

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)

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Abstract.—An understanding of the relative roles of historical and contemporary factors in structuring genetic variation is a fundamental, but understudied aspect of geographic variation. We examined geographic variation in microsatellite DNA allele frequencies in bull trout (*Salvelinus confluentus*, Salmonidae) to test hypotheses concerning the relative roles of postglacial dispersal (historical) and current landscape features (contemporary) in structuring genetic variability and population differentiation. Bull trout exhibit relatively low intrapopulation microsatellite variation (average of 1.9 alleles per locus, average $H_e = 0.24$), but high levels of interpopulation divergence ($F_{ST} = 0.39$). We found evidence of historical influences on microsatellite variation in the form of a decrease in the number of alleles and heterozygosities in populations on the periphery of the range relative to populations closer to putative glacial refugia. In addition, one region of British Columbia that was colonized later during deglaciation and by more indirect watershed connections showed less developed and more variable patterns of isolation by distance than a similar region colonized earlier and more directly from refugia. Current spatial and drainage interconnectedness among sites and the presence of migration barriers (falls and cascades) within individual streams were found to be important contemporary factors influencing historical patterns of genetic variability and interpopulation divergence. Our work illustrates the limited utility of equilibrium models to delineate population structure and patterns of genetic diversity in recently founded populations or those inhabiting highly heterogeneous environments, and it highlights the need for approaches incorporating a landscape context for population divergence. Substantial microsatellite DNA divergence among bull trout populations may also signal divergence in traits important to population persistence in specific environments.

Key words.—Genetic diversity, glaciation, landscape genetics, microsatellites, population structure, *Salvelinus*, spatial heterogeneity.

Received March 22, 2002. Accepted October 8, 2002.

One of the most fundamental observations in nature is that species are polytypic and vary phenotypically (and genetically) across their range (Mayr 1963; Ehrlich and Raven 1969). Since the end of typological thinking, studies of geographic variation have been fundamental to the study of evolution and have provided insights into the process of adaptation to local conditions, the divergence of populations, and ultimately on the origin of species (Gould and Johnston 1972; Mayr 1982; Futuyama 1986; Avise 1994). For the past 50 years, many evolutionary studies have described the geographic patterns of genetic structure within and between populations as well as the forces that have helped to shape them (Mayr 1963; Wright 1978; Ayala and Fitch 1997; Howard and Berlocher 1998; Turner and Trexler 1998). In some cases, the dominant factors acting to structure genetic variation are historical in nature, resulting from large-scale environmental processes such as glaciation or island formation (Mayr 1963; Grant 1998; Hewitt 2000). In others, they may be more a product of current environments, involving restricted gene flow, demographic processes (e.g., bottlenecks or founder events), or selection (Powers et al. 1991; Templeton et al. 1995; Latta and Mitton 1999; Turgeon and Bernatchez 2001).

The role of large-scale historical factors in organizing genetic variation has been relatively well studied, both theo-

retically and empirically (e.g., Slatkin 1993; Hewitt 1996; Latta and Mitton 1999; Turgeon and Bernatchez 2001), but a comparable understanding of the effects of local landscape and environmental factors is a more recent development (Keyghobadi et al. 1999; Sork et al. 1999; Castric et al. 2001; Michels et al. 2001). Assessing the relative influence of these historical and contemporary factors on extant geographic variation has clear implications for questions related to evolutionary change and has become an important consideration in terms of conservation. Knowledge of the historical connectedness and present-day relationships between populations can only further attempts to conserve populations at risk and the evolutionary potential of species as a whole (Avise and Hamrick 1996; Saccheri et al. 1998; Sork et al. 1999). Evolutionary and taxonomic studies of fishes have made significant contributions to research on the geographic structuring of intraspecific diversity and evolutionary change (Mayr 1963; Reznick and Endler 1982; Echelle and Kornfield 1984; Bernatchez and Wilson 1998). Salmonid fishes (salmon, trout, and char), in particular, tend to exhibit extensive genetic variability and population differentiation attributable, at least in part, to the physical discreteness of the aquatic habitats where they live and to natal stream fidelity, which

encourages reproductive isolation (Quinn and Dittman 1990; Taylor 1991).

Bull Trout (Salvelinus confluentus)

Bull trout (*Salvelinus confluentus*) are a salmonid fish endemic to the northwestern North America and exhibit considerable variation in size, coloration, and life-history characteristics across their range, a feature that has contributed to considerable taxonomic confusion in the genus (Behnke 1980; Nordeng 1983). Like many other taxa that have recolonized formerly glaciated areas, bull trout occupy a large geographic range, extending north from California into the Yukon and Northwest Territories of Canada and eastward to western Montana and the headwaters of the South Saskatchewan River in western Alberta (Cavender 1978). Generally, they are restricted to interior drainages but are also found on the Pacific Coast in the Puget Sound area of Washington and in the Fraser, Nass, Skeena, and Stikine drainages of British Columbia. Largely piscivorous, bull trout are often the top aquatic predator where they live, reaching up to 100 cm and 14 kg in size. They exhibit several life-history forms (including resident and migratory) and show strong spawning site fidelity (McPhail and Baxter 1996; Spruell et al. 1999; Nerass and Spruell 2001).

Geographic variation in bull trout has been identified from morphological analyses (Cavender 1978, 1997; Haas and McPhail 2001), and more recent genetic surveys have suggested that the species is subdivided into two or more clades that originated from distinct glacial refugia on either side of the Cascade/Coast Mountains (Leary et al. 1993; Spruell and Allendorf 1997; Taylor et al. 1999). Consequently, bull trout are inferred to have expanded their range postglacially from south of the Wisconsin glaciers (dispersing northward over a distance of several thousand kilometers), which suggests that (at the level of major intraspecific lineages) historical factors have played a significant role in organizing extant patterns of genetic variation in the species (cf. Bernatchez and Wilson 1998; Hewitt 2000). The signature of these historical events is also likely to persist in bull trout in terms of local population structure. Bull trout are a long-lived, late-maturing species with small effective population sizes (McPhail and Baxter 1996; Swanberg 1997; Taylor et al. 2001), which makes them particularly sensitive to the genetic effects of bottlenecks and founder events that likely accompanied postglacial dispersal.

Owing to their occupancy of subdivided habitats in dendritic stream and lake environments, as well as the evidence for historical isolation in distinct glacial refugia, bull trout represent an excellent system with which to test the relative influence of historical and contemporary factors in organizing genetic variation within and among populations. Specifically, we investigated the relative importance of glaciation, founder events, and aspects of the contemporary physical environment as factors shaping the distribution of intraspecific genetic variation by studying bull trout from the heart of their range in British Columbia, Canada. First, we tested the idea that postglacial dispersal from southern refugia has left a genetic signature on bull trout populations in terms of a progressive decline in genetic diversity with increasing distance

from putative Wisconsin refugia (cf. Sage and Wolff 1986; Hewitt 1996; Merilä et al. 1996; Armbruster et al. 1998; Bernatchez and Wilson 1998; Turgeon and Bernatchez 2001). Second, because recently founded populations may exhibit interrelationships that are more a function of historical associations than of a current balance between gene flow and drift (Sork et al. 1999), the relative strength of isolation by distance may also differ between geographic regions depending on how far constituent populations are from drift-migration equilibrium (Slatkin 1993; Hutchison and Templeton 1999). We examined the patterns of isolation by distance in two watersheds that were colonized postglacially at different times and by different routes to provide another test of the influence of historical events on current population structure. Third, the effects of contemporary factors such as watershed and habitat area or the presence of impassable migration barriers may mitigate or supersede those of glaciation, at least on smaller spatial scales (Angers et al. 1999; Keyghobadi et al. 1999; Scribner et al. 2001). Consequently, we gathered information on environmental variables at watershed to site-specific scales to test if any were associated with differences among sites in terms of genetic variation or levels of genetic differentiation among sites using canonical correspondence analysis (CCA; ter Braak 1988a; Angers et al. 1999).

To these ends, we assayed levels of genetic variation at seven microsatellite loci for 37 interior bull trout populations to test three predictions: (1) glaciation and subsequent recolonization have had a significant impact on current levels of variation in bull trout as evidenced by a negative correlation between genetic variation within sites and relative distance from glacial refugia; (2) contemporary watershed characteristics, particularly those reflecting measures of habitat quality or area, are correlated with genetic variation within sites; and (3) watershed characteristics that reduce interconnectedness among sites (e.g., presence of barriers) will promote genetic differentiation among sites.

MATERIALS AND METHODS

Sample Collection, DNA Extraction, and Species Diagnostics

During 1997–2000, tissue samples were collected from 37 interior bull trout populations ($N = 1188$) from five regions spanning roughly 21,000 km² including the Pine River (lower Peace River drainage) and the upper Kootenay and upper Columbia rivers in British Columbia. We also collected samples from the Red Deer and Bow River drainages (Banff National Park) and the Oldman River drainage (Waterton Lakes National Park), which are tributaries of the eastward-draining South Saskatchewan River originating in western Alberta (Table 1, Fig. 1). In the Pine and Kootenay River drainages, populations were sampled from several major tributaries spanning comparable within-drainage pairwise geographic distances (8.1–381.8 km, 1.4–351.5 km, respectively).

