Genetic Variation Between Collections of Hatchery and Wild Masu Salmon Inferred From Mitochondrial and Microsatellite DNA Analyses

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There has been very little effort to understand genetic divergence between wild and hatchery masu salmon (*Oncorhynchus masou*) in Japan. Polymorphisms in the mitochondrial DNA (mtDNA) NADH dehydrogenase subunit 5 gene and six nuclear microsatellite loci (msDNA) were used to assess the genetic variation within and among wild and hatchery collections of masu salmon in Hokkaido. Both DNA markers suggested genetic differentiation between putative wild populations and hatchery brood stocks, primarily owing to decreased genetic diversity in the latter. We compared genetic variability in three hatchery brood stocks of masu salmon with the variability in eight putative wild masu populations sampled in five rivers including one known source river for the hatchery brood stocks in Hokkaido.

A total of 762 masu salmon was examined including 141 from the former Mori Research Branch of the Hokkaido Fish Hatchery and 621 categorized into eight separate collections representing five rivers in Hokkaido from 1997 to 2007 (Yu et al. 2010a, b). The rivers sampled included the Shiribetsu (SHI) and Teshio (TES) Rivers on the Sea of Japan coast, and the Tokushibetsu (TOK), Shari (SHA), and Ichani (ICH) Rivers on the Sea of Okhotsk coast (Fig. 1). The Shiribetsu River was the source river of the hatchery fish, and four annual collections in this river were obtained and analyzed to detect any change in genetic diversity over time.

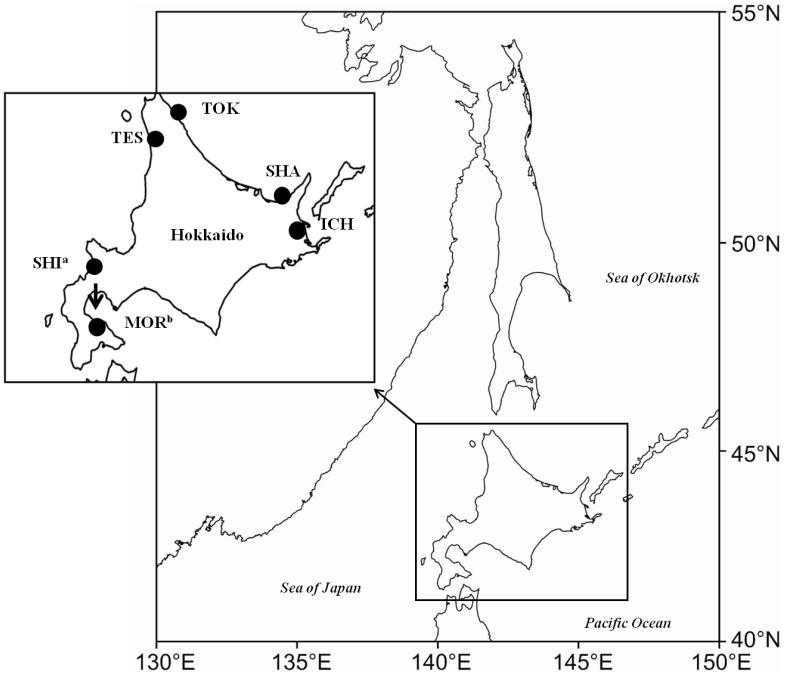

Fig. 1. Map showing the sampling sites for masu salmon in Hokkaido. The rivers sampled included the Shiribetsu (SHI[a]: four annual collections, 1997-2000), Teshio (TES), Tokushibetsu (TOK), Shari (SHA), and Ichani (ICH). Samples were also obtained from the Mori Hatchery (MOR[b]: three hatchery collections (Line 1, Line 2, and Line 3-RR).

Three different Mori Hatchery masu salmon collections were analyzed separately: (1) a captively bred (4-generation) line from the Shiribetsu River artificially selected for large size eggs since 1988 (hereafter referred to as Line 1), (2) a captively bred (5-generation) line from the Shiribetsu River without selection since 1987 (hereafter referred to as Line 2), and (3) 2nd generation fish derived from the wild fish of Line 3 (under selection for large body size and released at the 2nd generation after introduction) that originally homed to another adjacent river in 1989 (hereafter referred to as Line 3-RR).

