Appendix 86

Genetic Analysis of Bull Trout in Glacier National Park

Report 02/102
Wild Trout and Salmon Genetics Lab

to

The United States Fish and Wildlife Service
and
The National Park Service

9 August 2002

Paul Spruell*

Jonathan J. Huie

Marirose Spade

and

Fred W. Allendorf

Wild Trout and Salmon Genetics Lab
Division of Biological Sciences
University of Montana
Missoula, MT 59812

*Author to whom correspondence should be addressed
Phone (406) 243-6749
Fax (406) 243-4184
E-mail spruell@selway.umt.edu

Summary

We used seven microsatellite loci to describe the genetic population structure of bull trout within Glacier National Park and surrounding watersheds. Bull trout within the park were variable at five of these seven. We found consistent differences between bull trout east and west of the Continental Divide. We also found substantial genetic differentiation among lake populations in the western portion of Glacier National Park. These results indicate that each lake population is a separate demographic unit that is genetically distinct from adfluvial bull trout that use Flathead Lake during part of their life cycle. We found no genetic variation at all in the bull trout from Upper Kintla Lake. Less differentiation was observed among sample sites within the Saint Mary River basin. However, there appears to be significant genetic differences among Kennedy, Boulder, and Otatso Creeks. Management actions should consider that the limited gene flow among sites within the Saint Mary drainage may be important for the long term persistence of these populations.

Introduction

Conservation of threatened and endangered species requires accurate descriptions of the relationships among populations. Species are often subdivided into groups of populations that share evolutionarily important characteristics and often differ from other such groups of the same species. Describing this hierarchy is one of the first steps toward understanding the biology and recommending proper management actions for any species. Proper hierarchical groupings are essential for accurate ecological and genetic analyses. Descriptions of life history, estimates of a population's vital rates, and estimates of population size all assume that the bounds of a population can be defined. Similarly, estimates of genetic variation within samples, genetic differentiation among samples, and effective population size rely on the fact that representative samples are drawn from reproductively isolated groups of individuals.

The objective of this project was to describe the genetic relationships among bull trout occupying waters in Glacier National Park. We addressed two primary issues. First, we examined the relationship between populations on either side of the Continental Divide. We then estimated the amount of genetic variation found within these two groups of populations.

Materials and Methods

Sample Collection and DNA Extraction

Personnel from the United States Fish and Wildlife Service (USFWS) collected all samples. Fish from the Saint Mary drainage were captured either using electrofishing techniques or by fish traps placed near the mouth of each tributary as described in Mogen and Kaeding (2001). Fish from the lakes west of the Continental Divide were captured using gill nets (Wade Fredenberg, personal communication). A fin clip was taken non-lethally from each individual and stored in 95% ethanol. DNA was extracted from fin tissue with a Purgene kit (Gentra).

Data from bull trout samples from tributaries to Lake Pend Oreille, the Clark Fork River, the Flathead River, and the Kootenai River were included to increase the geographic range of the analysis and to provide a broader context for the Glacier National Park results.

Microsatellites

Seven microsatellite loci were amplified in an MJ Research PTC-100 thermocycler using the profiles described by the individuals who initially investigated each locus

(*ONEμ7*, Scribner et al. 1996; *SFO18*, Angers et al. 1995; *FGT3*, Sakamoto et al. 1994; *SSA311* and *SSA456*, Slettan et al. 1996; *OTS101*, Small et al. 1998; *BT73*, Estoup et al. 1993; *SCO19*, Taylor et al. 2001). Amplified products were separated on a 7% denaturing polyacrylamide gel and visualized using a Hitachi FMBIO-II fluorescent imager. Allele sizes were determined using standard base pair size ladders (MapMarkerLOW, Bioventures) and Hitachi FMBIO software. In order to achieve consistent scoring across gels, previously amplified individuals were included on each gel.

Data Analysis

Allele frequencies, expected heterozygosities (H_s), genetic divergence among population (F_{ST}), and deviations from expected Hardy-Weinberg genotypic proportions were calculated using GENEPOP (Raymond & Rousset 1995). We included all loci in our calculations of *Hs* in order to allow a more direct comparison with values from previously published work using these same loci (Neraas and Spruell 2001).

