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Genetic analysis of Arctic grayling population structure in the Williston Watershed

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SUMMARY

A previous study funded by the Peace / Williston Fish & Wildlife Compensation Program (PWFWCP) identified habitat use and migration patterns for Arctic grayling (*Thymallus arcticus*). The results indicated that grayling moved throughout large river systems and into tributaries with distinctive water signatures, but did not move into the reservoir. Lack of movement among populations within a single generation is suggestive of population structure, but genetic analysis is needed to resolve whether gene flow exists among these tributaries. During the summer of 2004, Arctic grayling were captured by angling from seven rivers within the Williston watershed; the Table, Anzac, Nation, Osilinka, Omineca, Mesilinka, and Ingenika Rivers. We also acquired scale samples from the PWFWCP for many of these watersheds and two other watersheds; Missinka and Fox Rivers. Fin clips and scale samples were analyzed from up to 39 samples taken from each population. Eleven polymorphic microsatellites were amplified and fragment size determined. The number of alleles per microsatellite was low and ranged from 2 to 8 across all populations examined. The frequency of alleles varied by river system and genetic structure was found among the rivers examined within the Williston Watershed.

INTRODUCTION

Among salmonids, reproductive isolation due to homing behaviour and spawning site fidelity creates genetic divergence. Straying among spawning populations, however, disrupts isolating effects and tends to homogenize genotypes of populations where gene flow exists. For the Williston watershed there is evidence for discrete spawning populations. Stamford & Taylor (2005) found significant genetic divergence among populations from the Upper Peace and a strong pattern of isolation by distance. A previous study examining elemental signatures in bony structures of grayling also suggests population structure (Clarke et al. 2005). Arctic grayling showed considerable differences in elemental signatures of strontium, barium, magnesium, and manganese among the major river systems that flow into the Williston Reservoir. Elemental signatures within rivers of the Williston Watershed were temporally stable and also showed substantial differences spatially. The data also indicated that use of the reservoir by grayling was unlikely. Elemental signatures characteristic of the reservoir were not found in any fish where reservoir chemistry differed from the rivers examined (Clarke et al. 2005).

In 2004, we collected tissue samples for genetic analysis. Our objective was to use genetic methods to determine if the putative stock structure indicated by chemical analysis of bones is consistent with phylogenetic structure. The results provide key information that will help guide management and restoration activities in watersheds that historically maintained healthy grayling populations. This project sought to characterize genetic structure of grayling populations in major tributaries that flow into the Williston Reservoir and whether these populations were discrete. We conducted genetic analyses using microsatellite markers for Arctic grayling within nine river systems of the Williston Watershed. From seven of these river systems, fish movements had previously been characterized based on chemical signatures in bony structures. That work suggested that grayling did not use the reservoir and population structure existed within the Williston Watershed for Arctic grayling. In the present study, our objective was to determine if population structure could be determined using neutral genetic markers.

METHODS AND MATERIALS

During the summer of 2004, Arctic grayling were captured by angling. We lethally sampled 10 fish from 7 rivers within the Williston watershed for bony structures to conduct Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS) analysis. The river systems selected were the Table, Anzac, Nation, Osilinka, Omineca, Mesilinka, and Ingenika Rivers. From each fish, we also collected a fin clip and preserved it in ethanol. Additional fish from each watershed were also caught and sampled non-lethally for an adipose fin clip. We also acquired scale samples from the PWFWCP for many of these watersheds and two other watersheds; the Missinka River and the Fox River (Figure 1; Table 1).

Fin clips and scale samples were analyzed from up to 39 samples taken from each population. DNA was extracted from fin clips preserved in ethanol or one to two dried uncleaned scales per fish. Tissues were digested to yield DNA, which was extracted and amplified. The use of Polymerase Chain Reaction (PCR) amplification of microsatellite markers allowed us to assess genetic status of many individuals from DNA extracted from the archived samples. Fragment size for microsatellites was determined on a Beckman CEQ 8000 sequencer at the University of Northern British Columbia. DNA was extracted and amplified from all the fin clips and all the available scales. Amplification of DNA using PCR was also successful for all samples, except one fish from the Nation River. Table 2 shows a list of microsatellite primers that were screened and used for this project. All were found to amplify DNA in Arctic grayling from the Williston watershed. We anticipated optimizing and using 10 primers for this study, but the initial 11 primers screened were found to show polymorphism and could be multiplexed for fragment analysis. The optimized PCR conditions for all primers used in this study are shown in Table 3.

Heterozygosity was calculated as an estimate of genetic variation using Tools for Population Genetic Analyses (TFPGA 1.3) software by Mark Miller (Biology Department; Arizona State University, PO Box 5640, Flagstaff, AZ 86011-5640, USA). An exact test for Hardy-Weinberg equilibrium using 10 000 permutations was calculated (using TFPGA) at each locus. The estimates of heterozygosity were corrected for significant departure from Hardy-Weinberg equilibrium using a sequential Bonferonni correction (Rice 1989).

