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Full Length Research Paper

Genetic structure of Japanese Spanish mackerel (*Scomberomorus niphonius*) in the East China Sea and Yellow Sea inferred from AFLP data

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The population genetic structure of Japanese Spanish mackerel (*Scomberomorus niphonius*) in the East China Sea and Yellow Sea was analyzed using amplified fragment length polymorphism (AFLP). A total of 247 putative loci were detected by four primer combinations among 83 individuals of Japanese Spanish mackerel collected from Ganyu, Ningbo, Wenzhou, Cheju Island and Nagasaki, 129 of which were polymorphic (52.23%). The proportion of polymorphic loci and Nei's genetic diversity for five populations ranged from 38.54% (Ganyu) to 45.70% (Nagasaki), and from 0.0808 (Cheju Island) to 0.0984 (Nagasaki). AMOVA analysis and pairwise F_{ST} revealed significant genetic differentiation among five populations. The UPGMA tree revealed the significant geographic structure in this species. Considering the high hydrological connectivity of this region and the species pelagic life history, retention of larvae, different migration route and different spawning season may be responsible for the significant genetic differentiation among populations in the East China Sea and Yellow Sea.

Key words: Japanese Spanish mackerel, *Scomberomorus niphonius*, genetic structure, AFLP.

INTRODUCTION

Understanding fish stock structure is an important component of successful and sustainable long-term management and has attracted considerable interest because of a fundamental interest in biotic evolution (Tudela et al., 1999). Determination of population genetic structure provides essential information to underpin resource recovery and to aid in delineating and monitoring populations for fishery management. Molecular genetic techniques offer the ability to identify and delineate fish stock structure where it may not be apparent from phenotypic or behavioural characteristics (Shaklee and Currens, 2003). Such techniques have been used successfully to understand the structure of marine fish species (Han et al., 2008a; Liu et al., 2006; Kochzius and Blohm, 2005).

Japanese Spanish mackerel *Scomberomorus niphonius* is an epipelagic, neritic species, widely distributed in subtropical and temperate waters of China, Japan and Korea. Being commercially and ecologically important, the Japanese Spanish mackerel becomes one of the main capturing targets of fishers in the East China Sea and Yellow Sea (Seikai National Fisheries Research Institute, 2001). Several preliminary studies related to Japanese Spanish mackerel mainly covered on ecology and fishery biology (Wei, 1980; Qiu and Ye, 1990; Qiu and Ye, 1991), but no information concerning the assessment of the genetic diversity of this species is available to date. It is unknown whether Japanese Spanish mackerel in the East China Sea and Yellow Sea forms a single stock, or is genetically subdivided into distinctly separate populations. For effective conservation and use of the Japanese Spanish mackerel in the East China Sea and Yellow Sea, it is first necessary to obtain knowledge of its genetic background.

Pelagic marine fishes usually have high fecundity, very large population size, and high dispersal potential at egg, larval and adult stages. These life-history features and the continuity of the pelagic environment in theory suggest little genetic divergence over large spatial scales (Borsa, 2003). There is some information about fishery

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biology of Japanese Spanish mackerel, which can give a clue to its population genetic structure. Japanese Spanish mackerel performs long seasonal migration in the East China Sea and Yellow Sea. However, different migration routes, different over-wintering grounds, different spawning grounds and spawning seasons are reported in the studied area (Seikai National Fisheries Research Institute, 2001). In the East China Sea, the species migrate from the over-wintering grounds in the offshore area of the East China Sea to coastal waters of Zhejiang and Fujian Provinces from March to April. In the Yellow Sea, the species migrate from the over-wintering grounds in southern Yellow Sea near Cheju Island to coastal waters of Yellow Sea and Bohai Sea in the late April. The spawning season varied greatly among different sea areas. In general, the spawning period in the north is later than that in the south. For example, the spawning period of Japanese Spanish mackerel in the East China Sea is from March to April, but that in Yellow Sea and Bohai Sea is from May to June (Qiu and Ye, 1993). Japanese Spanish mackerel has pelagic eggs and larvae, and adults are thought to undertake long distant seasonal migrations. These life-history features indicate that potential dispersal of larva and adult in this species is high. These may ensure connectivity between stocks and cause genetic homogenization between distant populations in the East China Sea and Yellow Sea.

