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Microsatellite DNA analysis of rainbow trout (*Oncorhynchus mykiss*) from western Alberta, Canada: native status and evolutionary distinctiveness of ''Athabasca'' rainbow trout

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Abstract Molecular genetic assays can contribute to conservation of aquatic taxa by assessing evolutionary and taxonomic distinctiveness, levels of genetic variation within and between populations, and the degree of introgression with introduced taxa. The Athabasca River drainage of western Alberta, Canada is one of only three (and the largest) drainages flowing east of the continental divide that contain native populations of rainbow trout (Salmonidae: *Oncorhynchus mykiss*). The ''Athabasca'' rainbow trout has been considered a preglacial relict worthy of special conservation measures. In addition, the native range of Athabasca rainbow trout has seen many instances of introductions of non-native populations since the beginning of the 20th century. We assayed rainbow trout from the Athabasca River drainage, from hatchery populations, and from representative populations in adjacent regions (N = 49 localities) for variation at 10 microsatelite loci to assess the level of evolutionary distinctiveness of Athabasca rainbow trout, and to assess the levels of introgression with non-native hatchery fish. We found that native Athabasca rainbow trout did not form a distinctive genetic assemblage and that the greatest amount of allele frequency variation was attributable to contemporary drainage systems (29.3%) rather than by a Athabasca/non-Athabasca distinction (12.6%). We found that 78% of all fish were confidently assigned to a ''wild'' rather than a ''hatchery'' genetic grouping and that most of the inferred introgression with hatchery fish was restricted to a few localities (N = 6). Our results suggest that: (i) Athabasca River rainbow trout are likely postglacial immigrants from adjacent populations of the Fraser River, and (ii) that there is no evidence of widespread introgression of hatchery alleles into native Athabasca River drainage rainbow trout.

Keywords Rainbow trout · Microsatellites · Conservation genetics · Artificial Propogation · Introgression taxonomy

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Introduction

Molecular genetic assays can contribute to conservation of taxa by assessing their evolutionary and taxonomic distinctiveness, the levels of genetic variation within and between populations, and the degree of introgression from introduced taxa (Frankham et al. 2002). Salmonid fishes (salmon, trout and char) have been the focus of many conservation studies because they are so important as food and recreational fishes and owing to their status in many areas as cultural icons (Allendorf and Waples 1996; Neilsen and Regier 2004). Two of the most important issues in salmonid conservation are: (i) the definition and identification of conservation units below the species level (e.g. ''evolutionarily significant units'', ESUs) to both safeguard the evolutionary legacy and potential of taxa, and (ii) the potential impacts of hatchery supplementation on the persistence of native gene pools (see review by Allendorf and Waples 1996). The identification of ESUs in salmonid conservation is problematic in that a single definition of

what constitutes an ESU across jurisdictions remains elusive (e.g. reviewed by Crandall et al. 2000). Notwithstanding this uncertainty, some level of genetic distinctiveness among groups of populations that reflects relatively old divergences (i.e., preglacial) is usually desired in such a definition. Hybridization between native and introduced salmonid taxa has long been recognized as a major conservation issue, especially for taxa in western North America (Leary et al. 1995). Although methods to document and study such hybridization are well established (e.g. Allendorf et al. 2001; Rubidge and Taylor 2005), how hybridized taxa are treated in setting conservation priorities in salmonids is an area of some debate (cf. Allendorf et al. 2005; Campton and Kaeding 2005).

The rainbow trout, *Oncorhynchus mykiss*, has a native distribution stretching from Kamchatka in the western Pacific Ocean, eastward to southern Alaska and coastal and interior regions of British Columbia, Washington, Oregon and southern California and Mexico down to the Baja Pennisula (Behnke 1992). Interior drainages of North America that contain native populations of rainbow trout are restricted to drainages flowing west of the continental divide to the Pacific Ocean with three exceptions. The Peace and Liard rivers in northern British Columbia flow to the Arctic drainage via the Mackenzie River and contain native rainbow trout in their headwaters. In addition, the Athabasca River in western Alberta also flows to the Arctic via the Mackenzie River and contains native rainbow trout (Behnke 1992; Nelson and Paetz 1992).

Behnke (1992) recognized up to five subspecies within rainbow trout based on a constellation of biogeographic, allozyme and chromosomal, and morphological data. One of these, the Columbia River redband trout (*O. m. gairdneri*) is thought to be the lineage that colonized the interior drainages of western Canada, after the most recent glaciation that ended approximately 12,000 years ago. This relatively recent colonization, principally via the Columbia River, into the Fraser River and eventually into the upper Laird, Mackenzie, and Athabasca rivers via postglacial headwater exchanges is thought to explain the origin of rainbow trout east of the continental divide, including those in Alberta (McPhail and Lindsey 1970; Nelson and Paetz 1992).

By contrast, Carl et al. (1994) reported allozyme and meristic data from a single population of rainbow trout (Wampus Creek) from the Athabasca River system that suggested that trout from this area were highly divergent from rainbow trout and steelhead trout (anadromous *O. mykiss*) sampled from parts of the Fraser River and from tributaries of the United States' portion of the middle and upper Columbia River. Carl et al. (1994) suggested that the depth of the divergence between Wampus Creek rainbow trout and adjacent inland rainbow trout indicated a pre-glacial origin of the Athabasca River rainbow trout. These data are at variance with mitochondrial DNA data (Wilson et al. 1985; McCusker et al. 2000) that showed that rainbow trout sampled from three localities in the upper Athabasca River were characterized by mtDNA haplotypes that were very similar or identical to those found in *O. mykiss* throughout the interior of British Columbia (BC). This latter result is consistent with hypothesized recent origin of Athabasca River rainbow trout via headwater transfer with the Fraser River.

Regardless of the age and origin of the Athabasca River rainbow trout these native populations have been subject to some intensive supplementation of hatchery-raised rainbow trout from a diversity of sources outside Alberta since the early 1920's (G. Sterling, Alberta Sustainable Resource Development, unpublished data). In addition, rainbow trout have been introduced and have established naturalized populations in other stocked Alberta watersheds (e.g. tributaries of the North and South Saskatchewan rivers). Over-harvest, habitat loss and degradation, and introgression with introduced genotypes are all considered factors that have contributed to the decline of native rainbow trout in western Alberta (Nelson and Paetz 1992).

In summary, there are two central issues relevant to the conservation of biodiversity of rainbow trout at the eastern periphery of its native range. First, the degree of phylogenetic distinction of Athabasca River rainbow trout is still somewhat uncertain. The most recent analysis of McCusker et al. (2000) raised doubts about its distinction from other inland rainbow trout, but this was a single locus (mtDNA) analysis based on relatively few samples. Second, significant stocking of non-native rainbow trout into the Athabasca River drainage raises the possibility that these gene pools have been affected by introgression of non-native genotypes (e.g. Utter 2000). To address these issues, we genotyped samples of Athabasca River rainbow trout at 10 microsatellite loci to: (i) provide a robust multilocus assessment of the level of divergence between these populations and a number of adjacent populations in the Fraser River basin, and (ii) test for introgression between native populations and a series of hatchery populations used in the Alberta stocking program. The results of our analyses provide important information concerning the prioritization of populations for conservation in terms of their degree of phylogenetic distinctiveness and the extent to which they represent native gene pools (Nelson and Paetz 1992).

