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Conservation genetics of bull trout: Geographic distribution of variation at microsatellite loci

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Abstract

We describe the genetic population structure of 65 bull trout (*Salvelinus confluentus*) populations from the northwestern United States using four microsatellite loci. The distribution of genetic variation as measured by microsatellites is consistent with previous allozyme and mitochondrial DNA analysis. There is relatively little genetic variation within populations (H_S = 0.000 – 0.404, average H_S = 0.186, but substantial divergence between populations (F_{ST} = 0.659). In addition, those populations that had low genetic variation for allozymes also tended to have low genetic variation at microsatellite loci. Microsatellite analysis supports the existence of at least three major genetically differentiated groups of bull trout: (1) "Coastal" bull trout populations, (2) "Snake River" populations, which also include the John Day, Umatilla, and Walla Walla Rivers and, (3) populations from the upper Columbia River, primarily from the Clark Fork basin. Within the major assemblages, populations are further subdivided, primarily at the river basin level. Most of the genetic similarities we have detected probably reflect patterns of historic isolation and gene flow. However, in some cases, genetic drift and low levels of variation appear to have influenced the relationships inferred from these data. Finally, we suggest using a hierarchical approach to direct management actions in species such as bull trout for which most of the genetic variation exists among populations and local populations in close proximity typically are genetically distinct.

Introduction

An understanding of the distribution of genetic variation within and among geographically isolated populations is necessary for species conservation. Without an explicit definition of populations, management actions could be detrimental to some populations by inappropriately initiating gene flow between historically isolated and genetically dissimilar populations. Conversely, limited resources could be used inefficiently by managing populations separately when, in fact, they represent a group of populations that exchange genetic information or did so historically.

In addition, the U.S. Endangered Species Act (ESA) allows populations or groups of populations to be protected as "distinct population segments" (DPSs). The first criterion for defining a DPS is "discreteness of the population segment in relation to the remainder of the species to which it belongs" (USFWS 1996). Although genetic data are not the sole measure for discreteness, they are perhaps the most powerful method available to quantify population divergence. Similarly, these data are valuable to help legally define the second criterion for listing, significance. Significance is define in part as differing "markedly from other populations in of the species in its genetic characteristics" (USFWS 1996).

Bull trout (*Salvelinus confluentus*) have a historic range from northern California to southern Alaska. Bull trout display a wide variety of life histories, including resident forms that complete their life cycle in small streams, fluvial forms that migrate from spawning and juvenile rearing streams to the mainstem of large rivers to feed and mature, and adfluvial forms that migrate to and from lakes (Pratt 1992). These forms may all occur in a single population. Migratory forms are typically larger (>300 mm) than resident forms (Pratt 1992). Bull trout are iteroparous, but individuals do not necessarily spawn annually (Shepard et al. 1984). In the case of fluvial and adfluvial individuals, a lengthy migration often accompanies each reproductive event.

Bull trout populations have declined throughout much of their range in the continental United States (Hesseldenz 1985; Johnson 1987; Ratliff and Howell 1992; Buchanan et al. 1997; USFWS 1998, 1999). Bull trout require very cold water and complex stream habitats and are especially susceptible to human activities that alter their habitat such as logging, mining, and road building (Howell and Buchanan 1992; Rieman and McIntyre 1993). In addition, many drainages that contain bull trout have hydroelectric dams that have limited or eliminated migratory life history forms and restrict gene flow (Neraas and Spruell 2001).

The United States Fish and Wildlife Service (USFWS) has listed all bull trout populations in the United States as threatened (USFWS 1999). The USFWS designated five DPSs for bull trout: Klamath River populations (southwestern Oregon), Columbia River populations (Washington, Oregon, Idaho, and Montana), Jarbidge River populations (a Columbia River tributary in northern Nevada), populations in the Olympic Peninsula/Puget Sound region (northwestern Washington), and populations in the Saskatchewan drainage (northern Montana; USFWS 1998). These designations were based primarily on geographic isolation. While DPS designations have already been made, these units can be amended as additional data become available. Recovery goals are also dependent on these units. A better understanding of the genetic relationships among and within populations in the five DPSs could be beneficial in conservation efforts.

Previous genetic studies provided limited resolution of bull trout population genetic structure. Using ten polymorphic allozyme loci to examine 21 bull trout populations from the northwestern U.S., Leary et al. (1993) reported a fixed allozyme difference between Klamath Basin bull trout and Columbia Basin bull trout. Restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) in 17 populations suggested genetic divergence between populations across broad geographic distances but provided limited divergence at a finer scale (Williams et al. 1997). Taylor et al. (1999) analyzed 47 populations of bull trout, primarily in British Columbia and Alberta, using both RFLP and sequence analysis of mtDNA and demonstrated substantial divergence between what they termed "Coastal" and "Inland" bull trout. The boundary between the two types generally corresponds to the Cascade Mountain crest.

The objective of this paper is to use microsatellite analysis to determine the genetic relationships among bull trout populations in the United States. We use four polymorphic microsatellite markers to analyze bull trout from 65 sites and compare these results to previous allozyme and mtDNA data. We also discuss the effects that random genetic drift and reduced heterozygosity may have on inferring genetic relationships among populations. Finally, we suggest a hierarchical approach to direct management of species in which populations are highly differentiated.

Methods

Samples

Approximately 30 bull trout from each of 65 sampling locations (1847 individuals, Table 1 and Figure 1) were non-lethally captured, the fork length of each individual was recorded to estimate age, and a fin clip (approximately 1 × 0.5 cm) was removed and stored in 95% ethanol. Samples were collected from several reaches within each site and from several age classes to minimize sampling related individuals. Since most fish sampled were less than 300 mm, most samples could have included a potential mix of juvenile migratory forms and juvenile and adult resident forms. Some samples were from likely resident populations (e.g., Little Minam River (L4), Silver Creek (P2), unpublished data).

