Genetic Influence of Radioactive Materials in Yamame (Onchorhynchus masou) from Fukushima Prefecture

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論文内容要旨

Genetic Influence of Radioactive Materials in Yamame (*Onchorhynchus masou*) from Fukushima Prefecture

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ABSTRACT

Fukushima Nuclear Power Plant accident in March 2011 has caused massive distribution of radiocesium through air, water and land. This raised the concern of radiocesium contamination in organism and massive evacuation from that area. Wild *yamame* is a form of masu salmon (*Onchorhynchus masou*) that non-anadromous, it means instead of moving to sea for grow and return to natal river for spawning, *yamame* remain in their natal in the entire life. Wild *yamame* in that area are living without influence of anthropogenic activities and exposed to radiation contamination in their entire life. Little is known about the wild population structure of yamame in that particular area after evacuation. Plus the radiocesium contamination effect on genetic, progenies and blood characteristics of contaminated fish is still undiagnosed. This study aim to resolve three main questions namely,

1) What is the current population genetic structure of *yamame* in contaminated area?

2) Is radiocesium contamination effect the performance of gynogenesis production in yamame?

3) Is there any mutation in next generation caused by radiocesium contamination?

Microsatellite markers, being codominant and proven to cross amplify in close species were used along with mitochondrial marker to elucidate the population genetic structure of yamame population from four rivers; Hirose River from Miyagi Prefecture), Mano River, Ukedo River and Abukuma River from Fukushima Prefecture. 28 pairs of microsatellite primers developed for various salmonid species were screened. 11 pairs of microsatellite primers were used in analysis, and 2 mtDNA markers (Cyt b and D-Loop) were added to support the result. The number of allele per locus ranged from 20 to 1. The expected heterozygosity ranged from 0.95 in Oke308 and lowest is 0 in Oke307. These markers, except Oke307 proven to be useful for population genetic

structure study in this species. Microsatellite markers and mtDNA markers showed concordant results in exhibiting genetic diversities and population structure of four river populations. Mtnda showed 13 haplotypes in Cyt b and 9 haplotypes in D-Loop. In Cyt b, haplotypes diversity ranged from 0.75 to 0.59 with highest nucleotide number in Hirose (6) and lowest in Abukuma (3), while in D-Loop from haplotypes diversity ranged from 0.74 to 0.63 with highest in Hirose (5) and lowest in Mano (3). In Cyt b, nucleotide diversity calculated, π ranged from 0.003 to 0.002 while in D-Loop ranged from 0.001 to 0.003. Population differentiation showed clear separation of Hirose river population from other rivers. Mano River, Ukedo River and Abukuma River showed no clear separation from each other. This might be due to sampling approach that concluded samples from many river branches as one population. Masu salmon has showed clear population structuring within rivers in previous study. (Table 1, Table 2, Table 3 and Fig. 1)

Mitotic gynogenesis is a process in producing homozygous diploid progenies with inherited genetic material from maternal. Gynogenesis is proven useful in reducing number of generation to produce clone. Lethal defect due to radiation will not pass down to next generation because the individual carrying this genotype will not survive in early development. Only non-lethal defect will be carried in mitotic gynogen and can be examined. In this study, mitotic gynogenesis performance of wild yamame exposed to radiation was compared with meiotic gynogenesis performance of captive stock from Fukushima Inland Fisheries. Putative gynogen were verified using four microsatellite markers. The offsprings genetic level were verified using four microsatellite markers. Survival rate (Hatching rate), and swim-up rate were measured in both population. Mean value of hatching rate, swim-up rate and normal rate ranging from 6% - 53 % , 2%-8% and 3%-9% respectively. Analysis of variance (ANOVA) in mitotic gynogen (Table 4.2) showed significant difference ($p<0.05$) between IC and other groups, whereas no significant difference between other groups. In meiotic gynogen, analysis of variance showed no significant difference between control and treatment. In meiotic gynogen, analysis of variance showed no significant difference between control and treatment. Microsatellite verification showed all mitotic gynogens receive alleles from maternal, while in control, some showed mutant microsatellite possibly derived from wild paternal parent. (Table 4)

