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Population subdivision in westslope cutthroat trout (*Oncorhynchus clarki lewisi*) at the northern periphery of its range: evolutionary inferences and conservation implications

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Abstract

Westslope cutthroat trout (*Oncorhynchus clarki lewisi*, Salmonidae) are native to the upper Columbia, Missouri, and South Saskatchewan river drainages of western North America and are at the northern periphery of their range in southeastern British Columbia, Canada. We examined geographical variation in allele frequencies at eight microsatellite loci in 36 samples of westslope cutthroat trout from British Columbia to assess levels of population subdivision and to test the hypothesis that different habitat types (principally mainstem vs. above migration barrier habitats) would influence levels of genetic diversity, genetic divergence among populations, and attainment of equilibrium between gene flow and genetic drift. Across all samples, the mean number of alleles per locus was 3.9 and mean expected heterozygosity was 0.56. Population subdivision was extensive with an overall F_{st} (θ) of 0.32. Populations sampled above migration barriers had significantly fewer alleles, lower expected heterozygosity, but greater average pairwise F_{st} than populations sampled from mainstem localities. We found evidence for isolation-by-distance from a significant correlation between genetic distance and geographical distance ($r = 0.31$), but the pattern was much stronger ($r = 0.51$) when above barrier populations and a population that may have been involved in headwater exchanges were removed. By contrast, isolation-by-distance was not observed when only above barrier populations were tested among themselves. Our data support the maintenance of separate demographic management strategies for westslope cutthroat trout inhabiting different river systems and illustrate how differing habitat structure (e.g. presence of migration barriers) may influence patterns of biodiversity and gene flow-drift equilibrium.

Keywords: conservation, isolation-by-distance, microsatellites, peripheral populations, population subdivision, westslope cutthroat trout

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Introduction

Understanding the nature of processes that generate and sustain biodiversity is a central problem in evolutionary biology (Mayr 1963; Ehrlich & Raven 1969; Gould & Johnston 1972; Mayr 1982). From the wealth of geographical surveys of genetic structure it is becoming clear both that historical factors, such as glaciation or island formation (Grant 1998; Hewitt 2000; Turgeon & Bernatchez 2001), and contemporary environmental conditions that influence gene flow, demographic processes (e.g. bottlenecks or founder events), or selection (Powers *et al*. 1991; Angers *et al*. 1999; Latta & Mitton 1999; Cassel & Tammaru 2003; Costello *et al*. 2003) are important in structuring diversity within and among natural populations. The existence of geographically peripheral populations, i.e. those found at the margins of the species' range separated spatially from central populations,

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can profoundly influence the nature of geographical variation within species. Such populations are also usually ecologically peripheral because of the differences in environmental conditions between the centre and margins of a species' range. The spatial and environmental distinction of peripheral populations may promote their evolutionary distinctiveness (Lesica & Allendorf 1995). Peripheral populations are also particularly important to conservation because they are often the most genetically divergent and they may harbour distinct traits important for adaptation to changing environments. Range peripheries are thought to be some of the most important areas for speciation, and at least some species have shown range collapse towards the periphery of their ranges, not the central portions, in response to environmental change (Lesica & Allendorf 1995; Channell & Lomolino 2000; Cassel & Tammaru 2003).

The westslope cutthroat trout (*Oncorhynchus clarki lewisi*) is one of up to 14 recognized subspecies of the polytypic cutthroat trout complex native to coastal and interior drainages of western North America (Allendorf & Leary 1988; Behnke 1992). Most cutthroat trout taxonomic diversity resides in interior drainages of the Columbia, Missouri, and Colorado river basins and is accompanied by substantial genetic and phenotypic divergence (Leary *et al*. 1987; Behnke 1992). The westslope cutthroat trout, first encountered in 1805 from the upper Missouri River during the Lewis and Clark expedition, was designated as *O. c. lewisi* to represent trout from the upper Columbia/Kootenay and Missouri rivers and to distinguish it from the Yellowstone River subspecies *O. c. bouvieri* (Behnke 1992). Westslope cutthroat trout are at the northern periphery of their native range in southeastern British Columbia (BC), Canada; the bulk of the range occurs in adjacent American states of Montana, Idaho, and Washington (see Allendorf & Leary 1988; Behnke 1992). Populations of westslope cutthroat trout in southeastern BC are also postglacial in origin because the current range of *O. clarki lewisi* in BC was completely glaciated during the Wisconsinan Period. The upper Pend d'Oreille, Missouri, and Clearwater river valleys, and associated glacial lakes Columbia and Missoula, constituted the principal refugium for freshwater fishes of southeastern BC from which westslope cutthroat trout dispersed after the ice sheets began to melt about 13 000 years ago (McPhail & Lindsey 1986; Pielou 1991). The peripheral and postglacial nature of BC populations of westslope cutthroat trout makes them an interesting model organism to study the effects of range expansion on genetic differentiation and, in particular, the relative roles of gene flow and genetic drift in structuring neutral molecular variation. For instance, westslope cutthroat trout inhabit aquatic environments of varying interconnectedness, from major tributaries to isolated headwater populations and analysis of genetic differentiation across such habitats may influence patterns of drift-migration equilibrium (e.g. Hutchison & Templeton 1999; Costello *et al*. 2003). Further, the interior cutthroat trout subspecies are at considerable conservation risk owing to restricted geographical distributions, extensive habitat degradation throughout these ranges, and displacement by and hybridization with introduced species (e.g. Allendorf & Leary 1988; Rhymer & Simberloff 1996; Dunham *et al*. 1997; Shepard *et al*. 1997). For the westslope cutthroat trout these threats are particularly pronounced, especially in Canada. The peripheral nature of Canadian populations means that they may be at greater demographic risk in marginal environments relative to populations at the centre of the range (Lawton 1993; Lesica & Allendorf 1995). In addition, a large portion of the US populations and some in Canada have been impacted to varying degrees by hybridization and introgression with introduced rainbow trout, *O. mykiss* (Leary *et al*. 1984; Deeds *et al*. 1999; Rubidge *et al*. 2001).

These concerns about the status and persistence of native westslope cutthroat trout in Canada led to its designation as a 'blue-listed' species of 'special concern' in British Columbia. This designation coupled with a lack of comprehensive information on genetic subdivision in BC precipitated the current analysis to illuminate historical relationships and provide a basis for inferences on current population interconnectedness and potential for quantitative divergence (Dunham *et al*. 1997; Saccheri *et al*. 1998; Sork *et al*. 1999; Moran 2002). We examined microsatellite variation in 36 samples of westslope cutthroat trout from a variety of watersheds and habitats ranging from interconnected systems to populations isolated above impassible (to upstream movement) barriers. Specifically, we wanted to assess levels of population subdivision and test the hypothesis that upstream migration barriers promote substantially different patterns of genetic variation in westslope cutthroat trout. Owing to their obvious greater isolation and occupancy of potentially more marginal, headwater habitats (e.g. Hilderbrand & Kershner 2000), we predicted that populations above migration barriers would show: lower levels of within population diversity, higher levels of among population diversity, and substantially greater deviation from migration-drift equilibrium than more interconnected populations found in larger tributary streams below natural migration barriers.

