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Genetic Diversity and Population Structure of a Pelagic Fish, Jack Mackerel (*Trachurus japonicus*), Based on AFLP Analysis

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Abstract.- *Trachurus japonicus* is a commercially important species in the East Asia, especially in China, Japan and Korea. We estimated the gene flow among six populations of *T. japonicus* from Chinese and Japanese coastal waters by using amplified fragment length polymorphism (AFLP) technology. A total of 531 bands were amplified for 81 individuals of 6 populations by 4 pair selective primers, 89.27% of which were polymorphic bands. The population Miyazaki showed the highest Nei genetic diversity and Shannon genetic diversity. The topology of UPGMA tree was very shallow and no significant genealogical branches or clusters corresponding to sampling localities. The pairwise F_{st} between populations ranged from 0.016 to 0.107 and most were not significant after sequential Bonferroni correction. The results of AMOVA showed that 95.61% of genetic variation existed within populations and 4.39% existed among populations, which indicated low level of genetic differentiation among populations and high level of gene flow between populations. The results suggested that *T. japonicus* around distribution area should be considered to be a single panmictic population.

Keywords: *Trachurus japonicas*; AFLP; genetic diversity; gene flow.

INTRODUCTION

The jack mackerel, *Trachurus japonicas*, is a pelagic fish belonging to Carangidae which is widely distributed on the continental shelf waters along the subtropical Kuroshio Current and the Tsushima Warm Current in the western North Pacific (Zhu *et al*., 1963). *T. japonicus* is a commercially important species in the East Asia, especially in China, Japan and Korea (Zhang and Lee, 2001). However, few studies on this species were conducted in China because the market landing was little before 2003 and fishing was not paid enough attention (Cao and Gao, 2006). Some analysis on market landing about *T. japonicus* was performed in China recently (Cao and Gao, 2006). In Japan, the resource of *T. japonicus* had been seriously destroyed due to recent overfishing. Until now studies have primarily focused on the distribution of *T. japonicus* larvae, juveniles, early growth and development (Sassa and Konishi 2006; Xie and Watanabe 2007; Kasai *et al*., 2008), and so on. Niu *et al.* (2011) and Zhang *et al.* (2014) analyzed genetic polymorphism of *T. japonicus* from Fujian coastal waters based on mitochondrial control region, cyt *b* and AFLP, respectively. No further population genetic studies were conducted for this species except that Song *et al*. (2013) reported the population genetic structure based on mitochondrial DNA.

Amplified fragment length polymorphism (AFLP) is a PCR-based, multi-locus fingerprinting technique that can detect genetic variations effectively. It combines the strengths and overcomes the weaknesses of the RFLP and RAPD methods (Vos *et al*., 1995). For instance, McCusker and Bentzen (2011) studied the population genetic structure of Atlantic wolffish throughout its North Atlantic range by AFLP loci and microsatellites, and AFLP loci revealed slightly higher F_{st} values but similar patterns of differentiation and isolation-by-distance estimates, compared to microsatellites. The significant population genetic structure of *Sardinella zunasi* in the Northwest Pacific has been detected successfully by AFLP marker (Ying *et al*., 2011). Moreover, AFLP marker has been used to analyze the genetic differentiation between wild and cultured populations of *Pseudosciaena crocea*, and the results showed that genetic variation of cultured populations was obviously lower than wild (Wang *et*

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al., 2002). Until now, AFLP has been proven to be successful in studying population genetic structure and differentiation of plants (Li *et al*., 2012), animals (Zhu *et al*., 2013; Kai *et al*., 2002; Takami *et al*., 2004) and some fish species such as *Carassius auratus* (Jung, 2013), *Pleuronectes yokohamae* (Zhang *et al*., 2012) and *Synechogobius ommaturus* (Song *et al*., 2010).

In the present study, AFLP was used to analyze the genetic structure of six populations which were collected from South China Sea and Japanese coastal waters and the results may provide useful information on the development of appropriate fishery management strategies.

MATERIALS AND METHODS

Sample collection

Five populations were collected from Japanese coastal waters and one population was collected from Beihai coastal water of South China Sea during Feb. 2009 to Sept. 2009 for this study (Fig. 1, Table I). All individuals were identified based on morphological characteristics, and a piece of muscle tissue was obtained from each individual and preserved in 95% ethanol or directly extracted from frozen samples.

