

Genetic Stock Identification of Chum Salmon in Highseas Test Fisheries in the Western North Pacific Ocean and Bering Sea.

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Substantial genetic variability among populations of chum salmon (*Oncorhynchus keta*) around the Pacific Rim make it possible to estimate the stock composition of complex mixed-stock fisheries using genetic stock identification (GSI) techniques. We briefly discuss the history of the use of portions or all of the Asian-North American baseline for protein genetic characters to resolve mixtures of chum salmon. We use the species-wide genetic database to analyze test fishery samples taken aboard Japanese research cruises in 1991-1992 in the Kuril Islands (145° E), the western North Pacific Ocean (165° E), and the North Pacific Ocean and Bering Sea at 179° 30' W. Mixture samples from the Kuril Islands and western North Pacific Ocean were dominated (>75%) by single stock groups, i.e., Japanese and Russian stocks, respectively. At 179° 30' W, the stock profile in the Bering Sea was 45% Japanese, 38% Russian, and 15% Alaskan and differed considerably from the samples (1991-1992) taken in the North Pacific Ocean (15% Japanese, 62% Russian, and 22% Alaskan). This protein genetic dataset has been standardized across international agencies and can be used across the species' distribution for accurate mixed-stock fishery analyses. Results from genetic stock identification can and are being used fisheries management and applied to biological models of stock distributions of salmon in the North Pacific Ocean.



INTRODUCTION

A genetic dataset for protein genetic (allozyme) characters exists for chum salmon populations throughout its distribution. The baseline dataset extends from Japan and Russia (Winans et al. 1994) eastward through western and southeast Alaska (Wilmot et al. 1994; Kondzela 1994; Seeb et al. 1995) to southern British Columbia and Washington (Phelps et al. 1994). The level and patterns of regional differentiation have been explored in the above studies and applied to questions of zoogeography, speciation, and fishery management.

Regional segments of this species-wide dataset have been used in several instances for coastal or

nearshore mixed-stock fishery analyses. Canadian biologists have used genetic stock identification to estimate the proportional contribution of Canadian and US stocks of chum salmon and for in season monitoring of Canadian stocks in several southern BC fisheries (Hop Wo et al. 1992). Their 41 stock/7 locus baseline is primarily BC stocks with representative Washington stocks. Since 1991, a 39 stock/30 locus baseline is being used in Washington by state and tribal agencies to monitor the timing and abundance of stocks in Puget Sound (NWIFC 1995). Stock composition estimates collected on a weekly basis through a portion of the fishery have been applied along with catch-per-unit effort for in-season and post-season models for management and

conservation of chum salmon (NWIFC 1995).

The species-wide dataset has been used to assess complex, international mixed-stock fisheries in two instances. Seeb et al (1995) estimated stock composition of chum salmon caught incidentally in sockeye salmon (*O. nerka*) fisheries in two areas of southern Alaska Peninsula in 1993 and 1994. Using available genetic data from Asia and North America, they constructed a 69 stock group/20 locus dataset. Wilmot et al (1995) added several Asian stocks to this baseline (77 stock groups total) and estimated the stock composition of chum salmon caught incidentally in Bering Sea trawl fisheries for walleye pollock (*Theragra chalcogramma*). Analyses of simulated high-seas mixtures indicate accurate estimates can be made to stock groups using slightly different versions of the species-wide baseline (Winans et al. 1994; Seeb et al 1995; Wilmot et al. 1995).

In this report we use the newly-assembled genetic baseline to describe test fisheries of chum salmon caught during several research cruises in the west Pacific Ocean, north Pacific Ocean, and Bering Sea.

MATERIALS AND METHODS

Tissues were collected from fish caught by longline or gillnet on high seas research vessels from 1991 to 1992 (Table 1). Samples of muscle, heart, liver, and retinal tissue were frozen and held onboard at approximately -20°C until transported to -80°C freezers, and ultimately shipped to the Northwest Fisheries Science Center, Seattle for processing and analysis.

