Genetic purity assessment of select Colorado River cutthroat trout populations in northwest Colorado

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Introduction

Early molecular methods proved capable of identifying introgression with rainbow trout (*Oncorhychus mykiss*) or Yellowstone cutthroat trout (*O. clarkii bouvieri)*, but separation of the three native cutthroat trout subspecies found in Colorado remained elusive (Rogers 2008a). AFLPs have been shown to be an efficient tool for measuring admixture and discriminating the source of an individual among putative populations making them especially beneficial in systems characterized by weak population structuring (Campbell et al. 2003). Colorado Parks and Wildlife has had good success using AFLPs to discriminate the three native cutthroat trout subspecies in the state (Metcalf et al. 2007, Rogers 2008a, Rogers et al. 2011)

This assessment continues ongoing work to evaluate purity of cutthroat trout in Colorado. The populations covered in this effort are restricted to Colorado River cutthroat trout (*O. c. pleuriticus; CRCT*) populations in northwest Colorado. Molecular testing on these populations was needed to determine their suitability for inclusion as conservation populations in the CRCT Assessment database (Hirsch et al. 2006) compiled by the Colorado River Cutthroat Trout Conservation Team.

Methods

Tissue collection

Fin tissues from 249 fish were collected from nine putative CRCT populations between 2008 and 2010 (Table 1). Fin collections were made as per Rogers (2007) with a small piece (one cm^2) of the top of the caudal fin removed from each fish in the sample. Fins were stored individually in 15 mL conical centrifuge tubes filled with 80% reagent grade ethanol.

TABLE 1: Tissue samples from nine putative Colorado River cutthroat trout populations of unknown purity were collected for subsequent genetic testing.

Location	Drainage	County	Water Code	Collection Date	Sample Size	Biologist
Beaver Creek	Colorado	Garfield	19097	10/21/10	17	Martin
Black Creek, S Fk	Blue	Summit	27551	07/14/10	30	Ewert
Butler Creek	Colorado	Garfield	19388	08/18/10	32	Martin
French Gulch	Blue	Summit	24179	10/07/10	29	Ewert
Hawxhurst Creek, EW	Colorado	Mesa	27993	06/24/10	30	Martin
Henderson Horseshoe	Williams Fork	Grand	NA	08/10/10	30	Ewert
Slater Creek, S Fk[a]	Little Snake	Routt	23286	07/31/08	25	Atkinson
Wheeler Creek	Yampa	Routt	21527	06/26/10	30	Atkinson
Whitewater Creek	Gunnison	Mesa	22765	10/19/10	26	Martin

[a] First ten fins from the tributary, and the last 15 fins from the upper part of the South Fork.

DNA isolation and evaluation

Tissue samples were delivered to Pisces Molecular (Boulder, CO) for DNA isolation and testing with the $\text{AFLP}_{\text{Standard}}$ procedure (Rogers 2008a). Cutthroat trout DNA was extracted from fin clips using a proteinase K tissue lysis and spin-column DNA purification protocols, then amplified using a polymerase chain reaction (PCR) to produce AFLP marker fragments. Fragment size was evaluated on an ABI 3130 sequencer (Applied Biosystems, Foster City, California). A genetic fingerprint was produced for each individual sample using the program Genemapper 4.0 (Applied Biosystems), by scoring for the presence or absence of a standardized set of 119 markers between 50 and 450 base pairs in size generated from reference cutthroat trout populations.

The genetic fingerprints of individuals in the test population were compared to those found in the reference populations (Table 2) using a Bayesian approach for identifying population clusters (Pritchard et al. 2000). This reference set is only slightly different than that used in Rogers (2008a). The program STRUCTURE 2.2 (Falush et al. 2007; Pritchard et al. 2007) was used to determine similarity between the test individuals and the reference populations. Reference populations were selected and grouped by their mtDNA lineage (Metcalf et al. 2007), and not necessarily by geographic or historic subspecies classifications. The similarity or dissimilarity was scored as the admixture proportion, or the probability that each test individual shares a genetic background with each of the cutthroat subspecies reference population groups. These proportions are expressed as q values for each subspecies. These q values were obtained by running STRUCTURE ten times for each population of interest using a burn-in of 50,000 steps followed by 50,000 Monte Carlo Markov Chain replicates. Average q values from the run with the highest log likelihood (Pritchard and Cowley 2007) were used to generate the admixture proportions for the unknown population. Confidence intervals around admixture were generated with the software application QSTRAP Version 3.1 (Rogers 2008b). This program uses a bootstrapping approach to derive confidence intervals around mean values of q.