Allele frequencies were determined for each population to establish population structure. An unrooted neighbour-joining cluster analysis was performed using two genetic distances; the microsatellite specific genetic distance D_{SW} of Shriver et al. (1995) which uses a stepwise mutation model and the genetic distance D_{CSE} of Cavalli-Sforza and Edwards (1967) which uses an infinite alleles model. Both analyses were boot strapped over loci 1000 times using Populations Version 1.2.24 (O. Langella, Centre National de la Recherche Scientifique, Laboratoire Populations, Genetique et Evolution, Gif sur Yvettev; http://www.cnrs-gif.fr/pge/bioinfo/populations) and viewed using Treeview (Page 1996) software.

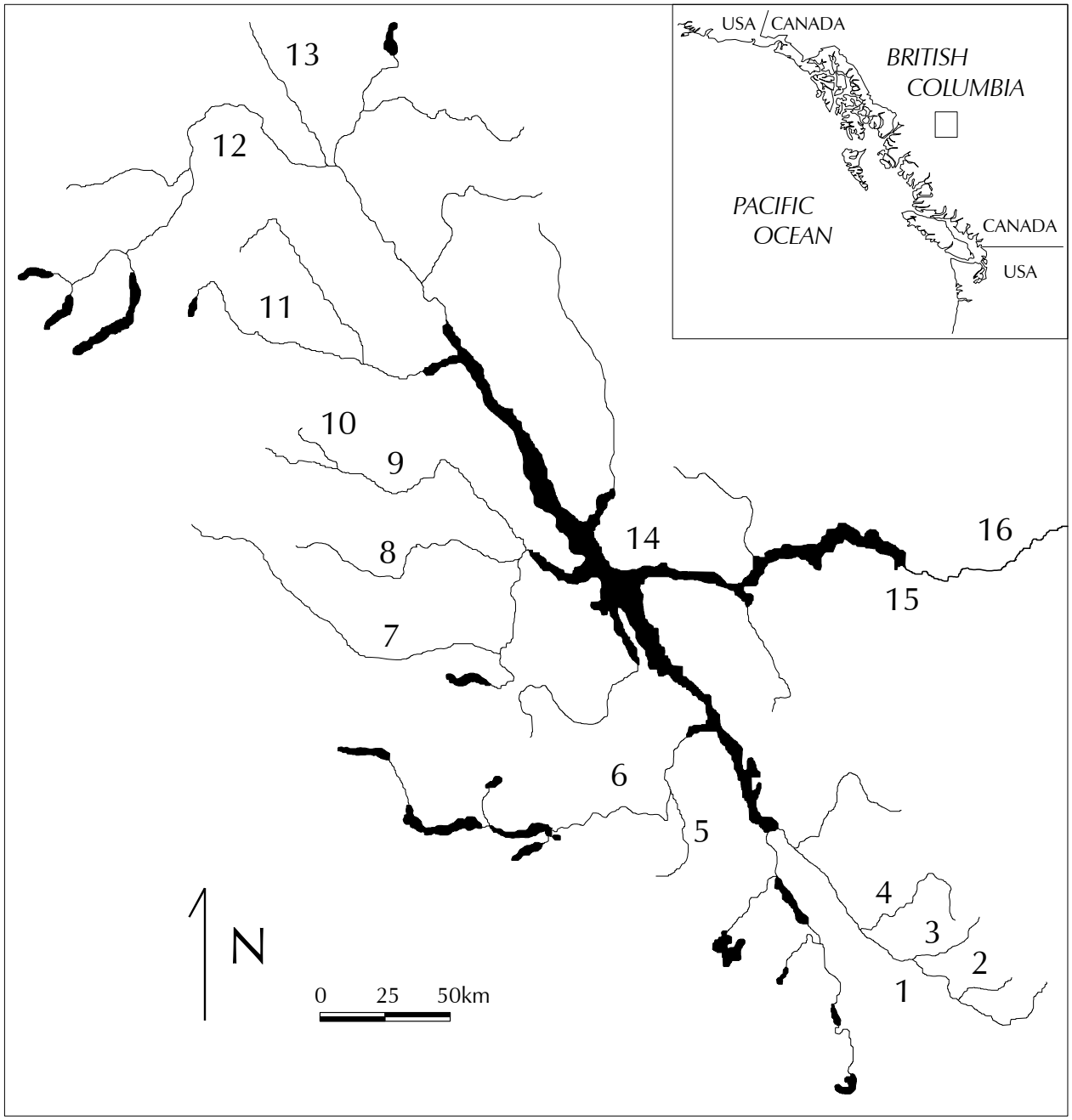

Figure 1. Map of major rivers and tributaries to the Williston Reservoir where Arctic grayling were sampled: 1, Parsnip River; 2, Missinka River; 3, Table River; 4, Anzac River; 5, Philip Creek; 6, Nation River; 7, Omineca River; 8, Osilinka River; 9, Mesilinka River; 10, Lay Creek; 11, Ingenika River; 12, Finlay River; 13, Fox River; 14, Williston Reservoir; 15, WAC Bennett Dam; and 16, Peace River.

