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ARTICLE

Fine-Scale Population Genetic Structure and Dispersal of Juvenile Steelhead in the Bulkley-Morice River, British Columbia

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Abstract

A knowledge of fine-scale population genetic structure and patterns of dispersal is an essential component of any action to conserve genetic diversity and maintain population viability. We genotyped 417 juvenile steelhead *Oncorhynchus mykiss* from the main stem and tributaries of the Bulkley–Morice River, British Columbia, at 10 microsatellite loci to assess fine-scale population structure and the patterns and magnitude of juvenile dispersal and mixing. We detected significant genetic structuring among juvenile steelhead from seven tributaries of the Bulkley–Morice River (pairwise F_{ST}: 0.008–0.156) and found significant isolation by distance among the tributary populations ($R^2 = 0.198$, $P = 0.038$). These results reflect the homing behavior of spawning adults as well as the temporal stability of those populations. Genotype assignment of tributary-caught juveniles showed that rates of juvenile dispersal varied among tributaries. The assignment of juveniles sampled from the main stem of the river to source tributary populations suggested that long-distance movement in juvenile steelhead is common and that juveniles are well mixed in the main stem. Dispersal and fine-scale genetic structure in pristine steelhead populations are more complex than previously thought. Therefore, actions to conserve Bulkley–Morice River steelhead must strive to maintain the genetic diversity of tributary populations.

The conservation of genetic diversity within and among natural populations, especially those that are exploited as a resource, is a priority for maintaining the health and viability of those populations. Genetic diversity is linked to population fitness and the ability of populations to evolve with changing environments (Reed and Frankham 2003). Knowledge of population substructure is thus a critical component of any plan to conserve genetic diversity as it identifies the limits of gene flow among subpopulations and determines the scale at which conservation actions will be most effective. In addition, population genetic structure is used for genetic stock identification and subsequent management actions to conserve declining or at-risk stocks in mixed fisheries (e.g., Shaklee et al. 1999; Beacham et al. 2004b). Finally, determining the

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spatial scale at which populations are structured is important because it generally defines the scale at which populations may experience local adaptation (Taylor 1991).

The highly specific homing behavior of spawning adult salmonids generally defines their population structure; however, the spatial scale of structuring that has been used in genetic stock identification is much coarser than the scale at which homing has been demonstrated (Quinn et al. 1999, 2006; Stewart et al. 2003; Neville et al. 2006). Recent studies have shown that fine-scale population structure exists for various salmonid species (Young et al. 2004; Grandjean et al. 2009; Kitanishi et al. 2009). Such studies highlight the potential impact of cryptic fine-scale population structuring on the effective conservation of salmonid populations (Dionne et al. 2009); however, dispersal of individuals leads to the homogenization of genetically divergent groups and can confound fine-scale genetic structure (Walter et al. 2009). Dispersal in salmonid fishes is species-, life stage-, and even population-specific (Quinn 2005). Upon emergence from the gravel as fry, stream-resident salmonids disperse locally (often downstream) to establish foraging territories. Further movement is associated with seasonal changes in stream flow (Quinn 2005), competitive displacement (Gowan and Fausch 1996), and habitat patch quality assessment (Kahler et al. 2001). Such movements lead to mixed groups of individuals that can confound traditional genetic approaches to detecting fine-scale structure among groups of juvenile salmonids.

Steelhead *Oncorhynchus mykiss* (anadromous rainbow trout) exhibit specific homing and low rates of straying (Shapovalov and Taft 1954) that result in population structure defined at multiple spatial scales for this species (Beacham et al. 1999, 2000, 2004a; Heath et al. 2001; Winans et al. 2004). There is some indication that fine-scale population structure exists in steelhead (Narum et al. 2006, 2008; Pearse et al. 2007); however, the studies reporting fine-scale structure in steelhead include populations of introduced hatchery strains and populations influenced by artificial barriers. Kahler et al. (2001) reported juvenile steelhead movements in coastal streams that indicate mixing occurs and has the potential to obscure fine-scale structure analyses of juvenile steelhead populations.

The Bulkley–Morice River is the largest tributary of the Skeena River in northwest British Columbia. It drains a watershed area of approximately 12,000 km^2 and supports important recreational and cultural steelhead fisheries. The river system is relatively pristine owing to low human population density and the absence of anthropogenic barriers to anadromy and dispersal. Steelhead spawn in the many tributaries of this river system, but spawning in the main stem of the river is thought to be rare. We used microsatellite markers to characterize fine-scale population structure among populations of juvenile steelhead from tributaries of the Bulkley–Morice River. We applied genotype assignment procedures, similar to those used in genetic stock identification, to assess the influence of mixing on fine-scale genetic structure in steelhead among tributaries and to examine patterns of juvenile movement in the main stem of the river.