Adipose or pelvic fin samples were taken and stored in 95% ethanol until DNA could be isolated from approximately 5 mg of tissue using the Puregene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN). Diagnostic polymerase

TABLE 1. Sampling locations for interior bull trout populations included in the study. Sample size (N), the presence or absence of a migration barrier, and year collected are given for each site. Numbers in parentheses correspond to locations in Figures 1 and 2.

Region	Site	Latitude	Longitude	N	Barrier	Year	
Upper Kootenay River (KOOT)	(1) Skookumchuck Creek	49.97	115.98	36	no	1997	
	(2) Bradford Creek	49.95	115.94	35	no	1997	
Elk River	(3) Sandown Creek	50.00	115.88	36	no	1997	
	(4) Wigwam River	49.05	114.83	35	no	1997	
	(5) Ram Creek	49.18	114.95	46	no	1997	
	(6) Lodgepole Creek	49.27	114.99	36	no	1997	
	(7) Line Creek	49.90	114.78	35	yes	1997	
	(8) South Line Creek	49.75	114.77	27	yes	1997	
Gold River	(9) Bloom Creek	49.06	115.50	19	no	1997	
St. Mary River	(10) Lower Redding Creek	49.56	116.59	33	no	1997	
	(11) Upper Redding Creek	49.48	116.58	51	yes	1997	
Kootenay River	(12) Wildhorse River	49.74	115.52	27	no	1997	
	White River	(13) Middle Fork White River	50.25	115.20	38	no	1997
		(14) Grave Creek	50.19	115.29	40	no	1997
Pine River (PINE)							
Upper Pine River mainstem	(15) Callazon Creek	55.58	112.82	30	no	1998	
	(16) Falling Creek	55.58	122.28	13	no	1998	
Sukunka River	(17) Chamberlain Creek	55.22	121.63	32	yes	1997	
	(18) Windfall Creek	55.10	121.69	32	yes	1998	
	(19) Burnt River	55.21	121.99	32	no	1997	
	(20) North Burnt River	55.30	122.00	32	no	1997	
	(21) Lower Brazion Creek	55.37	121.91	30	no	1997	
	(22) Upper Brazion Creek	55.39	122.20	40	putative	1997	
Murray River	(23) Bullmoose Creek	55.06	121.49	24	yes	1998	
	(24) Upper Wolverine Creek	54.93	121.48	29	no	1998	
	(25) Flatbed Creek	54.93	120.7	31	yes	1998	
	(26) Onion Creek	54.75	120.88	37	no	1998	
	(27) Hook Creek	55.86	121.52	28	yes	1998	
	(28) Perry Creek	55.09	121.20	30	no	1998	
	(29) Harrison Lake	51.33	115.48	43	yes	1997	
	(30) Cuthead Creek	51.26	115.45	19	yes	1998	
Red Deer River (RED)	Bow River (BOW)	(31) Cuthead Lake	51.26	115.45	51	yes	1999
		(32) Mystic Lake	51.16	115.44	28	yes	1999
		(33) Sawback Creek	51.20	115.46	10	no	1999
		(34) Yarrow Creek	49.11	113.59	12	no	1998
Oldman River (OLD)	(35) Belly River*	49.01	113.41	18	no	1998	
	(35) Belly River*	49.01	113.41	31	no	2000	
	(36) Blakiston Creek*	49.01	114.02	13	no	1998	
	(36) Blakiston Creek*	49.07	114.02	24	no	2000	
Upper Columbia River (UCOL)	(37) Ice River	51.11	116.26	25	no	1998	

* The temporal stability of allele frequencies was assessed for those populations where multiple-year collections were available (Belly River and Blakiston Creek, Oldman River drainage). There were no significant differences in terms of allele frequencies between collection years ($P = 0.9975$ and 0.3370 , respectively), and collections were combined for each location in all further analyses.

chain reaction (PCR) testing based on species-specific indels in type 2 growth hormone (GH2; McKay et al. 1996), was necessary for bull trout samples from Alberta as well as the Ice River population (Kicking Horse River tributary, upper Columbia River drainage) to determine the extent to which hybridization may have occurred with locally introduced brook trout (*S. fontinalis*; cf. Leary et al. 1993). Several microsatellite loci were also found to exhibit species-specific alleles that were further used to identify hybrids and backcrossed individuals (A. B. Costello, unpubl. data). All hybrid fish were removed from further analysis. Although GH2 species diagnostics were not performed on Pine River or Kootenay River samples, brook trout are not native to these watersheds and we avoided sampling localities where they have been introduced. None of the populations exhibited any brook trout-specific microsatellite alleles with the exception of one individual from the upper Wolverine Creek population (Pine River drainage). As a conservative measure, this individual was removed from further analysis.

Microsatellite Amplification and Scoring

After screening microsatellite loci for use in this study, seven loci were chosen for inclusion based on clarity of resolution and degree of polymorphism: *Omy77*, *Sfo18*, *Ssa197*, *Sco19*, *Sco23*, *Ssa456*, and *Ssa311* (Table 2). PCRs were carried out with ^{32}P -labeled primers in 10- μl volumes of 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl_2 , 0.8 mM dNTPs, and 0.1 units of *Taq* polymerase in MJ PTC 100 and 200 thermocyclers using a basic cycle profile of: one cycle (95°C for 3 min), five cycles (95°C for 1 min, T_A for 1 min, 72°C for 1 min), 27 cycles (92°C for 1 min, T_A for 1 min, 72°C for 1 min), and one cycle (72°C for 5 min), where T_A is the annealing temperature (see Table 2). PCR products were electrophoresed through 6% Long Ranger (FMC Corp., Philadelphia, PA) polyacrylamide gels and visualized on Kodak Biomax MS film (Kodak, Rochester, NY). Alleles were scored by eye with reference to standardized individuals run on every gel and to an M13 sequencing ladder (raw allele

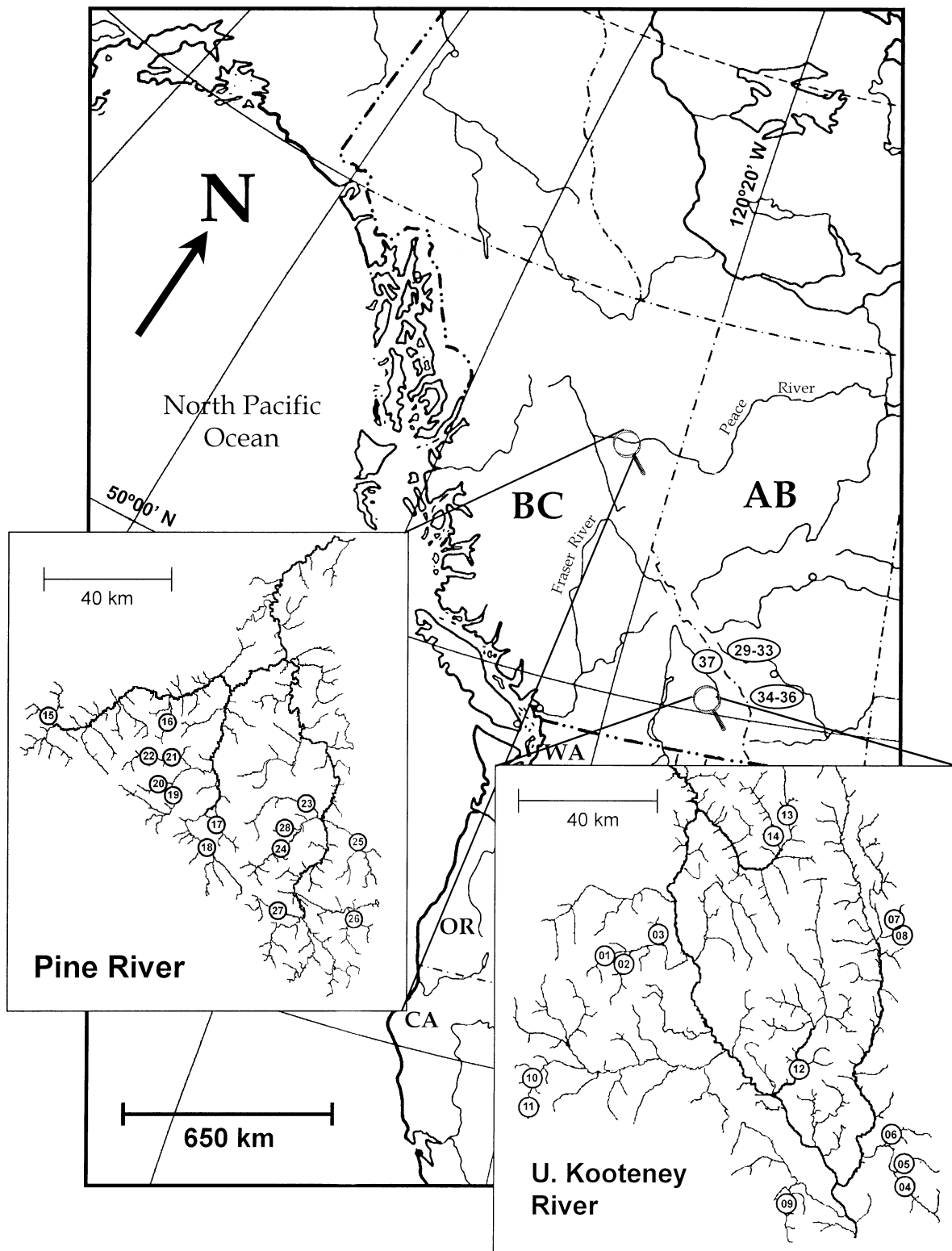


FIG. 1. Locations of sample sites in British Columbia and Alberta, Canada. Numbers refer to place names in Table 1.

