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PRELIMINARY INVESTIGATIONS OF GENETIC
STRUCTURE OF CHUM SALMON,
Oncorhynchus keta, IN THE NOATAK AND
KOBUK RIVER DRAINAGES OF
NORTHWESTERN ALASKA

by

Robert H. Davis, Jr.
and Carmen Olito
Number 62



Alaska Department of Fish & Game
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ABSTRACT

Tissues were sampled from spawning chum salmon, *Oncorhynchus keta*, from five sites in the Noatak River Drainage and from four sites in the Kobuk River Drainage. Samples were screened for genetic variation, using starch-gel electrophoresis. We were able to resolve 30 loci; six (20%) of these loci were polymorphic. Average heterozygosities were similar among populations (range .021 to .026) but lower than observed in populations from other areas of Alaska. Analysis of F-statistics indicated that only approximately 1% of the total genetic variation in the total population from the Noatak and Kobuk River drainages could be attributed to differences between samples; however, contingency table analysis revealed significant heterogeneity ($P < .01$) among Noatak River drainage samples.

Key Words: *Oncorhynchus keta*, genetic structure, electrophoresis, Noatak River, Kotzebue Sound, heterozygosity.

INTRODUCTION

The Kotzebue District commercial fishery (operating in Alaska since 1962) is the most northern commercial chum salmon, *Oncorhynchus keta*, fishery in the United States. The two major stocks intercepted in this fishery spawn in the Noatak River (75%) and Kobuk River (25%) drainages (Yanagawa 1969). Tagging studies have indicated that Kobuk River chums enter the fishery from mid- to late July and Noatak River chums from early to mid-August.

The commercial catch has averaged 182,566 chum salmon, ranging from a low of 29,400 in 1967 to a high of 627,900 in 1974 (Bird 1980). To stabilize production of salmon at a level that is high enough to support a commercial fish-processing plant in Kotzebue, in 1978 a hatchery was proposed for the Kotzebue Sound area. In 1980, after a survey of sites in the area, the Alaska Department of Fish and Game (ADF&G) proposed the construction of a chum salmon pilot hatchery (2 million eggs) at Sikusuilaq Springs, which is 46.7 km downstream from the village of Noatak. In the same year, the upper Noatak River drainage was designated a National Preserve by Congress, and the Noatak River above the confluence with the Kelly River (Fig. 1) was incorporated into the National Wild and Scenic River system.

Noatak River chum salmon stocks are the primary producers for the Kotzebue district commercial fishery. When developing a hatchery in a river that has a significant wild stock, concerns regarding interaction of hatchery and wild stocks must be addressed. The fact that the Noatak River has received

