

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 (completion)**

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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 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 continues to be devoted to genetic studies of selected 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 chinook salmon tissue samples from major and geographically diverse regions in Russia (the Kamchatka Peninsula). We have received most, but not all of those samples.
- 2) We have contacted laboratories along the Pacific coast and have acquired samples that we are using 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 was used to establish polymerase chain reaction (PCR) conditions that efficiently amplify specific microsatellite loci chosen from the literature and with the advice of other chinook genetics labs. We have data from many of the populations for six loci. We will obtain data from additional loci, which are used by as many labs as possible
- 4) Previously, 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. 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 mitochondrial 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.

- 5) 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.
- 6) 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. We are focusing our effort on 20 populations, six populations are from Kamchatka and the remainder includes populations ranging from central California to the Yukon River (Figure 1). The North American collections represent many of the presumed evolutionary lineages of chinook salmon in North America and, therefore, much of the genetic variation we would expect to see in bycatch samples.

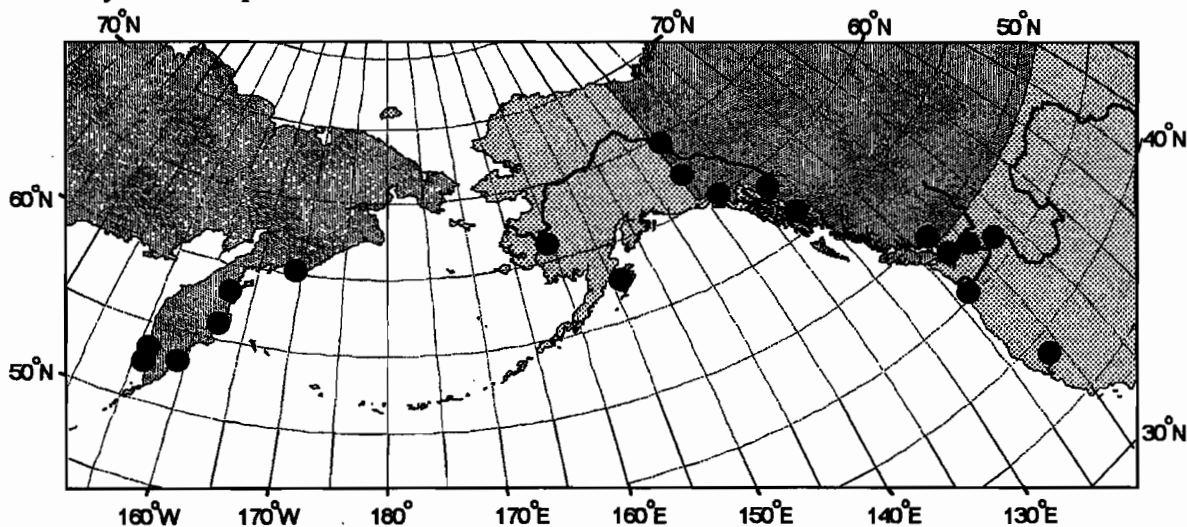


Figure 1. Locations of chinook salmon collections selected for analysis in this study.

We have not completed our mtDNA analysis, but the incomplete results show that chinook salmon have a much deeper "haplotype 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 little 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.

Haplotype trees are constructed from restriction site or nucleotide differences between haplotypes, that is, mutational differences. Mutations accrue over time, so a haplotype tree that estimates the evolutionary relationship (or genealogy) among

haplotypes provides a temporal scale. For example, an abundant haplotype in the center of a cluster is more likely to be ancestral than an infrequent haplotype at one of the tips of a tree. Nested clade analysis (Templeton 1998) contrasts the geographic distribution of haplotypes with their genealogical (temporal) relationships and provides a means for learning about the demographic history of a species. For example, the reduction in the number and sizes of populations caused by glacial advances also reduced the amount of genetic variation that could be carried and isolated many populations. In contrast, population expansion and colonization that occurred during interglacial periods produced increases in the number of new mutants that could be retained and provided opportunities for movement of haplotypes into new areas. Nested clade analysis of these preliminary data is consistent with episodes of population fragmentation and expansion that probably included more than one glacial advance.