TABLE 2. Microsatellite loci included in this study. The annealing temperature (T_A), number of alleles (N) and size range (in base pairs, bp) are given for each locus. The average expected heterozygosities (Avg. H_e) are average values from all 37 populations.

Locus	Source species	Reference	T_A (°C)	N	Range (bp)	Avg. H_e
<i>Omy77</i>	<i>Oncorhynchus mykiss</i>	Morris et al. (1996)	55	4	269–287	0.35
<i>Sfo18</i>	<i>Salvelinus fontinalis</i>	Angers et al. (1995)	61	2	150–156	0.49
<i>Ssa197</i>	<i>Salmo salar</i>	O'Reilly et al. (1996)	61	2	119–123	0.47
<i>Sco19</i>	<i>Salvelinus confluentus</i>	E. Taylor, unpubl. data	50	17	158–216	0.46
<i>Sco23</i>	<i>Salvelinus confluentus</i>	E. Taylor, unpubl. data	61	3	185–193	0.34
<i>Ssa456</i>	<i>Salmo salar</i>	Slettan et al. 1995	55	2	157–159	0.21
<i>Ssa311</i>	<i>Salmo salar</i>	Slettan et al. 1995	55	2	112–120	0.25

frequencies are available at <http://www.zoology.ubc.ca/~etaylor/btdata/>.

Genetic Data Analysis

The following tests were performed using GENEPOP ver. 3.1 (Raymond and Rousset 1995). 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. Tests for genotypic linkage disequilibrium for all combinations of locus pairs within a population were also made using a Markov chain method with GENEPOP default values. Tests for population differentiation between all possible pairs of populations was performed for each locus and over all loci combined using log-likelihood (G)-based exact tests (Goudet et al. 1996) with default values.

Basic descriptive statistics (N , H_e , H_o , etc.) were compiled using TFPGA ver. 3.2 (Miller 1997). This program was also used to test for population differentiation between pairwise comparisons of populations, river systems, and regions using a contingency table and Markov chain approach with 10 batches of 1000 dememorizations and 2000 permutations per batch. We tested for differences in average numbers of alleles and heterozygosity among regions and between bull trout and published values for other salmonids using rank-based Mann-Whitney U -tests or Kruskal-Wallis tests (cf. DeWoody and Avise 2000).

F_{ST} -values were calculated in ARLEQUIN ver. 2.0 (Schneider et al. 1997) with significance based on a permutation process. Cavalli-Sforza and Edward's (1967) chord distances were generated in the GENDIST program of the PHYLIP ver. 3.5 (Felsenstein 1993). From these distance matrices, the neighbor-joining algorithm in the NEIGHBOR module was used to generate trees, while CONSENSE was used to generate a consensus tree with bootstrap values from 1000 replicate datasets created in SEQBOOT. Maximum-likelihood distance matrices and dendrograms were generated in CONTML.

To determine the partitioning of genetic variation at variously nested levels, the program ARLEQUIN ver. 2.0 (Schneider et al. 1997) 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 genetic differences within populations (V_{IP}), among populations within groups (V_{IG}), and by differences between groups (V_{BG}) was calculated under a variety of hypotheses. For example, we tested if the five watersheds sampled (upper Kootenay,

Pine, Bow, Oldman, and upper Columbia rivers; watershed) represented distinct biological groupings sufficient to explain the patterns of variation observed. We compared those localities in east-flowing drainages against those in west-flowing drainages (the Pine River and Alberta populations vs. the Kootenay and upper Columbia populations, flow). We also tested if the distribution of genetic variation was best explained by separation into above- and below-barrier populations, collectively (i.e., all above-barrier localities in one group, all below-barrier localities in another group; barrier 1) and individually, with each barrier forming a distinct group (i.e., all below-barrier populations from one locality in one group, all above-barrier populations from the same locality forming a separate group; barrier 2). We performed these calculations using only F_{ST} , because R_{ST} -estimates 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 both GENEPOP and TFPGA was used for both F_{ST} and Cavalli-Sforza and Edwards (1967) chord distance (CSE) measures independently for the Pine and upper Kootenay watersheds. Geographic distances between tributaries within the hydrographic network within each watershed were determined using the Geographic Information System (GIS) program ArcView (ver. 3.1, Environmental Systems Research Institute). To determine whether populations have yet reached a drift-migration equilibrium, we applied the approach of Hutchison and Templeton (1999). Subsequent to a significant Mantel test result between genetic and geographic distances, a second Mantel test was performed using residuals from the initial fitted line (calculated using MINITAB student edition, Rel. 9, Addison-Wesley Publishing Reading, MA) against geographic distance. At equilibrium, scatter (residuals) should increase with increased geographic separation as drift, rather than gene flow, becomes the dominant force at larger distances. We also employed the approach of Hutchison and Templeton (1999) to assess the degree to which the Pine and upper Kootenay watersheds differed in the degree of scatter for the F_{ST} -geographic distance relationship. We calculated the ratio of the variances in averaged F_{ST} -values for independently grouped arrays of pairwise population comparisons (Hutchison and Templeton 1999). Differences in the F_{ST} variance components between regions would suggest that the two regions are at different stages of drift-migration equilibrium, perhaps owing to differences in the timing of postglacial colonization or to differing population dynamics during colonization. Specifically, we predicted that the Pine River watershed would show a greater degree of scatter (have a

higher F_{ST} -geographic distance variance) owing to its more distant location and the less direct physical connection to populations in known refuge areas of the Columbia River basin, south of the ice sheets (McPhail and Lindsey 1986; Pielou 1991; see Discussion).

MINITAB was also used to perform Spearman rank correlations of allele number (A) and heterozygosity (H) against ranked sample site latitude and longitude to determine the presence and significance of any spatial trends in the genetic data outward from the Columbia refuge. We also compared watersheds in terms of mean expected heterozygosities and mean number of alleles using an analysis of variance. Values were included for bull trout populations examined by Spruell and Allendorf (1997) and Nerass and Spruell (2001) from northeastern Oregon and the Clark Fork River in northern Idaho and western Montana. Both of these areas are south of the regions we sampled and represent areas closer in proximity to glacial refugia in the mid-Columbia River valley (Pielou 1991; Behnke 1992).

Environmental Data Analysis

We gathered information on a variety of environmental factors at watersheds to site-specific scales to determine the extent to which aspects of the contemporary physical environment could be related to the patterns of microsatellite variation in the upper Kootenay River and Pine River watersheds. This included several measures of habitat size or quality: average wetted stream width (m), average pool depth (m), stream order (1–5), watershed area (km²), stream length (km), percentage of watershed area forested, presence (1) or absence (0) of a migration barrier in system, average annual stream temperature (°C), total road density within watershed (km roads per watershed area in km²), and the numbers of other fish species present (Appendix 2; supplementary data tables and appendices are available on the web at <http://www.zoology.ubc.ca/~etaylor/btdata/>). These variables are hypothesized to influence bull trout demography (principally through population size effects) in terms of natural processes operating over longer evolutionary time frames (e.g., greater watershed areas should support higher population densities) or through anthropogenic effects operating over more recent time scales (e.g., intensive logging and associated road building within watersheds may degrade habitat and reduce population size; Rieman and McIntyre 1995; Dunham and Rieman 1999).

To assess the relative importance of these factors, the program CANOCO (ter Braak 1988b) was used to perform a canonical correspondence analysis (CCA). CCA incorporates both ordination and multiple regression techniques for direct analysis of the relationships between tables of multivariate data. It is one of the most efficient tools for relating species composition to different predictive variables (ter Braak 1988a; Magnan et al. 1995) and has recently been applied to describing the relationships between environmental variables and genetic diversity (e.g., Angers et al. 1999). In CCA, the number of alleles, expected heterozygosity, and allele frequencies for seven microsatellite loci act as dependent variables and are related separately to two sets of independent variables: drainage pattern and the set of environmental var-

iables. The drainage pattern matrix (Appendix 3) represents the spatial organization of British Columbian populations in terms of their connectivity through the hydrographic network (cf. Magnan et al. 1995; Angers et al. 1999; Fig. 2). Nodes were located at branching points and were numbered so that each population can be coded by the pattern of nodes traversed from sample site to the root (the root being defined as the common branching point for all populations; Lake Koocanusa Reservoir and the Pine River mainstem, respectively). Impassible migration barriers exist in both regions separating several populations from others in the hydrographic network. These barriers were coded as though they were distinct nodes in the drainage network. The presence or absence of a barrier was also included as a nominal environmental variable (0 or 1) in the environmental matrix.

Both matrices were related independently to the genetic data and for each, the variables most able to account for the distribution of genetic variation were extracted using the forward selection procedure available in CANOCO. Variables were selected on the basis of the proportion of variance in dependent variables that they explained and on their statistical significance, both individually and in linear combination with other such variables based on 1000 Monte Carlo permutations (cut-off point of $P = 0.10$). Selection of a particular drainage node, for example, would suggest a genetic discontinuity between populations located upstream and downstream of that node. The forward selected variables were then used to construct regression models whose contribution to explaining the genetic data and statistical significance was determined from the sum of canonical eigenvalues and P -values estimated using a permutation process.