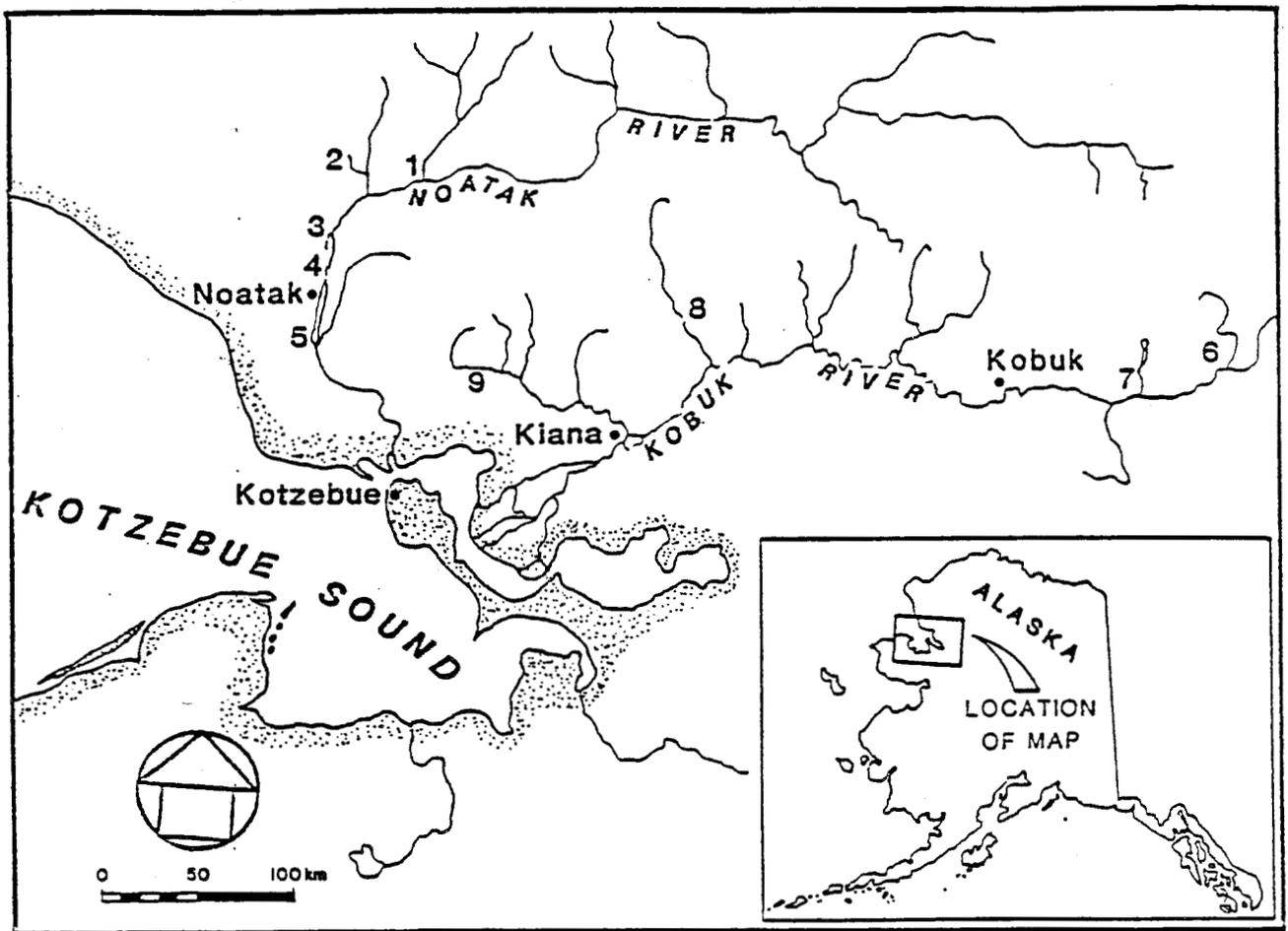


FIGURE 1. Collection sites on the Noatak and Kobuk River drainages.

Key: (1) Kugururok River, (2) Kelly River, (3) Kiyak Creek, (4) Noatak River (13 km north of Noatak Village), (5) Noatak River (6.5 km south of Noatak Village), (6) Beaver Creek, (7) Selby River, (8) Salmon River, and (9) Squirrel River.

"preserve" status underlines these concerns. Because they are subjected to a different environment within the hatchery and, therefore, to different selection pressures, hatchery stocks are expected to change genetically. In addition, chance factors (e.g., mutation, founder effect, and genetic drift) will tend to cause sexually isolated populations to accumulate genetic differences. Many believe that adaptation for hatchery survival reduces the ability to survive in the natural environment. If so, we might expect that the straying of hatchery stocks and their subsequent interbreeding with wild stocks would result in progeny with reduced fitness; consequently, this loss of survival potential would be detrimental to the wild stocks and could cause long-term losses of production. Another view is that hatchery strays will have little genetic impact on wild stocks because the exchange of genetic material is necessary for the development and expansion of salmon populations; however, the actual genetic effect probably lies between these extremes and is dependent on the genetic structure of wild stocks. Knowledge of this structure will allow better understanding of the implications of hatchery development and will provide the information needed to make rational decisions about hatchery broodstock development and management.

Utter et al. (1974) and Allendorf and Utter (1979) reviewed the applications of isozymic genetic data to studies of salmonid populations. They discuss many of the applications of isozymic gene frequency data to the culture and management of fish. Allendorf and Phelps (1981) discussed the assumptions necessary as well as the use of allelic frequencies to describe population structure. Utter et al. (1980) have determined the genetic structures of many salmonids in the Pacific Northwest. They describe evidence (based on two polymorphic loci) for three major groups of chum salmon populations that have been defined

as Asiatic, Alaskan, and American. Recently Japanese workers have used isozyme or allelic-frequency data to further define the structure of chum salmon populations in Japan (Okazaki 1979, 1982, 1983), (Kijima and Fujio 1982).

Our goal is not to determine major population groups but to determine if there is detectable evidence of population structuring within the two river systems studied. Specifically, the purpose of this phase of the study is to determine if electrophoretic analyses reveal evidence that genetically distinct populations exist in these river drainages and, if it is possible, to locate the boundaries of these populations.

