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Species identification of cutthroat trout

using diagnostic SNP markers

for Scott Opitz

by

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Objective

The goal of this analysis was to estimate the species composition of nine populations of trout cutthroat trout. Genetic analysis of single nucleotide polymorphisms (SNPs) was used to estimate the proportion of the genes in each population that were derived from rainbow trout (RBT), Westslope cutthroat trout (WCT), and Yellowstone cutthroat trout (YCT).

Species identification with SNP markers

A SNP is a nucleotide in a DNA sequence that is variable among individuals, populations, or species. As an example, consider a sequence fragment of cytochrome B in rainbow and cutthroat trout.

Westslope cutthroat	GATGTGGCA**G**ACAGAGGAGAAA
Yellowstone cutthroat	GATGTGGCA**G**ACAGAGGAGAAA
Rainbow trout	GATGTGGCA**A**ACAGAGGAGAAA

The DNA sequences for each taxon are identical except for the G/A polymorphism that distinguishes RBT from cutthroat trout. This is a single nucleotide polymorphism (SNP). Such polymorphisms are ideal for species identification because they have a very low mutation rate and can be accurately genotyped in the laboratory. In addition, standardization among different laboratories is generally easy. In order to use SNPs for species identification, diagnostic

YCT, 20 were hybrids, and two appeared to be RBT. Overall 23% of the genes in the sample were RBT. Hybridization in the population has progressed for at least two generations. This is evident because there are many post F1 hybrids in the sample.

The sample of four individuals from the 9th Street section of the Yellowstone River had three individuals that appear to be pure YCT and one individual with approximately 50% RBT ancestry.

The sample of 24 individuals form the Mill Creek section of the Yellowstone River showed no evidence of introgression with RBT.

The sample of 34 individuals from the Springdale section of the Yellowstone River had 24 individuals that appear to be pure YCT, 9 RBT hybrids, and one apparently pure RBT.

Eighty samples were collected from Peterson Creek between April 28th and June 10th. As Table 2 shows, the first half of the sample contained a low frequency of hybrid fish, but after May 25th, the rest of the fish appeared to be pure YCT.

The accuracy of field identification of hybrid fish varied (Table 3). The best results were obtained in the Corwin Springs sample. Twenty-one of the 43 fish in the sample were identified as possible hybrids in the field. Genetic data show that 17 of these 21 were hybrids, two were RBT, and two were YCT. Of the 22 fish in the Corwin Springs sample that were not flagged as potential hybrids, 20 appeared to be pure YCT, two showed low levels of introgression (1 RBT allele), and one was 40% RBT.

Three fish from Peterson Creek were identified as possible hybrids. Of these, one was a hybrid (40% RBT ancestry), and two appeared to be pure YCT.

Seven fish from the Springdale sample were identified as possible hybrids in the field. Of these three were hybrids and four appeared to be pure YCT. There were 27 other fish in the sample that were not flagged as potential hybrids. Of these, 20 appear to be pure YCT, 6 are hybrids, and one appears to be a RBT.

Table 2. Species identification for samples collected from Peterson Creek broken down by date. SNP's were used for all of these tests.

Collector	Date	N	Waterbody	Section	Sample Number	Lat.	Long.	% YCT	% RBT
Opitz	4-28-06	1	Yellowstone R	Peterson Creek	10008	45.69402	110.28203	100	
"	5-01-06	2	"	"	"	"	"	96.7	3.3
"	5-04-06	2	"	"	"	"	"	100	
"	5-10-06	2	"	"	"	"	"	100	
"	5-16-06	10	"	"	"	"	"	100	
"	5-17-06	19	"	"	"	"	"	97.9	2.1
"	5-18-06	7	"	"	"	"	"	97.0	3.0
"	5-19-06	1	"	"	"	"	"	100	
"	5-21-06	2	"	"	"	"	"	100	
"	5-24-06	2	"	"	"	"	"	100	
"	5-25-06	5	"	"	"	"	"	87.7	12.3
"	5-26-06	8	"	"	"	"	"	100	
"	5-27-06	4	"	"	"	"	"	100	
"	5-29-06	4	"	"	"	"	"	100	
"	5-30-06	1	"	"	"	"	"	100	
"	6-06-06	2	"	"	"	"	"	100	
"	6-07-06	1	"	"	"	"	"	100	
"	6-08-06	2	"	"	"	"	"	100	
"	6-09-06	1	"	"	"	"	"	100	
"	6-10-06	1	"	"	"	"	"	100	

YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_02	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_03	0.93	0.07
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_04	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_05	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_06	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_07	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_08	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_09	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_10	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_11	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_12	0.60	0.40
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_13	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_14	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_15	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_16	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_17	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_18	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_19	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_20	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_21	1.00	
YR_Opitz_R3_22samples_Corwin_Spring_Sect_4_26_07_22	1.00	
YR, 9thStreet		
YR_Opitz_R3_9th_Street_Sect_4_11_07_01	0.47	0.53
YR_Opitz_R3_9th_Street_Sect_4_11_07_02	1.00	
YR_Opitz_R3_9th_Street_Sect_4_11_07_03	1.00	
YR_Opitz_R3_9th_Street_Sect_4_11_07_04	1.00	
YR, MillCreek		
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_01	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_02	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_03	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_04	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_05	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_06	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_07	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_08	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_09	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_10	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_11	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_12	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_13	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_14	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_15	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_25_07_16	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_26_07_17	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_26_07_18	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_26_07_19	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_26_07_20	1.00	
YR_Opitz_R3_Mill_Creek_Sect_4_26_07_21	1.00	