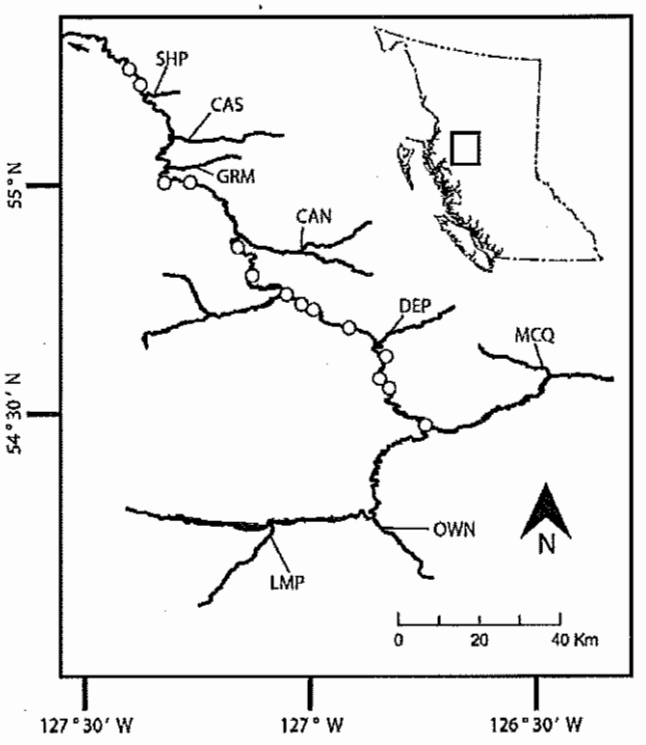

FIGURE 1. The Bulkley–Morice River, British Columbia, showing mainstem sampling sites (open circles) and tributary sampling sites for steelhead. CAN = Canyon Creek, CAS = Causqua Creek, DEP = Deep Creek, GRM = Gramophone Creek, LMP = Lamprey Creek, MCQ = McQuarrie Creek, OWN = Owen Creek, SHP = Sharpe Creek.

METHODS

Sample collection.—A total of 255 juvenile steelhead were collected from six Bulkley River and two Morice River tributary creeks in the late summer and early fall of 1998, and 162 juveniles were collected from 14 locations in the main stem of the river in late summer and early fall of 1999 (Figure 1). The closest tributary sites sampled were 14 km apart, measured as the shortest distance along the river using digital 1:400,000 scale maps. The most distant sites sampled were separated by 170 km (Figure 1). Fish were captured by using a combination of baited Gee minnow traps and electrofishing (Smith-Root BP15 electrofisher), and individuals were nonlethally sampled by taking an adipose or caudal fin clip, which was preserved in 95% ethanol for later DNA extraction.

Genotyping and data analysis.—The DNA was extracted from fin clips with the Promega Wizard Kit salt-based extraction. DNA was eluted in 100 μL of tris–EDTA buffer (10 mM tris, 1 mM EDTA, pH 8). Each fish was genotyped at 10 previously described microsatellite loci: *Omy87* (Olsen et al. 1996); *Ots4* (Banks et al. 1999); *RT36*, *RT119*, *RT191*, and *RT212* (Spies et al. 2005), and *OtsG83b*, *OtsG249*, *OtsG253b*, and *OtsG401* (Williamson et al. 2002). Polymerase chain reactions (PCRs) had a final volume of 11 μL and contained 1 μL DNA diluted to one-half its original concentration,

1.8 mM $MgCl_2$, 230 μM of each deoxynucleotide triphosphate, 0.91 ng/mL of dye-labeled forward primer, 1.8 ng/mL reverse primer, 0.4 units of *Taq* DNA polymerase (Sigma-Aldrich) in a 1 × PCR buffer. Amplification used the following protocol: 2 min at 95°C; 30 cycles of 15 s at 95°C, 15 s at 54°C (*RT36*), 55°C (*Ots4*), 56°C (*OtsG83b*), 58°C (*Omy87*, *RT119*, *RT212*, *OtsG249*, *OtsG253b*), 62°C (*OtsG401*), or 63°C (*RT191*); and 30 s at 72°C, followed by a 2-min extension at 72°C and a 4°C hold. Allele sizes were scored by means of a Li-Cor 4300 DNA analyzer and Gene ImagIR software (Scanalytics).