Table 1. Location and number of Arctic grayling samples collected throughout the Williston Watershed. All samples collected in 2004 were adipose fin clips, other samples were scales.

Watershed	River	# samples	Comments
Parsnip	Table	37	22 UNBC 2004, 15 PWFWCP 2003
	Anzac	35	30 UNBC 2004, 5 PWFWCP 2004
	Missinka	16	7 Triton 1998, 9 PWFWCP 2005
Nation	Nation	35	19 UNBC 2004, 16 PWFWCP 2004
Omineca	Omineca	14	10 UNBC 2004, 4 PWFWCP 2001
	Osilinka	23	UNBC 2004
	Mesilinka	39	10 UNBC 2004, 29 PWFWCP 1999
Ingenika	Ingenika	31	21 PWFWCP 2004, 10 PWFWCP 2003
Finlay	Fox	15	6 RL&L 1999, 9 Triton 2004
Total		245	

Table 2. Microsatellite loci used for analysis of Arctic grayling population genetic structure. Sequences for forward and reverse primers, species and source are given from the original publications.

Primer		Sequence	Species	Published source
BFRO004	F	GCTCCAGTGAGGGTGACCAG	European grayling	Koskinen & Primmer 2001
	R	GTTTAGGCCACTGATTGAGCAGAG		
BFRO005	F	CGCATCTGTATGAAAAACCT	European grayling	Koskinen & Primmer 2001
	R	GTTTTGGTTTGGTAGGAGTTTCGT		
BFRO010	F	GGACGGAGCCAGCATCAC	European grayling	Koskinen & Primmer 2001
	R	GTTTGCCCCCAGGTTATCATAGCT		
BFRO012	F	TCTGCACATCCAAAGCCATC	European grayling	Koskinen & Primmer 2001
	R	GTTTAATCTCTCTTAATGAATCGT		
BFRO013	F	GATGTAGTTGCATTGCTTGCTCT	European grayling	Koskinen & Primmer 2001
	R	GTTTGGCTTTACCATTATCATATGAGC		
BFRO015	F	GACTCAGTGAAGAACTAAAGTACA	European grayling	Koskinen & Primmer 2001
	R	GTTTGAAAAGTTATGAAGGTCAACCC		
BFRO018	F	AGAGGGGTCCAGCAACATCA	European grayling	Koskinen & Primmer 2001
	R	GTTTGGGGAACCAGTCTAAAGCCT		
Str85 *INRA*	F	GGAAGGAAGGGAGAAAGGT	Brown trout	Presa & Guyomard 1996
	R	GGAAAATCAATACTAACAA		
One2	F	ACATCGCACACCATAAGCAT	Sockeye salmon	Scribner et al. 1996
	R	GTTTCGACTGTTTCCTCTGTGTTGAG		
Ogo2	F	GGTGCCAAGGTTCAGTTTATGTT	Pink salmon	Olsen et al. 1998
	R	CAGGAATTTACAGGACCCAGGTT		
Tar1	F	ACATATCATTCTTAGCATATC	Arctic grayling	Stamford & Taylor 2005
	R	CAAAATAGTAATTGAAATGC		

Table 3. Microsatellite loci used for analysis of Arctic grayling populations structure. Protocols and annealing temperatures were optimized for Williston watershed Arctic grayling. The number of alleles and allele size range were calculated using data from all populations combined.

Primer	Protocol / Annealing Temperature	# alleles	Range
BFRO04	5 cycles @ 63 °C / 30 cycles @ 61 °C	3	166 – 170
BFRO05	35 cycles @ 56 °C	7	120 – 134
BFRO10	35 cycles @ 56 °C	2	98 – 104
BFRO12	35 cycles @ 56 °C	8	186 – 246
BFRO13	35 cycles @ 56 °C	3	200 – 232
BFRO15	35 cycles @ 56 °C	5	146 – 154
BFRO18	35 cycles @ 56 °C	4	180 – 196
Str85 *INRA*	20 cycles TD[A] @ 55 °C / 18 cycles @ 45 °C	4	180 – 186
One2	10 cycles TD[B] @ 60 °C / 30 cycles @ 50 °C	2	254 – 262
Ogo2	35 cycles @ 56 °C	5	212 – 228
Tar1	5 cycles @ 52 °C / 30 cycles @ 50 °C	7	72 – 86

[A]touchdown protocol, decreasing 0.5 °C with each cycle
[B]touchdown protocol, decreasing 1 °C with each cycle

RESULTS

All 11 microsatellites amplified were polymorphic, but were variable in revealing genetic diversity within and among populations (Figure 2). Loci and sites were generally in Hardy-Weinberg equilibrium (Table 4). Probability tests at each locus across populations revealed only one locus that deviated significantly from Hardy-Weinberg equilibrium. Tar1 differed from equilibrium in the Nation River and Mesilinka River populations due to a deficiency of heterozygotes. There were repeated PCR amplification failures for 21 fish across all populations except the Finlay River at BFRO12 that showed strong PCR product at all other loci; suggesting the possibility of a null allele. Only one fish (Nation 31) did not amplify well for all microsatellites and was removed from the analysis.

