

Annual Progress Report to:

**Pollock Conservation Cooperative
Research Center
School of Fisheries and Ocean Sciences
University of Alaska Fairbanks
Fairbanks, AK 99775-7220**

for Project:

**DNA Analysis of the Origins of Chinook Salmon Bycatch
in Alaskan Trawl Fisheries (continuation)**

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Overview

Chinook salmon bycatch in the Gulf of Alaska and Bering Sea creates problems for the groundfish fisheries, particularly the Bering Sea trawl fisheries. Those salmon destined to return to western Alaskan systems are critically important to the livelihood and culture of rural Alaskans; in recent years, salmon returns to have declined sharply. In addition, chinook salmon are the focus of a number of other issues ranging from Endangered Species Act concerns to allocations between the U.S. and Canada. Central to bycatch questions is the origin or destination of intercepted fish. Substantial effort has been and continues to be devoted to genetic studies of North American chinook stocks, with the objective of resolving stock mixtures to their component stocks. However without data from all potential contributors --at least the predominant ones --, stock mixture analyses are not reliable. Missing from the baseline are data from Russian chinook stocks. We are collaborating with Russian geneticists to obtain genetic information for Russian chinook populations and to examine the genetic divergence between those populations and North American chinook salmon lineages that represent much of the extant chinook salmon genetic diversity. We are quantifying genetic variation using both microsatellites and mtDNA to determine if there are markers that would assist in separating Russian salmon from North American fish in groundfish bycatches. We also plan to use the data to examine the recent evolutionary history of chinook salmon.

Approach

Several activities are necessary to accomplish our goals:

- 1) Our Russian colleague, Dr. Brykov (Institute of Marine Biology, Russian Academy of Sciences), has collected some chinook salmon tissue samples from major and geographically diverse regions in Russia (the Kamchatka Peninsula) and will collect additional samples in June 2003.
- 2) We have contacted geneticists along the Pacific coast and have acquired the samples we will need as reference samples. The samples come from the archives that many of the labs have maintained. The generosity of those labs makes this project possible.
- 3) A subset of samples from across the geographic range is being used to establish polymerase chain reaction (PCR) conditions that efficiently amplify specific microsatellite loci chosen from the literature and with the advise of other Chinook microsatellite labs. These samples will provide us an indication of the number and size diversity of microsatellite alleles throughout the geographic range. We intend to use loci that will generate data that are compatible with as many labs as possible
- 4) We have begun to improve the efficiency of the process as we screen selected North American populations for variation that can be compared to the variation in the Russian populations.
- 5) We surveyed mtDNA restriction site variation in the populations selected for preliminary microsatellite analysis [see 3)]. Our lab evaluates nearly the entire mtDNA genome (97%) using restriction endonucleases to sample the variation and determine the genomic location of variation that best defines the evolutionary history. We have shown that the region harboring useful variation varies among salmon species, so this step is necessary to maximize our chances of finding the

most useful stock markers. Preliminary analysis revealed that chinook salmon populations have undergone extensive divergence that includes most of the genome. Consequently, we expanded our initial survey from five to 12 populations (10 fish from each) ranging from the Sacramento River drainage to the Bolshaya River on southwestern Kamchatka.

- 6) Our intention was that when we had identified the mtDNA regions and restriction endonucleases that provided the most discriminating restriction sites, we would screen the populations for those sites. However, even with the additional populations, it appears that there is variation that requires additional evaluation.
- 7) Data analysis, interpretation, and reporting are a critical step, and include several phases.

Where are we?

We now have obtained samples from 25 rivers ranging from the Sacramento River drainage in the south through the Yukon drainage in the north (Figure 1), seven of which are from Kamchatka. We have conducted a preliminary evaluation of the restriction site variation in the mtDNA of 12 of those populations. These collections represent many of the presumed evolutionary lineages of chinook salmon in North America and, therefore, much of the genetic variation we expect to see. Although samples provided to us by the US Fish and Wildlife Service from Russian systems were not usable, Dr. Brykov obtained tissues in June 2001 and 2002, which were in excellent condition, and he plans to obtain more samples in 2003.