Because many environmental variables exhibit some type of spatial heterogeneity, the variation explained by environmental and spatial (drainage) models may be correlated and, therefore, partly redundant. To determine whether the selected environmental variables still explain a significant proportion of the variation once spatial trends are removed, we used the method of variation partitioning suggested by Bo-card et al. (1992). We calculated the pure component of variation explained by environmental variables after removing the effects of drainage pattern using partial CCA. Similarly, we calculated the pure drainage component by removing the effects of environmental variables, the variation shared between environmental and spatial variables, and the residual variance. In this way we could determine the relative and independent influence of spatial and environmental factors in structuring the variation in numbers of alleles, heterozygosity, and allele frequencies that we observed. The statistical significance of the pure components was assessed in CANOCO by permuting the sum of all eigenvalues ($N = 1000$) and applying Bonferroni corrections (initial $\alpha = 0.05/8$ in the upper Kootenay, $\alpha = 0.05/7$ in the Pine River).

RESULTS

Variability at Microsatellite Loci

The number of alleles per microsatellite locus was low, ranging from one to 10 in individual populations with an average of 1.9 alleles across all loci (Suppl. Table 1; supplementary tables and appendices are available on the web

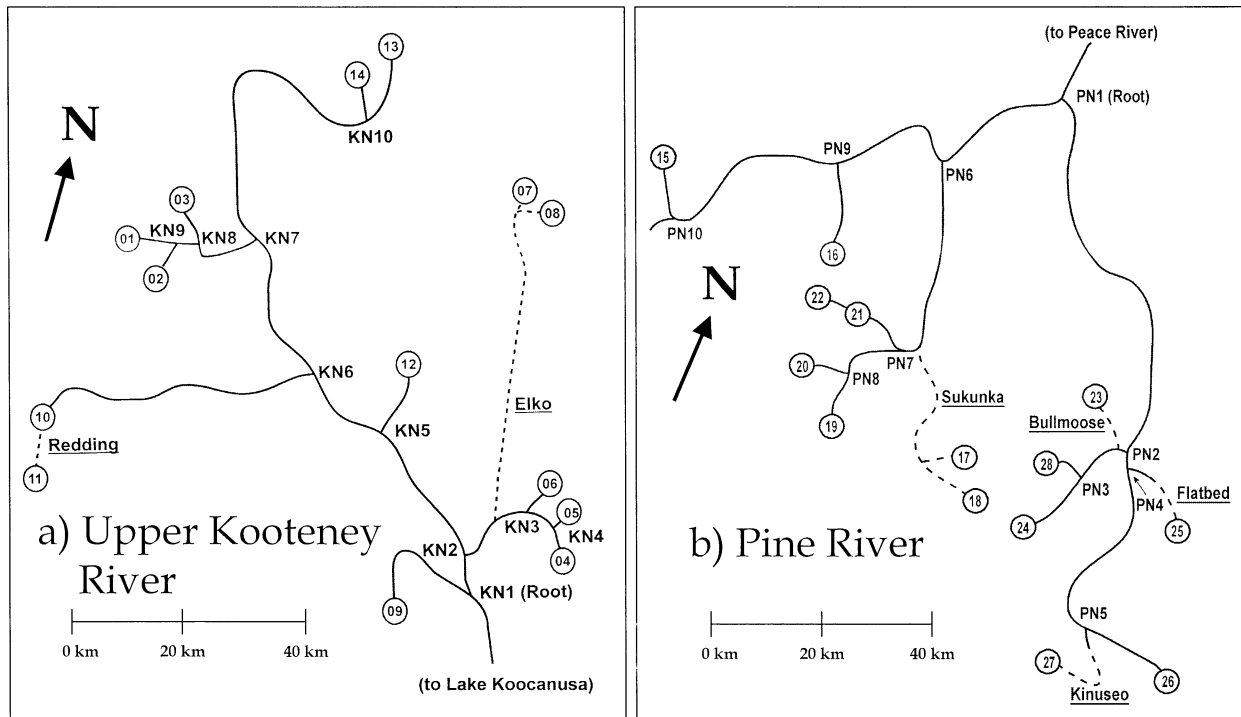


FIG. 2. Drainage matrices for the upper Kootenay and Pine River regions illustrating the spatial arrangement of sample locations in the hydrographic matrix. Population numbers correspond to those in Figure 1 and the names in Table 1. Nodes are numbered from (a) KN1–KN10 (Kootenay River) and (b) PN1–PN10 (Pine River), except for barrier nodes that are named and underlined. Above-barrier populations are connected to the remainder of the hydrographic network by dashed lines.

at <http://www.zoology.ubc.ca/~etaylor/btdata/>). Generally, most populations had a common allele and one other variant with the exception of *Sco19*. A total of 17 alleles were detected across all populations at this locus, with individual populations having as many as 10 alleles. The percentage of polymorphic loci for individual populations ranged from 0% to 100% while observed heterozygosities ranged from 0.00 to 0.78, averaging 0.24. This is significantly less than an average of 8.0 alleles and heterozygosity of 0.62 observed in other studies of freshwater salmonids using heterologous loci and similar sample sizes: Arctic char, *Salvelinus alpinus* (Brunner et al. 1998); kokanee, *Oncorhynchus nerka* (Taylor

et al. 2000); European whitefish, *Coregonus* spp. (Douglas et al. 1998); rainbow trout, *O. mykiss* (P. Tamke and E. B. Taylor, unpubl. data); and westslope cutthroat trout, *O. clarki lewisi* (C. Landry, pers. comm.), with $P < 0.005$ for both variables. Tests for conformity to Hardy-Weinberg equilibrium indicated no significant deficit of heterozygotes in 259 cases (7 loci \times 37 populations) when analyzing individual loci across populations with Bonferroni corrections ($\alpha = 0.05/37 = 0.0014$) and in only two cases (0.77%) across loci within each population ($\alpha = 0.05/7 = 0.007$). Tests for genotypic disequilibrium reject the null hypothesis of independence in four of 777 comparisons (0.51%, $\alpha = 0.05/37 = 0.0014$) but the significant results were not restricted to any single locus pair.

TABLE 3. Spearman rank correlation coefficients for comparisons of latitude/longitude, the number of alleles, and expected heterozygosities at the seven microsatellite loci in Pine and upper Kootenay River populations. Ranking and the calculation of correlation coefficients were performed in MINITAB. Significant values are bold and were judged relative to the critical value for significance in the one-tailed test with $\alpha = 0.05$ ($r_{crit} = 0.275$, $n = 37$).

Locus	Mean number of alleles		Heterozygosity	
	Latitude	Longitude	Latitude	Longitude
<i>Omy77</i>	0.020	0.250	-0.141	0.103
<i>Sfo18</i>	0.094	0.371	0.320	0.512
<i>Ssa197</i>	0.024	0.282	-0.126	0.001
<i>Sco19</i>	-0.235	0.043	-0.233	0.014
<i>Sco23</i>	0.300	0.574	0.407	0.652
<i>Ssa456</i>	- 0.383	- 0.276	- 0.360	- 0.266
<i>Ssa311</i>	- 0.629	- 0.412	- 0.623	- 0.396
Across all loci	-0.220	0.100	-0.117	0.202

Measures of within-population genetic diversity (A , H_e) differed among regions (Suppl. Table 1, maximum $P = 0.001$) with those populations from east of the continental divide (Red Deer and Bow rivers) showing the lowest levels of genetic variability. Spearman rank correlations between allele number (A), heterozygosity (H), and site coordinates (latitudes and longitudes) indicated trends of decreasing numbers of alleles and heterozygosity with increasing latitude, and increasing diversity with increasing longitude (i.e., east to west, Table 3). The correlations averaged across loci, however, were not significant (both $P > 0.05$) and correlations often showed reversals of sign at different loci (e.g., compare *Ssa456* and *Sco23* for number of alleles and latitude correlation, Table 3). When incorporating allelic variation from other regions to the south (Spruell and Allendorf 1997;

Spruell et al. 1999; Nerass and Spruell 2001), no simple relationship between latitude and intrapopulation genetic variability was apparent. Rather, populations nearest the periphery of the range of bull trout to the south (mid-Columbia River), east (Red Deer, Bow, and Oldman Rivers), and the north (Pine River) tended to have lower diversity than populations nearer the central portions of the species' range (upper Kootenay River, Clark Fork River; Fig. 3). At the three loci that are in common between our study and those in more southern portions of the Columbia River (*Sfo18*, *Ssa311*, 456), the number of populations that had become fixed for the most common allele at each of the loci was significantly higher at the northern and eastern extremes of our sample coverage (Pine and Alberta populations, 65%) than in the upper Kootenay drainage (11%, $\chi^2 = 31.7$, $P < 0.001$).

