GENETIC INVESTIGATION OF SNAKE RIVER AND YELLOWSTONE CUTTHROAT TROUT

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✦ SUMMARY

The relationship between Yellowstone cutthroat trout, *Oncorhynchus clarki bouvieri*, and Snake River finespotted cutthroat trout, *O. clarki behnkei,* was examined with two mitochondrial DNA fragments, COI and ND5. No variation was found within either subspecies, and just one (out of 1069) nucleotide differed between subspecies. Thus, these subspecies are very closely related.

Samples for this study were obtained from hatcheries, and may not be representative of the subspecies. *O. c. bouveri* were sampled from the Clark Fork Hatchery, in Powell, WY, and *O. c. behnkei* were sampled from the National Fish Hatchery in Jackson, WY. Further sampling, preferably from natural populations, is needed to more thoroughly survey the variation within subspecies, and to measure the differences between subspecies.

✦ INTRODUCTION

In the upper portion of the Snake River drainage, two subspecies of cutthroat trout can be distinguished by the sizes of their spots. The Snake River finespotted cutthroat, *Oncorhynchus clarki behnkei*, and Yellowstone cutthroat trout, *O. clarki bouveri*, both occupy the Snake River drainage between Jackson Lake and Palisades Reservoir, but Yellowstone occupies the smaller streams, while finespotted are in the main stem of the Snake River. Despite contact in several streams, the subspecies remain distinct for their spotting pattern. However, none of the meristic characters differ between these subspecies, and geneticists have not yet reported any diagnostic differences of any kind.

The purpose of this study was to survey these subspecies for diagnostic genetic markers. Mitochondrial sequences were chosen for their high rates of evolution and their maternal inheritance without recombination.

✦ MATERIALS AND METHODS

Samples

Kerry Grande provided Snake River cutthroat from the National Fish Hatchery in Jackson, WY. Dave Miller provided Yellowstone cutthroat taken from the Lahar Rapids of the Yellowstone River, and currently in production at the Clark Fork Hatchery in Powell, WY. Arlene Ganek provided Rio Grande cutthroat, *O. c. virginalis*, from the Colorado Division of Wildlife Poudre River fish hatchery, which are a mixture of fish from different rivers. Brood fish from Mindano Creek, West Indian Creek and Placer Creek were all crossbred to create the hatchery stock at the Poudre River facility. The Rio Grande cutthroat in those three streams are considered to be "pure" Rio Grande cutthroat. Colorado Division of Wildlife personnel, including Douglas Krieger, Tom Nesler, and Jim Melby, provided samples of DNA from greenback cutthroat trout, *O. c.*

stomias, from Severy Creek, Como Creek, and Graneros Creek. Biologists in the US Fish and Wildlife Service and Rocky Mountain National Park supplied Colorado River cutthroat, *O. c. pleuriticus,* from Ptarmigan Creek and Columbine Creek in Rocky Mountain National Park.

Molecular methods

Genomic DNA was extracted from a small piece of the adipose fin, using the manufacturer's instructions for the AIAamp Tissue Kit, marketed by Qiagen.

MtDNA sequences were obtained for COX I and ND5. The primers for COX I were COIared, (5' CACAGTGTGTAGGCGTCTGG 3') and COIf (5' CCTGCAGGAGGAGGAGACCC 3'); both primers were modified from Palumbi (1996) to better fit cutthroat trout sequences. The primers for ND5 were ND5-2 (5' AATAGTTTAT CCGTTGGTCTTAGG 3') and ND5R1 (5' GGCC AAGATCTCCTACTCG 3'). Amplifications were conducted in a total volume of 50µl using 50 mM KCL, 10 mM Tris-HCL (pH 8.3), 0.01% gelatin, 2 mM $MgCl_2$, 200 µM dNTPs, 1.5 units Taq polymerase, 0.3 µM of each primer, 100ng template DNA and water to the final volume. PCR cycle conditions for COX I consisted of an initial denaturing step at 95°C for one minute followed by 39 cycles of 1 minute at 95°C, 1 minute at 55°C and 3 minutes at 72°C, capped off by a final step at 72°C for 7 minutes. The ND5 cycle conditions were modified from this program with an annealing temperature of 50°C, an extension time of 1 minute, and 35 cycles.

All sequences were generated using Big Dye chemistry and an ABI377 in the sequencing facility in the Department of Molecular, Cellular, and Developmental Biology at the University of Colorado in Boulder. Sequences were edited, assembled into contigs, and arranged into correct reading frame with the software Sequencher.

The COX I and ND5 sequences were trimmed to 560 bp and 509 bp, respectively. These were amplified, sequenced, edited and aligned separately, but the sequences for each fish were combined into a single 1069 bp sequence for comparison.

