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Genetic analysis of Arctic grayling population structure in the Williston Watershed: samples from the Finlay River

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SUMMARY

In a previous study funded by the Peace / Williston Fish & Wildlife Compensation Program (PWFWCP) we examined genetic population structure of Arctic grayling (*Thymallus arcticus*) from nine river systems flowing into the Williston Reservoir. In this report, we expand on the analysis with inclusion of samples collected from the Finlay River system and two major tributaries. Fin clips and scale samples were analyzed from up to 40 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 9 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. Our results also suggest population structure within larger watersheds as samples from the Fox River differed from fish collected in the upper Finlay watershed. For populations sampled throughout the watershed, we found a highly significant isolation-by-distance relationship.

INTRODUCTION

For the Williston watershed there is evidence for discrete spawning populations of Arctic grayling. Elemental signatures of bony structures from Arctic grayling show little overlap among river systems, suggesting that discrete populations exist within the Williston Watershed (Clarke et al. 2007). Stamford & Taylor (2005) found significant genetic divergence among populations from the Upper Peace and a strong pattern of isolation-by-distance. In an earlier project, we used microsatellite analysis to determine genetic structure and found a pattern that strongly reflected geographic location (Shrimpton et al. 2012). This study however focussed on populations of grayling south of the Ingenika River systems. In August 2006 and August 2007, the Peace / Williston Fish & Wildlife Compensation Program conducted sampling programs for fish throughout the Finlay River watershed. These samples represent fish from populations over a considerable geographic range and we have included the samples into our previous analysis.

Our objective was to use genetic methods to determine if stock structure also exist for the Finlay River watershed. 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 the Finlay River in comparison to other 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 five of the major river systems of the Williston Watershed.

METHODS AND MATERIALS

Adult Arctic grayling were collected from rivers flowing into the Williston Watershed. For samples collected before 2004, scales were removed and stored dry. For samples collected in 2004 and after, a fin clip was collected and stored in ethanol (Figure 1; Table 1). Adult grayling were mainly captured in the mainstems of each river and correspondingly grouped by river with the exception of the Finlay River. Fish were caught over a wide geographic range in the Finlay and grouped by major tributary within that section of the river; fish from the Fox River and Finlay

River near the Fox were grouped together, fish upstream of Bower Creek and below the falls were referred to as Finlay, fish above the falls and below the Fishing Lakes were grouped as Mid Finlay, fish collected in the Toodoggone River and near the confluence with the Finlay were grouped as Toodoggone, and fish caught in the Finlay River above the confluence with the Toodoggone River were grouped as Upper Finlay.

Fin clips and scale samples were analyzed from up to 40 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 using 11 polymorphic microsatellite primers (Table 2). 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 the microsatellite specific genetic distance D_{SW} of Shriver et al. (1995) which uses a stepwise mutation model. Analysis was boot strapped over loci 4000 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.

Pairwise Fst values were calculated for all population comparisons using GenAlEx (Peakall and Smouse 2006). Geographic distances between each population were calculated using the Geographic Information System ArcView and the network application for tracing distances along river lengths. Isolation by distance relationships were determined by plotting Fst against geographic distance.