Microsatellite data were tested for linkage disequilibrium and departures from Hardy–Weinberg equilibrium (HWE) in Genepop 4.0.10 (10,000 dememorization steps, 100 batches, 5,000 permutations per batch; Raymond and Rousset 1995; Rousset 2008). To correct for multiple tests, significance was determined with the sequential Bonferroni method (Rice 1989). The data were also tested for null alleles and large allele dropout with Microchecker version 2.2.3 (Van Oosterhout et al. 2004). Exact tests of allele frequency distributions were used to test for tributary population differentiation (Genepop 4.0.10; 10,000 dememorization steps, 20 batches, 5,000 permutations per batch). Significance for multiple tests was again corrected by means of the sequential Bonferroni method (Rice 1989). Weir and Cockerham's (1984) unbiased estimator of F_{ST} was calculated for all pairs of tributary populations and the 95% confidence intervals were determined with a bootstrap analysis in Arelquin 3.11 (Excoffier et al. 2005). To correct for differences in heterozygosity among loci we also calculated G'_{ST} (Hedrick 2005) for each population pair in SMOGD (version 1.2.5; Crawford 2010). Pairwise chord distances (D_C; Cavalli-Sforza and Edwards 1967) and a neighbor-joining cluster analyses based on D_C with 1,000 bootstrapped replicates were calculated with the software package Phylip 3.69 (Felsenstein 2005). Chord distances were chosen because they have been demonstrated to provide accurate tree topology for closely related populations (Angers and Bernatchez 1998).

An isolation-by-distance analysis was conducted with a Mantel test to test for a correlation between pairwise geographic distances and linearized pairwise genetic distances [$F_{ST}/(1 - F_{ST})$] in Tools for Population Genetics Analyses (TFPGA; Mark Miller, U.S. Geological Survey). We used the recommended F_{ST} transformation [$F_{ST}/(1 - F_{ST})$] for isolation-by-distance analyses when habitats are distributed in one dimension (Rousset 1997), as they are in this study. Isolation by distance analysis was also conducted with G'_{ST}.

The fish sampled from Causqua Creek produced results in all analyses that were not consistent with other tributary populations. Allelic richness and observed heterozygosity at all loci were severely reduced in this population compared with those in the other tributaries. We calculated pairwise relatedness coefficients by using a method-of-moments estimator approach (RI; Ritland 1996) in GenAlEx 6.4 (Peakall and Smouse 2006) for individuals within each tributary population and all fish sampled from the main stem to assess whether sampling-related individuals may explain the anomalous results. All analyses were subsequently conducted both with and without the Causqua Creek population.

Nonnatal-rearing individuals (hereafter, dispersers) in the tributary samples were detected by using partial Bayesian genotype assignment (Rannala and Mountain 1997) with the leave-one-out approach in the program GeneClass 2.0.h (Piry et al. 2004). Monte Carlo resampling (10,000 replicates; Paetkau et al. 2004) was used to exclude individuals if their probability of occurring in the population from which they were sampled was less than $\alpha = 0.05$. These individuals were identified as dispersers and were assigned to another tributary based on likelihood values (see below).

Assignment of main-stem fish followed the same methodology and used partial Bayesian genotype assignment (Rannala and Mountain 1997) with Monte Carlo resampling (10,000 replicates; Paetkau et al. 2004) to exclude main-stem fish that did not originate from any of the tributary populations based on a threshold of $\alpha = 0.05$. Such excluded fish would probably have come from other, unsampled, tributary populations. The remaining fish were assigned by means of a rank-based method of Bayesian posterior probabilities (Rannala and Mountain 1997) that assigned an individual to a population if they had a four times or greater probability of originating from that population relative to the next highest likelihood. The threshold of four times was chosen based on a sensitivity analysis (not shown) and its successful use in other studies (e.g., Beneteau et al. 2009). A Chi-square test was used to test whether juvenile dispersal within the main-stem Bulkley River steelhead departed from random expectations for upstream versus downstream dispersal.

RESULTS

All 10 microsatellite loci were moderately to highly polymorphic (Table 1) and the number of alleles per locus ranged from 5 to 51. A substantial proportion (21%, 17 of 80) of locus by population comparisons showed heterozygote deficiency that caused departures from HWE. Microchecker results indicated the possible presence of null alleles in multiple populations; however, no particular locus drove this pattern, suggesting that null alleles probably only contribute slightly to the observed departures from HWE. Tests for linkage disequilibrium revealed 13 out of 360 (3.6%) locus-by-population combinations to be out of equilibrium. Over one-half of these combinations occurred in the population from McQuarrie Creek and two loci (*RT191* and *OtsG249*) were responsible for all significant linkage pairs. The analyses used in this study are fairly robust to departures from HWE and linkage disequilibrium; however, to ensure the validity of our results we removed the most HWE problematic loci (*RT119* and *Ots G253B*) as well as the two loci (*RT191* and *OtsG249*) that were the cause of linkage disequilibrium, and repeated the analyses. The removal of these loci