MATERIALS AND METHODS

Although the Noatak River chum salmon were of primary interest, we assumed that allelic-frequency differences among drainages might be larger than those among samples within drainages. The Kobuk River drainage was sampled to test this assumption and to serve as a control, against which the Noatak River samples could be compared. Tissues were sampled (heart, eye, liver, and muscle) from spawning chum salmon collected at five sites in the Noatak River drainage and four sites in the Kobuk River drainage during 1983 (*see* Figure 1). Spawning locations were initially located by aerial survey; later, boats were used to capture the fish for sampling. Fish were captured either by seine or by "snagging" with rod and reel. Depending on the remoteness of the sampling location, tissues were kept on ice for a period of from several hours to a week before they could be frozen. Because of the sampling methods used, we were only able to approximate a sex ratio of one male to one female. This was not considered a critical problem, because the ratio did not vary

much from 1:1. Moreover, we detected no differences between males and females, except for somewhat reduced enzymatic activity in the liver tissues of females.

Starch-gel electrophoresis and histochemical staining were performed on the tissues, using methods described by May (1975, 1980) and Harris and Hopkins (1977). An allele can be maintained in a population at low frequency by recurrent mutation. Therefore, we normally assume that a population is polymorphic at a locus only if the frequency of the common allele is equal to or less than 0.99. Because we sampled tissues from 50 adult salmon at each sample site, a locus was considered polymorphic if only one heterozygote was observed in the population.

Polymorphic loci observed in our study are those described by May (1975, 1980); however, there are two exceptions. The malate dehydrogenase (Mdh) locus described in this report is considered a duplicate locus and is commonly designated Mdh-B (May 1975, 1980). To aid our data entry and analysis, our convention is to treat the Mdh-B locus as two loci and to divide observed variant genotypes among the two loci. For example, if there are four heterozygotes in the population, two are assigned to Mdh-3 and two to Mdh-4. When an odd number of variants are observed, the odd genotype is assigned to the Mdh-3 locus. Creatine kinase (Ck) is the other exception. Although it has been described as a dimer (May 1975, 1980), the variable pattern in our study appears to be a monomer in which the variant mobility is 111, relative to the common allele.

Data were analyzed using BIOSYS-1, which is a computer program developed by Swofford and Selander (1981). BIOSYS-1 is a

multi-purpose program capable of performing most types of data analyses that are commonly used in population genetics. Data were entered as integers specifying all genotypes at a locus. Allelic frequencies and standard measures of genetic variability were calculated. The usual chi-square goodness-of-fit test and an exact significance probabilities test were used to test polymorphic loci for conformance of genotypes to Hardy-Weinberg expected frequencies. F-statistics (Wright 1965, 1978) were calculated using the basic formula:

$$1-F_{IT} = (1-F_{IS}) (1-F_{ST})$$

where F_{IT} the overall inbreeding coefficient of an individual includes an effect F_{IS} due to nonrandom mating within subpopulations and an effect F_{ST} due to differences between subdivisions.

Contingency table analyses were performed to test the hypothesis that differences in the genotypic proportions among the sample populations collected are no greater than would be expected among samples taken from a large, randomly mating population. Heterogeneity was evaluated among the nine populations sampled in the study; the five Noatak River and four Kobuk River sample populations were then independently tested.

RESULTS

Seventeen enzymes encoded by 30 loci were resolved and used in the analyses (Table 1). Genetic variation was observed at six (20%) of these loci (Tables 2 and 3). Standard chi-square goodness-of-fit tests indicated deviations from the Hardy-Weinberg frequencies expected for 6-phosphogluconate dehydrogenase (6Pgdh) in the Kiyak Creek sample ($P=.009$) and

Table 1. Enzyme systems and genetic loci resolved in tissue samples from Noatak and Kobuk River chum salmon.

Enzyme	No. of loci
alpha-glycerophosphate dehydrogenase (Agp)	1
sorbitol dehydrogenase (Sdh)	2
lactate dehydrogenase (Ldh)	5
malate dehydrogenase (Mdh)	4
malic enzyme (Me)	1
isocitrate dehydrogenase (Idh)	1
6-phosphogluconate dehydrogenase (6Pgdh)	1
glyceraldehyde phosphate dehydrogenase (Gap)	1
diaphorase (Dia)	1
superoxide dismutase (Sod)	1
pyruvate kinase (Pk)	2
creatine kinase (Ck)	3
phosphoglucose mutase (Pgm)	1
acid phosphotase (Acp)	1
peptidase (Pep)	2
phosphoglucose isomerase (Pgi)	2
phosphomannose isomerase (Pmi)	1
TOTAL	30

Table 2. Gene frequency and population size for each polymorphic locus for samples from the Noatak River drainage.