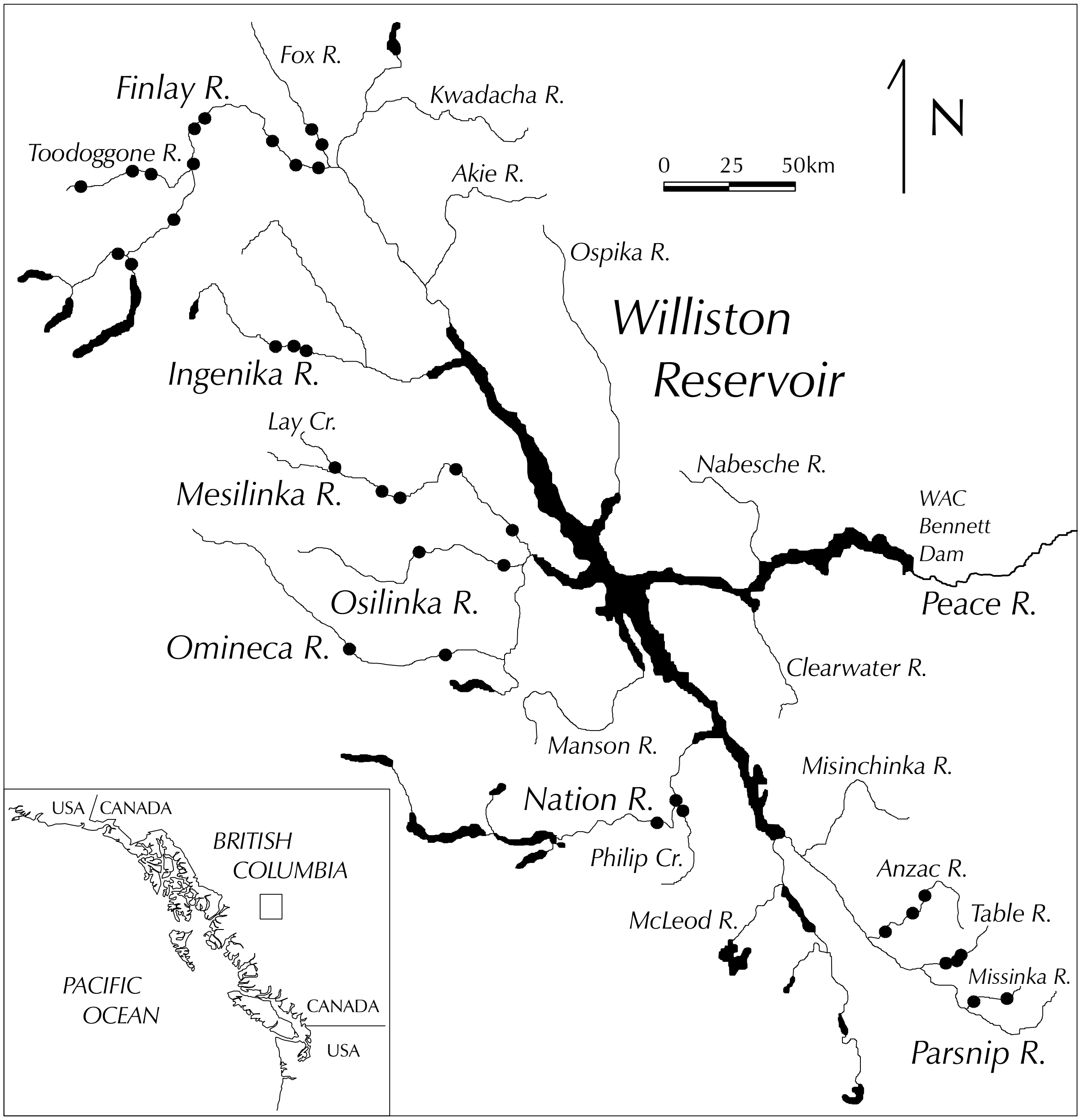

Figure 1. Map of major rivers and tributaries to the Williston Reservoir where Arctic grayling were sampled. Closed circles indicate locations where fish were caught.

Table 1. Location and number of Arctic grayling samples collected throughout the Williston Watershed. All samples collected in 2004-2007 were adipose fin clips, other samples were scales.

Watershed	River	# samples	Comments
Parsnip	Table	37	22 UNBC '04, 15 PWFWCP '03
	Anzac	35	30 UNBC 2004, 5 PWFWCP '04
	Missinka	16	7 Triton '98, 9 PWFWCP '05
Nation	Nation	35	19 UNBC '04, 16 PWFWCP '04
Omineca	Omineca	23	10 UNBC '04, 13 PWFWCP '01
	Osilinka	23	UNBC '04
	Mesilinka	39	10 UNBC '04, 29 PWFWCP '99
Ingenika	Ingenika	31	21 PWFWCP '04, 10 PWFWCP '03
Finlay	Finlay	24	PWFWCP '07
	Fox	27	6 RL&L '99, 9 Triton '04, 12 PWFWCP '07
	Mid Finlay	16	PWFWCP '06
	Upper Finlay	28	PWFWCP '06
	Toodoggone	40	PWFWCP '06
Total		374	

