African Journal of Biotechnology Vol. 10(60), pp. 12833-12839, 5 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1231
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Genetic differentiation in Japanese flounder in the Yellow Sea and East China Sea by amplified fragment length polymorphism (AFLP) and mitochondrial DNA markers

Sanlei Li[1], Dongdong Xu[1]*, Bao Lou[1], Hanxiang Xu[1], Feng You[2], Wei Zhan[1] and Zhi Geng[1]

[1]Marine Fishery Institute of Zhejiang Province, Key Lab of Mariculture and Enhancement of Zhejiang Province, Zhoushan 316100, People's Republic of China.
[2]Zhejiang Ocean University, College of Marine Science, Zhoushan 316100, People's Republic of China.
[3]Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, People's Republic of China.

Accepted 1 September, 2011

The population structure of Japanese flounder (*Paralichthys olivaceus*) in the Yellow and East China Seas were analyzed using amplified fragment length polymorphism (AFLP) and cytochrome c oxidase subunit I (*COI*) gene sequencing. A total of 390 reproducible bands were generated by 10 AFLP primer combinations in two populations collected from the coasts of Qingdao (located at the Yellow Sea) and Zhoushan (located at the East China Sea). The percentage of polymorphic loci (*P*), Nei's genetic diversity (*H*) and Shannon's information index (*I*) values were higher in the Qingdao population (*P* = 72.85%, *H* = 0.243 and *I* = 0.364) than those in the Zhoushan population (*P* = 56.35%, *H* = 0.189 and *I* = 0.284). The genetic diversity reduction in the Zhoushan population may be attributed to fishing pressure and habitat loss in this area. Based on the *COI* sequencing analysis, a total of 25 polymorphic sites were examined, and 15 haplotypes were identified in the two populations. The haplotype diversity (*h*) and nucleotide diversity (π) values in the Qingdao population were 0.746 ± 0.0728 and 0.00334 ± 0.00103, respectively. The corresponding values in the Zhoushan population were 0.712 ± 0.0470 and 0.00318 ± 0.00049. Both the AFLP and mtDNA data revealed significant genetic differentiation between the two populations. The present study discussed the factors that may result in genetic differentiation between the populations in the Yellow and East China Seas.

Keywords: Japanese flounder, amplified fragment length polymorphism (AFLP), cytochrome c oxidase subunit I (*COI*) gene, genetic diversity, population structure.

INTRODUCTION

Japanese flounder (*Paralichthys olivaceus*) is an important commercial species that is widely cultured in China. However, the natural resources of Japanese flounder are declining owing to changes in the environment and fishing pressure. Moreover, aquaculture production of this species has not increased greatly over the last decade despite the extensive fishery management efforts because of inbreeding depression, viral and bacterial disease problems (Hulata, 2001; You et al. 2007). Thus, maintaining long-term resource sustainability is of concern for the Japanese flounder in China. Understanding the fish population structure is an important component of successful and sustainable long-term management, and it is critical for the rational use of the exploitable resources. Therefore, clarifying the population structure of Japanese flounder is crucial in formulating fishery management and aquaculture development programs for this species.

Genetic assessment of Japanese flounder has been performed using several types of genetic markers. You et al. (2001) compared the genetic variation between wild populations in the coastal area of the Yellow Sea in China using allozymes. Sekino and Hara (2001) applied

*Corresponding author. E-mail: xudong0580@163.com. Tel: 86-580-3080985. Fax: 86-580-3080417.

microsatellite markers to analyze the genetic structure of populations collected from coastal sea areas around Japan. Kim et al. (2010) also employed microsatellite markers to analyze the genetic structure of populations collected from coastal sea areas around Korea. Moreover, several studies have compared the genetic variation in hatchery populations to wild populations using random amplified polymorphic DNA (RAPD) (You et al., 2007), microsatellite markers (Sekino and Hara, 2001) and amplified fragment length polymorphism (AFLP) (Zhang et al., 2004). The genetic variation of selected stocks has also been reported (Sekino et al., 2002; Liu et al., 2005).

