BIOCHEMICAL AND CYTOGENETIC ANALYSIS OF CUTTHROAT TROUT POPULATIONS IN NORTHWESTERN WYOMING

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Objectives

Prior to 1900 cutthroat trout (*Salmo clarki*) inhabited most drainages of the intermountain west including the Columbia, South Saskatchewan, Missouri, Colorado, Arkansas, Rio Grande, and South Platte Rivers and the Great Basin. Because of overfishing, introductions of nonnative fish, and careless use of water resources, 99% of the original populations of *Salmo clarki* are believed to have gone extinct (Behnke, 1971). Recently, however, management agencies have become increasingly aware of the precarious situation the cutthroat trout is in and have implemented special regulations to help restore these fisheries.

One problem facing fisheries managers is to identify pure populations of cutthroat tourt; another is to assign these populations to the proper subspecies. The problem of subspecies designation is particularly difficult because meristic variation can be as great within a subspecies as between them. Moreover, Pleistocene glaciation influenced the dispersal patterns of the existing subspecies complicating our understanding of their zoogeography.

During the past three summers I have studied the distribution of chromosomal and protein variants among *Salmo clarki* subspecies. The objective of my work within Yellowstone National Park and Teton County, Wyoming, is to: reassess the subspecies status of cutthroat trout in the Snake, Yellowstone, and Missouri drainages; develop base line criteria for determining the purity of cutthroat trout populations; and assess the purity of suspected hybrid populations.

Procedures

Populations studied. During the summers of 1976 and 1977, Le Hardy's Rapids (Yellowstone drainage), Pelican Creek (Yellowstone drainage) and Sedge

Creek (Yellowstone drainage) from Yellowstone National Park and Dime Creek (Snake drainage), Snake River (Snake drainage), Blacktail Ponds (Snake drainage), Bar B-C Spring Creek (Snake drainage), Skull Creek (Snake drainage), and Spread Creek (Snake drainage) from Teton County, Wyoming, were examined electrophoretically and cytogenetically. In the summer of 1978, Cascade Creek (Yellowstone drainage), Sylvan Lake (Yellowstone drainage), Little Thumb Creek (Yellowstone drainage), Thumb Creek (Yellowstone drainage), Cougar Creek (Missouri drainage), Gibbon River (Missouri drainage), Firehole River (Missouri drainage) and Ted Creek (Colorado River drainage) were sampled and are being examined electrophoretically and cytogenetically. These samples represent three subspecies of *Salmo clarki*, *S. c. lewisi*, *S. c. bouvieri*, and *S. c. pleuriticus*; and possible hybrids between *Salmo clarki* and *Salmo gairdneri*.

Cytogenetic methods. Chromosome preparations followed the method of Gold (1974) with modification. Briefly, after 6 hours of in-vivo incubation in colchicine, homogenized anterior head kidney was treated with a 0.075 N KCl hypotonic solution and fixed in Carnoy's solution (3:1 methanol:glacial acetic acid). One or two drops of the fixed cell suspension was dropped onto cold, wet microscope slides which were then plate dried. Slides were stained in dilute (4-6%) Giemsa in phosphate buffer or C-banded using the $Ba(OH)_2$ technique (Salamanca and Armendares, 1974).

Electrophoretic methods. Samples of blood, heart, skeletal muscle and liver were removed from freshly killed specimens or frozen carcasses and stored in liquid nitrogen. Tissue extracts, prepared by homogenization and high speed centrifugation, were analyzed electrophoretically in horizontal starch gels. The enzymes studies are lactate dehydrogenase (LDH), malate dehydrogenase (MDH), alpha-glycerol phosphate dehydrogenase (aGPDH), sorbitol dehydrogenase (SDH), xanthine dehydrogenase, (XDH), esterase (EST), aspartate-amino-transferase (AAT), superoxide dismutase (SOD), and creatin kinase (CK), as in previous years. In addition, phosphohexose isomerase (PHI), phosphoglucomutase (PGM), fumerase (FUM), malic enzyme (ME), isocitrate dehydrogenase (IDH), and alcohol dehydrogenase (ADH) were added to the study.

Genetic loci were considered polymorphic if the frequency of the predominant allele was less than 0.99. Genetic heterozygosity was estimated by calculating the expected heterozygote frequency from the observed allele frequencies.

