

## CUTTHROAT TROUT TAXONOMY: EXPLORING THE HERITAGE OF COLORADO'S STATE FISH

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**ABSTRACT** — Prior to recent molecular testing, larger spots and more scales above the lateral line were phenotypic traits associated with greenback cutthroat trout *Oncorhynchus clarkii stomias* as compared to Colorado River cutthroat trout *O. c. pleuriticus*. However, these two subspecies could not be separated consistently on the basis of those characteristics. As a result, geographic range had become the default approach for establishing subspecies designation. In 2007, researchers at the University of Colorado and others used mitochondrial DNA (mtDNA), microsatellites, and amplified fragment length polymorphisms (AFLPs) to suggest there was a genetic basis for separating greenback cutthroat trout from Colorado River cutthroat trout. The primary concern raised by that study was that five of the nine greenback cutthroat trout populations they examined actually displayed genetic fingerprints more similar to Colorado River cutthroat trout from Trappers Lake, Colorado (Lineage CR). A finding that attracted less attention was the discovery of an alleged greenback cutthroat trout population west of the Continental Divide near Gunnison, Colorado, in West Antelope Creek. Recovery Team partners used the same AFLP test to canvass cutthroat trout populations for genetic purity across Colorado. Results indicate that the West Antelope Creek population is not unique, as 46 additional populations sharing the “greenback” genetic fingerprint (Lineage GB) have now been identified west of the Continental Divide. It is therefore questionable whether West Antelope Creek fish are really greenback cutthroat trout as suggested in the 2007 study, or whether they simply represent diversity within Colorado River cutthroat trout. Given their broad geographic distribution and lack of a robust East Slope source from which to stock, it seems unlikely that these Lineage GB populations were established by anthropogenic means. A more parsimonious explanation would suggest they are aboriginal to Colorado's West Slope. Interestingly, no Lineage GB populations have been discovered in the White and Yampa river basins. This region appears to be a stronghold for Lineage CR cutthroat trout and coincides with the native range of Colorado's only other native salmonid, the mountain whitefish *Prosopium williamsoni*. Perhaps Lineage GB fish were already present in southwestern Colorado when Lineage CR and mountain whitefish invaded the state from the Green River drainage.

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### INTRODUCTION

There has been a long-standing interest in the taxonomy of cutthroat trout in Colorado ever since the rediscovery and listing of greenback cutthroat trout *Oncorhynchus clarkii stomias* as endangered under the Endangered Species Act in 1973 and subsequent down-listing to threatened status in 1978. No fewer than 72 reports have been published since then discussing genetic purity, introgression, and relatedness. Much of this work has been summarized

in several books (Gresswell 1988; Behnke 1992; Behnke 2002). Phenotypic traits typically associated with greenback cutthroat trout are larger spot size and more scales above the lateral line and in the lateral series as compared to Colorado River cutthroat trout *O. c. pleuriticus* (Behnke 1992). However, these two subspecies cannot be separated definitively on the basis of these characteristics (Behnke 1992; Behnke 2002). Further, Behnke maintains that a modern taxonomist would not separate the greenback cutthroat trout from Colorado River cutthroat

trout (R. J. Behnke, personal communication). Given the difficulty separating the two subspecies using visual characteristics, geographic range became the default criterion for establishing subspecies designation.

Though early molecular work also failed to reliably distinguish these two subspecies (Behnke 2002), more recent studies of molecular data, including mitochondrial DNA (mtDNA), microsatellites, and amplified fragment length polymorphisms (AFLPs) suggested that indeed there was a genetic basis for separating greenback cutthroat trout from Colorado River cutthroat trout (Metcalf et al. 2007). An important finding of the Metcalf et al. study was that many of the putatively pure greenback cutthroat trout populations east of the Continental Divide were genetically more similar to Colorado River cutthroat trout from Trappers Lake, Colorado (Lineage CR), than to other greenback cutthroat trout populations such as those found in Severy Creek, Colorado (Lineage GB). This was particularly troubling since mechanisms were in place to deliver pure Lineage CR to the East Slope. From 1903 through 1938 what could have been well in excess of 40 million pure Colorado River cutthroat trout were produced at Trappers Lake, Colorado (Rogers 2008). Millions more were produced on the south slope of Pikes Peak (Rogers and Kennedy 2008). The fate of many of these fish remains a mystery. The long-standing notion that fishless waters above barriers to immigration were likely stocked from nearby waters may not hold, as fingerlings produced at Trappers Lake and other state and federal spawn operations were readily available, and obviated the need for capturing wild fish. It is certainly conceivable that some pure cutthroat trout populations east of the Continental Divide were founded in historically fishless waters from progeny of distant spawn operations.