The mean number of alleles across the eleven microsatellites within the nine populations ranged from 2.45 to 3.55 (Table 4). The total number of alleles across populations was higher, averaging 4.55 and ranged from two to eight at any single locus. The mean observed heterozygosity across loci within populations varied from 0.27 to 0.47 and expected heterozygosity from 0.32 to 0.45. Frequency of allele size varied by river system for each locus (Figure 3).

Analyses using genetic distances to construct neighbour-joining phylograms provided striking resolution of Arctic grayling genetic structure in the Williston Watershed. The unrooted neighbour-joining cluster analysis based on the D_{SW} genetic distance for microsatellite markers revealed a tree topology that was similar to geographic separation for each river (Figure 4). More southerly populations tended to group together and more northerly populations tended to group together. The exception was the position of the Mesilinka, Omineca and Osilinka River populations which showed considerable genetic difference yet are close together geographically.

In fact the Mesilinka River appears to be more closely related to the Finlay and Ingenika River populations than the Omineca River population. Interestingly the Nation River population grouped closely with the Osilinka and Omineca River populations. Unrooted neighbour-joining cluster analysis based on D_{CSE} genetic distance revealed a similar tree topology (Figure 5) that again reflected the geographic separation of the watersheds. Although the percent support for each branch between the southern populations is not strong, the D_{CSE} genetic distance again indicates considerable difference between the Mesilinka River and the Omineca / Osilinka River populations.