Samples were typically collected from areas in which brook trout (*Salvelinus fontinalis*) were absent. Brook trout and bull trout do not naturally occur sympatrically. Therefore, any hybridization would be recent and not a natural part of the bull trout's evolutionary history. In locations that were known to contain brook trout, fish were screened visually to

Table 1. Sample locations and sample sizes (*N*) of bull trout populations. Letters indicate drainages. Numbers indicate sample sites within each drainage

Location	*N*
COASTAL DRAINAGES	
A. Skagit River, WA	
1. Sauk River	25
B. Queets River, WA	
1. Queets River	20
C. Klamath River, OR	
1. South Fork Sprague River	15
2. Long Creek	29
COLUMBIA RIVER DRAINAGES	
D. Lewis River, WA	
1. Merwin Reservoir	24
2. Swift Reservoir	30
E. Willamette River, OR	
1. South Fork McKenzie River	21
2. Anderson Creek	30
F. Hood River, OR	
1. Clear Branch Creek	33
2. Compass Creek	19
G. Deschutes River, OR	
1. Warm Springs River	27
2. Shitike Creek	29
3. Whitewater River	30
4. Jefferson Creek	30
5. Jack Creek	31
H. John Day River, OR	
1. Upper John Day River	16
2. Indian Creek	16
3. Call Creek	32
4. Granite Boulder Creek	25
5. Big Creek	30
6. Clear Creek	25
7. South Fork Desolation Creek	17
8. Baldy Creek	30
9. South Fork Trail Creek	26
10. Clear Creek	30
I. Umatilla River, OR	
1. North Fork Umatilla River	33
J. Walla Walla River	
1. North Fork Touchet River, WA	32
2. Mill Creek, OR	30
3. South Fork Walla Walla River, OR	32
K. Clearwater River, ID	
1. North Fork Clearwater*	20
L. Grande Ronde River, OR	
1. South Fork Wenaha River	30
2. South Fork Butte Creek	26
3. Elk Creek	30
4. Little Minam River	31
5. Bear Creek	30
6. Lostine River	25
7. Hurricane Creek	30
8. North Fork Catherine Creek	26
9. Indian Creek	29
10. Limber Jim Creek	22
11. Clear Creek	31
M. Imnaha River, OR	
1. North Fork Imnaha	31
2. Lick Creek	30
N. Pine Creek, OR	
1. East Fork Pine Creek	30
2. Elk Creek	30
O. Indian Creek, ID	
1. Indian Creek	32
P. Powder River, OR	
1. North Fork Powder River	30
2. Silver Creek	30
Q. Malhuer River, OR	
1. Beaula Reservoir	15
2. Swamp Creek	31
3. Meadow Fork Big Creek	30
R. Boise River, ID	
1. 18 tributaries*	40
S. Jarbidge River, NV	
1. Dave Creek	13
2. West Fork Jarbidge River	24
T. Lake Pend Oreille, ID	
1. Trestle Creek	35
2. East Fork Lightning Creek	35
3. Gold Creek	35
U. Clark Fork River, MT	
1. East Fork Bull River	35
V. Flathead River, MT	
1. Elk Creek	65
2. Big Salmon Creek	55
3. Dolly Varden Creek	25
4. Whale Creek	29
W. Blackfoot River, MT	
1. Copper Creek	25
X. Kootenai River, MT	
1. O'Brien Creek	25
SASKATCHEWAN RIVER DRAINAGE	
Y. Milk River, BC	
1. Belly River	20

*Samples were obtained from various tributaries throughout the drainage. The Boise River samples were obtained from 18 tributaries to the Boise River above Arrowrock Dam. The North Fork Clearwater samples were obtained from eight tributaries.

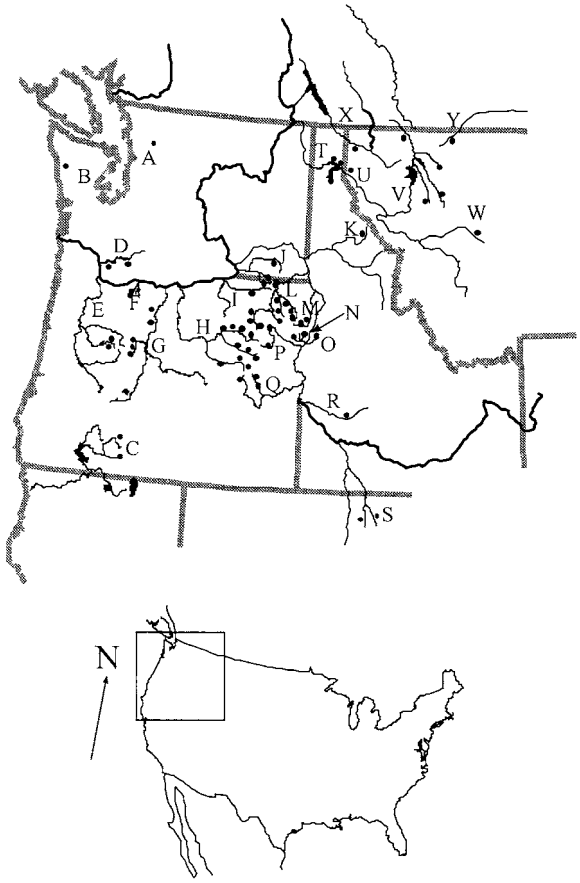

Figure 1. Drainages and approximate sample locations from which bull trout were sampled. Letters designate drainages and circles correspond to sampling locations described in Table 1.

eliminate apparent hybrids. The remaining individuals were tested using species-specific nuclear DNA fragments (Spruell et al. 2001) to identify and eliminate brook trout or hybrids from the analysis. No sample included more than three F1 hybrids. Most F1 hybrids of bull trout and brook trout appear to be sterile, making identification of hybrids and their removal from subsequent analyses fairly straightforward (Leary et al. 1993). Thus, it is unlikely that the estimated allele frequencies are biased by the presence of brook trout alleles.

Most samples represent juvenile individuals from a single tributary. Exceptions include the Boise River (R1) with 47 bull trout from 18 different tributaries, the North Fork of the Clearwater River (K1) with 20 individuals collected from eight tributaries, and Beulah Reservoir (Q1) in which 8 of the individuals were adfluvial migrants potentially of mixed origin from tributaries of the North Fork Malheur River. These samples were treated as collections from a single site for subsequent analyses.