Population Differentiation and Genetic Structure

Log-likelihood (G)-based exact tests of population differentiation (e.g., Goudet et al. 1996) suggested that all five regions included in our study are significantly differentiated in terms of allele frequencies, both simultaneously and in all pairwise combinations ($P < 0.0001$; Suppl. Table 2). Within regions, most populations were genetically differentiated, but there was some evidence for regional substructuring (Suppl. Table 3). Generally, populations isolated above the same migration barrier were undifferentiated from each other, but were distinct from downstream populations (e.g., Line and South Line Creeks in the upper Kootenay drainage; Chamberlain and Windfall Creeks in the Pine River drainage). In the upper Kootenay drainage, most populations closely associated with the Lake Kocanusa Reservoir (Wigwam River, Ram Creek, Bloom Creek) were not significantly differentiated from one another. Overall, population subdivision within regions was moderate with F_{ST} -estimates for the Pine River being slightly higher than in the upper Kootenay drainage (0.2426 and 0.2260, respectively; Suppl. Table 4)

A hierarchical analysis of the distribution of genetic diversity (AMOVA) revealed that the grouping of populations that explained the greatest amount of variation across the sampling area was the barrier 2 comparison; that is, populations below barriers form one collective group and populations above different barriers form separate groups. Over half of the total variation (56.4%) was found to reside within populations themselves; 22.7% of the total variation was due to differences between the putative barrier 2 groups and 20.9% due to differences existing within groups (Table 4). The large percentage of variation due to differences within groups suggests that further regional and subregional population structure exist. With nearly as much genetic variability observed within groups as between them, we performed similar hierarchical analyses for individual regions.

We tested whether the distribution of genetic variability within each region was best explained by grouping populations by major tributary drainage into above- and below-barrier groups (collectively and individually; barrier 1 and barrier 2), or by the a posteriori groups identified as distinct in TFGA tests for population differentiation. In both the Pine and upper Kootenay rivers, the groupings by major tributary or simply into above- and below-barrier categories (bar-

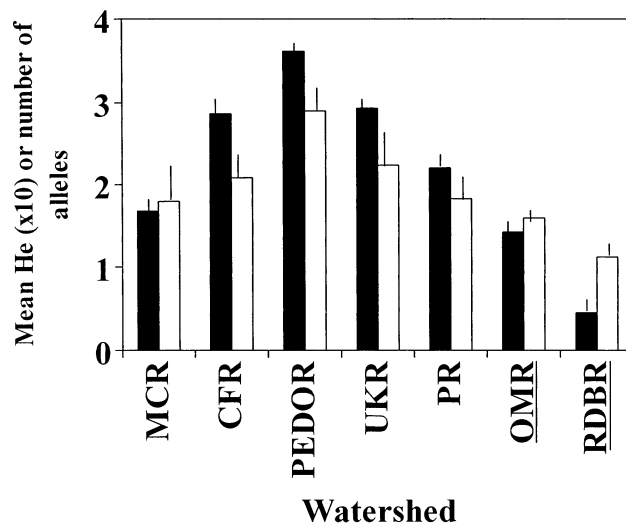


FIG. 3. Mean (+ SD) expected heterozygosity ($\times 10$, closed bars) or numbers of alleles (open bars) in bull trout from seven major watersheds in western North America. Values are based on allelic variation at seven microsatellite loci. MCR, middle Columbia River ($N = 34$ populations); CFR, Clark Fork River ($N = 10$ populations); PEDOR, Pend d'Oreille River ($N = 21$ populations); UKR, upper Kootenay River (current study, $N = 14$ populations); OMR, Oldman River (current study, $N = 3$ populations); RDBR, Red Deer/Bow Rivers (current study, $N = 5$ populations); PR, Pine River (current study, $N = 14$ populations). Values for MCR, CFR, and PEDOR are from Spruell and Allendorf (1997), Spruell et al. (1999), and Nerass and Spruell (2001). Underlined watersheds drain east of the continental divide.

rier 1) explained little of the observed genetic variation (-2.0% to 8.0% , Table 4). Again, grouping populations by individual barriers (barrier 2) explained the greatest amount of variation between groups ($\sim 23\%$). These results, therefore, suggest that both between and within the regions, individual barriers (i.e., those segregating habitats within individual streams) are important factors in organizing localized patterns of genetic diversity in bull trout.

Genetic Relationships among Populations

Tests for isolation by distance in the upper Kootenay River show a highly significant association with geographic distance for both F_{ST} ($r = 0.40$, $P = 0.0002$) and Cavalli-Sforza and Edward's genetic distance (CSE, $r = 0.47$, $P = 0.0002$; Fig. 4). The removal of above-barrier populations from these analyses, however, made the relationship with F_{ST} nonsignificant ($r = 0.22$, $P = 0.0672$) and reduced the significance for CSE ($r = 0.33$, $P = 0.0080$). In the Pine River region, no significant association was found to exist between either genetic distance metric and geographic distance until above-barrier resident populations were removed from the analysis; only the relationship with CSE was significant ($r = 0.33$, $P = 0.0343$; Fig. 4). Residuals (scatter) from standard linear regressions of pairwise F_{ST} and CSE values on geographic distance did not increase with increasing geographic distance in either the upper Kootenay or Pine River drainages (not shown, $P > 0.05$) as expected for populations at drift-migration equilibrium. As well, the degree of scatter in F_{ST} -geographic distance relationships was greater in the Pine Riv-

TABLE 4. Hierarchical analysis of the regional and subregional distribution of genetic diversity in bull trout populations included in this study under various hypotheses. Calculated using ARLEQUIN version 2.0; V_{BG} represents the percentage of variation existing between regions; V_{IG} is the amount existing among populations within regions; and V_{IP} is the percentage of variation existing within populations. All values are highly significant ($P < 0.0001$) except where italic.

Comparison	V_{BG}	V_{IG}	V_{IP}	F_{ST}
Pine River and upper Kootenay River (combined)				
Watersheds: Pine, Koot, UCol, Bow, Old	18.57	23.71	57.73	0.4228
Flow: east- vs. west-flowing drainages	19.68	25.12	55.21	0.4479
Barrier 1: above vs. below barriers (collectively)	10.88	31.29	57.83	0.4217
Barrier 2: above vs. below individual barriers	22.71	20.85	56.44	0.4355
Pine River (only)				
Trib: by major tributary	<i>-1.87</i>	15.69	86.17	0.1383
Barrier 1: above vs. below barriers (collectively)	5.23	20.90	73.87	0.2613
Barrier 2: above vs. below different barriers	23.35	8.13	68.52	0.3148
TFPGA: a posteriori differentiated groups	14.17	0.78	85.05	0.1495
Upper Kootenay River (only)				
Trib: by major tributary	<i>0.92</i>	7.87	91.21	0.8786
Barrier 1: above vs. below barriers (collectively)	8.45	18.16	73.38	0.2662
Barrier 2: above vs. below different barriers	24.68	9.17	66.15	0.3385
TFPGA: a posteriori differentiated	23.15	0.64	76.21	0.2378

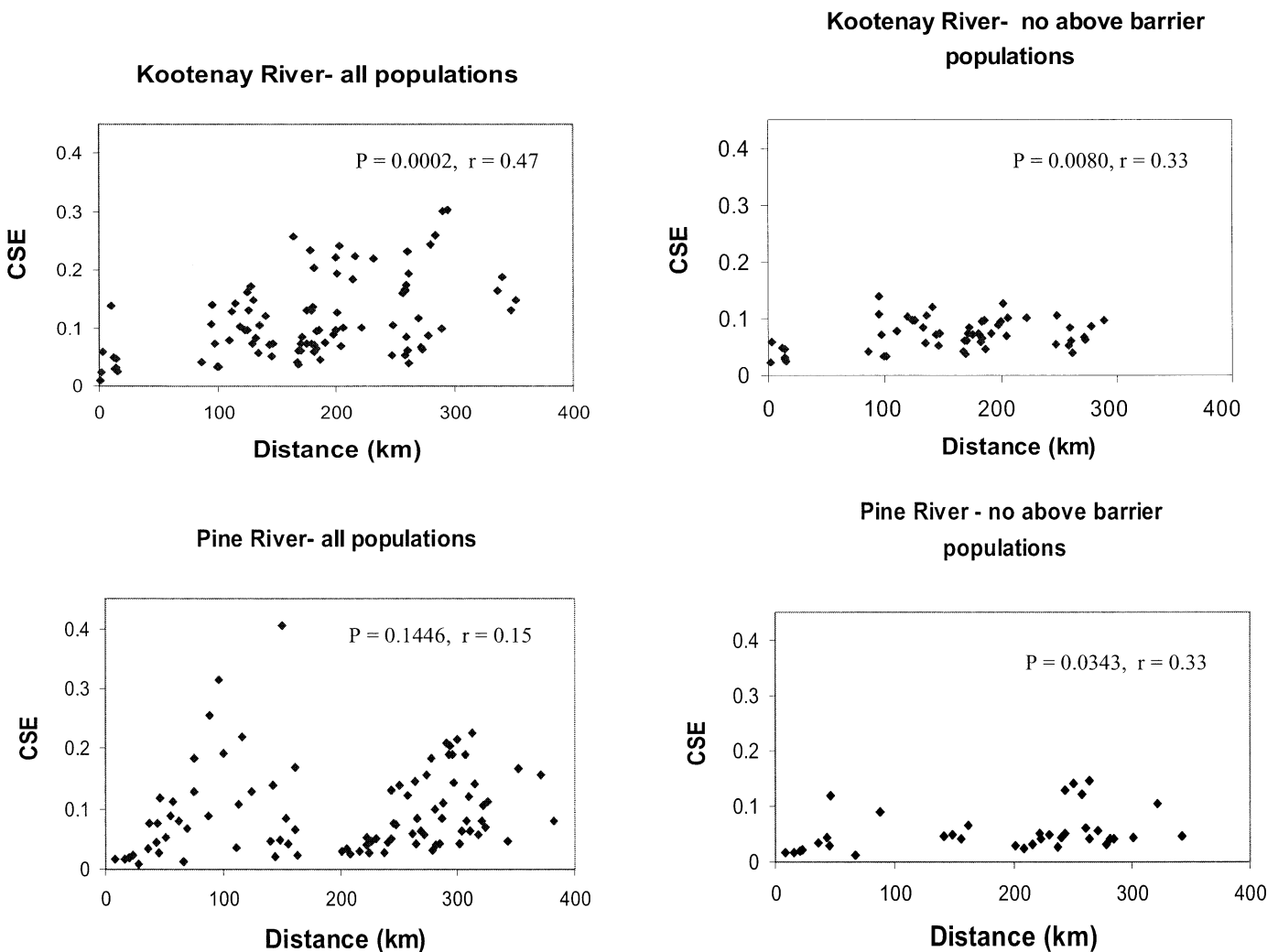


FIG. 4. Isolation-by-distance analyses for the upper Kootenay River (top) and Pine River (bottom). Pairwise Cavalli-Sforza and Edwards (1967) distances (y-axis) are plotted against pairwise geographic distances (x-axis) for all populations within each watershed (left panels) and for comparisons with populations above migration barrier excluded (right panels).

