

## Genetic differences of two chum salmon (*Oncorhynchus keta*) populations returning to the Tokachi River

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### Abstract

This investigation examines two peaks of chum salmon returning to the Tokachi River in 1977 relative to the allelic frequencies of polymorphic proteins of collections from both peaks, and the timing of runs and allelic frequencies of chum salmon populations from other rivers that have been introduced to the Tokachi River. Prior to 1973, the chum salmon run of the Tokachi River exhibited a single peak during late September and early October. Both the timing and the allelic frequencies of isocitrate dehydrogenase (ICD) variants of the second peak during late November and early December corresponded with chum salmon runs from the Abashiri and Yubetsu Rivers; transplantation from both of these rivers to the Tokachi River were made in 1970. Both lines of evidence independently support the hypothesis that the second peak of chum salmon return to the Tokachi River is largely a reflection of these transplantations. The significance of timing as a major variable to be considered in transplantations and the value of gene frequency data in monitoring the success of transplantations is discussed.

### Introduction

Chum salmon have been transplanted with varying intensities among many rivers of Japan. It is difficult to determine the initial success of these transplantations through traditional methods such as tagging or fin clipping because of the necessity of releasing chum salmon at a very early age and -consequently- small size. Even if traditional methods were feasible, the information is limited to the immediate generation and thus yields no data regarding the consequences of transplantation on subsequent generations.

Biochemical genetic methods offer an alternative to traditional marking procedures that is unaffected by the size of fish at release and that yields useful information concerning the success of transplantation beyond the initial generation (UTTER *et al.*, 1974; ALLENDORF and UTTER, 1978). Allelic frequencies of polymorphic protein systems often differ significantly among populations of a species. Stable frequencies are useful to characterize populations and tend to remain similar in transplanted and parent stocks.

The present report uses biochemical genetic data to examine the returns of transplanted and native fish to the Tokachi River. The data verify the success of an initial transplantation over two generations on the basis of both allelic frequencies and the time of return. The

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Received November 25, 1978. Contribution No. 192 from the Far Seas Fisheries Research Laboratory.

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applicability of this procedure to the study of transplated populations in general is discussed.

### Materials and methods

Samples of liver and heart were collected during the fall of 1977 from mature chum salmon returning to the Tokachi, Yubetsu, Abashiri and Shari Rivers (see Figure 1 for locations and Table 1 for dates of collections and sample sizes). Tissues were taken at hatcheries during the process of artificial spawning, and were frozen immediately and remained frozen at  $-20^{\circ}\text{C}$  for up to four months until processed for electrophoresis. Extraction of tissues and methods of electrophoresis followed procedures outlines in NUMACHI (1974). Genetic data were collected from four polymorphic systems; malate dehydrogenase (MDH, 1.1.1.37) and  $\alpha$ -glycerophosphate dehydrogenase ( $\alpha$ -GDH, 1.1.1.8) from extracts of the heart, and isocitrate dehydrogenase (ICD, 1.1.1.42) and phosphogluconate dehydrogenase (6-PGD, 1.1.1.44) from extracts of liver.



**Fig. 1.** Map of Hokkaido showing the rivers and hatcheries (●) from which chum salmon were sampled. (1) Tokachi River; (2) Shari River; (3) Abashiri River; (4) Yubetsu River.

## Results

Each of the four polymorphic protein systems used in this study has been identified in chum salmon by other workers. Variation of MDH-B (the system that predominates in the skeletal muscle) has been described by NUMACHI *et al.* (1972), ALTUKHOV *et al.* (1972) and SEEB and GRANT (1976); three alleles have been identified for this duplicated locus. Two allele polymorphisms were reported for  $\alpha$ -GDH by ALTUKHOV *et al.* (1972) and for 6-PGD by SEEB and WISHARD (1977). A four-allele polymorphism for ICD has been identified by NUMACHI *et al.*; this variation is extended to five alleles through the present report.

Allelic frequencies for three of the polymorphic systems (MDH-B,  $\alpha$ -GDH, 6-PGD) were statistically indistinguishable among the collections of this study, but significant differences were detected at the ICD locus between the early and late collections of the Tokachi River ( $\chi^2=18.85$ ,  $P<.001$ ; Table 1). The remainder of this report examines this difference relative to other known aspects of the Tokachi River chum salmon runs and runs of chum salmon returning to rivers entering the Sea of Okhotsk.