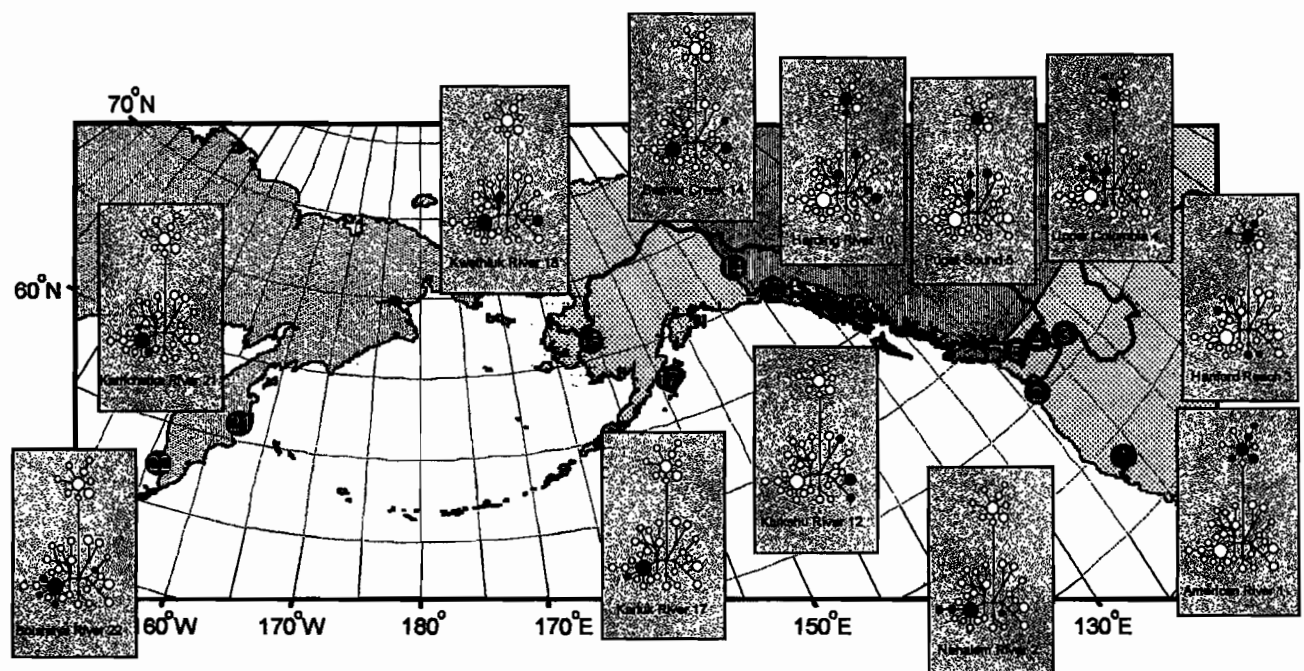


Figure 2. The geographic distribution of mtDNA haplotypes. The gene trees shown are for the entire sample of 120 fish. Corresponding to each population is a gene tree in which the haplotypes present in that at population are indicated by solid fill. It should be apparent that the upper cluster is strongly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

The haplotype gene tree has three major clusters that can be distinguished from the variation at two restriction sites. Single nucleotide polymorphism (SNP) sites can be developed for the sites. The variation observed at those sites has a geographic distribution that is easier to see than the variation presented in Figure 2, but shows that (except for the single Oregon coastal sample) the southern populations are distinct from the northern populations, probably a consequence of post glacial colonization (Figure 3).