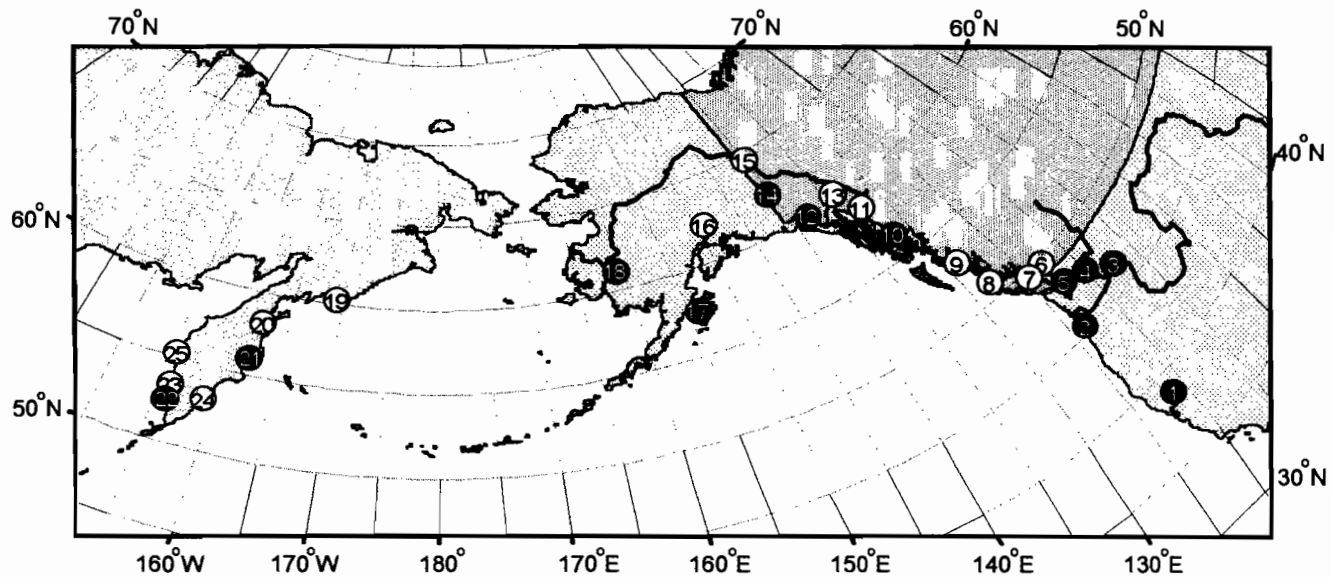


Figure 1. Locations of chinook salmon collections for this study. Filled circles are collections for which we have analyzed mtDNA variation. The quality of the DNA for the other North American populations has not yet been fully evaluated.

Our mtDNA analyses show that chinook salmon have a much deeper "gene tree" than other species of Pacific salmon we have studied (pink, chum, sockeye, and coho). The southeastern populations from Yakutat to California exhibit substantial divergence that indicates that they have been reproductively isolated for a very long time and that virtually no gene flow connects them (Figure 2). These populations are generally distinct from western Alaskan and Asian populations. It appears as if the two Asian populations emerged much more recently than the southern North American populations and may be related to western Alaskan populations. We requested a small amount of additional support from the Pollock Conservation Cooperative Research Center to investigate the unexpectedly large mtDNA divergences more thoroughly.

