

Genetic Variation in Chum Salmon in the Sanriku Region, Japan, Inferred from Mitochondrial DNA Analysis

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Abstract: Genetic variation in about 500 chum salmon (*Oncorhynchus keta*) representing seven populations in the Sanriku region and one population in Fukushima, on the Pacific coast of northern Honshu, Japan, was estimated by mitochondrial DNA analysis. A total of nine haplotypes of 479–481 bp in the 5' first half of the control region were found in the chum populations examined. The observed haplotype diversity ranged from 0.424 ± 0.084 to 0.805 ± 0.056 , which was lower than previously reported diversity in Japan's Hokkaido populations, but higher than Russian and North American populations. AMOVA suggested moderate genetic differentiation among local populations within Sanriku and Fukushima, but no large-scale regional differences were detected.

Keywords: chum salmon, genetic diversity, mitochondrial DNA, Sanriku region

INTRODUCTION

Chum salmon (*Oncorhynchus keta*) are widely distributed from the Far East to North America around the Pacific Rim, and are an important fisheries resource around the range. They are semelparous and anadromous with homing migrations to spawn in natal rivers or tributaries within major river systems, leading regional populations to partial genetic isolation as seen in other Pacific salmon species such as pink salmon (Beacham et al. 2012) and Chinook salmon (Templin et al. 2011).

Understanding the genetic characteristics of chum salmon is important in planning conservation measures and managing sustainable fisheries. Although genetic variation among chum salmon populations has been demonstrated with a variety of genetic markers (e.g., Winans et al. 1994; Sato et al. 2001, 2004; Yoon et al. 2008; Beacham et al. 2008, 2009; Moriya et al. 2009; Yokotani et al. 2009; Sato et al. 2014), genetic features of chum salmon in the marginal regions of the most easterly limit of their distribution remain to be elucidated.

Here, we conducted a population genetic analysis of chum salmon using nucleotide sequence variation of the mitochondrial DNA (mtDNA) control region (CR) in about 500 specimens representing eight locations in the Sanriku region and Fukushima, on the Pacific coast of northern Hon-

shu, Japan, that are the southernmost limits of the natural range of chum salmon.

MATERIALS AND METHODS

A total of 486 specimens of chum salmon was collected from seven locations in the Sanriku region, i.e., four coastal hatcheries including the Mabuchi River (MB), Akka River (AK), Tsugaruishi River (TG), and Kesen River (KS), three hatcheries in the tributaries of the inland Kitakami River including the Yana River (YN), Satetsu River (ST), and Hienuki River (HE), and one location in Fukushima, the Uda River (UD) (Fig. 1, Table 1). The samples used were homing adult fish, with the exception that fry were used from HE and ST in the Kitakami River tributaries (Table 1). Early and late runs were tentatively designated as those that homed to natal rivers from September to October and those that homed from November to December, respectively, both of which were sampled from AK, TG and KS. A fin clip obtained from each fish was fixed in 99.5% ethanol and stored at room temperature until use.

Total DNA was extracted from fin clip samples using the DNeasy Tissue kit (QIAGEN, Hilden, DE, USA). The 5' first half of the mtDNA CR was amplified by the polymerase chain reaction (PCR) using the previously designed primers,