Amplified fragment length polymorphism (AFLP) analysis (Vos et al., 1995) is a PCR-based multilocus fingerprinting technique that combines the strengths and overcomes the weaknesses of PCR-RFLP and RAPD-PCR. AFLP analysis has been used for indirect examination of levels of genetic diversity in several species (Liu et al., 2005; Chen et al., 2005; Kim et al., 2007). The major strengths of the AFLP method include simultaneous screening of a large number of polymorphic loci, high reproducibility due to high stringency of PCR, and relative cost effectiveness (Liu and Cordes, 2004). Moreover, it does not require any prior molecular information about sequences under investigation and is thus especially applicable to species in which the genome sequences are not well characterized, like Japanese Spanish mackerel. The objectives of this study are determination of genetic diversity and intraspecific population differentiation of Japanese Spanish mackerel in the East China Sea and Yellow Sea using AFLP analysis for which no data are available at present.

MATERIALS AND METHODS

Sampling

A total of 83 adults of Japanese Spanish mackerel were collected in five localities from the East China Sea and Yellow Sea, including three populations from China, one population from Japan and one population from Korea from 2007 to 2008 through local fishing boats (Figure 1; Table 1). Sampling locations (identification abbreviation) were as follows: Ganyu (GY), Ningbo (NB), Wenzhou (WZ), Cheju Island (CH) and Nagasaki (NA). These populations were all spawning populations. Muscle samples were preserved in 70 - 90% ethanol before DNA extraction.

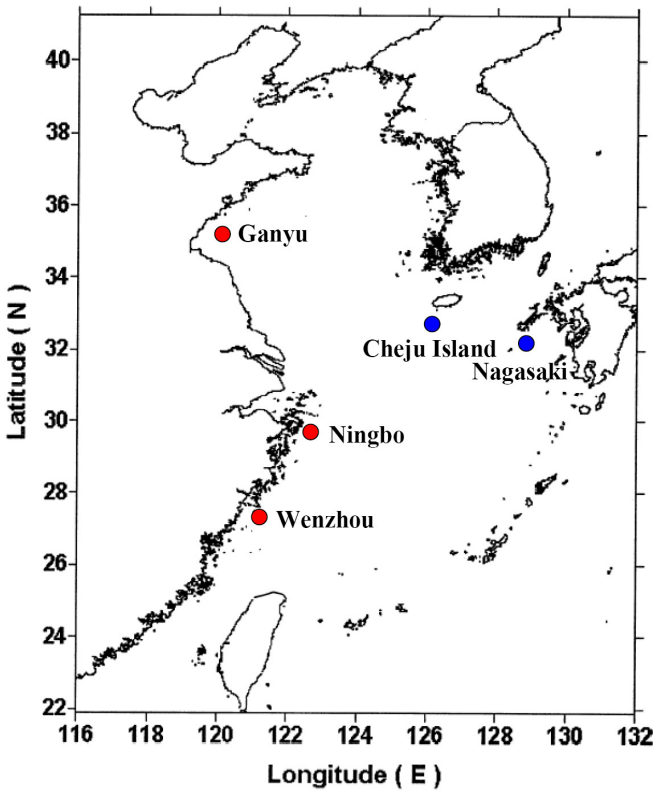

Figure 1. Sample sites for Japanese Spanish mackerel. Two groups of Japanese Spanish mackerel were identified by the red and blue colors.

AFLP analysis

Genomic DNA was isolated from muscle tissue by proteinase K digestion followed by a standard phenol–chloroform method. DNA was subsequently resuspended in 100 µL of TE buffer (10 mmol/L Tris-Cl, 1 mmol/L EDTA, PH=8.0). Procedures of AFLP were essentially based on Vose et al. (1995) and Wang et al. (2000). About 100 ng genomic DNA was digested with 1 unit of *Eco*RI and *Mse*I (NEB) at 37°C for 6 h. Double-stranded adapters were ligated to the restriction fragments at 20°C overnight after adding 1 µL 10 × ligation buffer, 5 pmol *Eco*RI adapter (*Eco*R I-1/*Eco*RI-2; Table 2), 50 pmol *Mse* I adapter (*Mse*I-1/*Mse*I-2; Table 2), 0.3 unit of T4 DNA ligase (Promega) with a final volume of 10 µL. Preamplification PCR reaction was conducted using an Eppendorf Thermocycler (Mastercycler 5334) with a pair of primers containing a single selective nucleotide. Amplification was performed at an annealing temperature of 53°C for 30 s. The 20 µL PCR product mixture was diluted 10-fold with distilled water and used as templates for the subsequent selective PCR amplification. The selective amplifications were carried out in 20 µL PCR reaction volume containing 1 µL productions of preamplifications, 1 × PCR reaction buffer, 150 µM of each dNTP, 30 ng of each selective primer, and 0.5 unit of Taq DNA polymerase on a gradient thermal cycler (Mastercycler 5334) with a touchdown cycling profile of nine cycles of 30 s at 94°C, 30 s at 65°C (–1°C at each cycle), and 30 s at 72°C followed by the cycling profile of 28 cycles of 30 s at 94°C, 30 s at 56°C, and 1 min at 72°C. The final step was a prolonged extension

Table 1. Parameters of genetic diversity for populations of Japanese Spanish mackerel.