Materials and methods

Sample watersheds

Fin clips or whole fry were collected from various tributaries of the Athabasca River drainage during the summers of 2000, 2003, and 2004 (Table 1, Fig. 1). The samples

Table 1 Localities and population status, Number code (NC) for wild-collected populations in Fig. 1, collection dates, and sample sizes for rainbow trout collected from western Alberta

Locality	NC	Year collected	Years stocked	*N*
Non-stocked populations				
Athabasca River				
Oldman Creek	1	2000	NA	13
Lynx Creek	2	2000	NA	10
Emerson Creek	3	2000	NA	10
Windfall Creek	4	2000	NA	14
Pine Creek	5	2000	NA	4
Sakawatamau River	6	2000	NA	5
Buffalo Prairie	35	2002	NA	40
McLeod River				
Felton Creek	18	2000	NA	10
Deerlick Creek	19	2000	NA	10
Anderson Creek	21	2000	NA	25
Wampus Creek	20	2004	NA	20
Wildhay/Berland River				
Hightower Creek	33	2000	NA	10
Barbara Creek	31	2000	NA	10
Moberly Creek	32	2000	NA	10
Cabin Creek	16	2000	NA	4
Cabin Creek	16	2004	NA	20
Jessie Creek	17	2004	NA	20
Hatchery populations				
Beity /Beaver Lake fry		2000	NA	30
Mount Lassen alevins		2000	NA	30
Mount Lassen fry		2000	NA	30
Mount Lassen /Bow River adults		2000	NA	30
Mount Lassen adults		2004	NA	20
Stocked populations				
Athabasca River				
Athabasca River[a]	7	2000	1950–1952	10
Two Creeks[b]	8	2000	1985	10
Fish Creek[c]	10	2000	1940	10
Katy Creek[c]	11	2000	1952	10
Chickadee Creek[b]	12	2000	1985	13
Sandstone Creek[b]	13	2000	1985	10
Canyon Creek[b]	14	2000	1985–1988	4
Wabasso Creek[d]	36	2003	1925, 1951	41
Lac Beauvert[d]	38	2003	1950–1952	34
Tekarra Creek[d]	37	2003	1941–1956	5
Cottonwood Creek[d]	39	2003	1947	40
McLeod River				
Moose Creek[c]	22	2000	1928–1940	10
Luscar Creek[c]	23	2000	1934	17
White Creek[c]	24	2000	1953	10
McKenzie Creek[c]	25	2000	1935–1936	10
Edson River[c]	26	2000	1926–1953	10
Trout Creek[c]	27	2000	1926–1947	24
Mitchell Creek[c]	28	2000	1932–1934	1
Prest Creek[c]	29	2000	1935–1953	3
Lac des Roches[c]	30	2000	?	36
Wildhay/Berland				
Rainbow Lake[b]	9	2000	1985–1997	10
Rainbow Lake outlet[b]	15	2000	1985–1997	1
Muskeg River				
Muskeg River[c]	34	2004	1970–2004	17
Snaring River				
Harvey Lake[d]	40	2003	1962, 1967	36
British Columbia populations				
Queen Charlotte Islands				

Table 1 continued

Locality	NC	Year collected	Years stocked	*N*
Copper River[e]	41	2003	NA	21
Mamin River[e]	41	2003	NA	31
upper Fraser River				
Glatheli Lake	42	2000	NA	32
Fenten Lake	42	2000	NA	32
Columbia River				
Kootenay River[f]	43	2003	NA	52
Salmo River	43	2003	NA	60

''Non-stocked populations'' are native populations with no known introductions of hatchery populations. ''Stocked populations'' are those known to have one or more introductions of hatchery fish from the sources listed under ''Hatchery populations''. ''NA'' for not applicable. Also shown are localities of six British Columbia populations included for comparative purposes. Localities in bold were used in population-level analyses

[a]Mainstem Athabasca River current population likely derived from a number of unknown sources

[b]Stocked with Mount Lassen, California (CA) captive population derived from crosses between Clearwater River, CA stock and Kamloops (BC) stock. Beity hatchery strain is a mixture of various populations established in Washington State

[c]Fry and/or fingerlings shipped from rearing facilities at the Calgary Brewing Company, Calgary or the Maligne River Hatchery, Jasper National Park. It is unknown from where these two facilities acquired eggs. Eggs likely shipped via rail from south/central BC or northern California. Eggs were also supplied from hatcheries in Idaho and Montana in the 1930's and from the Banff Park hatchery before this date. From the 1940's to the 1980's most rainbow trout were derived from a broodstock program at the Trout Lodge Facility in Washington State

[d]Source of hatchery fish unknown

[e]Anadromous rainbow trout (steelhead trout)

[f]Mainstem, near Castlegar, BC

Date of hatchery stocking unknown

consisted of indigenous (''non-stocked'') populations from the Athabasca River and from the McLeod and Wildhay/Berland, and Snaring river subdrainages (*N*=245 samples). These represent fish for which there are no documented records of hatchery fish being stocked and represent the suspected range of indigenous populations (see Nelson and Paetz 1992). Fish tissue was also obtained from tributaries of these same three major drainages, but where non-indigenous hatchery populations of rainbow trout have been stocked at least once (''stocked samples'', $N = 482$ samples, Table 1). Samples were also obtained from major sources of Alberta hatchery populations ($N = 170$). The tissue samples were either stored in 95% ethanol or frozen at −20°C and genomic DNA was extracted using standard laboratory protocols.

Microsatellite analysis

We scored individuals using 10 microsatellite loci that had been isolated from salmonids: *Oki*3a (coho salmon, *Oncorhynchus kisutch*, P. Bentzen, Dept.of Biology, Dalhousie University, Halifax, NS, unpublished data), *Ssa*85, 197, and 456 (Atlantic salmon, *Salmo salar*, O'Reilly et al. 1996; Sletten et al. 1995), *One*8, *One*14 (sockeye salmon, *O. nerka*, Scribner et al. 1996), *Ots* 3, 100, and 103 (chinook salmon, *O. tshawytscha*, Banks et al. 1999; Nelson and Beacham 1999), and *Omy*77 (rainbow trout, Morris et al. 1996). These loci were chosen for their ease of PCR-based allele scoring (i.e. they gave ''clean signals'' with little to no artifact stutter bands) and because they have been assayed in other rainbow trout populations throughout BC (P. Tamkee and E.B. Taylor, unpublished data). Analyses for the 2000 year samples were completed using $^{32/33}$P end-labeled primers and PCR products were scored from autoradiographic films after polyacrlyamide electrophoresis using 6% Long Ranger gels (see Costello et al. 2003). Allele sizes were obtained by comparison to M13 sequencing ladders. Samples from 2003 and 2004 were examined using fluorescently labeled primers and assayed on a Beckman-Coulter CEQ 8000 automated genotyper. Representative genotypes from the initial isotopic analyses were re-run on the CEQ system to ensure consistent scoring of alleles across platforms. All raw allele frequency summary data can be obtained from an electronic database (http://www.zoology.ubc.ca/~etaylor/nfrg/rbtr/athabasca/appendix.html) or from the senior author upon request.

Data analysis

The following tests were performed using GENEPOP ver. 3.1 (Raymond and Rousset 2001) for all collections with sample sizes of at least 15. Tests for deviations from Hardy-Weinberg equilibrium were performed for each locus-population combination using an exact test in which *P*-values were estimated using a Markov chain method. Tests for genotypic linkage disequilibrium for all combinations of locus pairs within a population were also made

using a Markov chain method with GENEPOP default values. Basic descriptive statistics of sample size (N), number of alleles (N_a), observed (H_o) and expected (H_e) heterozygosity were compiled using Fstat ver 2.9 (Goudet 2001). $F_{st}(\theta)$ values were calculated in ARLEQUIN ver. 2.0 (Schneider et al. 2000) based on allelic frequency differences with significance of overall and pairwise comparisons based on 1000 permutations.

