A CYTOGENETIC ANALYSIS OF CUTTHROAT TROUT IN THE GRAND TETON AND YELLOWSTONE NATIONAL PARKS

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Objectives

The cutthroat trout, Salmo clarki, is the trout species native to the Rocky Mountains on both sides of the Continental Divide. The widespread distribution of cutthroat trout in several independent drainages has resulted in the formation of considerable morphological and behavioral diversity both within and between cutthroat trout populations. Behnke has described several different subspecies of Salmo clarki on the basis of their meristic serological characteristics. However, because the genetic basis of these characteristics is unknown, the results of these studies have been inconclusive for the taxonomic designations of cutthroat trout subspecies in Grand Teton and Yellowstone National Parks.

One of the primary goals of fisheries managers is the preservation of the breeding populations of native species. The cutthroat trout in Grand Teton and Yellowstone National Parks are the last representatives of a gene pool which has become well adapted over thousands of years to the environmental conditions prevalent in this area. However, several non-native trout species have been introduced into these areas in the past with which native cutthroat trout might hybridize. The objectives of this research project were (1) to compare cytogenetically the cutthroat trout populations present in Grand Teton and Yellowstone National Parks, (2) to determine the extent of within individual and between individual variation in the chromosomal constitution of cutthroat trout, and (3) to assess the purity of the presently existing cutthroat trout populations by looking for cytogenetic evidence of introgression between cutthroat trout and non-native rainbow trout.

Procedures

Specimens were collected from three populations: (1) a Yellowstone Lake population collected from fish traps in Pelican Creek during their spawning run, (2) a Yellowstone River population collected by dip-netting from Le Hardy's Rapids during their spawning run, and (3) a fine-spotted cutthroat trout population collected by seining from Blacktail Pond in Grand Teton National Park.

Cutthroat trout were injected with colchicine and placed in cold, vigorously

aerated spring or stream water for 2 hrs. The fish were then killed and muscle and liver tissue was removed for biochemical analysis. The head kidney was removed, minced and ground with a dounce homogenizer. The resulting cell suspension was centrifuged and resuspended in a hypotonic KCl solution for 20 min. The kidney cells used for cytogenetic analysis were fixed in 3:1 methanolglacial acetic acid fixative, placed drop-wise on a cold moist microscope slide, and dried on a hot plate at 60°C. Slides were stained by conventional Giemsa staining techniques or with recently developed heterochromatin staining (C-banding) or Giemsa banding (G-banding) techniques. The total number of chromosomes and the chromosome arm number was determined from as many well-spread metaphase plates as possible for each individual. In cases in which the chromosomes were poorly spread, camera lucida drawings were made to facilitate accurate chromosome counts.

Constitutive heterochromatin was localized by using the BaOH C-Banding technique. This procedure involves removal of chromosomal proteins by immersing the slide for 1 hr in 0.2 N HCl. The slides are then carefully rinsed in tap water and the chromosomal DNA is rapidly denatured by placing the slide for 5 min in a saturated BaOH solution at 50°C. After thoroughly washing the slides in running tap water they are incubated for 1 hr in 2xSSC (0.3 M NaCl and 0.03 M sodium citrate, pH 7.0) at 65°C. The slides are then rinsed, air dried, and stained in 4% Giemsa stain in pH 6.8 Sorensen's buffer for 20 min.

Three different Giemsa banding techniques were used on cutthroat trout chromosome preparations. These included the ASG (acid-saline-Giemsa), urea, and trypsin Giemsa banding techniques. Although each of these techniques has proven successful in our laboratory with small mammalian species, none of these G-banding techniques worked on chromosome preparations made from head kidney of cutthroat trout.

Results

Both the total chromosome number and the fundamental number of chromosome arms (FN) were determined for 22 of the 24 cutthroat trout specimens studied cytogenetically from our summer field collections in Grand Teton and Yellowstone National Parks. The modal chromosome number was 2n = 64 and the fundamental number was FN = 104 for both populations sampled from Yellowstone National Park (Pelican Creek and Le Hardy's Rapids) and the population studied from Grand Teton National Park (Blacktail Ponds). The cytogenetic data are summarized in Table 1. One medium-sized marker metacentric chromosome pair was observed which had a long, lightly staining region in the distal half of its long arm. This marker was detectable in about 20% of the cells examined in most individuals collected from each of the three populations.

Many of the chromosomes, but by no means all of the chromosomes, had small, dark-staining C-bands in their centromeric regions. In addition, the marker metacentric chromosome had a large, darkly staining C-band in the distal half of its long arm that corresponds in its position to the lightly stained region after conventional Giemsa staining. C-bands were also observed in a few cells at the very end (telomeric C-bands) of one small submetacentric chromosome in two individuals sampled from Pelican Creek.

