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Population Genetics of Chinook Salmon in the Salmon River System

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Abstract

Chinook salmon (Oncorhynchus tshawytscha) from the Salmon River system of Northern California was studied to determine whether or not fall and spring runs were discrete breeding populations/stocks using nuclear DNA as the basis for the analysis, and delineate the physical extent of the habitat occupied by the samples. The project was funded by USFWS through the "The Klamath Act".

Three institutions that participated in the study: USFS, California State University Northridge, and California Polytechnic State University (SLO). Adult Chinook samples were collected from four reaches of the Salmon River system. Samples from Big Lake Hatchery, Big Lake Alaska were used for an "out" population comparison. Three independent methodologies were used to examine the nuclear DNA: Ribosomal DNA, Restriction Fragment Length Polymorphism's, and Randomly Amplified Polymorphic DNA fragments. A digital base map of the Salmon River was created using the ARC/INFO Geographic Information System. Adult salmon sample point locations were entered into a GIS database along with digital data identifying sample characteristics and the results of the DNA analysis.

The DNA analysis revealed distinct 1) differences between Alaska and California fish, and 2) genotypes between fall and spring runs in the Klamath river. More extensive sampling will be required to determine the degree to which stocks

are genetically distinct and to delineate the exact physical habitats occupied. Analysis of juveniles collected in the Klamath River estuary demonstrated that there are more genotypes present in estuary fish than those observed in the Salmon River populations samples.

Introduction

This report presents findings from a study on stock identification of Chinook salmon from the Salmon River watershed in Northern California. It is a part of efforts on the part of fisheries scientists and managers to study, and restore, the anadromous fisheries in the Klamath River Basin. The research was conducted by an interdisciplinary team, with expertise in the areas of Fisheries and Wildlife Management, Regional Resources Management and Planning, Biology, and Research Genetics.

This work demonstrates a professional commitment to the preservation of anadromous fisheries resources of the Klamath River Basin and to the belief that these noble creatures are must be saved and helped to prosper in the 21st century. The Klamath River Fisheries Symposium demonstrates that we, as a group, are willing to accept the challenges facing fisheries managers in the Klamath Basin and commit our efforts to meeting those challenges.

Chinook salmon populations in the Salmon River System have suffered a severe decline over the years, brought on by the effects of agriculture and other human activity on the natural habitat. In attempts to save these populations, steps are being taken to protect the remaining fish by limiting the number that can be caught and to identify and correct the environmental factors most important to survival and successful reproduction of the fish stocks.

Fundamental to any fishery management strategy is the need to know the genetic structure of the population in question and to understand the causes and effects of genetic changes that occur. As populations decline, there is a corresponding loss of genetic diversity. A manager needs to know the extent to which this has occurred in order to assess the genetic health of the stock under his or her control. Also important is the need to know the geographical limits of the breeding population. Knowledge of the range and distribution of the stock is necessary in order to know where to implement management procedures.

Relatively little is known about the genetic structure of salmon populations at the level of the DNA itself. Little, if any, work has been done to identify genetic

stocks, to determine the degree of genetic diversity in them, or to determine the extent to which the stocks are genetically isolated from one another. Genetic differences accumulate between breeding stocks as a result of mutation and genetic isolation. In species that display natal site fidelity, as occurs in salmon, one would expect genetic differences to develop that could be exploited to reveal the degree of genetic variation within and between populations, the extent to which interbreeding goes on between populations, and changes in genetic composition that occur over time. Importantly, genetic differences can provide markers for identifying the breeding stocks of salmon caught away from their spawning sites, thus permitting the implementation of management activities to adult populations at sea.

Method

The current study applied DNA technology to the study of the genetic structure of Chinook salmon from the Salmon River system of Northern California. Recent developments make it feasible to find genetic markers and to study the genetic structure of the populations with a reasonable expenditure of time and money.

DNA Analysis

The study involved three approaches: 1) amplification by the polymerase chain reaction (PCR) and restriction digestion of a variable region of the DNA, 2) amplification of random regions of DNA with single primers used at low stringency (RAPDs), and 3) probing restriction fragments separated by electrophoresis to find restriction fragment length polymorphisms (RFLPs).