TABLE 2: Reference populations used to assess relatedness and purity of unknown populations using AFLPs in this report. Colorado River cutthroat trout are divided into LineageCR and LineageGB clades.

Trout Subspecies	Water	County	Water Code	Collection Date	Sample Size
LineageCR[a]	Williamson Lake #3	Inyo	NA	07/31/06	22
	Piedra, E Fk	Hinsdale	42096	02/07/06	20[f]
	Slater Crk, S Fk	Routt	23286	NA	14[g]
	Parachute Crk, E Fk	Garfield	21460	NA	10[g]
LineageGB[b]	Severy Crk	El Paso	31312	NA	10[f]
	Antelope Crk, W	Gunnison	48016	02/21/03[e]	21
	Bobtail Crk	Grand	23026	09/03/03	19
Rio Grande cutthroat	Canones Crk	Rio Arriba	329	03/29/06	19
	Columbine Crk	Taos	1026	09/17/02	20[h]
	Osier Crk	Conejos	44444	09/22/04	11
	Cuates Crk	Costilla	38141	07/25/05	10
Yellowstone cutthroat	Dog Crk[i]	Teton	813220	06/28/01	20[f]
	Willow Crk[i]	Teton	813350	10/26/02	14[f]
	Yellowstone R[c]	Park	TenSleep	03/01/05	12
Rainbow	Colorado R[d]	Grand	21298	NA	10
	Bellaire	Garfield	RifleFalls	03/06/08	9
	Eagle Lake	Garfield	RifleFalls	03/06/08	9
	Erwin	Garfield	RifleFalls	03/06/08	9
	Fish Lake	Garfield	RifleFalls	03/06/08	9
	Kamloops	Garfield	RifleFalls	03/06/08	9
	Tasmanian	Garfield	RifleFalls	01/12/08	9

[a] Lineage reported as Colorado River cutthroat trout by Metcalf et al. (2007)
[b] Lineage reported as greenback cutthroat trout by Metcalf et al. (2007)
[c] These derived from the LeHardy rapids stock, now at Ten Sleep hatchery in Wyoming
[d] Colorado River rainbow stock from the Glenwood hatchery in Colorado
[e] These fish were taken into captivity on 8/21/02 to avoid desiccation during extreme drought; fins were collected from 19 on 2/21/03, and 7 on 1/1/03
[f] These DNAs obtained from J. Metcalf and were used in her dissertation work at CU Boulder.
[g] These DNAs obtained from D. Shiozawa via J. Metcalf, and used in her dissertation work at CU
[h] These DNAs obtained from V. Pritchard and were used in her dissertation work at New Mexico State University

[i] J. Metcalf obtained these DNAs from Mark Novak as putative fine-spotted forms of the Yellowstone cutthroat trout. Dog Creek is a direct tributary of the Snake River (full water code JN813220TN) and Willow Creek lies in the Hoback drainage (full water code JN813350TN).

Results

Of the nine putative CRCT populations examined, six looked better than 90% pure (Table 3) using the AFLP fingerprint and reference populations described in Table 2. Butler Creek (Figure 1) and South Fork Slater Creek (Figure 2) appeared to be very pure populations, with Whitewater Creek (Figure 3) and South Fork Black Creek (Figure 4) close behind. Two of the six displayed the LineageGB CRCT fingerprint that appears to belong to trout native to southwestern Colorado (Rogers 2010). Henderson Horseshoe Pond (Figure 5) and Beaver Creek (Figure 6) both are pure enough to be considered as conservation populations (Hirsch et al. 2006). Interestingly, one of the cutthroat trout populations suspected of being pure (French Gulch, Figure 7) turned out to be more closely aligned with Yellowstone cutthroat trout. The remaining two populations look heavily admixed with non-native trout, with Hawxhurst Creek (Figure 8) trending toward LineageGB but with substantial rainbow trout introgression, and Wheeler Creek (Figure 9) suggesting evidence of both rainbow trout and Yellowstone cutthroat trout admixture.