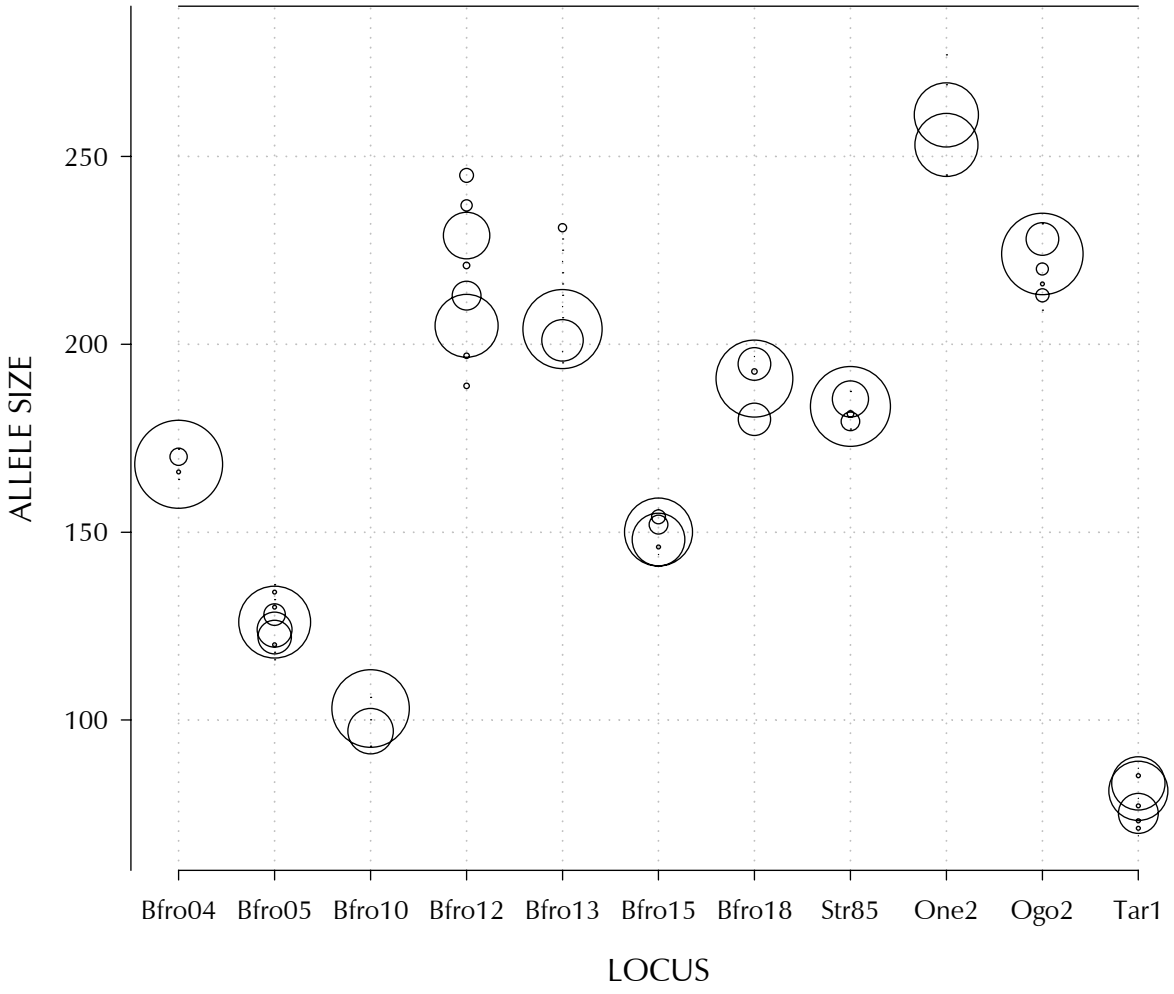

Figure 2. Allele frequencies and size distributions of 11 microsatellite loci in Arctic grayling from the Williston watershed. Areas of the bubbles correspond to the frequencies of the respective alleles.

Table 4. Sample sizes for each loci (N), allele numbers (A), and observed and expected heterozygosity (Ho and He) at 11 microsatellite loci for Williston Watershed Arctic grayling populations. Significant departures from Hardy–Weinberg equilibrium (after Bonferroni correction) are shown in bold italics.

	POP	Table	Anzac	Missinka	Nation	Omineca	Osilinka	Mesilinka	Ingenika	Fox
BFRO04	N	37	35	16	34	14	23	39	31	15
	A	1	1	2	1	1	2	2	2	2
	Ho	0.00	0.00	0.06	0.00	0.00	0.04	0.10	0.23	0.20
	He	0.00	0.00	0.06	0.00	0.00	0.04	0.09	0.29	0.18
BFRO05	N	37	35	16	34	14	23	39	31	15
	A	5	4	4	4	4	4	4	5	3
	Ho	0.59	0.54	0.50	0.65	0.57	0.73	0.44	0.45	0.33
	He	0.62	0.49	0.41	0.58	0.59	0.67	0.42	0.44	0.37
BFRO10	N	37	35	16	34	14	23	39	31	15
	A	2	2	2	2	2	2	2	2	2
	Ho	0.57	0.63	0.38	0.09	0.71	0.26	0.56	0.16	0.27
	He	0.48	0.49	0.43	0.08	0.46	0.29	0.44	0.29	0.23
BFRO12	N	35	31	14	32	12	22	37	25	15
	A	7	4	4	4	3	4	5	5	3
	Ho	0.77	0.35	0.50	0.38	0.42	0.50	0.46	0.28	0.47
	He	0.70	0.53	0.60	0.49	0.58	0.59	0.51	0.55	0.53
BFRO13	N	37	35	16	34	14	23	39	31	15
	A	3	2	2	2	2	2	2	2	2
	Ho	0.32	0.20	0.19	0.20	0.50	0.26	0.54	0.45	0.47
	He	0.36	0.22	0.17	0.26	0.43	0.29	0.40	0.48	0.36
BFRO15	N	37	35	16	34	14	23	39	31	15
	A	4	3	2	3	4	4	4	4	4
	Ho	0.68	0.43	0.50	0.38	0.57	0.43	0.62	0.74	0.67
	He	0.50	0.44	0.43	0.31	0.54	0.53	0.54	0.65	0.67
BFRO18	N	37	35	16	34	14	23	39	31	15
	A	4	3	2	3	4	3	3	4	3
	Ho	0.32	0.31	0.25	0.23	0.64	0.35	0.51	0.42	0.53
	He	0.28	0.37	0.22	0.30	0.49	0.45	0.45	0.60	0.46
Str85	N	37	35	16	34	14	23	39	31	15
	A	3	3	2	2	3	3	3	3	3
	Ho	0.38	0.43	0.19	0.06	0.64	0.35	0.38	0.39	0.33
	He	0.41	0.43	0.26	0.06	0.56	0.33	0.37	0.35	0.29
One2	N	37	35	16	34	14	23	39	31	15
	A	2	2	2	2	2	2	2	2	2
	Ho	0.38	0.26	0.50	0.15	0.53	0.48	0.38	0.29	0.27
	He	0.49	0.44	0.50	0.34	0.46	0.47	0.45	0.47	0.23
Ogo2	N	37	35	16	34	14	23	39	31	15
	A	3	2	2	3	3	3	2	4	3
	Ho	0.43	0.31	0.38	0.62	0.21	0.26	0.10	0.19	0.27
	He	0.37	0.30	0.38	0.50	0.19	0.23	0.09	0.18	0.24
Tar1	N	37	35	16	34	14	23	39	31	15
	A	5	3	3	3	4	3	5	3	3
	Ho	0.49	0.51	0.50	***0.20***	0.36	0.65	***0.49***	0.65	0.60
	He	0.68	0.60	0.55	0.65	0.61	0.62	0.56	0.62	0.62