Microsatellites

DNA was extracted using the Pure Gene® kit (Gentra) following the manufacturer's instructions. We amplified four microsatellite loci following the conditions described by the original authors (*ONEμ7*, Scribner et al. 1996; *SFO18*, Angers et al. 1995; *μSAT73*, Estoup et al. 1993; *FGT3*, Sakamoto et al. 1994). PCR was conducted in a MJ Research PTC-100 thermocycler with a total reaction volume of 10 μl.

Amplification products were size fractionated in 7% denaturing polyacrylamide gels and visualized using either autoradiography or fluorescent imagery. DNA fragments visualized using autoradiography were sized by comparison to the M13 control DNA in the Sequenase® DNA sequencing kit (United States Biochemical). Fluorescently labeled products were visualized using a Hitachi FMBIO100® fluorescent imager and were sized using Hitachi's FMBIO® analysis software (version 5.0) and either the PRISM® GeneScan 350 ladder (Applied Biosystems Incorporated) or MapMarker LOW (BioVentures). In addition, previously analyzed individuals were included on each gel to insure consistent scoring across gels and to be certain that the product sizes determined by the fluorescent imager were consistent with the lengths previously assigned by autoradiography.

Data analysis

Allele frequencies, exact probabilities for conformity with Hardy-Weinberg proportions, F-statistics, and expected heterozygosities were calculated using GENEPOP (version 3.1a; Raymond and Rousset 1995).

We chose two methods to display the relationships among populations. First, we used PHYLIP (Felsenstein 1992) to calculate Cavalli-Sforza and Edwards' (1967) chord distance (D_{CSE}) between all pairs of populations. We used the resulting pair-wise distance matrix to construct dendrograms using both the UPGMA and Neighbor-Joining option of PHYLIP. Both dendrograms were visualized and printed using TreeView PPC (Page 1996).

To project the relationships among populations without the limitations of a bifurcating tree, we used MINITAB (release version 11) to conduct principal components analysis (PCA). We computed the PC

scores based on the covariance among allele frequencies, omitting the largest allele at each locus to account for the non-independence of allele frequencies within each locus. We then plotted the first two principal component scores to estimate genetic divergence as the relative linear distance between points representing each population.

Results

Variation within population

All of the bull trout populations we examined contain relatively little genetic variation in comparison to other salmonid fishes (Table 2). Few alleles were detected at each locus, low mean heterozygosities were observed in all populations (Table 2), and no single population was polymorphic at all four loci. We found a maximum of ten alleles at locus *FGT3* (159–179 bp). Locus *μSAT73* had four alleles (138–148 bp) and both *ONEμ7* (218 and 244 bp), and *SFO18* (150 and 156 bp) had two. The two alleles observed at *SFO18* appear to be identical to the two alleles identified by Angers and Bernatchez (1996); however, there is a one base pair difference in the size assigned to each allele.

The range of mean heterozygosities (H_S = 0.000 – 0.404, mean = 0.180) among bull trout populations was quite large (Table 2). The expected heterozygosity is less than 0.010 in four populations (C1, C2, L3, and P1), including both populations from the Klamath Basin. Eight additional populations have H_S less than 0.100; whereas samples from nine sites exceed 0.300.

Genotypic frequencies generally conformed to expected Hardy-Weinberg proportions. The 13 significant deviations from the expected proportions ($P < 0.05$) were close to the 12 of the 240 comparisons expected to exceed $\propto = 0.05$ level by chance alone. No single population deviated from expectations at more than one locus. We also did not observe a particular locus causing the deviations as would be expected if null alleles were responsible for the departures from the expected Hardy-Weinberg proportions. After correcting for multiple tests (Lessios 1992), only the Boise River (R1) deviated significantly from expected Hardy-Weinberg proportions with a significant excess of homozygotes at *ONEμ7*. This distribution of genotypes is not unexpected from samples pooled from multiple populations.

Divergence among populations

Broad geographic subdivisions

Microsatellite analysis indicates three major genetic groups of bull trout (Figures 2 and 3) that generally correspond to the geographic regions from which samples were collected (Figure 1). Populations found along the Pacific Coast form one group. This group (A-G) includes tributaries to the Columbia River downstream of and including the Deschutes River. The samples from Puget Sound (A1) and the Olympic Peninsula (B1) also appear to belong to this group (Figure 2). The PCA (Figure 3) suggests that the sample from the Queets River (B1) may be an outlier in this group but it is more closely related to these populations than to those in other groups. Populations in the Klamath basin (C1 and C2) also belong to this group. We will refer to this population assemblage as "Coastal" following the terminology of Taylor et al. (1999).

Populations from tributaries to Lake Pend Oreille (T) and the Clark Fork River (U, V, and W) in Montana form a second discrete cluster we will call "Upper Columbia."

The third major group of populations supported by the microsatellite data are principally from tributaries in the Snake River Basin (K-S). However, three of the drainages that fall within this group (John Day, H; Umatilla, I; and Walla Walla, J) are tributaries of the Columbia River. This group includes samples (L5, R1, and S2) that were categorized as "Inland" by Taylor et al. (1999). However, substantial divergence in microsatellite allelic frequencies occurs between this group and the Upper Columbia populations that are also geographically inland. We will use the term "Snake River" to differentiate the former.

Variation within major groups

There are some notable differences in allele frequencies among Coastal populations, primarily between drainages. These differences are reflected in a F_{ST} of 0.635 among Coastal populations. Populations in the Willamette drainage (sites E1 and E2) are fixed for *FGT-3∗159*. This allele is present in six other sites (A1, H8, L1, L4, L9, and Y1) but exceeds a frequency of 15% only in the Sauk (A1) sample. Similarly, Merwin and Swift Reservoirs (D1 and D2) are fixed for *FGT-3∗165*. This allele is common in the Upper Columbia group but is not found in any other Coastal site.