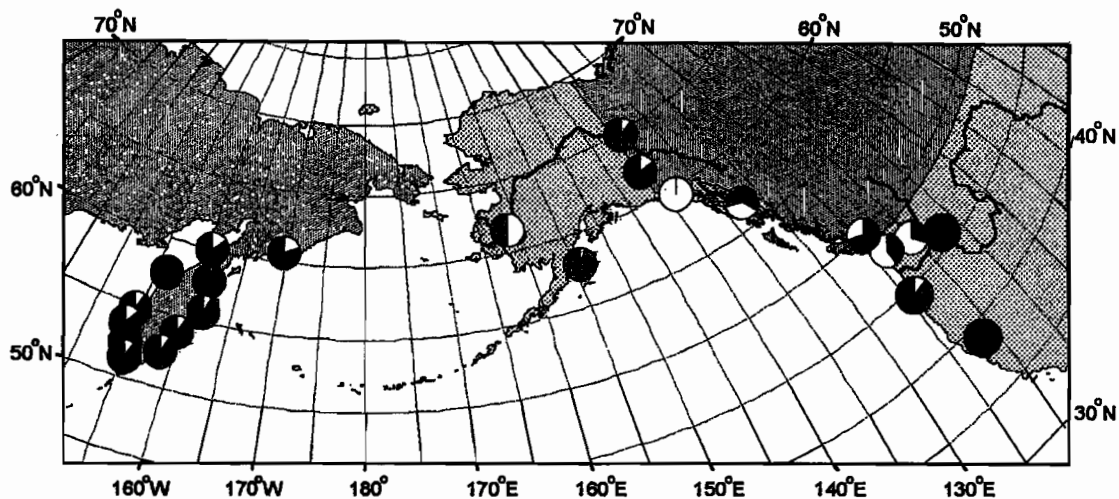


Figure 3. Distribution of haplotype variation resulting from variation two mtDNA restriction sites that can be developed as SNP's. V. Brykov provided data from additional Russian samples.

No standard suite of microsatellite loci is used by labs involved in chinook salmon genetics. The issue is both dynamic and contentious. We have data for six loci that include most of the samples we plan to analyze: μOne 13 (Scribner et al. 1996), μOgo 4 (Olsen et al. 1998), μOke 4 (Buckholz 1999), μOts 1 and μOts 10 (Banks et al. 1999), and μOts 107 (Nelson and Beacham 1999). The data are preliminary because they have not been thoroughly "groomed" to ensure they were read and interpreted correctly, however, they should be substantially correct. One of the loci, μOne 13, appears to have numerous null alleles, and we have designed new primers for that locus that should reduce the problem. The locus μOts 107 is highly variable and may be too variable for use. The other loci appear well behaved. We plan to select several additional loci, whose choice will be made to assure compatibility with other labs, particularly those in Alaska and the Pacific Northwest.

We analyzed the microsatellite data in several ways. First, we examined histograms of the data to look for obvious marker alleles. We observed substantial variability that appeared to include both inter- and intra-regional differences in the frequencies of many of the more abundant alleles. Subsequently, we conducted principal components analysis (PCA) using only alleles that had a frequency, which exceeded at least 0.2 in at least one population or averaged more than 0.1 over all populations. Application of PCA to the arcsine-square root transformation of those allele frequencies treated all the other alleles at each locus as a pool. Plots of the first and second principal components cleanly resolved the Asian populations from North American populations (Figure 4). The component loadings, which describe the relative importance of each allele in plotting the populations, can be used to identify the loci and alleles that are most useful in resolving populations and regional aggregations of populations.

Pairwise tests of heterogeneity between populations were significant except for some pairs of Kamchatka populations. A neighbor joining tree constructed from chord distances that used all the allele frequency information showed strong geographic

structure. The divergence among populations was strongly ($P < 10^{-6}$) correlated with the geographic distances separating the populations.

Conclusions

From the small number of samples for which mtDNA analysis is complete, there appears to be substantial divergence among southern populations, but much less among northern populations, which suggests that the western Alaskan and Asian populations probably reflect a large post Pleistocene expansion. In contrast, the southern populations appear to be much older and to have been isolated for long times. Mitochondrial variation appears to provide markers that permit distinction of northern and southern populations, but not between Asian and North American populations.