**Table 1.** Gene frequencies at ICD, 6-PGD, MDH and  $\alpha$ -GDH loci in each river population of chum salmon.

River name	Collected date	Number of specimens	ICD					6-PGD		MDH			$\alpha$ -GDH	
			A	B	C	D	E	F	S	B	B'	B''	F	S
Tokachi R.	Oct. 12 th	100	.530	.435	.010	.025	.000	.985	.015	.973	.028	.000	.985	.015
Tokachi R.	Nov. 24 th	60	.408	.442	.075	.042	.033	.992	.008	.996	.004	.000	1.000	.000
Shari R.	Nov. 26 th	100	.439	.408	.061	.082	.010	.985	.015	.970	.028	.003	.985	.015
Abashiri R.	Nov. 27 th	100	.490	.381	.046	.062	.021	.985	.015	.975	.028	.003	.985	.015
Yubetsu R.	Nov. 25 th	100	.480	.395	.025	.080	.020	.995	.005	.980	.013	.008	.990	.010

The timing and magnitude of chum salmon runs returning to the rivers investigated (outlined in Figs. 2 and through 5) indicates differences in some rivers in different years (Annual Report of Hokkaido Salmon Hatchery). Some of these differences may reflect intrinsic fluctuations among year classes of natural runs; others may be a result of transplantation. The greatest year to year fluctuation in timing is seen for the Tokachi River (Fig. 2) where an early peak during late September and early October occurs every year, and a second peak in December is also seen in 1973, 1974, 1976 and 1977. It is necessary to look into the recent records of transplantation among these streams (Table 2) before an attempt is made to correlate this fluctuation with transplantation. Large numbers of eggs were transferred to and from the Tokachi River during 1970 and 1971. The 1970 transfers from the Abashiri and Yubetsu Rivers to the Tokachi River are particularly pertinent because of the subsequent increases of late returning fish to the Tokachi River which correspond in timing with the runs of the Abashiri and Yubetsu Rivers. These returns correspond exactly with returns that would be expected from three and four year old fish resulting directly from the 1970 transplantations

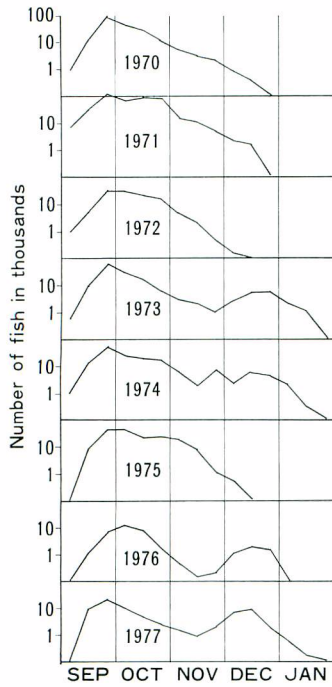


Fig. 2. Seasonal abundance of chum salmon catch in the Tokachi River from 1970 to 1977.

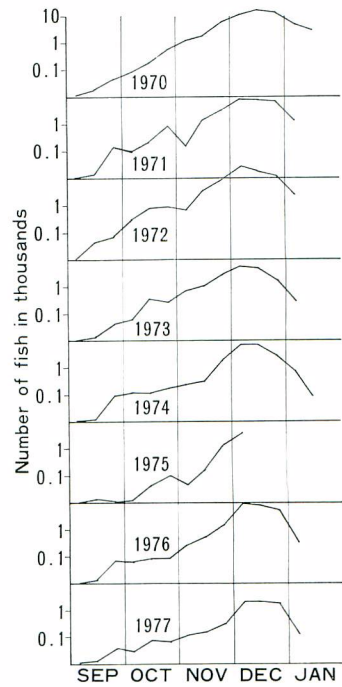


Fig. 3. Seasonal abundance of chum salmon catch in the Yubetsu River from 1970 to 1977.