Locus	Allele	Population				
		Kugururok River	Kelly Lake	Kiyak Creek	N.Noatak River	S.Noatak River
MDH-3	N	50	50	50	60	50
	100	1.000	1.000	1.000	1.000	1.000
	80	0.000	0.000	0.000	0.000	0.000
IDH-2	N	49	43	46	56	48
	100	0.643	0.663	0.609	0.571	0.646
	40	0.296	0.337	0.370	0.357	0.281
	80	0.061	0.000	0.022	0.071	0.063
	30	0.000	0.000	0.000	0.000	0.000
6PGDH	N	50	50	50	50	50
	100	1.000	1.000	0.950	0.958	0.990
	90	0.000	0.000	0.050	0.042	0.010
CK-1	N	50	50	50	60	50
	100	1.000	1.000	1.000	1.000	1.000
	110	0.000	0.000	0.000	0.000	0.000
PEP-B	N	46	49	50	53	46
	100	0.924	0.918	1.000	0.953	0.967
	80	0.076	0.082	0.000	0.047	0.033
PMI	N	47	49	45	60	43
	100	0.947	0.929	0.922	1.000	0.942
	92	0.053	0.071	0.078	0.000	0.058
Mean Heterozygosity		0.025	0.024	0.024	0.024	0.023

Table 3. Gene frequency and population size for each polymorphic locus for samples from the Kobuk River drainage.

Locus	Allele	Population			
		Beaver Creek	Selby River	Salmon River	Squirrel River
MDH-3					
	N	50	50	50	50
	100	1.000	0.990	1.000	1.000
	80	0.000	0.010	0.000	0.000
IDH-2					
	N	50	50	50	50
	100	0.530	0.540	0.630	0.568
	40	0.390	0.420	0.340	0.375
	80	0.080	0.040	0.020	0.057
	30	0.000	0.000	0.010	0.000
6PGDH					
	N	50	50	50	49
	100	0.960	0.970	0.970	0.969
	90	0.040	0.030	0.030	0.031
CK-1					
	N	50	50	50	50
	100	1.000	0.990	1.000	0.990
	110	0.000	0.010	0.000	0.010
PEP-B					
	N	50	50	50	50
	100	1.000	1.000	1.000	0.980
	80	0.000	0.000	0.000	0.020
PMI					
	N	50	50	50	50
	100	0.950	0.980	0.900	0.960
	92	0.050	0.020	0.100	0.040
Mean Heterozygosity		0.024	0.022	0.024	0.024

Pep-B in the North Noatak sample ($P=.007$). In both cases the occurrence of a homozygote variant in the sample resulted in a significant chi-square value. When exact significance probabilities were calculated, neither population deviated significantly from that expected ($P=.098$ and $P=.092$, respectively).

Average heterozygosities were relatively uniform among all populations sampled, ranging from .022 to .025 (Table 2 and 3). Nonhierarchical F-statistics (Wright 1965) are given for each polymorphic locus for all samples combined (Table 4) and for the five Noatak River samples alone (Table 5). These statistics indicate that there is little heterogeneity among sample genotypes.

Contingency table analysis over all samples (Table 6) yielded a significant total chi-square ($P=.003$). Chi-square values for two of the six polymorphic loci (Pep-B [$P<.01$] and Pmi [$P<.05$]) were significant. Heterogeneity among Kobuk River drainage samples (Table 7) was not significant ($P=0.303$). Analysis of Noatak River drainage samples revealed significant ($P=.007$) heterogeneity among samples for total chi-square (Table 8). In Noatak River samples, heterogeneity was significant for two polymorphic loci, 6Pgdh ($P=.023$) and Pep-B ($P=.046$). A third locus (Pmi) produced a large but nonsignificant chi-square value ($P=.061$).

Table 4. Summary of F-statistics at all loci for populations sampled in the Noatak and Kobuk River drainages.

Locus	F(IS)	F(IT)	F(ST)
MDH-3	-.010	-.001	.009
IDH-2	.048	.056	.009
6PGDH	.051	.062	.012
CK-1	-.010	-.002	.008
PEP-B	.011	.046	.035
PMI	-.026	-.009	.016
MEAN	.034	.046	.012

Table 5. Summary of F-statistics at all loci for populations sampled in the Noatak River drainage.

Locus	F(IS)	F(IT)	F(ST)
IDH-2	0.044	0.050	0.006
6PGDH	0.161	0.180	0.023
PEP-B	0.014	0.034	0.020
PMI	0.013	0.028	0.015
MEAN	0.043	0.052	0.010

Table 6. Contingency table analysis at all loci for samples taken in the Noatak and Kobuk River drainages.

Locus	No. of alleles	D.F.	Chi-Square
MDH-3	2	8	8.209
IDH-2	4	24	26.040
6PGDH	2	8	10.712
CK-1	2	8	7.216
PEP-B	2	8	31.268 **
PMI	2	8	16.020 *
TOTALS		64	99.464**

* Significant ($P \leq .05$)

** Significant ($P \leq .01$)

Table 7. Contingency table analysis at all loci for samples taken in the Kobuk River drainage.