Table 2. Allele frequencies and expected heterozygosity for bull trout from the northwest United States. Locations correspond to Table 1 and Figure 1

Location	*ONEμ7*		*BT73*				*SFO18*		FGT3										H_S
	∗218	∗244	∗138	∗140	∗144	∗148	∗150	∗156	∗157	∗159	∗163	∗165	∗167	∗169	∗171	∗173	∗175	∗179	
Coastal																			
A1	1.000	—	—	—	1.000	—	—	1.000	0.604	0.396	—	—	—	—	—	—	—	—	0.119
B1	1.000	—	—	0.765	0.235	—	—	1.000	1.000	—	—	—	—	—	—	—	—	—	0.091
C1	1.000	—	—	—	1.000	—	—	1.000	1.000	—	—	—	—	—	—	—	—	—	0.000
C2	1.000	—	—	—	0.983	0.017	—	1.000	1.000	—	—	—	—	—	—	—	—	—	0.009
D1	0.220	0.780	—	—	0.590	0.410	—	1.000	—	—	—	1.000	—	—	—	—	—	—	0.211
D2	0.589	0.411	—	—	0.426	0.574	—	1.000	—	—	—	1.000	—	—	—	—	—	—	0.249
E1	1.000	—	—	—	0.711	0.289	—	1.000	—	1.000	—	—	—	—	—	—	—	—	0.106
E2	0.870	0.130	—	—	0.450	0.550	—	1.000	—	1.000	—	—	—	—	—	—	—	—	0.183
F1	0.224	0.776	—	0.030	0.970	—	0.367	0.633	1.000	—	—	—	—	—	—	—	—	—	0.238
F2	0.167	0.833	—	—	1.000	—	0.132	0.868	1.000	—	—	—	—	—	—	—	—	—	0.130
G1	0.558	0.442	—	—	—	1.000	—	1.000	0.426	—	—	—	—	—	0.556	0.019	—	—	0.256
G2	0.911	0.089	—	—	0.086	0.914	—	1.000	1.000	—	—	—	—	—	—	—	—	—	0.082
G3	0.800	0.200	—	—	0.950	0.050	—	1.000	1.000	—	—	—	—	—	—	—	—	—	0.106
G4	0.767	0.233	—	0.100	0.683	0.217	—	1.000	1.000	—	—	—	—	—	—	—	—	—	0.207
G5	0.810	0.190	—	—	0.850	0.150	0.033	0.967	1.000	—	—	—	—	—	—	—	—	—	0.158
Snake River																			
H1	1.000	—	—	1.000	—	—	1.000	—	—	—	0.781	—	—	—	0.063	0.156	—	—	0.091
H2	1.000	—	—	1.000	—	—	1.000	—	—	—	0.563	—	—	—	—	0.344	0.094	—	0.144
H3	1.000	—	—	1.000	—	—	1.000	—	—	—	0.867	—	—	—	—	0.083	0.050	—	0.056
H4	1.000	—	—	1.000	—	—	1.000	—	—	—	0.560	—	—	—	0.020	0.200	0.220	—	0.152
H5	1.000	—	—	1.000	—	—	1.000	—	—	—	0.276	—	—	—	0.724	—	—	—	0.100
H6	1.000	—	—	0.580	0.420	—	1.000	—	—	—	—	—	—	—	1.000	—	—	—	0.124
H7	1.000	—	—	1.000	—	—	1.000	—	—	—	0.735	—	—	—	0.265	—	—	—	0.100
H8	1.000	—	0.034	0.862	0.103	0.001	1.000	—	0.233	0.033	0.600	—	—	—	0.033	0.033	0.067	—	0.227
H9	1.000	—	—	0.942	0.058	—	1.000	—	—	—	0.775	—	—	—	0.025	0.125	0.075	—	0.125
H10	1.000	—	—	1.000	—	—	1.000	—	0.083	—	0.700	—	—	—	0.083	0.133	—	—	0.122
I1	1.000	—	—	0.803	0.197	—	1.000	—	—	—	0.482	—	—	—	—	0.054	0.464	—	0.227
J1	0.683	0.317	—	0.391	0.609	—	1.000	—	—	—	0.733	0.083	—	—	—	0.150	0.033	—	0.341
J2	0.839	0.161	—	0.776	0.224	—	1.000	—	—	—	0.400	0.067	—	—	—	0.017	0.517	—	0.302
J3	0.724	0.276	—	0.578	0.422	—	1.000	—	—	—	0.767	—	—	—	—	—	0.233	—	0.316
K1	0.971	0.029	0.050	0.475	0.475	—	1.000	—	—	—	0.750	0.056	0.111	—	—	—	0.083	—	0.262
L1	1.000	—	0.083	0.900	0.017	—	1.000	—	0.250	0.033	0.383	0.083	—	—	—	—	0.250	0.001	0.230
L2	1.000	—	—	0.980	0.020	—	1.000	—	—	—	0.750	—	—	—	—	—	0.250	—	0.106
L3	1.000	—	—	1.000	—	—	1.000	—	—	—	1.000	—	—	—	—	—	—	—	—
L4	1.000	—	0.484	0.516	—	—	1.000	—	0.210	0.113	0.677	—	—	—	—	—	—	—	0.250
L5	0.933	0.067	—	0.983	0.017	—	1.000	—	0.100	—	0.817	—	—	—	—	—	0.083	—	0.120
L6	1.000	—	—	0.435	0.565	—	1.000	—	0.104	—	0.896	—	—	—	—	—	—	—	0.174
L7	1.000	—	—	0.117	0.883	—	1.000	—	0.983	—	0.017	—	—	—	—	—	—	—	0.061
L8	1.000	—	—	0.769	0.231	—	1.000	—	0.154	—	0.788	—	—	—	—	—	0.058	—	0.180
L9	1.000	—	0.276	0.724	—	—	1.000	—	0.034	0.017	0.948	—	—	—	—	—	—	0.001	0.127
L10	1.000	—	0.023	0.977	—	—	1.000	—	0.091	—	0.909	—	—	—	—	—	—	—	0.054
L11	1.000	—	0.032	0.968	—	—	1.000	—	0.048	—	0.952	—	—	—	—	—	—	—	0.039
M1	0.984	0.016	0.048	0.903	0.048	0.001	1.000	—	0.032	—	0.597	0.065	—	—	—	0.145	0.161	—	0.204
M2	0.923	0.077	—	0.750	0.250	—	1.000	—	0.133	—	0.633	—	—	—	—	0.167	0.067	—	0.275
N1	1.000	—	0.067	0.933	—	—	1.000	—	0.517	—	0.483	—	—	—	—	—	—	—	0.159
N2	0.950	0.050	0.172	0.603	0.224	0.001	1.000	—	0.083	—	0.917	—	—	—	—	—	—	—	0.205
O1	0.964	0.036	0.950	0.050	—	—	1.000	—	0.117	—	0.883	—	—	—	—	—	—	—	0.094
P1	1.000	—	—	1.000	—	—	1.000	—	0.017	—	0.983	—	—	—	—	—	—	—	0.008
P2	1.000	—	0.054	0.946	—	—	1.000	—	0.483	—	0.517	—	—	—	—	—	—	—	0.153
Q1	0.719	0.281	0.125	0.031	0.844	—	1.000	—	0.600	—	0.400	—	—	—	—	—	—	—	0.297
Q2	0.967	0.033	0.533	0.033	0.433	0.001	1.000	—	0.550	—	0.450	—	—	—	—	—	—	—	0.276
Q3	0.517	0.483	0.300	0.633	0.067	—	1.000	—	0.700	—	0.300	—	—	—	—	—	—	—	0.359
R1	0.387	0.613	—	—	1.000	—	1.000	—	0.843	—	0.086	—	—	—	—	—	0.071	—	0.187
S1	0.420	0.580	0.220	0.060	0.720	—	1.000	—	0.080	—	0.170	—	—	—	—	—	0.750	—	0.369
S2	0.188	0.812	0.500	0.166	0.333	0.001	1.000	—	0.750	—	—	—	—	—	—	—	0.210	0.040	0.329