Total genomic DNA was extracted from liver or fin samples from adult hatchery and wild fish using the Gentra Puregene Tissue Kit (QIAGEN). Nucleotide sequence variation in a PCR-amplified 561 bp fragment from the 5' end of mtDNA

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ND5 and allelic variation at six polymorphic msDNA loci were examined as previously described by Yu et al. (2010a). For genetic variability within and among collections, haplotype diversity (h) and nucleotide diversity (π) were calculated for *ND5*. Allelic richness (*Ar*), number of alleles per locus (*Na*), observed (*Ho*) and expected heterozygosity (*He*) per locus and sample collection, and mean heterozygosity within collections were computed, together with the departure from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium, for msDNA in ARLEQUIN version 3.1 (Excoffier et al. 2005). The population structure was estimated with pairwise F_{ST} and the hierarchical nesting of the genetic diversity among eight wild and three hatchery collections were analyzed using the analysis of molecular variance (AMOVA) in the ARLEQUIN program. The cluster of the collections was examined using the neighbor-joining (NJ) method (Saitou and Nei 1987) with NEIGHBOR in the PHYLIP program version 3.67 (Felsenstein 2004) based on the genetic distance of the Kimura-2-parameter method (Kimura 1980) for mtDNA and the Cavalli-Sforza and Edwards (1967) chord distances for msDNA. The bootstrapped consensus tree was generated with 1,000 replications using CONSENSUS in the PHYLIP program.

Average haplotype and nucleotide diversity were higher in the wild collections (h, 0.5577; π, 0.0016) than in the hatchery broodstocks (h, 0.457; π, 0.0011). The *He* and *Ho* for all collections ranged from 0.728 (Line 3-RR) to 0.817 (ICH) and 0.685 (Line 3-RR) to 0.817 (ICH), respectively, and no linkage disequilibrium was observed among all loci examined. Mean *He* was significantly greater in the wild (0.786) than in the hatchery (0.751) collections ($p = 0.02$), but there was no difference in the level of *Ho* ($p = 0.07$). Also, mean *Ar* was greater in the wild (11.27) than in the hatchery (9.47) collections ($p = 0.04$), suggesting a decreased genetic diversity in hatchery samples. Statistically significantly deviation from HWE was observed in some wild and hatchery collections at some microsatellite loci.

Table 1. F_{ST} estimates of putative wild populations and hatchery brood stocks of masu salmon (mtDNA, above diagonal; msDNA, below diagonal). Statistical significance at $p < 0.005$ (*) and $p < 0.001$ (**) after sequential Bonferroni adjustments. No significant differences observed in the annual (1997-2000) collections of the SHI source population.

	TOK	SHA	ICH	TES	SHI97-00	Line 1	Line 2	Line 3-RR
TOK		-0.007	0.079**	0.221**	0.161**-0.224**	0.226**	0.173**	0.213**
SHA	0.023**		0.072**	0.236**	0.167**-0.235**	0.241**	0.192**	0.232**
ICH	0.016**	0.026**		0.093**	0.033-0.073**	0.080**	0.067**	0.097**
TES	0.044**	0.074**	0.052**		0.000-0.021	0.080**	0.015	0.003
SHI97-00	0.027**-0.035**	0.052**-0.064**	0.029**-0.041**	0.006**-0.015**		0.038*-0.055*	0.000-0.027*	0.000-0.034*
Line 1	0.043**	0.062**	0.054**	0.045**	0.031**-0.041**		0.085**	0.132**
Line 2	0.026**	0.051**	0.039**	0.032**	0.019**-0.025**	0.042**		0.024*
Line 3-RR	0.029**	0.049**	0.038**	0.058**	0.037**-0.048**	0.030**	0.044**	

Pairwise population F_{ST} estimates using both *ND5* and microsatellites suggest significant heterogeneity in the allele frequency distribution ($p < 0.001$) in almost all pairwise comparisons among collections, confirming their genetic divergence (Table 1). In the four annual SHI source river collections no significant differences were observed in both DNA markers, suggesting a common gene pool. The significant F_{ST} estimates with both DNA markers suggested genetic differentiation between the hatchery and the wild collections including those from the source population (Table 1). In addition, as shown in Table 2, AMOVAs revealed a small but significant genetic differentiation between all eight wild and three hatchery collections (*ND5*, 12.3%, $p < 0.001$; microsatellites, 2.31%, $p < 0.001$). Differentiation between all the SHI collections and all the hatchery collections was apparent with microsatellites but not with *ND5* (*ND5*, -0.70%, $p > 0.1$; microsatellites, 1.53%, $p < 0.05$). The three hatchery collections were distinctly separated from the wild collections (including the source river collections) on the NJ tree using both *ND5* and microsatellites (Fig. 2). The

Table 2. Analysis of molecular variance (AMOVA) based on mtDNA and msDNA data from putative wild populations and hatchery brood stocks of masu salmon. Analysis I: among stocks of the Sea of Okhotsk, Sea of Japan and Mori Hatchery. Analysis II: between stocks of the Shiribetsu River and Mori Hatchery.