Materials and methods

Sample collection, DNA extraction, and species diagnostics

During 1998–2001, tissue samples were collected from a total of 32 localition river system (N = 1100, Table 1). These included samples from the same locality collected in different years (e.g. Gold Creek, Wigwam River) and collections from different sites within the same (Elk River, Gold Creek, Kirkup Creek). The collections spanned an area of some

Table 1 Sample localities, the major watersheds to which they belong, year of collection, Northing (No), Easting (Ea), sample size (*N*), ave average across locus allele number (N_a), average across locus allele richness (N_r), and average across locus expected heterozygosity (H_E). The numeral proceeding each locality name refers to the map code in Fig. 1 and italicize localities are found above upstream migration barriers

Population	Code	Watershed	Year	No	Ea	*N*	N_a	N_r	H_E
1 Sage Cr.	SG	Flathead R.	2001	5439378.72	685384.76	30	4.8	2.8	0.45
2. *Upper Lodgepole Cr.*	UL	Wigwam R.	2001	5462158.83	656899.95	30	2.3	1.8	0.24
3. Wigwam R.	WW99	Kootenay R.	1999	5452675.49	654112.07	30	4.0	2.6	0.42
3. Wigwam R.	WW00	Kootenay R.	2000	5452675.49	654112.07	30	6.0	3.4	0.53
3. Wigwam R.	WW01	Kootenay R.	2001	5452675.49	654112.07	31	5.8	3.1	0.46
4a. Coal Cr.	ERCC	Elk R.	2000	5484284.93	641469.33	30	3.9	2.6	0.44
4b. Michel Cr.	ERMC	Elk R.	2000	5503796.70	662220.23	30	5.1	3.1	0.49
4c. *Fording R.*	ERFR98	Elk R.	1998	5530224.36	654785.63	28	2.8	2.3	0.37
4c. *Fording R.*	ERFR00	Elk R.	2000	5530224.36	654785.63	27	6.9	2.3	0.54
4d. Upper Elk R.	ER99	Kootenay R.	1999	5537629.19	650387.60	30	3.8	2.5	0.38
4e. *Connor L.*	CON	Elk R.	2001	5576676.64	636122.35	32	2.3	1.8	0.29
5. Gold Cr.	GC99	Kootenay R.	1999	5445903.35	618792.62	30	7.8	3.9	0.60
5. Gold Cr.	GC00	Kootenay R.	2000	5445903.35	618792.62	40	4.9	3.4	0.51
6a. Lower Bull R.*	LB	Kootenay R.	2001	5481122.69	612329.33	25	5.3	3.3	0.58
6b. *Upper Bull R.*	UB	Kootenay R.	1999	5491733.96	621183.21	30	3.8	2.5	0.37
7. Wild Horse Cr.	WDH	Kootenay R.	2000	5498701.46	600839.16	30	5.6	3.3	0.56
8. Lussier R.	LUS	Kootenay R.	2000	5541181.69	596834.01	30	6.3	3.6	0.61
9. White R.	WR	Kootenay R.	1999	5558792.74	621234.67	30	4.5	2.8	0.50
10. *Cross R.*	CRS	Kootenay R.	2000	5612854.95	585556.97	30	5.8	2.8	0.62
12. Findlay Cr.	FIN	Kootenay R.	2000	5553110.79	567930.29	30	3.2	2.1	0.29
13a. Lower St. Mary R.	LSM	Kootenay R.	1999	5494914.15	578518.25	30	5.0	2.6	0.47
13b. Upper St. Mary R.	USM	Kootenay R.	1999	5503828.31	545892.26	30	4.4	2.9	0.41
14. *Mather Cr.*	MATH	Kootenay R.	2000	5506183.09	580958.77	30	5.8	3.6	0.62
15a. Lower Skookumchuck Cr.	LSK	Kootenay R.	1999	5535695.35	580267.27	30	4.4	3.0	0.51
15b. Upper Skookumchuck Cr.	USK	Kootenay R.	1999	5535067.97	564667.08	30	3.9	2.8	0.46
16. *Goat R.*	GR	Kootenay R.	2001	5448091.31	548359.65	30	4.1	3.1	0.52
17. *Mat Cr.*	MAT	Kootenay L.	2001	5574556.82	496965.89	30	1.9	1.6	0.21
18. *Sitkum Cr.*	SIT	Kootenay L.	2001	5499349.77	484025.55	30	3.9	2.3	0.36
19. *Swift Cr.*	SC	Salmo R.†	2001	5437948.68	484025.55	30	1.3	1.2	0.05
20. Bitter Cr.	BC	Kettle R.†	2001	5430148.36	418780.45	29	1.9	1.5	0.17
21. Sutherland Cr.	SUTH	Christina L.†	2001	5435588.42	413274.02	18	4.3	3.1	0.55
22. *Upper Norns Cr.*	UNC	Columbia R.	2001	5477485.43	445093.26	20	1.6	1.4	0.16
23. *Ladybird Cr.*	LDB	Upper Norns Cr.	2001	5479202.12	437927.77	30	1.9	1.7	0.21
24. *Akolkolex R.*	AKX	Columbia R.	2001	5635816.76	433412.15	32	2.3	1.7	0.19
25a. *Lower Kirkup Cr.*‡	LKC	Columbia R.	2001	5654631.40	406494.64	26	1.8	1.5	0.19
25b. *Upper Kirkup Cr.*‡	UKC	Columbia R.	2001	5654679.85	405586.00	27	1.6	1.5	0.18
26. *Crazy Cr.*‡	CRZ	South Thompson R.§	2001	5654171.42	390697.99	30	3.3	2.4	0.34

*Rainbow trout (*Oncorhynchus mykiss*).
†Tributary of Columbia River downstream of confluence of Columbia-Kootenay rivers.
‡Described as *O. clarki alpestris* by Dymond (1931).
§Part of Fraser River drainage.

98 000 km² and included the mainstem upper Kootenay River, the Columbia River both upstream and downsteam of the confluence of the Columbia and Kootenay rivers, the Flathead River, and the upper Fraser River (Fig. 1; Table 1). The Fraser River sample was included to cover the western most distributed populations in BC and because Dymond (1931) described cutthroat trout from this area as a distinct subspecies; the mountain cutthroat trout, *O. m. alpestris*. By contrast, Behnke (1992) argued that this form is a synonym of *lewisi* owing to its recent (i.e. < 10 000 years) divergence from the main line of westslope cutthroat trout following postglacial flooding and dispersal from the upper Columbia and Kootenay rivers. The samples also included a range of habitats from the mainstem upper Kootenay River and mainstem reaches of large tributaries (e.g. Elk River, White River, St. Mary River), one lake (Connor Lake) and above migration barrier, headwater reaches of smaller tributaries (e.g. Kirkup Creek, Sitkum Creek, Table 1). In addition, the study area is fragmented by a number of hydroelectric developments on the different rivers. The

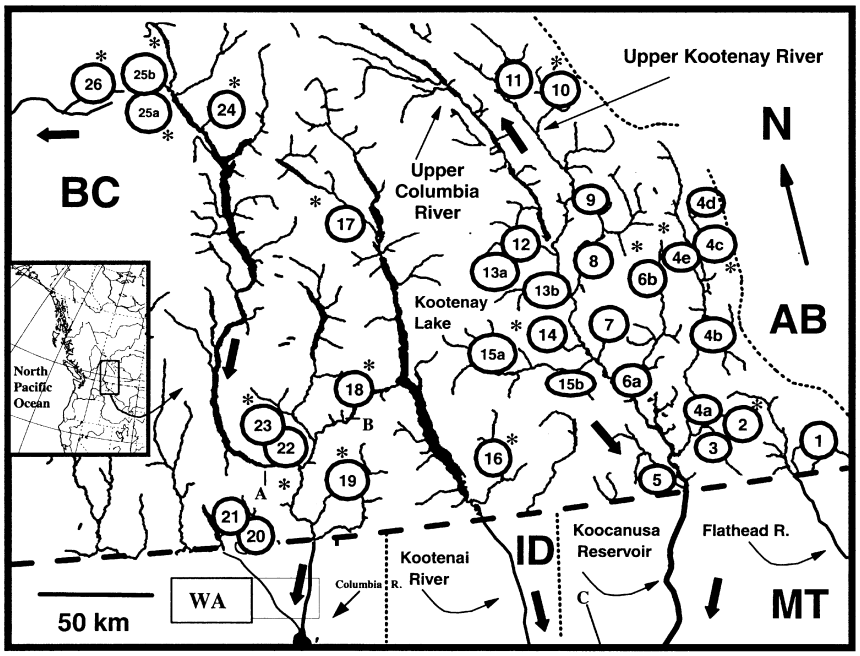

Fig. 1 Localities from which westslope cutthroat trout were assayed for variation in microsatellite DNA. 1 = Sage Creek, 2 = upper Lodgepole Creek, 3 = Wigwam River, 4a = Coal Creek (Elk R), 4b = Michel Creek (Elk R), 4c = upper Fording River (Elk R), 4d = upper Elk River, 4e = Connor Lake (Elk R), 5 = Gold Creek, 6a = lower Bull River, 6b = upper Bull River, 7 = Wild Horse Creek, 8 = Lussier River, 9 = White River, 10 = Cross Creek, 11 = upper Kootenay River, 12 = Findlay Creek, 13a = upper Skookumchuk Creek, 13b = lower Skookumchuk Creek, 14 = Mather Creek, 15a = upper St. Mary River, 15b = lower St. Mary River, 16 = Goat River, 17 = Mat Creek, 18 = Sitkum Creek, 19 = Swift Creek, 20 = Bitter Creek, 21 = Sutherland Creek, 22 = upper Norns Creek, 23 = Ladybird Creek, 24 = Akolkolex River, 25a = lower Kirkup Creek, 25b = upper Kirkup Creek, 26 = Crazy Creek. 'A', 'B', and 'C' represent approximate locations of major hydroelectric dams. 'BC' = British Columbia, 'AB' = Alberta, 'MT' = Montana, 'ID' = Idaho, 'WA' = Washington State. The heavy dashed line represents the Canada — United States border, and heavy arrows indicate direction of river flows. Inset shows study area in the context of British Columbia and adjacent northwestern United States. Asterisks (*) indicate above migration barrier populations.