Restriction Digestion of PCR-amplified DNA. The internal transcribed spacer (ITS) sequences of ribosomal DNA repeat units have been shown to contain a relatively high frequency of nonconserved restriction sites in several species (Saiki, 1988; Baker, Honeycutt, & Van Den Bussche, 1991). The ribosomal DNA coding sequences that lie on either side of the ITS region are highly conserved, providing a basis for PCR primer synthesis that does not require prior sequence information specifically from salmon (Sogin & Gunderson, 1987) (Figure 1). Using published mouse and frog primer sequences (Hillis & Dixon, 1991), we have amplified the Chinook salmon ITS regions by PCR. The product was cleaved with restriction endonucleases and the size of the resulting fragments determined from their mobility on agarose electrophoresis gels. Variation in fragment size have been found among the fish used in this study.

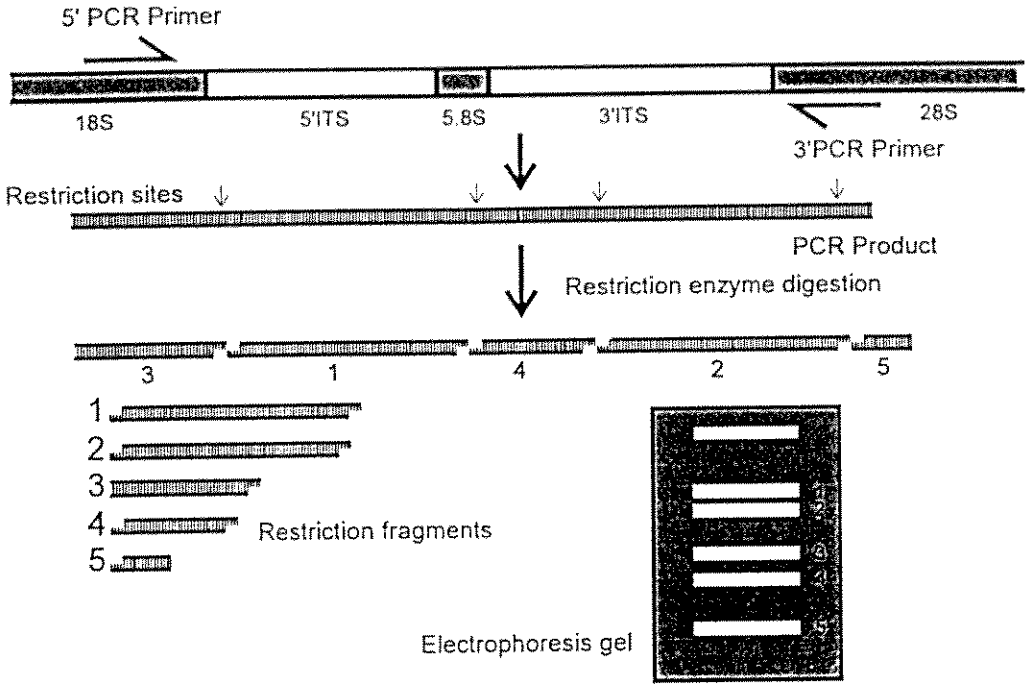

Figure 1. PCR amplification and restriction digestion of the salmon ribosomal DNA internal transcribed sequences (rDNA ITS regions). There are several hundred of these regions in the salmon DNA. The ITS regions are more likely to be variable (and thus a source of polymorphisms) than the neighboring (grey) regions that code for functional product. The PCR is used to amplify the ITS regions, and the resulting products are cut with selected restriction enzymes to give restriction fragments that may vary in length from one individual to another.

Randomly Amplified Polymorphic DNA. Randomly amplified polymorphic DNA (RAPD) fragments are produced when single primers are used in a PCR-like reaction that employs relatively low annealing temperatures (Williams, et al., 1991). Lower annealing temperatures allow a limited amount of mismatching between primer and template DNA. When two of the primers happen to anneal in opposite orientation and close enough to permit the production of amplification fragments, the fragments are often polymorphic and can be used to distinguish genetically different populations. RAPD technology requires that careful attention be paid to several factors in order to obtain reproducible results. The optimum conditions required for each of several primers was determined and these primers were used for stock identification studies.