Mitochondrial DNA of mitotic gynogen masu salmon were examine to identify possible mutation by utilising Cyt b and D-Loop region. The mitotic gynogens were derived from wild parent (Mano River) which live in contaminated area in Fukushima prefecture and captive parent (double haploid) which bred in Fukushima Inland Fisheries Station. By utilising two loci (Cyt b and D-Loop) in mtDNA, we analyse the DNA sequence of every gynogen to observe any mutation occur and estimate the mutation rate for that group. 24 mitotic gynogen eggs and 50 control larvae from each two maternal wild parent (m5,m6) and 50 meiotic gynogen larvae each derived from two maternal captive parent (PF9,PF23) were used in this study. Forward and reverse sequence obtained was ecorded in 3500XL Genetix and aligned using MEGA v6. In this study, polymorphic sites are treated as mutation site. Mutation rate estimation were calculate following (Haag-Liautard et al., 2008). Direct sequencing of Cyt b region from these four families (M5,M6, PF9,PF23) disclose 3 point mutations in M5 and M6 families and no mutation observed in PF9 and PF23. All mutation in M5 family is from mitotic gynogen eggs, whereas in M6, both larvae and eggs show point mutation. In D-Loop, sequences 671 nucleotides from 46 samples (M5 family) and 34 samples (M6) family has been used. Mutation has been observed in mitotic gynogen eggs of M5 at base 299 and base 624 whilst in M6, no mutation observed. All samples from PF9 and PF23 showed neither variation nor mutation. The point mutation caused amino acid substitution (ie Ala > Thr) or just synonymous mutation. The overall mutation rate estimation is high in Cyt b compared to D-Loop and many mutations occurred in M5 family than in M6. Point mutation or single-nucleotide mutations showed synonymous in base 324 of Cyt b while the other non-synonymous. However, since no observation of morphology and phenotype in larvae was done, no strong evidence to expect that radiocesium contamination in eggs caused morphological changes in yamame offspring. (Table 5)

Blood exhibits signs of alteration in its properties if any stress occurred. Contamination of heavy metal in water system caused reducing erythrocytes performance and viability. The need to understanding effect of radiocesium contamination in blood characteristics is important because it considered as suitable indicator of health condition in vertebra. The haematological analysis of

yamame from control showed significantly higher numbers of haemoglobin, haematocrit and MCV in comparison to yamame treated with 50 000bq/kg ($p<0.05$). However the values of erythrocytes, MCH and MCHC were not significant between two groups. Decrease in hemoglobin may result from high concentrations of radiocesium. Although RBC content showed no significant changes, the values of HCT and MCV were low. RBC is function to transport hemoglobin which transports oxygen. Although RBC is not affected, significant lower number of hemoglobin reduce the effectiveness oxygen transport for oxidative metabolism. (Table 6)

Table 1 : Average allelic richness (Ar), Observed Heterozygosity (Ho) and Expected Heterozygosity (He) per population.

Locus	Ar	Ho	He
Hirose	9.8	0.63	0.68
Mano	10.8	0.7	0.76
Ukedo	10.9	0.65	0.78
Abukuma	9.8	0.52	0.68

Table 2: Pairwise Fst estimation among four populations

	Hirose	Mano	Ukedo	Abukuma
Hirose	-			
Mano	0.04	-		
Ukedo	0.06	0.02	-	
Abukuma	0.05	0.02	0.04	-

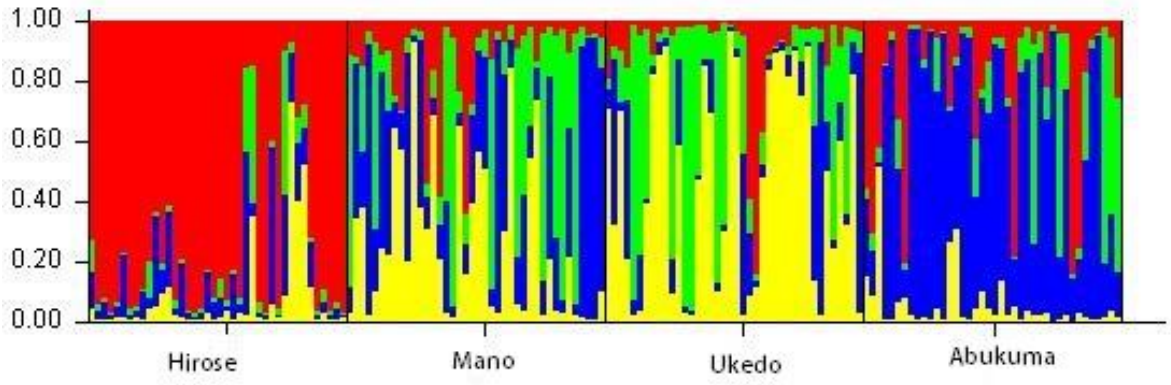

Figure1 Population genetic structure of 160 individuals based on 11 microsatellite markers using STRUCTURE program.