major dams are the Hugh Keenleside Dam at the outlet of the Lower Arrow Lake, a series of dams on the lower Kootenay River between the confluence of the Kootenay and Columbia rivers, and Libby Dam on the Kootenai River which forms Koocanusa Reservoir upstream of Libby, Montana (Fig. 1, labelled A, B, and C, respectively).

Adipose or pelvic fin samples were taken and stored in 95% ethanol until DNA could be isolated from ~5 mg of tissue using the Puregene™ DNA isolation kit (Gentra Systems, Inc). Exotic rainbow trout have been introduced sporadically into tributaries of the study area where they can hybridize with native westslope cutthroat trout (Rubidge *et al*. 2001). Consequently, samples were screened with two nuclear DNA PCR-RFLP markers that distinguish westslope cutthroat trout and rainbow trout using the methods outlined in Rubidge *et al*. (2001). Any fish heterozygous at either locus were discarded from further analyses. As a further precaution, we compared microsatellite DNA profiles for each fish using known allelic profiles for 65 populations of rainbow trout assayed with the same set of loci (P. Tamkee and E. Taylor, unpubl. data) and a subset of populations of westslope cutthroat trout with no history of stocking with rainbow trout. We used the Bayesian assignment procedures implemented in GENECLASS (Cornuet *et al*. 1999) to assign fish to species. Any fish that was not at least 10 times more likely to be classified as a westslope cutthroat trout rather than rainbow trout was also removed from subsequent analyses. These precautions make it very unlikely that hybrid genotypes, except for perhaps advanced generation backcrosses to westslope cutthroat trout or hybrids, influence our estimates of microsatellite variation in westslope cutthroat trout.

Microsatellite amplification and scoring

After screening microsatellite loci for use in this study, eight loci were chosen for inclusion based on clarity of resolution and degree of polymorphism: *Omy*77, *Ssa*85, *Ssa*197, *Ssa*456, *Ots*3, *Ots*103, *One*14, and *Oki*3a (Table 2). Polymerase chain reactions (PCR) were carried out with ^{32}P-labelled primers in 10 μL volumes of 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.8 mM dNTP's, and 0.1 units of *Taq* DNA polymerase in a MJ PTC 100 thermocycler using a basic cycle profile of: 1 cycle (95 °C/3 min), 5 cycles

Table 2 Microsatellite locus names, source species, annealing temperature (Ta, °C) total numbers of alleles, average expected heterozygosity, and authority for each locus used to assay variation in westslope cutthroat trout

Locus	Source species	Ta	No. alleles	H_E	Size range (base pairs)	Citation
*Ots*3	Chinook salmon	50	10	0.69	77–96	Banks *et al*. (1999)
*Ots*103	Chinook salmon	57	12	0.73	66–112	Nelson & Beacham (1999)
*Ssa*85	Atlantic salmon	57	20	0.70	100–162	O'Reilly *et al*. (1996)
*Ssa*197	Atlantic salmon	60	6	0.32	110–124	O'Reilly *et al*. (1996)
*Ssa*456	Atlantic salmon	55	5	0.14	152–160	Slettan *et al*. (1995)
*Oki*3a	Coho salmon	50	19	0.87	127–203	P. Bentzen, Dalhousie U., unpubl.
*Omy*77	Rainbow trout	55	18	0.38	80–140	Morris *et al*. (1996)
*One*14	Sockeye salmon	60	20	0.61	119–199	Scribner *et al*. (1996)

(95 °C/1 min, T_a/1 min, 72 °C/1 min), 27 cycles (92 °C/1 min, T_a/1 min, 72 °C/1 min), and 1 cycle (72 °C/5 min), where T_a is the annealing temperature (see Table 2). PCR products were electrophoresed through 6% Long Ranger™ (Mandel Scientific) polyacrylamide gels and visualized on Kodak Biomax™ MS film. Alleles were scored by eye with reference to standardized individuals run on every gel and to an M13 sequencing ladder. Raw allele frequencies are available from an electronic appendix (electronic Appendix Table 1 available at http://www.zoology.ubc.ca/etaylor/nfrg/wsctdata/ukrallelefreq2.htm)

Genetic data analyses

Tests for deviations from Hardy–Weinberg equilibrium were performed for each locus-population combination using an exact test in which *P*-values were estimated using a Markov chain method performed using GENEPOP version 3.1 (Raymond & Rousset 1995). Tests for genotypic linkage disequilibrium for all combinations of locus pairs within populations were also made using a Markov chain method with GENEPOP default values. Tests for population differentiation between all pairs of populations were performed over all loci combined using log-likelihood (G)–based exact tests (Goudet *et al*. 1996) with default values implemented in GENEPOP. Significance for all tests was declared after sequential Bonferroni multiple test adjustments (Rice 1989).

The basic descriptive statistics sample size (N), number of alleles (N_a), expected heterozygosity (H_E) and observed heterozygosity (H_O) were compiled using FSTAT version 2.9.3.2 (Goudet 2001). We tested for differences in allelic richness (N_r, adjusted for differences in sample size), expected multilocus heterozygosity, and pairwise F_{ST}, estimated using θ (Weir & Cockerham 1984), between populations isolated above migration barriers (waterfalls) and other populations using the permutation approach in FSTAT. Specifically, because above barrier populations are isolated and their headwater locations are typically associated with smaller drainage areas and smaller or more variable population sizes (Hilderbrand & Kershner 2000), we predicted that they would show lower intrapopulation genetic diversity relative to interconnected and larger downstream tributaries. We also predicted that such processes acting randomly in isolated, headwater populations should generate greater interpopulation divergence (F_{ST}) in above barrier populations and that they would be farther from migration-drift equilibrium than interconnected systems.

Pairwise comparisons among population F_{ST} values were calculated in ARLEQUIN version 2.0 (Schneider *et al*. 1997) with significance based on a permutation process. The allele frequency data were also subject to principal components analysis using PCA-Gen (available at http://www.unil.ch/izea/softwares/pcagen.html) to resolve relative similarities among populations without the constraints of forcing them into a set of bifurcations.

To determine how genetic variation was partitioned, ARLEQUIN was used to estimate the hierarchical nesting of genetic diversity using the Analysis of Molecular Variance (AMOVA) approach of Excoffier *et al*. (1992). The percentage of the total genetic variation explained by allele frequency variation within populations, among populations within groups, and by differences between groups was calculated under a variety of grouping hypotheses. For example, we tested for a major genetic division between above and below barrier populations, for a difference between populations upstream and downstream of Bonnington Falls on the Kootenay River, a major barrier to fish passage during deglaciation, just upstream of the confluence between the Kootenay and Columbia rivers, and for differentiation among major watersheds relative to variation within watersheds. We performed these calculations using only F_{ST}, rather than R_{ST}, because estimates of the latter are subject to higher variance and typically underperform F_{ST} in recently diverged populations (e.g. Gaggiotti *et al*. 1999).