✦ RESULTS AND DISCUSSION

Sequences were obtained for six Yellowstone and six finespotted cutthroat. No variation was found within either subspecies, but it is worth noting that each subspecies was sampled from a single hatchery. One diagnostic difference was noted between these subspecies, in the portion of the sequence from the COXI gene (position #12, table 1).

For comparison to the subspecies occurring in further south, a sequence is presented that is shared by Colorado River, greenback, and Rio Grande cutthroat. The sequences in Yelllowstone and Snake River finespotted differ from this sequence by 9 and 10 substitutions, respectively (Table 1).

This study is the first to find a genetic difference between Yellowstone and Snake River finespotted cutthroat, but this result is offered tentatively. The Snake River cutthroat all came from one hatchery, and the Yellowstone all came from a different hatchery. It is likely that those hatchery stocks do not represent all of the variation within their subspecies. The stocks in those two hatcheries have different haplotypes, but does that indicate that the two subspecies are different? I recommend that each of these subspecies be sampled more thoroughly, from natural populations, if possible. This report also provides the incentive to survey further in the mitochondrial in the mitochondrial genome, focusing on the fastest-evolving genes, such as ND2.

✦ LITERATURE CITED

Palumbi, S. R. 1996. Nucleic acids II: The polymerase chain reaction. pp. 205-247 in D. M. Hillis, C. Moritz, and B. K. Mable (eds.) Molecular Systematics: Second Edition. Sinauer Associates, Inc. Sunderland, MA.

Table 1. Mitochondrial DNA sequences from cutthroat trout. The sequences are from COXI (first 560 bp) and ND5 (509 bp) in Yellowstone, Snake River finespotted, and three subspecies in Colorado; greenback, Colorado River, and Rio Grande cutthroat.

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G CR RG       #1     GTCTATATTC TTATCCTCCC AGGCTTTGGT ATGATTTCAC
Snake River   #1     GTCTATATTC TCATCCTCCC AGGCTTTGGT ATGATTTCAC
Yellowstone   #1     GTCTATATTC TTATCCTCCC AGGCTTTGGT ATGATTTCAC
                     ..........................................
              #1     GTCTATATTC TTATCCTCCC AGGCTTTGGT ATGATTTCAC
                                *

G CR RG       #41    ATATCGTTGC ATACTACTCC GGCAAAAAAG AACCCTTCGG
Snake River   #41    ATATCGTTGC ATACTACTCC GGCAAAAAAG AACCCTTCGG
Yellowstone   #41    ATATCGTTGC ATACTACTCC GGCAAAAAAG AACCCTTCGG
                     ..........................................

G CR RG       #81    ATATATAGGA ATAGTCTGAG CTATAATAGC CATCGGATTA
Snake River   #81    ATATATAGGA ATAGTCTGAG CTATGATAGC CATCGGATTA
Yellowstone   #81    ATATATAGGA ATAGTCTGAG CTATGATAGC CATCGGATTA
                     ..........................................
              #81    ATATATAGGA ATAGTCTGAG CTATGATAGC CATCGGATTA
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G CR RG       #121   TTAGGATTTA TCGTTTGAGC CCACCATATG TTTACTGTCG
Snake River   #121   TTAGGATTTA TCGTTTGAGC CCACCATATG TTTACTGTCG
Yellowstone   #121   TTAGGATTTA TCGTTTGAGC CCACCATATG TTTACTGTCG
                     ..........................................
              #121   TTAGGATTTA TCGTTTGAGC CCACCATATG TTTACTGTCG

G CR RG       #161   GGATAGACGT GGACACTCGT GCCTACTTTA CATCTGCCAC
Snake River   #161   GGATAGACGT GGACACTCGT GCCTACTTTA CATCTGCCAC
Yellowstone   #161   GGATAGACGT GGACACTCGT GCCTACTTTA CATCTGCCAC
                     ..........................................
              #161   GGATAGACGT GGACACTCGT GCCTACTTTA CATCTGCCAC

G CR RG       #201   CATGATTATC GCTATCCCTA CAGGAGTAAA AGTATTTAGC
Snake River   #201   CATGATTATC GCTATCCCTA CAGGAGTAAA AGTATTTAGT
Yellowstone   #201   CATGATTATC GCTATCCCTA CAGGAGTAAA AGTATTTAGT
                     ..........................................
              #201   CATGATTATC GCTATCCCTA CAGGAGTAAA AGTATTTAGT
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G CR RG       #241   TGACTAGCCA CGCTACACGG AGGCTCGATC AAATGAGAAA
Snake River   #241   TGACTAGCCA CACTACACGG AGGCTCGATC AAATGAGAAA
Yellowstone   #241   TGACTAGCCA CACTACACGG AGGCTCGATC AAATGAGAAA
                     ..........................................
              #241   TGACTAGCCA CACTACACGG AGGCTCGATC AAATGAGAAA
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G CR RG       #281   CACCACTTCT TTGAGCCCTC GGGTTCATTT TCCTATTTAC
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Snake River     #281    CACCACTTCT TTGAGCCCTC GGGTTCATTT TCCTATTTAC
Yellowstone     #281    CACCACTTCT TTGAGCCCTC GGGTTCATTT TCCTATTTAC
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                #281    CACCACTTCT TTGAGCCCTC GGGTTCATTT TCCTATTTAC


