Analyses of genetic stock structure of the southern bluefin tuna (*Thunnus maccoyii*) using nuclear DNA variation

Motoki Nakadate, Nobuaki Suzuki, Tomoyuki Itoh, Hiroyuki Kurota, Sachiko Tsuji and Seinen Chow

Summary

Seven polymorphic nuclear DNA markers found in the southern bluefin tuna were used to investigate genetic differentiation between samples (n=87 in total) collected from off Cape of Good Hope, Southeast Indian Ocean and off Tasmania. Number of alleles and expected heterozygosity per locus ranged from 2 to 4 and 0.15 to 0.58, respectively. No significant difference in allele and genotype frequencies among samples was observed and no apparent population structuring was indicated by *F*st index. However, significant departure from Hardy-Weinberg equilibrium was observed in several loci, indicating further investigation to be necessary.

要約

ミナミマグロの多型的核遺伝子マーカー7 種を用いて、ケープ沖、インド洋南東部及びタスマニア海域 3 標本、計 87 個体の比較を行った。遺伝子座ごとの対立遺伝子数は 2 から 4、平均ヘテロ接合体率は 0.15 から 0.58 であった。海域標本間の遺伝子頻度及び遺伝子型頻度に有意差はみられず、集団の分化を示す遺伝的分化指数（*F*st）も有意ではなかった。しかしながら、いくつかの遺伝子座においてHardy-Weinberg平衡からの乖離が見られたことから、さらに多くの標本を分析する必要性が示された。

Introduction

The southern bluefin tuna (*Thunnus maccoyii*) is a highly migratory large pelagic fish distributed throughout southern hemisphere south of 30˚S. Since the spawning of this species is thought to occur within limited area south of Java to off northwestern Australia (Ueyanagi, 1966) and the spawning season extends throughout the summer from September to March, no genetic population structuring has been expected. Fujino and Kang (1968) performed allozyme analysis of the southern bluefin tuna and observed no spatial genetic differentiation between the three samples collected off Australia. More recently, using restriction fragment length polymorphism (RFLP) analysis on entire mitochondrial DNA (mtDNA) molecule, Grewe *et al*. (1997) detected no heterogeneous haplotype distribution among southern bluefin tuna samples collected from Western and South Australia, South Africa and Tasmania. The allozyme analysis and RFLP analysis on mtDNA molecule were widely used techniques, but they are no longer common for fish population genetics because of the apparent limit to detect genetic variation and technical tediousness. Although methods to detect genetic variation rapidly advanced by

incorporating polymerase chain reaction (PCR), no further attempt has been performed to investigate genetic population structure of the southern bluefin tuna since the analysis by Grewe *et al*. (1997). We have attempted to isolate polymorphic nuclear DNA markers to investigate genetic stock structure of the southern bluefin tuna. Here, we introduce polymorphic nuclear gene loci in this species and report the results of population genetic analysis using these genetic markers.

Materials and methods

Three local samples used in this study were collected off Tasmania, Southeast Indian Ocean and off Cape of Good Hope (Table 1). Of seven gene loci used, four were introns of four protein-coding genes and three were anonymous regions. Methods to detect variation at the seven loci are shown in Table 2. Using Genepop v3.4 (Raymond and Rousset, 1995a), Fisher exact test for allele frequency (Raymond and Rousset, 1995b), log-likelihood (G) based exact test for genotype frequency (Goudet *et al*., 1996), and estimation of *F*st (Weir and Cockerham, 1984) were performed.

Results and discussion

Allele frequencies at each locus are shown in Table 3. All seven loci were polymorphic, and number of alleles per locus ranged from 2 to 4. No significant difference in allele and genotype frequencies among samples was observed and no apparent population structuring was indicated by *F*st index (Table 4). The results obtained in this study conform to previous population genetic analyses using allozyme and mtDNA (Fujino and Kang, 1968; Grewe *et al*., 1997). However, significant departure from Hardy-Weinberg equilibrium was observed in five loci. This may be due to the small sample size, null allele, and/or mixture of different populations. Analysis for much larger number of individuals is necessary to further investigate genetic differentiation not only for comparing between local samples but also between age classes.

References

Fujino, K. and Kang, T. (1968): *Genetics* **59**, 79-91.

Goudet, J., Raymond, M., De Meeüs, T. and Rousset, F. (1996): *Genetics* **144**, 1933-1940.