To test for isolation-by-distance, the Mantel test option in FSTAT was used to assess the significance of correlations between geographical (fluvial) distance and genetic distance (F_{ST}). Geographic distances between tributaries within the

study area was determined using the Geographic Information System (GIS) program, ARCVIEW (version 3.1, ESRI). To determine whether populations have reached drift-migration equilibrium, we applied the approach of Hutchison & Templeton (1999). Subsequent to a significant Mantel test result between genetic and geographical distances, a second Mantel test was performed using residuals from the initial fitted line (calculated using FSTAT) against geographical distance. At equilibrium, scatter (residuals) should increase with increased geographical separation as drift, rather than gene flow, becomes the dominant force at larger distances (see Costello *et al*. 2003 for an additional example in bull trout, *Salvelinus confluentus*).

As a final measure of distinctiveness among populations, we conducted 'assignment tests' to assess the accuracy with which individual fish could be classified to their known sample population based on their composite eight locus microsatellite genotypes (Hansen *et al*. 2001). This analysis was conducted using the program GENECLASS incorporating the 'jackknife' or 'leave one out' option to reduce assignment bias.

Results

Hybridization with rainbow trout

Morphological examination of individual trout during collection of samples suggested the presence of exotic rainbow trout, hybrid, or introgressed individuals at some localities. In particular, the sample from the lower Bull River appeared to have a large number of hybrids and rainbow trout. Screening these fish with nuclear markers (introns) classified most of the lower Bull River fish as rainbow trout (24/29 fish) or hybrids between rainbow trout and westslope cutthroat trout (5/29; see Rubidge 2003 for details). The assignment tests using microsatellite allele frequency data for the eight loci also identified these fish as rainbow trout or hybrids. In addition, several fish from Gold Creek (5), Michel Creek (upper Elk River, 5), lower St. Mary River (2), Sutherland Creek (2), Wigwam River (2000 year sample, 4) and the upper Kootenay River mainstem (3) were identified as rainbow trout or hybrids between westslope cutthroat trout and rainbow trout and were removed from subsequent analyses (see also Rubidge 2003).

Within-population variation

We assayed microsatellite variation across almost 1100 individuals at eight microsatellite loci (electronic Appendix Table 1). The numbers of alleles observed across all populations ranged from 5 (*Ssa*456) to 20 (*Ssa*85 and *One*14) with an average of 13.8 alleles per locus. Expected heterozygosity averaged 0.56 across all loci and populations and ranged from 0.14 (*Ssa*456) to 0.87 (*Oki*3a).

Virtually all samples were in Hardy–Weinberg equilibrium with 11 (out of a possible 304) tests showing significant heterozygote deficits. These exceptions were found at several separate loci in 11 different populations and therefore do not compromise subsequent analyses. Results of tests for linkage disequilibrium resulted in significant departures in 12 out of a possible 769 tests. The significant results were not concentrated on particular locus pairs or within specific populations.

Variation within populations ranged widely (Table 1); expected heterozygosity, averaged across the 8 loci, ranged from a low of 0.05 (Swift Creek) to a high of 0.53–0.61 (upper Kootenay River mainstem, White River, Lussier River, Gold Creek, Mather Creek). Across all loci and populations, the average number of alleles per locus was 3.9 (Table 1). Some populations displayed extremely low levels of variation. Swift Creek westslope cutthroat trout, for instance, were fixed for single alleles at 7 of the 8 loci. Also, Bitter Creek, and upper and lower Kirkup creek fish displayed no more than 4 alleles at any one locus and were often fixed for single alleles (electronic Appendix Table 1 available at http://www.zoology.ubc.ca/~etaylor/nfrg/wsctdata/ukrallelefreq2.htm)

By contrast, samples from Gold Creek or the Wigwam River often displayed 8–10 alleles per locus. When the sample localities were grouped into those residing above migration barriers ($N = 16$) and those below barriers ($N = 19$), permutation tests demonstrated that above barrier sites have significantly lower average allele richness (2.1 vs. 2.9, respectively) and expected heterozygosity (0.303 vs. 0.463, respectively), but significantly greater pairwise F_{ST} (0.45 vs. 0.18, respectively) than populations sampled from below upstream migration barriers (all $P < 0.005$).

Among population variation

Variation among populations in our survey was extensive. There were 703 pairwise comparisons made between populations for differences in allele frequencies summed across all eight loci. Only *four* of these comparisons were not significant (i.e. $P \geq 0.0002$ when adjusting for multiple comparisons); the comparison between upper and lower Kirkup creek samples, between the upper and lower Skookumchuk Creek samples, between samples collected from the Fording River in 1998 and 1999, and from Gold Creek in 1999 and 2000. The proportion of the total molecular variance in allele frequencies attributable to differences between westslope cutthroat trout populations was 0.32 (95% CI 0.28–0.38) and was significantly greater than zero ($P < 0.001$). Differentiation among populations varied amongst specific populations. Pairwise F_{ST} estimates ranged from lows of 0.0 (between upper and lower Kirkup creek), 0.02 (between upper and lower St. Mary River), 0.03 (between Mather Creek and Wild Horse Creek), and 0.05

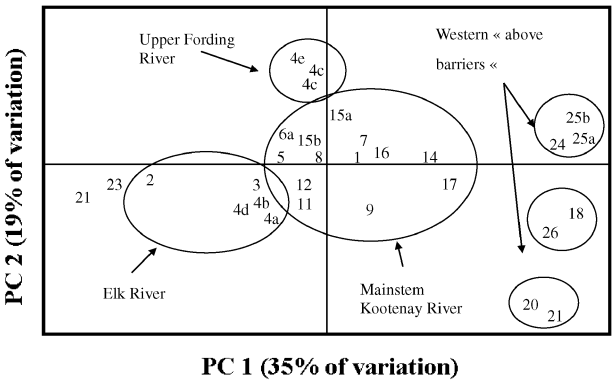

Fig. 2 Results of principal components analysis of allele frequency variation in westslope cutthroat trout assayed at eight microsatellite loci depicted as plots of mean component scores for each population along axes 1 and 2. Population number codes are defined in Table 1. Note that for clarity, some temporally replicated samples within streams and which overlapped with each other have been removed. Putative groups of populations are included within ellipses.

Table 3 Hierarchical analysis of the regional and subregional distribution of genetic diversity in westslope cutthroat trout populations included in this study under various hypotheses. Calculated using ARLEQUIN version 2.0, V_{bg} represents the percentage of variation existing between groups, V_{ap}, the amount existing among populations within groups, and V_{wp} is the percentage of variation existing within populations. The stated *P*-value refers to the probability that the observed value for V_{bg} is equaled or exceeded by chance determined from 1000 permutations. Probability values for all observed values of V_{ap} and V_{wp} were 0.0001 Among watersheds groupings include the Wigwam, Skookumchuck Creek, St. Mary River, Elk River, and Kirkup and Norns creeks. PCA groups refers to those population groups shown in Fig. 2

Comparison	V_{bg}	V_{ap}	V_{wp}	*P*
Above vs. below Bonnington Falls:	6.7	26.7	66.6	0.0001
East vs. West Kootenay:	4.8	27.4	67.7	0.0001
Above vs. Below barriers:	2.3	28.9	68.8	0.0030
Among watersheds:	21.1	10.5	68.4	0.0001
Among PCA groups:	12.2	21.2	66.6	0.0001

(between upper Kootenay River and White River) to highs of 0.67 (between Mat Creek and Bitter Creek), 0.78 (between upper Norns Creek and Swift Creek) and 0.82 (between Swift Creek and Bitter Creek). Non-significant pairwise F_{ST} were limited to tests between years for the same locality (Gold Creek 1999 and 2000, Fording River 1998 and 2000), between localities within the same small drainage (upper and lower Kirkup Creek, upper and lower Skookumchuk Creek).

Projection of populations in principal component space (Fig. 2) suggested substantial differentiation among four major sets of populations: samples from the Fording River (upper Elk River) and its tributaries, a group of mainstem upper Kootenay River populations, a group of populations from the Elk River, but below the barrier on the Fording River, and a heterogenous group of above barrier populations located west of the Kootenay River-Columbia River confluence. Above barrier populations (e.g. Swift, Bitter, and Kirkup creeks) were highly distinct both amongst themselves and from all other populations. In addition, there is a general association between geographical proximity and genetic similarity. For instance, the majority of Elk River sites grouped together and with the nearby Wigwam River (Fig. 2). The Fording River site on the Elk River grouped closely with the sample from Connor Lake, whose outlet also drains into the upper Elk River. Bitter and Sutherland creeks are both part of the Kettle River drainage and grouped together. The general correspondence between geographical proximity and genetic similarity was also reflected in the grouping of upper Kootenay River, White River, Mather Creek, Wild Horse Creek and Lussier River samples with one another (Figs 1 and 2). By contrast, two populations near the western extreme of the study area (Ladybird and upper Norns Creek) grouped along PC 1 nearest group 3 populations located near the eastern extreme of the study area (Fig. 2).