Previous studies on the genetic diversity of Japanese flounder in China were mainly focused on hatchery stocks (Zhang et al., 2004; Liu et al., 2005; You et al., 2007). The genetic background of the wild Japanese flounder populations in the China Sea is limited. Therefore, the baseline information about the Japanese flounder should be acquired to meet the demands of natural resource protection and genetic breeding studies. In the present study, the genetic diversity and differentiation between two wild populations collected from the Yellow Sea and East China Sea were compared using AFLP and mitochondrial cytochrome c oxidase subunit I (*COI*) gene sequencing analyses to investigate the genetic resource status.

MATERIALS AND METHODS

Fish samples and genomic DNA extraction

A total of 100 wild individuals were sampled. Among these individuals, 50 individuals were collected from the coast of Qingdao (120°38'N, 36°09'E, located at the Yellow Sea) in 2006, and the other 50 individuals were collected from the coast of Zhoushan (122°30'N, 30°08'E, located at the East China Sea) in 2008 (Figure 1). Muscle tissue samples were preserved in 95% ethanol and stored frozen at -20°C until DNA extraction. Genomic DNA was isolated from muscle tissue using DNA extraction kits (TaKaRa No. D305, Dalian, China) following the manufacturer's instruction.

AFLP analysis

AFLP analysis was performed as described by Xu et al. (2009). Sequences of AFLP adapters and primers are listed in Table 1 and the following 10 primer combinations were employed to generate the bands: E-AGA/M-CAT, E-ACT/M-CAT, E-AAG/M-CAA, E-ACT/M-CTG, E-ACT/M-CAA, E-ACC/M-CAA, E-ACT/M-CTT, E-ACT/M-CTC, E-AAG/M-CTG and E-ACT/M-CCA. PCR products were separated using 6% denaturing polyacrylamide gels (acrylamide/ bisacrylamide, 19:1; 7 mol/L urea; and 1×TBE buffer) and sized with the DL2000 DNA marker (Promega, Shanghai China). DNA bands were visualized with silver staining.

AFLPs were scored as dominant markers and bands were scored as "1" for present or "0" for absent. Using this method, the markers were transformed into a 0/1 binary data matrix. The percentage of polymorphic loci (*P*), Nei's genetic diversity (*H*) (Nei, 1978), Shannon's information index (*I*) (Lewontin, 1972), gene flow ($Nm = 0.5(1 - G_{ST}) / G_{ST}$) (McDermott and McDonald, 1993) and Nei's genetic distance (*D*) (Nei, 1978) were calculated using the Popgene 1.32 software package (Yeh et al., 1997). Molecular variances within and among populations of Japanese flounder were estimated by analysis of molecular variance (AMOVA) using the Arlequin 3.1 software package (Excoffier et al., 2005). To quantify the genetic differentiations between populations, F_{ST} values were calculated, and the significance of these values were tested with 1000 permutations using the Arlequin 3.1 software package (Excoffier et al., 2005).

COI sequencing

A fragment of 3′ end of the mitochondrial cytochrome oxidase subunit one (*COI*) gene was PCR amplified in a subset of 80 individuals. The fragments of *COI* gene sequences were obtained by PCR amplification using the following primer set: COI-F (5'-CCT GCA GGA GGA GGA GAY CC-3') and COI-R (5'-AGT ATA AGC GTC TGG GTA GTC-3'). PCR amplification was performed in a reaction volume of 50 µL containing 29.6 µL high-performance liquid chromatography (HPLC) water, 5 µL 10× PCR buffer, 2 µL 2.5 mM dNTPs, 5 µL 25 mM $MgCl_2$, 2 µL of each primer/10µM, 2U Taq DNA polymerase (TaKaRa Biotechnology Co., Ltd.) and 40 ng diluted DNA. The PCR reaction was performed as follows: an initial incubation at 94°C for 2 min, followed by 35 cycles of PCR (denaturing at 94°C for 45 s, annealing at 52°C for 1 min, and extension at 72°C for 1 min), and a final extension at 72°C for 7 min. The PCR products were purified with an agarose gel DNA purification Kit (Tiangen No. DP204-02, Beijing) following the manufacturer's introduction. The purified of fragment were sequenced on an ABI prism 3730 automatic sequencer with forward or reverse primers.