To summarize genetic differentiation among all samples, a factorial correspondence analysis (FCA) was conducted on allele frequency data using GENETIX (Belkhir et al. 2001). Factorial Correspondence Analysis is a type of factor analysis that seeks to find the best linear combination of variables (in this case allele frequencies at different loci) that best describe variation between individual observations (fish). In general terms, FCA is best suited for categorical data, such as allele frequency counts, and determines the first K axes of an orthogonal number of axes that describe the most variance from a ''cloud'' of observations

To determine whether or not stocked samples differed from indigenous samples in a manner that could be attributed to introgression from hatchery populations, we used maximum likelihood-based classification procedures. These analyses make use of multilocus genetic profiles of individual fish (i.e., stocked fish) to assign them to *a priori* groups (i.e., known indigenous fish or known hatchery fish) that had been characterized at the same loci (e.g. reviewed by Hansen et al. 2001). The program GENECLASS (version 2.0, Piry et al. 2004) was used to classify the stocked fish samples to one of either indigenous or hatchery origin using the Bayesian assignment methodology of Rannala and Mountain (1997). In these analyses, we used the known hatchery and indigenous samples as our baseline

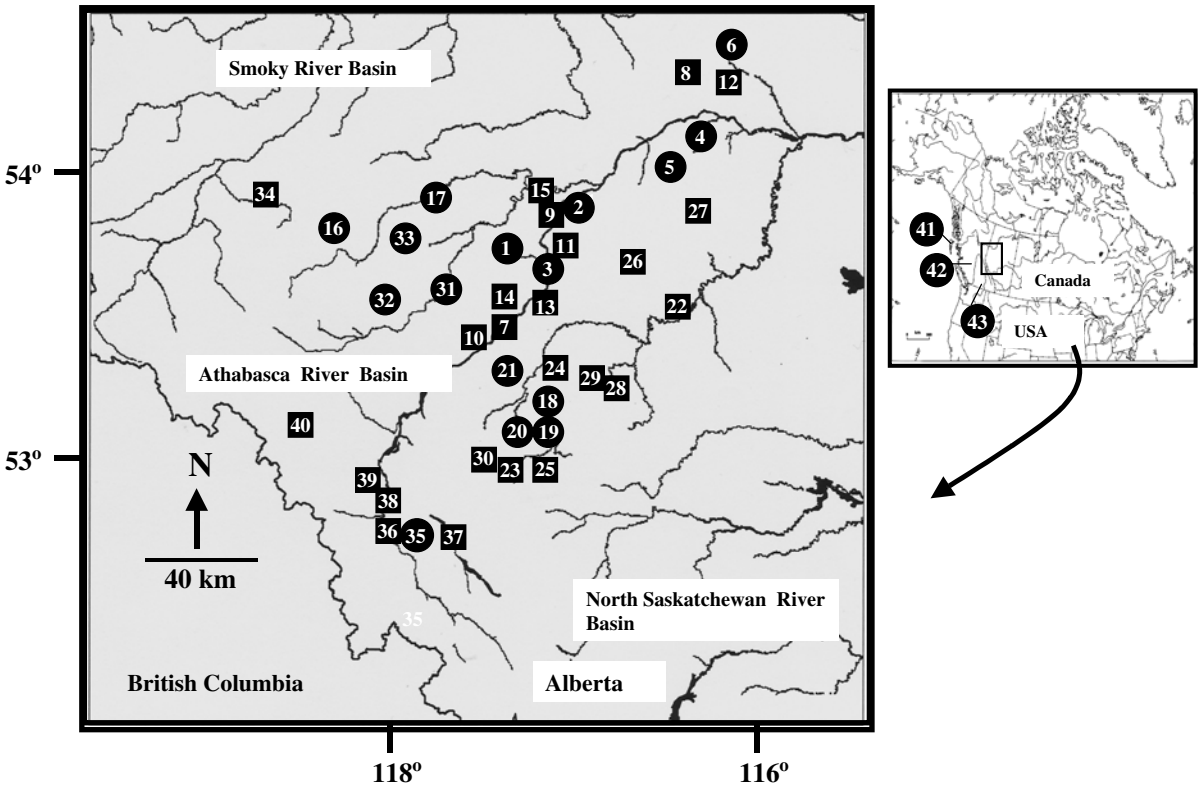

Fig. 1 Map of localities sampled for rainbow trout (*Oncorhynchus mykiss*) in western Alberta, Canada. Localities known to have been stocked with rainbow trout and those free from known stocking are denoted by squares and circles, respectively. Boxed area on small map shows the position of the study area (inset map) in western Canada. 1 = Oldman Creek, 2 = Lynx Creek, 3 = Emerson Creek, 4 = Windfall Creek, 5 = Pine Creek, 6 = Sakawatamau River, 7 = mainstem Athabasca River, 8 = Two Creeks, 9 = Rainbow Lake (outlet), 10 = Fish Creek, 11 = Katy Creek, 12 = Chickadee Creek, 13 = Sandstone Creek, 14 = Canyon Creek, 15 = Rainbow Lake, 16 = Cabin Creek, 17 = Jessie Creek, 18 = Felton Creek, 19 = Deerlick Creek, 20 = Wampus Creek, 21 = Anderson Creek, 22 = Moose Creek, 23 = Luscar Creek, 24 = White Creek, 25 = McKenzie Creek, 26 = Edson Creek, 27 = Trout Creek, 28 = Mitchell Creek, 29 = Prest Creek, 30 = Lac des Roches, 31 = Barbara Creek, 32 = Moberly Creek, 33 = Hightower Creek, 34 = Muskeg River, 35 = Buffalo Prairie, 36 = Wabasso Creek, 37 = Tekarra Creek, 38 = Lac Beauvert, 39 = Cottonwood Creek, 40 = Harvey Lake, 41 = Queen Charlotte Islands (Mamin and Copper rivers), 42 = upper Fraser River (Glatheli and Fenten lakes), 43 = upper Columbia River (Kootenay and Salmo rivers)

data with which to assign the populations of unknown status. We included a more stringent ''exclusion analysis'' in GENECLASS to statistically assess the probability of an individual being of indigenous or hatchery origin. Individuals were excluded from indigenous or hatchery samples if their observed multilocus genotype was observed in <5% of 10,000 simulated genotypes that were generated by randomly sampling from the reference populations (Cornuet et al. 1999; Piry et al. 2004).

In addition, we used the program STRUCTURE (Pritchard et al. 2000) to probalistically estimate the proportion of an individual fish's genome that originated from the indigenous and/or hatchery reference populations. The method clusters individuals, based on their genotypes, into K randomly interbreeding groups by minimizing departures from Hardy–Weinberg and linkage disequilibrium within groups. We used STRUCTURE to calculate posterior distributions of the admixture coefficient, q, or the proportional contribution of each reference sample to an individual's genotype (Pritchard et al. 2000; Koskinen et al. 2002). We employed 50,000 replications during the pre-simulation (''burn-in'') period and then estimated q after a subsequent 450,000 replications.

We also applied the analysis of molecular variance (AMOVA) approach of Excoffier et al. (1992) to partition microsatellite variation into its various components (among populations within coastal and interior regions versus between coastal and interior regions) using ARLEQUIN. We pooled samples using several hierarchical arrangements: by major watershed within Alberta, by watersheds across the entire sample area, and by ''Athabasca'' and ''non-Athabasca'' samples. We based our inferences on allele frequency variation among populations assuming no mutation-based differentiation because estimates based on the former appear to be more appropriate for recently diverged populations (e.g. Gaggiotti et al. 1999). In addition, all samples were used in GENECLASS assignment-based analyses to try and identify hatchery fish or their recent descendents sampled from streams. Population level analyses, however, where conducted using individual collections with sample sizes of at least 15 (Table 1). Where appropriate, all statistical tests were conducted at tablewide significance levels of $\alpha = 0.05$ using the sequential Bonferroni adjustment (Rice 1989).