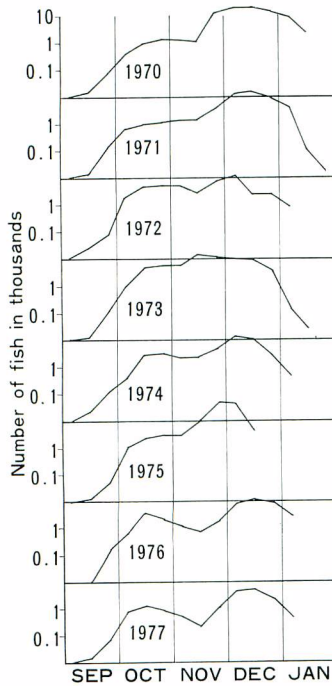


Fig. 4. Seasonal abundance of chum salmon catch in the Abashiri River from 1970 to 1977.

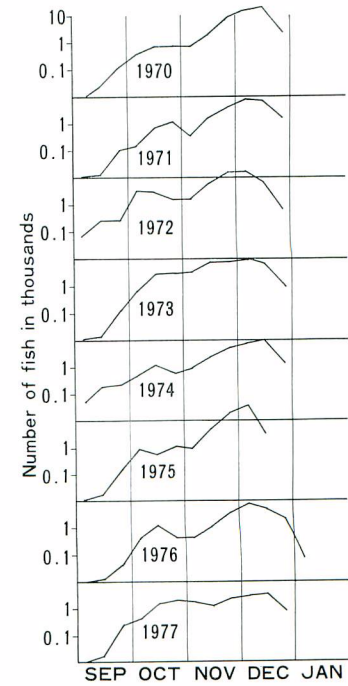


Fig. 5. Seasonal abundance of chum salmon catch in the Shari River from 1970 to 1977.

to the Tokachi River (as outlined in Fig. 6); and it is known that chum salmon returning to the Abashiri and Yubetsu Rivers are predominantly three and four year old fish.

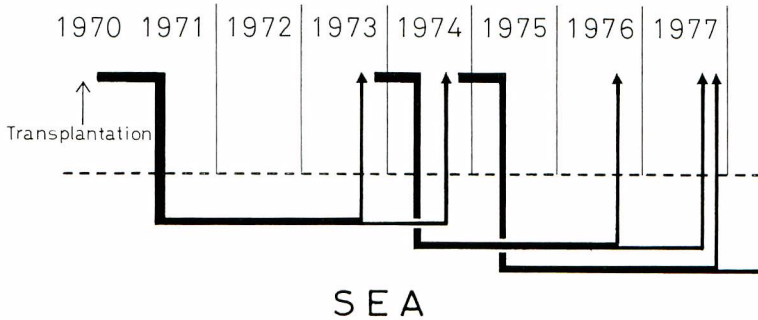


Fig. 6. Schematic model of the relation between returning year and age composition of the transplanted chum salmon in the Tokachi River.

The genetic data for the allelic frequencies of ICD (Table 1) also suggests that the late peak of Tokachi River chum salmon is a reflection of the 1970 transplantations. It is particularly noteworthy that the *Icd-e* allele was present in the late peak of the Tokachi River and in all three collections from rivers entering the Sea of Okhotsk, but not in the early peak of the Tokachi River. The genetic similarity and similar timing of the runs returning to the three rivers entering the Sea of Okhotsk are presumably a reflection of gene flow either resulting from their proximity to one another or frequent transplantations among them in the past (reported verbally by hatchery personnel).

The data presented to this point independently indicate that the late peak of the Tokachi River run is a direct result of 1970 transplantations from the Abashiri and Yubetsu Rivers. It is pertinent to consider the absence of an early peak reflecting the reciprocal transplantations from the Tokachi River to rivers entering the Sea of Okhotsk made at the same time (Table 2). The answer is that the transplanted Tokachi River fish did approach the Okhotsk Sea streams as adult in considerable number but were harvested by the set net fishery so that they did not contribute significantly to subsequent generations (Fig. 7). Chum salmon approaching large rivers (e.g. the Tokachi River) remain silvery upon entry to fresh water in contrast to fish approaching small rivers (e.g. the rivers entering the Sea of Okhotsk) which take on nuptial coloration in salt water. Bright silver chum salmon were, in fact, intercepted in the set net fishery on the coast of the Okhotsk Sea starting in September and October of 1973. Some of the transplanted Tokachi River fish had been tagged prior to release, and some of these silver colored chum salmon taken in the set net fishery contained these tags. In contrast, the set net fishery of the estuary of the Tokachi River closes early in November. Thus the transplanted fish from the Okhotsk Sea rivers, whose spawning peak occurs after this time, returned in sufficient number to continue the transplanted lineage for another generation.