Comparatively little microsatellite variation was found between major geographical regions across the study area. For instance, Bonnington Falls are located in the lower Kootenay River just upstream of the confluence of the Kootenay and Columbia rivers and formed a natural barrier to upstream fish movement postglacially. We divided all populations into those above and below Bonnington Falls and following an analysis of molecular variance, this division represented 6.7% of the total variation, compared to 26.7% of the microsatellite variation that was explained by differences among populations within these groups, and 66.6% within individual populations (all $P < 0.001$, Table 3). Interestingly, these results were very similar to those obtained when all populations were divided into the two British Columbia fisheries management regions that span the study area: 'East Kootenay', populations 1–15, Fig. 1, and 'West Kootenay', populations 16–26 (4.8%, 27.4%, 67.7%, respectively, all $P < 0.001$). By contrast, larger amounts of variation were resolved among watersheds located across the study area compared to variation among multiple samples within watersheds. Among the Wigwam River (three localities), Skookumchuck Creek (two localities), St. Mary River (two localities), Elk River (four localities) and Kirkup and Norns creeks (two localities each), twice as much variation was found among watershed groups than within them (21 vs. 10%, both $P < 0.001$). In addition, when all samples were partitioned into the four groups suggested by the principal components analysis (Fig. 2), variation among groups was 12.1%, among populations within groups was 21.2%, and variation within

Population grouping	r F_{ST}	P	r Residuals	P
All populations	0.32	0.005	0.02	0.66
Above barrier populations removed	0.37	0.001	–0.53	0.001
Sage Creek removed	0.50	0.001	0.22	0.001
Above barrier populations and Sage Creek removed	0.50	0.001	0.29	0.001
Only above barrier populations	0.11	0.250	–0.15	0.100

Table 4 Results of analysis of isolation-by-distance for different sets of populations of westslope cutthroat trout. The P-value reported is the probability of obtaining the observed correlation (r) between pairwise comparisons of geographical distance and: (i) F_{ST} (θ), or (ii) the residuals of the geographical distance — F_{ST} regression, by chance assessed using Mantel tests (2000 permutations)

populations was 66.6% (all $P < 0.001$). Finally, samples were arranged into two groups: those above migration barriers and those below. There was a small, but statistically significant distinction between these 'headwater' and 'mainstem' categories; 2.3% of the variation was attributable to differences between them compared to 28.9% among populations within above and below barrier groupings ($P = 0.003$ and < 0.001, respectively).

Finally, assignment tests also indicated the highly unique genetic composition of several of the populations. For instance, populations such as Bitter Creek, Akolkolex River, Sitkum Creek, and Swift Creek showed 100% correct classification to their known stream of origin, and several others showed assignment success of over 90% (e.g. Sage Creek, Goat River, Connor Lake, Fording River, and Crazy Creek). A few samples, however, were notable in having low classification success. For instance, only 46–60% of Gold Creek, Mather Creek, Wild Horse Creek, Wigwam River, and St. Mary River trout were correctly classified to their known sample stream. Each of these populations contained many individuals that were classified as more likely to be members of a number of other populations rather than their known sample location.

Isolation-by-distance

Westslope cutthroat trout displayed a strong pattern of isolation-by-distance ($r = 0.32$, $P < 0.001$, Fig. 3), but residuals were not significantly correlated with geographical distance ($r = 0.02$, $P = 0.66$). Within this overall pattern, however, there are some notable deviations. First, a cluster of comparisons represented substantial divergence at relatively low geographical distances (100–400 km), and another cluster of comparisons involved relatively low divergences at the highest geographical distances (> 1000 km, Fig. 3). The former involved mostly comparisons when one of the populations was isolated above a migration barrier, and when they were removed from the analyses, evidence for isolation-by-distance increased ($r = 0.37$, $P < 0.001$, Table 4), and the residuals were highly negatively correlated with geographical distance ($r = –0.53$, $P < 0.001$). By contrast, the comparisons showing relatively low F_{ST} at high geographical distances were those involving the Sage Creek population which, by river kilometre, is the most isolated

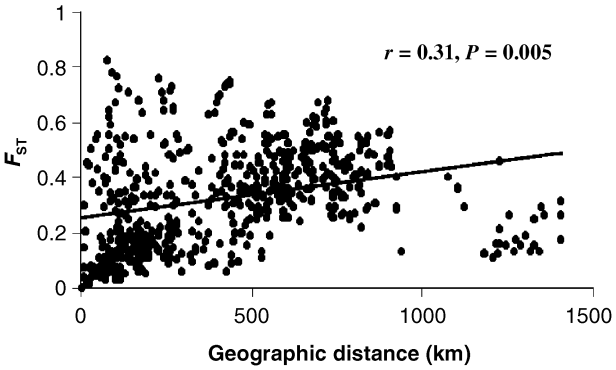

Fig. 3 Isolation by distance analyses for the upper Kootenay River westslope cutthroat trout assayed at 8 microsatellite loci. Pairwise F_{ST} (θ) distances (y-axis) are plotted against pairwise geographical distances (x-axis) for all populations.

population. When comparisons involving Sage Creek were removed, evidence for isolation-by-distance increased greatly ($r = 0.50$, $P < 0.001$), and the residuals were positively correlated with geographical distance ($r = 0.22$, $P < 0.001$). When both above barrier populations and the Sage Creek population were removed, this left only populations separated by no known natural migration barriers. In this case, evidence for isolation-by-distance was strong ($r = 0.50$, $P < 0.001$) and the residuals showed the highest correlation with geographical distance ($r = 0.29$, $P = 0.004$, Table 4). Considering only those pairwise comparisons involving at least one above barrier population, isolation-by-distance was again evident ($r = 0.23$, $P = 0.005$), but residuals showed no trend with geographical distance ($r = –0.06$, $P = 0.7$). By contrast, there was no evidence of isolation-by–distance when above barrier populations were compared only amongst themselves ($r = 0.11$, $P = 0.25$) and residuals showed no trend with geographical distance ($r = –0.15$, $P = 0.10$).

Discussion

Population subdivision and postglacial dispersal in westslope cutthroat trout

Our analysis of microsatellite DNA clearly establishes that there is substantial genetic differentiation among the various

westslope cutthroat trout populations sampled. All but four pairwise comparisons of F_{ST} indicated significant genetic divergence between populations, a result not unexpected for isolated headwater populations. The significant genetic divergence among populations with at least some potential for exchange (e.g. most mainstem upper Kootenay River tributaries) suggests considerable demographic independence among these populations despite extensive movements that can be made by fluvial adult westslope cutthroat trout (e.g. Schmetterling 2001). This key result supports the treatment of these populations as distinct biological units, in excess of any decisions to manage them separately in terms of habitat considerations or fishery values. In some cases, however, substantial differentiation between populations may be a function of strong genetic drift in the face of migration and gene flow between populations. For instance, although most temporal replicate samples showed non-significant F_{ST}, those within the Wigwam River were relatively high and significant (F_{ST} range: 0.03–0.08). This suggests that genetic drift within populations owing to low effective population sizes could be substantial and contribute to underestimation of movement among localities inferred from F_{ST}.