Table 2. Continued

Location	*ONEμ7*		*BT73*				*SFO18*		FGT3										H_S
	∗218	∗244	∗138	∗140	∗144	∗148	∗150	∗156	∗157	∗159	∗163	∗165	∗167	∗169	∗171	∗173	∗175	∗179	
Clark Fork																			
T1	0.956	0.044	—	—	1.000	—	0.843	0.157	—	—	—	0.281	0.063	0.078	—	0.578	—	—	0.241
T2	1.000	—	—	—	1.000	—	0.729	0.271	—	—	—	0.470	0.197	0.106	0.015	0.212	—	—	0.315
T3	0.800	0.200	—	—	1.000	—	0.790	0.210	—	—	—	0.529	0.114	0.043	—	0.314	—	—	0.372
U1	0.758	0.242	—	0.014	0.986	—	0.857	0.143	—	—	—	0.671	0.200	—	—	0.114	—	0.015	0.373
V1	0.125	0.875	—	—	1.000	—	1.000	—	0.023	—	—	0.656	0.320	—	—	—	—	0.001	0.404
V2	0.143	0.857	—	—	1.000	—	0.991	0.009	0.268	—	—	0.089	0.643	—	—	—	—	—	0.322
V3	0.423	0.577	—	—	1.000	—	0.942	0.058	—	—	—	0.192	0.769	0.019	—	0.019	—	0.001	0.257
V4	0.138	0.862	—	—	1.000	—	0.534	0.466	0.017	—	—	0.190	0.690	0.103	—	—	—	—	0.228
W1	0.917	0.083	—	—	1.000	—	0.979	0.021	—	—	—	0.840	—	—	—	0.160	—	—	0.118
Kootenai																			
X1	1.000	—	—	—	1.000	—	0.870	0.130	—	—	—	0.476	0.405	—	—	0.119	—	—	0.210
Saskatchewan																			
Y1	1.000	—	—	—	1.000	—	0.050	0.950	0.725	0.100	—	0.175	—	—	—	—	—	—	0.135

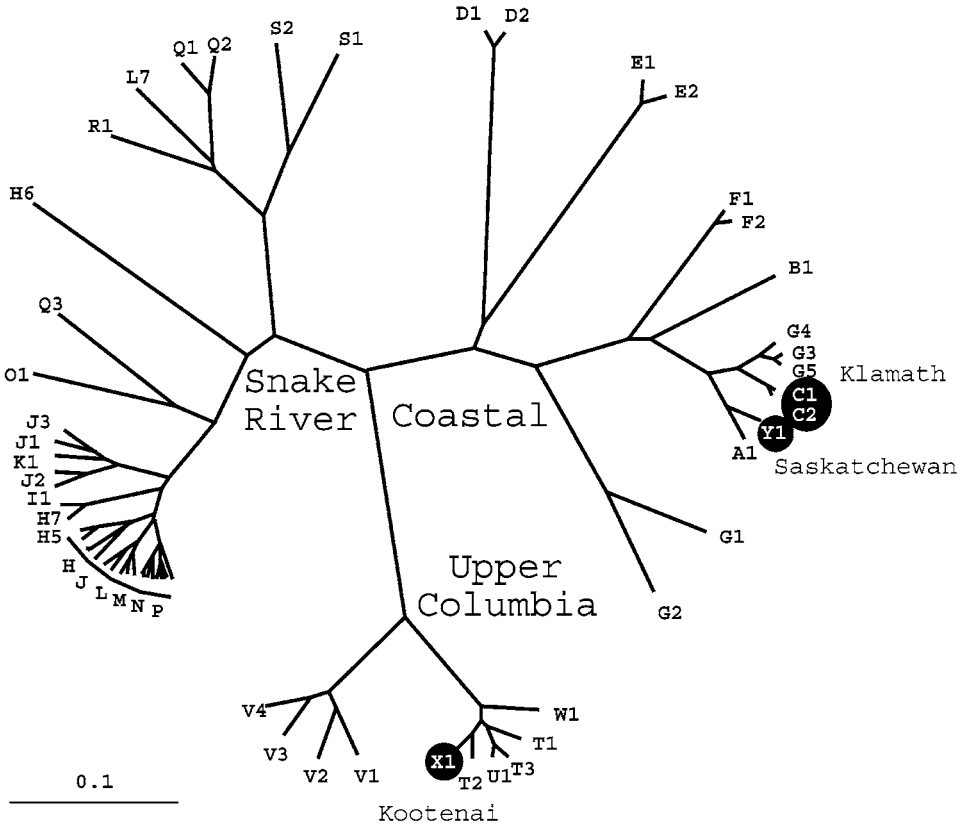

Figure 2. UPGMA dendrogram of bull trout populations based on Cavalli-Sforza and Edwards chord distance calculated using allele frequencies at four polymorphic microsatellite loci. Drainage abbreviations and location numbers correspond to sample locations from Table 1 and Figure 1. Locations shaded by black ovals are assigned to groups from which they are geographically isolated.