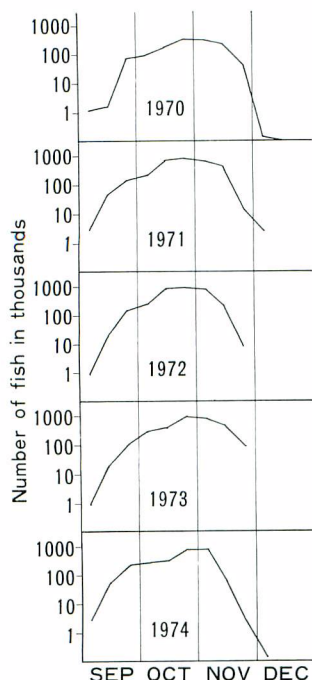


Fig. 7. Seasonal abundance of chum salmon catch on the coast of the Okhotsk Sea from 1970 to 1974.

Table 2. Summary of chum salmon egg transplants during 1970 and 1971. Number represents thousands of eggs.

Area transplanted	Donor stream		
	Tokachi River	Abashiri River	Yubetsu River
Tokachi River	—	4,773(1970)	5,324(1970)
Abashiri River	4,373(1970)	—	0
Yubetsu River	4,470(1970) 4,919(1971)	0	—
Shari River	4,401(1971)	0	0

### Discussion

This report describes only a portion of the transplantations that have been attempted with chum salmon in Japanese rivers. Transplantations to the Tokachi River were attempted in 1959 with fish from streams entering the Strait of Nemuro (Fig. 1), whose timing coincides with that of the runs returning to the streams of the Sea of Okhotsk. These plantings apparently did not produce a second generation because they did not result in the establishment of a second peak such as that observed in this study. This failure was presumed to be the result of poor survival of the transplanted eggs (reported verbally by hatchery personnel).

However, other successful transplantations have been reported. A new late peak—similar to that of the Tokachi River—was temporarily established in the Okawa River (of the Miyagi Prefecture) three and four years after transplantations of fish of a late run from the Otsuchi River. This late peak eventually disappeared and the Okawa River presently consists of a single early peak—as it did prior to the transplantations (NOSE, 1970).

Natural chum salmon runs consisting of two peaks are known in the Ishikari and Tsugaruishi Rivers. The two peaks of the Ishikari River (September-October and November-December) were initially differentiated by sex ratio and growth measurements. These runs have been altered through such factors as transplantations and stream bed modifications and

the early run is presently the major component (KOBAYASHI, 1961). An early run of the Tsugaruishi River has occurred since the early 1940's. This run precedes the regular run by more than a month and its causes have not yet been determined (NOSE, 1970).

A general characteristic of annual chum salmon runs returning to different rivers is their definite timing. This timing tends to be later proceeding southward along Honshu to 39°N. latitude where the trend reverses and runs to streams further to the south again tend to return earlier (MACHIDORI, 1978). It is presumed that this distinct timing of different groups is the result of fairly long periods of natural selection and that this characteristic has therefore a high genetic component.

This trait of distinct timing apparently persists in transplanted fish, and permits monitoring of the relative success of these transplantations over time intervals such as has been done with runs of the Tokachi and Okawa Rivers. Monitoring the success of transplantations by time of return alone is not possible, however, if the transplanted and native stocks do not differ with regard to this trait. Other means of identifying populations such as by scale patterns, fecundity and number of vertebrae are similarly limited because of the tendency of overlap of characters between populations.

Gene frequency data, on the other hand, permit monitoring of the relative success of transplanted runs having similar timing to native runs if differences of allelic frequencies exist for the two groups, and if data pertaining to these differences are available prior to transplantation. Such data are available for the runs presently returning to the Tokachi River. These data will permit continued monitoring of possible changes occurring in these runs at different times relative to their genetic makeup. If the second peak disappears at some time (as occurred in the Okawa River) it will be possible to determine if this disappearance was the result of actual loss of this run, or the continuation of the run with a change in its time of return.