TABLE 3: AFLP results from nine putative Colorado River cutthroat trout populations analyzed in this report. Percent admixture was given by taxa where CR = LineageCR, GB = LineageGB, RG = Rio Grande cutthroat trout, YS = Yellowstone cutthroat trout, and RB = rainbow trout.

Location	**Water Code**	**Collection Date**	**Sample Size**	**% Lineage by AFLP**				
				CR	**GB**	**RG**	**YS**	**RB**
Beaver Creek	19097	10/21/10	17	5	94	-	-	-
Black Creek, S Fk	27551	07/14/10	30	98	-	-	1	-
Butler Creek	19388	08/18/10	32	100	-	-	-	-
French Gulch	24179	10/07/10	29	1	-	1	98	-
Hawxhurst Creek, EW	27993	06/24/10	30	3	48	2	1	46
Henderson Horseshoe	NA	08/10/10	30	-	99	-	1	-
Slater Creek, S Fk	23286	07/31/08	25	100	-	-	-	-
Wheeler Creek	21527	06/26/10	30	66	-	1	21	11
Whitewater Creek	22765	10/19/10	26	99	-	-	-	-

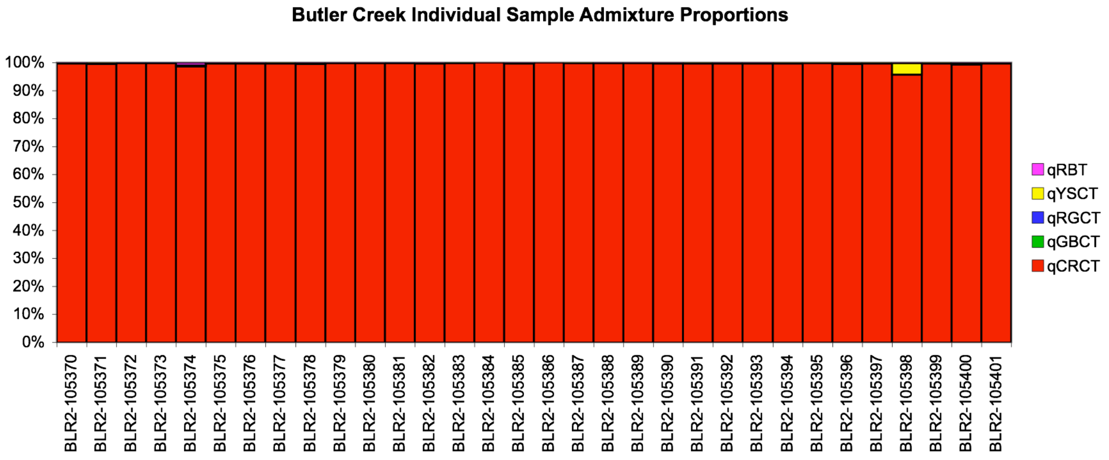

FIGURE 1: AFLP$_{\text{Standard}}$ results obtained from 32 samples collected on August 18, 2010 from Butler Creek (Pisces sample numbers 105370-105401). First 23 samples collected from above confluence with George Creek. Samples 24-30 were collected on USFS lands above BLM property, while the remaining two fins came from upstream of Big Parker Creek and from Middle Rifle Creek just downstream of Butler Creek.