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 ºC / 30 cycles @ 61 ºC	3	166 – 170
BFRO05	35 cycles @ 56 ºC	7	120 – 134
BFRO10	35 cycles @ 56 ºC	2	98 – 104
BFRO12	35 cycles @ 56 ºC	8	190 – 246
BFRO13	35 cycles @ 56 ºC	3	202 – 232
BFRO15	35 cycles @ 56 ºC	5	146 – 154
BFRO18	35 cycles @ 56 ºC	5	180 – 196
Str85 *INRA*	20 cycles TD[A] @ 55 ºC / 18 cycles @ 45 ºC	4	180 – 186
One2	10 cycles TD[B] @ 60 ºC / 30 cycles @ 50 ºC	3	254 – 270
Ogo2	35 cycles @ 56 ºC	5	212 – 228
Tar1	5 cycles @ 52 ºC / 30 cycles @ 50 ºC	8	70 – 88

[A]touchdown protocol, decreasing 0.5 ºC with each cycle
[B]touchdown protocol, decreasing 1 ºC 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. One fish (Nation 31) did not amplify well for all microsatellites and was removed from the analysis. Additionally, five fish from the Upper Finlay and six fish from the Toodoggone River did not amplify well and were also removed from the analysis.

The mean number of alleles across the eleven microsatellites within the 13 populations ranged from 2.75 to 3.60 (Table 4). The total number of alleles across populations was higher, averaging 3.91 and ranged from two to eight at any single locus. The mean observed heterozygosity across loci within populations varied from 0.28 to 0.50 and expected heterozygosity from 0.34 to 0.46.

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 2). More southerly populations tended to group together and more northerly populations tended to group together. There was more variation for rivers that flow into the middle part of the reservoir. The northern rivers separated onto two branches; rivers below the falls on the Finlay River (Finlay, Fox and Ingenika) and rivers above the falls (Mid Finlay, Upper Finlay and Toodoggone).

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	Finlay	Fox	MFinlay	UFinlay	Toodoggone
BFRO04	N	37	35	16	34	23	23	39	31	24	27	16	23	33
	A	1	1	2	1	1	2	2	2	2	2	2	2	2
	Ho	0.00	0.00	0.06	0.00	0.00	0.04	0.10	0.23	0.08	0.22	0.19	0.09	0.12
	He	0.00	0.00	0.06	0.00	0.00	0.04	0.09	0.29	0.16	0.20	0.18	0.08	0.11
BFRO05	N	37	35	16	34	23	23	39	31	24	26	14	23	34
	A	5	4	4	4	4	4	4	5	3	3	2	3	3
	Ho	0.59	0.54	0.50	0.65	0.65	0.73	0.44	0.45	0.50	0.42	0.43	0.57	0.44
	He	0.62	0.49	0.41	0.58	0.61	0.67	0.42	0.44	0.49	0.45	0.48	0.48	0.46
BFRO10	N	37	35	16	34	23	23	39	31	24	27	16	23	34
	A	2	2	2	2	2	2	2	2	2	2	2	2	2
	Ho	0.57	0.63	0.38	0.09	0.65	0.26	0.56	0.16	0.29	0.26	0.31	0.17	0.12
	He	0.48	0.49	0.43	0.08	0.47	0.29	0.44	0.29	0.25	0.28	0.35	0.34	0.11
BFRO12	N	35	31	14	32	21	22	37	25	22	26	15	23	32
	A	6	4	6	6	6	6	6	6	5	5	4	4	5
	Ho	0.77	0.35	0.50	0.38	0.57	0.50	0.46	0.28	0.55	0.58	0.40	0.61	0.47
	He	0.67	0.53	0.60	0.49	0.62	0.59	0.51	0.55	0.51	0.64	0.49	0.58	0.47
BFRO13	N	37	35	16	34	23	23	39	31	24	27	16	23	34
	A	3	2	2	2	2	2	2	2	2	2	2	2	2
	Ho	0.32	0.20	0.19	0.20	0.30	0.26	0.54	0.45	0.50	0.44	0.31	0.35	0.24
	He	0.36	0.22	0.17	0.26	0.31	0.29	0.40	0.48	0.38	0.39	0.27	0.42	0.45
BFRO15	N	37	35	16	34	23	23	39	31	24	27	16	23	32
	A	4	3	2	3	4	4	5	4	4	4	4	4	4
	Ho	0.68	0.43	0.50	0.38	0.61	0.43	0.62	0.74	0.83	0.70	0.69	0.78	0.66
	He	0.50	0.44	0.43	0.31	0.58	0.53	0.54	0.65	0.75	0.72	0.68	0.63	0.67

Table 4. continued.