Results

Microsatellite polymorphism within populations

Thirteen of the wild populations had sufficient sample sizes (i.e., N of at least 15) for population-level analyses as did the six hatchery populations (Table 1). In general, microsatellite polymorphism in rainbow trout across all loci was variable across loci and populations (i.e. expected heterozygosities ranged between 0.10 and 0.80, Table 2). The most variable loci were *Oki*3a, *Ssa*85, and *Ots*100 with 32, 30, and 24 alleles, respectively (allele frequencies are available at http://www.zoology.ubc.ca/~etaylor/nfrg/rbtr/athabasca/appendix.html). The hatchery samples were significantly more variable in terms of expected heterozygosity, than either the wild-collected populations (permutation of observed differences, P=0.002). Virtually all samples were in Hardy-Weinberg equilibrium with 18 out of a possible 190 (10 loci × 19 sample populations) tests showing statistically significant heterozygote deficits (Table 2, $P < 0.0003$). Twelve of these deviations occurred in the five hatchery samples with the remainder being scattered among loci and wild populations (Table 2). There were two significant departures from linkage disequilibrium between loci within populations; one in the Mt. Lassen fry sample (*Ssa*85 and *One*14) and the other in Lac Beauvert (*Omy*77 and *Ots*100).

Microsatellite polymorphism among populations

There was significant variation in allele frequencies (Table 3) among populations (i.e., those samples with N of at least 15). Fixation indices(θ) ranged from 0.104 (*Ots*103) to 0.59 (*Ssa*456). The overall level of subdivision was high($\theta = 0.33$, 95% CI 0.27–0.40) among all populations as well as just among the wild-collected samples ($\theta = 0.30$, 95% CI 0.26–0.35). Pairwise differences in θ were substantial and most were statistically significant (Table 3). There were, however, several comparisons that were not significant and these were largely found in comparisons between populations within the McLeod River drainage and between two creeks in the Wildhay/Berland River system (Table 3).

In the FCA, four broad groupings of rainbow trout were resolved (Fig. 2). One group (''A'') consisted of a tight clustering of wild-collected (both non-stocked and stocked samples) rainbow trout from Alberta. A second group (''B'') consisted of four Alberta populations (Lac des Roches, Wabasso, Cottonwood, and Rainbow creeks) and the two upper Fraser River populations (Glatheli and Fenton lakes). A third group (''C'') consisted of the Muskeg River (Alberta) population, two steelhead populations from the Queen Charlotte Islands, and two Columbia River populations. The final group (''D'') consisted of all hatchery populations and the Harvey Lake (Alberta) sample (Fig. 2).

The distinction between hatchery and non-stocked and stocked wild-collected rainbow trout was also evident in

Table 2 Population genetic statistics summarizing variation at 10 microsatellite loci in populations of rainbow trout (*Oncorhynchus mykiss*) sampled from western Alberta

Population	Locus									
	*Oki*3a	*Omy*77	*One*8	One14	*Ots*3	Ots100	*Ots*103	*Ssa*85	*Ssa*197	*Ssa*456
Non-stocked populations										
Anderson Cr.										
N	25	25	25	25	25	25	25	25	25	25
H_O	0.368	0.263	0.471	0.211	0.158	0.000	0.000	0.474	0.053	0.316
H_E	0.383	0.234	0.480	0.241	0.149	0.000	0.000	0.591	0.053	0.279
N_A	3	2	6	3	2	1	1	4	2	3
N_R	2.3	1.9	3.4	2.1	1.7	1.0	1.0	2.9	1.3	2.2
Buffalo Prairie										
N	40	40	40	38	39	38	40	38	38	40
H_O	0.000	0.375	0.103	0.605	0.436	0.000	0.000	0.447	0.000	0.000
H_E	0.000	0.372	0.099	0.548	0.468	0.000	0.000	0.528	0.000	0.000
N_A	1	3	2	3	3	1	1	3	1	1
N_R	1.0	2.1	1.5	2.7	2.1	1.0	1.0	2.8	1.0	1.0
Jessie Cr.										
N	20	19	17	20	20	20	20	20	20	19
H_O	0.474	0.316	0.647	0.526	0.133	0.000	**0.053**	0.650	0.000	0.000
H_E	0.465	0.440	0.669	0.553	0.129	0.000	0.392	0.722	0.000	0.000
N_A	3	3	3	4	2	1	3	6	1	1
N_R	2.8	2.3	3.0	2.6	1.6	1.0	2.3	4.1	1.0	1.0
Wampus Cr.										
N	20	20	20	20	20	17	20	20	20	20
H_O	0.278	0.438	0.375	0.176	0.000	0.000	0.000	0.500	0.000	0.250
H_E	0.412	0.517	0.460	0.261	0.125	0.000	0.209	0.610	0.000	0.317
N_A	3	4	2	2	2	1	2	4	1	2
N_R	2.5	2.0	2.9	1.9	1.6	1.0	1.8	3.5	1.0	2.0
Cabin Cr.										
N	24	24	24	24	18	24	24	20	24	16
H_O	0.188	0.588	0.563	0.471	0.474	0.000	0.056	0.600	0.000	0.000
H_E	0.342	0.539	0.679	0.515	0.436	0.067	0.163	0.691	0.000	0.000
N_A	4	3	3	2	3	2	3	4	1	1
N_R	2.8	2.7	3.0	2.0	2.3	1.4	1.9	3.6	1.0	1.0
Stocked populations										
Wabbaso Cr.										
N	41	41	41	40	38	41	41	41	41	41
H_O	0.537	0.641	0.700	0.415	0.447	**0.146**	0.000	0.195	0.268	0.415
H_E	0.650	0.696	0.658	0.495	0.514	0.209	0.000	0.267	0.334	0.391
N_A	5	7	5	4	3	8	1	4	2	3
N_R	3.2	3.6	3.9	2.4	2.2	2.3	1.0	2.4	2.0	2.1
Muskeg R.										
N	17	17	17	17	17	17	17	17	17	17
H_O	0.455	0.833	0.286	0.300	0.700	0.167	0.333	0.235	0.300	0.286
H_E	0.723	0.800	0.893	0.672	0.861	0.767	0.567	0.213	0.267	0.262
N_A	5	4	6	3	7	4	3	2	2	2
N_R	4.4	4.0	5.7	3.0	5.8	4.0	3.0	1.8	2.0	2.0
Lac Beauvert										
N	34	27	31	34	33	34	27	32	34	33
H_O	0.640	0.759	0.516	0.400	**0.217**	0.407	0.000	0.563	0.424	0.565
H_E	0.703	0.756	0.772	0.539	0.584	0.499	0.000	0.535	0.509	0.584
N_A	7	7	6	5	4	8	1	5	2	3
N_R	4.5	4.7	4.7	2.8	2.9	3.9	1.0	3.4	2.0	2.2
Cottonwood Cr.										
N	40	33	40	37	40	40	40	38	39	38
H_O	0.333	0.545	0.622	0063	**0.238**	0.621	0.000	0.553	0.487	0.632
H_E	0.389	0.664	0.637	0.061	0.655	0.744	0.000	0.661	0.507	0.559
N_A	2	4	4	4	1	5	1	5	2	3
N_R	2.0	3.2	3.2	1.3	3.7	4.3	1.0	3.6	2.0	2.6
Lac des Roches										
N	36	36	36	36	36	36	36	36	36	36

