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Genetic Analysis of Asian Chum Salmon Populations Based on Microsatellite DNA Variation

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We examined the genetic variability of Asian chum salmon (*Oncorhynchus keta*) populations using nuclear microsatellite (ms) DNA analysis with four polymorphic loci (OKM4, OKM5, OKM7, and OKM8) in 397 individuals from nine populations, including one in Korea, seven in Japan, and one in Russia. The msDNA gene diversity was highest in the Japanese populations, suggesting greater genetic variation in the populations in Japan than in populations in Korea and Russia. The pairwise F_{ST} estimates based on our msDNA data showed that the Korean population was genetically different from the Japanese and Russian populations, and there were higher F_{ST} estimates between Hokkaido and Honshu populations than between other population pairs. A neighbor-joining tree showed that the Korean population was distinct from two other clusters, representing the populations in Honshu and the populations in Hokkaido and Russia. These results suggest that the observed population genetic patterns of Asian chum salmon might be influenced by low or restricted gene flow.

Key words: Chum salmon, Genetic variation, Microsatellite DNA, *Oncorhynchus keta*

Introduction

Chum salmon, *Oncorhynchus keta*, have received considerable attention due to their high commercial importance and wide geographic distribution in the Pacific Rim (Quinn, 2005). Despite their significance, the ecology and life history of Asian chum salmon populations are poorly understood. In particular, little is known about their genetic variation, population structure, and migration patterns. Information on the genetic variation and population structure of chum salmon is therefore important for understanding population history, patterns of ocean migration, and the stock composition of mixed populations in the high seas. Recently developed mitochondrial (mt) and nuclear DNA markers are expected to provide a powerful means of estimating the genetic variation and population structure of salmon with increased accuracy and resolution (Park et al., 1993; Taylor et al., 1994; Sato et al., 2001, 2004; Beacham and Candy, 2005; Habicht et al., 2005). However, sufficient information has not yet accumulated to conclude which DNA markers improve discrimination among populations. A single locus of mtDNA might not explain historical events such as genetic drift in lower hierarchical levels of populations. Genetic drift involves random change in gene frequencies, and these changes will not occur in the same way for independent loci (Slatkin and Hudson, 1991). In addition, mtDNA data allow only the reconstruction of maternal lineages (Wilson et al., 1985; Avise et al., 1987). Males and females of one species may differ in the mechanisms of or behavior related to dispersal. Thus, the population structure estimated with maternally inherited mtDNA may differ from that assessed with biparentally inherited DNA markers, such as microsatellite (ms) DNA. msDNA markers, with tandem repeats of 2- to 4-base motifs, are a class of highly polymorphic nuclear DNA markers that are suitable for studies of intraspecific genetic diversity and population structure (Burford and Wayne, 1993). Regions flanking the msDNA can be used to design suitable primers, so that specific loci are amplified with PCR. Analysis of variation at msDNA loci therefore requires only a small amount of tissue, which can be conveniently stored in alcohol at room temperature. A variety of polymorphic msDNA loci

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have been isolated as genetic markers for population analysis of Pacific salmon (Scribner and Fields, 1996; Beacham and Candy, 2005; Habicht et al., 2005), with the aim of using them for genetic stock identification in mixed ocean salmon aggregations (Beacham et al., 2000; Beacham and Candy, 2005; Habicht et al., 2005). Here we aimed to clarify the genetic variation and genetic structure among and within Asian chum salmon populations using four polymorphic msDNA loci (Abe et al., 2002).

Materials and Methods

Sampling profile

Blood, liver, fin, or muscle samples were collected from 397 chum salmon for msDNA analysis. Salmon were collected from nine populations: one in Korea, seven in Japan, and one in Russia (Table 1). Liver samples were stored at -80°C, and fin and muscle samples were kept in ethanol at room temperature until DNA extraction.

Table 1. Sampling locations, date of collection, the number of chum salmon samples (N), and gene diversity used for msDNA analysis

Sampling location	Date of collection	N	Gene diversity
Korea			
1. Namdae River	13 Nov. 2000	46	0.65±0.39
Japan			
Hokkaido Island			
2. Tokushibetsu	23 Sept. 1997	51	0.74±0.43
3. Tokoro River	20 Nov. 1998	44	0.64±0.38
4. Nishibetsu River	25 Sept. 1997	41	0.71±0.42
Honshu Island			
5. Tsugaruishi River	10 Dec. 1997	44	0.70±0.41
6. Koizumi River	21 Nov. 1996	47	0.73±0.43
7. Gakko	10 Dec. 1996	45	0.73±0.43
8. Uono River	23-24 Oct. 1996	49	0.71±0.42
Russia			
9. Avakumovka	1994	30	0.56±0.35