Samples were examined for protein electrophoretic variation on horizontal starch gels using standard procedures described by Aebersold et al. (1987) and Winans et al. (1994). Standard nomenclature for loci and alleles was used as outlined in Shaklee et al. (1990). Alleles were compared and

standardized for 20 polymorphic loci (Seeb et al. 1995).

Baseline Data

Data are available for approximately 200 stocks species-wide. We used the simplified baseline dataset formulated in Seeb et al. (1995) for 69 stock groups that was augmented by Wilmot et al. (1995) to a 77 stock group/20 locus dataset. Seeb et al. (1995) simplified the entire Pacific Rim baseline by selecting representative stocks from southeast Alaska, British Columbia, and Washington and by pooling statistically nonsignificant regional stock pairs or groups for a total of 69 stock groups. Wilmot et al. (1995) added two stocks from China and six stocks from Russia for a total of 77 stock groups. Mixed-stock fishery results were no more precise when assessing contributions by geographic region when additional populations from southeast Alaska, British Columbia, and Washington were added for a total of 190 stocks (R. Wilmot, pers. comm., National Marine Fisheries Service). Our analyses were based on a 19-locus baseline; we dropped *PEPA* from our analyses because it was frequently missed in the electrophoretic screening of mixture samples. Because of the low level of *PEPA* variation, its exclusion from the mixture analyses had minimal to no effect on results reported herein. The 19 loci are: *sAAT-1,2*, *mAH-3*, *mAAT-1*, *ALAT*, *ESTD*, *G3PDH-2*, *GPI-A*, *GPI-B1,2*, *mIDHP-1*, *sIDHP-2*, *LDH-A1*, *LDH-B2*, *PEPB-1*, *sMDH-A1*, *sMDH-B1,2*, *sMEP-1*, *mMEP-2*, *MPI*, and *PGDH*. Allele frequencies for the isoloci were computed over both loci and treated as a single locus.

For a multilocus estimate of population differentiation, Cavalli-Sforza and Edwards' chord distances (1967) and Nei's unbiased genetic distance values (Nei 1978) were calculated for all pair-wise comparisons of populations with the computer program BIOSYS (Swofford and Selander 1981), and

Table 1. Summary of research cruises with test fisheries of chum salmon for which tissues were available for genetic analysis. Number of fish refers to number of fish successfully processed for protein genetic data.

Research Cruise	Date	Latitude	Longitude	No. of fish	Gear	Reference
<i>Wakatake maru</i> 1991: Bering Sea	7/2-7/9	56-57 N [§]	179 W-178 30 W	155	longline & gillnet	Ishida et al. 1991
<i>Wakatake maru</i> 1992: Bering Sea	7/5-7/6	56-58 N	179 30 W	196	longline & gillnet	Ishida et al. 1992
<i>Wakatake maru</i> 1992: N. Pac. Ocean	6/25-6/27	46-48 N	179 30 W	102	longline & gillnet	Ishida et al. 1992
<i>Hokuho maru</i> 1992 (Kurul Islands)	9/13-10/5	42-50 N	145-160 E	234	longline	Ishida et al. 1994
<i>Hokko maru</i> 1992	7/3-7/13	42-50 N	165 E	207	gillnet	Azuma & Yoshida 1992

§ stations 20 and 27 only.

genetic relationships among populations were depicted in three-dimensional space with a multidimensional scaling (MDS) technique using the NTSYS-pc program (Rohlf 1994).

Mixed-stock Analysis

Estimates of stock contributions were made with a conditional maximum likelihood algorithm (Pella and Milner 1987) using the GIRLSEM program of Masuda et al. (1991). Precision of the composition estimates was calculated by bootstrap resampling (Efron 1981). Estimates were computed repeatedly from resampled observations of the baseline and mixture samples (Pella and Milner 1987). Standard deviations of estimates were calculated after 500 bootstrap resamplings. Estimates were made to individual stocks and then pooled to regional stock groups used by Seeb et al. (1995) and Wilmot et al. (1995). The regional stock groups are Japan, Russia, Western Alaska (summer run stocks), Yukon River (fall run stocks), Alaska Peninsula/Kodiak, SE Alaska and Prince William Sound, British Columbia, and Washington. Simulation studies of Wilmot et al. (1995) indicated that when true group contributions were 100%, average estimates were greater than 80% accurate for each of the eight groups.