Levels of genetic variation and differentiation were consistent with studies of westslope cutthroat trout in other portions of their range (e.g. Leary *et al*. 1987; Landry *et al*. 2000; Shaklee & Young 2000). The low levels of genetic variation within some of the headwater populations (e.g. Bitter Creek, Swift Creek) are characteristic of isolated and presumably small populations that have likely undergone repeated bottlenecks in population size within their relatively small habitat areas (Nei *et al*. 1975; Costello *et al*. 2003). In addition, it is known that reduced variation within populations tends to exaggerate measures of inter-population variation such as F_{ST} (Hedrick 1999). Consequently, the high average F_{ST} (0.32) is to some extent driven by the dependence of F_{ST} on heterozygosity (and many populations had low heterozygosities). It is also likely that the current barriers to movement between many of the populations (e.g. waterfalls, cascades) contribute to the high levels of population subdivision observed in westslope cutthroat trout. Such high levels of F_{ST} are consistent with other salmonid species where natural restrictions to movement between populations are abundant (e.g. Currens *et al*. 1990; Angers *et al*. 1999; Bouza *et al*. 1999; Carlsson & Nilsson 1999; Costello *et al*. 2003). Hendry *et al*. (2003) reviewed molecular and biochemical genetic studies of population subdivision in salmonids and found that when comparisons among groups of populations included at least some that were completely isolated, the average microsatellite-based F_{ST} was 0.27 (0.14–0.37). Interior subspecies of cutthroat have not been well studied using microsatellites, but allozyme surveys have demonstrated strong geographical subdivision with F_{ST} values ranging from 0.08 to 0.45 (Loudenslager & Gall 1980; Allendorf & Leary 1988). By contrast, microsatellite-based assays of coastal cutthroat trout show F_{st} that are consistently lower, ranging from 0.03 to 0.12, and may reflect the greater connectivity of coastal cutthroat trout promoted by their anadromous behaviour (Wenburg *et al*. 1998; Wenburg & Bentzen 2001; Roberge *et al*. 2002; see also Bouza *et al*. 1999 for brown trout, *Salmo trutta*). In sum, our results demonstrate the substantial geographical subdivision that occurs in westslope cutthroat trout for the poorly studied northern periphery of its range and how geographical isolation, both by distance or migration barriers, promotes such differentiation.

Our documentation of substantial subdivision among populations in British Columbia coupled with isolation-by-distance is consistent with predictions both from 'pioneer' and 'phalanx' type expansions of populations from Wisconsinan refugia using riverine dispersal routes (Ibrahim *et al*. 1996). Headwater, above barrier populations likely resulted from pioneer type colonization by few individuals and produced severe founder events leading to reduced genetic diversity and strong differentiation from other populations. Such initial reductions in genetic diversity have probably been compounded by isolation and relatively small population sizes of above barrier populations. By contrast, mainstem populations were more likely colonized by phalanx or stepping-stone dispersal where more persistent connection along founding and source populations has maintained greater variation within populations and reduced among population differentiation.

Two hypotheses have been posited to explain the origin of westslope cutthroat trout populations in headwaters of the Fraser River (upper South Thompson River drainage) that are isolated from the main portion of the species range in the Columbia River basin. Given that the heart of the range of westslope cutthroat trout is the Columbia/Kootenay drainage, one hypothesis to account for upper fraser River Populations is headwater capture from western tributaries of the Columbia-Kootenay area (e.g. transfer of fish from Kirkup Creek to Crazy Creek, Fig. 1). Alternatively, populations in the easternmost regions of the Fraser River may be remnants of a formerly more widespread Fraser River drainage ancestral population and, consequently, colonized the Fraser River postglacially directly from the Columbia rather than by headwater exchanges. In fact, Dymond (1931) suggested that westslope cutthroat trout were historically more widespread in the Fraser River before it was recolonized by rainbow trout. Subsequent colonization by rainbow trout and interspecific competition may have led to the widespread extirpation of westslope cutthroat trout in the Fraser River drainage, particularly when they occurred below migration barriers (Dymond 1931). Our data support this latter hypothesis because westslope cutthroat trout from Crazy and Kirkup creek were very

distinct from one another. These populations did not cluster together (Fig. 2) and there was no cross-assignment between them. In addition, some alleles common in one population (e.g. *Ots**76 at 0.28 in Crazy Creek, or *Ssa*85*152 at 0.42 in Kirkup Creek) were absent in the other (frequency of 0.0 in Kirkup Creek, 0.0 in Crazy Creek, respectively, see electronic appendix). By contrast, other populations in close proximity that probably do share a recent common origin, or experience current gene flow between them were more similar to one another (e.g. Sutherland and Bitter creeks, Ladybird and upper Norns creeks).

Estimates of equilibrium conditions

Our data also provide one of the few quantitative examples in freshwater fishes of how regional equilibrium can vary as a function of the spatial scale of analysis and habitat. Hutchison & Templeton (1999) demonstrated how recent postglacial colonization influenced the attainment of equilibrium conditions between gene flow and drift in the eastern collared lizard *Crotaphytus collaris collaris*. Populations sampled from areas only inhabited postglacially in the north central United States (Kansas and Ozark Mountains area) were not in drift-migration equilibrium, whereas populations in Texas, where lizards persisted during glaciation, were in equilibrium (Hutchison & Templeton 1999). Costello *et al*. (2003) demonstrated a similar phenomenon for comparisons between bull trout from the Pine River and upper Kootenay River systems in British Columbia which are farther and closer, respectively, to areas of the upper Columbia River that served as glacial refugia during the Wisconsinan.

We found that westslope cutthroat trout over our entire study area were not in drift-migration equilibrium, despite a significant correlation between F_{ST} and geographical distance, because of a lack of increase in residuals with increasing distance between populations. This result is likely due to the large spatial scale of our study and the unrealistic assumption that over this area, fish populations would follow a simple stepping-stone or island model of population structure. Rather, the situation is more complex as revealed by inspection of our regional isolation-by-distance analysis. Similar to results for the collared lizards, we found the pattern of isolation by distance was not consistent across the full range of geographical distances (Hutchison & Templeton 1999); many comparisons showed high divergence at low geographical distances and low divergences at large geographical distances. This effect in westslope cutthroat trout was generated by comparisons between above barrier populations (even when geographically close to each other) generating relatively large pairwise F_{ST} values and illustrates the effect of local environments on attainment of equilibrium (see also Bouza *et al*. 1999; Costello *et al*. 2003). By contrast, the geographically most remote population (Sage Creek, riverine distance ranges from 740 to 1408 km) had relatively low divergence from many other populations. The unexpectedly low divergence of this population may reflect a footprint of historical, rather than contemporary, gene flow. For instance, Sage Creek is part of the Flathead River system which is one of the eastern most tributaries of the Columbia River. Although their headwaters are in close proximity (Fig. 1), the Flathead River and upper Kootenay River are isolated from each other by the Whitefish and MacDonald mountain ranges in southeastern BC. During deglaciation, however, both areas were probably recolonized by westslope cutthroat trout from populations inhabiting areas associated with Glacial Lake Missoula, a large proglacial lake formed by the damming of the Clark Fork River by the Purcell Trench Ice Lobe (Pielou 1991; Behnke 1992). In addition, the close proximity of Sage Creek to adjacent tributaries in the headwaters of the upper Kootenay River (e.g. Wigwam River, Elk River) may have facilitated headwater transfers between these drainage. Hutchison & Templeton (1999) suggested a similar situation for collared lizards where, owing to changes in vegetation cover, gene flow between some populations was historically higher than it is currently. Similarly, longer term historical changes in connectedness between adjacent fish populations are known to have left footprints on extant genetic variability (e.g. Hurwood & Hughes 1998; Waters & Wallis 2000).