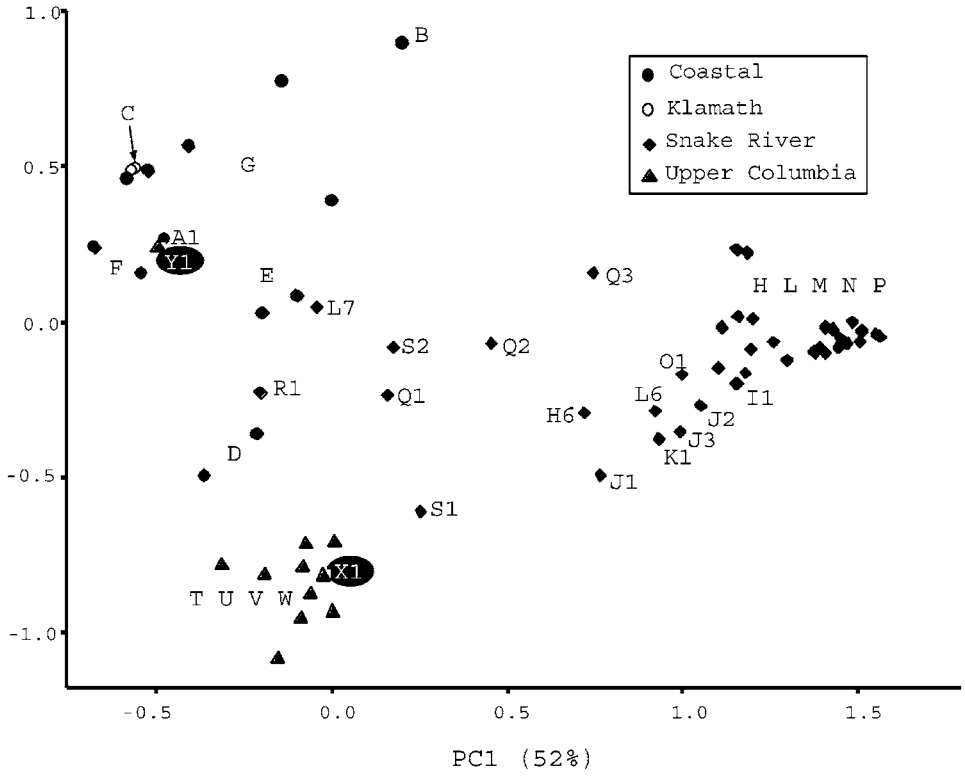

Figure 3. Plot of first two principal component scores derived from allele frequencies for all population samples. Major geographic clusters are labeled using drainage abbreviations from Table 1 and Figure 1. Single points are numbered using the drainage abbreviation and sample location number as described in Table 1. Percentages are the proportion of the total variation attributable to each axis.

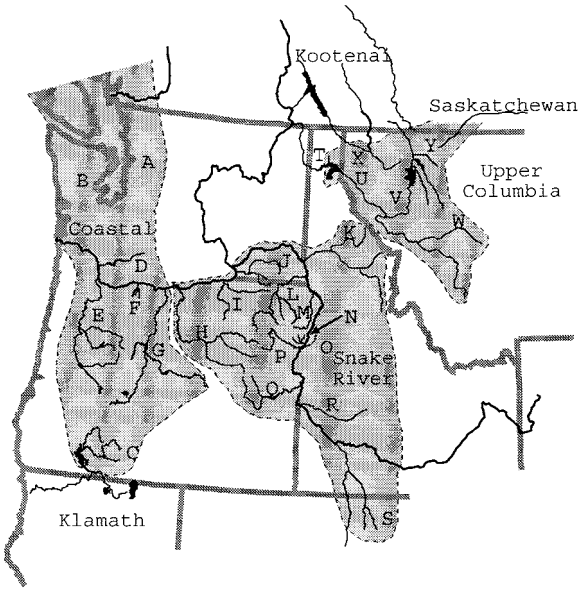

Figure 4. Map showing three major groups of bull trout based on microsatellite data.

Although there is substantial differentiation within the Snake River samples, they are less differentiated (F_{ST} = 0.405) than those in the Coastal samples (F_{ST} = 0.635), despite covering approximately the same geographic area. With the exception of Hurricane Creek (L7), tributaries to the John Day (H) and Grande Ronde (L) Rivers tend to be similar in allele frequencies. However, *FGT-3∗173* is found in seven of ten John Day populations and was not present in any of the 11 Grande Ronde populations (Table 2). Samples collected in the Malheur (Q), Boise (R), and Jarbidge (S) Rivers consistently form a cluster discrete from other Snake River sites.

Discussion

Distribution of genetic variation

The amount and distribution of genetic variation estimated by microsatellite analysis is consistent with previous studies using allozymes in which approximately 60% of the variation was due to population

subdivision (Leary et al. 1993; Kanda et al. 1997). For mitochondrial DNA analysis the corresponding value was approximately 55% (Taylor et al. 1999). This genetic population structure probably reflects the biological characteristics of bull trout (Taylor et al. 1999). Bull trout are an apex predator that have highly specific spawning locations (Swanberg 1997). We expect such species to exist at relatively low abundance and, therefore, to contain reduced genetic variation within populations due to the effects of genetic drift in small isolated populations. The high level of fidelity to spawn in natal streams, on the other hand, should produce a high level of divergence between populations.