Microsatellite DNA analyses

DNA was isolated with the conventional phenol-chloroform method (Sambrook et al., 1989) from the stored tissue samples. Four polymorphic msDNA loci (OKM4, OKM5, OKM7, and OKM8; Abe et al., 2002) were examined by PCR amplification. One primer of each primer set was labeled with one of the following fluorescent dyes: FAM, NED, NIC, or PET. PCR conditions included 1 min of pre-cycling denaturation at 95°C, followed by 30 cycles of denaturation at 95°C for 15 sec, annealing at 57°C (55°C for OKM5) for 15 sec, and extension at 72°C for 60 sec. PCR products were analyzed using an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems).

Population genetic data analysis

Departure from Hardy-Weinberg equilibrium (HWE) and genotype frequency at each locus were assessed in each population using Genepop version 3.4. Nei's genetic diversity (Nei, 1987) and pairwise population F_{ST} estimates were obtained using Arlequin version 2.000 (Schneider et al., 2000). Populations were grouped by the neighbor-joining method (Saitou and Nei, 1987) using pairwise nucleotide divergence. The topology obtained was tested for stability by a consensus analysis using 1,000 replicates of the original population divergence matrix obtained by bootstrap resampling of individuals from each population. A neighbor-joining tree was constructed for each replicate with NEIGHBOR, and the consensus tree was generated using CONSENSUS in PHYLIP version 3.5c, available at http://www.evolution.genetics.washington.edu/phylip.html.

Results and Discussion

Variation among the four msDNA loci

Considerable variation in allele frequency was observed at the four microsatellite loci examined. As shown in Table 2, 58 alleles were observed across the four loci, ranging from 13 alleles of OKM5 to 27 alleles of OKM8, over all populations. The observed heterozygosity for all populations ranged from 0.499 at OKM7 to 0.770 at OKM4. These results reflect the large difference in the number of alleles among these loci.

Genetic variation within and among populations

Two of the 36 tests for HWE showed significant deviation from the observed allele frequencies (Table 2). Significant heterozygote excess was only observed at OKM8 in Namdae River, Korea, and Nishibetsu River, Japan (Table 2). Although no historical data were available for genetic profiles of chum salmon in these two areas, this finding might be associated with non-random mating or a demographically unstable condition that has not yet reached genetic equilibrium. The genetic diversity of the Japanese populations was higher than that of the Korean and Russian populations (Table 1), suggesting that genetic variation is higher in the Japanese populations than the Korean and Russian populations. Lower genetic variation in the Korean and Russian populations may result from extensive hatchery operations in these areas. The potential genetic problems connected to hatchery operations have been dis-

Table 2. Total number of alleles (A_T), observed (H_O), and expected (H_E) heterozygosity by msDNA locus for 9 chum salmon populations. *Departure from Hardy-Weinberg equilibrium by Markov chain procedure with 1,000 permutation ($p<0.01$)

Population		Locus			
		OKM4	OKM5	OKM7	OKM8
Namdae	A_T	11	11	5	13
	H_O	0.954	0.767	0.186	0.930*
	H_E	0.846	0.709	0.215	0.832
Tokushibetsu	A_T	11	8	8	12
	H_O	0.903	0.807	0.548	0.839
	H_E	0.819	0.754	0.506	0.872
Tokoro	A_T	12	9	5	14
	H_O	0.651	0.605	0.372	0.791
	H_E	0.708	0.682	0.347	0.861
Nishibetsu	A_T	10	11	9	11
	H_O	0.586	0.741	0.535	0.672*
	H_E	0.671	0.798	0.498	0.865
Tsugaruishi	A_T	7	10	8	10
	H_O	0.859	0.766	0.500	0.750
	H_E	0.786	0.763	0.444	0.827
Koizumi	A_T	7	9	5	8
	H_O	0.917	0.750	0.625	0.667
	H_E	0.814	0.777	0.518	0.816
Gakko	A_T	10	12	7	12
	H_O	0.833	0.688	0.604	0.708
	H_E	0.832	0.638	0.611	0.870
Uono	A_T	11	11	5	13
	H_O	0.816	0.551	0.592	0.776
	H_E	0.856	0.678	0.488	0.839
Avakumovka	A_T	10	9	4	13
	H_O	0.406	0.375	0.531	0.719
	H_E	0.442	0.369	0.643	0.824
Overall	A_T	14	13	14	27
	H_O	0.770	0.672	0.499	0.761
	H_E	0.751	0.676	0.468	0.841

cussed previously (Allendorf and Phelps, 1980; Ryman and Laikre, 1991).