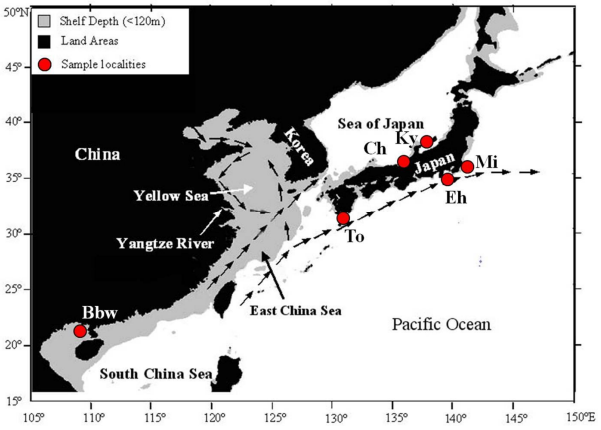

Fig.1. Sample sites of *T. japonicus* in the present study.

Genomic DNA extraction and AFLP method

Genomic DNA was isolated from the muscle tissue by proteinase K digestion followed by a standard phenol–chloroform method. Procedures of AFLP were essentially based on Vos *et al*. (1995) and Wang *et al*. (2000). About 100 ng genomic DNA was digested with 1 unit of *Eco*R I 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 *EcoR* I adapter (*Eco*R I-1/*Eco*R I-2; Table II), 50 pmol *Mse* I adapter (*Mse*I-1/*Mse*I-2; Table II), 0.3 unit of T4 DNA ligase (Takara) with a final volume of 10 µL. Preamplification PCR was conducted using an Takara Thermocycler with a pair of primers containing a single selective nucleotide. Amplification was performed at an annealing temperature of 53°C for 30s. 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 thermocycler with a touchdown cycling profile of 9 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, each of 30 s at 94°C, 30 s at 56°C, and 1 min at 72°C. The final step was a prolonged extension 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 II. Four primer combinations (E-AAG/M-CTC, E-AGG/M-CTC, E-AGA/M-CTC, E-ACG/M-CTT) were chosen for AFLP analysis (Table II).

Data analysis

Clear and unambiguous bands in length ranging from 50 to 1200 bp were considered as usable. AFLP bands were scored for presence (1) or absent (0) excluding the smeared and weak ones by visual inspection, and transformed into 0/1 binary character matrix. Percentages of polymorphic loci, Nei's genetic diversity and Shannon diversity index

Table I.- Sample information of *T. japonicus* including sampling sites, date of collection, sample size and several genetic diversity indices.

ID	Sampling site	Date of collection	Sample size	Number of loci	Number of polymorphic loci	Proportion of polymer-phic loci	Nei's gene diversity	Shannon's information index
Eh	Ehime, Japan	2009.06	16	394	197	50.00 %	0.107±0.156	0.176±0.222
Ky	Kyoto, Japan	2009.03	17	407	325	79.85 %	0.179±0.170	0.288±0.234
To	Toyama, Japan	2009.05	14	385	298	77.40 %	0.185±0.168	0.296±0.235
Bbw	Beihai, China	2009.09	13	390	300	76.92 %	0.175±0.166	0.283±0.231
Ch	Chiba, Japan	2009.06	14	374	288	77.01 %	0.191±0.172	0.305±0.239
Mi	Miyazaki, Japan	2009.06	7	357	253	70.87 %	0.210±0.182	0.325±0.255
Total	\	\	81	531	465	87.57%	0.144±0.151	0.244±0.210

Table II.- Adapters and primer combinations sequences used in the study.

Primers		Sequence
Adapter	EcoRI-adapter	5'-CTCGTAGACTGCGTACC-3' 5'-AATTGGTACGCAGTCTAC-3'
	MseI-adapter	5'-GACGTGAGTCCTGAG-3' 5'-TACTCAGGACTCAT-3'
Pre-amplification primer	EcoRI-preprimer	5'-GACTGCGTACCAATTC-3'
	MseI-preprimer	5'-GATGAGTCCTGAGTAA-3'
Selective amplification primer	E-AAG/M-CTC	5'-GACTGCGTACCAATTCAAG-3' 5'-GATGAGTCCTGAGTAACTC-3'
	E-AGG/M-CTC	5'-GACTGCGTACCAATTCAGG-3' 5'-GATGAGTCCTGAGTAACTC-3'
	E-AGA/M-CTC	5'-GACTGCGTACCAATTCAGA-3' 5'-GATGAGTCCTGAGTAACTC-3'
	E-ACG/M-CTT	5'-GACTGCGTACCAATTCACG-3' 5'-GATGAGTCCTGAGTAACTT-3'