G CR RG         #321    AGTGGGCGGA CTTACGGGTA TTGTCCTTGC TAACTCCTCA
Snake River     #321    AGTGGGCGGA CTTACGGGTA TTGTCCTTGC TAACTCCTCA
Yellowstone     #321    AGTGGGCGGA CTTACGGGTA TTGTCCTTGC TAACTCCTCA
                        ...........................................
                #321    AGTGGGCGGA CTTACGGGTA TTGTCCTTGC TAACTCCTCA


G CR RG         #361    TTAGACATTG TTCTACATGA CACTTACTAC GTAGTTGCTC
Snake River     #361    TTAGACATTG TTCTACATGA CACTTACTAC GTAGTTGCTC
Yellowstone     #361    TTAGACATTG TTCTACATGA CACTTACTAC GTAGTTGCTC
                        ...........................................
                #361    TTAGACATTG TTCTACATGA CACTTACTAC GTAGTTGCTC


G CR RG         #401    ATTTCCACTA CGTATTATCC ATAGGAGCTG TGTTTGCTAT
Snake River     #401    ATTTCCACTA CGTATTATCT ATAGGAGCTG TGTTTGCTAT
Yellowstone     #401    ATTTCCACTA CGTATTATCT ATAGGAGCTG TGTTTGCTAT
                        ...........................................
                #401    ATTTCCACTA CGTATTATCT ATAGGAGCTG TGTTTGCTAT
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G CR RG         #441    TATAGGCGCT TTCGTACACT GATTTCCCCT ATTCACAGGA
Snake River     #441    TATAGGCGCT TTCGTACACT GATTTCCCCT ATTTACAGGA
Yellowstone     #441    TATAGGCGCT TTCGTACACT GATTTCCCCT ATTTACAGGA
                        ...........................................
                #441    TATAGGCGCT TTCGTACACT GATTTCCCCT ATTTACAGGA
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G CR RG         #481    TATACCCTTC ACAGCACATG GACCAAAATC CATTTTGGAA
Snake River     #481    TACACCCTTC ACAGCACATG GACCAAAATC CATTTTGGAA
Yellowstone     #481    TACACCCTTC ACAGCACATG GACCAAAATC CATTTTGGAA
                        ...........................................
                #481    TACACCCTTC ACAGCACATG GACCAAAATC CATTTTGGAA
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G CR RG         #521    TTATATTTAT CGGCGTAAAT TTAACCTTTT TCCCACAGCA
Snake River     #521    TTATATTTAT CGGCGTAAAT TTAACCTTTT TCCCACAGCA
Yellowstone     #521    TTATATTTAT CGGCGTAAAT TTAACCTTTT TCCCACAGCA
                        ...........................................
                #521    TTATATTTAT CGGCGTAAAT TTAACCTTTT TCCCACAGCA


G CR RG         #561    TCCGACTACA CTCATCTTAA GCTCATCCCT TTTAACAATC
Snake River     #561    TCCGACTACA CTCATCTTAA GCTCATCCCT TTTAACAATC
Yellowstone     #561    TCCGACTACA CTCATCTTAA GCTCATCCCT TTTAACAATC
                        ...........................................
                #561    TCCGACTACA CTCATCTTAA GCTCATCCCT TTTAACAAT
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G CR RG       #601    TTCGCACTTC TAATTTATCC TCTTGTTACC ACCCTCACCC
Snake River   #601    TTCGCACTTC TAATTTATCC TCTTGTTACC ACCCTCACCC
Yellowstone   #601    TTCGCACTTC TAATTTATCC TCTTGTTACC ACCCTCACCC
                      ..........................................
              #601    TTCGCACTTC TAATTTATCC TCTTGTTACC ACCCTCACCC

G CR RG       #641    CAACCCCCCA GCACAAAGAC TGAGCCCTCA CTCACGTAAA
Snake River   #641    CAACCCCCCA GCACAAAGAC TGAGCCCTCA CTCACGTAAA
Yellowstone   #641    CAACCCCCCA GCACAAAGAC TGAGCCCTCA CTCACGTAAA
                      ..........................................
              #641    CAACCCCCCA GCACAAAGAC TGAGCCCTCA CTCACGTAAA