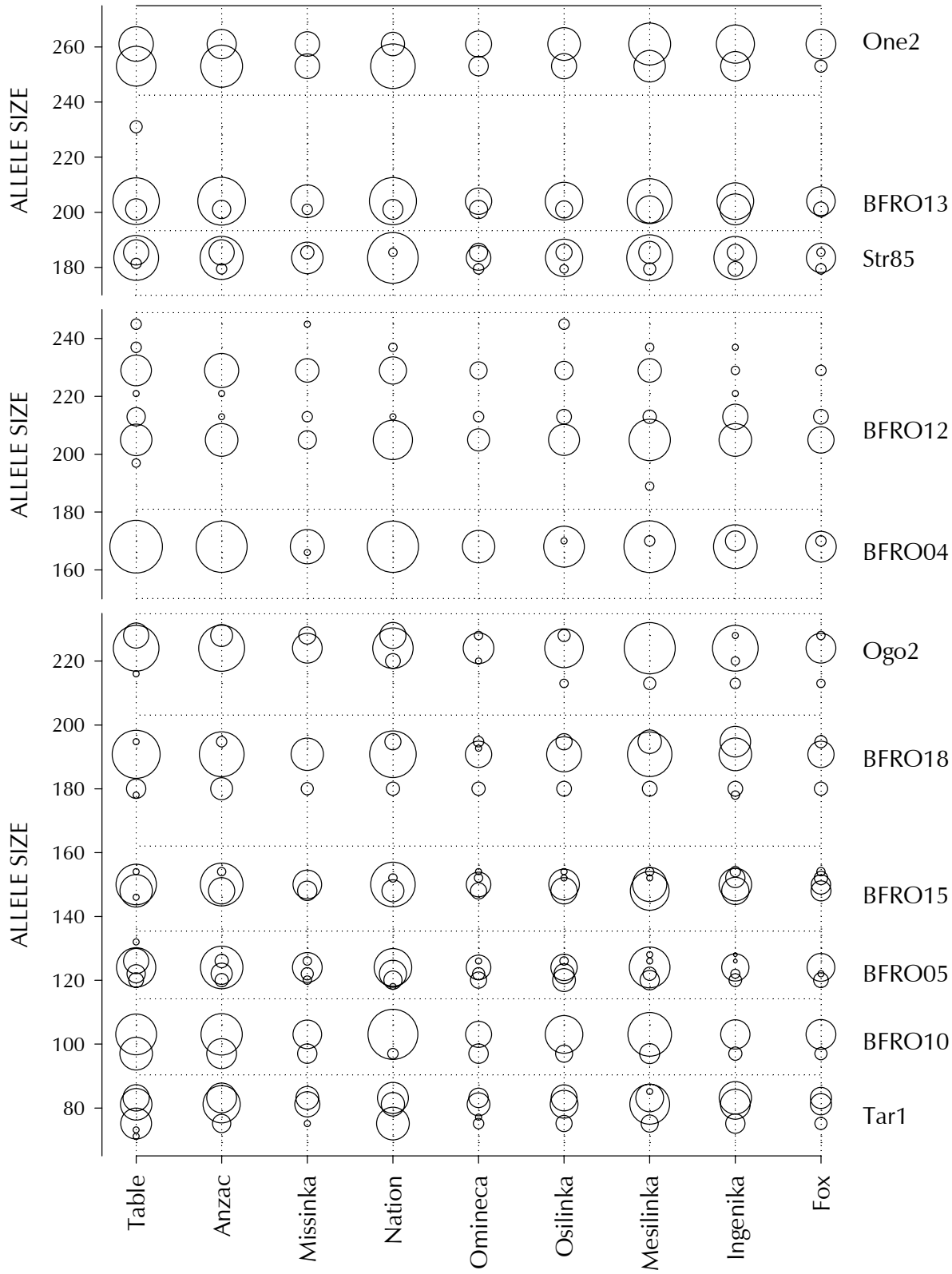

Figure 3. Allele frequencies and size distributions of 11 microsatellite loci in Arctic grayling from nine Williston watershed river systems. Areas correspond to the number of the respective alleles; allele frequency per population multiplied by number of fish per population.