Probing Restriction Enzyme Digested DNA. Restriction fragment length polymorphisms have traditionally been identified by probing restricted genomic DNA after the DNA fragments have been separated by electrophoresis and transferred to nylon or nitrocellulose membranes (Southern, 1975). This technique was used in the search for polymorphisms, initially using the ITS-containing PCR product as a probe rather than as a source of restriction fragments. When used as a probe, the PCR product can identify polymorphic restriction sites that lie in the DNA surrounding the ITS region. For this purpose, the researchers labeled PCR product during amplification with digoxigenin-11-dUTP, and detected its presence with an antibody-alkaline phosphatase system (Genius Non-radioactive Labeling System, Boehringer Mannheim Corp).

DNA Collection

DNA used in this study was obtained from gill filaments removed from adult fish that had returned to spawn. About 1 cm of filaments were placed in approximately 1 ml 5X NET buffer, which preserves the DNA in the tissues. DNA was subsequently extracted from the tissue using standard phenol/chloroform/isoamyl alcohol extraction procedures. Because salmon die after spawning, the sampling procedure did not affect their survival potential.

Project Description

This project investigated Spring and Fall runs of the Chinook Salmon. The project was conducted from 1 October 1991 through July 1993.

Study Team

Three institutions participated in the study. The USFS (Klamath National Forest) provided streams, soils, vegetation, and geologic data for the GIS data base, attribute data for stream habitats, and fish samples for the nuclear DNA analysis. California State University Northridge conducted the nuclear DNA analysis; and California Polytechnic State University (SLO) mapped the sample point data and integrated and analyzed the DNA results.

Objectives

There were 5 questions addressed by the study: 1) Are there different stocks of Chinook Salmon; 2) Can the reaches occupied by different stocks within the basin be identified; 3) Can we identify the habitat types of these stocks and optimize habitat management within reaches; 4) Can the at risk stocks in the fishery be identified; and 5) Is it possible to identify in the "out" migration of juveniles the proportion of the known "stocks" to unknown stocks?

Study Area

Six sampling reaches of the Salmon River were established as study areas: 1) the Mainstem, 2) the Lower North Fork, 3) the Upper North Fork, 4) the South Fork, the Lower South Fork; and, 5) the East Fork of the South Fork.

Sampling

Fifty-seven adult Chinook salmon spent spawners were collected from four reaches of the Salmon River system: the Mainstem, the Lower North Fork, and the Upper and Lower South Forks. All spent spawners observed were collected.

Juveniles were not sacrificed in the study area. Thirty-one caudal fin samples were collected in September of 1993 from the Mainstem, Lower North Fork, and the Lower South Fork. In July of 1992, 15 gill and tail samples, from the same organism, were collected from fingerlings in the Klamath Estuary.

An "out" population comparison was made with samples of adult Alaskan Chinook salmon from Big Lake Hatchery, Big Lake Alaska.

Results

DNA Analysis

Ribosomal DNA (rDNA) revealed six unique phenotypes in the 52 California animals tested. Forty-six of the salmon shared a common phenotype. Five polymorphisms were found in the six other animals. Probes of membrane bound DNA revealed no polymorphic fragments (RFLP's) among 16 animals from all reaches when tested with six enzymes. RAPD analysis revealed polymorphism's between Upper South Fork animals and animals from the other reaches. DNA extracted from caudal fin samples was not of the quality that could be used for comparison analysis.

PCR-amplified internal transcribed spacer (ITS) sequences from the rDNA repeat unit of Chinook salmon were digested with the restriction enzymes Hha I, Hinf I, and Msp I. DNA of 53 individuals from the four regions of the Salmon River System (Upper South Fork, Lower South Fork, Lower North Fork, and Mainstream regions) in Northern California were tested for polymorphic bands. Of the 10 samples from the Upper South Fork, there were no polymorphisms when using any of the three enzymes. Five genetically distinct individuals were identified in other portions of the system (Figure 2). The total number of restriction sites represented by the fragments that were obtained is close to 5000. If all the polymorphic fragments were due to single nucleotide substitutions, the number of polymorphic bands would correspond to 17 substitutions, and the nucleotide substitution frequency would correspond to about 3×10^{-3}.