	POP	Table	Anzac	Missinka	Nation	Omineca	Osilinka	Mesilinka	Ingenika	Finlay	Fox	MFinlay	UFinlay	Toodoggone
BFRO18	N	37	35	16	34	23	23	39	31	24	27	16	23	32
	A	3	3	2	3	4	3	3	4	3	3	3	3	2
	Ho	0.32	0.31	0.25	0.23	0.61	0.35	0.51	0.42	0.58	0.56	0.25	0.39	0.44
	He	0.28	0.37	0.22	0.30	0.48	0.45	0.45	0.60	0.50	0.49	0.32	0.43	0.40
Str85	N	37	35	16	34	23	23	39	31	24	27	15	23	33
	A	3	3	2	2	4	3	3	3	3	3	4	3	3
	Ho	0.38	0.43	0.19	0.06	0.52	0.35	0.38	0.39	0.50	0.22	0.33	0.52	0.55
	He	0.42	0.43	0.26	0.06	0.61	0.33	0.37	0.35	0.44	0.40	0.49	0.59	0.57
One2	N	37	35	16	34	19	23	39	31	17	26	11	21	28
	A	2	2	2	2	2	2	2	2	2	2	2	3	3
	Ho	0.38	0.26	0.50	0.15	0.42	0.48	0.38	0.29	0.53	0.35	0.27	0.38	0.36
	He	0.49	0.44	0.50	0.34	0.50	0.47	0.45	0.47	0.51	0.29	0.45	0.49	0.50
Ogo2	N	37	35	16	34	23	23	39	31	24	27	16	23	34
	A	3	2	2	3	3	3	2	4	3	3	2	3	3
	Ho	0.43	0.31	0.38	0.62	0.13	0.26	0.10	0.19	0.46	0.30	0.25	0.30	0.26
	He	0.37	0.30	0.38	0.50	0.13	0.23	0.09	0.18	0.37	0.27	0.39	0.26	0.33
Tar1	N	37	35	16	34	23	23	39	31	24	25	16	23	33
	A	5	3	3	3	4	3	5	3	3	3	2	2	2
	Ho	0.49	0.51	0.50	***0.20***	0.39	0.65	***0.49***	0.65	0.58	0.56	0.62	0.52	0.48
	He	0.68	0.60	0.55	0.65	0.55	0.62	0.56	0.62	0.60	0.59	0.44	0.45	0.48