Genetic differentiation and population structure

As shown in Table 3, pairwise F_{ST} estimates tended to be low within groups of Honshu and Hokkaido populations but elevated between groups. F_{ST} was larger between Korea and Russia than between either Korea or Russia and the Japanese populations. The high F_{ST} values reflect increased genetic isolation among Korean, Russian, and Japanese populations compared to other pairwise estimates. As shown in Fig. 2, the neighbor-joining tree showed two groups of populations in Japan, suggesting low or restricted gene flow between Honshu and Hokkaido, which is consistent with a previous mtDNA analysis (Sato et al., 2001). Furthermore, the Korean population was distinctly separated from the Honshu and Hokkaido populations, as well as the Russian population, on the

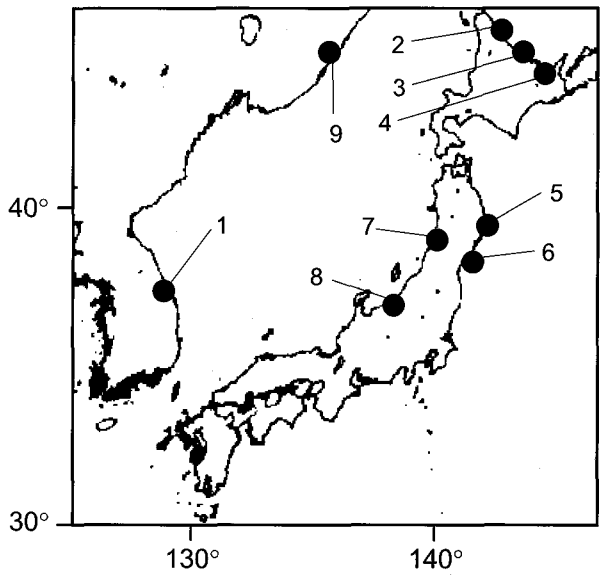

Fig. 1. Geographical locations of sampling sites (See table 1 for the site names).

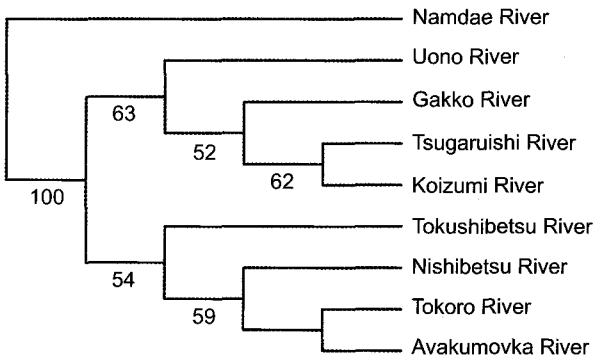

Fig. 2. Topology of the consensus tree shown with nodal values for bootstrap support over 50% of the 1,000 replicated trees.

neighbor-joining tree. Gene flow between the Korean and foreign populations might be restricted by differences in their spawning migration routes. In fact, a previous allozyme study suggested that separation by at least 600 km is required for genetic differentiation to occur (Kijima and Fujio, 1982).

In conclusion, these msDNA data suggest that the current genetic population structure of Asian chum salmon populations is grouped into Korea, Honshu, and Hokkaido (including the Avakumovka population in Russia). Moreover, the msDNA analysis provided increased resolution in the geographic differentiation between Korean and other Asian chum salmon populations. Our data suggest that msDNA can be used to estimate the genetic population structure of chum salmon in Asian populations, which will be useful for obtaining baseline data for a conservative

Table 3. Pairwise F_{ST} estimates among and between chum salmon populations based on the msDNA analysis data. Symbol "*" indicates significant support for F_{ST} values at $p<0.05$

	TOKU	TOKO	NISH	TSUG	KOIZ	GAKKO	UONO	NAMD	AVAK
Tokushibetsu	0.000								
Tokoro	0.096	0.000							
Nishibetsu	0.012	0.004	0.000						
Tsugaruishi	0.021*	0.025*	0.022*	0.000					
Koizumi	0.032*	0.043*	0.031*	0.009	0.000				
Gakko	0.022*	0.032*	0.036*	0.009	0.014	0.000			
Uono	0.024*	0.021*	0.028*	0.018*	0.026*	0.004	0.000		
Namdae	0.030*	0.032*	0.037*	0.033*	0.060*	0.043*	0.030*	0.000	
Avakumovka	0.082*	0.049*	0.061*	0.085*	0.122*	0.082*	0.079*	0.104*	0.000

approach to stocking programs, genetic conservation, and biodiversity conservation.

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