RESULTS

The genetic relationships among the 77 stock groups are depicted in Figure 1. As seen previously (Seeb et al. 1995; Wilmot et al. 1995), regional groups of stocks are genetically similar. For example, the western Alaskan stocks cluster together, as do the Washington, Yukon, and Japan stocks. Although the majority of the Russian and Alaskan Peninsula stocks form distinctive, separate clusters, outliers are seen in

both of these groups. A similar pattern of genetic differentiation was obtained with Nei's genetic distance values (results not shown).

Estimates for the mixed stock fisheries are summarized in Table 2. As expected, estimated stock contributions differed among the test fishery locales. The Bering Sea samples from the *Wakatake maru* cruises in early July for 1991 and 1992 were a mix of Japanese and Russian chum salmon, and differed considerably from the mixture samples taken approximately two weeks earlier from the North Pacific Ocean which were predominantly Russian-origin chum salmon. The three predominant stock groups in the Bering Sea over the two sampling periods were Japan (34-56%), Russia (32-44%), and Alaska (11-20%). The Alaska Peninsula/Kodiak Island or Western Alaska stock group constituted approximately half of the Alaskan contributions in the Bering Sea samples in 1991 and 1992, respectively (Table 2).

The North Pacific Ocean mixture from the *Wakatake maru* cruise in 1992 was 62% Russian stocks. Alaskan stocks made up 22.3% and Japanese stocks constituted 14.9% of the mixture. At 14.3%, the Alaska Peninsula/Kodiak Island stock group was the largest Alaska stock group.

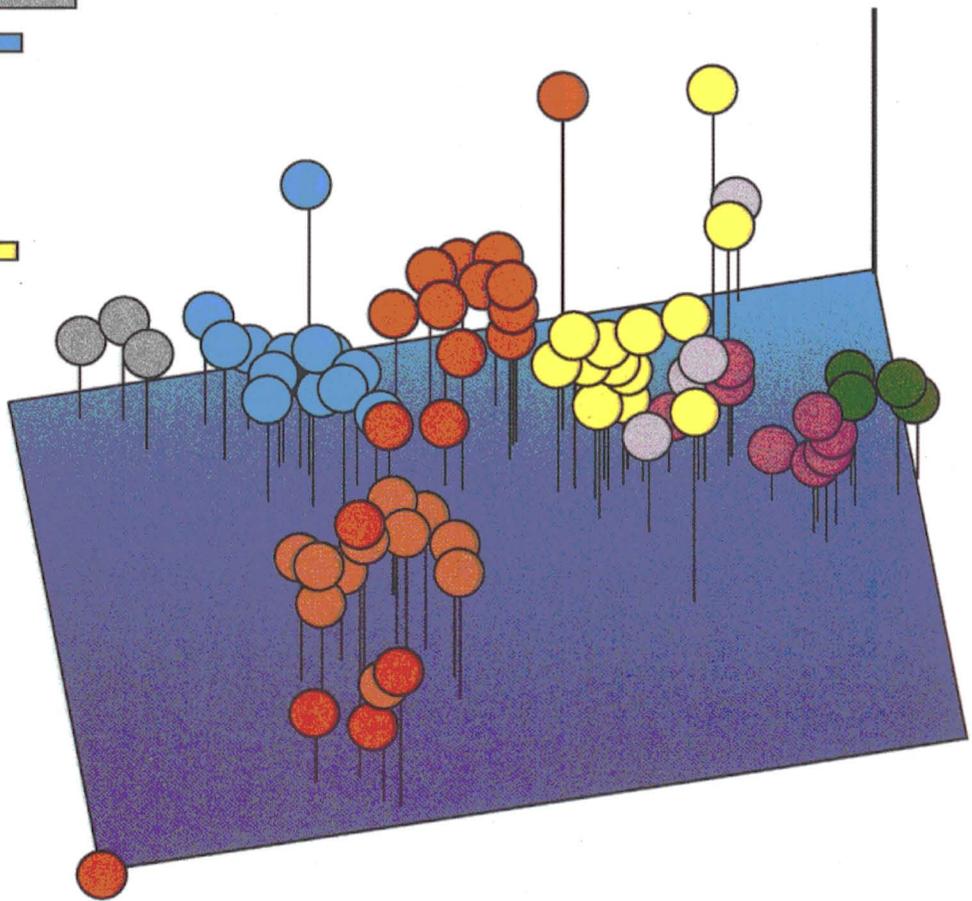
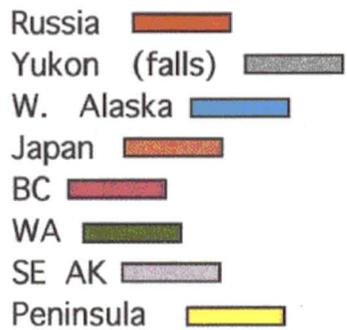
The two mixtures in the western North Pacific were each dominated by one stock group. The *Hokuho maru* 1992 mixture was 78% Japanese stocks, with smaller contributions from Russia (15.9%) and Alaska (5.5%). The *Hokko maru* 1992 was 86% Russian stocks, with lesser contributions from Alaska (10.9%) and Japan (1.9%).