were calculated by POPGENE (Yeh *et al*., 1999). Similarity indices were calculated using the formula $S=2N_{ab}/(N_a+N_b)$ (Nei and Li, 1979), where N_a and N_b are the number of bands in individuals a and b, respectively and N_{ab} is the number of sharing bands. Genetic distances between individuals were computed using the formula D=-ln S (Nei and Li, 1979). Genetic relationships among individuals were constructed based on unweighted pair-group method analysis (UPGMA; Sokal and Michener, 1958) by Mega 3.0 based on Nei's genetic distance (Nei and Li, 1979). Genetic differentiation between pairs of population samples was evaluated by pairwise fixation index F_{st} and the significance of the F_{st} was tested by 10,000 permutations for each pairwise comparison in ARLEQUIN (Excoffier *et al*., 1992). When multiple comparisons were performed, *P* values were adjusted using the sequential Bonferroni procedure (Rice, 1989). Analysis of molecular variance (AMOVA) (Excoffier *et al*., 1992) was employed to further examine hierarchical population structure as well as the geographical pattern of population subdivision (Excoffier *et al*., 1992). The gene flows were estimated using the equation: $N_m= (1-F_{st})/4F_{st}$ (Wright, 1951), where Nm is the number of effective immigrants per generation.

RESULTS

A total of 531 bands were amplified for 81 individuals of 6 populations by 4 pair selective primers. Four hundred and seventy-four polymorphic sites were detected and the percentage was 89.27%. The average polymorphic sites for 4 pair primers were 119 with a range of 103-147 (Table III). The percentage of polymorphic sites for 6 populations was 50.0%-77.0%. The population Mi showed the highest Nei genetic diversity and Shannon genetic diversity, the population Eh showed the lowest Nei genetic diversity and Shannon genetic diversity.

Table III. Number of bands generated by four primer combinations.

	No. of loci	No. of poly-morphic loci	Proportion of poly-morphic loci
E-AAG/M-CTC	128	106	82.81%
E-AGG/M-CTC	142	118	83.10%
E-AGA/M-CTC	154	147	95.45%
E-ACG/M-CTT	107	103	96.26%
Total	531	474	89.27%

Table IV.- Pairwise F_{st} (below diagonal) and gene flow (above diagonal) among populations of *T. japonicus.*

	Eh	Ky	To	Bbw	Ch	Mi
Eh		6.417	6.693	4.838	4.164	2.087
Ky	0.038		15.326	6.269	8.609	2.764
To	0.036	0.016		14.702	7.485	2.914
Bbw	0.049*	0.038	0.017		10.167	2.352
Ch	0.057*	0.028	0.032	0.024		5.329
Mi	0.107*	0.083	0.079	0.096	0.045	

The pairwise F_{st} was ranged from 0.016 to 0.107, the largest being between populations Ky and To and the lowest between population Eh and Mi (Table IV). Most F_{st} value was not significant after sequential Bonferroni correction. The results of AMOVA showed that 95.61% of genetic variation existed within populations and 4.39% among populations, which indicated that genetic differentiation among populations was low (Table V). The population pairwise gene flow estimates (Nm) among the five populations (Eh, Ky, To, Bbw and Ch) were very high (ranged from 4.164 to 15.326), suggesting frequent gene flow. The population Mi showed weak genetic differentiation with other populations, but the value of N_m was still more than 1, which indicated that there still was gene flow between population Mi and other populations. The result of the Mantel test did not reveal a significant association between the geographic and genetic distances. The genetic differentiation between Chinese population and Japanese populations was not obviously larger than that among Japanese populations.

Table V.- The results of AMOVA for *T. japonicus.*

	Among populations	Within populations
Degrees of freedoms	5	75
Sum of squares	355.904	3311.355
Variance components	2.02496 Va	44.15140 Vb
Percentage of variation (%)	4.39	95.61

The UPGMA tree based on genetic distance among 81 individuals showed that there were no significant genealogical branches or clusters corresponding to sampling localities. The topology of UPGMA tree was very shallow (Fig. 2).