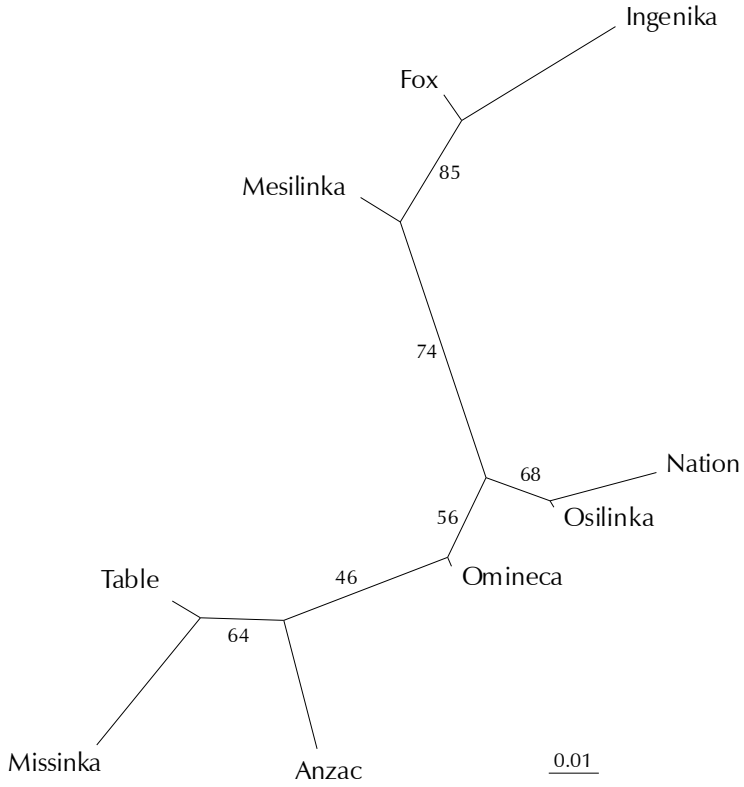

Figure 4. Unrooted neighbour-joining cluster analysis diagram based on D_{SW} genetic distance from Shriver et al. (1995) for microsatellite markers. The data were bootstrapped over loci, with replacement for 1000 replicates; the numbers represent the percent support of the branch.

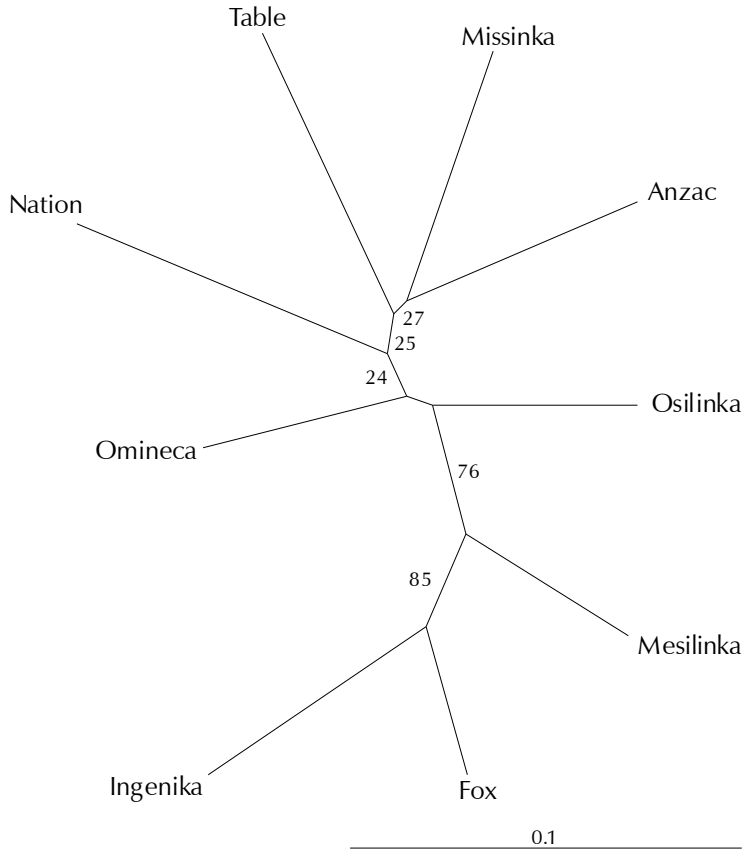

Figure 5. Unrooted neighbour-joining cluster analysis diagram based on the chord distance D_{CSE} from Cavalli-Sforza and Edwards (1967). The data were bootstrapped over loci, with replacement for 1000 replicates; the numbers represent the percent support of the branch.

DISCUSSION

Our data demonstrates genetic structure for Arctic grayling populations across the Williston watershed. Arctic grayling are widely distributed in freshwaters from the Hudson Bay to central Asia. In an examination of mitochondrial DNA (mtDNA) and microsatellite DNA variation in Arctic grayling from North America, Stamford and Taylor (2004) tested for genetic signatures of postglacial dispersal from glacial refugia. They suggest that extant North American Arctic grayling consist of at least three major lineages that originated in distinct Pleistocene glacial refugia. Grayling found in the upper Peace River dispersed from a South Beringia lineage which includes populations found from western Alaska to northern British Columbia. The fish collected in our sample, therefore, represent populations that have diverged from a common ancestral lineage. Smaller scale population structure has also been resolved for Williston watershed grayling. In a recent study examining upper Peace River Arctic grayling populations, Stamford and Taylor (2005) found significant microsatellite divergence among populations and strong isolation by distance among samples. A significant proportion of the microsatellite variation was attributable to differences between samples above and below the historic natural barrier to upstream fish migration, the Peace River Canyon. This region now represents a permanent barrier to fish passage with the construction of the Peace Canyon Dam and the WAC Bennett Dam further upstream.