Table 2 continued

Population	Locus									
	*Oki*3a	*Omy*77	*One*8	One14	*Ots*3	Ots100	*Ots*103	*Ssa*85	*Ssa*197	*Ssa*456
H_O	0.278	0.667	0.528	0.361	0.306	0.000	0.000	0.611	0.278	0.194
H_E	0.273	0.698	0.535	0.458	0.421	0.000	0.000	0.606	0.242	0.203
N_A	4	7	7	4	2	1	1	5	2	3
N_R	2.3	4.0	3.8	2.7	2.0	1.0	1.0	3.1	1.9	1.9
Luscar Cr.										
N	17	17	17	17	17	17	17	17	17	17
H_O	0.267	0.588	0.412	0.412	0.600	0.000	0.000	0.588	0.118	0.060
H_E	0.410	0.542	0.417	0.393	0.476	0.000	0.000	0.520	0.114	0.169
N_A	2	4	7	4	3	1	1	4	2	2
N_R	2.0	2.8	3.6	2.6	2.4	1.0	1.0	2.9	1.6	1.7
Trout Cr.										
N	23	24	22	24	24	24	24	24	24	24
H_O	0.522	0.625	0.417	0.522	0.042	0.000	0.000	0.417	0.000	0.083
H_E	0.590	0.665	0.492	0.645	0.042	0.000	0.000	0.492	0.000	0.082
N_A	4	4	4	4	2	1	1	3	1	2
N_R	2.9	3.2	2.7	3.3	1.2	1.0	1.0	2.7	1.0	1.4
Harvey Lake										
N	30	33	36	36	36	28	28	36	33	35
H_O	0.867	0.676	0.333	**0.308**	0.259	0.750	0.000	**0.212**	0.472	0.571
H_E	0.821	0.824	0.360	0.601	0.584	0.886	0.000	0.444	0.488	0.492
N_A	10	10	5	4	3	11	1	5	2	4
N_R	5.6	5.5	2.7	2.9	2.6	6.8	1.0	2.5	2.0	2.8
Hatchery populations										
Mt. Lassen[a]										
N	30	30	30	30	30	30	30	30	30	30
H_O	0.567	**0.533**	0.665	**0.353**	0.567	0.967	0.167	0.800	0.567	0.067
H_E	0.775	0.659	0.553	0.710	0.557	0.843	0.157	0.846	0.493	0.066
N_A	10	5	4	5	4	9	3	11	2	2
N_R	5.1	3.5	2.9	3.8	3.1	5.8	1.8	6.4	2.0	1.4
Mt. Lassen[b]										
N	30	30	30	30	30	30	30	30	30	30
H_O	0.633	0.655	0.567	**0.190**	0.733	0.724	0.207	0.615	0.533	**0.154**
H_E	0.750	0.737	0.521	0.652	0.710	0.674	0.188	0.804	0.499	0.597
N_A	6	6	4	3	4	8	2	9	2	3
N_R	4.3	4.3	3.3	2.9	3.7	4.5	1.6	5.9	2.0	2.8
Mt. Lassen[c]										
N	30	30	30	30	30	30	30	30	30	30
H_O	0.704	**0.464**	**0.379**	**0.400**	0.593	0.852	0.300	0.833	0.467	0.034
H_E	0.917	0.793	0.654	0.723	0.749	0.906	0.270	0.904	0.500	0.102
N_A	16	12	8	8	6	12	3	20	2	3
N_R	8.1	5.5	4.1	5.0	4.1	7.4	2.3	8.0	2.0	1.6
Mt. Lassen[c]										
N	20	20	20	20	20	20	20	20	20	20
H_O	0.850	0.400	0.250	**0.158**	0.800	0.850	0.300	0.850	0.450	0.350
H_E	0.643	0.646	0.558	0.608	0.629	0.761	0.329	0.878	0.513	0.303
N_A	6	4	4	4	3	4	2	10	2	3
N_R	3.6	3.4	2.6	3.6	3.2	3.2	5.5	6.7	2.0	2.2
Beattie/Beaver[b]										
N	30	30	30	30	30	30	30	30	30	30
H_O	0.586	**0.667**	0.467	**0.308**	**0.500**	0.897	0.357	0.700	0.600	**0.000**
H_E	0.826	0.732	0.434	0.752	0.690	0.856	0.437	0.801	0.498	0.131
N_A	13	7	4	6	6	10	3	8	2	3
N_R	6.2	4.4	2.8	4.5	3.9	6.3	2.5	5.5	2.0	1.7

N = sample size, H_O = observed heterozygosity, H_E = expected heterozygosity, N_A = numbers of alleles, N_R = Allelic richness. Statistics are presented for localities with sample sizes of at least 15. Values of H_O that are in bold type represent significant deviations from H_E

[a]alevins (see Table 1)

[b]fry

[c]adults

Table 3 Pairwise $F_{st}(\theta)$ values between thirteen wild-collected populations of rainbow trout from western Alberta

	Buf	Wab	LacB	Harv	Cott	Wamp	And	LacR	Trout	Lusc	Cab	Jess
Wab	0.16547											
LacB	0.28311	0.09035										
Harv	0.55862	0.28656	0.20937									
Cott	0.41473	0.29049	0.15602	0.34689								
Wamp	0.22480	0.26028	0.24503	0.49716	0.31349							
And	0.26043	0.31576	0.28774	0.52293	0.26807	**0.05599**						
LacR	0.24319	0.28921	0.24903	0.45309	0.25077	0.04498	**0.01060**					
Trout	0.30386	0.34836	0.32660	0.54509	0.34429	**0.05308**	**0.05323**	0.05467				
Lusc	0.27329	0.32736	0.29396	0.52582	0.31195	**0.01966**	**0.01060**	**0.00173**	**0.02399**			
Cab	0.28287	0.17103	0.17419	0.40123	0.19054	0.19943	0.17063	0.16972	0.23558	0.22641		
Jess	0.34931	0.25415	0.23316	0.44188	0.20286	0.20231	0.16086	0.16680	0.22302	0.21743	**0.00891**	
Musk	0.59254	0.16880	0.00312	0.14753	0.21753	0.56172	0.56743	0.45608	0.59436	0.58993	0.42462	0.46832

F_{st} values are based on variation in allele frequencies at 10 microsatellite loci. Bold values are not significantly different from one another (at a tablewide P value of 0.0006). Buf = Buffalo Prairie, Wab = Wabasso Creek, LacB = Lac Beauvert, Harv = Harvey Lake, Cott = Cottonwood Creek, Wamp = Wampus Creek, And = Anderson Creek, LacR = Lac des Roches, Trout = Trout Creek, Lusc = Luscar Creek, Cab = Cabin Creek, Jess = Jessie Creek, Musk = Muskeg River

the distribution of alleles at individual loci. For example, the *Ssa*456*153, *Ots*100*174, *One*8*174, and *Oki*3a*140 alleles were at near identical frequencies in all wild-collected samples (averages of 0.862, 0.931, 0.510, and 0.698, respectively), but these alleles were almost absent in the hatchery samples (averages of 0.008, 0.006, 0.003, and 0.009, respectively) Within hatchery samples, *One*8*154, *Ssa*456*157, *Omy*77*100 and *116 alleles were generally common (averages of 0.201, 0.724, 0.400, and 0.245, respectively) compared to wild-collected populations where these same alleles were absent or rare (averages of 0.009, 0.004, 0.018, and 0.005, respectively). Similar trends were also found among the Harvey Lake fish which possessed many alleles that were common in the hatchery samples (see electronic appendix), but that were rare or absent in the other wild-collected samples.