The publication of the Metcalf et al. (2007) paper was followed by an international media firestorm critical of recovery program efforts for allegedly using the “wrong” fish for founding new populations of cutthroat trout in greenback cutthroat trout recovery waters. The Greenback Recovery Team has always used the best available science to guide recovery efforts, recognizing that science is a dynamic field. As such, the most prudent management direction can and often does change over time. Although the multi-agency Greenback Recovery Team had initiated development of a long-range greenback cutthroat trout management plan that

would allow delisting after decades of progress, unresolved taxonomic issues regarding what constitutes a greenback cutthroat trout ground recovery efforts to a halt.

A finding of Metcalf et al. (2007) that attracted less attention was the discovery of an alleged greenback cutthroat trout population west of the Continental Divide near Gunnison, Colorado, in West Antelope Creek. Early results from concurrent studies conducted by the Colorado Division of Wildlife through a private genetics lab using AFLP markers indicated that in fact the West Antelope Creek population was not unique. By 2008, a dozen additional populations displaying the Lineage GB fingerprint were identified west of the Continental Divide (Rogers 2008). Those findings led the Recovery Team to question whether the West Antelope Creek fish were really greenback cutthroat trout as suggested by Metcalf et al. (2007), or whether they represented a diverse lineage endemic to the western slope of Colorado. Interest in resolving that dilemma led to the major molecular survey efforts by Greenback Recovery Team and Colorado River Cutthroat Trout Conservation Team members discussed here.

## METHODS

### Tissue Collection

Fin tissues from over 8,200 fish were collected from 366 populations of cutthroat trout in Colorado and southern Wyoming. A small piece (1 cm<sup>2</sup>) fin was collected from the top of the caudal fin from each fish, as this fin regenerates rapidly, is assumed to be less critical for digging redds, and provides adequate tissue volume even on small fish (Rogers 2007). Fins were stored individually in 15-mL conical centrifuge tubes filled with 80% reagent grade ethanol until processing. In addition, UTM coordinates, photographs, and fish lengths were recorded.

### DNA Isolation and Evaluation

Tissue samples were delivered to Pisces Molecular (Boulder, Colorado) for DNA isolation and testing with the same AFLP procedure describe in Metcalf et al. (2007). Cutthroat trout DNA was extracted from fin clips using a proteinase K tissue lysis and spin-column DNA purification protocol (DNeasy Tissue Kit, Qiagen, Inc. Chatsworth, CA).

The DNA was then amplified using a polymerase chain reaction (PCR) to produce AFLP marker fragments. These fragments were separated and sized on an ABI 3130 sequencer (Applied Biosystems, Foster City, California).

Using Genemapper 4.0 (Applied Biosystems), a genetic fingerprint was produced for each individual sample by scoring for the presence or absence of a standardized set of 119 markers between 50 and 450 base pairs in size generated from a reference set of cutthroat trout populations (Rogers 2008). The genetic fingerprints of individuals in the test population were compared to those found in the reference populations using a Bayesian approach for identifying population clusters (Pritchard et al. 2000). The program STRUCTURE 2.2 (Falush et al. 2007; Pritchard et al. 2007) was used to evaluate 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 trout 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 population in question.

Populations showing greater than 20% admixture (where  $q$  values were less than 0.80) with Yellowstone cutthroat trout *O. c. bouvieri*, rainbow trout *O. mykiss* or the two lineages native to Colorado (Lineage CR and Lineage GB) were excluded from further consideration. Locations of the populations were plotted to discern spatial relationships. The number of populations represented by each lineage was calculated for each major watershed-based geographic management unit (GMU) across the range of Colorado River cutthroat trout (Hirsch et al. 2006).

## RESULTS

Extensive molecular surveys of the 366 populations of cutthroat trout in Colorado and the Little Snake River drainage in southern Wyoming have identified 156 streams containing cutthroat trout west of the Continental Divide that displayed  $q$  values higher than 0.80 as measured with AFLP markers (Rogers 2008). This approach identified 37 populations as Lineage GB. When combined with an additional 9 populations identified from molecular work conducted at Brigham Young University (D. Shiozawa, Brigham Young University, personal communication) and one population at the University of Colorado (Martin 2008), it appears that at least 47 populations of Lineage GB exist west of the Continental Divide at the time of this writing. These populations are distributed across 14 counties in western Colorado and one in San Juan County, Utah (Figure 1). Populations are concentrated around the Grand Mesa (Figure 1) but range into the headwaters of the Colorado, Gunnison, and Dolores drainages as well. No Lineage GB populations were found in the Yampa, White, or Little Snake river basins where Lineage CR is pervasive (Figure 2). In fact, 59 of the 109 Lineage CR populations identified in this survey were located in the Yampa GMU, though good numbers (36) were also found in the Upper Colorado GMU (Figure 3).