Our study was restricted to populations of grayling upstream of the historic barrier to fish migration in the Peace River Canyon, but there is evidence that the flooding of the Williston Reservoir has further fragmented the population. Clarke et al. (2005) used otolith microchemistry analysis to determine Arctic grayling movement and suggested that the Williston Reservoir now limits movement. They argued that grayling in tributaries to the Williston Reservoir are fluvial and do not use habitat within the reservoir. Grayling may rear in the embayments where fluvial habitat still exists. There was no overlap between water chemistries of the Parsnip, Omineca, and Ingenika watersheds and the reservoir based on expected Sr and Ba concentrations measured in the otolith. Water chemistry of the Nation system was similar to the reservoir and use of the reservoir for this population could not be ruled out. It is not likely, however, that grayling from the Nation River would be the only adfluvial population in the Upper Peace watershed. The fact that grayling do not appear to leave their respective watersheds throughout their entire life history suggests that the reservoir limits movement for fluvial grayling and isolates the populations. Consequently, the lack of grayling throughout the reservoir represents a barrier to gene flow and lack of migration among the major river systems has forced geographic separation of grayling populations.

The genetic structure indicates that the grayling populations examined were likely distinct before the flooding of the watershed. Additionally we have shown that microgeographic population structure exists for Arctic grayling in this watershed. For example at the southern end of the reservoir, we analyzed three river systems within the Parsnip River drainage; the Table, Anzac and Missinka Rivers. We found that the allele frequency differed among these rivers (Table 4), distinctive rare alleles were present in some of these river populations (Figure 3), and neighbour-joining trees based on two different genetic distances provided support for population separation (stepwise mutation model, Figure 4; infinite alleles model, Figure 5). Similarly, there were differences within the Omineca River watershed. The Osilinka River consistently grouped with the Omineca, but the Mesilinka River tended to group with the

more northern river systems (Figures 4 and 5). We expect that similar microgeographic population structure also exists for the Nation, Ingenika and Finlay River watersheds. In this report, the Finlay River samples represent a group of fish captured from the Fox River. In 2006, additional Arctic grayling were captured from the Upper Finlay River and a major tributary to the Upper Finlay River, the Toodoggone River by the PWFWCP. With the analysis of these samples, we will be able to determine if a similar pattern of genetic separation exists within this watershed and our overall analysis will be strengthened. Three of the populations had fairly small numbers of samples; Missinka 16, Omineca 14, and Fox 15. The sample sizes may not have been adequate to reflect variation in some of the more variable markers and the number of alleles for the most variable microsatellite, BFRO12, was less for these small samples (Figure 3, Table 4). The number of loci used in the analysis, however, would help to offset the small sample size. Additionally, our genetic distances among populations were large. Kalinowski (2005) using computer simulations found that for large genetic distances among populations sampling fewer than 20 individuals per population should be sufficient.

Number of alleles within the nine populations of Williston watershed grayling in this study was low (2.45 to 3.55, Table 4), but similar to values reported by Stamford and Taylor (2005). It is interesting to note that other interior salmonids also show relatively low numbers of alleles and low values of heterozygosity. Westslope cutthroat trout (*Onocorhynchus clarki lewisi*) from British Columbia and Montana had low numbers of alleles per microsatellite; average 3.9 and 3.7, respectively (Taylor et al. 2003; Boyer et al. 2008). Bull trout (*Salvelinus confluentus*) sampled from a similar geographic range also show relatively low numbers of alleles; 2.9 from Idaho (Spruell et al. 1999) and 4.6 from British Columbia (Costello et al. 2003). Additionally, Costello et al. (2003) found a decrease in the number of alleles and heterozygotes in populations on the periphery of the range relative to populations closer to the putative glacial refugia suggesting historical influences on microsatellite variation. Williston grayling dispersed from South Beringia and would be considered to be on the periphery of their range. Consequently, low numbers of alleles and levels of heterozygosity is not surprising.

The clear structure of Arctic grayling populations suggests that for management purposes each population should be treated as an isolated discrete unit. Our data indicates that the population structure observed with the otolith micro-element analysis by Clarke et al. (2005) has a genetic basis that existed before the formation of the reservoir. Clarke et al. (2005) also suggest that reservoir use is not occurring and the reservoir is effectively a barrier to movement. The Arctic grayling populations in the Williston watershed, therefore, are more isolated than usual inland salmonids. It is unlikely that genetic exchange exists among the populations, making them more susceptible to stochastic events associated with small population size. These populations will also be more vulnerable to extinction as natural re-colonization is unlikely to occur.

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