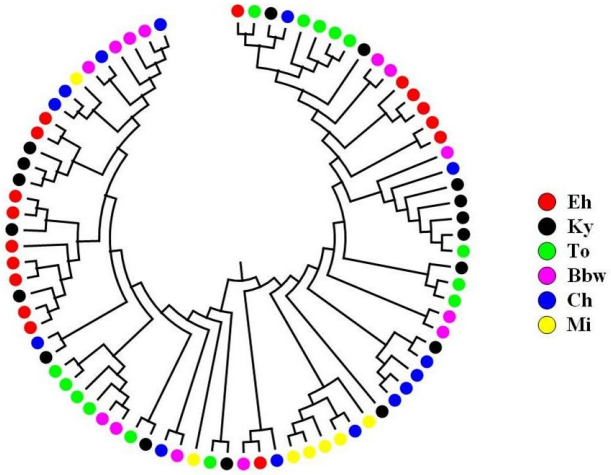

Fig. 2. UPGMA tree of 81 individuals of *T. japonicus* based on Nei's genetic distance.

DISCUSSION

AFLP marker has been widely used in population genetic studies and reflected the genetic status of some species sensitively, because it is easy, fast, inexpensive, robust and owns large numbers of polymorphisms, high reproducibility and so on (Dorenbosch *et al*., 2006; Liu *et al*., 2009). In the present study, 531 loci have been detected by 4 pair primers for *T. japonicus* and the percentage of polymorphic sites was 89.27%, which indicated rich polymorphism and high sensitivity for this nuclear marker. The percentage of polymorphic sites for 6 populations was 50.0-77.0%, which was in accordance with the results of two Fujian

populations based on AFLP markers (63.28% and 61.89%, respectively) (Zhang *et al*., 2014). Compared with other fishes reported, *T. japonicus* showed high level of polymorphism (Wang *et al*., 2007; Liu *et al*., 2009; Lin *et al*., 2009; Song *et al*., 2010). The results indicated that the genetic diversity of *T. japonicus* in the present study is above the middle level and the population genetic structure had not been destroyed.

The shelf-break regions of the East China Sea were the primary spawning ground of *T. japonicas*. The eggs and larvae of *T. japonicus* can be transported by Kuroshio Current and Tsushima Current over large distance within 2 months (Kasai *et al*., 2008). The results of mitochondrial DNA for *T. japonicus* showed that no significant genetic differentiation was detected among populations from East China Sea, Japanese Sea and the west coastal waters of Japanese Sea (Song *et al*., 2013).The results of AMOVA in the present study also revealed that most of genetic variation existed within populations. Low genetic differentiation index was detected among the different populations and indicated strong gene flow existed along the western North Pacific. Compared with freshwater and anadromous fishes, marine fish usually do not have strong population partitions due to the high dispersal potential of different life-history stages coupled with an absence of physical barriers to movement (Ward *et al*., 1994; Grant and Bowen, 1998). The Kuroshio Current and its branch play an important part in gene exchange of *T. japonicus* because it can transport large numbers of egg and larvae which spawned in the East China Sea to their nursery grounds (Kasai *et al*., 2008). The shallow waters off the west coast of Kyushu and the Pacific coast of southern Japan would be full of larvae and juveniles that spawned in the Southern East China Sea within one month (Katoh *et al*., 1996; Ichikawa and Beardsley, 2002; Lie and Cho, 2002). The recruitment from East China Sea to the coastal waters of Japan was estimated to be larger than that from the local areas (Sassa *et al*., 2008; Kasai *et al*., 2008). Our results suggested genetic homogeneity among different populations and it may be closely related with the transport of eggs and larvae. The results of mitochondrial DNA also supported this deduction (Song *et al*., 2013).

According to the value of gene flow, population MI showed weak genetic differentiation with other populations while no genetic differentiation among other populations was detected. It was confusing to get this conclusion because the other five populations from Chinese coastal waters and Japanese coastal waters showed high genetic homogeneity with each other. In the present study we deduced that less individuals of MI may lead to particularity of this population. Moreover, no genetic differentiation was detected by mitochondrial DNA (Song *et al*., 2013). More sensitive molecular markers like microsatellite DNA should be used to do further study to examine this population in the future.

The results of mitochondrial DNA and AFLP implied that around distribution area should be considered to be a single panmictic population, which is opposed to the conclusion of sagittal otoliths analysis by Xie and Watanabe (2007). Different answers of genetic studies and early life character suggested different management strategy for *T. japonicus*. More sensitive molecular markers should be used to investigate the genetic variation among populations to make a proper strategy for this species.

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