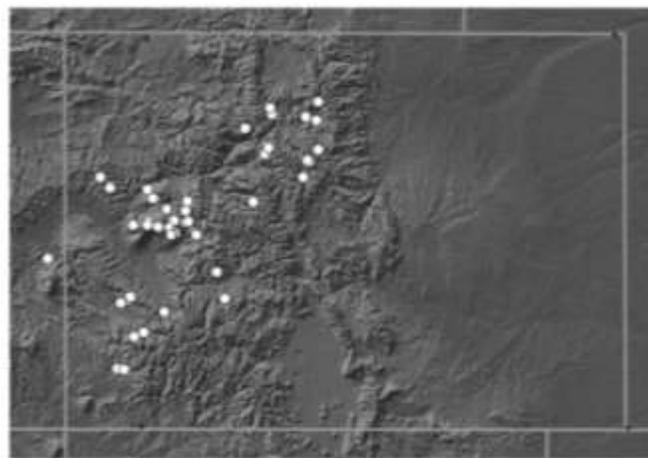


Figure 1: Geographic location of 47 Lineage GB cutthroat trout populations identified west of the Continental Divide as of August 2010.

## DISCUSSION

Given their broad geographic distribution and lack of a robust East Slope wild spawn operation from which to gather fertilized eggs, it seems unlikely that Lineage GB populations were established west of the Continental Divide by anthropogenic means as suggested by Metcalf et al. (2007). A more parsimonious explanation is that these fish are aboriginal at least to the Gunnison and Dolores basins, establishing east of the Continental Divide either on their own or with the aid of early fish culturists. As such, Lineage GB may represent a divergent lineage reflecting a complex past within Colorado River cutthroat trout. This scenario is consistent with Evans and Shiozawa's (2000) assertion that Colorado River cutthroat trout were

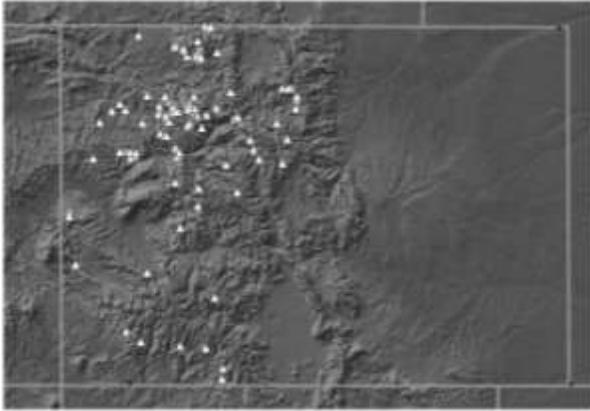


Figure 2: Geographic location of 109 Lineage CR cutthroat trout populations identified west of the Continental Divide as of August 2010.