The hybridization approach, using the PCR product as a probe against total genomic DNA revealed no polymorphisms among the fish tested in this study.

Total genomic DNA was tested with two RAPD primers. This technique is thought to offer higher sensitivity than traditional approaches, but failed to reveal any intrapopulational polymorphisms among the California populations. However, two interpopulation polymorphisms were detected in comparisons of Alaska and California salmon using two different primers (Figure 3).

GIS Data Base

A digital base map of the Salmon River was created using the ARC/INFO Geographic Information System (GIS). Fifty-seven adult salmon sample point locations were entered into a GIS database along with digital data identifying sample characteristics and the results of the DNA analysis.

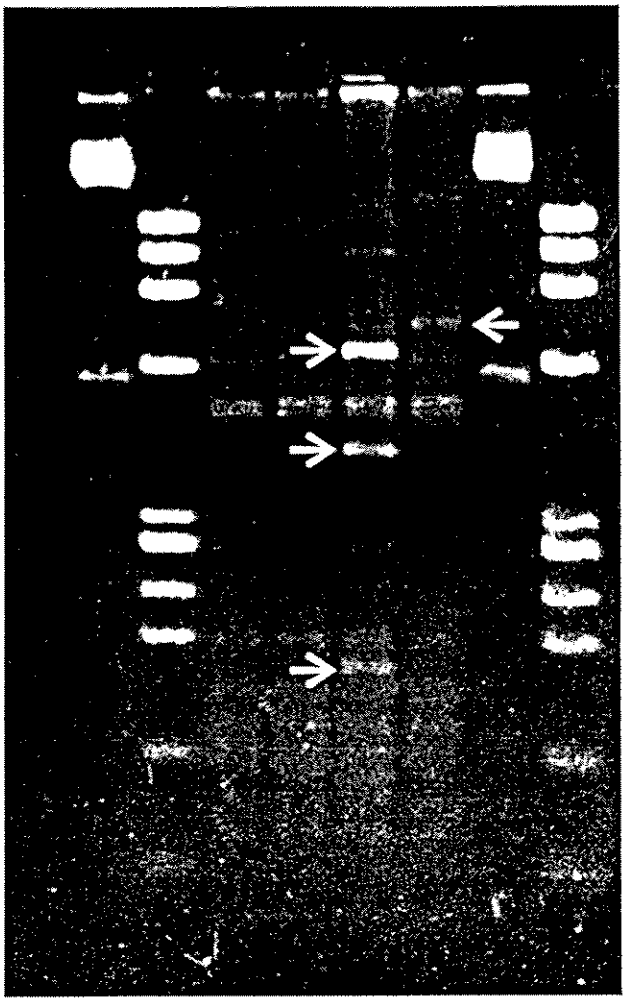

Figure 2. Photograph of an electrophoresis gel showing restriction fragment length polymorphisms in PCR-amplified ITS regions. The PCR product was digested with the restriction enzyme PstI. Arrows indicate examples of polymorphic bands. Lanes 1, 2, 7, and 8 include DNA size markers.