Using AMOVA, when all Athabasca River drainage samples were pooled and compared against all other rainbow trout (''non-Athabasca'') to simulate two distinct glacial refugia groups, 12.6% of the variation in allele frequencies was attributable to this grouping (Table 4, $P < 0.01$). This was comparable to simply pooling all populations into ''eastern'' (Columbia River and eastward) and ''western'' (Fraser River and Queen Charlotte Island samples) were 10.2% of the variation was attributable to differences between these groupings ($P = 0.01$). Separation of populations into three putative refugial groups (Athabasca, Fraser/Columbia, and Queen Charlottes Islands)

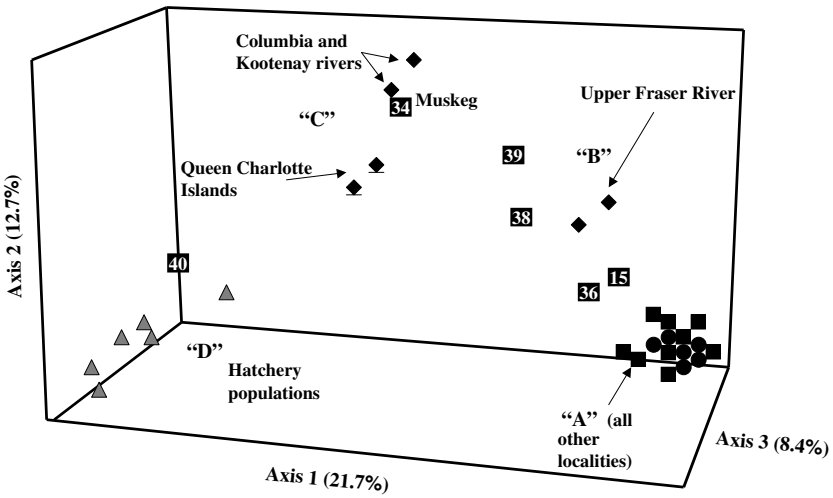

Fig. 2 Plots of mean factorial correspondence analysis (FCA) scores for each sample of western Alberta rainbow trout (*Oncorhynchus mykiss*) assayed for variation at 10 microsatellite loci. Populations with no history of stocking are shown as circles, those with histories of stocking by squares, hatchery populations used in the Alberta stocking program as gray triangles. Rainbow trout and steelhead (underlined) populations from British Columbia are shown as diamonds. Four general groupings of populations are denoted by ''A'' – ''D'' (see text for details). Only those populations grouping away from the main cluster of Alberta populations (''A'') are denoted by locality numbers defined in Fig. 1. The percentage of the total variation in allele frequencies explained by each FCA axis is shown along each axis

Table 4 Results of analysis of molecular variance on allele frequencies across 10 microsatellite loci assayed in rainbow trout (*Oncorhynchus mykiss*) sampled from the Athabasca River area of western Alberta and British Columbia

Grouping	V_{bg}	V_{ap}	V_{wp}
1. Athabasca vs. non-Athabasca	12.6**	21.7***	65.8***
2. Eastern vs. western	10.2*	23.9***	65.9***
3. Athabasca vs. Columbia/Fraser vs. Queen Charlotte Islands	13.8**	20.4***	65.9***
4. Athabasca vs. Columbia vs. Fraser vs. Queen Charlotte Islands	23.4***	13.6***	63.0***
5. Athabasca River vs. McLeod River vs.Wildhay/Berland rivers	12.6***	11.5***	76.0***

The analyses presented represent the hierarchical dissection of variation when samples were arranged into different geographic groupings. V_{bg} = percentage variation between groups, V_{ap} = variation among populations within groups, V_{wp} = variation within populations

*$P < 0.05$

**$P < 0.01$

***$P < 0.001$

increased the among group component of variation slightly to 13.8% ($P < 0.001$). By contrast, when ''non-Athabasca'' samples were further subdivided into their component contemporary drainages (Columbia River, Fraser River, Queen Charlotte Islands) and compared to Athabascan populations a greater percentage of among group variation was resolved (23.4%, $P < 0.001$). Finally, within the Alberta populations only, 12.6% of the microsatellite variation was attributable to differences among the Athabasca River mainstem, McLeod River, and Wildhay/Berland River systems (Table 4, $P < 0.001$).

Classification of individual fish

We observed excellent discrimination between wild-collected and hatchery rainbow trout as all but four of the 416 learning sample fish were correctly self-assigned (99.5%). Three wild-collected, non-stocked rainbow trout were assigned to the hatchery sample (two from Wampus Creek and one from Emerson Creek) and one hatchery fish was assigned to a wild population (to Cabin Creek). When all of the wild-collected, stocked and non-stocked rainbow trout from Alberta were examined ($N = 613$ fish), all but four (0.65%) were assigned to their sample population, or another wild-collected population rather than to any of the hatchery samples. The four fish assigned to a hatchery population were all collected from lakes; three from Harvey Lake (posterior probability of hatchery origin >0.90) and one from Rainbow Lake (posterior probability of hatchery origin = 1.0). We also assigned all wild-collected fish from populations that had experienced some hatchery supplementation to either ''native'' (all wild-collected fish from localities that had experienced no known hatchery introductions) or ''hatchery'' groups. In this case, 80 of the 367 (22%) rainbow trout from localities subject to some supplementation were assigned to the hatchery grouping. The fish classified as hatchery fish included all those from Harvey Lake and the one fish from Rainbow Lake. In addition, two fish from Wabasso Creek (4.8% of all fish from Wabasso Creek), 13 from Lac Beauvert (41%), 17 from Cottonwood Creek (43%), and 11 fish from the Muskeg River (65%) were assigned to the hatchery group. In addition, when we applied the more stringent exclusion criterion, all 80 of these ''hatchery assigned'' fish were excluded as being indigenous ($P < 0.05$; i.e., we had a 5% chance of erroneously excluding any individual from the indigenous group). By contrast, only 73 fish of the wild-collected fish from hatchery-supplemented localities could be excluded as hatchery fish or their recent descendants at a confidence level of 0.05.

The admixture analysis was broadly consistent with the assignment tests in that most of the indigenous rainbow trout has q-values at or near $q = 1.0$ (mean ± SD = 0.98 ± 0.11) with most hatchery fish having q-values near $q = 0$ (mean = 0.017±0.062). Fish from localities that have received some stocking of hatchery fish had lower q-values than indigenous fish and were more variable (mean = 0.72 ± 0.32, t-test, $P < 0.001$) indicating a greater degree of similarity to, or admixture with, hatchery samples. For example, the previously described Harvey Lake fish all had q-values of < 0.01 (mean = 0.07 + 0.16, Fig. 3).