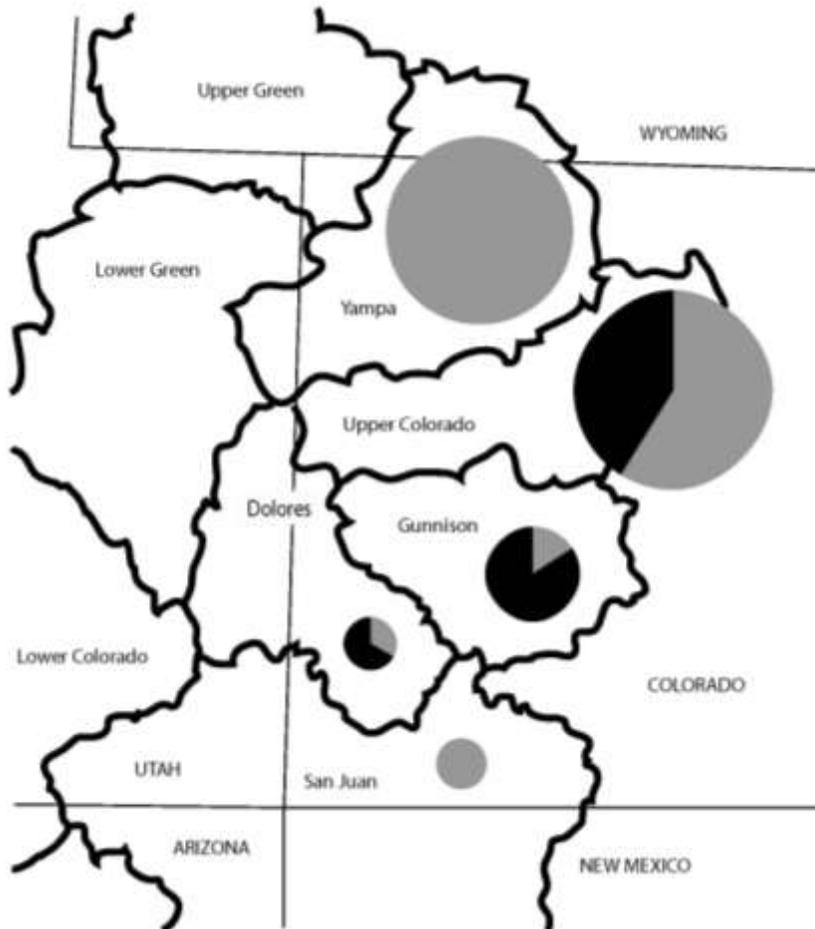


Figure 3: The area of each pie is proportional to the number of cutthroat trout populations from each GMU that displayed less than 20% admixture with rainbow trout or Yellowstone cutthroat trout. Wedges from each pie represent the number that were Lineage CR (Gray) or Lineage GB (black). This study did not cover the Upper Green, Lower Green, or Lower Colorado GMUs.

established first by an ancient invasion from the west which was then masked in many populations by subsequent invasions from the north out of Wyoming down the Green River drainage.

Of particular interest is the lack of Lineage GB populations in the Yampa GMU (White, Yampa, and Little Snake river drainages in northwestern Colorado and southern Wyoming). This area appears to be a stronghold for Lineage CR cutthroat trout and coincides with the native range of Colorado's only other native salmonid – the mountain whitefish *Prosopium williamsoni*. Whiteley et al. (2006) conducted a range-wide survey of mountain whitefish and found five genetically divergent assemblages that were organized around major drainages. Mountain whitefish in Colorado are likely most closely related to the Snake River assemblage perhaps invading from the north down the Green River drainage along with Lineage CR following the recession of the last ice age. It is noteworthy that mountain whitefish have become very common in the Colorado and Roaring Fork rivers following introductions in the 1940s, even though they are not native to those drainages (Feast 1938). Clearly, the habitat is not limiting, so the absence of mountain whitefish historically suggests that something else excluded them. Perhaps the desert confluence of the Colorado and Green Rivers provided a thermal barrier to movement out of the Green River into the Colorado River. Interestingly, mountain suckers *Catostomus platyrhynchus* are similarly restricted to the Yampa GMU (Woodling 1985), perhaps reflecting the invasion of an entire assemblage of fish species at the same time.

Whether Lineage GB, Lineage CR, or both are native to the Upper Colorado GMU is still difficult to discern, as both lineages are present in large numbers (Figure 3). Many of the Lineage GB populations are found in remote locations that might not have attracted much attention from early fish managers. Much of the Upper Colorado River is prime trout habitat, and it was those prime habitats that apparently attracted the subsequent stocking of potentially hybridizing species leaving remnant populations of pure cutthroat trout spread around the periphery of the range (Hirsch et al. 2006). While that might argue for Lineage GB as the aboriginal fish to the Upper Colorado GMU, it is true that unlike mountain whitefish, cutthroat trout are capable of invading the uppermost tributaries of headwater streams. That ability makes them much

better candidates for successful headwater transfers. Certainly there are numerous shallow divides near the headwaters of the White and Yampa rivers that could have acted as conduits for the natural migration of Lineage CR into the Colorado River Basin.

The presence of only Lineage CR fish in the San Juan River drainage was curious since that part of the state lies farthest from the Lineage CR stronghold in the Yampa GMU, and close to the Dolores and Gunnison GMUs where Lineage GB is pervasive (Figure 3). Metcalf et al. (2007) already suggested that one of these populations (East Fork Piedra River) was likely founded by stocking given the similarity of its genetic fingerprint to geographically remote populations to the north. Further research will be necessary to determine if the seven other populations in the San Juan GMU identified in this report also share DNA similar to more northerly populations such as Trappers Lake that provided large numbers of pure cutthroat trout to the state hatchery system, or whether they harbor unique haplotypes, suggesting remnant genetic diversity across the landscape.

Given the illustrious stocking history in Colorado over the last century (Wiltzius 1985), it will be challenging to completely resolve the native distribution of these lineages. Ongoing research at the University of Colorado studying museum specimens of cutthroat trout collected in the late 1800s will hopefully shed some light on the historic distributions of these fish prior to the bulk of fish culture activity in the state. A Colorado State University study exploring different visual characteristics between the lineages will also be illuminating. Piecing together the taxonomic past of these fish will be critical for providing information that will allow recovery efforts to resume.

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