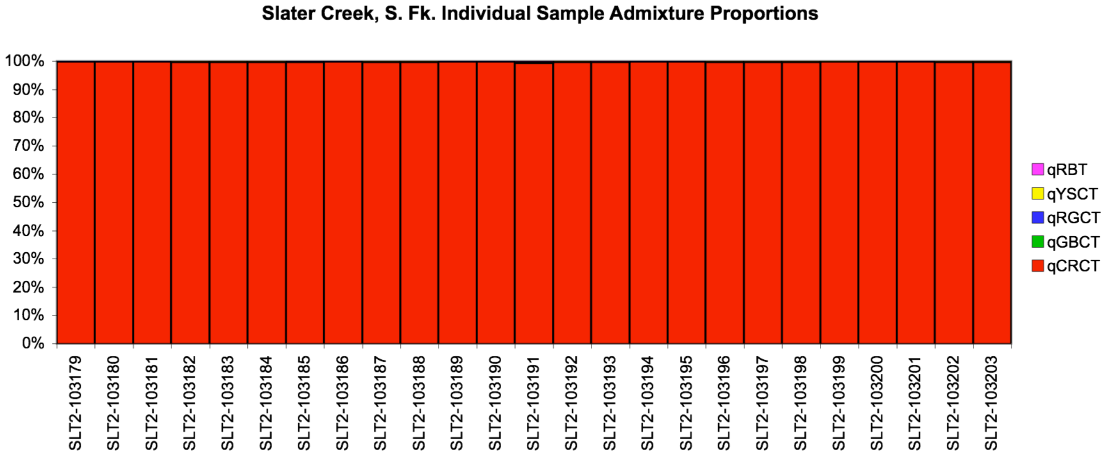

FIGURE 2: AFLP$_{\text{Standard}}$ results obtained from 25 samples collected on July 31, 2008 from Slater Creek, S Fk (Pisces sample numbers 103179-103203). The first 10 fins came from the tributary of the South Fork and the remaining 15 came from the upper reaches of the South Fork.

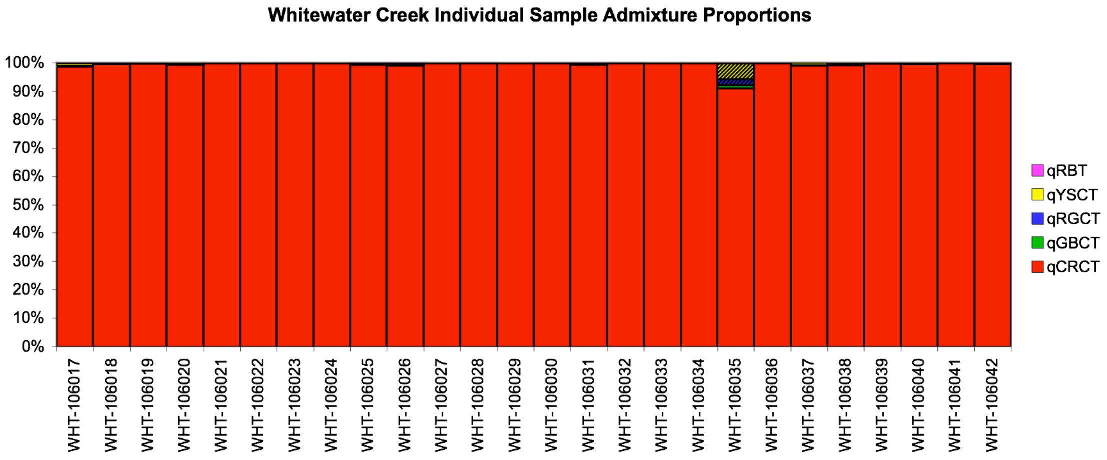

FIGURE 3: AFLP$_{\text{Standard}}$ results obtained from 26 samples collected on October 19, 2010 from Whitewater Creek upstream of the Brandon Ditch on City of Grand Junction property (Pisces sample numbers 106017-106042). Black bar indicates Genemapper ambiguity.