DNA sequences were aligned by Clustal X 1.83 (Thompson et al., 1997), and they were manually refined. The MEGA version 4.0 (Tamura et al., 2007) and DnaSP 4.0 (Rozas et al., 2003) software packages were used to calculate statistical values including the nucleotide composition, number of haplotypes, number of polymorphic sites, nucleotide diversity (π) (Lynch and Crease, 1990) and haplotype diversity (*h*) (Nei, 1987) for each population. The gene flow (*Nm*) between populations (Hudson et al., 1992) was calculated using the DnaSP 4.0 software package (Rozas et al., 2003). Molecular variances within and among populations of Japanese flounder were estimated by AMOVA using the Arlequin 3.1 software package (Excoffier et al., 2005). The F_{ST} values were calculated and the significance of these values were tested with 1000 permutations using the Arlequin 3.1 software package (Excoffier et al., 2005).

RESULTS

AFLP analysis

A total of 390 bands were produced from 100 individuals using 10 primer combinations and 341 (87.44%) of these bands were polymorphic. There were 279 and 204 polymorphic bands in the Qingdao and Zhoushan populations, respectively. The percentage of polymorphic bands was significantly lower in the Zhoushan population (56.35%) than that in the Qingdao population (72.85%) ($df = 1$, $\chi^2 = 22.20$, $P < 0.01$). The genetic diversity values in the Qingdao population in terms of Nei's genetic diversity (*H*) and Shannon diversity indices (*I*) were 0.243 and 0.364, respectively. The corresponding values in the Zhoushan population were 0.189 and 0.284, respectively (Table 2). The genetic diversity in the Qing- dao