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TABLE 1. Sample size (N), number of alleles (A), and observed (H_o) and expected (H_e) heterozygosity at 10 microsatellite loci for eight tributary populations of juvenile steelhead in the Bulkley–Morice River, British Columbia. Asterisks indicate significant differences from Hardy–Weinberg expectations after Bonferroni correction. See Figure 1 for the abbreviations of the sampling sites.

	Sampling site							
Locus	CAN	CAS	DEP	GRM	LMP	MCQ	OWN	SHP
Ots4								
N	39	23	38	21	24	43	31	21
A	10	1	7	8	9	7	18	7
H_o	0.67*	0	0.82	0.86	0.46	0.67	0.61*	0.90
H_e	0.86	0	0.82	0.83	0.67	0.84	0.84	0.83
RT191								
N	38	23	40	26	29	43	32	20
A	16	2	20	15	13	18	20	18
H_o	0.95	0.57	0.93	1.00	0.79	0.91*	0.81	1.00
H_e	0.93	0.45	0.94	0.93	0.90	0.88	0.91	0.94
RT36								
N	37	23	39	26	29	43	31	20
A	21	5	8	7	22	10	31	14
H_o	0.78*	0.74	0.72	0.81	0.86	0.86	0.87*	0.90
H_e	0.92	0.69	0.83	0.81	0.94	0.81	0.96	0.93
Omy87								
N	39	23	41	26	29	43	33	21
A	19	4	10	6	15	7	20	11
H_O	0.85	0.35	0.90	0.35	0.62	0.91	0.82	0.90
H_E	0.89	0.31	0.86	0.58	0.80	0.80	0.92	0.76
RT212								
N	36	23	40	25	29	42	33	21
A	22	3	17	13	18	15	21	13
H_o	0.89	0.35	0.85	0.96	0.93	0.90	0.88	0.95
H_e	0.95	0.30	0.93	0.88	0.91	0.86	0.95	0.92
RT119								
N	39	23	41	26	29	43	31	21
A	12	2	15	7	5	5	9	7
H_o	0.62*	0.13	0.80*	0.65	0.10*	0.84	0.29*	0.67
H_e	0.80	0.12	0.89	0.66	0.23	0.64	0.53	0.64
OtsG401								
N	38	23	41	26	28	43	33	21
A	11	11	12	12	14	11	12	11
H_o	0.89*	0.87	0.83*	0.81	0.89	0.84*	0.79	0.81
H_e	0.91	0.88	0.86	0.91	0.92	0.84	0.90	0.91
OtsG253B								
N	39	23	41	25	28	42	32	21
A	22	1	19	12	20	12	24	11
H_o	0.87	0	0.88	0.72	0.64*	0.55*	0.72	0.86
H_e	0.94	0	0.94	0.91	0.94	0.75	0.96	0.91
OtsG249								
N	37	20	41	23	29	38	31	19
A	16	3	14	13	13	13	21	14
H_o	0.89	0.25	0.71*	0.87	0.72	0.53*	0.84	0.89
H_e	0.89	0.23	0.85	0.92	0.84	0.75	0.91	0.90
OtsG83B								
N	38	17	41	25	28	41	33	17
A	29	4	19	16	26	13	31	15
H_o	0.87	0.47	0.88	1.00	0.86	0.59*	1.00	0.94
H_e	0.95	0.44	0.92	0.92	0.97	0.83	0.97	0.93

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TABLE 2. Genetic distances of eight Bulkley–Morice River tributary steelhead populations. Pairwise F_{ST} estimates are based on Weir and Cockerham's (1984) θ (below the diagonal; all estimates significant at $P < 0.05$ after Bonferroni correction) and a standardized distance estimate, G'_{ST} (Hedrick 2005), to account for differences in heterozygosity among loci (above the diagonal). See Figure 1 for the abbreviations of the sampling sites.

	Sampling site							
Site	SHP	CAS	GRM	CAN	DEP	MCQ	LMP	OWN
SHP		0.676	0.064	0.131	0.211	0.252	0.674	0.501
CAS	0.296		0.730	0.728	0.709	0.670	0.860	0.830
GRM	0.01	0.322		0.286	0.354	0.379	0.725	0.618
CAN	0.019	0.271	0.043		0.215	0.336	0.418	0.240
DEP	0.03	0.28	0.06	0.024		0.328	0.638	0.499
MCQ	0.039	0.299	0.07	0.052	0.054		0.693	0.651
LMP	0.134	0.391	0.151	0.064	0.11	0.156		0.101
OWN	0.077	0.335	0.098	0.027	0.064	0.111	0.008	

did not significantly alter the results of our analyses (results not shown).