A UPGMA dendrogram based on Cavalli-Sforza and Edwards chord distance (CSE) was generated using PHYLIP (Felsenstein 1993; Cavalli-Sforza and Edwards 1967). As an alternative projection of the genetic relationships among samples, we also completed a principal components analysis (PCA) using MINITAB (version 11). For this analysis, we omitted the largest allele at each locus to account for the nonindependence of allele frequencies.

Results

Variation within samples

We observed no significant deviations ($P<0.05$) from expected Hardy-Weinberg proportions in any of the samples collected in Glacier National Park. The expected average heterozygosity at across all loci (H_s) ranged from 0.000 in Upper Kintla Lake to 0.344 in Upper Quartz Lake. The mean H_s for samples from the Saint Mary drainage (0.166) was similar to the mean H_s for the samples from the lakes west of the divide (0.181). However, the variation observed among lake samples was great and included both the minimum and maximum values observed (Table 1).

Comparisons between East and West of the Continental Divide

There are clear differences in allelic composition between samples collected east of the Continental Divide and those collected west of the Continental Divide (Table 2). Three of the five western lakes contain the *ONEμ7*244* allele that is not found in any of the Saint Mary samples.

Similarly *SCO19*174* is the common allele in three of the five lake samples and is present in a fourth. This allele is not found in any of the of the Saint Mary samples. Finally, *SCO19*204* is found in all samples from the Saint Mary drainage but is not found in any of the lake populations west of the divide. These allele frequency differences are also obvious in the dendrogram (Fig. 1) and PCA (Fig. 2) that both cluster those samples from the Saint Mary drainage to the exclusion of any samples from lakes in the western portion of the Park but the lake samples, rather than forming a discrete group, are scattered among other samples from the Flathead drainage (Fig. 3)

Differentiation Among Samples West of the Continental Divide

The five different lakes sampled west of the Continental Divide are all significantly different from each other. In fact, over 40% of the genetic variation observed in these samples is attributable to differences among lakes. There are several examples of alleles being found in one lake but not others (Table 1). In addition, there are substantial frequency differences among alleles that are shared by sample sites.

The differences among lake samples are also apparent in the dendrogram (Fig. 1) and the PCA (Fig. 2). Within the dendrogram, the branch lengths connecting most lake samples are relatively long, reflecting a high level of genetic differentiation (Fig. 1). A similar pattern is observed in the PCA in which the five lake populations are scattered throughout the two-dimensional space. Upper Kintla Lake and Trout Lake appear fairly similar in both the dendrogram (Fig. 1) and the PCA (Fig. 2). However, this similarity is most likely attributable to random genetic drift causing fixation for the same allele at six loci in both samples. However, *SSA456*159* is present at a frequency of 0.514 in Trout Lake but is absent in Upper Kintla Lake illustrating that there is limited exchange between these two sites as would be expected based on their geographic isolation.

Differentiation Among Samples in the Saint Mary River

Samples within the Saint Mary drainage are more similar to each other than those samples in the western lakes. Among the sample sites in the Saint Mary drainage, 18.4% (*Fst* = 0.184) of the genetic variation observed is attributable to differences among sample locations. The only obvious difference in allele distribution among the three sites is that *SSA456*159* is found in both samples from Boulder Creek but none from either Kennedy or Otatso.

The samples show similar patterns of differentiation in both the dendrogram and the PCA. Samples from Otatso Creek are more differentiated from Kennedy and Boulder in both

cases. Samples from Kennedy and Boulder Creeks are fairly similar to each other based on both analyses. The PCA, however, displays similarity among lower sites that is not obvious in the dendrogram. This pattern is consistent with the relatively common movement among tributaries observations of Mogen and Kaeding (2001)

Discussion

Comparisons Across the Continental Divide

We expected substantial differences between bull trout from either side of the Continental Divide. Our observations are consistent with that expectation. In several cases, the alleles found on one side of the divide are absent from the other. Therefore, as we would assume based on the geographic separation of the two systems, the bull trout in the Saint Mary drainage should be managed independently from bull trout found in Glacier National Park west of the Continental Divide.