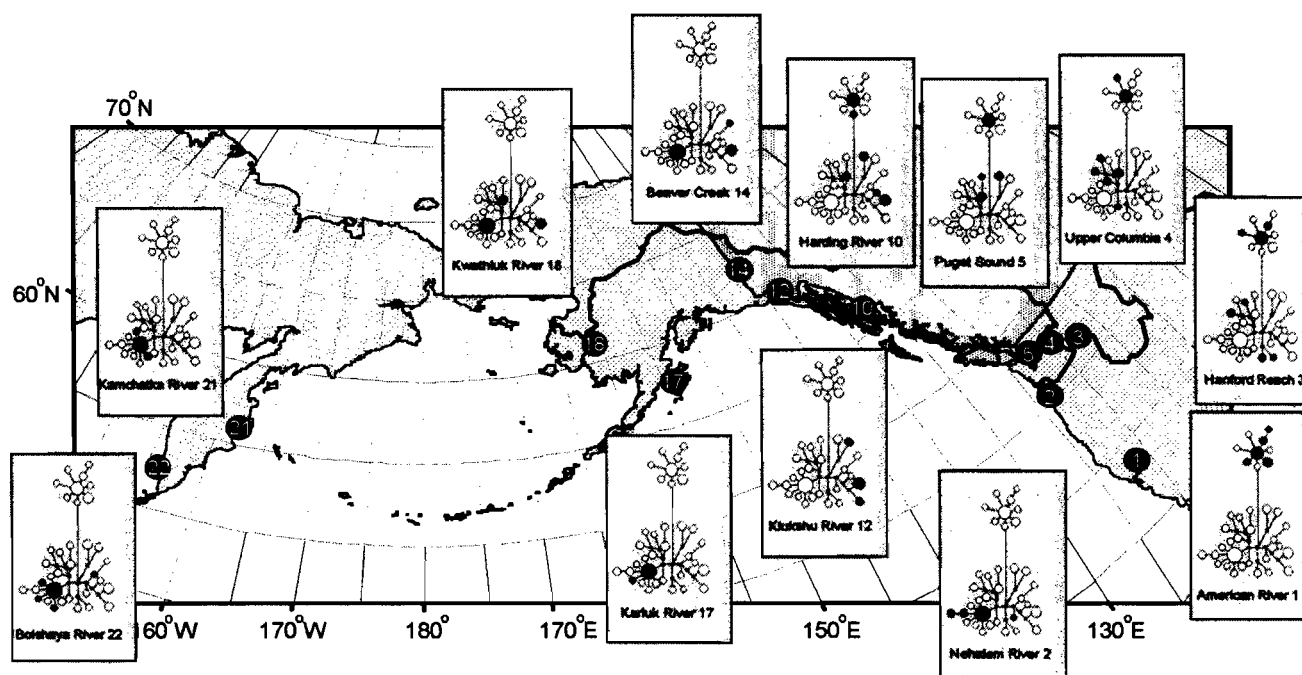


Figure 2. The geographic distribution of mtDNA haplotypes. The genetrees shown are for the entire sample of 120 fish. Corresponding to each population is a gene tree in which the haplotypes present in that at population are indicated by solid fill. It should be apparent that the upper cluster is strongly represented in the eastern range and the lower left cluster in the Asian and central Alaskan ranges.

No standard suite of microsatellite loci is used by labs involved in chinook salmon genetics. The issue is both dynamic and contentious. We have preliminary data for three loci, and are choosing the others so that they will be as compatible as possible with other labs, particularly those in Alaska and the Pacific Northwest (Table 1).

Table 1. Microsatellite loci used by laboratories conducting chinook salmon genetics work. X denotes loci used routinely and x denotes loci recommended but not used. DFO is the Department of Fisheries and Oceans, Canada; ADFG is the Alaska Department of Fish and Game; USFWS is the US Fish and Wildlife Service, Anchorage; NMFS-NW is the National Marine Fisheries Service Northwest Science Center; and UCD is the University of California Davis. We have preliminary data from three loci.

Microsatellite locus	DFO	ADFG	USFWS	NMFS-NW	UCD	Preliminary data
<i>One</i> 2		X				
<i>One</i> 9		X				
<i>One</i> 10		X				
<i>One</i> 13		X	X		X	X
<i>One</i> 103		X				
<i>One</i> 120		X				
<i>Ots</i> 1		X				X
<i>Ots</i> 2	X	X		X	X	
<i>Ots</i> 3				X	X	
<i>Ots</i> 4				X		
<i>Ots</i> 9	X				X	
<i>Ots</i> 10				X	X	
<i>Ots</i> 100	X	X				
<i>Ots</i> 101	X					
<i>Ots</i> 102	X					
<i>Ots</i> 104	X			X	X	
<i>Ots</i> 107	X	X			X	X
<i>Ots</i> 311			X			
<i>Ots</i> A5				X		
<i>Ots</i> B1				X		
<i>Ots</i> B3				X		
<i>Ots</i> D8				X		
<i>Ots</i> D9				X		
<i>Ots</i> E1				X		
<i>Ots</i> G3			X			
<i>Ots</i> G68			X			
<i>Ots</i> G243				X		
<i>Ots</i> G253b			X			
<i>Ots</i> G409			X			
<i>Ots</i> G432			X			
<i>Ots</i> G474			X	X		
<i>Oki</i> 3				X		
<i>Oki</i> 10			X			
<i>Oki</i> 11			X			
<i>Oki</i> 100	X					
<i>Oke</i> 1			X			
<i>Oke</i> 2			X			
<i>Oke</i> 4	X	X	X	X		
<i>Ssa</i> 197	X			X		
<i>Ssa</i> 408				X		
<i>Ogo</i> 2	X					
<i>Ogo</i> 4	X			X		
<i>Omy</i> 325	X					
<i>Omy</i> 1011				X		
<i>mSat</i> 73		X				

Preliminary microsatellite analyses at three loci, *One* μ 13, *Ots* μ 1, and *Ots* μ 107 show substantial allele frequency differences among four North American populations

(Figure 3). Those differences suggest that it is likely that there may be useful microsatellite markers. At this time we are processing Chinook salmon for microsatellite variation. I anticipate that I will have more to report at the January 2003 meeting in Anchorage.

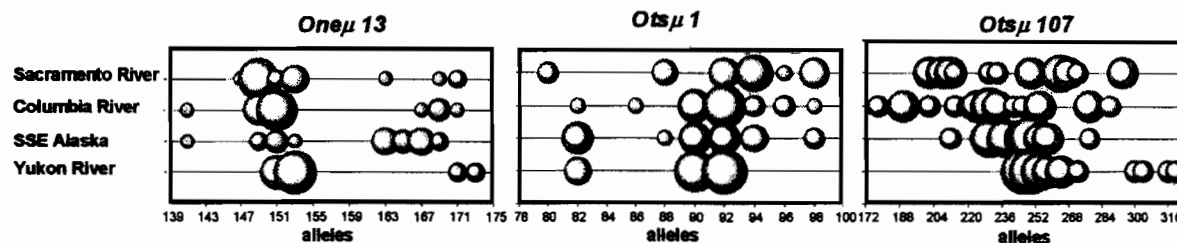


Figure 3. Microsatellite variation at three microsatellite loci for four North American populations. Each ball along the line corresponding to a population represents the presence of a class of allele. The allele is defined by its size (along the X-axis), and the frequency of an allele is reflected by the size of the ball.

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