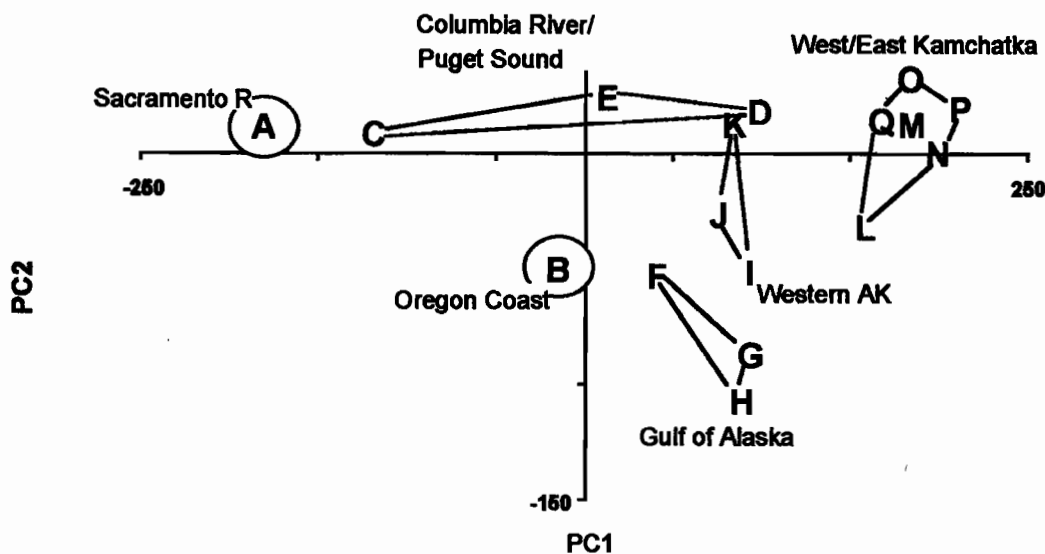


Figure 4. Plot of the first two principal components of (arcsine square root-transformed) microsatellite allele frequencies of chinook salmon populations.

Microsatellite variation, in contrast, reflects substantial divergence between Asian and North American populations as well as among the North American populations. Consequently, there appears to be a genetic basis for resolving stock mixtures, including estimating the contribution of Asian chinook salmon to groundfish bycatch. The concern here is that the divergence among populations within a region are not large relative to populations from other regions. The development of chinook salmon microsatellite baseline information by Alaska Department of Fish and Game, US Fish and Wildlife Service, National Marine Fisheries Service, and Department of Fisheries and Oceans, Canada will provide the baseline for addressing this question.

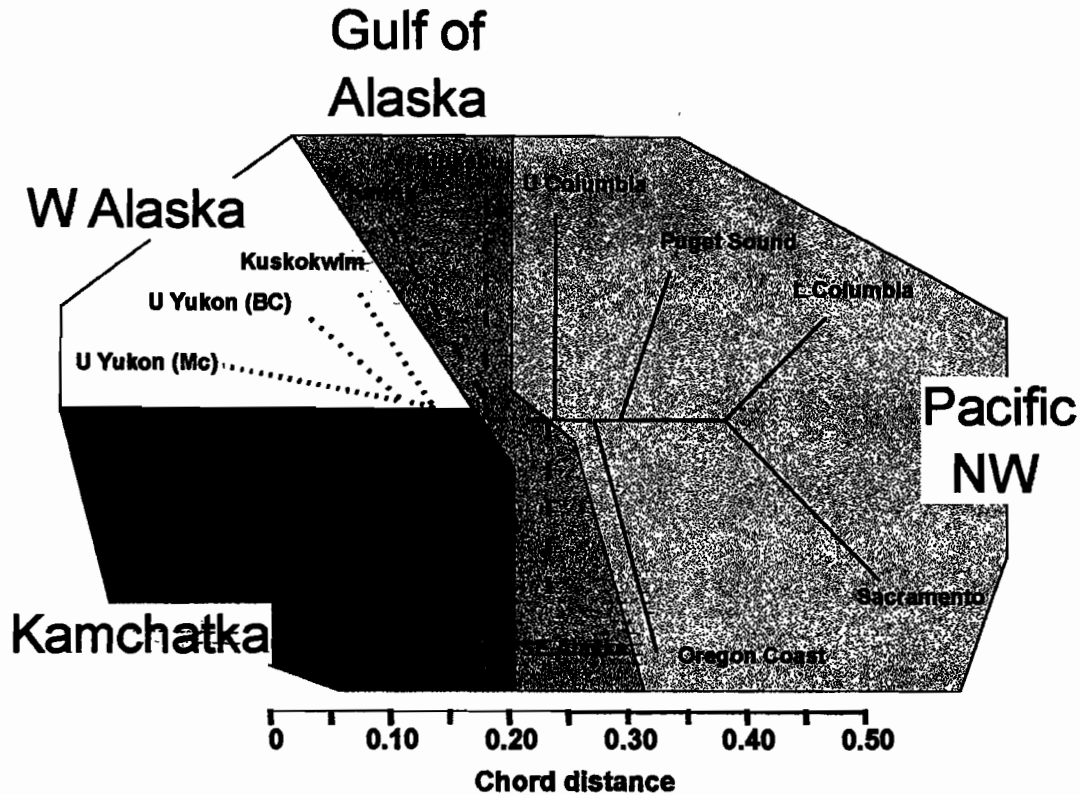


Figure 5. Neighbor-joining tree based for microsatellite variation in chinook salmon. Regional groupings are emphasized using differently shaded backgrounds.

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