Le Hardy's Rapids (Yellowstone National Park). Eight specimens captured from Le Hardy's Rapids were studied cytogenetically. The modal chromosome number was 2n = 64 and the modal fundamental number was FN = 104 in 7 of 8 specimens. No analyzable metaphase cells were present in either of two slides examined from the eighth specimen (LR77-2). About 60% of the cells analyzed from these individuals displayed the normal diploid chromosome constitution (2n = 64, FN = 104) and 28% had a lower chromosome number which most likely could be attributed to random chromosome loss during slide preparation. Chromosomal mosaicism was evident in 4 of 7 specimens: LR77-6 had two chromosomally different populations, and LR77-1, LR77-3 and LR77-5 each had 3 chromosomally distinguishable cell populations.

Pelican Creek (Yellowstone National Park). Eleven specimens obtained from fish traps during their spawning run from Yellowstone Lake were studied cytogenetically. No analyzable metaphase cells were found in the slides prepared from specimen PC77-1. The modal chromosome number was 2n = 64 (FN = 104) in the other ten individuals. About 32% of the cells analyzed from this population had a lower chromosome number than 2n = 64. In six of these cells the reduction in the chromosome number was inconsistent with the fundamental number (e.g., 2n = 62, FN = 103 or 104; 2n = 63, FN = 104). This observation suggests that in these instances some genetic mechanism other than random chromosome loss during slide preparation is responsible for the chromosome loss observed in these cells. Chromosomal mosaicism was observed in 9 of 10 specimens studied cytogenetically from Pelican Creek.

Blacktail Ponds (Grand Teton National Park). A small population of fine-spotted cutthroat trout was collected from redds by seining at Blacktail Ponds. Although a relatively high proportion of cells (42%) showed a chromosome number lower than 64, the modal chromosome complement for each of the five specimens was 2n = 64 (FN = 104). One specimen, BP77-3, did have 1 cell with a chromosome complement similar to that expected of a rainbow x cutthroat hybrid (2n = 62, FN = 104), but 11 other cells had a normal karyotype. Sixty percent of the specimens (3/5) exhibited chromosomal mosaicism.

Conclusions

The two cutthroat trout populations collected from Yellowstone National Park and the single population sampled from Grand Teton National Park were indistinguishable from one another cytogenetically. Each of the 22 specimens examined during this study had a normal diploid chromosome constitution (2n = 64, FN = 104). No chromosomal polymorphisms were detected which might serve as cytological markers to distinguish individuals of one population from those of another population.

Chromosomal mosaicism was observed in a relatively high proportion of the specimens from each of the three populations. Variation in both chromosome number and/or fundamental number may result from many possible mechanisms, including (1) Robertsonian centric fusions or centric fission, (2) pericentric inversions, (3) chromosomal nondisjunction during mitosis, (4) saltatory replication of chromosomal DNA, (5) artifacts during slide preparation, or (6) simple counting errors. The only definitive means of

determining the mechanism(s) responsible for the chromosomal mosaicism observed in cutthroat trout is a detailed study of the Giemsa banding patterns of many cells from each individual. This was not possible, however, because trout chromosomes did not band with any of the three Giemsa banding techniques used in this study. Hopefully, short term lymphocyte cultures will provide suitable material for G-banding studies in the future.

The primary objective of this study was to use cytogenetic techniques to assess the purity of cutthroat trout populations in Yellowstone and Grand Teton National Parks. Since cutthroat trout have a diploid chromosome number (2n = 64) different from that of rainbow trout (2n = 60), rainbow x cutthroat F_1 hybrids are expected to have a chromosome complement of 2n = 62 (FN=104). The chromosome complement of 22 cutthroat trout specimens obtained from two populations in Yellowstone National Park and one population in Grand Teton National Park was 2n = 64 (FN = 104). There was no cytogenetic evidence for the impurity of any of the cutthroat trout populations studied resulting from interspecific hybridization between cutthroat and rainbow trout.

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Table 1. The number of chromosomes and the fundamental number (FN) of analyzable cells from cutthroat trout collected from Le Hardy's Rapids (LR) and Pelican Creek (PC) in Yellowstone National Park and from Blacktail Ponds (BP) in Grand Teton National Park

		Chromosome Number																	
		62	62					63			64			65					
Specimen	(FN)		100	101	102	103	104	102	103	104	103	104	105	104	105	106	107	108	109
LR77-1		2		1	1				1		1	10	2						
LR77-2*																			
LR77-3		2										6	3				1		
LR77-4		2						1	1			7							
LR77-5		4			1						1	5				1			
LR77-6					1				1		1	5							
LR77-7								3	2			13							
LR77-8								1	1			9							
PC77-1*																			
PC77-2											1	3	1						
PC77-3		2										7	1						
PC77-4		1						1	2	1		16							
PC77-5		1	1									4		1		2			
PC77-6		1					1		2			5							
PC77-7		2	1					2	1			14			1				
PC77-8		1						1	2			4							
PC77-9		2							1	2		7	1				1		
PC77-10		1				1						4							1
PC77-11		1	1			1			1			3							
BP77-1		2		1					2		3	3							
BP77-2		3		1				1			3	4							
BP77-3		6		1		1		3	1			15	1						
BP77-4				1	1				1			9							
BP77-5		2			1							4							

*No analyzable metaphases were present in this individual.