Conservation genetic implications

Our microsatellite data have several implications for the conservation of native westslope cutthroat trout. First, our data demonstrate substantial genetic divergence among populations which is consistent with earlier allozyme-based studies of interior cutthroat (e.g. Loudenslager & Gall 1980; Allendorf & Leary 1988) and which appears characteristic of interior basin salmonids in general (e.g. Nielsen & Fountain 1999; Costello *et al*. 2003). Such high levels of subdivision suggest that westslope cutthroat trout populations are largely demographically independent as well. For example, even populations not separated by natural migration barriers in the mainstream Kootenay River showed average F_{ST} of 0.12 which suggests relatively low levels of exchange of trout between watersheds. Although low levels of inferred movement of individuals among streams may be sufficient to sustain genetic variability in westslope cutthoat trout (e.g. Madsen *et al*. 1999) they are perhaps insufficient to represent demographic 'rescue effects' should particular populations suffer large declines in population size. Consequently, our data support management and regulation of fishery activities that consider populations in different rivers to be composed of distinct demographic units. Dunham *et al*. (1997), however, found

that connectivity among stream basins was the most important factor explaining variation in the presence of Lahontan cutthroat trout (*O. c. henshawi*) among a set of streams in Nevada. These data clearly imply some movement and demographic interactions along stream populations of Lahontan cutthroat trout. Our finding of some cross-assignment among the larger tributaries to the upper Kootenay River as well as a strong pattern of isolation-by-distance suggests movement among streams across contemporary, in addition to historical, time frames. Consequently, it will be important to define the spatial and temporal scale of demographic interactions (movement and reproduction) among river systems for better informed management of watersheds (cf. Wenburg & Bentzen 2001). Second, two observations argue that in order to conserve representative westslope cutthroat trout biodiversity, at least that reflected by microsatellites, as many populations as possible should be maintained. This is because of the large fraction of the total molecular variance that was observed among populations (32%), and because of the high frequencies of some alleles that appear to be restricted to a single or few populations. [e.g. the restriction of several alleles to Ladybird Creek, cf. Allendorf & Leary (1988) for allozyme variation in US populations]. This means that considerable allelic variation, while locally abundant, may be rare across the total range (i.e. found in only one or a few populations). Third, molecular divergence among populations can provide an indication, and often a conservative one, of the extent of differences among populations in behavioural, morphological, physiological, or life history traits that are important to survival of populations in particular environments (e.g. Lynch *et al*. 1999; Merilä & Crnokrak 2001). Consequently, our microsatellite data suggest such differences may exist within westslope cutthroat trout that are important to persistence of populations across a broad array of habitats throughout their range. Novel colour phenotypes and life histories in westslope cutthroat trout, and physiological adaptations in related interior cutthroat are examples of such variation that exists and that should be maintained (Dymond 1931; Carl & Stelfox 1989; Wilkie 1994). Fourth, our data and those on other stream salmonids demonstrate that significant portions of within species diversity are partitioned within streams, and particularly between populations living above and below migration barriers (Northcote 1981; Bouza *et al*. 1999; Carlsson & Nilsson 1999; Costello *et al*. 2003). Significant molecular divergence between above and below barrier populations is consistent with their obvious physical isolation and their demographic independence, and emphasizes that conservation and recovery plans for such species must take account of isolated populations within streams for conservation to be truly representative. This is important for above barrier populations which are typically smaller in size and often considered to be of lower value to recreational fisheries than below barrier populations. Fifth, our data provide evidence for the colonization of exotic rainbow trout in tributaries of the study area and in particular the upper Kootenay River (e.g. lower Bull River) as well as for hybridization and introgression between westslope cutthroat trout and rainbow trout in this area. Rubidge *et al*. (2001) and Rubidge (2003) reported increased (from the mid-1980s) levels of hybridization across a number of watersheds in the upper Kootenay River. Our study corroborates this finding using microsatellite data. The characterization of westslope cutthroat trout and rainbow trout microsatellite allele frequency variation, coupled with assignment tests, provides a convenient and nonlethal technique to study the level of introgression and a mechanism to help prioritize populations for conservation of native westslope cutthroat trout gene pools.

Conclusions

Our study illustrates the complex nature of population structure that is probably characteristic of most aquatic organisms inhabiting heterogenous environments. Over our whole study area, westslope cutthroat trout populations are likely far from migration-drift equilibrium which may be unobtainable owing to migration barriers. In such cases, population structure reflects, in large part, the remnants of historical island-like postglacial recolonization and/or the formation of migration barriers. Within smaller spatial scales and more interconnected habitats such as the mainstem upper Kootenay River, however, populations may more closely approximate equilibrium conditions and reflect contemporary interactions in an isolation-by-distance, stepping-stone model of population structure. Taken together, examination of different spatial and temporal scales and incorporation of landscape genetic approaches to sampling (Manel *et al*. 2003) are necessary for a comprehensive understanding of population structure and its implications for conservation in organisms that inhabit variable environments. Our results and those of others (Bouza *et al*. 1999; Castric *et al*. 2001; Costello *et al*. 2003) illustrate the value of freshwater salmonids as model systems in such analyses.

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References

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

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*). *Molecular Ecology*, **8**, 1043–1053.

ARCVIEW GIS *Version 3.1*. Environmental Systems ResearchInstitute, Inc.

Banks MA, Blouin MS, Baldwin BA *et al.* (1999) Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Heredity*, **90**, 281–288.

Behnke RJ (1992) *Native Trout of Western North America*. American Fisheries Society Monograph, American Fisheries Society, Bethesda, MD.

Bouza C, Arias J, Castro J, Sanchez L, Martinez P (1999) Genetic structure of brown trout, *Salmo trutta* L., at the southern limits of the distribution of the anadromous form. *Molecular Ecology*, **8**, 1991–2002.

Carl LM, Stelfox JD (1989) A meristic, morphometric and electrophoretic analysis of cutthroat trout *Salmo clarki* from two mountain lakes in Alberta Canada. *Canadian Field Naturalist*, **103**, 80–84.

Carlsson J, Nilsson J (1999) Effects of geomorphological structures on genetic differentiation among brown trout populations in a northern Boreal river drainage. *Transactions of the American Fisheries Society*, **130**, 36–45.

Cassel A, Tammaru L (2003) Allozyme variability in central, peripheral, and isolated populations of the scarce heath (Coenonympha hero: Lepidoptera, Nymphalidae): implications for conservation. *Conservation Genetics*, **3**, 86–93.

Castric V, Bonney F, Bernatchez L (2001) Landscape structure and hierarchical genetic diversity in the brook charr, *Salvelinus fontinalis*. *Evolution*, **55**, 1016–1028.

Channell R, Lomolino M (2000) Dynamic biogeography and conservation of endangered species. *Nature*, **403**, 84–96.

Cornuet J-M, Piry S, Luikart G, Estoup A, Solignac M (1999) Comparison of methods to select or exclude populations as origins of individuals. *Genetics*, **153**, 1989–2000.

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.

Currens KP, Schreck CB, Li HW (1990) Allozyme and morphological divergence of rainbow-trout (*Oncorhynchus mykiss*) above and below waterfalls in the Deschutes River, Oregon. *Copeia*, **1990**, 730–746.

Deeds SA, Kaeding LR, Lohr SC, Young DA (1999) Status review for the westslope cutthroat trout in the United States. Department of the Interior, United States Fish and Wildlife Service Portland, OR.

Dunham JB, Vinyard GL, Reiman BE (1997) Habitat fragmentation and extinction risk of Lahontan cutthroat trout. *North American Journal of Fisheries Management*, **17**, 1126–1133.

Dymond JR (1931) Description of two new forms of British Columbia trout. *Contributions to Canadian Biology and Fisheries*, **6**, 393–395.

Ehrlich PH, Raven PH (1969) Differentiation of populations. *Science*, **165**, 1228–1232.

Excoffier L, Smouse P, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Gaggiotti OE, Lange O, Rassmann K, Gliddon C (1999) A com-parison of two indirect methods for estimating levels of gene flow from microsatellite data. *Molecular Ecology*, **8**, 1513–1520.

Goudet J (2001) FSTAT, *a Program to Estimate and Test Gene Diversities and Fixation Indices Version 2.9.3*. Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995).

Goudet J, Raymond G, Demeeus T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics*, **144**, 1933–1940.

Gould SJ, Johnston RF (1972) Geographic variation. *Annual Review of Ecology and Systematics*, **3**, 457–498.

Grant PR, ed. (1998) *Evolution on islands*. Oxford University Press, Oxford.

Hansen MM, Kenchington E, Nielsen EE (2001) Assigning individual fish to populations using microsatellite DNA markers. *Fish and Fisheries*, **2**, 93–112.

Hedrick PW (1999) Perspective: Highly variable loci and their interpretation in evolution and conservation. *Evolution*, **53**, 313–318.

Hendry AP, Castric V, Kinnison MT, Quinn TP (2003) The evolution of philopatry and dispersal: homing vs. straying in salmonids. In: *Evolution in Salmonids* (eds Hendry AP, Stearns SE), in press. Oxford University Press, Oxford, UK.

Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Hilderbrand RH, Kershner JL (2000) Conserving inland cutthroat trout in small streams: how much stream is enough? *North American Journal of Fisheries Management*, **20**, 513–520.