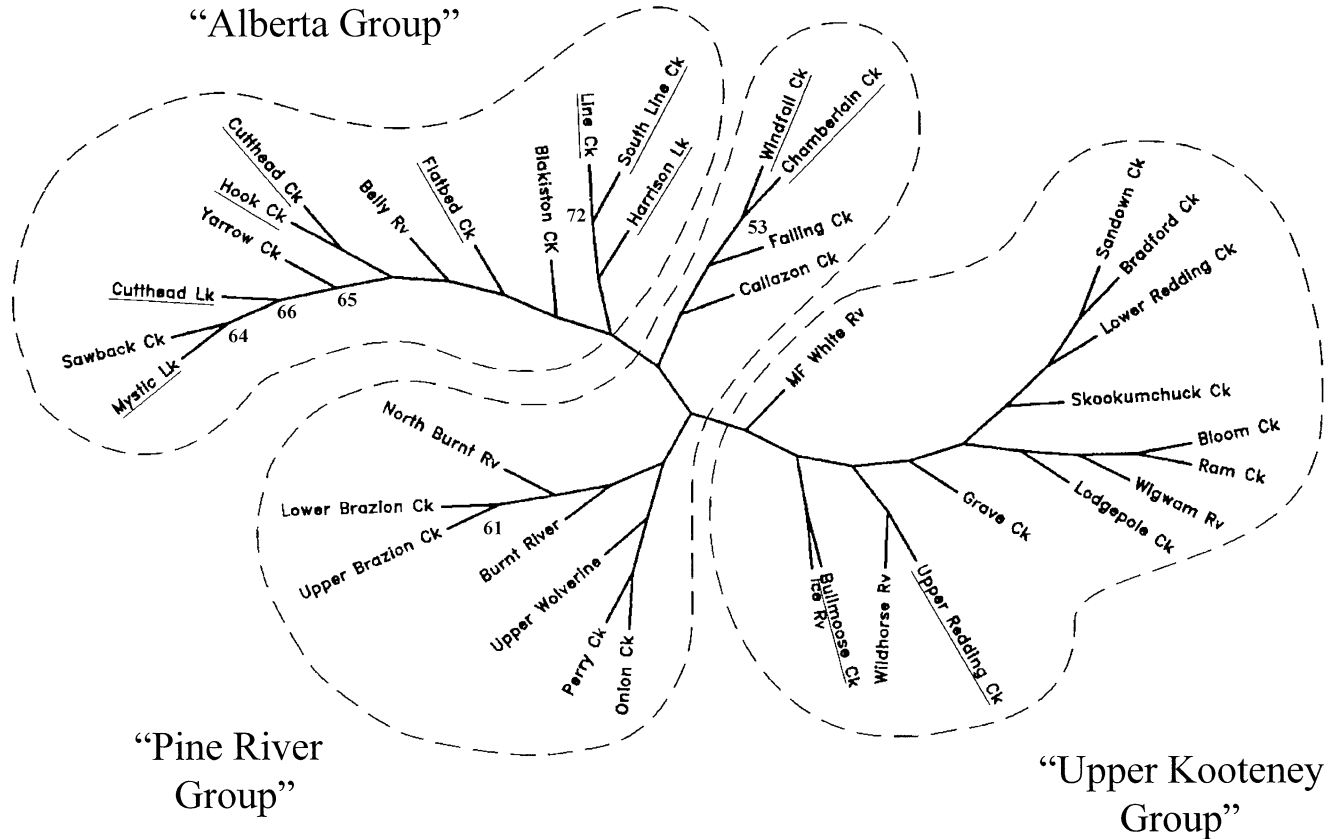


FIG. 5. Neighbor-joining tree of relationships among populations of bull trout from British Columbia and Alberta. Clustering was based on Cavalli-Sforza and Edwards' (1967) chord distances (CSE) derived from allelic variation at seven microsatellite loci. Populations above migration barriers are underlined. Numbers at branch points represent bootstrap percentages from 1000 replicates (only those values $\geq 50\%$ are shown).

er region ($V_p = 0.0340$) than in the upper Kootenay River region ($V_k = 0.0095$). The ratio of the variance components between regions ($V_k/V_p = 0.2794$) suggests that the Pine River region may be further out of drift-migration equilibrium than the upper Kootenay watershed.

We chose to examine genetic divergence between populations using Cavalli-Sforza and Edward's distance (CSE) because it is purely drift based and should outperform measures such as Nei's D (which are based on mutational processes) in recently diverged populations (Goldstein et al. 1995; Paetkau et al. 1997). In the upper Kootenay River, CSE values ranged from 0.0093 to 0.3005 (average = 0.1152). In the Pine River, there was a greater range of values (0.0081–0.4871), although populations were on the whole less differentiated than in the upper Kootenay River (average = 0.0959). In Alberta, values ranged from near identity (0.0002) to 0.4618 (Suppl. Table 5). Again, barriers appear to be important in structuring variation. In all regions, the smallest genetic distances existed between populations isolated above a common migration barrier, whereas the greatest distances existed between populations isolated above different barriers.

A majority consensus unrooted neighbor-joining tree based on CSE tended to group populations by physiogeographic region, although bootstrap support was generally low (Fig. 5). Consistent with the AMOVA results, populations found above migration barriers tended to cluster within distinct re-

gional groups. In some cases, however, above-barrier populations from different regions clustered together rather than with nearby below-barrier populations; for example Line, South Line Creeks (upper Kootenay River) with Harrison Lake (Red Deer River); Hook Creek (Pine River) with Cutthead Creek (Bow River; Fig. 5). The maximum-likelihood tree (not shown), also closely grouped rivers by physiogeographic regions.

Environmental Analysis

The canonical correspondence analyses revealed that spatial connectivity appears to have a stronger influence on the distribution of genetic variation in bull trout than do the selected environmental variables we studied. In the upper Kootenay River, nine of the 12 drainage nodes were forward selected as being significant predictors of genetic diversity, explaining 32.5–95.0% of the total variation ($0.001 < P < 0.025$; Suppl. Table 6). Node KN5 and the Elko and Redding barrier nodes were each selected in four separate instances (i.e., as significantly explaining four separate dependent genetic variables). The importance of KN5 is in agreement with tests for population differentiation that suggested a genetic discontinuity between the Koocanusa Reservoir group and other tributary populations of the upper Kootenay River mainstem (see also Fig. 5). In the Pine River region, drainage

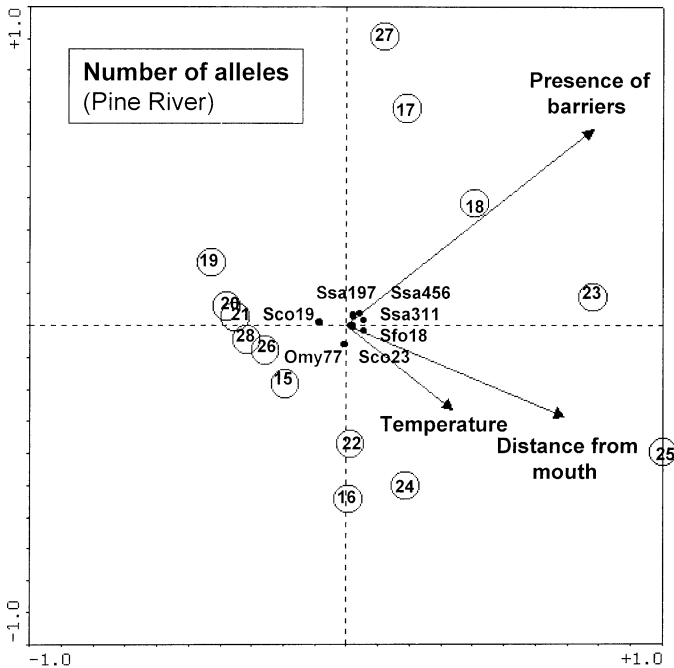


FIG. 6. Canonical correspondence ordination triplot of populations (open circles; numbering as in Table 1), loci (closed circles), and selected environmental variables (arrows) for the number of alleles per population within the Pine River. The length of the arrows, drawn from the centroid of population dispersion, represents the strength of the correlation between population variation and the ordination axes (ter Braak 1995). Populations are mainly discriminated along the first axis (i.e., by the presence/absence of barriers). Loci appear tightly clustered at the center of the diagram, reflecting the low levels of variation at individual loci.

pattern explained more of the genetic variation (31.0–100.0%; $0.001 < P < 0.078$) with nine of the 14 nodes being significantly associated with heterozygosity levels or micro-satellite allele frequencies (Suppl. Table 7). Barrier nodes were forward selected at seven of the nine genetic measures (Bullmoose on five occasions, Sukunka and Kinuseo on three, and so on).

