between above-barrier populations. However, mutation, selection, and/or gene flow may also have contributed to genetic diversity in these populations. The significant correlation that we observed between available habitat and genetic diversity indicates that the amount of habitat available in headwater stream networks is an important consideration for maintaining the evolutionary potential of isolated populations.

Acknowledgments E. Tucker assisted with map creation and fieldwork. S. Friedman assisted with field collections. L. Chikhi provided the modified version of BAYESASS. We thank S. McCairns and N. Ryman for helpful discussions. Three anonymous reviewers provided valuable comments on an earlier draft of this manuscript. Support was provided by the U.S. Fish and Wildlife Service (Alaska Region) and the Tongass National Forest. KH also received support from a National Science Foundation Graduate Research and Training grant to the University of Montana (NSF-GRT-9553611 to P. Kukuk, C. Brewer and FWA). ARW was supported by the National Science Foundation (NSF-OISE-0601864) during the preparation of this manuscript. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

References

Allendorf FW, Leary RF (1988) Conservation and distribution of genetic variation in a polytypic species: the cutthroat trout. Conserv Biol 2:170–184

Allendorf FW, Leary RF, Hitt NP, Knudsen KL, Lundquist LL, Spruell P (2004) Intercrosses and the US Endangered Species Act: should hybridized populations be included as westslope cutthroat trout? Conserv Biol 18:1203–1213

Angers B, Magnan P, Plante M, Bernatchez L (1999) Canonical correspondence analysis for estimating spatial and environmental effects on microsatellite gene diversity in brook charr (*Salvelinus fontinalis*). Mol Ecol 8:1043–1053

Balloux F (2001) EASYPOP (Version 1.7): a computer program for population genetics simulations. J Hered 92:301–302

Bensch S, Andren H, Hansson B, Pedersen HC, Sand H, Sejberg D, Wabakken P, Akesson M, Liberg O (2006) Selection for heterozygosity gives hope to a wild population of inbred wolves. PLoS ONE 1:e72

Bouza C, Arias J, Castro J, Sanchez L, Martinez P (1999) Genetic structure of brown trout, *Salmo trutta* L., at the southern limit of the distribution range of the anadromous form. Mol Ecol 8: 1991–2001

Bryant MD (2002) Estimating fish populations by removal methods with minnow traps in southeast Alaska streams. N Am J Fish Manag 20:923–930

Burnham KP, Anderson DR (1998) Model selection and inference: a practical information-theoretic approach. Springer, New York

Carlsson J, Nilsson J (1999) Effects of geomorphological structures on genetic differentiation among brown trout populations in a northern Boreal river drainage. Trans Am Fish Soc 130:36–45

Cavalli-Sforza LL, Menozzi P, Piazza A (1993) Demic expansions and human evolution. Science 259:639–646

Cheylan G, Granjon L (1998) Distribution of genetic diversity within and between western Mediterranean island populations of the black rat *Rattus rattus* (L 1758). Biol J Linn Soc 63:393–408

Clague JJ (1989) Quaternary sea levels. In: Fulton RJ (eds) Quaternary geology of Canada and Greenland. Geological Survey of Canada, Geology of Canada, No. 1. (Also Geological Survey of America, The Geology of America, v. K-1.), pp 43–45

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014

Costello AB, Down TE, Pollard SM, Pacas CJ, Taylor EB (2003) The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: an examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus* (Pisces: Salmonidae). Evolution 57:328–344

Crispo E, Bentzen P, Reznick DN, Kinnison MT, Hendry AP (2006) The relative influence of natural selection and geography on gene flow in guppies. Mol Ecol 15:49–62

Crnokrak P, Roff DA (1999) Inbreeding depression in the wild. Heredity 83:260–270

Crow JF, Kimura M (1970) An introduction to population genetics theory. Burgess Publishing Company, Minneapolis

Deiner K, Garza JC, Coey R, Girman DJ (2007) Population structure and genetic diversity of trout (*Oncorhynchus mykiss*) above and below natural and man-made barriers in the Russian River, California. Conserv Genet 8:437–454

DiRienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB (1994) Mutational processes of simple-sequence repeat loci in human populations. Proc Natl Acad Sci USA 91:3166–3170

Dunham JB, Vinyard GL, Rieman BE (1997) Habitat fragmentation and extinction risk of Lahontan cutthroat trout. N Am J Fish Manag 17:1126–1133

Ellegren H (2004) Microsatellites: simple sequences with complex evolution. Nat Rev Genet 5:435–445

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587

Faubet P, Waples RS, Gaggiotti OE (2007) Evaluating the performance of a multilocus Bayesian method for the estimation of migration rates. Mol Ecol 16:1149–1166

Frankham R (1997) Do island populations have less genetic variation than mainland populations? Heredity 78:311–327

Frankham R (1998) Inbreeding and extinction: island populations. Conserv Biol 12:665–675

Fraser DJ, Lippe C, Bernatchez L (2004) Consequences of unequal population size, asymmetric gene flow and sex-biased dispersal on population structure in brook charr (*Salvelinus fontinalis*). Mol Ecol 13:67–80

Fukushima M, Kameyama S, Kaneko M, Nakao K, Steel EA (2007) Modeling the effects of dams on freshwater fish distributions in Hokkaido, Japan. Freshw Biol 52:1511–1524

Gaggiotti OE, Lange O, Rassmann K, Gliddon C (1999) A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. Mol Ecol 8:1513–1520

Goudet J (2001) FSTAT version 2.9.3, a program to estimate and test gene diversities and fixation indices. www.unil.ch/izea/softwares/fstat.html (Updated from Goudet (1995))

Guy TJ, Gresswell RE, Banks MA (2008) Landscape-scale evaluation of genetic structure among barrier-isolated populations of coastal cutthroat trout, *Oncorhynchus clarkii clarkii*. Can J Fish Aquat Sci 65:1749–1762

Hagenblad J, Olsson M, Parker HG, Ostrander EA, Ellegren H (2009) Population genomics of the inbred Scandinavian wolf. Mol Ecol 18:1341–1351

Hansen MM, Skaala O, Jensen LF, Bekkevold D, Mensberg KLD (2007) Gene flow, effective population size and selection at major histocompatibility complex genes: brown trout in the Hardanger Fjord, Norway. Mol Ecol 16:1413–1425

Harig AL, Fausch KD (2002) Minimum habitat requirements for establishing translocated cutthroat trout populations. Ecol Appl 12:535–551

Hastings K (2005) Long-term persistence of isolated fish populations in the Alexander Archipelago. Unpublished Ph.D. dissertation, The University of Montana, Missoula, MT

Hedrick PW (2005) A standardized genetic differentiation measure. Evolution 59:1633–1638

Hedrick PW, Kalinowski ST (2000) Inbreeding depression in conservation biology. Annu Rev Ecol Syst 31:139–162

Heller R, Siegismund HR (2009) Relationship between three measures of genetic differentiation G_{ST}, D_{EST}, and G'_{ST}: how wrong have we been? Mol Ecol 18:2080–2083

Hinten G, Harriss F, Rossetto M, Baverstock PR (2003) Genetic variation and island biogeography: microsatellite and mitochondrial DNA variation in island populations of the Australian bush rat, *Rattus fuscipes greyii*. Conserv Genet 4: 759–778

Hitt NP, Frissell CA, Muhlfeld CC, Allendorf FW (2003) Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarki lewisi*, and nonnative rainbow trout, *Oncorhynchus mykiss*. Can J Fish Aquat Sci 60:1440–1451

Jones AG, Rosenqvist E, Berglund A, Avise JC (1999) Clustered microsatellite mutations in the pipefish *Syngnathus typhle*. Genetics 152:1057–1063

Jorde PE, Ryman N (1996) Demographic genetics of brown trout (*Salmo trutta*) and estimation of effective population size from temporal change of allele frequencies. Genetics 143:1369–1381