The primary consideration regarding the relative success of transplantations in the past has been the number of eggs transferred, and little attention has been paid to qualitative differences that may exist regarding the adaptation of runs of two areas. Differential timing of runs is a major variable that must also be considered. The continued study of the chum salmon runs returning to the Tokachi River through the use of genetic markers provides a model for the general study of transplanted salmonid populations.

### Acknowledgements

The author wishes to express many thanks to Dr. Tetsuo KOBAYASHI and Mr. Shinichi ABE of Hokkaido Salmon Hatchery for valuable advice and their kind help in providing specimens. The author is also indebted to Assoc. Prof. Kenichi NUMACHI, Ocean Research Institute, Tokyo University, for his interest and encouragement. Grateful acknowledgement is to Dr. UTTER, Northwest Fisheries Center, National Oceanic and Atmospheric Administration in USA, for his critical proofreading of the manuscript.

## References

- ALLENDORF, F. W. and F. M. UTTER 1978: Population genetics., In: Fish Physiology, Vol. VII, 407-454. Ed. W. S. HOAR and D. J. RANDALL. Academic Press, New York.
- ALTUKHOV, Y. P. 1975: Population genetics of fishes., Fish Mar. Serv. Transl. Ser., No. 3548. Annual Report of Hokkaido Salmon Hatchery., 1974-77, (in Japanese).
- KOBAYASHI, T. 1961: Biology of chum salmon, *Oncorhynchus keta* (Walbaum), by the growth formula of scale. *Sci. Rep. Hokkaido Salmon Hatchery*, (16), 1-102, (in Japanese with English Summary).
- MACHIDORI, S. 1978: Water temperature problem and enhancement of chum salmon in the Japan Sea coast region of Honshu. *Sake to Masu*, 20 (33), 6-23, (in Japanese).
- NOSE, Y. 1970: Upstream migration mode of chum salmon and improvement of breed. *Kagaku to Seibutsu*, 8 (12), 6-23, (in Japanese).
- NUMACHI, K. 1974: Genetic characteristics of fish population., In: Shigenseibutsuron., 5-36. Ed. M. NISHIWAKI. Tokyo University Press, Tokyo, (in Japanese).
- NUMACHI, K., Y. MATSUMIYA and R. SATO 1972: Duplicate genetic loci and variant forms of Malate Dehydrogenase in chum salmon and rainbow trout., *Bull. Japan. Soc. Sci. Fish.*, 38 (7), 699-706.
- NUMACHI, K., T. OKAZAKI and M. OHYA: Genetic distance among rivers populations of chum salmon., in preparation.
- SEEB, J. and W. S. GRANT 1976: Biochemical genetic variation in coho, chinook, chum and pink salmon: the use of electrophoretic markers in stock identification., Final Report Service Contract, No. 711, Washington State Department of Fisheries.
- SEEB, J. and L. N. WISHARD 1977: The use of biochemical genetics in the management of Pacific salmon stocks: genetic marking and mixed fishery analysis., Final Report Service Contract, No. 792, Washington State Department of Fisheries.
- UTTER, F. M., H. O. HODGINS and F. W. ALLENDORF 1974: Biochemical genetic studies of fishes: potentialities and limitations., In: Biochemical and biophysical perspectives in marine biology. Vol. I, 213-237, Academic Press, London, New York.

十勝川へ回帰する遺伝的に異なる2つのサケ  
(*Oncorhynchus keta*) 集団について

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要 約

十勝川にそ上回帰したサケの遺伝的特性を前期と後期の各集団について検討した結果、イソクエン酸脱水酵素に関与する遺伝子頻度に著しい差異が認められた。十勝川へのサケのそ上は、従来は9月下旬～10月上旬を盛期とする単峰型の傾向を示していたが、1973年からは新たに12月にも小さなピークが認められるようになった。当河川には、1970年にオホーツク海に注ぐ河川(網走川と湧別川)からの移殖が行なわれており、そ上時期に変化がみられるようになったことと移殖群の回帰との関連が想定されていた。

この想定を裏付けるものとして、十勝川での後期そ上群と、移殖群を供給した前記河川へそ上するサケ集団間には遺伝的特性に大きな相関があることが判明した。その結果、十勝川で新たに生じたそ上のピークは、これらの河川からの移殖群の影響によるものであることが確認された。