Results

Chromosome numbers in somatic cells of cutthroat trout from several locations in Yellowstone National Park and Teton County, Wyoming, are presented in table 1. Intraindividual variation is believed to be caused by chromosome loss during preparation and counting errors. Table 2 presents a comparison of the karyotypes of various cutthroat trout subspecies. Two distinctly different karyotypes are found within the Yellowstone Region. The first karyotype is observed in fish from the Yellowstone and Snake River drainages and is characterized by a 2n chromosome number of 64

with a chromosome arm number of 104. This karyotype consists of 38 metacentric chromosomes, two of which often have satellites, 2 small submetacentric chromosomes, and 24 acrocentric chromosomes. The second karyotype is observed in native cutthroat trout from the Missouri River drainage and is characterized by a 2n chromosome number of 66 with 104 chromosome arms. This karyotype consists of 34 metacentric chromosomes, 4 small submetacentric chromosomes, 12 subtelocentric chromosomes, and 16 acrocentric chromosomes.

The two karyotypes differ by a 2n chromosome number of two, without a change in chromosome arm number. The simplest explanation for this is the fusion or fission of acrocentric or subtelocentric chromosomes termed a robertsonian rearrangement. There is also a difference in the number of small submetacentric chromosomes. In addition, the 2n=66 karyotype contains several subtelocentric chromosomes which are absent in the 2n=64 karyotype.

Electrophoretic analysis is completed for all the populations sampled in 1976 and 1977. In addition, 1978 samples from Ted Creek, Cougar Creek and Sylvan Lake have been analyzed and the results are presented in table 3. Cascade Creek, Thumb Creek, Little Thumb Creek, Gibbon River and Firehole River are currently being analyzed.

The Ted Creek population which represents the subspecies *S. c. pleuriticus* exhibited no electrophoretic variation within the sample (n=30). This population was divergent from Yellowstone cutthroat trout (*S. c. bouvieri*) at the SDH locus. And divergent from west slope cutthroat (*S. c. lewisi*) at the SDH, IDH, ME and PHI-1 loci.

The Sylvan Lake population is polymorphic for AAT, MDH (1,2) and PHI-3. The AAT polymorphism is identical to the AAT polymorphism described in Yellowstone Lake and River (Loudenslager and Kitchin, 1979). The low frequency MDH polymorphism was not detected previously in any Yellowstone populations. However, the detection of the variant allele may have been facilitated by an improved separation technique. Currently it is unknown if this is a widespread polymorphism throughout the Yellowstone drainage. If it is common, we should detect it in the Cascade Creek, Thumb Creek and Little Thumb Creek populations. PHI is a new enzyme system, and a low frequency variant was found. Because this enzyme was not examined previously, no data is available on the geographic distribution of the variant. Again, final analysis of Cascade Creek, Thumb Creek and Little Thumb Creek should give some indication of how widespread this polymorphism is.

The Cougar Creek population is polymorphic for low frequency variants at the ME, SDH, PHI and IDH loci. In addition, the Cougar Creek population is differentiated from populations in the Yellowstone drainage at IDH, ME, PHI-3, SDH and an EST locus (see table 3).

Discussion

A comparison of the karyotypes and electrophoretic profiles of the populations sampled within Yellowstone National Park and Teton and Carbon

counties, Wyoming, to those available for other S. clarki subspecies suggest some interesting affinities. The karyotype of the individuals examined from Cougar Creek are identical to that described from Salmo clarki lewisi from the Jocko River State Trout Hatchery, Arlee, Montana (Loudenslager and Thorgaard, 1979). The electrophoretic profile is similar to that of Salmo clarki lewisi presented by Reinitz (1977). Thus, Cougar Creek and presumably other native cutthroat populations in the Missouri drainage are west-slope cutthroat trout, Salmo clarki lewisi, not Yellowstone cutthroat trout, Salmo clarki bouvieri. Salmo clarki lewisi, both electrophoretically (Allendorf and Phelps, personal communication) and karyotypically (Loudenslager and Thorgaard, 1979), is more closely related to the coastal cutthroat trout (Salmo clarki clarki) than other interior subspecies. Cutthroat trout populations from the Yellowstone and Snake River drainage are identical electrophoretically and karyotypically. Their karyotype (2n=64) is similar to other interior subspecies, including S. c. pleuriticus, S. c. utah, S. c. henshawi and S. c. seleneris. Electrophoretically, Yellowstone cutthroat trout can be differentiated from these subspecies but are very closely related to S. c. pleuriticus and S. c. utah. The genetic similarities between Cougar Creek cutthroat trout and west-slope cutthroat trout and between Yellowstone-Snake River cutthroat trout and Bonneville Basin cutthroat trout suggest that two well differentiated cutthroat trout lineages have made postglacial invasions into the headwater streams on the eastern side of the Continental Divide.