All measures of population differentiation indicated structuring of tributary populations. Allele frequency distributions were significantly different between all pairs of populations after Bonferroni correction. All pairwise F_{ST} values were also significant after correction (Table 2). The Causqua Creek (CAS) population was highly divergent from the others (average pairwise $F_{ST} = 0.31$; range = 0.27–0.39), while the pairwise F_{ST} among all other populations ranged from 0.008 to 0.156 (Table 2). Global estimates of F_{ST} changed substantially when the CAS population was excluded: F_{ST} estimates dropped from 0.114 to 0.067 when this highly divergent population was excluded.

The topology of the neighbor-joining (NJ) tree of chord distances (D_C, Cavalli-Sforza and Edwards 1967) roughly mirrored the geographic distribution of the sampling locations (Figure 2). The NJ analysis provided support for the pairwise F_{ST} estimates by grouping the three weakly divergent populations together with strong bootstrap support. The NJ analysis also identified the CAS population as being highly divergent, consistent with our other analyses (not shown). The highly differentiated nature of this steelhead population probably results from the reduced allelic diversity and heterozygosity in this tributary. All pairs of individuals in the CAS tributary were found to have a relatedness coefficient (Ritland 1996) of 0.06 or greater, indicating that sampling individuals of a kin group or inbreeding may explain the divergent nature of this population.

The Mantel test including all pairwise population comparisons resulted in a nonsignificant relationship between genetic and geographic distances ($R^2 = 0.01$, $P = 0.156$). However, the highly divergent CAS population, which had anomalously high genetic divergence from all other populations, distorted the relationship between pairwise genetic and geographic distances. Removing this population caused the isolation by distance correlation to become significant ($R^2 = 0.198$, $P = 0.038$; Figure 3A). The use of a standardized genetic distance measure (G'_{ST}) produced similar results ($R^2 = 0.205$, $P = 0.035$; Figure 3B), although the magnitude of the difference between CAS and the other tributaries was reduced (data not shown).

Genotype assignment of individuals from the tributary samples identified 11 (4.7%) individuals as being excluded from the tributary where they were sampled. Four (1.7%) of them were positively assigned as dispersers among tributaries and seven (3%) other individuals failed to assign with enough confidence

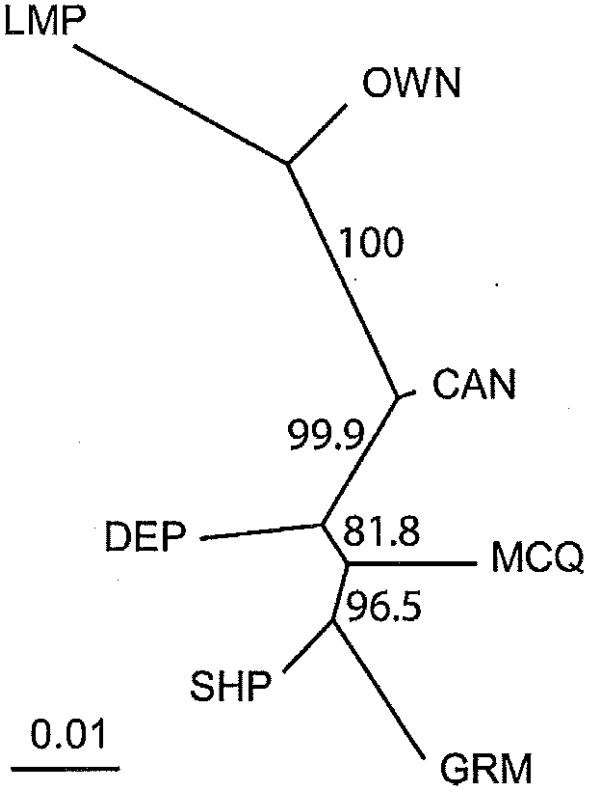

FIGURE 2. Unrooted neighbor-joining tree of Cavalli-Sforza and Edwards (1967) chord distances among juvenile steelhead sampled in five Bulkley River and two Morice River tributaries (CAS not shown). Node labels indicate proportion of support from 1,000 bootstrap replicates.