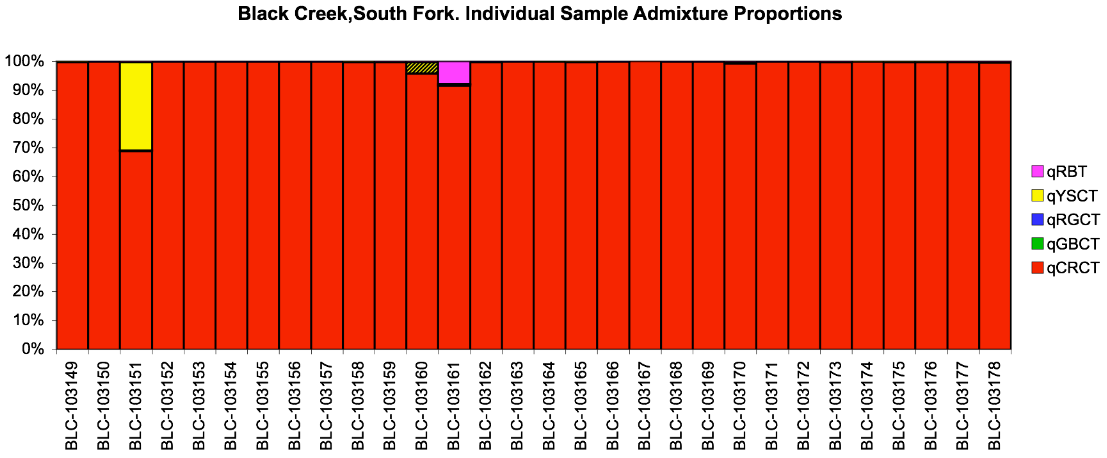

FIGURE 4: AFLP$_{\text{Standard}}$ results obtained 30 samples collected on July 14, 2010 from Black Creek, S Fk (Pisces sample numbers 103149-103178). Black bar indicates Genemapper ambiguity.

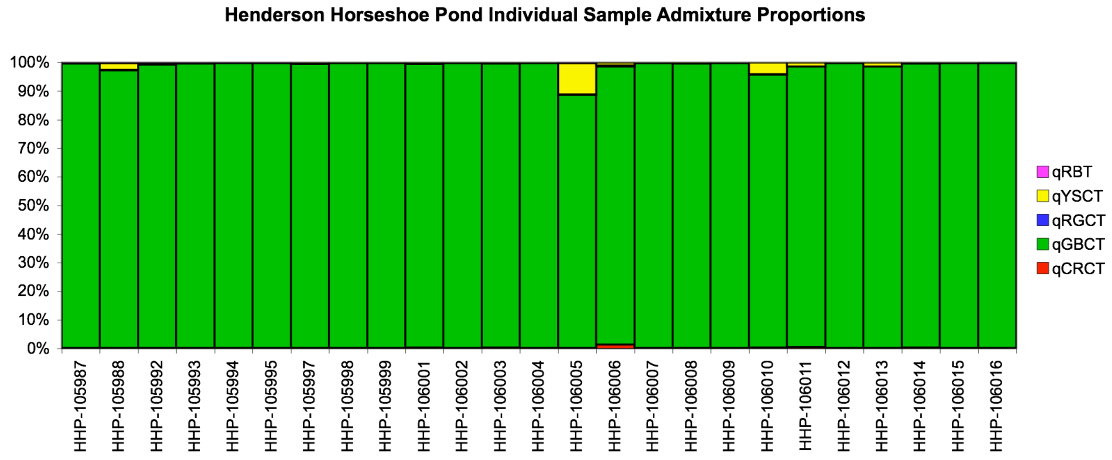

FIGURE 5: AFLP$_{\text{Standard}}$ results obtained from 30 samples collected on August 10, 2010 from Henderson Horseshoe Pond (Pisces sample numbers 105987-106016).

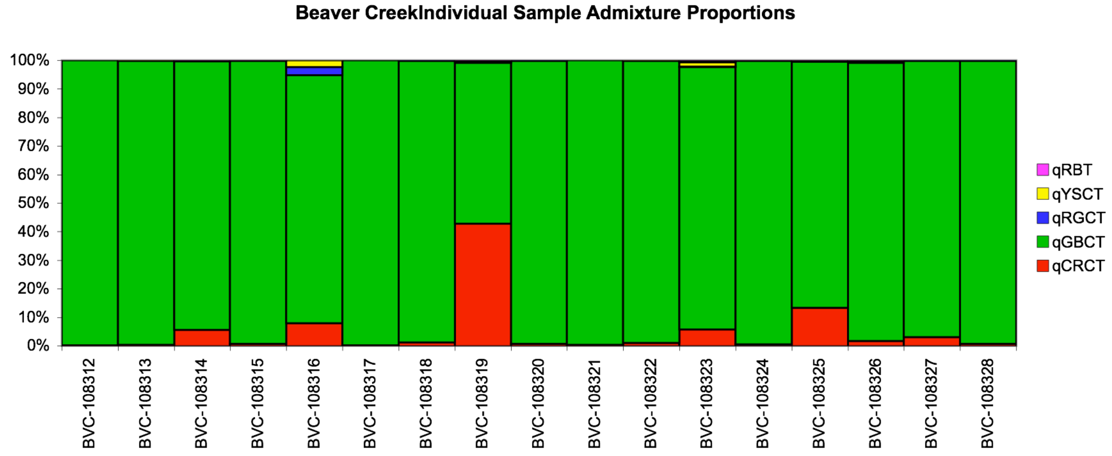

FIGURE 6: AFLP$_{\text{Standard}}$ results obtained from 17 samples collected on October 21, 2010 from Beaver Creek on USFS property (Pisces sample numbers 108312-108328).