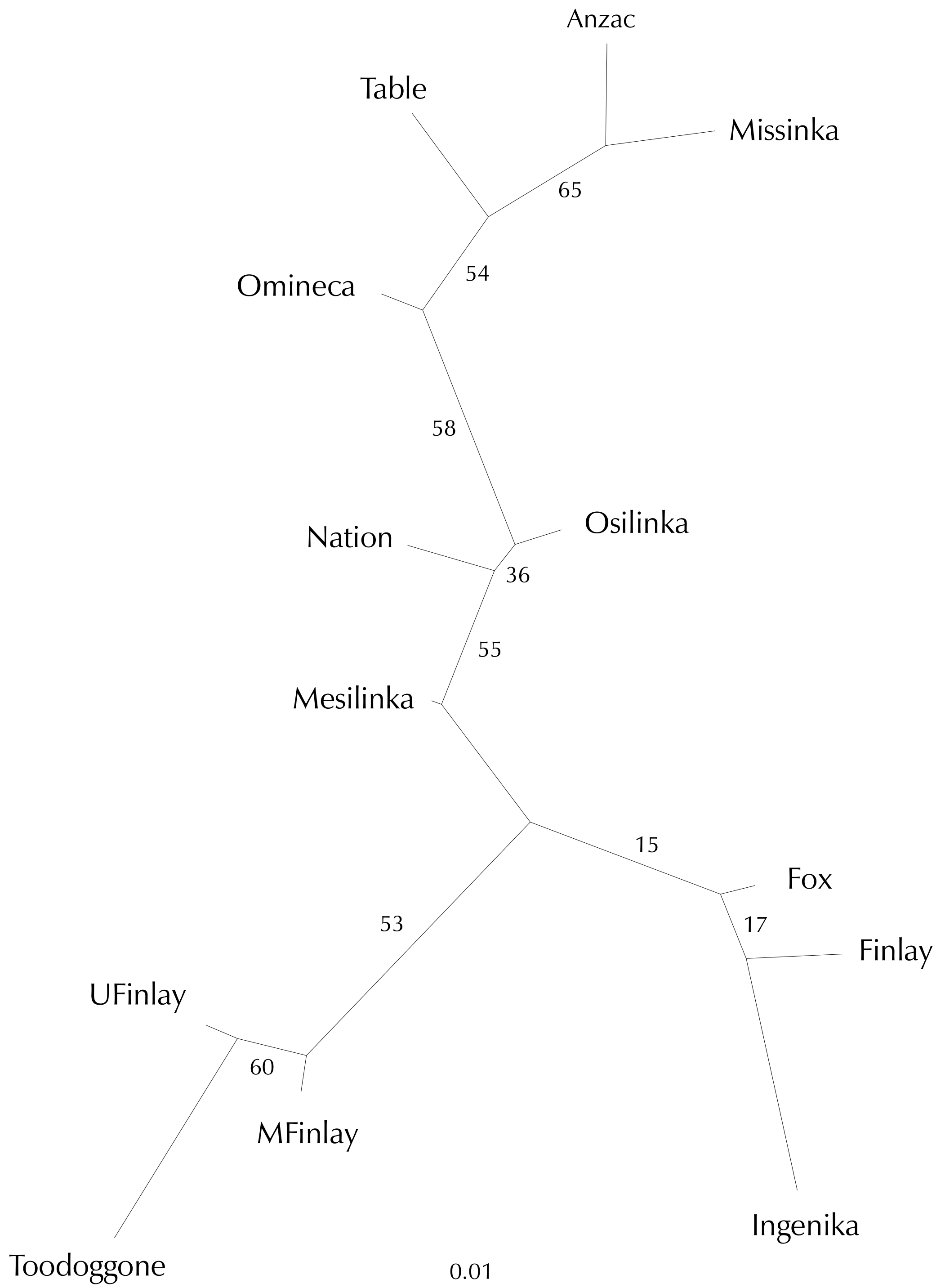

Figure 2. Unrooted neighbour-joining cluster analysis diagram based on D_{SW} genetic distance from Shriver et al. (1995) for microsatellite markers. The data were bootstrapped over loci, with replacement for 4000 replicates; the numbers represent the percent support of the branch.

