

Comparative Population Genetics of Chum and Masu Salmon Using Mitochondrial DNA Sequence Variation

Rizalita R. Edpalina¹, Shunpei Sato², Shigehiko Urawa²,
Vladimir Brykov³, Deuk-Hee Jin⁴, Akihisa Urano⁵, and Syuiti Abe¹

¹Division of Marine Biosciences, Graduate School of Fisheries Sciences, Hokkaido University,
Hakodate, Hokkaido 041-8611, Japan

²National Salmon Resources Center, Sapporo, Hokkaido 062-0922, Japan

³Institute of Marine Biology, Russian Academy of Science, Vladivostok, Russia

⁴Faculty of Marine Bioscience and Technology, Kangnung National University, Kangnung, Korea

⁵Division of Biological Sciences, Graduate School of Science, Hokkaido University,
Sapporo, Hokkaido 060-0810, Japan



Keywords: Chum salmon, masu salmon, mitochondrial DNA control region, nucleotide sequence variation, genetic population structure

Using nucleotide sequence variations found in the 5' half of the control region of the mitochondrial (mt) DNA, genetic diversity of chum (*Oncorhynchus keta*) and masu salmon (*O. masou*) was compared to get an insight into the specific difference, if any, in the genetic population structure of these two Pacific salmon species with different lifetime characteristics. Polymerase chain reaction (PCR)-based direct sequence analysis was successfully performed with the primers, tRNAt_{thr}-2 and tRNAp_{he}-2, originally designed for chum salmon (Sato et al. 2001) and other sequencing primers for each species. Occasional occurrence of larger PCR products than regular-sized amplified fragments of about 1.0 kb were found in each population of masu salmon, whereas no size polymorphisms of PCR products were observed in chum salmon which constantly showed the regular PCR fragments. The occurrence of such irregular-sized fragments in masu salmon ranged from 3.3% to 12.5% in each population examined. Sequence analysis with primers of tRNA_{pro}-3, OkdL-L1, Omdl-H1 (designed for masu salmon), and tRNAp_{he}-2 revealed that the irregular-sized PCR fragments were due to the occurrence of one to five repeats of an 80 bp unit in the 3' portion of the control region. Except for the observed sequence repeats, the structure of masu salmon control region and its flanking gene order were in keeping with the previous findings (Shedlock et al. 1992). Thus, sequence analysis excluded the 3' portion occasionally containing varying number of the 80 bp repeats in masu salmon.

As reported previously, chum salmon showed 20 variable nucleotide sites in the target mtDNA region, which defined a total of 30 haplotypes in more than 3,200 individuals representing 76 populations in the Pacific Rim (Yoon et al. 2004). On the other hand, the same mtDNA region disclosed 128 variable nucleotide sites, which defined a total of 51 haplotypes in 688 individuals from 11 Japanese, six Russian populations and one Korean population in masu salmon. Parsimony network analysis of the 30 haplotypes in chum salmon showed the presence of three clades (A, B and C) (Sato et al. 2001, 2004). However, phylogenetic relationship of the 51 haplotypes in masu salmon was not well-resolved using available network programs employed, probably because of an excess of non-informative indels. Instead, an unrooted NJ consensus tree suggested at least nine clades of masu salmon mtDNA haplotypes.

The observed haplotype diversity was larger in the populations of masu salmon (range 0.72–0.93) than chum salmon (range 0.24–0.61) (Yoon et al. 2004) (Table 1). The analysis of molecular variance (AMOVA), contingency χ^2 test, and pairwise population F_{ST} estimation showed a distinct genetic differentiation among Japanese, Russian

Table 1. Haplotype and nucleotide diversities of chum and masu salmon.