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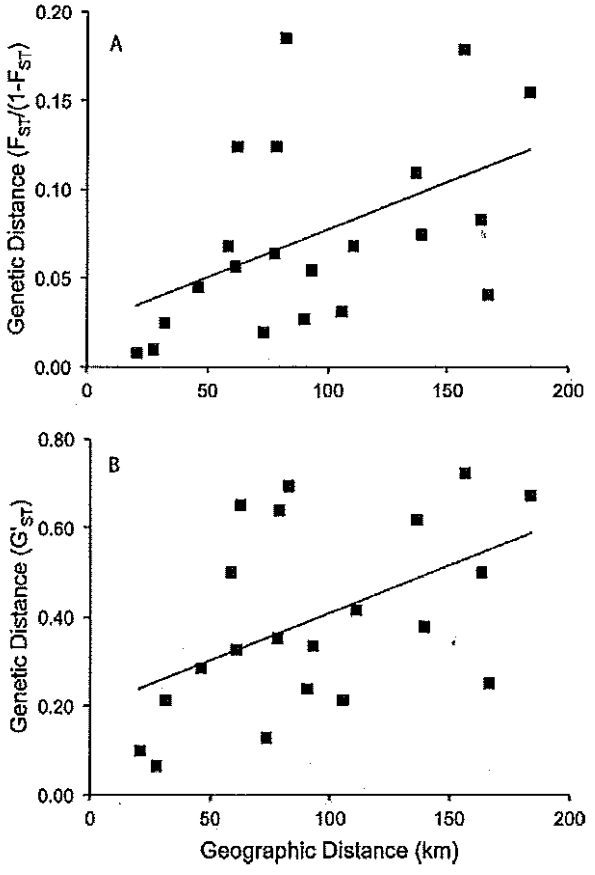

FIGURE 3. Relationship between genetic distances and fluvial distance among seven Bulkley–Morice River tributary steelhead populations. (**A**) F_{ST}/(1 − F_{ST}) (R^2 = 0.198, P = 0.038) and (**B**) G'_{ST} (R^2 = 0.210, P = 0.035). Probabilities are from Mantel tests.

to determine their tributary of origin (Table 3). The proportion of dispersing fish detected in each tributary population ranged from 0% to 6%. No juveniles sampled from the main stem of the river were excluded from all of the sampled reference populations, which suggested that we sampled most of the source genetic groups in the Bulkley–Morice River. The rank-based assignment assigned 121 main-stem individuals (74.5%) successfully to a single tributary population. The results of the genotype assignment (Table 4) suggest that juvenile steelhead are not simply aggregating near the mouth of their natal stream, but are mixing in the main-stem of the river and moving significant distances in both directions. The direction of dispersal (upstream versus downstream) did not depart from random expectation ($\chi^2_{0.05,1} = 1.09$, $P = 0.296$). Juveniles had a tendency to stay reasonably close to natal streams (Figure 4). However, 67 fish (55% of successfully assigned main-stem juveniles) did move in excess of 50 km in the main river.

DISCUSSION

Juvenile steelhead are significantly genetically structured among Bulkley–Morice River tributaries. The pairwise F_{ST} values for most population pairs presented in this study are among the largest published for steelhead. These results indicate substantially stronger structuring than that reported by Narum et al. (2006) who used 20 microsatellites to describe structure among one hatchery and three natural populations spaced from 10 to 60 km apart in the Grande Ronde River, Oregon (range of pairwise F_{ST} = 0.005–0.016). Our results are more similar to those of a study that used 16 microsatellites to describe structure among 33 steelhead populations separated by 5–450 km in the Klamath–Trinity River, California (range of pairwise F_{ST} = 0.0008–0.1830, overall F_{ST} = 0.0395, Pearse et al. 2007). However, the four most highly differentiated populations studied by Pearse et al. (2007) were either known or suspected to be above barriers to anadromy, which suggested that they represent resident rainbow trout populations and not true steelhead populations. Narum et al. (2008) discovered a similar situation among 21 populations in the Klickitat River, Washington, and determined that anadromous populations were limited to the lower reaches of streams because of high gradients and other barriers. None of the tributaries in our study are restricted in this way; however, our anomalous population (CAS) has characteristics similar to the resident populations studied by

TABLE 3. Genotype assignment of juvenile steelhead sampled in five Bulkley River (SHP, GRM, CAN, DEP, and MCQ) and two Morice River (OWN and LMP) tributaries. The numbers on the diagonal are self-assigned (gray shading), while the off-diagonal values are dispersers. Tributaries are ordered from downstream to upstream (SHP to LMP). See Figure 1 for the abbreviations of the tributaries.