West of the Divide

The bull trout inhabiting lakes west of the Continental Divide in Glacier National Park are all significantly different from each other. This is also expected based on geographic isolation. The lack of a defined "Glacier National Park lakes" grouping in the PCA and the high level of differentiation in the dendrogram probably reflects the strong effect of random genetic drift in small isolated populations. This same effect probably accounts for the similarity between Upper Kintla Lake and Trout Lake as displayed by the dendrogram and PCA (Figs. 1 and 2). It is likely that populations in both lakes drifted to fixation for the same allele at six of the seven loci examined. However, *SSA456*159* is the common allele in Trout Lake. This allele is not found in Upper Kintla Lake bull trout.

Saint Mary Basin

Samples from the Saint Mary basin have had more opportunity for genetic exchange than the populations inhabiting the western lakes. This is reflected in an *Fst* that is approximately half that observed for lake populations. However, despite the potential for exchange, there are still substantial differences among sites. Most notably, the samples from Otatso Creek appear to be different from those in either Kennedy or Boulder Creek. This may not be unexpected in the upper two reaches of Otatso due to at least partial fish passage barriers. However, tagging studies (Mogen and Kaeding 2000) would suggest substantial exchange between streams.

There are two possible explanations for this apparent discrepancy. First, there may be sufficient downstream migration from upper to lower Otatso to cause those samples to form a discrete group. Alternatively, tagged fish may be moving among tributaries to feed or to seek preferred habitat conditions but return to spawn in their natal streams.

Several allele frequency patterns may support the former explanation. For example, *SFO18*150* is found at a frequency of 0.813 in upper Otatso and 0.550 in the middle reach but is almost absent from either Boulder or Kennedy Creeks (maximum value of 0.032). In lower Otatso, this allele is found at 0.296, a level that might be expected if fish are moving downstream and mixing with bull trout from Boulder and Kennedy Creeks.

It is important to recognize that adult bull trout moving among the lower reaches of different tributaries does not equate to gene flow. Even if the lower Otatso sample is comprised of a mixture of adults from various tributaries, there may be little or no genetic exchange among spawning aggregates. The presence of *SSA456*159* exclusively in Boulder Creek is one indication that exchange may be limited.

Conservation Implications

Bull trout pose a particularly difficult conservation problem as they typically display limited genetic variation within populations but substantial differentiation between populations (Spruell et al. 2002; Neraas and Spruell 2001; Spruell et al. 1999; Taylor et al 2001). In addition, many populations occupy habitats that impose strict requirements for migration timing, spawning location, and spawning timing. Given this situation, virtually every bull trout population could be considered its own management unit.

The bull trout in Glacier National Park follow this general pattern. There is a major geographic and genetic division between populations on either side of the Continental Divide. This distinction has been legally recognized by placing those populations in the Saint Mary basin in their own DPS.

Bull trout inhabiting the lakes in the western portion of the park should be considered independent management units. There is little or no opportunity for current migration among lakes. Based on the genetic data, this has been the case for centuries. In addition, many of these populations are likely to exhibit genetically based local adaptations to the lake in which they are found. For example, bull trout in Upper Kintla Lake spawn and rear in

the lake outlet, a somewhat unusual strategy for bull trout. Therefore, the populations of bull trout that are isolated in headwater lakes and streams of the Flathead drainage are substantially genetically differentiated from the populations of migratory bull that use Flathead Lake during part of their life cycle.

Bull trout in the Saint Mary drainage may provide the greatest challenge to managers. There is some genetic evidence suggesting that there is restricted gene flow among sites even though the fish appear to be highly mobile. One way migration within the Otatso system may also complicate the management of this system. More importantly, the majority of this drainage lies in Canada where they are not protected by the ESA or any other Federal actions. We are left facing the possibility of trying to manage isolated headwater populations but having no control over much of the mainstem corridor that would have historically connected the Saint Mary metapopulation.

Literature Cited

Angers, B., Bernatchez, L., Angers, A., and Desgroseillers, L. (1995) Specific microsatellite loci for brook char reveal strong population subdivision on a microgeographic scale. Journal of Fish Biology 47(suppl A):177-185.

Cavalli-Sforza, L.L. and Edwards, A.W.F. (1967) Phylogenetic analysis: models and estimation procedures. Evolution 21:550-570.

Estoup, A., Presa, P., Kreig, F., Vaiman, D., and Guyomard, R. (1993) (CT)n and (GT)n microsatellites: a new class of genetic markers for *Salmo trutta* L. (brown trout). Heredity 71:488-496.