Discussion

Conservation issues in salmonids

Owing to their long association with humans as food and recreational fishes and because they are often conspicuous in nature (e.g. during spawning migrations), salmonid fishes (salmon, trout, and char) are the focus of many conservation programs and can serve as useful models for other taxa (Behnke 1992; Huppert 1999; Myers et al. 2003). Two major conservation issues in salmonid fishes concern: (i) the identification of taxa and the definition of conservation units most suitable to preserve the

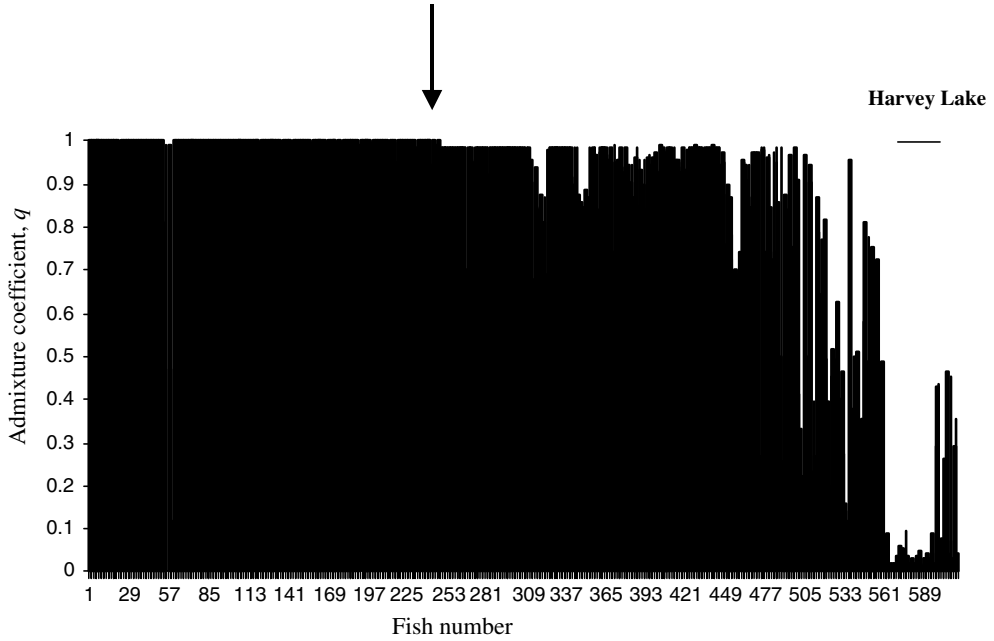

Fig. 3 Posterior distribution of admixture coefficients, *q*, for individual wild-collected rainbow trout (*Oncorhynchus mykiss*) assayed with 10 microsatellite loci. Non-stocked fish (left of the vertical arrow) are those that have no history of hatchery supplementation, stocked samples (right of the arrow) are those subject to hatchery supplementation. Each fish is represented by a thin, black vertical line. Each such line represents the proportion of an individual's genotype that is derived from indigenous trout (black portion) or hatchery trout (white portion). A *q*-value of 1.0 indicates a pure indigenous rainbow trout genotype and a *q*-value of 0.0 indicates a pure hatchery genotype

evolutionary legacy and potential of the group, and (ii) the potential impacts of hatchery supplementation on the persistence of native gene pools (see review by Allendorf and Waples 1996). Both of these issues have a long history of study in rainbow trout, *Oncorhynchus mykiss* (reviewed in Behnke 1992). Our results are directly relevant both aspects of the diversity of rainbow trout; the distinctiveness of the ''Athabasca'' rainbow trout relative to the other forms of *O. mykiss*, and the genetic integrity of native gene pools in the Athabasca River drainage of western Alberta. Behnke (1992) considered rainbow trout native to the Athabasca River system to be representatives of the Columbia River ''redband trout'' *O. m. gairdneri* that had come to colonize the Athabasca River by way of postglacial dispersal from temporary connections between upper Columbia, Fraser, and Athabasca rivers (see also McPhail and Lindsey 1970, 1986; Nelson and Paetz 1992). This view, however, was challenged by Carl et al. (1994) who presented morphological and allozyme data that showed that fish sampled from Wampus Creek in Alberta were highly distinctive from samples of trout from the upper Columbia River. These authors used these data to suggest that the extant Athabasca River rainbow trout were preglacial in origin, stemming from isolation beginning at least 65,000 years ago in a glacial refuge, the southwestern Alberta refuge, that was independent from the Columbia (or Pacific) refuge (Carl et al. 1994). McCusker et al. (2000), however, provided mtDNA data and marshaled arguments that supported the view of Behnke (1992) and Nelson and Paetz (1992); i.e., that Athabasca River rainbow trout were post-Wisconsinan colonists. Our current data provide strong substantiation of the conclusions of Behnke (1992) and McCusker et al. (2000). Our multilocus microsatellite DNA analysis showed that multiple populations of Athabasca rainbow trout were very similar to nearby Fraser River populations (from which Athabasca River trout are though to have been derived). In addition, a hierarchical analysis of microsatellite diversity showed that the greatest amount of among group variation was accounted for when populations were arranged in contemporary watershed groupings rather than in a grouping reflecting a putative separate southwestern Alberta glacial refuge. Our conclusion that Athabasca River rainbow trout do not represent preglacial relict populations is also consistent with the zoogeography of the species. The putative southwestern Alberta refuge extended well south into the South Saskatchewan River basin, yet rainbow trout are not native to this drainage. This drainage, however, is clearly suitable for salmonids as evidenced by the presence of westslope cutthroat trout, *O. clarkii lewisi*, and bull trout, *Salvelinus confluentus* (Nelson and Paetz 1992).

The postglacial origin of Athabasca River rainbow trout does not necessarily reduce their importance to conservation. For instance, the Athabasca River assemblage of populations is one of only three such assemblages (the others being those in the headwaters of the Peace and Liard rivers) that occupy rivers the drain the east slopes of the Rocky Mountains and flow to the Arctic watershed (Behnke 1992). In addition, the Athabasca River populations have by far the more extensive distributions within these eastward-flowing drainages (McPhail and Lindsey 1970; Behnke 1992). Consequently, the Athabasca River rainbow trout are found in the ''Western Arctic'' freshwater aquatic ecoregions in Canada, one of 14 such ecoregions recognized by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2004). Occupancy in distinctive biogeographic regions is one of the criteria used to establish ''designatable units'' (at or below the species level) for conservation status review and potential listing under Canada's Species at Risk Act (COSEWIC 2004). By this criterion alone, Athabasca River rainbow trout warrant separate assessment of conservation status in Canada.

Average expected heterozygosities of 0.17 to 0.62 in the wild-collected rainbow trout samples are consistent with values reported for rainbow trout from other regions in western Canada (Tamkee and Taylor, unpublished data). The expected heterozygosities, however, were generally lower than reported for anadromous steelhead trout (Beacham et al. 1999; Heath et al. 2001; Hendry et al. 2002). Of some note is the observation that Alberta hatchery samples exhibited expected heterozygosities almost twice that of indigenous and stocked populations. Hatchery populations of salmonids often carry lower levels of genetic variation than wild populations (e.g. Nielsen et al. 1997). The enhanced variation in Alberta hatchery samples, however, may stem from several donor populations contributing to hatchery samples (e.g. Mt. Lassen hatchery) and active cross breeding of different strains by Alberta hatchery personnel (G. Sterling, unpublished observations). Large numbers of deviations from Hardy–Weinburg expectations in the hatchery samples support the suggestion of mixed origins of hatchery fish as they would not represent a single, randomly mating population. Alternatively, deviations from HW expectations in the hatchery samples could result from selective breeding or differential survival among families within the hatchery. These diversity data are the first hint that stocking of fish into Alberta streams has had little detectable influence on the genetic structure of trout in the recipient streams; clearly stocked streams more closely resemble indigenous streams in terms of variation within samples.