June JA (1981) Life history and habitat utilization of cutthroat trout (*Salmo clarki*) in a headwater stream on the Olympic Peninsula. University of Washington, Seattle, Washington, p 112

Kanda N, Leary RF, Spruell P, Allendorf FW (2002) Molecular genetic markers identifying hybridization between the Colorado River greenback cutthroat trout complex and Yellowstone cutthroat trout or rainbow trout. Trans Am Fish Soc 131:312–319

Keyghobadi N, Roland J, Matter SF, Strobeck C (2005) Among- and within-patch components of genetic diversity respond at different rates to habitat fragmentation: an empirical demonstration. Proc R Soc B Biol Sci 272:553–560

Laikre L, Jorde PE, Ryman N (1998) Temporal change of mitochondrial DNA haplotype frequencies and female effective size in a brown trout (*Salmo trutta*) population. Evolution 52:910–915

Letcher BH, Nislow KH, Coombs JA, O'Donnell MJ, Dubreuil TL (2007) Population response to habitat fragmentation in a stream-dwelling brook trout population. PLoS ONE 2:e1139

MacKiewicz M, Fletcher DE, Wilkins SD, DeWoody JA, Avise JC (2002) A genetic assessment of parentage in a natural population of dollar sunfish (*Lepomis marginatus*) based on microsatellite markers. Mol Ecol 11:1877–1883

Mann D (1986) Wisconsin and Holocene glaciation in Southeast Alaska. In: Hamilton TD, Reed KM, Thorson RM) (eds) Glaciation in Alaska, the geological record. Alaska Geological Society, Anchorage, pp 237–265

Mann DH, Hamilton TD (1995) Late pleistocene and Holocene paleoenvironments of the North Pacific coast. Quat Sci Rev 14:449–471

Milner AM, Robertson AL, Monaghan KA, Veal AJ, Flory EA (2008) Colonization and development of an Alaskan stream community over 28 years. Front Ecol Environ 6. doi:10.1890/060149

Morita K, Yamamoto S (2002) Effects of habitat fragmentation by damming on the persistence of stream-dwelling charr populations. Conserv Biol 16:1318–1323

Neville HM, Dunham JB, Peacock MM (2006) Landscape attributes and life history variability shape genetic structure of trout populations in a stream network. Landsc Ecol 21:901–916

Newman D, Pilson D (1997) Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. Evolution 51:354–362

Nicholas JW (1978) A review of literature and unpublished information on cutthroat trout *Salmo clarki clarki* of the Willamette watershed. Oregon Department of Fish and Wildlife, Research and Development Section, Corvallis

Nieminen M, Singer MC, Fortelius W, Schops K, Hanski I (2001) Experimental confirmation that inbreeding depression increases extinction risk in butterfly populations. Am Nat 157:237–244

Novinger DC, Rahel FJ (2003) Isolation management with artificial barriers as a conservation strategy for cutthroat trout in headwater streams. Conserv Biol 17:772–781

Ortego J, Aparicio JM, Cordero PJ, Calabuig G (2008) Individual genetic diversity correlates with the size and spatial isolation of