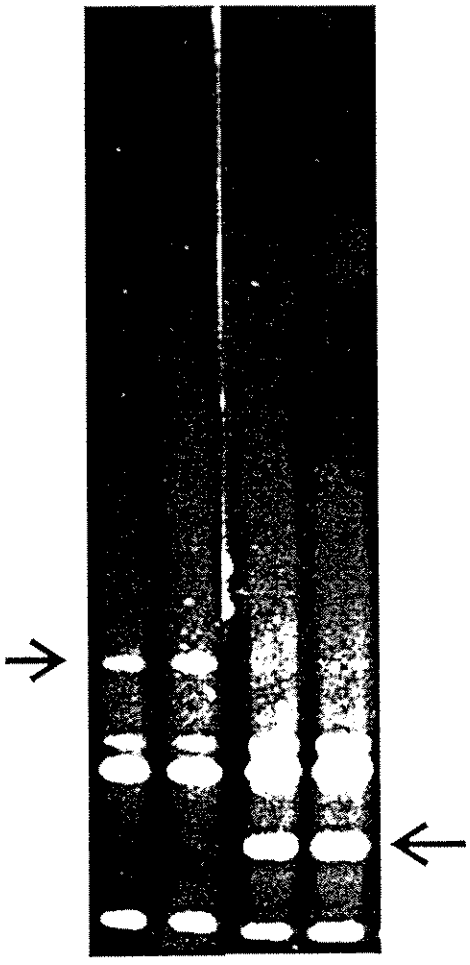

Figure 3. Composite photograph of electrophoresis gels comparing RAPD products produced from a Salmon river individual (left two lanes) and an Alaskan individual (right two lanes). None of the California samples generated the second fragment from the bottom of the Alaska salmon lanes. All Alaska samples did. The band on the upper left in the California fish was not present in all Califonia samples, but was never fond in the Alaskan samples.

Spatial examination revealed that the simplified rDNA analysis produced the most clearly structured results. In the Upper South Fork, 100% of the samples tested were of a common genotype (see Figure 4).

Soil, geology and vegetation characteristics were extracted for sample points. For a section of the Lower South Fork, the GIS stream data was enhanced by attaching habitat typing tables to the stream data (see Figure 5).

Discussion

Findings

Distinct polymorphic differences between the study area fish and the "out population" sample were found.

DNA analysis of adults reveled distinct genotypes in four reaches of the Salmon River. The Upper South Fork fish (spring fish) are genetically uniform. Genetic differences have been found among fish from the remaining reaches.

Juveniles collected in the Klamath River estuary revealed fragment DNA patterns demonstrating that there are more genotypes present that those observed in the Salmon River populations. Due to the lack of juveniles in the Mainstem sample the proportion of identified stocks to unidentified stocks could not be determined.

Future Research

The current study revealed important genetic and spatial characteristics of the salmon population, but the need for further research is indicated by the shortcomings of the study.

A small sample size for adults and juveniles reduces the generalizability of the current study. In addition, not all areas identified for sampling produced fish.

Spent spawners did not necessarily reflect the spawning area. Adult genotypes were not verified with juvenile fin clip samples.

Stream characteristics for each sample point were not available. Currently, the stream locations are maintained in a GIS database, but the stream characteristics are maintained in a separate computerized habitat inventory. This inventory could be linked to the GIS database to provide detailed information on stream habitats, and their relation to the genetic identity of the samples.