Contributions from southern North American stocks were minimal in these test fisheries. The largest estimated contributions from the Southeast Alaska/Prince William Sound stock group was approximately 3.5% in both 1992 *Wakatake maru*

Table 2. Estimates of percent stock composition by region in test fisheries of chum salmon. The regional groups are: Japan, Russia, Alaska total, Western Alaska (summer run stocks), Yukon River (fall run stocks), Alaska Peninsula/Kodiak, Southeast Alaska/Prince William Sound, British Columbia, and Washington. Standard deviations, in parentheses, were calculated from 500 bootstrap samples of the baseline and mixture samples.

Research Cruise	Japan	Russia	Alaska total	Pen/Kodiak	W. Alaska	Yukon Fall	SE/PWS	BC	WA
<i>Wakatake maru</i> 1991/Bering Sea	56.0 (6.6)	32.4 (7.3)	11.4 (5.8)	5.6 (4.4)	4.0 (3.2)	0.2 (0.5)	1.6 (2.2)	0	0
<i>Wakatake maru</i> 1992/Bering Sea	34.4 (6.1)	43.9 (7.7)	19.7 (6.8)	5.9 (3.5)	10.3 (5.7)	0	3.5 (2.4)	1.9 (1.7)	0.1 (0.4)
<i>Wakatake maru</i> 1992/N. Pac. Ocean	14.9 (6.9)	62.1 (8.3)	22.3 (7.8)	14.3 (6.8)	4.3 (3.8)	0	3.8 (4.2)	0.6 (1.4)	0
<i>Hokuho maru</i> 1992 (Kurils)	78.0 (4.7)	15.9 (4.1)	5.5 (3.1)	2.0 (1.9)	3.5 (2.5)	0	0	0.5 (0.8)	0
<i>Hokko maru</i> 1992	1.9 (2.4)	86.1 (5.7)	10.9 (5.2)	4.9 (3.7)	5.6 (3.7)	0.1 (0.3)	0.2 (0.5)	1.0 (1.0)	0

Fig. 1 Multidimensional scaling (MDS) plot of Cavalli-Sforza and Edwards' chord distances among 77 stock groups of chum salmon based on allele frequencies at 19 loci. Stock groups are: Russia, Yukon River (fall run stocks), Western Alaska (summer run stocks), Japan, British Columbia, Washington, SE Alaska and Prince William Sound, and Alaska Peninsula/Kokiak.



mixtures. British Columbia stock contributions were all 1.9% or less. Estimates of contributions for Washington stocks were essentially 0; only one estimate was actually non-zero (0.1% in the *Wakatake maru* Bering Sea mixture, 1992).