Hurwood DA, Hughes JM (1998) Phylogeography of the freshwater fish, *Mogurnda adspersa*, in streams of northeastern Queensland, Australia: evidence for altered drainage patterns. *Molecular Ecology*, **7**, 1507–1517.

Hutchison DW, Templeton AR (1999) Correlation between pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, **53**, 1898–1914.

Ibrahim KM, Nichols RA, Hewitt GM (1996) Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity*, **77**, 282–291.

Landry C, Papillon L, Bernatchez L (2000) *Analysis of Genetic Diversity in Cutthroat and Rainbow Trout from Waterton Lakes and Banff National Parks*. Report from Department of Biology, Laval University. Laval, QC.

Latta RG, Mitton JB (1999) Historical separation and present gene flow through a zone of secondary contact in Ponderosa pine. *Evolution*, **53**, 769–776.

Lawton JH (1993) Range, population abundance, and conservation. *Trends in Ecology and Evolution*, **8**, 409–413.

Leary RF, Allendorf FW, Knudsen KL (1984) Introgression between westslope cutthroat trout and rainbow trout in Clark Fork River drainage, Montana. *Proceedings of the Montana Academy of Sciences*, **43**, 1–18.

Leary RF, Allendorf FW, Phelps Knudsen KL (1987) Genetic divergence among populations of westslope cutthroat trout in the upper Kootenay River drainage, *British Columbia*. Report from

the Population Genetics Laboratory, Department of Zoology, University of Montana, Missoula.

Leary RF, Allendorf FW, Phelps SR, Knudsen KL (1987) Genetic divergence among seven subspecies of cutthroat trout and rainbow trout. *Transactions of the American Fisheries Society*, **116**, 580–587.

Lesica P, Allendorf FW (1995) When are peripheral populations valuable for conservation? *Conservation Biology*, **9**, 753–760.

Loudenslager EJ, Gall GAE (1980) Geographic patterns of protein variation and subspeciation in cutthroat trout, *Salmo clarki*. *Systematic Zoology*, **29**, 27–42.

Lynch M, Pfrender ME, Spitze K *et al.* (1999) The quantitative and molecular genetic architecture of a subdivided species. *Evolution*, **53**, 100–110.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution*, **18**, 189–197.

Madsen T, Shine R, Olssen M, Wittzell H (1999) Restoration of an inbred adder population. *Nature*, **402**, 34–35.

Mayr E (1963) *Animal Species and Evolution*. The Belknap Press of Harvard University, Cambridge.

Mayr E (1982) *The Growth of Biological Thought*. The Belknap Press of Harvard University, Cambridge.

McPhail JD, Lindsey CC (1986) Zoogeography of the freshwater fishes of Cascadia (the Columbia River and rivers north to the Stikine). In: *The Zoogeography of North American Freshwater Fishes* (eds Hocutt CH, Wiley EO), pp. 615–637. John Wiley and Sons, New York.

Merilä J, Crnokrak P (2001) Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology*, **14**, 892–903.

Moran P (2002) Current conservation genetics: building an ecological approach to the synthesis of molecular and quantitative genetic methods. *Ecology of Freshwater Fish*, **11**, 30–55.

Morris DB, Richard K, Wright JM (1996) Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study of salmonids. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 120–126.

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variation in populations. *Evolution*, **29**, 1–10.

Nelson RJ, Beacham TD (1999) Isolation and cross species amplification of microsatellite DNA useful for study of Pacific salmon. *Animal Genetics*, **30**, 228–229.

Nielsen JL, Fountain MC (1999) Microsatellite diversity in sympatric reproductive ecotypes of Pacific steelhead (*Oncorhynchus mykiss*) from the Middle Fork Eel River, California. *Ecology of Freshwater Fish*, **8**, 159–168.

Northcote TG (1981) Juvenile current response, growth, and maturity of above and below waterfall stocks of rainbow trout, *Salmo gairdneri*. *Journal of Fish Biology*, **18**, 741–751.

O'Reilly PT, Hamilton L, McConnell S, Wright JM (1996) Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 2292–2298.

Pielou E (1991) *After the Ice Age*. *The Return of Life to Glaciated North America*. University of Chicago Press, Chicago.

Powers DA, Lauerman T, Crawford D *et al.* (1991) The evolutionary significance of genetic variation at enzyme synthesizing loci in the teleost *Fundulus heteroclitus*. *Journal of Fish Biology*, **39**, 169–184.

Raymond M, Rousset F (1995) GENEPOP Version 1.2.: Population genetics software for exact tests and ecumenism. *Journal of Heredity*, **86**, 248–249.

Rhymer JM, Simberloff D (1996) Extinction and hybridization and introgression. *Annual Review of Ecology and Systematics*, **27**, 83–109.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Roberge M, Taylor EB, Slaney TL (2002) *Movement and Genetic Differentiation of Coastal Cutthroat Trout from Tributaries of the Lower Fraser River*. Report for the BC Ministry of Water, Land and Air Protection. Aquatic Resources Limited and Department of Zoology, University of BC, Vancouver.

Rubidge E (2003) A genetic analysis of hybridization between native westslope cutthroat trout (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*) in the upper Kootenay River drainage of southeastern British Columbia. MSc Thesis, University of British Columbia.

Rubidge E, Corbett P, Taylor EB (2001) A molecular analysis of hybridization between native westslope cutthroat trout (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*) in southeastern British Columbia. *Journal of Fish Biology*, **59**, 42–54.

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature*, **392**, 491–494.

Schmetterling DA (2001) Seasonal movements of westslope cutthroat trout in the Blackfoot River drainage, Montana. *North American Journal of Fisheries Management*, **21**, 507–520.

Schneider S, Kueffer J-M, Roessli D, Excoffier L (1997) ARLEQUIN, *Version 2.0: an Exploratory Population Genetics Software Environment*. Genetics and Biometry Laboratory. University of Geneva, Switzerland.

Scribner KT, Gust J, Fields RL (1996) Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 833–841.

Shaklee JB, Young SF (2000) *A Microsatellite DNA-Based Analysis of Population Structure of Cutthroat Trout (*Oncorhynchus clarki*) in the Pend Oreille Basin in Washington*. Wash. Department Fish and Wildlife Report, Olympia, WA.

Shepard BB, Sanborn B, Ulmer L, Lee DC (1997) Status and risk extinction for westslope cutthroat trout in the Upper Missouri River basin, Montana. *North American Journal of Fisheries Management*, **17**, 1158–1172.

Slettan A, Olsaker I, Lie O (1995) Atlantic salmon, *Salmo salar*, microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. *Animal Genetics*, **26**, 281–284.

Sork VL, Nason J, Campbell DR, Fernandez JF (1999) Landscape approaches to historical and contemporary gene flow in plants. *Trends in Ecology and Evolution*, **14**, 219–224.

Turgeon J, Bernatchez L (2001) Clinal variation at microsatellite loci reveals historical secondary intergradation between glacial races of *Coregonus artedi* (Teleostei: Coregoninae). *Evolution*, **55**, 2274–2286.

Waters JM, Wallis GP (2000) Across the Southern Alps by river capture? Freshwater fish phylogeography in South Island, New Zealand. *Molecular Ecology*, **9**, 1577–1582.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wenburg JK, Bentzen P (2001) Genetic and behavioral evidence

for restricted gene flow among coastal cutthroat trout populations. *Transactions of the American Fisheries Society*, **130**, 1049–1069.

Wenburg JK, Bentzen P, Foote CJ (1998) Microsatellite analysis of population structure in an endangered salmonid: the coastal cutthroat trout (*Oncorhynchus clarki clarki*). *Molecular Ecology*, **7**, 733–750.

Wilkie MP (1994) The physiological adaptations of the Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) following transfer from well water to the highly alkaline waters of Pyramid Lake, Nevada (pH 9.4). *Physiological Zoology*, **67**, 355–380.

This study was completed as part a comprehensive review of the general status of westslope cutthroat trout in British Columbia as an aid to conservation planning for the subspecies. EB Taylor's interests span from vertebrate genetics, biogeography, and speciation to the use of molecular markers in conservation. MD Stamford recently completed an MSc thesis on population subdivision and conservation in Arctic grayling and is interested in aquatic ecology and conservation. JS Baxter has broad interests in aquatic conservation, particularly for native fishes of British Columbia.