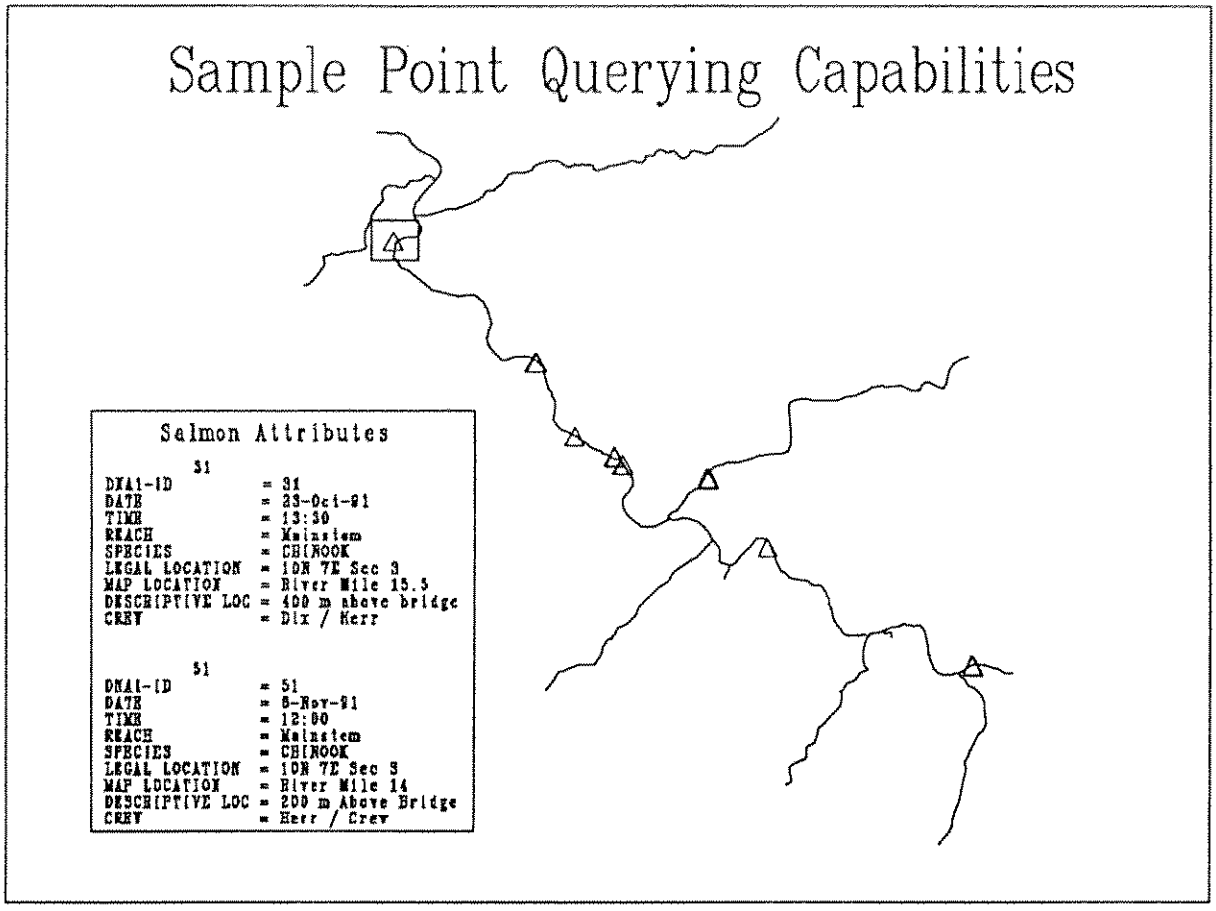

Figure 4. Spatial distribution of rDNA samples.

Due to the small sample size and the distribution area of collection, it was not feasible to ascertain with any degree of reliability the proportion of stocks missed.

Many nuclear DNA-based techniques have been developed over the past few years for studying the structure and dynamics of natural populations. These techniques vary widely in cost, convenience, and resolution. The most appropriate technique to use depends on the degree of genetic diversity in the population under study, which must be determined empirically before continuing a study on a wider scale. We have conducted a variety of pilot studies to find a suitable approach to study Salmon River Chinook salmon, and have found DNA polymorphisms using a number of techniques.

The internal transcribed spacer (ITS) sequences within the rDNA repeat unit have been shown to contain a relatively high frequency of polymorphic restriction sites in several species. Our present study indicates that polymorphic restriction sites, as evidenced by RFLPs, are present in the ITS region of Chinook salmon in the Northern California Salmon River System, albeit in low frequency. The study will continue using additional enzymes, and additional regions will be examined for polymorphic restriction sites using the same approach (i.e., PCR amplification of other nonconserved regions followed by restriction digestion and electrophoresis of the PCR product).

RFLPs have not been found when the ITS PCR product itself is used as a probe against genomic DNA, possibly because the probe hybridizes to fragments that terminate in the neighboring conserved 18S and 28S subunit regions and because we have not tested enough enzymes. We have successfully used digoxygenin-labeled anonymous probes to detect RFLPs in genomic DNA from rockfish and hagfish, and that approach might be productive in salmon. However, hybridization-based RFLP studies involve considerably more time and expense than analysis of the restriction fragments themselves, and are more prone to error because many precise steps are involved. Consequently, this technique is less desirable as a means of finding polymorphisms.

Although the RAPD procedure has not yet revealed intrapopulational diversity in our study, it has produced clearly different fragments from Salmon River and Alaska salmon DNA. The procedure requires that careful attention be paid to reaction conditions in order to obtain reliable results. Nevertheless, numerous investigators have used RAPDs effectively in population studies, and our results show promise that the procedure may provide an effective method for discriminating genetic stocks.

including layers for the various physical and biological attributes that impact the fisheries.

This database would be used to coordinate a region-wide effort to achieve two ends: 1) Identify stocks within the entire Klamath Basin (including the Trinity River); 2) Identify stocks in the watersheds north and south of the Klamath.

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