Variation among samples

Microsatellite variation demonstrated considerable divergence among the population samples analyzed. Pooled across loci, all sample populations were highly divergent from one another. The level of subdivision(θ) across these populations averaged 0.31. This is considerably higher than reported for rainbow trout introduced into Lake Ontario, $\theta = 0.012$ (O'Connell et al. 1997) and of 0.09 to 0.12 reported for coastal cutthroat trout (*Oncorhynchus clarkii clarkii*) for a similar number of loci and heterozygosity levels (Wenburg et al. 1998). Interestingly, the θ value of 0.21 when hatchery fish from Alberta were removed from our analysis, is very similar to that reported among populations of wild rainbow trout collected from Arrow Lakes' drainage in eastern BC rainbow trout, $\theta = 0.18$, (Taylor and McLean 1999), but somewhat lower than 0.39 for a broader survey of populations across BC (Tamkee and Taylor, unpublished data), 0.33 reported for bull trout and 0.32 for westslope cutthroat trout (Costello et al. 2003; Taylor et al. 2003). The higher θ values in these latter studies probably stem from their wider geographic coverage and the fact that many of the populations examined were isolated above upstream migration barriers (e.g. Costello et al. 2003; Taylor et al. 2003). The relatively high level of subdivision of the Alberta ''stocked'' populations of rainbow trout, and its similar value to that reported for other native populations is the second indication that these western Alberta populations have not been unduly ''homogenized'' by past stocking with hatchery trout (cf. Wishard et al. 1984).

Individual classification and conservation implications

Our results strongly suggest that there has been little detectable genetic introgression of hatchery rainbow trout alleles into most of the Alberta populations that have received introductions of hatchery fish. Allele frequencies were markedly different between stocked and hatchery samples, stocked samples more closely resembled indigenous trout, and over 99% of the wild-collected trout that had experienced no known hatchery supplementation were assigned as ''native'' trout. By contrast, up to 21% of wild-collected trout that had experienced some hatchery supplementation were assigned to the ''hatchery'' group rather than the ''native'' grouping. Almost half of these fish (37/80) came from lake populations, Harvey and Rainbow lakes, and all of the Harvey Lake fish (36) were assigned to the hatchery category. Harvey Lake was fishless before it was stocked between 1962 and 1967 and our data indicate that a self-sustaining population has become established in the lake. Only two streams, Cottonwood Creek and Muskeg

River, had appreciable numbers of wild-collected trout assigned to the hatchery grouping. Interestingly, Muskeg River is part of the Smoky River drainage that has no known indigenous populations of *O. mykiss* (G. Sterling, unpublished data). The Muskeg River is, therefore, one of the few localities in our survey that are known to have been colonized by hatchery fish introduced into adjacent watersheds. Although the river itself has not been supplemented, a complex of small lakes just upstream (Pierre Greys Lakes) were stocked repeatedly beginning in 1970 and most recently in 2004 (G. Sterling, unpublished data). Consequently, the presence of hatchery genotypes within the Muskeg River samples probably stems from downstream dispersal of hatchery fish from these lake sources (cf. Rubidge and Taylor 2005). Further, Harvey Lake is connected to the mainstem Athabasca River via about 30 km of the Snaring River and could provide a conduit for hatchery alleles to spread to downstream native populations.

Effects of introductions of hatchery fish on native fish in other species have been variable (Leary et al. 1995; Utter 2000). Clear cases of genetic introgression have been documented in cutthroat trout when one subspecies has been introduced into the geographic range of another (e.g. westslope cutthroat trout and Yellowstone cutthroat trout, *O. c. bouvieri*, hybrid swarms) (Leary et al. 1995). In addition, populations of steelhead trout in California, Oregon, and Washington State tend to show less genetic population subdivision than do populations in British Columbia, an effect attributed to greater homogenization from hatchery transplantation in the American states (Leary et al. 1995). A similar scenario has been offered to explain low levels of genetic differentiation among chinook salmon (*O. tshawytscha*) in certain California watersheds (Bartley and Gall 1990). On the other hand, there are several studies that have demonstrated little or no introgression of hatchery alleles into indigenous populations after stocking, particularly for European salmonid populations (e.g. Moran et al. 1994; Hansen et al. 1995). Wishard et al. (1984) also indicated that introgression between inland and introduced coastal forms of rainbow trout has not occurred in areas of southern Idaho. There are instances where natural selection against hybrids (between indigenous and hatchery salmonids) appears to limit the extent of introgression of hatchery alleles into indigenous populations (e.g. Wishard et al. 1984; Chilcote et al. 1986; Skaala et al. 1996; Poteaux et al. 1998). This could certainly be occurring in western Alberta if hatchery populations originate from trout collected from other geographic areas (e.g. Mt. Lassen hatchery population). It is also possible that introgression of hatchery alleles may be limited by higher angling mortality suffered by hatchery trout (e.g. Garcia-Marin et al. 1998). Freshwater resident salmonids appear to be more susceptible to introgression of hatchery alleles than anadromous fish (Hansen et al. 2000; Utter 2000). Despite this generalization, most Alberta populations of rainbow trout in stream habitats appear to have been little affected by hatchery introductions, at least in terms of genetic structure. By contrast, the greatest concentration of hatchery genotypes was found in a formerly fishless lake population (e.g. Harvey Lake) or in stream populations directly downstream of stocked, lake habitats (Muskeg River). Our data, therefore, suggest that even within freshwater habitats, lake and adjacent stream habitats ecosystems may be more susceptible to naturalization of hatchery genotypes than spatially more complex stream habitats, particularly when they previously contained no indigenous populations of rainbow trout.

A further factor to consider is the time that has elapsed since the most recent introduction. Most of the stocking of rainbow trout in Alberta occurred from the 1920–1960s with little or no hatchery input subsequently, at least in streams (stocking still occurs annually in many isolated lake habitats). The only exceptions were Canyon Creek (Athabasca River), Rainbow Lake (Wildhay/Berland), and Pierre Greys Lakes/Muskeg River (2004) which received hatchery introductions in 1985–1988 and 1985–1997, and 2004, respectively. Consequently, in most stocked streams, there has been considerable opportunity over 30–80 years for natural selection, or immigration from nearby indigenous populations, to eliminate hatchery genotypes in these streams. It is possible, however, that the hatchery samples that we analyzed (collected in 2000, 2004) are not representative, genetically, of the hatchery fish that were stocked into Alberta streams prior to 2000. This would make discrimination of hatchery and indigenous fish more difficult and could mean that some introgression of hatchery alleles into stocked populations could have occurred and be undetected by us. Three observations, however, make this potential complication unlikely. First, the clear evidence of the poor survival performance of hatchery fish in other species and areas in nature (see above), reduces the likelihood of geographically widespread and sustained introgression of hatchery alleles. Second, the clear similarity of Harvey Lake trout, which was last stocked in 1967, with our 2000–2004 hatchery samples suggests that the latter are broadly representative of historical hatchery genotypes that were used to stock Harvey Lake and other habitats in the study area. Finally, the vast majority of fish used for stocking into Alberta (including those used to stock at least 19 of our 23 ''stocked'' populations) stem from the US-derived hatchery populations that we sampled (Alberta stocking records, unpublished data).

In conclusion, our multilocus microsatellite analysis indicates that Athabasca rainbow trout are most likely of

postglacial origin and do not represent preglacial relicts. In addition, except in a few localities we found little to no influence of past stocking of hatchery rainbow trout on the genetic diversity or structure of native populations; the latter are very similar to indigenous trout with no record of past stocking. These results strongly suggest that native Alberta trout gene pools have persisted, in spite of past stocking with genetically divergent populations. Increased efforts to conserve these native gene pools should be made as they are the genotypes most likely to persist in local environments (Taylor 1991). Finally, our analyses suggest that hatchery trout do not survive well in western Alberta streams at least when exotic populations are used as hatchery broodstock. This result calls into question of efficacy of hatchery introductions as a long-term population management technique, at least for stream and river samples. Efforts and resources may be better directed towards effective habitat and fishery regulation management to better conserve Alberta's native rainbow trout. If, however, hatchery supplementation is to continue in Alberta streams, our results, and those for studies of salmonids in general (Reisenbichler 1988; Taylor 1991), strongly suggest that the use of indigenous populations for artificial propagation and introductions would result in higher survival of hatchery fish.

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