Populations	n	Date of collection	Number of loci	Number of polymorphic loci	Proportion of polymorphic loci	Nei's genetic diversity
Ningbo	19	March 2007	216	88	40.74%	0.0823
Ganyu	11	May 2007	205	79	38.54%	0.0886
Wenzhou	13	May 2008	209	83	39.71%	0.0876
Cheju Island	17	March 2008	211	86	40.76%	0.0808
Nagasaki	23	March 2008	221	101	45.70%	0.0984

Table 2. Adaptor and primer sequences used in AFLP analysis.

Primer	Sequence
Adapters	
*Eco*RI-adapter	5'-CTCGTAGACTGCGTACC-3' 5'-AATTGGTACGCAGTCTAC-3'
*Mse*I-adapter	5'-GACGTGAGTCCTGAG-3' 5'-TACTCAGGACTCAT-3'
Pre-amplification primer	
*Eco*RI	5'-GACTGCGTACCAATTC-3'
*Mse*I	5'-GATGAGTCCTGAGTAA-3'
Selective amplification primer	
E-AAG/M-CAC	5'-GACTGCGTACCAATTCAAG-3' 5'-GATGAGTCCTGAGTAACAC-3'
E-AAC/M-CTT	5'-GACTGCGTACCAATTCAAC-3' 5'-GATGAGTCCTGAGTAACTT-3'
E-AGT/M-CTA	5'-GACTGCGTACCAATTCAGT-3' 5'-GATGAGTCCTGAGTAACTA-3'
E-AGA/M-CTG	5'-GACTGCGTACCAATTCAGA-3' 5'-GATGAGTCCTGAGTAACTG-3'

of 7 min at 72°C. PCR products were run on 6.0% denaturing polyacrylamide gel electrophoresis (PAGE) for 2.5 h at 50°C on the Sequi-Gen GT Sequencing Cell (Bio-Rad, USA), and finally detected using the silver staining technique modified from Merril et al. (1979). Sequences of AFLP adapters and primers are listed in Table 1. Four primer combinations (E-AAG/M-CAC, E-AAC/M-CTT, E-AGT/M-CTA, and E-AGA/M-CTG) were chosen for AFLP analysis (Table 2).

Data analysis

AFLP bands were scored for presence (1) or absent (0), and transformed into 0/1 binary character matrix. Proportion of polymorphic loci and Nei's genetic diversity were calculated by POPGEN. Genetic distances between populations were calculated by Nei's (1978) unbiased genetic distance. Genetic relationships among populations were estimated by constructing UPGMA tree based on Nei's unbiased genetic distance (Nei, 1978) in Mega 3.0. Population structure of Japanese Spanish mackerel was investigated using the molecular variance software package (AMOVA) and *F*-statistics in ARLEQUIN 2.000 (Schneider et al., 2000).

RESULTS

A total of 247 putative loci were detected by the four primer combinations, 129 of which were polymorphic (52.23%, Table 3). The average number of bands scored per primer pair was 61.75, ranging from 54 to 76. The number of polymorphic loci amplified by each primer combination over all populations ranged from 22 to 40, with the average of 32.25 polymorphic loci per primer combination (Table 3). The population with the highest proportion of polymorphic loci (45.70%) and number of polymorphic loci (101) was Nagasaki, whereas that with the lowest values was Ganyu, in which the proportion of polymorphic loci and number of polymporphic loci was 38.54% and 79, respectively. The population with the highest Nei's genetic diversity was also Nagasaki population, with a value of 0.0984, the lowest Nei's genetic diversity was found in Cheju Island population, only with

Table 3. Number of bands generated by primer combinations.