Felsenstein, J. (1993) PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.

Mogen, J.T. and Kaeding, L.R. (2001) Population biology of bull trout (*Salvelinus confluentus*) in the Saint Mary River drainage. Progress Report. Bozeman, Montana: U.S. Fish and Wildlife Service, Branch of Native Fishes Management.

Neraas, L.P. and Spruell, P. (2001) Fragmentation of riverine systems: the genetic effects of dams on bull trout (*Salvelinus confluentus*) in the Clark Fork River system. Molecular Ecology 10:1153-1164.

Raymond, M. and Rousset, F. (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 83:248-249.

Sakamoto, T., Okamoto, N., and Ikeda, Y. (1994) Dinucleotide repeat polymorphism of rainbow trout, FGT3. Journal of Animal Science 72:2766.

Scribner, K.T., Gust, J., and Fields, R.L. (1996)

Isolation and characterization of novel microsatellite loci: cross-species amplification and population genetic applications. Canadian Journal of Fisheries and Aquatic Sciences 53:883-841.

Slettan, A., Olsaker, I., and Lie, O. (1996) Atlantic salmon, *Salmo salar*, microsatellites at the SSOSL25, SSOSL311, SSOSL417 loci. Animal Genetics 27: 57-64.

Small, M.P., Beacham, T.D., Withler, R.E., and Nelson, R.J. (1998) Discriminating coho salmon (*Oncorhynchus kisutch*) populations within the Fraser River, British Columbia, using microsatellite DNA markers. Molecular Ecology 7:141-155.

Spruell, P., Rieman, B.E., Knudsen, K.L., Utter, F.M., and Allendorf, F.W. (1999) Genetic population structure within streams: microsatellite analysis of bull trout populations. Ecology of Freshwater Fish 8:114-121.

Spruell P., Hemmingsen A.R., Howell P.J., Kanda N., and Allendorf F.W. (2002) Conservation genetics of bull trout: geographic distribution of variation at microsatellite loci. Conservation Genetics (in press).

Taylor, E. B., Z. Redenbach, A. B. Costello, S. M. Pollard, and C. J. Pacas. (2001) Nested analysis of genetic diversity in northwest North American char, Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*). Canadian Journal of Fisheries and Aquatic Sciences 58:406-420.

Table 1. Summary of data for bull trout from Glacier National Park. Sample number corresponds to Figures 1 and 2. Allele frequency at each locus, average heterozygosity (*Hs*) at seven loci, and average number of alleles at the five polymorphic loci (*A*) are given.

			ONEμ7		SFO18	
Sample Location	Sample Size	Sample Number	*218	*244	*150	*156
Flathead River						
Harrison Lake	19	1	0.346	0.654	1.000	--
Trout Lake	39	2	1.000	--	1.000	--
Upper Quartz	28	3	0.482	0.518	0.429	0.571
Bowman Lake	10	4	0.800	0.200	0.700	0.300
Upper Kintla Lake	30	5	1.000	--	1.000	--
Saint Mary River						
Boulder Fish Trap	60	6FT	1.000	--	0.019	0.981
upper Boulder Creek	12	6UP	1.000	--	--	1.000
Otatso Fish Trap	27	7FT	1.000	--	0.296	0.704
Middle Otatso	20	7MD	1.000	--	0.550	0.450
upper Otatso (Slide Lake)	16	7UP	1.000	--	0.813	0.187
Kennedy Fish Trap	31	8FT	1.000	--	0.032	0.968
upper Kennedy	10	8UP	1.000	--	--	1.000

	FGT3				SSA456		
Sample Location	*157	*165	*167	*169	*157	*159	*161
Flathead River							
Harrison Lake	--	0.423	0.577	--	0.808	0.192	--
Trout Lake	--	--	1.000	--	0.486	0.514	--
Upper Quartz	0.179	0.482	0.285	0.054	0.732	0.250	0.018
Bowman Lake	0.250	0.400	0.300	0.050	0.950	0.050	--
Upper Kintla Lake	--	--	1.000	--	1.000	--	--
Saint Mary River							
Boulder Fish Trap	0.164	0.647	0.078	0.111	0.974	0.026	--
upper Boulder Creek	0.083	0.626	0.083	0.208	0.958	0.042	--
Otatso Fish Trap	0.296	0.186	0.241	0.277	1.000	--	--
Middle Otatso	0.150	0.025	0.400	0.425	1.000	--	--
upper Otatso (Slide Lake)	--	0.094	0.438	0.468	1.000	--	--
Kennedy Fish Trap	0.194	0.419	0.113	0.274	1.000	--	--
upper Kennedy	0.333	0.389	0.056	0.222	1.000	--	--