G CR RG       #681    AACTGCTATC AAAATGGCCT TCCTAGTGAG CTTACTCCCC
Snake River   #681    AACTGCTATC AAAATGGCCT TCCTAGTGAG CTTACTCCCC
Yellowstone   #681    AACTGCTATC AAAATGGCCT TCCTAGTGAG CTTACTCCCC
                      ..........................................
              #681    AACTGCTATC AAAATGGCCT TCCTAGTGAG CTTACTCCCC

G CR RG       #721    CTTTTTATAT TCCTAGACCA AGGAACCGAA ACTATCGTCA
Snake River   #721    CTTTTTATAT TCCTAGACCA AGGAACCGAA ACTATCGTCA
Yellowstone   #721    CTTTTTATAT TCCTAGACCA AGGAACCGAA ACTATCGTCA
                      ..........................................
              #721    CTTTTTATAT TCCTAGACCA AGGAACCGAA ACTATCGTCA

G CR RG       #761    CTAATTGACA GTGAATAAAC ACCACAACCT TTGACATTAA
Snake River   #761    CTAATTGACA GTGAATAAAC ACCACAACCT TTGACATTAA
Yellowstone   #761    CTAATTGACA GTGAATAAAC ACCACAACCT TTGACATTAA
                      ..........................................
              #761    CTAATTGACA GTGAATAAAC ACCACAACCT TTGACATTAA

G CR RG       #801    CCTTAGCTTT AAATTTGACC ACTACTCCGT TATTTTCACC
Snake River   #801    CCTTAGCTTT AAATTTGACC ACTACTCCAT TATTTTCACC
Yellowstone   #801    CCTTAGCTTT AAATTTGACC ACTACTCCAT TATTTTCACC
                      ..........................................
              #801    CCTTAGCTTT AAATTTGACC ACTACTCCAT TATTTTCACC
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G CR RG       #841    CCTATTGCCC TGTACGTAAC CTGATCTATT CTAGAATTCG
Snake River   #841    CCTATTGCCC TGTACGTAAC CTGATCTATT CTAGAATTCG
Yellowstone   #841    CCTATTGCCC TGTACGTAAC CTGATCTATT CTAGAATTCG
                      ..........................................
              #841    CCTATTGCCC TGTACGTAAC CTGATCTATT CTAGAATTCG

G CR RG       #881    CATCCTGGTA TATACATGCT GACCCCAACA TAAACCGGTT
Snake River   #881    CATCCTGGTA TATACATGCT GACCCCAACA TAAACCGGTT
Yellowstone   #881    CATCCTGGTA TATACATGCT GACCCCAACA TAAACCGGTT
                      ..........................................
              #881    CATCCTGGTA TATACATGCT GACCCCAACA TAAACCGGTT
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G CR RG       #921    CTTTAAGTAC CTCCTCCTCT TCCTGATTGC CATAATTATT
Snake River   #921    CTTTAAGTAC CTCCTCCTCT TCCTGATTGC CATAATTATT
Yellowstone   #921    CTTTAAGTAC CTCCTCCTCT TCCTGATTGC CATAATTATT
                      ..........................................
              #921    CTTTAAGTAC CTCCTCCTCT TCCTGATTGC CATAATTATT

G CR RG       #961    TTGGTAACCG CCAACAACAT GTTCCAACTG TTTATCGGCT
Snake River   #961    TTGGTAACCG CCAACAACAT GTTCCAACTG TTTATCGGCT
Yellowstone   #961    TTGGTAACCG CCAACAACAT GTTCCAACTG TTTATCGGCT
                      ..........................................
              #961    TTGGTAACCG CCAACAACAT GTTCCAACTG TTTATCGGCT

G CR RG       #1001   GAGAGGGAGT TGGAATTATA TCGTTCCTCC TTATCGGGTG
Snake River   #1001   GAGAAGGAGT TGGAATTATA TCATTCCTCC TTATCGGGTG
Yellowstone   #1001   GAGAAGGAGT TGGAATTATA TCATTCCTCC TTATCGGGTG
                      ..........................................
              #1001   GAGAAGGAGT TGGAATTATA TCATTCCTCC TTATCGGGTG
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G CR RG       #1041   GTGGCACGGT CGAGCCGACG CTAACACAG
Snake River   #1041    GTGGCACGGT CGAGCCGACG CTAACACAG
Yellowstone   #1041   GTGGCACGGT CGAGCCGACG CTAACACAG
                      ..........................................
              #1041   GTGGCACGGT CGAGCCGACG CTAACACAG
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