Of the 20 environmental variables, nine were selected as contributing significantly to an explanation of the genetic data in the upper Kootenay drainage (only four in the Pine region), but these variables explained less of the variation in genetic data than did the spatial components (20.3–92.5% and 21.8–84.9%, respectively; Suppl. Tables 8, 9). In the upper Kootenay River the presence of barriers and stream order were each selected on three occasions, whereas in the Pine River the presence of barriers was selected on four separate instances as significantly predicting the partitioning of genetic variation (e.g., Fig. 6).

Combined analysis of environmental and spatial components indicated that the spatial component was the more powerful descriptor of genetic variation (Table 5). On average, pure environmental components explained only 7–9% of the observed genetic variation in both the upper Kootenay and Pine River drainages (and none significantly so). In contrast, pure drainage components explained an average of 29.2% of the observed genetic variation in the upper Kootenay River and 45.2% in the Pine River, most with statistical significance. In the Pine River, purely spatial (i.e., drainage) components account for nearly all of the observed variation at *Ssa197* (90.9%) and *Ssa456* (86.4%) as well as 60.6% of the variation in heterozygosity (51.9% in the upper Kootenay River). Often, it is the barrier nodes that are driving these relationships, which is in agreement with our AMOVA results that demonstrated significant genetic discontinuities between populations located upstream and downstream of barriers.

TABLE 5. Partitioning (%) of the total variation into pure environmental, pure spatial (drainage pattern), shared, and unexplained components for the upper Kootenay and Pine River drainages. Values in parentheses after the pure environmental and drainage components refer to *P*-values based on 1000 permutations of the sum of all eigenvalues. Significant values after the application of Bonferroni corrections ($\alpha = 0.05/8$ in the upper Kootenay River, 0.05/7 in the Pine River) are italic. The average value reported is across all nine measures.

Measure	Pure drainage	Pure environmental	Shared	Unexplained
Upper Kootenay River				
Alleles	36.2 (0.0010)	14.9 (0.0320)	29.8	19.1
Heterozygosity	51.9 (0.0020)	4.2 (0.1668)	26.4	17.5
<i>Omy77</i>	6.3 (0.0080)	2.3 (0.0440)	53.4	37.9
<i>Sfo18</i>	39.3 (0.0010)	0.8 (0.6114)	55.7	4.2
<i>Ssa197</i>	30.6 (0.0010)	2.5 (0.2617)	59.3	7.7
<i>Sco19</i>	19.5 (0.0999)	25.9 (0.0330)	12.9	41.7
<i>Sco23</i>	7.5 (0.1489)	7.5 (0.3407)	53.2	31.9
<i>Ssa456</i>	na	na	na	na
<i>Ssa311</i>	42.0 (0.0220)	0.6 (0.6494)	20.4	36.9
Average	29.2	7.3	38.9	24.6
Pine River				
Alleles	2.5 (0.0050)	11.8 (0.1250)	66.1	19.6
Heterozygosity	60.6 (0.0010)	1.2 (0.1050)	36.0	2.2
<i>Omy77</i>	0.5 (0.8801)	23.7 (0.0130)	61.2	14.6
<i>Sfo18</i>	19.0 (0.0240)	23.8 (0.0819)	25.0	32.1
<i>Ssa197</i>	90.9 (0.0010)	0.4 (0.5455)	3.5	5.1
<i>Sco19</i>	na	na	na	na
<i>Sco23</i>	56.5 (0.0030)	1.0 (0.4585)	28.1	14.5
<i>Ssa456</i>	86.4 (0.0010)	0.0 (0.7592)	13.6	0.0
<i>Ssa311</i>	na	na	na	na
Average	45.2	8.8	33.4	12.6

DISCUSSION

Historical Impacts on Microsatellite Variation

Several studies of molecular variation have demonstrated the profound influence of postglacial colonization on standing genetic variability in contemporary populations (e.g., Green et al. 1996; Merilä et al. 1996; Bernatchez and Wilson 1998; Taylor and McPhail 1999, 2000). The result is typically one of reduced levels of variation, attributable in part to bottlenecks and founder events associated with serial dispersal from source areas (Nei et al. 1975; Sage and Woolf 1986). Patterns of recolonization further influence extant levels of interpopulation differentiation both directly, via connectivity and gene flow, and indirectly, via influences on intrapopulation variability (McCauley 1993). Such historical patterns are often further modified by the ecological response of individual species (and organisms) to local contemporary environments to produce existing patterns of genetic variation and differentiation (e.g., Lugon-Moulin et al. 1999). Although few empirical studies have previously attempted to determine the relative influence of these historical and contemporary forces, our study of microsatellite DNA variation illustrates how these factors may act together to influence the evolution of genetic population structure in postglacial populations of a widespread freshwater fish species.

The majority of British Columbia's southern interior fish fauna is derived from ancestral lineages that dispersed from the upper Columbia River in the wake of receding glaciers (McPhail and Lindsey 1986; Behnke 1992). Fish recolonizing British Columbia at that time would have faced harsh and changing environmental conditions: cold, silt-laden water, fast and erratic flow, and many barriers to movement (Pielou 1991; Rempel and Smith 1998). Through chance founding events, and perhaps selection, populations surviving on the periphery of the expansion front would probably be composed of small, isolated groups of related individuals with reduced genetic variability (i.e., fewer alleles and lower heterozygosities) than the source population, leading to low variability within newly founded populations. In an analysis of mtDNA variation throughout their range, Taylor et al. (1999) found that individual bull trout populations exhibited low levels of variation and were often monomorphic for a single haplotype. Allozyme variation in Montana, Idaho, and Oregon bull trout populations is also low (Leary et al. 1993; Kanda et al. 1997), and a recent microsatellite-based assay of bull trout in northern Idaho revealed only marginally higher heterozygosities (Spruell et al. 1999; Nerass and Spruell 2001). Postglacial bull trout populations in our study exhibited similarly low levels of genetic variation at microsatellite loci, yet were well differentiated over small spatial scales in terms of allele frequencies.

This combination of low within-population variation and high levels of interpopulation differentiation observed in bull trout may be consistent with what would be expected if recolonization followed a propagule-type dispersal model (cf. McCauley 1993). According to the propagule model, alleles are drawn from one (or a few) of a series of possible source populations during recolonization of vacant (recently deglaciated) habitats rather than as independent samples from all possible source populations (migrant pool model). This ef-

fectively enhances the loss of intrapopulation variation but increases interpopulation differentiation, particularly if recolonization occurs in a stepping-stone manner (McCauley 1993), as is likely in stream fish populations. This is especially true when demographic characteristics tend to further reduce effective population size. Bull trout are a long-lived species and typically do not reach sexual maturity until five years of age (McPhail and Baxter 1996). As a top aquatic predator, they have relatively small population sizes (i.e., tens to a few hundred breeders in the largest systems; e.g., Hagen and Taylor 2001), making the effects of founder events and bottlenecks especially pronounced (Leary et al. 1993; Avise 1994; Swanberg 1997).

There were few instances of alleles being unique to particular bull trout populations. Instead, populations were typically differentiated in terms of the frequency of alleles common to the whole area, which suggests founding from a small number of source populations. The paucity of private alleles in bull trout also suggests, at least in this species, that *de novo* mutation has not been an important force in structuring the levels of genetic variability within and among populations that were founded postglacially. Rather, our data suggest that founder effects associated with postglacial recolonization of northwestern North America are primarily responsible for the levels of extant variation in bull trout. A historical signature of the effects of postglacial colonization was evident from our analyses as a significant decrease in heterozygosity and allelic diversity in populations on the periphery of the bull trout's range to the north, south, and east (e.g., Fig. 3).

As expected, average numbers of alleles and average heterozygosities tended to be lower in the Pine River relative to the upper Kootenay River (Suppl. Table 1). Pine River populations showed losses of alleles that were common in more southern localities (i.e., upper Kootenay and Columbia River drainages) and a less developed pattern of isolation by distance with higher variance components that we interpret as reflecting its more recent colonization from refugia. Given its more northerly location, the Pine River would have been colonized later in deglaciation than the upper Kootenay River (McPhail and Lindsey 1986). Furthermore, it was recolonized more indirectly (compared to the upper Kootenay River) via headwater exchanges; first between the Columbia and Fraser Rivers and then between the upper Fraser and upper Peace Rivers (McPhail and Lindsey 1986). Alternatively, differences in the regional patterns of isolation by distance may reflect differences in the degree to which current dispersal is limited within each region. Five of the 14 populations in the Pine River were sampled above impassable migration barriers, whereas only three above-barrier populations were sampled in the upper Kootenay River. However, our results are consistent with relationships documented for a terrestrial vertebrate, the eastern collared lizard (*Crotaphytus collaris collaris*). In this species, populations from habitats in Texas that have been occupied more continuously in the past had stronger patterns of isolation by distance and less scatter in these relationships than more northerly areas in Kansas and the Ozark Mountains that were colonized more recently (post-Wisconsinan; Hutchison and Templeton 1999).