	SCO19							
	*172	*174	*200	*202	*204	*212	A	Hs
Flathead River								
Harrison Lake	--	--	1.000	--	--	--	1.60	0.163
Trout Lake	--	1.000	--	--	--	--	1.20	0.063
Upper Quartz	0.036	0.625	0.286	0.036	--	0.017	3.20	0.344
Bowman Lake	0.050	0.350	0.100	0.500	--	--	2.80	0.336
Upper Kintla Lake	--	1.000	--	--	--	--	1.00	0.000
Saint Mary River								
Boulder Fish Trap	--	--	0.623	0.140	0.237	--	2.40	0.146
upper Boulder Creek	--	--	0.583	0.250	0.167	--	2.20	0.157
Otatso Fish Trap	--	--	0.538	0.077	0.385	--	2.20	0.219
Middle Otatso	--	--	0.605	--	0.395	--	2.00	0.207
upper Otatso (Slide Lake)	--	--	0.906	--	0.094	--	1.80	0.136
Kennedy Fish Trap	--	--	0.581	0.129	0.290	--	2.20	0.168
upper Kennedy	--	--	0.833	0.111	0.056	--	2.00	0.129

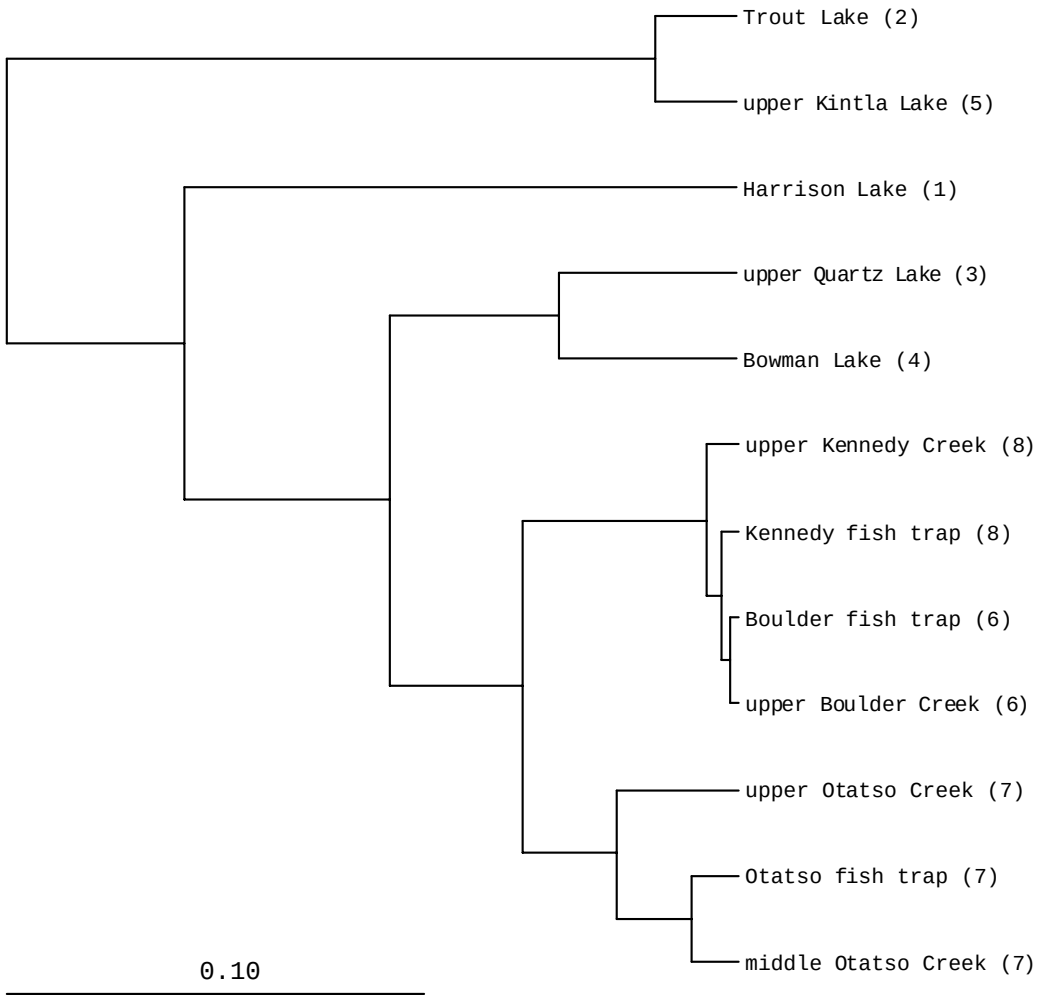

Figure 1. UPGMA dendrogram based on Cavalli-Sforza & Edwards chord distance. Samples and numbers correspond those described in Table 1.

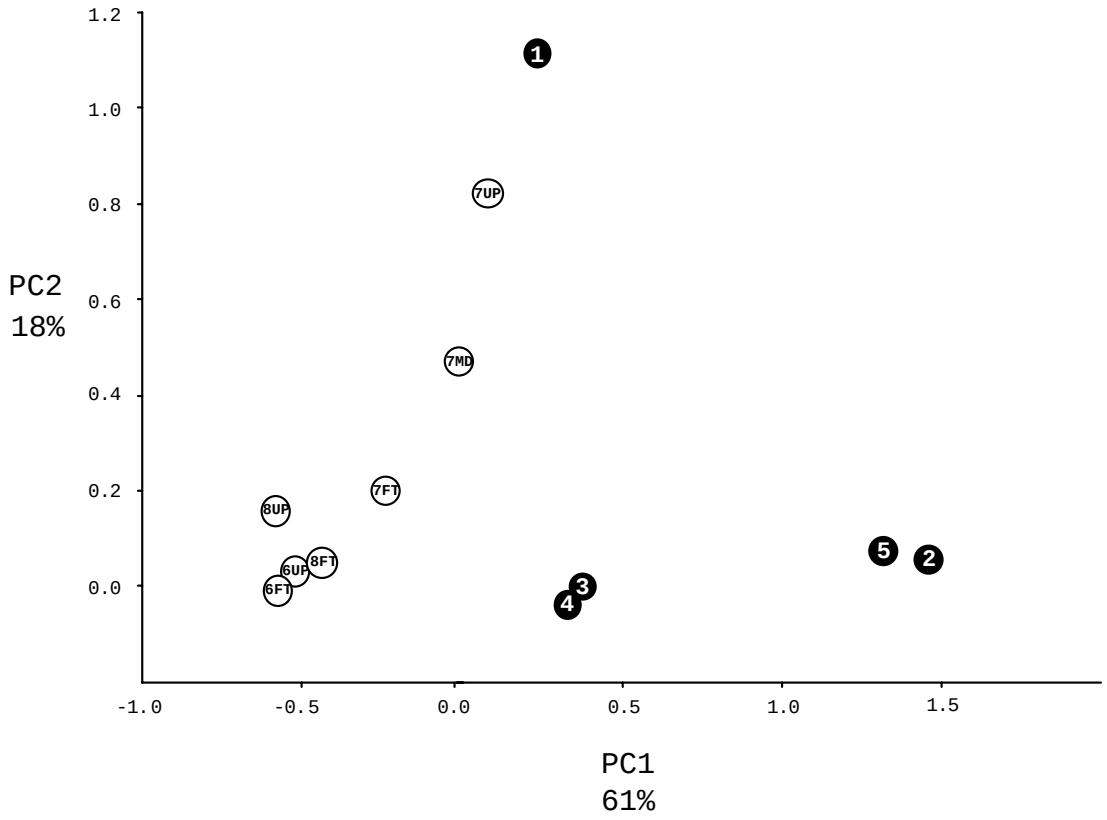

Figure 2. Principle components analysis of bull trout from Glacier National Park based on five polymorphic loci. White circles represent samples from the Saint Mary drainage. Black circles represent samples from western Glacier. Numbers correspond to Table 1. Percentages are the proportion of the overall genetic variation attributable to each axis.