	Assigned tributaries							
Sampled tributary	SHP	GRM	CAN	DEP	MCQ	OWN	LMP	Unknown
SHP	21							
GRM		26						
CAN		1	37					1
DEP				40				1
MCQ	1				40			2
OWN	1				1	30		1
LMP							27	2

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TABLE 4. Distribution of juvenile steelhead genotype assignments from main-stem Bulkley River samples to source tributary populations. Individuals have a four times greater likelihood of assignment to the identified tributary than to any other tributary. Main-stem and tributary sites are both ordered from downstream to upstream. See Figure 1 for the abbreviations of the tributaries; BR = Bulkley River.

	Assigned tributary							
Main-stem site	SHP	GRM	CAN	DEP	MCQ	OWN	LMP	Unknown
BR01	1			4	2			4
BR02	1		2	3	3			3
BR03	1		1	3	2			5
BR04	1			6				3
BR05	2		2	5	1			2
BR06	2	8	1					1
BR07	7	4		1				3
BR08	4		2	1				4
BR09	4			3	2			3
BR10	5		1	2	1			1
BR11	1			4	2			4
BR12	2			2	3			3
BR13			2	4	4			2
BR14	2			2	4	1		3

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Narum et al. (2008) (low heterozygosity, allelic richness, does not follow a pattern of isolation by distance), which suggests that this sample represents the resident form of rainbow trout. Our genetic distance estimates (F_{ST}) are also near the upper reaches of published estimates for much larger spatial scales (e.g., Heath et al. 2001; Beacham et al. 2004). Indeed, it appears that genetic structure in steelhead populations is more pronounced at local rather than regional scales, as suggested by Parkinson (1984).

The correlation between genetic distance and geographic distance indicates that this system is approaching, or has reached, equilibrium between drift and gene flow, based on the adherence to the isolation-by-distance model of genetic structure (Slatkin 1993). The isolation-by-distance pattern implies reasonably specific homing by spawning adults to tributaries within the Bulkley–Morice River watershed, and straying most frequently occurs to nearby tributaries. A similar pattern of isolation by distance was reported by Pearse et al. (2007) and Narum et al. (2008) for steelhead in the Klamath–Trinity River and Klickitat River, respectively. No significant extinction or colonization events appear to have occurred in the sampled tributaries in the recent past, suggesting that steelhead populations in Bulkley–Morice River tributaries have been relatively stable through time.

The use of a standardized genetic distance measure (G'_{ST}) did not appreciably alter the isolation by distance relationship (Figure 3). While it did diminish the magnitude of difference between CAS and the other tributary populations by correcting for the differences in heterozygosity, the overall pattern of their relationship did not change. The CAS population still failed to show an isolation-by-distance relationship with the other tributaries.

Proportions of dispersed juvenile steelhead reached 6% in some tributary populations. This indicates that juveniles are in fact moving and mixing among tributaries, which could possibly confound the detection of genetic structuring by homogenizing allele frequencies. Given the magnitude of population divergence presented in this study it is unlikely to have masked our results. Paetkau et al. (2004) proposed a genetic distance

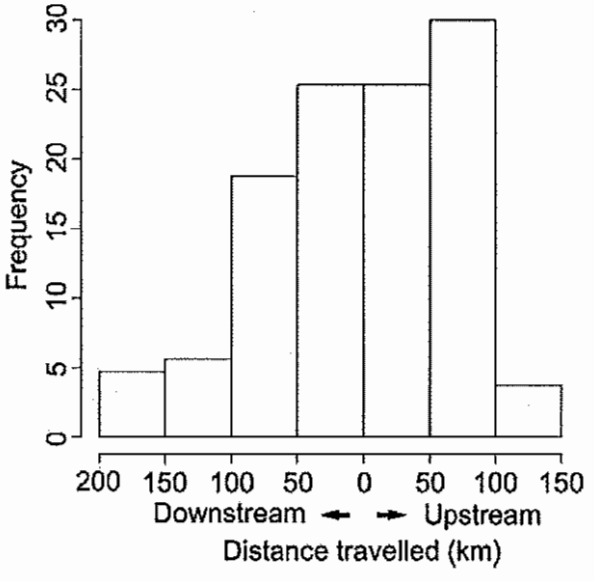

FIGURE 4. Frequency distribution of the distances traveled by juvenile steelhead sampled in the main-stem Bulkley River based on genotype assignment of individuals and the linear river distance between the assigned tributary site and the sample site in the main stem.