Grewe, P., Elliott, N., Innes, B. and Ward, R. (1997): *Mar. Biol.* **127**, 555-561.

Raymond, M. and Rousset, F. (1995a): *J. Hered.* **86**, 248-249.

Raymond, M. and Rousset, F. (1995b): *Evolution* **49**, 1280-1283.

Ueyanagi, S. (1966): *Bull. Jap. Soc. Sci. Fish.* **32**, 739-751.

Weir, B. S. and Cockerham, C. C. (1984): *Evolution* **38**, 1358-1370.

Table 1 Details of three southern bluefin tuna samples analyzed in the present study.

Local sample	Latitude	Longitude	Year sampled	Fork length (cm)	Number of individuals
Off Tasmania	43S	154E	1999	116-183	24
Southeast Indian Ocean	39S	104E	1994	70-191	29
Off Cape of Good Hope	40S	24E	1992	99-187	34

Table 2 Nuclear DNA loci analyzed in the present study and methods to detect variations.

Loci	Gene	Methods to detect variations
G6PD 4th intron	Glucose-6- phosphate dehydrogenase	Amplified fragment length polymorphism (605 - 715 bps)
CaM 4th intron	Calmodulin	Amplified fragment length polymorphism (550 - 600 bps)
GH2 2nd intron	Growth hormone	Amplified fragment length polymorphism (290 - 310 bps)
S7RP 2nd intron	S7 ribosomal protein	SSCP (354bps)
F2	Anonymous	Amplified fragment length polymorphism (290 - 300 bps)
F3	Anonymous	Amplified fragment length polymorphism (200 - 400 bps)
ANOM231	Anonymous	SSCP (280 bps)

Ranges of PCR-amplified fragment length are shown in parentheses.

Table 3 Allelic frequencies of 3 local samples in each locus analyzed in our study

		Oceanic samples		
Loci	Allele	Off Tasmania	Southeast Indian Ocean	Off Cape
G6PD	A	0.66	0.59	0.65
	B	0.34	0.41	0.35
	N	22	29	34
	ho	0.33	0.21*	0.36
	he	0.47	0.50	0.47
CaM	A	0.05	0.07	0.13
	B	0.92	0.87	0.84
	C	0.03	0.06	0.03
	N	19	27	30
	ho	0.05*	0.19	0.20
	he	0.15	0.24	0.29
GH2	A	0.06	0.08	0.04
	B	0.75	0.76	0.82
	C	0.19	0.16	0.14
	N	24	25	25
	ho	0.43	0.41	0.29
	he	0.42	0.41	0.32
RP2	A	0.91	0.88	0.80
	B	0.09	0.12	0.20
	N	17	21	30
	ho	0.06	0.14	0.07*
	he	0.18	0.22	0.34
F2	A	0.82	0.62	0.72
	B	0.18	0.38	0.28
	N	20	26	29
	ho	0.05*	0.39	0.35
	he	0.31	0.49	0.42
F3	A	0.59	0.56	0.60
	B	0.31	0.36	0.36
	C	0.06	0.08	0.04
	D	0.04	0	0
	N	24	25	25
	ho	0.43	0.57	0.45
	he	0.58	0.57	0.53
Anom231	A	0.75	0.61	0.70
	B	0.23	0.39	0.30
	C	0.02	0	0
	N	22	22	20
	ho	0.51	0.14*	0.10*
	he	0.40	0.50	0.44
Average	*Ho*	0.27	0.29	0.26
	He	0.36	0.42	0.40

ho, Ho: Observed heterozygosity in each locus and average value of ho, respectively.

he, He: Expected heterozygosity in each locus and average value of he, respectively.

*: Value of *ho* significantly differs from he at P=0.05.

Table 4 Results of homogeneity tests in 3 samples of south bluefin tuna conducted by Genepop v3.4

	Allele frequency		Genotype frequency
Loci	P−value	Fst	P−value
G6PD	0.738	−0.019	0.792
CaM	0.681	−0.011	0.737
GH2	0.875	−0.014	0.882
S7RP2	0.317	−0.009	0.486
F2	0.092	0.023	0.159
F3	0.738	−0.020	0.671
Anom231	0.293	−0.007	0.433

Methods of homogeneity tests among 3 samples were as follows:

Allelic frequencies: Using Fisher exact test (Raymond and Rousset, 1995).

Fst: According to Weir and Cockerham (1984).

Genotypic frequencies: Using Log-likelihood (G) based exact test (Goudet et al., 1996).