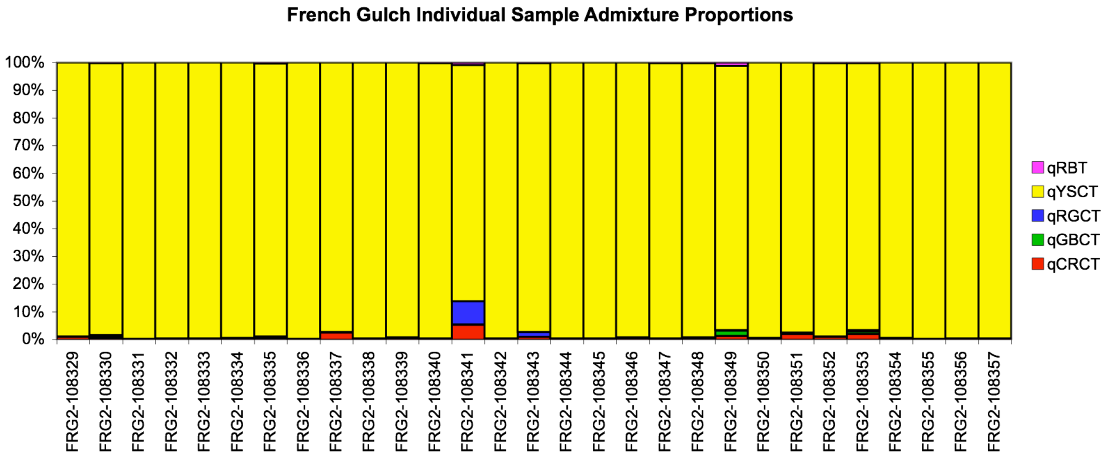

FIGURE 7: AFLP$_{\text{Standard}}$ results obtained from 29 samples collected on October 7, 2010 from French Gulch (Pisces sample numbers 108329-108357).

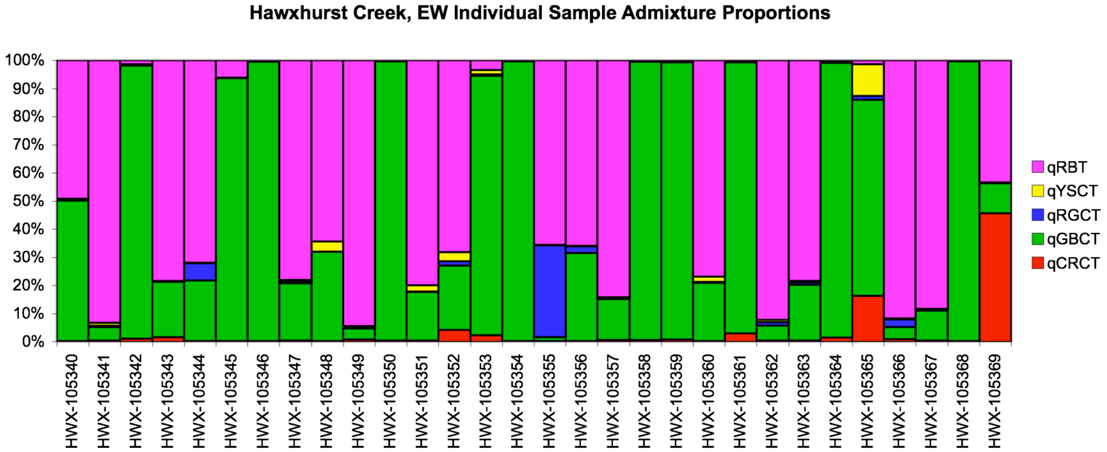

FIGURE 8: AFLP$_{\text{Standard}}$ results obtained from 30 samples collected on June 24, 2010 from the East and West Forks (last 6 fish) of Hawxhurst Creek (Pisces sample numbers 105340-105369).