Population	Diversities	
	Haplotype	Nucleotide
Chum		
Japan	0.61+/-0.001	0.0021
Russia	0.36+/-0.001	0.0017
North America	0.24+/-0.001	0.0006
Masu		
Japan	0.93+/-0.003	0.1076
Russia	0.85+/-0.003	0.0110
Korea	0.72+/-0.003	0.0123

and North American populations in chum salmon and a weak but significant differentiation between local populations within each region (Sato et al. 2004; Yoon et al. 2004). Similar population genetic analyses in masu salmon also showed a genetic differentiation between Japanese and Russian populations. However, the population structure of masu salmon within Japan was notably different from the structure of chum salmon. In chum salmon, the AMOVA inferred a genetic differentiation among the populations from three regions, i.e. Hokkaido, Pacific Ocean coast in Honshu and Sea of Japan coast in Honshu, with a substantial statistical support (Sato et al. 2001). On the other hand, in masu salmon, no such genetic differentiation was observed in the sets of populations between the Hokkaido and the Honshu populations. Although there was a highly significant heterogeneity in the haplotype frequency ($p < 0.001$) revealed with the contingency χ^2 test for the entire set of populations in Japan, such significant heterogeneity was not observed for the sets of populations from Honshu and the Sea of Okhotsk coast in Hokkaido ($p > 0.05$). Likewise, AMOVAs revealed the population structure between the two geographic groups of the Sea of Japan coast and Okhotsk-Pacific Ocean coasts, but failed to show the structure between Hokkaido and Honshu, within Hokkaido, and within Honshu, as shown in Fig. 1. These results suggest a distinct genetic differentiation in masu salmon along the two coastal sides from north to south of Japanese Archipelago.

The high genetic divergence and the significant differences in haplotype frequencies in masu salmon river populations which is in contrast with that of chum salmon populations that exhibit a high degree of genetic similarity, suggest that each river of masu salmon has its own genetic traits probably due to a more precise homing ability than chum salmon. Further, this result supports Waples (1995) who claims that population structures of Pacific Salmon are strongly influenced by the ability of salmon to home to their native stream.

In conclusion, the present mtDNA sequence analysis revealed notable differences in both the structure of the control region at a molecular level and the genetic population structure between chum and masu salmon. It remains to be elucidated whether the observed difference in the population structure of these two species is related to their evolutionary history.

REFERENCES

- Sato, S., J. Ando, S. Urawa, U. Urano, and S. Abe. 2001. Genetic variation among Japanese populations of chum Salmon inferred from the nucleotide sequences of the mitochondrial DNA control region. *Zool. Sci.* 18: 99–106.
- Sato, S., H. Kojima, J. Ando, H. Ando, R.L. Wilmot, L.W. Seeb, V. Efremov, L. LeClair, W. Buchholz, D.-H. Jin, S. Urawa, M. Kaeriyama, A. Urano, and S. Abe. 2004. Genetic population structure of chum salmon in the Pacific Rim inferred from mitochondrial DNA sequence variation. *Environ. Biol. Fish.* 69: 37–50.
- Shedlock, A.M., J.D. Parker, D.A. Crispin, T.W. Pietsch, and G.C. Burmer. 1992. Evolution of the salmonid mitochondrial DNA control region. *Mol. Phy. Evol.* 1: 179–192.
- Waples, R.S. 1995. Evolutionary significant units and the conservation of biological diversity under the Endangered Species Act. *Am. Fish. Soc. Symp.* 17:8–27.
- Yoon, M.G., V. Brykov, N. Varnavskaya, L.W. Seeb, S. Urawa, and S. Abe. 2004. Mitochondrial DNA analysis of genetic variation in the Pacific Rim populations of chum salmon. (NPAFC Doc. 792) 25 p. Graduate School of Fisheries Sciences, Hokkaido University, 3-1-1 Minato, Hakodate 041-8611, Japan.

Fig. 1. The results of AMOVAs in chum and masu salmon. In chum salmon, the AMOVA inferred a distinct genetic differentiation among Japanese, Russian and North American populations and a weak but significant differentiation between local populations within each region (Sato et al. 2004, Yoon et al. 2004). Similarly, a genetic differentiation between Japanese and Russian populations of masu salmon was apparent. However, the population structure of masu salmon within Japan was notably different from the structure of chum salmon. In chum salmon, a genetic differentiation was found among the populations from Hokkaido, Pacific Ocean coast in Honshu and Sea of Japan coast in Honshu (Sato et al. 2001). On the other hand, in masu salmon, AMOVAs revealed the population structure between the two geographic groups of the Sea of Japan coast and Okhotsk-Pacific Ocean coasts, but failed to show the structure between Hokkaido and Honshu, within Hokkaido, and within Honshu.