Based on allozyme analysis, bull trout have relatively low levels of genetic variation within populations when compared to other salmonids (Leary et al. 1993). This same pattern is observed at microsatellite loci and is supported by the few alleles identified across the range of the species. This pattern of few alleles has also been observed at a microsatellite isolated from bull trout (Spruell et al. 1999; Neraas and Spruell 2001). Similarly, although there is a wide range of H_S estimates, the mean H_S (0.186) for the 65 populations we have analyzed is very low compared to other microsatellite-based estimates of H_S for salmonids. For example, in their analysis of five brook trout populations, Angers et al. (1995) observed a mean H_S of 0.391.

There is an exceptionally large range of heterozygosities among the bull trout populations sampled (Table 2). These differences probably reflect historic patterns of gene flow and isolation. Bull trout in the Klamath Basin (C) contain virtually no genetic variation at either microsatellite or allozyme loci (Leary et al. 1993; Williams et al. 1997). These populations have been geographically isolated for thousands of years and were perhaps founded by a limited number of individuals or went through a severe bottleneck. Conversely, bull trout in the Pend Oreille (T) and Flathead (V) systems consistently have above average heterozygosities. Both of these systems contain large natural lakes that may have enabled populations to persist at higher number than those confined to small stream systems. Different populations have been analyzed using various techniques making direct comparisons of the estimated heterozygosities are difficult. However, the relative levels of heterozygosity in various geographic regions estimated using microsatellites are consistent with the allozyme-based estimates (Leary et al. 1993). For example, both studies found essentially no variation in bull trout from the Klamath Basin.

Among population divergence

Despite the limited amount of within-population variation, the microsatellite data suggest genetic structuring that was not apparent in earlier studies. Although we have only analyzed four loci, our data should be more robust than the earlier studies. Twelve of the 51 loci surveyed by Leary et al. (1993) and five of the 45 loci surveyed by Kanda et al. (1997) were polymorphic but few loci displayed regionally informative variation. The studies using mtDNA are based on a single genetic locus. In addition, we have analyzed more populations from a broader geographic range than most of the previous studies.

While considerable debate regarding the most appropriate technique to analyze microsatellite data continues, the major geographic patterns we suggest are apparent by examining the raw data (Table 2). For example, *SFO18* alone is almost sufficient to differentiate the three genetic groups. Coastal populations, excluding the Hood River (F), are fixed for *SFO∗156*. Snake River populations are uniformly fixed for *SFO∗150*. Upper Columbia populations, with a single exception (V1), have both alleles. Similar differences in the distribution and frequency of alleles among groups are obvious for the remaining three loci as well. These results illustrate that loci with few alleles nevertheless may be very valuable for differentiating large-scale geographic patterns, as has been suggested by simulations (Ferguson and Danzmann 1998).

Although most populations group with others that are in the same geographic region, there are a few exceptions. The O'Brien Creek (X1) sample in the Kootenai drainage clusters very tightly with samples from Lake Pend Oreille in the Upper Columbia group. Headwater exchanges between these drainages during the glacial Lake Missoula floods seems a plausible explanation for the similarities. Finally, the single site from the Belly River (Y1) in the Saskatchewan drainage clusters with the Coastal group despite being on the eastside of the continental divide. This relationship results from the high frequency of *SFO18∗156* that is common to Coastal samples and is probably attributable to genetic drift (see below).

Zoogeography

Based on mitochondrial DNA analysis, Taylor et al. (1999) suggested that the major divergence observed in bull trout reflects the existence of two glacial refugia. Our data are largely consistent with their interpretations. The same Inland/Coastal division observed at mtDNA is apparent in the microsatellite data. Like the mtDNA analysis, samples from lower Columbia tributaries are genetically similar to samples from the Olympic Penninsula and Puget sound and are distinct from more inland forms at microsatellite loci. These data are consistent with postglacial dispersal of bull trout into the lower Columbia Basin from the Chehalis refuge as suggested by Taylor et al. (1999).

Within the coastal group, the Hood River samples are differentiated from other lower Columbia populations based on both data sets. Taylor et al. (1999) identified "Inland" haplotypes in Clear Branch Creek (F1), a tributary of the Hood River. Both Hood River samples (F1 and F2) cluster with Coastal populations based on microsatellite analysis. However, *SFO18∗150* is present in bull trout from the Hood River. This allele is absent from all other Coastal populations except G5 and found in high frequency in all Snake River and Upper Columbia samples. This observation, coupled with the mtDNA data, suggests that colonizers from both refugia have contributed to bull trout populations in this drainage.

Both data sets also suggest that bull trout in the Deschutes River (G) represent the upstream limit of the Coastal lineage in the Columbia Basin. A striking level of divergence between bull trout in Deschutes (G) and adjacent John Day (H) drainages was observed (see Figures 2 and 3). Despite the proximity of the mouths of the rivers (approximately 25 km), we find greater genetic divergence between bull trout in the John Day River and bull trout in the Deschutes River than has been reported between North American and European Atlantic salmon using three microsatellite loci (McConnell et al. 1995).

Surprisingly, bull trout in tributaries to the John Day River (H) are genetically more similar to samples collected in tributaries in the Snake River group. The most direct current migratory route from the John Day to any of these sites is hundreds of kilometers. However, there are few consistent differences in allelic frequency that discriminate between the two basins.

There are several explanations that could account for this observation. (1) Some geologic event may have connected the two systems. Although these basins are widely separated via current migratory routes, spawning and rearing areas are in adjacent headwater drainages. (2) There may be enough gene flow among populations to homogenize allelic frequencies. Bull trout are capable of migrating more than 200 km (Bjornn and Mallet 1964; Shepard et al. 1984). However, given our data indicating large differences between John Day and Deschutes populations despite their close proximity and that spawning areas in the Snake River tributaries and John Day are about 1,200 km apart fluvially, this explanation does not seem plausible. (3) The limited number of loci used in this study may not have been sensitive enough to discriminate some fine-scale distinctions among populations. However, analysis using six additional loci also failed to detect allele frequency differences that consistently distinguished tributaries to the Grande Ronde from tributaries to the Snake River (unpublished data).