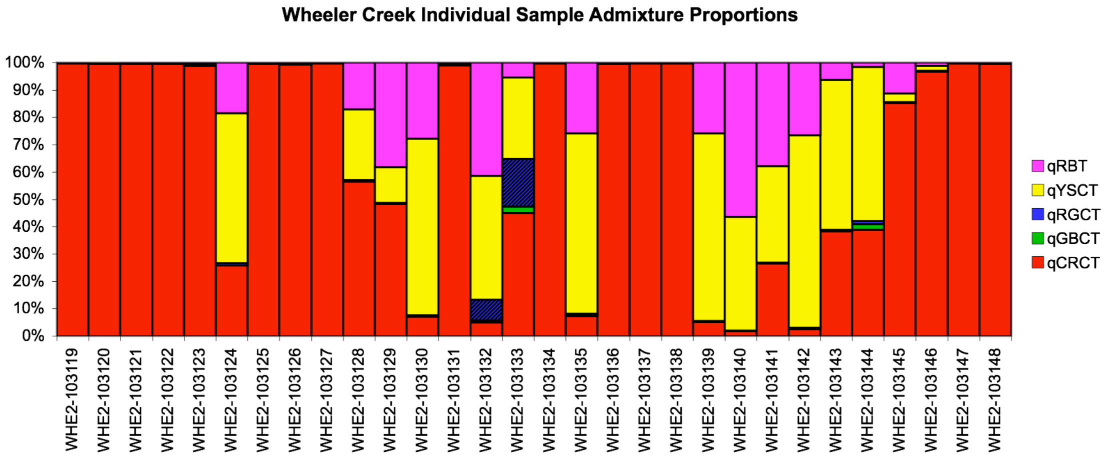

FIGURE 9: AFLP$_{\text{Standard}}$ results obtained using 30 samples collected from Wheeler Creek on June 26, 2010 (Pisces sample numbers 103119-103148). Dark bars represent Genemapper ambiguities.

Discussion

It is important to emphasize that the calculation of admixture used in this report is not the same as the method of calculating introgression adopted by the Greenback Recovery Team or the Colorado River and Rio Grande Conservation Teams in 2000 (UDWR 2000). AFLP markers while numerous, are not generally diagnostic but merely informative. That is, rather than being present in 100% of one group and none of the other, a marker may simply be underrepresented in one group and over represented in another. This type of informative marker does not lend itself to calculation of introgression as described in the "Genetic considerations associated with cutthroat trout management" position paper (UDWR 2000). Instead, the presence or absence of each marker is evaluated in a Bayesian analysis framework to assign a probability that each fish belongs to each of the given reference population groups. While anecdotal evidence suggests that in practice, the results are similar (at least for introgression with Yellowstone cutthroat trout or rainbow trout), an empirical test of the similarity between the classical calculation of introgression and this surrogate for admixture is ongoing.

Based on admixture levels calculated in this report, 6 of the 9 populations considered look to be pure enough to include as conservation populations in the CRCT recovery effort (Hirsch et al. 2006). Two of the remaining populations (Hawxhurst Creek and Wheeler Creek) show substantial admixture with rainbow trout or Yellowstone cutthroat trout that coincides with fairly elaborate stocking histories in each drainage. Finally, a molecular test of tissue samples collected from French Gulch in September 2008 suggested that the population was predominantly *O. c. bouvieri* (88%) rather than *O. c. pleuriticus* (11%). Tissue samples gathered in October 2010 and covered in this report were collected to confirm those results, which they did (Figure 7). The NADH subunit 2 (ND2) mitochondrial gene was also sequenced on the 2008 collection (17 fish) to further evaluate these results. Gene sequencing was conducted as per Rogers et al.

(2011), and indeed 6 of 17 haplotypes were characteristic of *O. c. bouvieri*, but not identical to haplotypes identified to date in Yellowstone Lake (Figure 10). The remainder represented one of the common haplotypes found in Trappers Lake (Figure 10). It should be noted that the 2008 samples suggested 11% admixture with CRCT while the 2010 samples from this report only suggested 1% CRCT admixture by $\text{AFLP}_{\text{Standard}}$. It might be useful to sequence the ND2 gene in the 29 fish from the 2010 collection to further evaluate haplotype diversity to identify potential source populations. In any case, this population appears to have been compromised by stocking efforts early in the 20th century, both from Trappers Lake and potentially eggs derived from LeHardy Rapids cutthroat trout stock in Yellowstone National Park that were regularly imported into the state via both state and federal hatcheries. A thorough review of recently available stocking histories does confirm that this stream was stocked with 2000 cutthroat trout in 1951 and again in 1953 by the Colorado Game and Fish Department. This stocking record is not necessarily comprehensive, nor is the actual stocking location or subspecies specified. Although not good news, it is hoped that this new information will prove useful toward guiding future conservation efforts in French Gulch.