natal colonies in a bird metapopulation. Proc R Soc B 275:2039–2047
Palstra FP, Ruzzante DE (2008) Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? Mol Ecol. doi:10.1111/j.1365-294X.2008.03842.x
Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. J Hered 90:502–503
Pritchard K, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959
Rahel FJ (2007) Biogeographic barriers, connectivity and homogenization of freshwater faunas: it's a small world after all. Freshw Biol 52:696–710
Raymond M, Rousset F (1995) GENEPOP (version 3.2): population genetics software for exact tests and ecumenicism. J Hered 86: 248–249
Rehe WG (2007) Influence of landscape-scale variables on the age and growth of coastal cutthroat trout *Oncorhynchus clarkii clarkii* in headwater streams. Oregon State University, Corvallis
Rexstad E, Burnham KP (1991) Users guide for interactive program CAPTURE. Colorado Cooperative Fish & Wildlife Research Unit, Colorado State University, Fort Collins
Rice WR (1989) Analyzing tables of statistical tests. Evolution 43: 223–225
Riddihough RP (1982) Contemporary movements and tectonics on Canada's west coast: a discussion. Tectonophysics 86:319–341
Ryman N, Leimar O (2009) G_{ST} is still a useful measure of genetic differentiation—a comment on Jost's *D*. Mol Ecol 18:2084–2087
Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I (1998) Inbreeding and extinction in a butterfly metapopulation. Nature 392:491–494
Shimoda N, Knapik EW, Ziniti J, Sim C, Yamada E, Kaplan S, Jackson D, de Sauvage F, Jacob H, Fishman MC (1999) Zebrafish genetic map with 2000 microsatellite markers. Genomics 58:219–232
Soule ME, Mills LS (1998) No need to isolate genetics. Science 282:1658–1659
Spruell P, Bartron ML, Kanda N, Allendorf FW (2001) Detection of hybrids between bull trout (*Salvelinus confluentus*) and brook trout (*Salvelinus fontinalis*) using PCR primers complementary to interspersed nuclear elements. Copeia 2001(4):1093–1099
Steinberg EK, Lindner KR, Gallea J, Maxwell A, Meng J, Allendorf FW (2002) Rates and patterns of microsatellite mutations in pink salmon. Mol Biol Evol 19:1198–1202
Tallmon DA, Koyuk A, Luikart G, Beaumont M (2008) ONeSAMP: a program to estimate effective population size using approximate Bayesian computation. Mol Ecol Notes 8:299–301
Taylor EB, Stamford MD, Baxter JS (2003) Population subdivision in westslope cutthroat trout (*Oncorhynchus clarki lewisi*) at the northern periphery of its range: evolutionary inferences and conservation implications. Mol Ecol 12:2609–2622
Trizio I, Crestanello B, Galbusera P, Wauters LA, Tosi G, Matthysen E, Hauffe HC (2005) Geographical distance and physical barriers shape the genetic structure of Eurasian red squirrels (*Sciurus vulgaris*) in the Italian Alps. Mol Ecol 14:469–481
Trotter PC (1989) Coastal cutthroat trout: a life history compendium. Trans Am Fish Soc 118:463–473
USFS (1997) Tongass National Forest land and resource management plan. USDA Forest Service, Juneau
Wang SZ, Hard J, Utter F (2001) Salmonid inbreeding: a review. Rev Fish Biol Fish 11:301–319
Waples RS (2005) Genetic estimates of contemporary effective population size: to what time periods do the estimates apply? Mol Ecol 14:3335–3352
Waples RS (2006) A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conserv Genet 7:167–184
Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. Mol Ecol Resour 8:753–756
Warren ML, Pardew MG (1998) Crossings as barriers to small-stream fish movement. Trans Am Fish Soc 127:637–644
Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370
Wenburg JK, Bentzen P, Foote CJ (1998) Microsatellite analysis of genetic population structure in an endangered salmonid: the coastal cutthroat trout (*Oncorhynchus clarki clarki*). Mol Ecol 7: 733–749
White TA, Searle JB (2007) Genetic diversity and population size: island populations of the common shrew, *Sorex araneus*. Mol Ecol 16:2005–2016
White GC, Burnham KP, Otis DL, Anderson DR (1978) Users manual for program CAPTURE. Utah State University Press, Logan
Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191
Wofford JEB, Gresswell RE, Banks MA (2005) Influence of barriers to movement on within-watershed genetic variation of coastal cutthroat trout. Ecol Appl 15:628–637
Wyatt B (1959) Observations on the movements and reproduction of the Cascade form of cutthroat trout. Oregon State University, Corvallis
Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. Trends Ecol Evol 11:413–418
Yue GH, David L, Orban L (2007) Mutation rate and pattern of microsatellites in common carp (*Cyprinus carpio* L.). Genetica 129:329–331