Hierarchical structure	mtDNA			msDNA		
	%	Φ	*P*	%	Φ	*P*
Analysis I	12.3	0.120	0.000	2.31	0.023	0.000
Analysis II	-0.70	-0.006	0.828	1.53	0.016	0.027

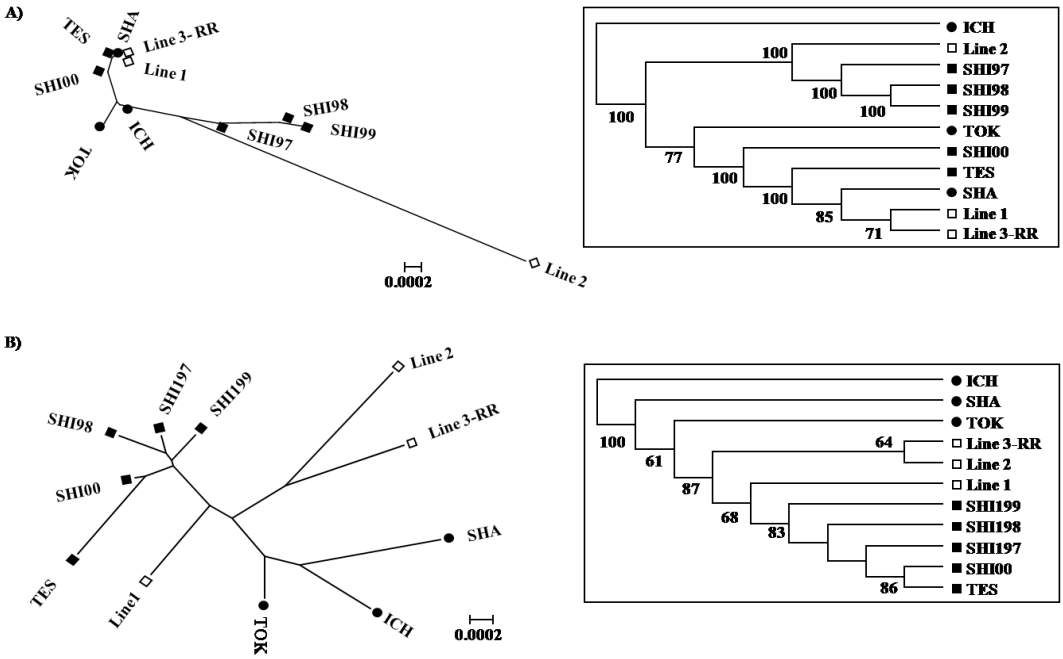

Fig. 2. Unrooted neighbor-joining tree showing genetic distance among collections based on mtDNA (A) and msDNA (B). Inset shows the topology of the consensus tree with nodal values for bootstrap support of over 50% of the 1000 replicated trees. Filled circle: samples from coastal rivers of the Sea of Okhotsk (Tokushibetsu, TOK; Shari, SHA; Ichani, ICH). Filled square: samples from coastal rivers of the Sea of Japan (Shiribetsu, SHI 1997-2000; Teshio, TES). Open square: Mori Hatchery samples (Line 1, Line 2, and Line 3-RR).

msDNA tree also suggested differentiation between masu salmon collections from the Sea of Okhotsk (TOK, SHA and ICH) and the Sea of Japan (SHI and TES), but the *ND5* tree failed to show a clear differentiation (Fig. 2).

The present results suggested that genetic diversity estimated with both DNA markers was low in three captive brood stocks relative to putative wild masu salmon populations. Such decrease in diversity occurred rapidly after introduction of wild masu salmon to the hatchery, i.e., within five generations, and caused genetic differentiation between the captive brood stocks and putative wild populations including the source population. These findings also suggest that careful hatchery operations are necessary for maintenance of genetic diversity in captive brood stocks and for safe-guarding against release of these fish that can potentially lower the fitness of wild masu salmon.

REFERENCES

Cavalli-Sforza, L.L., and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Am. J. Human Genet. 19: 233–257.

Excoffier. L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinform. Online 1: 47-50.

Felsenstein, J. 2004. PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111-120.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Ecol. 4: 406-425.

Yu J-N., N. Azuma, V. Brykov, S. Urawa, M. Nagata, D-H. Jin, and S. Abe. 2010a. Genetic population structure and phylogeography of masu salmon *(Oncorhynchus masou masou*) inferred from mitochondrial and microsatellite DNA analyses. Zool. Sci. 27: 375-385.

Yu J-N., N. Azuma, V. Brykov, S. Urawa, K. Ohkuma, and S. Abe. 2010b. Genetic relationships between anadromous and non-anadromous masu salmon (*Oncorhynchus masou*) inferred from mitochondrial and microsatellite DNA variation. Fish Genet. Breed. Sci. 39: 75-85.