Locus	No. of alleles	D.F.	Chi-Square
MDH-3	2	3	3.01
IDH-2	4	9	8.77
6PGDH	2	3	0.23
CK-1	2	3	2.01
PEP-B	2	3	6.03
PMI	2	3	6.99
TOTALS		24	27.04

* Significant ($P \leq .05$)

** Significant ($P \leq .01$)

Table 8. Contingency table analysis at all loci for samples taken in the Noatak River drainage.

Locus	No. of alleles	D.F.	Chi-Square
IDH-2	4	12	14.509
6PGDH	2	4	11.380 *
PEP-B	2	4	9.693 *
PMI	2	4	8.998
TOTALS		24	44.581 **

* Significant ($P \leq .05$)

** Significant ($P \leq .01$)

DISCUSSION

Although we assume that reproductive isolation of subpopulations will in time lead to genetic differentiation, there are no clear gene-frequency differences between sample populations (*see* Tables 2 and 3). Average heterozygosities are consistent from sample to sample, ranging from .022 to .025. These average heterozygosities are lower than those reported for southeast and southcentral Alaska chum salmon populations by Davis and Olito (1982). Allendorf and Utter (1979) and Okazaki (1982) have also reported larger heterozygosities for chum salmon populations, $H=.045$ and $H=.062$, respectively. Okazaki argues that it is inappropriate to compare estimates obtained from different studies because average heterozygosity will fluctuate depending on the number and type of loci examined. However, in comparing the present study to our earlier work (in which similar methods were used), the proportion of polymorphic loci examined are similar for both studies, 20% and 18.2%, respectively. Lower estimates of average heterozygosity in the present study appear to be due to lower heterozygosities at the polymorphic loci. This may be an example of a general observation that populations at the periphery of their range tend to be less variable (Mayr 1971).

Fixation indices (F_{ST}) revealed little genetic differentiation among sample populations. Analysis was applied to the nine populations sampled from the Noatak and Kobuk River drainages (*see* Table 4). Mean fixation index was $F_{ST}=.012$, indicating that 1.2% of the total genetic variation could be attributed to differentiation of sample groups. Wright (1978) states that values as small as .05

or even smaller can not be considered negligible. It is difficult to interpret the meaning of a fixation index as small as .01, but it should be noted that the largest fixation indices were for the 6Pgdh (.012), Pep-B (.035), and Pmi (.016) loci.

Because we are primarily concerned with the genetic structure of populations in the Noatak, we applied F-statistic analysis to the five Noatak River populations alone. The mean value of the fixation index (.010) differed little from that for both drainages combined (Table 5). Values for the 6Pgdh (.023), Pep-B (.020), and Pmi (.015) loci were again relatively high.

Mean levels of F_{ST} are low, and it is clear that most of the genetic variation at sampled loci is within samples. However, apparent genetic differentiation at three loci among sample populations is confirmed by contingency table analysis. This analysis indicates that there is significant variation among sample populations at these loci that can be attributed primarily to variation among Noatak River sample populations. Contingency table analysis of combined Noatak and Kobuk samples yielded a significant total chi-square (P .01) (see Table 6). Further analysis revealed that total chi-square for Kobuk samples (see Table 7) was nonsignificant (P 0.3), but there is significant (P .01) heterogeneity among Noatak samples (see Table 8).

On the basis of F-statistics, there appears to be little genetic diversity among sample populations. However, variation at two loci in the Noatak River samples is

evidence that the Noatak population is structured. Although the evidence will not support definition of the breeding units, characteristics of the Noatak River and the population suggest a working hypothesis.

Most spawning of chum salmon in the Noatak River takes place in spring-fed sloughs and side channels below the confluence of the Kelly River, where the Noatak is very braided. In the upper river, chum salmon populations are small, and they spawn in tributaries. We suggest that, because these spawning areas are physically separate and ecologically distinct, it is reasonable to expect that gene flow has been restricted between the upper and lower Noatak populations. The small size of the upriver populations would contribute to differentiation between these groups. Therefore, it seems reasonable to assume that fish from below the Kelly River confluence and those of the Kelly River and tributaries of the upper Noatak River are from two genetically discrete breeding units. This hypothesis will be tested as the study continues.

The sampling program is continuing on both the Noatak and Kobuk systems. We have increased the sample size at each sample location and expanded the number of sample sites. We hope to resolve the genetic structure of the Noatak River with three additional years of sampling.

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