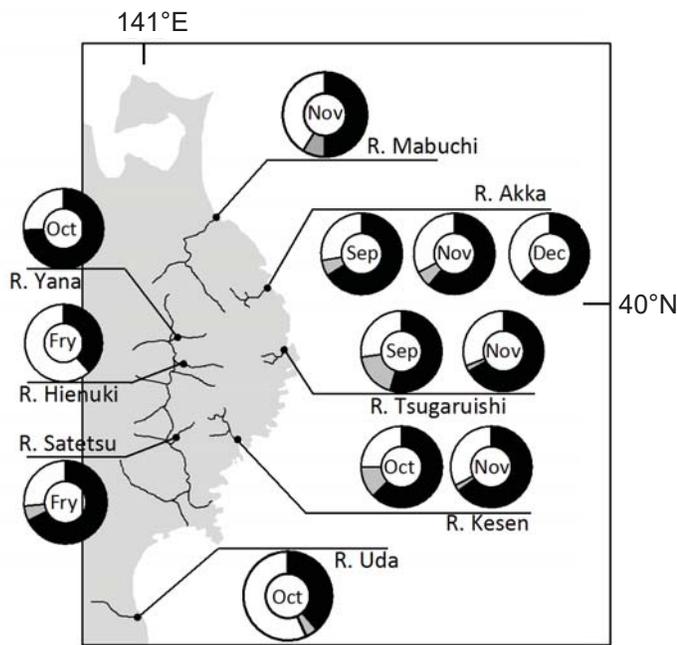


Fig. 1. Map showing locations for genetic sampling of chum salmon from eight locations along the Pacific coast of northern Honshu, Japan (●). Pie graphs show approximate frequency of the haplotypes found (■, A groups; □, B groups; ▨, C groups). The grouping of haplotypes follows Sato et al. (2004).

tRN^Athr-2 and tRN^Aphe-2, and established PCR conditions reported in Sato et al. (2001). Purified PCR products were subjected to sequencing reactions using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) and the previously designed primers tRN^Athr-3 and Okdl-L1 (Sato et al. 2001), and analyzed using an Applied Biosystems 3500xl Genetic Analyzer.

Sequences were aligned using Clustal X (Thompson et al. 1997) with default settings, and manually checked by eye. Haplotype diversity (*h*) and nucleotide diversity (π) were calculated using the program Arlequin version 3.5 (Excoffier et al. 2005). Genetic differentiation within and among populations were statistically tested through hierarchical analyses of molecular variance (AMOVA) in Arlequin, based on both molecular distances and haplotype frequencies. We tested groups by geographic location i.e., Sanriku vs. Fukushima, and coastal vs. inland populations in the Sanriku region.

RESULTS AND DISCUSSION

We sequenced 479–481 bp fragments of the mtDNA CR for the 486 specimens collected from the eight locations (Table 1), which defined a total of nine haplotypes from A, B and C clades reported in Sato et al. (2004). Over all samples,

Table 1. Sampling location, date of collection (year-month-day), number of chum salmon analyzed (*N*), frequency of haplotypes, haplotype diversity (*h* ± SD), and nucleotide diversity (π ± SD). Based on the nucleotide sequence variation of the 5' half of the mtDNA control region (479–480 bp).

Region/ Area	Sampling locality	Date	<i>N</i>	The frequency of mtDNA haplotype indicated										<i>h</i>	π	
				A1	A2	A3	A6	A8	B3	C1	C3	C5				
Sanriku	Coastal collections															
	R. Mabuchi (MB)	14/11/19	46	23						4	17	1	1	0.618 ± 0.044	0.0029 ± 0.0020	
	R. Akka (AK)	12/9/14	47	29			1		1	3	13			0.550 ± 0.062	0.0025 ± 0.0018	
		12/11/5	46	25	1		2		3	15				0.605 ± 0.052	0.0027 ± 0.0019	
		13/1/11	35	20			2			13				0.548 ± 0.051	0.0030 ± 0.0021	
	R. Tsugaruishi (TG)	12/9/3–13	22	8			1	1	2	4	5		1	0.805 ± 0.056	0.0041 ± 0.0028	
		12/11/28	42	28						1	13			0.470 ± 0.058	0.0021 ± 0.0016	
	R. Kesen (KS)	12/10/15	48	29				1		6	11		1	0.578 ± 0.063	0.0029 ± 0.0020	
		12/12/20	40	26						1	12		1	0.499 ± 0.063	0.0022 ± 0.0017	
	Inland collections in the Kitakami river system															
R. Yana (YN)		12/10/17	43	32							7	1	3	0.424 ± 0.084	0.0020 ± 0.0015	
R. Satetsu (ST)		13/2/5 (fry)	34	23					2	9				0.483 ± 0.076	0.0022 ± 0.0017	
R. Hienuki (HE)		13/2/5 (fry)	39	15							16		8	0.659 ± 0.031	0.0027 ± 0.0019	
Fukushima		R. Uda (UD)	14/10/29	44	16				1	2	23		2	0.604 ± 0.048	0.0027 ± 0.0019	

Table 2. Results of the AMOVA for chum salmon. Genetic differentiation was examined for a total of 486 individuals from 12 collections in this study. *Statistical significance at $P < 0.001$.