Although the subspecies S. c. bouvieri, S. c. lewisi and S. c. pleuriticus are quite distinct electrophoretically, indicating genetic variability and differentiation among Salmo clarki subspecies, my stuides have failed to detect extensive biochemical genetic variation within populations. Estimates of heterozygosity for Ted Creek, Cougar Creek and Sylvan Lake are 0.00, 0.034 and 0.020, respectively. These values are in the range observed in other cutthroat trout populations in the Yellowstone region (0.00 to 0.026) (Loudenslager and Kitchin, 1979), but much lower than other vertebrates. In addition, current studies with Salmo clarki henshawi generally reveal a similar low level of genetic polymorphism. Stochastic effects could account for low variation in relatively small populations with homing tendencies. However, the evolutionary consequences of this monomorphism is unknown.

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Table 1. Chromosome numbers in somatic cells of cutthroat trout.

Population	No.	58	59	60	61	62	63	64	65	66	67	68
Le Hardy's Rapids	3	-	-	2	-	5	1	23	2	11	-	-
Pelican Creek	7	-	1	1	1	1	3	46	2	6	-	-
Snake River	21	3	-	11	-	18	1	83	1	8	-	-
Blacktail Ponds	10	-	-	-	3	1	5	48	7	-	-	-
Skull Creek	3	-	2	-	-	1	1	12	2	-	-	-
Spreak Creek	5	1	-	1	5	1	4	27	5	-	-	-
Dime Creek	10	9	-	11	-	7	5	42	-	1	-	-
Cascade Creek	1[a]	-	-	-	-	1	3	6	-	-	-	-
Thumb Creek	1[a]	-	-	-	1	2	-	7	-	-	-	-
Cougar Creek	2[a]	-	-	-	-	-	2	1	5	8	3	1
Jocko Hatchery	5	-	4	-	-	1	2	-	3	47	-	2

[a]All individuals are not yet analyzed.

Table 2. A comparison of karyotypes of _Salmo clarki_ subspecies.

Subspecies	2n	NF[1]	M[1]	SM[1]	ST[1]	A[1]
S. c. clarki	70	106	38	0	32	0
S. c. clarki	68	104	36	0	18	14
S. c. lewisi	66	104	34	4	12	16
S. c. bouvieri	64	104	38	2	0	24
S. c. pleuriticus	64	104	38	2	0	24
S. c. henshawi	64	104	38	2	0	24

[1] NF is fundamental number or chromosome arm number; M is metacentric chromosome; SM is submetacentric chromosome; ST is subtelocentric chromosome; and A is acrocentric chromosome.

Table 3. Allele frequencies of variant proteins for 9 genetic loci[1].

Enzyme	Allele	TED CREEK *S. c. pleuriticus* n=30		SYLVAN LAKE *S. c. bouvieri* n=43		COUGAR CREEK *S. c. lewisi* n=40	
ME	A	1.00		1.00		.07	(.135)
	B					.93	
PHI-3	A	1.00		.91	(.163)	.05	(.092)
	B			.09		.95	
PGM	A	1.00		1.00		.80	(.320)
	B					.20	
SDH-1	A						
	B	1.00					
	C			1.00		1.00	
	D						
	E						
SDH-2	A						
	B					.09	(.163)
	C					.91	
	D	1.00		1.00			
MDH-3,4	A	1.00		.98	(.040)	1.00	
	B			.02			
IDH-1	A	1.00		1.00		.85	(.255)
	B						
	C					.15	
IDH-2	A	1.00		1.00			
	B					1.00	
AAT	A			.03			
	B	1.00		.75	(.380)	1.00	
	C			.22			
$\bar{H}$ =			(0.00)		(.020)		(.034)

[1]Numbers in parenthesis are heterozygosity estimates for individual genetic loci ($h=1-\Sigma x^2$). h for monomorphic proteins is 0.00. $\bar{H}=\Sigma h/r$; where r= number of loci examined and includes monomorphic loci.