	E-AAG/M-CAC	E-AAC/M-CTT	E-AGT/M-CTA	E-AGA/M-CTG	Total
Number of loci	76	61	54	56	247
Number of polymorphic loci	40	31	36	22	129
Proportion of polymorphic loci	52.63%	50.82%	66.67%	39.29%	52.23%

Table 4. Nei's genetic distance (above) and pairwise F_{ST} (below) between populations.

	Ningbo	Ganyu	Wenzhou	Cheju Island	Nagasaki
Ningbo		0.0093	0.0129	0.0260	0.0197
Ganyu	0.0570*		0.0076	0.0190	0.0175
Wenzhou	0.0971*	0.0442*		0.0148	0.0142
Cheju Island	0.2348*	0.2111*	0.1456*		0.0073
Nagasaki	0.1901*	0.1681*	0.1164*	0.0705*	

*Significant (P<0.01).

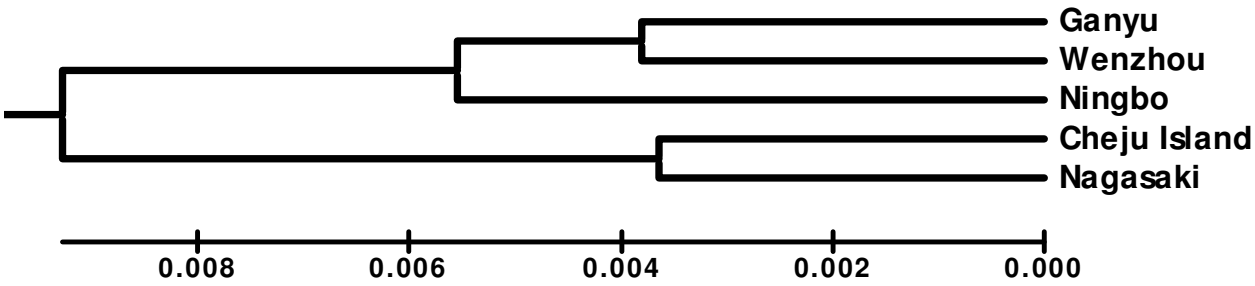

Figure 2. UPGMA cluster analysis based on Nei's genetic distances among five populations.

a value of 0.0808 (Table 1).

According to AMOVA analysis, overall genetic differentiation among Japanese Spanish mackerel from the five populations was high and significant (F_{ST} = 0.1436 P < 0.001), suggesting significant genetic differentiation among localities. Moreover, pairwise F_{ST} values among populations were high and significant (P < 0.01), ranging from 0.0442 to 0.2348 (Table 4). These analyses indicated that several distinct populations of the Japanese Spanish mackerel existed in the East China Sea and Yellow Sea. Additionally pairwise F_{ST} analysis indicated the largest genetic difference among populations existed in locations Ningbo and Cheju Island (F_{ST} = 0.2348, P <0.01), whereas the difference between Ningbo and Ganyu was the smallest (F_{ST} = 0.0442, P < 0.01). Nei's genetic distance analysis also suggested that populations Ningbo and Cheju Island were the most different genetically (D = 0.0260), whereas the populations Cheju Island and Nagasaki were the most similar genetically (D = 0.0073) (Table 4). Furthermore, cluster analysis revealed two geographic groups, populations from Ningbo, Wenzhou and Ganyu as one group, while other populations Cheju Island and Nagasaki as another group (Figure 2).

DISCUSSION

The present study provided the first molecular evidence for the existence of separate Japanese Spanish mackerel populations in the East China Sea and Yellow Sea. Contracting to our expectation of strong connectivity among localities, significant genetic differentiation was detected in Japanese Spanish mackerel by pairwise F_{ST} and AMOVA analysis. The null hypothesis that Japanese Spanish mackerel in the East China Sea and Yellow Sea constitutes a single panmictic stock was rejected. The F_{ST} values among five populations were all significant (P < 0.01), indicating limited gene flow among populations in the lack of dispersal barriers. The AMOVA analysis also supported the significant genetic structure in Japanese Spanish mackerel (F_{ST} = 0.1436 P < 0.01). Two geographic groups were revealed by UPGMA tree based on Nei's genetic distance (Figure 2), suggesting significant geographic structure between two sides of the East China Sea and Yellow Sea.

The significant geographic structure of populations in marine species is usually affected by local oceanographic characteristics and species life history (Borsa et al., 1997). The ocean current circulation between the Yellow

and the East China seas consists of inflow from the East China Sea to the Yellow Sea along the west coast of Korea (Yellow Sea Warm Current), and outflow of water from the Yellow Sea to the East China Sea along the China coast (China Coastal Current) (Li, 2000). Previous studies found that these currents potentially interconnect distant populations in the East China Sea and Yellow Sea (Yu et al., 2005; Han et al., 2008b). So the ocean currents might be not responsible for the significant genetic differentiation in Japanese Spanish mackerel.