YR_Opitz_R3_Peterson_Creek_5_21_06_45	1.00	
YR_Opitz_R3_Peterson_Creek_5_24_06_01	1.00	
YR_Opitz_R3_Peterson_Creek_5_24_06_02	1.00	
YR_Opitz_R3_Peterson_Creek_5_25_06_01	0.31	0.69
YR_Opitz_R3_Peterson_Creek_5_25_06_02	1.00	
YR_Opitz_R3_Peterson_Creek_5_25_06_03	1.00	
YR_Opitz_R3_Peterson_Creek_5_25_06_04	1.00	
YR_Opitz_R3_Peterson_Creek_5_25_06_05	1.00	
YR_Opitz_R3_Peterson_Creek_5_26_06_01	1.00	
YR_Opitz_R3_Peterson_Creek_5_26_06_02	1.00	
YR_Opitz_R3_Peterson_Creek_5_26_06_03	1.00	
YR_Opitz_R3_Peterson_Creek_5_26_06_04	1.00	
YR_Opitz_R3_Peterson_Creek_5_26_06_12	1.00	
YR_Opitz_R3_Peterson_Creek_5_26_06_13	1.00	
YR_Opitz_R3_Peterson_Creek_5_26_06_14	1.00	
YR_Opitz_R3_Peterson_Creek_5_26_06_15	1.00	
YR_Opitz_R3_Peterson_Creek_5_27_06_16	1.00	
YR_Opitz_R3_Peterson_Creek_5_27_06_17	1.00	
YR_Opitz_R3_Peterson_Creek_5_27_06_18	1.00	
YR_Opitz_R3_Peterson_Creek_5_27_06_19	1.00	
YR_Opitz_R3_Peterson_Creek_5_29_06_20	1.00	
YR_Opitz_R3_Peterson_Creek_5_29_06_21	1.00	
YR_Opitz_R3_Peterson_Creek_5_29_06_22	1.00	
YR_Opitz_R3_Peterson_Creek_5_29_06_23	1.00	
YR_Opitz_R3_Peterson_Creek_5_30_06_24	1.00	
YR_Opitz_R3_Peterson_Creek_5_30_06_25	1.00	
YR_Opitz_R3_Peterson_Creek_6_06_06_26	1.00	
YR_Opitz_R3_Peterson_Creek_6_06_06_27	1.00	
YR_Opitz_R3_Peterson_Creek_6_07_06_28	1.00	
YR_Opitz_R3_Peterson_Creek_6_08_06_29	1.00	
YR_Opitz_R3_Peterson_Creek_6_08_06_30	1.00	
YR_Opitz_R3_Peterson_Creek_6_09_06_31	1.00	
YR_Opitz_R3_Peterson_Creek_6_10_06_32	1.00	
YR_Opitz_R3_Peterson_Creek_6_13_06_33	1.00	
YR_Opitz_R3_Peterson_Creek_6_16_06_34	1.00	
YR, Springdale		
YR_Opitz_R3_Springdale_Sect_4_14_06_04		1.00
YR_Opitz_R3_Springdale_Sect_4_14_06_05	0.60	0.4
YR_Opitz_R3_Springdale_Sect_4_17_06_18	1.00	
YR_Opitz_R3_Springdale_Sect_4_17_06_19	1.00	
YR_Opitz_R3_Springdale_Sect_4_17_06_20	1.00	
YR_Opitz_R3_Springdale_Sect_4_17_06_21	1.00	
YR_Opitz_R3_Springdale_Sect_4_17_06_22	1.00	
YR_Opitz_R3_Springdale_Sect_4_17_06_23	1.00	
YR_Opitz_R3_Springdale_Sect_4_17_06_24	1.00	
YR_Opitz_R3_Springdale_Sect_4_17_06_25	1.00	
YR_Opitz_R3_Springdale_Sect_4_18_06_26	1.00	
YR_Opitz_R3_Springdale_Sect_4_19_06_06	1.00	
YR_Opitz_R3_Springdale_Sect_4_19_06_07	0.47	0.53

Appendix 1. Diagnostic SNP loci used in this study.The column "Diagnostic taxon" indicates which taxon has a unique allele that is not found in the other taxa.

Locus Name	Abbreviation	Diagnostic taxon
Cytochrome b	CytB	RBT
Thymosin beta	Thymo-b	RBT
Recombination activating locus	RAG1	RBT
Vimentin	Vim	RBT
Calreticulin	Cal	RBT
Carbonyl reductase	CBR1	WCT
Proto-oncogene	P53	WCT
Transferrin exon 5 to 8	Tnsf	WCT
Prolactin II	PrL2-R1	YCT
Metallothionein B	MT1B	YCT
Trypsin III precursor	Try-III	YCT