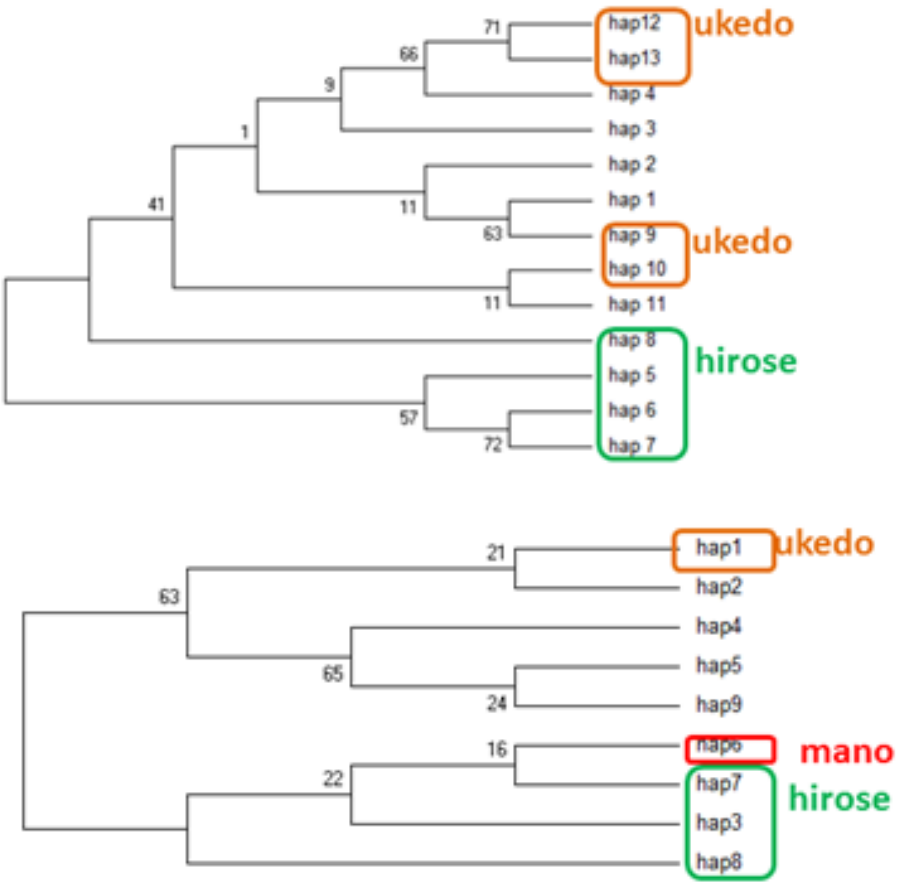

Figure 2 Phylogenetic tree of *O.masou* haplotypes from four rivers using Maximum Likelihood method. Upper part (Cyt b), lower part (D-Loop).

Table 3. Mean value for hatching rate,swim-up rate and normal rate for mitotic gynogen.

	IC	GC	56	58	60	62	64
Hatching rate	0.75*	0.06	0.35	0.53	0.20	0.18	0.13
Normal rate	0.73*	0.03	0.09	0.06	0.05	0.05	0.03
Swim-up rate	0.71*	0.02	0.08	0.05	0.06	0.04	0.03

Table 4 : Genotypic segregation of 4 microsatellite loci in putative mitotic gynogen of masu salmon in locus Oke308.

Locus	**Family**	**Control genotypes**	**56**	**58**	**60**	**62**	**64**
Oke308	M5	**274/274** (4)	-	**246/246**(1)	-	**246/246** (4)	**246/246**(4)
	246/246	**274**/**246** (2)					
		246/**246** 3)					
		***250/234 (1)**					
		***246/234 (1)**					
	M6	**290**/234 (4)	**290/290** (3)	-	**290/290** (2)	**290/290** (3)	**290/290** (0)
	290/234	**290**/278 (7)	**234/234** (2)	-	**234/234** (4)	**234/234** (2)	**234/234** (3)
		234/234 (5)					
		234/278 (1)					
		***298/290 (2)**					
		***298/234(1)**					

mutant alleles are labeled with asterisk (*).