Source of variation	df	Sum of squares	Variance components	Percentage variations	Fixation index
Overall populations					
Among populations	11	145.6	0.186	3.2	$F_{st} = 0.0316^*$
Within populations	474	2704.1	5.705	96.8	
Between Sanriku region and Fukushima					
Among groups	1	5.0	-0.123	-2.1	$F_{ct} = -0.0212$
Among populations within groups	10	140.6	0.209	3.6	$F_{sc} = 0.0353^*$
Within populations	474	2704.1	5.705	98.5	$F_{st} = 0.0148^*$
Between Coastal and inland population in Sanriku region					
Among groups	1	18.6	0.030	0.5	$F_{ct} = 0.0053$
Among populations within groups	9	122.0	0.206	3.7	$F_{sc} = 0.0372^*$
Within populations	431	2293.1	5.320	95.8	$F_{st} = 0.0423^*$

the frequencies of A1 (56.4 %), and C1 (31.7%) haplotypes were higher than those of the other haplotypes (0.2–5.3%). The distribution of haplotypes was similar to that observed in Honshu populations in a previous study (Sato et al. 2004). Although the haplotypes A6 and C5 were found at low frequencies in the previous study (Sato et al. 2004), these haplotypes occurred at substantially higher frequencies in the present investigation, suggesting they are haplotypes that characterize chum salmon populations in the Sanriku region and Fukushima. The observed haplotype diversity (h) and nucleotide diversity (π) ranged from 0.424 ± 0.084 to 0.805 ± 0.056 and from 0.0020 ± 0.0015 to 0.0041 ± 0.0028 , respectively. The h of most Hokkaido chum salmon populations was > 0.6 , with a range from 0.57 ± 0.06 to 0.75 ± 0.04 (Sato et al. 2004). In addition, the h of Russian and North American chum populations was from 0.04 to 0.79 and from 0.00 to 0.053, respectively (Yoon et al. 2008). Thus, the haplotype diversity was slightly lower in the southernmost chum salmon populations than in Hokkaido populations, but generally higher than Russian and always higher than North American populations.

The early-run Tsugaruishi collection tended to have high genetic diversity in the Sanriku coastal region. The h of early-run Tsugaruishi collection was 0.805 ± 0.056 , whereas the h of the other Sanriku coastal collections ranged from 0.470 ± 0.058 to 0.618 ± 0.044 . Also, a high h in the early-run Tsugaruishi collection in the 1990s was reported in Sato et al. (2004).

The hierarchical AMOVA indicated molecular variations mostly within populations (96.8%) and a small portion among populations (3.2%, $F_{st} = 0.0316$ with $P < 0.001$), but no such variations among regional groups of populations (Table 2). In addition, the result of AMOVA showed no differentiation among groups when tested between the Sanriku region and Fukushima, and between coastal and inland populations in the Sanriku region. These results suggest a moderate genetic differentiation among local populations, but no such differentiation between geographical groups of populations, within

Sanriku and Fukushima. However, nonrandom haplotype distribution in these regions, e.g., A6 mostly in Iwate (the Akka, Tsugaruishi and Kesen rivers) and C5 mostly in the southern coast (the Kesen and Uda rivers) and the Kitakami River tributaries (Table 1), may encourage us to further investigate a possible geographical differentiation within or between Sanriku and Fukushima, using competent biparental nuclear DNA markers such as polymorphic microsatellite DNA (msDNA) loci (Tsukagoshi et al. 2015). Genetic variation based on mtDNA and msDNA markers will provide important information (e.g., effect(s) of using hatchery populations on genetic diversity) for sustainable adaptive management of Sanriku chum salmon at the southernmost limit of its natural range. This, in turn, may also provide a clue to understanding the mechanism underlying thermal resistance to enhance survival under climate change conditions.

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