The reported standard deviations for estimates > 10% were small (Table 2). In general, the 95% confidence intervals for these estimates were approximately 1-2% around each estimate, e.g., for *Hokko maru* 1992, the confidence intervals of the estimates were 85.6%-86.6% Russian, 10.4-11.3% Alaskan, and 1.7-2.2% Japanese. On the other hand, estimates of < 10% had relatively greater standard deviations, as is frequently seen (Pella and Milner 1987).

DISCUSSION

The observed level of genetic differentiation among groups of chum salmon across the species distribution permits accurate mixed-stock fishery estimates with protein genetic markers. The use of regional genetic baselines for stock identification has been demonstrated in British Columbia (Hop Wo et al. 1992) and in the United States and Canada (NWIFC 1995) in efforts to monitor the composition of near-shore fisheries of chum salmon. Determining stock compositions of more complex fisheries has been demonstrated in the Bering Sea and the Alaska Peninsula (Wilmot et al. 1995; Seeb et al. 1995) using a species-wide baseline.

We used the a 19-locus version of the 20-locus species-wide baseline established recently by Seeb et al. (1995), as augmented by Wilmot et al. (1995). This baseline represents our current best possible genetic profile for the major contributing stocks across the species' distribution. We recognize, however, that it is subject to refinement, with the addition of more polymorphic, stock-discriminating loci (protein or DNA-based), and more representative stocks. For more localized management applications (Hop Wo et al. 1992; NWIFC 1995), additions of regionally-important stocks and deletions of geographically-distant stocks groups are warranted. For example, for future analyses of mixtures from the Sea of Okhotsk, additional baseline samples from Sakhalin Island and Japan need to be evaluated and considered for a more precise mixed-stock fishery application (S. Urawa, unpublished data).

Mixture samples from the western portion of the north Pacific Ocean were dominated by single stock groups. The *Hokko maru* mixtures (1992 and 1995) were primarily Russian stocks (86-87%), with approximately 10% Alaskan and 2% Japanese stocks. On the other hand, the sample from the Kuril Islands (*Hokuho maru* 1992) was primarily Japanese stocks (78%) with approximately 16% Russian stocks and

6% Alaskan stocks. Stocks from British Columbia were detected in 6 of the 8 mixtures at a level of \leq 1.9%. Washington stocks were essentially absent in these samples.

After two years of sampling at 179° 30'W in early July, we have evidence of a broad difference in the relative abundance of the three primary stock groups between the Bering Sea and North Pacific Ocean. The stock profile for Japanese/Russian/Alaskan stocks in the Bering Sea was approximately 45:38:15% and differed substantially from the data available from the same cruise survey at 179° 30'W in the North Pacific Ocean. The approximate profile for Japanese/Russian/Alaskan stocks in the North Pacific Ocean was 15:62:22%. This trend is also apparent in 1995 data (S. Urawa, unpublished data). Similarly, the *Hokko maru* sample which was primarily Russian chum salmon in 1992 was predominantly Russian stocks in 1995 (S. Urawa, unpublished data). These suggestions of stock abundance trends for specific time and places will be examined in future studies.

These broad differences presumably represent distributional patterns inherent to the stocks at a particular life history stage in a particular water mass. Therefore stock composition estimates will vary with water mass characteristics, stock age, stock maturity, etc. This study demonstrates that protein genetic markers can be used to accurately identify the composition of mixtures to a regional level. We recognize that the same biotic and abiotic conditions in the ocean that establish these broad differences may also be responsible for the between-year difference within one area, e.g., compare 1991 and 1992 results in the Bering Sea (Table 2). As more information on water mass characteristics (e.g., Davis et al. 1996) are collected, further canonical comparisons will add information about stock abundance variables.

Other genetic markers may someday augment or replace these protein genetic markers. Some of the leading contenders are DNA-based characters (Park and Moran 1994) that can be derived, in theory, from fins, scales, and tissues preserved with alcohol--thus, eliminating the need for ultracold preservation of the four tissues required here. In light of these potential developments, protein genetic profiles are available now across the species' distribution and can be implemented in accurate mixed-stock fishery analyses. Protein genetic characters are available species wide, are economically processed in the laboratory, and have been standardized across international agencies. Results like those reported here can be used in programs of highseas stock assessment.

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