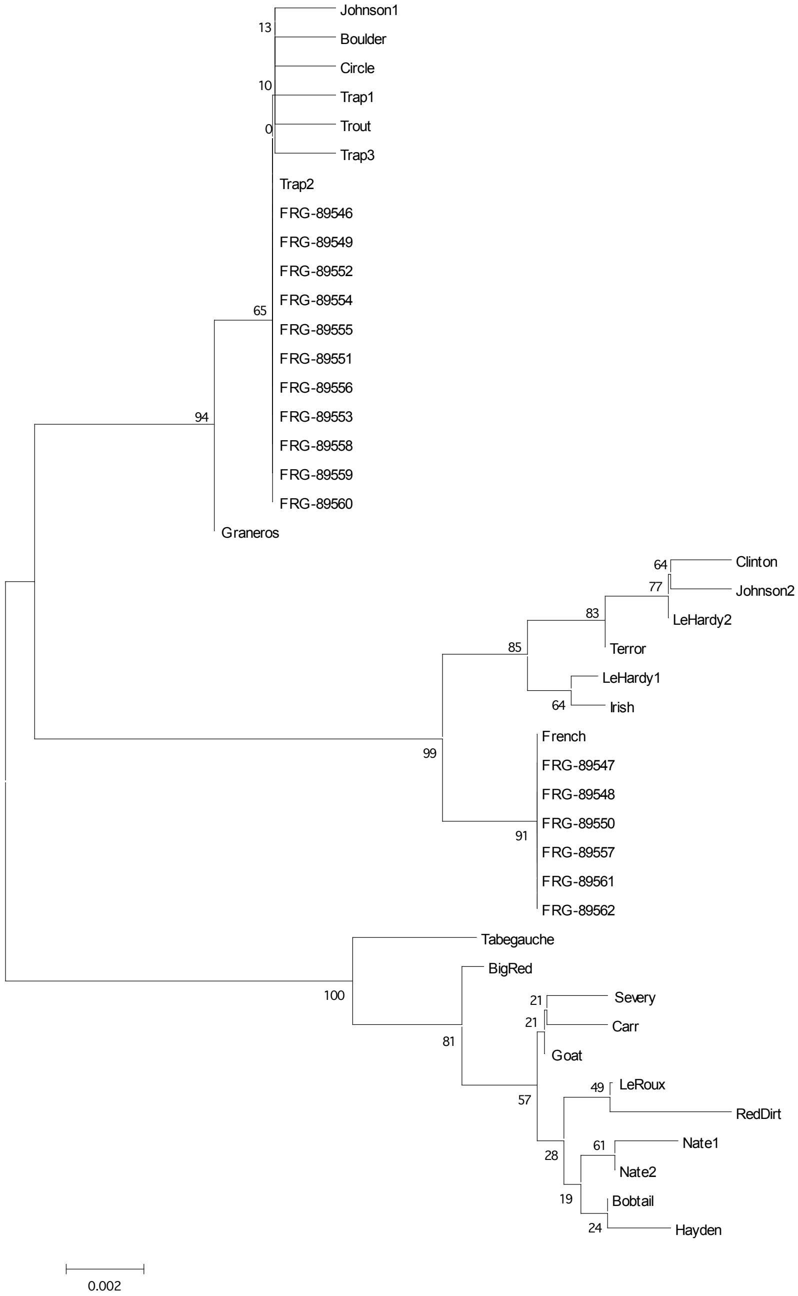

FIGURE 10: The evolutionary history of 2008 French Gulch specimens (FRG) are compared to type haplotypes for LineageCR CRCT (top branch), Yellowstone cutthroat trout (middle branch), and LineageGB CRCT (bottom branch) inferred using a Minimum Evolution method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method, and are in the units of the number of base substitutions per site.

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