Japanese Spanish mackerel utilizes near-shore or estuarine habitat as nursery areas for larvae and juveniles after wintering migrations following predictable route. 5-6 days after hatching, the larvae can swim initiatively and begin to prey (Qiu and Ye, 1993). This suggested the potential dispersal ability of larvae is high. The strong swimming ability of larvae might counteract the passive transportation along coast by ocean currents. The species may choose the strategy to retention of larvae in spawning grounds. This strategy may limit the gene flow between distant spawning populations. The significant genetic differentiation among five populations of Japanese Spanish mackerel supported this strategy. Furthermore, the significant geographic structure revealed by UPGMA tree supported the retention of larvae in coastal waters to minimize offshore transplant of larvae. Retention of larvae is also reported in Indian scad mackerel *Decapterus russelli* (Borsa, 2003). Indian scad mackerel showed similar biological characteristics like Japanese Spanish mackerel with high dispersal potential for adults and juveniles. Both nuclear and mitochondrial markers showed sharp geographic structure in *D. russelli* in the Indo-Malay archipelago. The rehomogenization of allele frequencies between different populations did not happen in Indian scad mackerel, indicating either reproductive homing, or the choice by adults of spawning areas that also constitute retention zones for eggs and larvae, or both.

Besides larvae disperse strategy, the migratory behavior including the different migration routes and different over-wintering grounds, and different mating period in the East China Sea and Yellow Sea might also be responsible for the significant genetic differentiation among populations of Japanese Spanish mackerel. These biological characteristics predispose Japanese Spanish mackerel to genetic structuring along its geographical distribution, although the species showed strong dispersal ability. The observation of sharp geographic differences in Indian scad mackerel and some other highly mobile, pelagic species (Borsa, 2003; Kotoulas et al., 1995; Ward et al., 1997) shows that migratory abilities may also be associated with increased potential for homing, hence for reproductive isolation.

Genetic analysis of fish species in the East China Sea and Yellow Sea is still few in number, which can be used for comparison to our present study. Genetic study on the *Nibea albiflora* revealed no significant genetic structure in China coastal waters by mtDNA and AFLP markers, suggesting high gene flow among populations (Han et al., 2008b). The Ocean currents in the East China Sea and Yellow Sea were responsible for the lack of genetic structure in *N. albilfora*. A study on the redlip mullet, *Chelon haematocheilus* in the Northwestern Pacific, found no significant genetic structure between the Yellow and the East China Seas, although there were significant genetic differences between the three marginal seas of Northwestern Pacific (the Sea of Japan, East China Sea, and South China Sea) (Liu et al., 2007). Recent range expansion and insufficient time to attain migration-drift equilibrium were reasons for the lack of phylogeographical structure in redlip mullet. However, similar to our study, AFLP analysis of yellowback sea bream *Dentex tumifrons* in China coastal waters also revealed significant genetic differentiation among populations in the East China Sea and Yellow Sea (Xia and Jiang, 2006). The explanation for significant genetic differentiation in this species might result from the differences of geographic conditions and oceanographic characteristics among the populations. mtDNA analysis of white croaker *Pennahia argentata* in the East China Sea and Yellow Sea also revealed two geographic groups, with the same geographic distribution of two groups in Japanese Spanish mackerel (Han et al., 2008c). This geographic structure of white croaker was partly due to the biological characteristics of this species.

Although sample size from each geographic site in this study was limited, specimens were collected from different geographic locations on either side of the East China Sea and Yellow Sea. This should be sufficient to generate the preliminary data on genetic diversity and population differentiation of Japanese Spanish mackerel in the East China Sea and Yellow Sea. Moritz (1994) proposed the concept of a management unit (MU), which was defined as a conservation unit that had statistically significant divergence in allele frequencies (nuclear or mitochondrial). Therefore, the Japanese Spanish mackerel in the East China Sea and Yellow Sea should be considered as at least five management units, which provides a guideline for further effective conservation and management of the species, and would help greatly in understanding the biology of this species. These results have important implications for fisheries management of this species in China, Japan and Korea. However, the boundaries of the populations remain unknown and should be identified for effective conservation, management and utilization of the species.

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