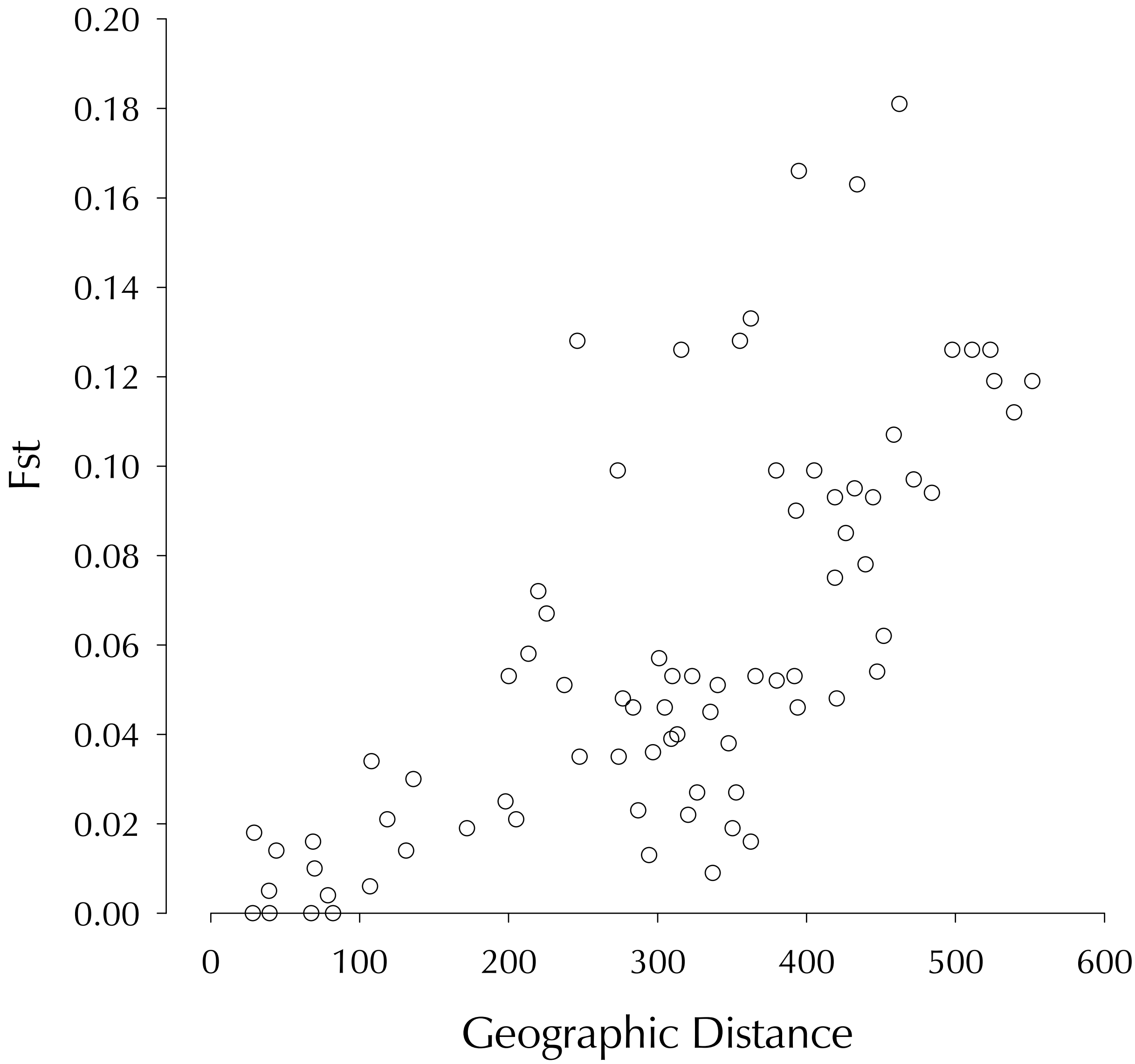

Figure 3. Isolation-by-distance in Arctic grayling inferred from Fst estimated from variation at eleven microsatellite loci for all pairwise measurements compared to geographic distance between populations measured in km.

DISCUSSION

The present analysis has expanded on our previous work and data demonstrates genetic structure for Arctic grayling populations across the Williston 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) and neighbour-joining trees based on two different genetic distances provided support for population separation (stepwise mutation model, Figure 2). Similarly, there were differences within the Omineca River watershed. The Osilinka River, the Omineca, and the Mesilinka River did not group particularly closely together. Within the Finlay River system, two branches on the neighbour-joining tree included rivers from this system. The separation of these two branches is consistent with a flow constriction and high gradient reach on the mid section of the Finlay River. Although, Fst values did not differ significantly between the Finlay River samples from populations above and below this reach, the Fox River Fst values differed significantly from fish collected above the high gradient reach. A barrier to upstream gene flow in the Finlay River, therefore, may exist.

Genetic population structure has been shown to exist among a wide diversity of salmonids, in both migratory and resident forms. When genetic distances are correlated with geographical distance (isolation by distance model of gene flow), populations have believed to be in migration-mutation equilibrium (Heath et al. 2001 for steelhead, *Oncorhynchus mykiss*; Stamford and Taylor 2005 for Arctic grayling, *Thymallus arcticus*). Not all populations, however, have been found to be in equilibrium. Work on brook charr, *Salvelinus fontinalis*, did not find equilibrium in genetic structure. The lack of a relationship was hypothesized to be due to landscape rearrangement as population differentiation was strongly correlated with historic landscape features (Poissant et al. 2005). Despite the large impact by the creation of the reservoir, our finding of a highly significant isolation-by-distance relationship indicates that Williston Arctic grayling populations are in equilibrium.

Our data indicate that Arctic grayling in the Williston Watershed exhibit significant levels of genetic subdivision at microsatellite loci. This finding is consistent with previous work reported by Stamford and Taylor (2005), but also supports the findings by Clarke et al. (2007) using elemental analysis. Given the significant population subdivision within the watershed and the strong pattern of isolation-by-distance, Arctic grayling should be managed to maintain these levels of restricted gene flow.

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