Genetic Stock Identification of Chum Salmon (*Oncorhynchus keta*) in the Bering Sea Using DNA Microarray

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Molecular techniques to assess the genetic variation of fish populations are a promising means of genetic stock identification (GSI) of salmon (Ferguson et al. 1995). We recently showed 20 polymorphic nucleotide sites in the 5' half of the control region of the mitochondrial (mt) DNA (Sato et al. 2001). The observed single nucleotide polymorphisms (SNPs) have defined 30 haplotypes of three genealogical clades (A, B, and C) in more than 2,100 individuals from 48 populations in the Pacific Rim (Sato et al. 2004). The observed haplotypes were mostly associated with geographic regions. The haplotypes that belong to clade A and C characterized Asian populations, and clade B haplotypes distinguished North American populations. We developed a DNA microarray technique for rapid detection of 20 SNPs and accurate identification of 30 haplotypes in the mtDNA control region (Moriya et al. 2004, in press). In this study we estimate the stock composition of chum salmon in the Bering Sea using the DNA microarray method.

The DNA microarray analysis includes; 1) simultaneous PCR amplification and labeling of the 5' variable portion of the mtDNA control region with a pair of 5'-biotinylated and non-biotinylated primers, 2) two-hour hybridization of biotinylated PCR fragments with DNA microarray and subsequent short washing, and 3) visualization of hybridization signals colored by the conventional ABC (Avidin-Biotin Complex) method and computer-assisted comparison of scanner-taking signal images. The entire process of hybridization and detection was completed within eight hours.

Samples of chum salmon were collected from the Bering Sea during research cruises of RV *Kaiyo maru* during September 2002 and 2003. A total of 978 chum salmon was sampled in 2002 from 18 stations in the Bering Sea (172°30'E to 172°30'W, 51°30' to 58°30'N), whereas a total of 1,254 chum salmon was collected in 2003 from 23 stations in the Bering Sea and the North Pacific Ocean (175°30'E to 165°00'W, 50°00' to 58°00'N).

Stock contributions of the mixed samples were estimated by a conditional maximum likelihood method (Pella and Milner 1987). A conjugate-gradient searching algorithm using a square root transformation was employed (Pella et al. 1996). Standard deviations were estimated by 1,000 bootstrap resamplings of the baseline and mixture samples. Estimates were made to individual stocks and then pooled to regional stock groups of Japan, Russia, and North America. Computations were performed with the Statistics Programs for Analyzing Mixtures (SPAM version 3.7, ADF&G 2003). For SPAM, we used the baseline data set of 48 populations of chum salmon in the Pacific Rim reported by Sato et al. (2004).

Japanese and Russian stocks were widely distributed in the survey areas (Fig. 1). The Japanese stocks were predominant in the central Bering Sea, comprising over 50% of sampled chum salmon between 180°00 to 177°30'W, 54°00' to 58°30'N. Russian stocks were predominant in the western Bering Sea (from 172°30'E to 177°30'E) and in the southern Bering Sea (from 51°30' to 53°30'N), exceeding 40% of the composition. Japanese stocks were abundant in the central Bering Sea (from 175°00'E to 172°30'W) (Fig. 2). Japanese stocks exceeded over 60% of chum salmon sampled in the northern Bering Sea (from 180°00' to 177°30'W, from 54°00' to 58°00'N). Russian stocks were predominant in the eastern Bering Sea (from 175°00'E to 177°30'E) and, increased in proportion southerly, reaching over 70% (from 50°00' to 51°30'N). North American stocks increased in proportion eastward, reaching over 70% of sampled salmon in the area around the Aleutian Islands (from 170°00'W to 165°00'W, from 50°00'N to 53°30'N).

These results suggest non-random distribution of Asian and North American stocks of chum salmon, in that the Japanese stocks were abundant in the central and northern areas of the Bering Sea, the Russian stocks were in the western area of the Bering Sea, the North American stocks were in the area around the Aleutian Island.

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Fig. 1. Estimated stock composition of chum salmon mixtures collected in the Bering Sea in September 2002. The Japanese and Russian stocks were widely distributed in the survey areas. The Japanese stocks were predominant in the northern Bering Sea (from 54º00' to 58º30'N), the Russian stocks were in the southern Bering Sea (from 51º30' to 53º30'N). The Russian stocks were also predominant in the eastern Bering Sea.

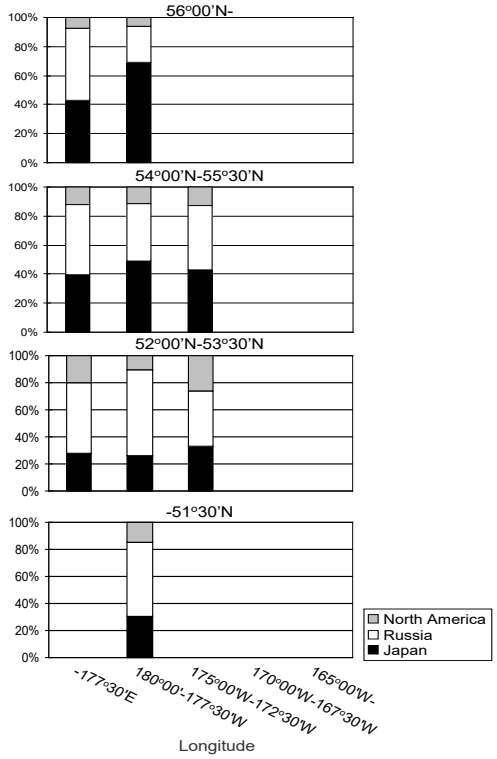
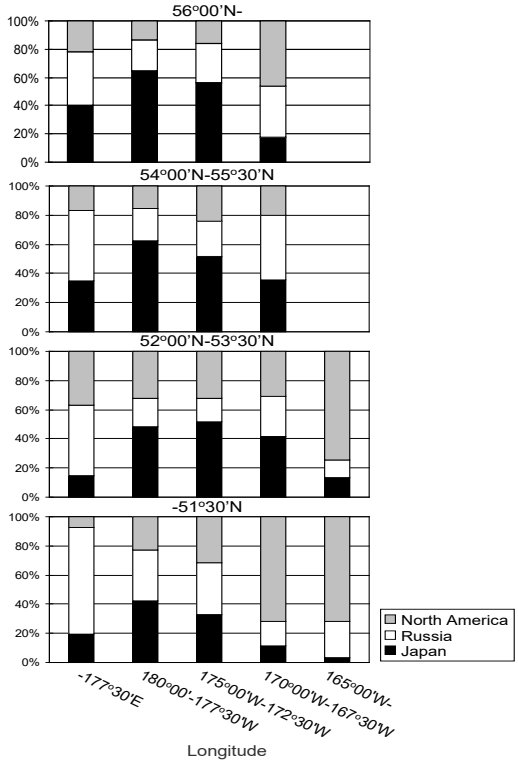


Fig. 2. Estimated stock composition of chum salmon mixtures collected in the Bering Sea in September 2003. The Japanese stocks were predominant in the central and northern Bering Sea. The Russian stocks were predominant in the eastern Bering Sea (from 175 º00'E to 177º30'E). The North American stocks were predominant in the area around the Aleutian Islands (from 170º00W, to 53º30'N).



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