Table 5 : Mutation position, effect, frequency and mutation rate in Cyt b and D-Loop of *O. masou*.

Gene	Family	Position	Mutation	Effect	Frequency	Mutation rate
Cyt b	M5	324	T > A	Leu > Leu	0.08	8.04×10^{-6}
Cyt b	M5	324	T > G	Leu > Leu	0.08	8.04×10^{-6}
Cyt b	M6	324	T > G	Leu > Leu	0.06	4.9×10^{-6}
Cyt b	M5	697	G > A	Ala > Thr	0.08	8.04×10^{-6}
Cyt b	M6	697	A > G	Thr > Ala	0.06	4.9×10^{-6}
Cyt b	M5	795	G > A	Pro > Pro	0.15	3.1×10^{-5}
Cyt b	M6	795	A > G	Pro > Pro	0.06	4.9×10^{-6}
D-Loop	M5	299	G > A	Arg >Glu	0.11	1.76×10^{-5}
D-Loop	M5	623	T > C	Ile > Thr	0.11	1.76×10^{-5}

Table 6 Effect of 50 000bq/kg radiocesium intake on hematological variables in yamame O. masou on 0 day and 40 days pooled with 130 days. The mean concentrations denoted with different letters within same exposure period are statistically different ($p < 0.05$)

		RBC counts (/mm3)	HGB (g/dL)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
0 days	control	1186000	10.34	19.2	163.2	93.0	56.9
40days +	control	1061428	7.2	17.67	166.6	68.1	36
130 days	treatment	946666	6[a]	14.1[a]	147.4[a]	63.9	43.5

論文審査の結果の要旨及び担当者

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論　文　審　査　の　結　果　の　要　旨

本論文は福島県内の河川に生息する淡水魚であるヤマメにおいて放射能汚染の遺伝的影響について取り組んだものである。2011 年 3 月 11 日の東日本大震災の際に発生した東京電力福島第一原子力発電所事故により膨大な量の放射性物質が放出され、阿武隈山地をはじめとする福島県内の山林に降り注いだ。河川は周辺環境より放射性物質が流入し、蓄積する場所であり、淡水魚はそのような環境において長期間生息していることになる。ヤマメは福島県の山間部における食料であるとともに遊漁として重要な産業対象種である。これまでの放射線障害に関する研究は短時間の高線量被曝が中心であり、今回の事故のように比較的低線量の放射線に長時間曝されることは想定していない。従って、長期間の低線量被曝の遺伝的影響の把握は魚類だけではなく生物全体において重要な課題と言える。

本論文では序章において事故の経緯と研究の重要性について述べた後、第二章において遺伝マーカーであるマイクロサテライト DNA(MsDNA)マーカーの他種における利用の可能性について検討を行っている。更に第三章において MsDNA マーカーとミトコンドリア(MtDNA)マーカーを用い福島県内河川から採集されたヤマメの遺伝的多様性の比較を行い有意な変化が生じていないことを示した。第四章では放射能汚染地域である真野川雌性発生技術を用いて全ホモ個体を作成し、孵化率、浮上率、奇形率の比較を行い、コントロールとの差異が無いことを示している。第 5 章では雌性発生二倍体個体を用いて MtDNA の D-Loop 領域と Cytb 領域における突然変異率を調べ、汚染地域から採集した個体において突然変異個体を観察している。第六章では雌性発生二倍体から作成したクローンを用いて放射線の血液性状に及ぼす影響を調べている。クローンを比較することにより、遺伝的影響を排除した比較実験が可能となる。この実験において赤血球数、ヘモグロビン量、ヘマトクリット値が被曝区においてコントロールより有意に低下していた。

これらの結果は低線量の長期間にわたる放射線被曝がヤマメ生体に何らかの影響及ぼしていることを示している。

本論文は低線量放射線の長期的な影響を魚類において初めて検証した。また、雌性発生個体を比較実験に用いたことも画期的であると言える。

以上のことから審査員一同は、本論文提出者に対し、博士（農学）の学位を授与するに値するものと認定した。