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measure based on likelihood ratios (D_{LR}; Paetkau et al. 1997) as a proxy measure of genotype assignment power to detect dispersers. The average D_{LR} value for each population in our study was well in excess of 5; a value suggested by Paetkau et al. (2004) to provide maximal power to detect dispersers at a variety of sample sizes and numbers of loci. Thus, we conclude that, while some individual fish identified as dispersers may be artifacts of the assignment process, it is probably not a common occurrence.

Juvenile steelhead distribution in the main stem of the Bulkley River indicates that juvenile fish are leaving their natal streams, mixing in the river, and dispersing significant distances both upstream and downstream. In contrast, most Morice River juveniles appear to be staying within the Morice River system. The farthest upstream main-stem sample we have is located at the Bulkley–Morice confluence, which thus does not allow conclusions to be drawn about juvenile steelhead distributions in the Morice River main stem. Given the genetic differentiation of the two Morice River tributaries (Lamprey Creek and Owen Creek) we would expect to have ample power to detect Morice River dispersers in the Bulkley main stem in addition to the Bulkley River tributaries (see discussion above). Of the 41 main-stem steelhead that failed to assign to a tributary with confidence, only one individual had a Morice tributary as a putative source indicating that Morice River juveniles were truly absent from our main-stem samples. The failure of any main-stem fish to assign to CAS further reinforces the status of these fish as either a family group or as a group of resident rainbow trout. Other differences among the numbers of main-stem fish assigned to tributary sites may reflect differences in the relative contribution of these tributaries to overall steelhead production in the Bulkley–Morice River system.

Juvenile steelhead emigration data from Toboggan Creek (another Bulkley River tributary) during the spring also supports juvenile emigration from natal areas and recruitment to the Bulkley River main stem (D. Atagi, unpublished data). This is not surprising given that the ideal habitat for egg incubation (cold, well-oxygenated, and clean water) is generally not as suitable for the growth and development of juvenile salmonids that need warmer, more productive areas to feed (Quinn 2005). Use of nonnatal rearing habitats has been previously demonstrated in juvenile Fraser River (Murray and Rosenau 1989) and Yukon River (Daum and Flannery 2011) Chinook salmon *O. tshawytscha*. Nonnatal rearing increases the amount of available habitat through juvenile dispersal to habitats that are either unsuitable or inaccessible to spawning adults, allowing the river system to support greater numbers of fish. Given that their freshwater residency time can be as long as 4 years (owing to low temperatures and a short growing season at higher latitudes; Withler 1966), the tendency of juvenile steelhead in the Bulkley–Morice River system to share dispersal and rearing habitat is probably vital for them to survive and grow to sufficient size to smolt.

Competitive exclusion from rearing and foraging territories by more dominant individuals (Gowan and Fausch 1996) as well as patch assessment in order to maximize time spent in the highest quality patches (Kahler et al. 2001) might have been factors in the surprisingly long-distance movement of stream-resident juvenile steelhead in our study. During the fall, upstream movements of juvenile salmonids have been associated with (1) accessing off-channel and low-velocity habitats during freshets, (2) avoiding high turbidity during increased flows, (3) movement to warmer waters, and (4) dispersal to habitats with appropriately sized substrate (reviewed in Kahler and Quinn 1998). However, there is no obvious reason to suspect that such movements would require the dispersal distances we observed in this study. It is thus still unclear why these fish would undertake such an energetically expensive dispersal process when appropriate habitat and flow refugia are probably available to them closer to their natal tributaries.

The small sample sizes presented in this study were limited by the small population sizes of the tributaries we sampled. In these systems with low effective population sizes, genetic drift can have a considerable effect on genetic structure through time (Shrimpton and Heath 2003). Although temporal samples were not available for this study, our results highlight the need for a detailed analysis of temporal genetic stability and an assessment of effective population size in small-scale, genetically structured populations of salmonids. Low effective population sizes are likely for systems such as that presented here, and would represent an important management and conservation concern for the future viability of the small tributary populations.

Here, we provide evidence that steelhead populations from tributary streams in the Bulkley–Morice River watershed are highly structured, even at very small spatial scales (14 km). This is not the first study to demonstrate population structure at this scale for this species; however, it is the first time it has been demonstrated in a pristine system, with the implication that such levels of genetic structuring are natural. The significant isolation-by-distance relationship indicates that natal homing by spawning adults is very specific and that rates of adult straying are low. On the contrary, nonnatal rearing by juveniles appears to be common in this system and mixed populations of juveniles that exhibit habitat sharing may be common in steelhead. Furthermore, the population structure described for the Bulkley–Morice River steelhead indicates that small tributary populations have the necessary isolation for local adaptation, making the conservation of spawning tributaries a priority for the maintenance of genetic diversity in natural systems.

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