Influence of Contemporary Factors

Although founder events and serial postglacial dispersal likely played large roles in determining the broad-scale general patterns of genetic diversity in bull trout, our results suggest that contemporary factors can strongly modulate historical patterns. For instance, our hierarchical analysis of genetic variation points to the importance of migration barriers in structuring genetic variation within and between watersheds in bull trout. In all regions, grouping populations isolated above different barriers against those without barriers to movement explained more of the differences between populations than any other type of grouping. In addition, although populations isolated above common migration barriers were typically undifferentiated, the greatest pairwise genetic distances within regions always occurred between populations isolated above different migration barriers, and these distances were often greater than the average genetic distances between regions (Suppl. Table 5). The importance of barriers to the structuring of genetic variation in bull trout was further supported by our interpretation of the canonical correspondence analysis. In both spatial and environmental matrices, barriers were selected more often than any other variable as significant predictors of the structuring of genetic diversity in the regions. Migration barriers, therefore, appear to be an important factor influencing patterns of genetic variability among populations both over a large geographic area as well as within single drainages (e.g., Avise and Felley 1979; Currens et al. 1990; Preziosi and Fairburn 1992) and may indicate how spatial heterogeneity, through its influence on dispersal, can disrupt or modulate expected patterns of drift-migration equilibrium (Britten et al. 1995; Hutchinson and Templeton 1999; Gerlach and Musolf 2000).

In contrast to the apparently strong influence of barriers, less than half of the 20 environmental variables were selected as contributing significantly to the explanation of genetic diversity; altogether, pure environmental components were able to explain only minor amounts of the observed genetic variation in regions (7–9%, none significantly). The apparent lack of stronger interactions may be attributed to stochastic factors related to sampling efforts or to simply not having included the appropriate variables. For instance, Angers et al. (1999) and Castric et al. (2001) found that differences in the altitude between different localities were able to explain significant amounts of variation in genetic diversity within and among populations of brook trout (*Salvelinus fontinalis*) from northeastern North America. Altitude (which is thought to reflect the difficulty of recolonization or reduction in founding population size via its influence on stream gradient) was not included in our analyses because of the difficulty in assigning a single altitude measure to samples collected from streams relative to lakes. Our few lake samples, however, suggest that altitude is likely to be an important factor in structuring variation in bull trout. The lake populations that we sampled from the Red Deer and Bow River drainages were all found at high altitude (> 2000 m) and showed some of the lowest genetic diversities we observed (Fig. 3, Suppl. Table 3). As well, the watershed area and habitat complexity existing above barriers may be an important factor for populations isolated above them. Larger, more complex habitats

will likely support larger population sizes and buffer the stochastic loss or fixation of alleles. Although most above-barrier populations did show reduced variability and were often monomorphic at several loci, populations isolated above barriers but in larger watersheds (e.g., Chamberlain and Windfall Creeks above the Sukunka Falls in the Pine River drainage) did retain higher levels of genetic variation.

Alternatively, the generally low levels of genetic variability in bull trout (or the degree of variation in environmental characteristics at sample sites) may not have been sufficiently great to reveal an interaction. It may be that the various habitat and environmental factors have a more direct and immediate relationship with site occupancy and population demographics than with genetic parameters. Attempts to use such indices of habitat quality and/or complexity as predictors of standing genetic variation in wild populations do not generally appear able to explain the partitioning of diversity (this study; Jorde and Ryman 1996; Angers et al. 1999; Castric et al. 2001) and may reflect our incomplete understanding of the complex nature of the relationship between habitat quality and population size (e.g., Hanski and Ovaskainen 2000; Smith and Hellmann 2002).

Ultimately, it is largely the response of individual species to dispersal challenges (or opportunities) that determines the distribution of genetic variation in natural populations (cf. McCauley 1993; Hewitt 1996), both those occurring historically and those of contemporary origin. For example, intrapopulation diversity (A, H) in bull trout appears to be largely a product of historical factors accumulating over time (repeated founder effect associated with postglacial recolonization). In contrast, interpopulation diversity, while partially influenced by the diversity existing in ancestral populations and the particular mode of recolonization, appears to be most influenced by the degree of spatial connectivity between sites and by those contemporary factors affecting dispersal and gene flow. Barriers to movement, be they partial (e.g., forest cover; Keyghobadi et al. 1999) or nearly complete (e.g., waterfalls, dams; this study; Currens et al. 1990; Keyghobadi et al. 1999 and references therein), appear to promote genetic divergence by contributing to habitat heterogeneity (e.g., Rainey et al. 2000). More generally, our evidence for the importance of local landscape structure on molecular variation suggests how environmental structure (via its effect on dispersal) can compromise the application of certain models of population structure. Established models of gene flow, such as the isolation-by-distance model, may be too simplistic for hierarchically structured or dendritic habitats such as those inhabited by many freshwater faunas. For such systems, more inclusive measures of geographic isolation and landscape permeability (e.g., Kudoh and Whigham 1997; Lugon-Moulin et al. 1999) across appropriate spatial scales (Fausch et al. 2002) will be needed to better understand patterns of geographic structure in the wild.

Conservation Implications of Evolutionary Inferences

Our study has investigated the role of historical factors and contemporary watershed characteristics as determinants of the evolution of microsatellite diversity within and between natural populations of a native salmonid fish. Given that the

bull trout is listed as threatened in the United States (U.S. Department of Interior *Federal Register*, July 1998) and is a blue-listed species of special conservation concern in British Columbia, the heart of its range, it is important to consider possible conservation implications of our analyses. First, our data extend previous analyses that have indicated that bull trout tend to show high levels of population subdivision in localized areas (cf. Leary et al. 1993; Spruell et al. 1999). Such high levels of subdivision indicate high levels of demographic independence and suggest that human-induced reductions in population size will not likely be offset by immigration from nearby populations, at least over the short term of a few generations or less. Second, as drift tends to predominantly fix those alleles most common in ancestral populations, the tendency for locally rare alleles to be fixed in several above-barrier populations raises the possibility that the area was recolonized by previously differentiated groups of bull trout. This could involve recolonization from distinct subrefugia (cf. Angers and Bernatchez 1998) or by some type of double invasion by successive waves of fish at different meltwater recessional stages (cf. Latham 2001). This type of heretofore undescribed biodiversity may be of significant conservation interest in the area and represents an important area for further study.

The apparently low levels of intrapopulation molecular variation in bull trout (this study; Leary et al. 1993; Taylor et al. 1999; Spruell et al. 1999), however, do not necessarily translate into low variability at fitness-related traits (Lynch 1996; Pfrender et al. 2000; Reed and Frankham 2001). In fact, low levels of molecular variation stemming from post-glacial range expansion have been associated with increased levels of quantitative genetic variation (e.g., Armbruster et al. 1998). If however, low molecular variation does reflect characteristically low effective population sizes in bull trout, then this may indicate high susceptibility to local extinctions from: (1) increased probability of stochastic demographic or genetic factors playing a role in population persistence; (2) increased rate of loss of genetic variability; or (3) reduced efficiency of selection (and an increased role of drift) in populations that must cope with environmental change.

Third, Pfrender et al. (2000) demonstrated a broad concordance between measures of microsatellite-based (F_{ST}) and quantitative genetic-based (Q_{ST}) measures of subdivision in two species of *Daphnia* (see also Lynch et al. 1999) and suggested that molecular markers may, in fact, provide conservative estimates of interpopulation divergence in quantitative traits (those related to morphology, life history, or behavior). If also true in bull trout, then our results suggest that considerable variation may exist among population in terms of quantitative traits, characteristics that are likely more directly involved in population persistence in current and future environments. This may be particularly relevant for populations isolated above migration barriers. Although barriers can reduce effective population size and leave populations vulnerable to stochastic factors, they also act to isolate populations from the homogenizing effects of gene flow and, in doing so, can become important reservoirs for locally rare and novel phenotypes (A. B. Costello and E. B. Taylor, unpubl. data). Downstream migration from such populations (where possible) may be important in maintaining hetero-

zygosity levels in recipient populations (e.g., Latham 2001) and may contribute significantly to the evolutionary potential of the species to adapt and evolve to changing environmental conditions (cf. Turpeinen et al. 2001). As such, one-way migration from above-barrier populations may represent an unappreciated aspect of models of metapopulation dynamics for species like the bull trout (e.g., Reiman and Dunham 2000).

ACKNOWLEDGMENTS

This research was funded by the British Columbia Habitat Conservation Trust Fund administered by the British Columbia Ministry of Fisheries, as well as NSERC and Parks Canada grants awarded to EBT. We thank the British Columbia Ministry of Water, Land, and Air Protection in Victoria for gathering spatial and environmental data from digital maps (British Columbia Watershed Atlas, unpubl. data). We also thank R. Watt (Parks Canada) for assistance in sample collection; R. Devlin (Canadian Department of Fisheries and Oceans) for assistance during the early stages of this project; and M. Whitlock, L. Bernatchez, and an anonymous reviewer for comments on an earlier version of this manuscript.

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Corresponding Editor: G. Wallis