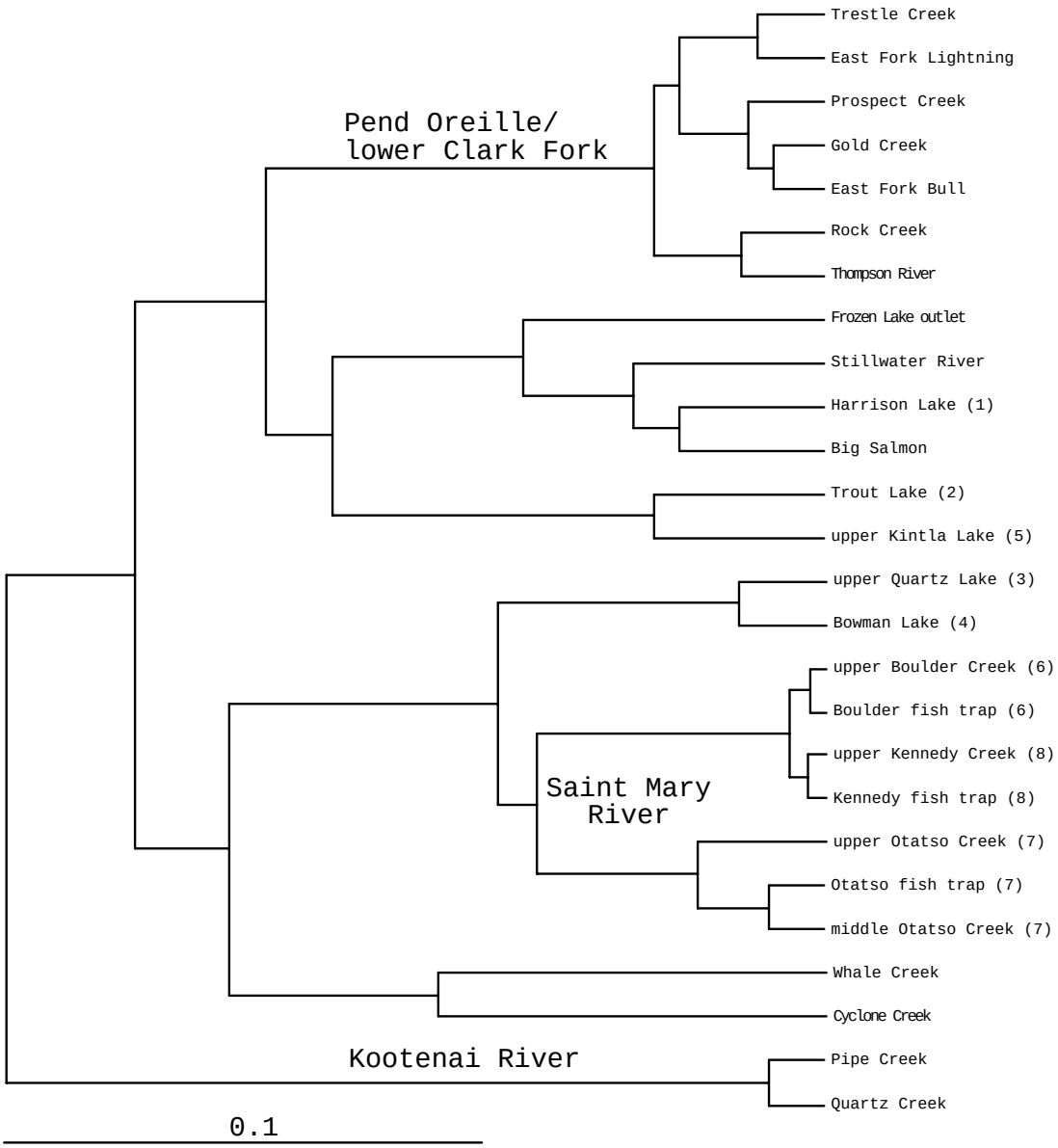

Figure 3. UPGMA dendrogram for bull trout from northwestern Montana and northern Idaho. Major geographic groupings are indicated on branches where appropriate. Numbers in parentheses correspond to Table 1.