Genetic drift and population divergence

Allelic frequencies are influenced by the opposing influences of migration among populations that tends to increase genetic similarity and genetic drift that leads to divergence. Analysis of a limited number of loci may lead to erroneous conclusions about the relationships among populations if both of these forces are not considered. Most of the groups we suggest correspond to geographically proximate locations. However, in some cases the effects of genetic drift may obscure the true genetic relationships.

Many bull trout spawning populations are small (fewer than 50 mature adults, Leary et al. 1993; Rieman and Allendorf, 2001) and exist in isolation from other populations, making genetic drift a particularly important factor to consider when interpreting these data. In the absence of mutation and migration, genetic drift will eventually lead to fixation. As a result, a population may be grouped with other populations to which it is genetically differentiated if, by genetic drift, it becomes fixed at one or all loci for the most common allele of another group. Similarly, distinct populations could appear to be fixed for the same allele due to homoplasy. This phenomenon may be more common in microsatellites that have higher mutations rates than many other techniques. In the absence of other major allele frequency differences, such populations will incorrectly appear to belong within that group. The assignment of the Belly River sample (Y1) to the Coastal group is influenced by

the high frequency of *SFO18∗156* that is probably the result of genetic drift in an isolated population. We suspect that if additional loci were analyzed, differences between populations in the Saskatchewan Basin and Coastal populations would become apparent (Utter et al. 1992). Based on mtDNA data suggesting that mtDNA haplotypes from the Belly River clustered with their Inland group (Taylor et al. 1999; N. Kanda unpublished data) and geographic proximity, we have tentatively assigned the Belly River to the Upper Columbia group.

We also suspect there is at least one case in which populations divergent from other are included within the same group. We did not detect any unique alleles differentiating samples from the Coastal group and those from the Klamath Basin. However, using protein electrophoresis Leary et al. (1993) found an allele, *GPI-B2∗135*, that is fixed in the Klamath Basin and found in none of the other samples they analyzed. The upper Klamath Basin has probably been geologically isolated for at least 10,000 years, precluding any gene flow between Klamath Basin and Columbia Basin populations (Leary et al. 1993). Our results probably reflect the fixation of the most common Coastal allele at each microsatellite locus due to genetic drift rather than the homogenization of the Klamath and other Coastal populations by migration. The geographic isolation and the presence of a unique allozyme allele fixed in the Klamath drainage is compelling evidence that populations in the Klamath Basin represent a unique evolutionary lineage of bull trout within the Coastal group.

Analysis of additional loci decreases the likelihood that drift will cause spurious results. However, a single locus may be sufficient to cause errors in the inferred relationships among populations. This is particularly true if there is no significant divergence at any of the other loci.

Genetic divergence and DPSs

Designation of conservation units depends upon determining evolutionary relationships among populations and then determining which populations, or groups of populations, should be the appropriate focus for conservation actions (Waples 1995). The appropriate hierarchical level for specific actions must then be identified. The current bull trout DPSs do not define groups with a consistent biological hierarchy.

The most obvious example of this inconsistency is the Jarbidge DPS. The other four DPSs represent collections of many geographically widespread populations. The Jarbidge DPS, on the other hand, consists of a single basin in which the USFWS estimates that only 100 bull trout spawn annually (USFWS 1999). The designation of the bull trout in the Jarbidge River as a separate DPS was based upon its unusual setting (it is the southernmost bull trout population) and geographical separation (USFWS 1999). However, neither our microsatellite data nor the mtDNA data of Taylor et al. (1999) suggest that bull trout in the Jarbidge system (S) are distinct from other bull trout populations in the upper Snake River Basin. Therefore, based on the genetic evidence, there is no justification for not including Jarbidge bull trout in the same DPS as other Snake River populations.

The Olympic Peninsula-Puget Sound DPS is another example of populations being separated from the group to which they naturally belong. The samples from the Olympic Peninsula (A) and Puget Sound (B) fall within the Coastal group. However, they do not appear to be more closely related to each other than to other coastal populations. Like the Jarbidge population, this DPS was based on geographic proximity but the genetic data do not support this group as being distinct from other populations in the lower Columbia basin.

The Columbia River DPS appears to combine several evolutionarily distinct lineages. The microsatellite data presented in this paper (Figure 2) and mtDNA analysis (Taylor et al. 1999) document substantial divergence between populations in lower Columbia River tributaries and upstream tributaries. There are also substantial differences in microsatellite allele frequencies between bull trout populations in the Upper Columbia group and those from Snake River tributaries. It appears that the Columbia River DPS is actually a combination of at least three distinct groups that have historically had little exchange of migrants.

The genetic data suggest major groups that are largely consistent with the geographic distribution of bull trout. For example, populations within the Lake Pend Oreille/Clark Fork system occupy historically continuous habitat isolated from other Columbia River populations by a natural barrier falls. Therefore, these populations may for an appropriate unit for regional recovery and planning. There are likely to be additional levels of complexity within any conservation unit that may be important for the evaluation of management actions. For example, the data presented in this paper suggest that tributaries to Flathead Lake form a discrete group within the Upper Columbia

group. Neraas and Spruell (2001) also report substantial genetic divergence between populations from Lake Pend Oreille tributaries and those from lower Clark Fork River tributaries. Furthermore, there is evidence to support significant genetic divergence among bull trout populations from different tributaries within a single tributary to Lake Pend Oreille, Idaho (Spruell at al. 1999). Thus, depending upon the action being considered, a different biologic level of the hierarchy may be affected.

The current bull trout DPSs do not reflect our understanding of the genetic population structure of bull trout and may increase the likelihood of inappropriate management actions. Management based on those DPSs is likely to be inadequate for conserving the genetic and ecological diversity of this species. Using a consistent hierarchical approach based on the available genetic data would be a more effective method to direct efforts to conserve bull trout.

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