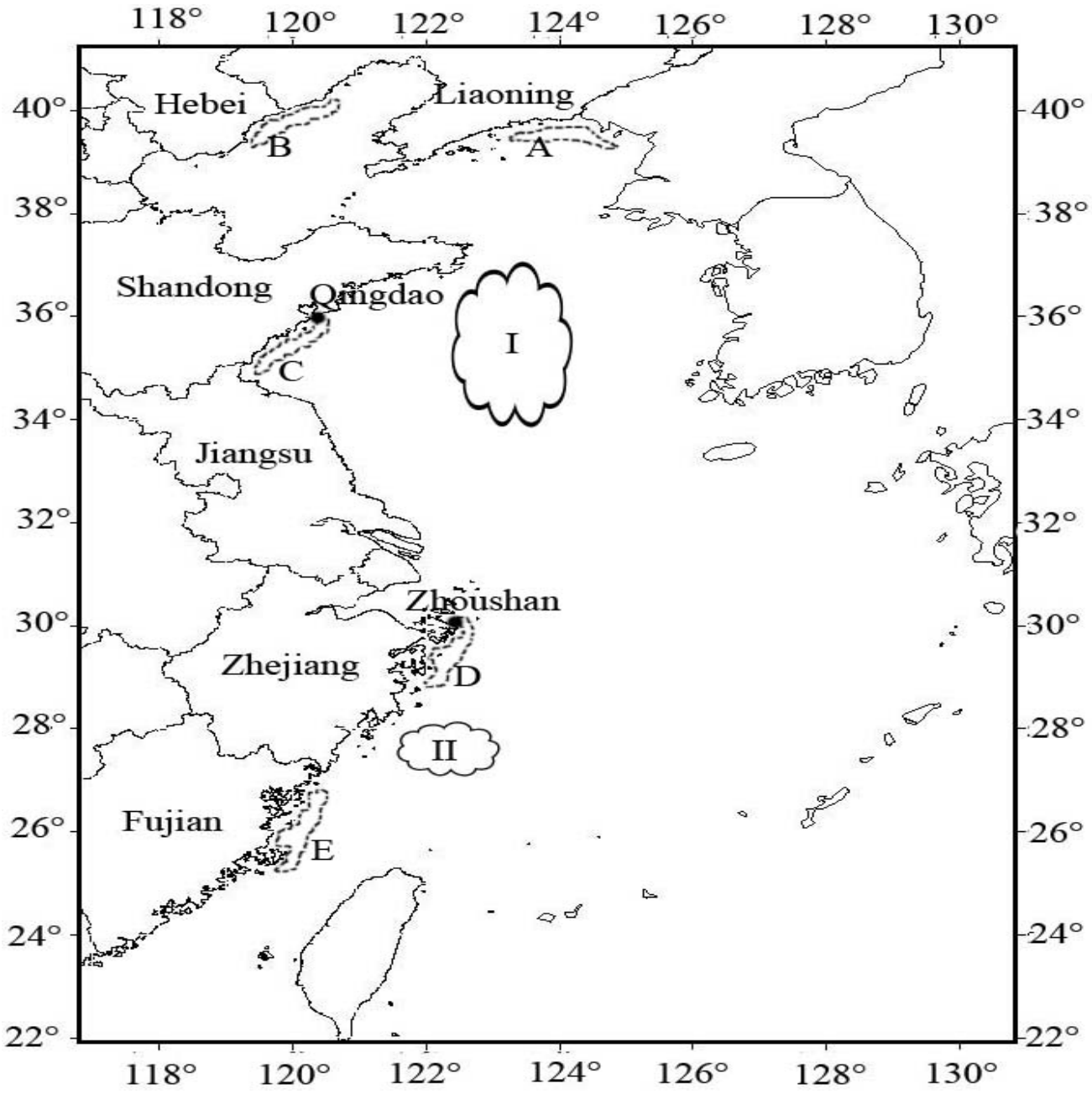

Figure 1. Sample locations and overwintering grounds for Japanese flounder. The overwintering grounds (area I, 33°30′ - 37°30′N, 122°30′ - 124°00′E; area II, 27°00′ - 27°30′N, 121°30′ - 122°30′E) and spawning grounds (area A, B, C, D and E)are according to Li (1995).

population was therefore higher than that in the Zhoushan population.

More also, the genetic variation between the two populations was as follows: D = 0.0849, Nm = 3.084, and F_{ST} = 0.195. The F_{ST} values indicated significant genetic differentiation between the two populations (P < 0.001). Analysis of molecular variance (AMOVA) was conducted to describe the variance components of Japanese flounder populations (Table 3), and it revealed that 80.53% of the genetic variation occurred within samples and 19.38% of the genetic variation occurred among populations.

COI gene sequencing

The *COI* sequences were corrected and aligned and 528 bp consensus sequences were obtained. The average base composition was as follows: T = 29.10%, C = 25.10%, A = 25.30%, and G = 20.50%. Among the sequences

Table1. Oligonucleotide adapters and primers used in AFLP analysis.

Adapters or primer	Sequence (5′ — 3′)
Adapters	
EcoR Ⅰ 1	CTC GTA GAC TGC GTA CC
EcoR Ⅰ 2	AAT TGG TAC GCA GTC TAC
Mse Ⅰ 1	GAC GAT GAG TCC TGA G
Mse Ⅰ 2	TAC TCA GGA CTC AT
Primer of pre-amplification	
EcoR Ⅰ 1	GAC TGC GTA CCA ATT C
Mse Ⅰ 1	GAT GAG TCC TGA GTA A
Primer for selective amplification	
E-AGA	GAC TGC GTA CCA ATT C AGA
E-ACT	GAC TGC GTA CCA ATT C ACT
E-AAG	GAC TGC GTA CCA ATT C AAG
E-ACC	GAC TGC GTA CCA ATT C ACC
M-CAT	GAT GAG TCC TGA GTA A CAT
M-CAA	GAT GAG TCC TGA GTA A CAA
M-CTG	GAT GAG TCC TGA GTA A CTG
M-CCA	GAT GAG TCC TGA GTA A CCA
M-CTC	GAT GAG TCC TGA GTA A CTC
M-CTT	GAT GAG TCC TGA GTA A CTT

Table 2. Parameters of genetic diversity for populations of Japanese flounder.

Parameter	*n*	Total band	Polymorphic band	*P* (%)	*H*	*I*
Qingdao	50	383	279	72.85	0.243	0.364
Zhoushan	50	362	204	56.35	0.189	0.284
Total	100	390	341	87.44	0.240	0.379

The letter "*P*", "*H*" and "*I*" indicate percentage of polymorphic loci, Nei's genetic diversity (H) and Shannon's information index, respectively.

Table 3. Analysis of molecular variance (AMOVA) within and among the populations of Japanese flounder.

Source of variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation (%)
Among populations	1	355.0	6.56	19.47
Within populations	98	2658.32	27.13	80.53
Total	99	3013.32	33.68	

examined, the A/T base contents were higher than the C/G base contents. Sequence comparisons of the *COI* sequences revealed 25 polymorphic sites in two populations. Of these polymorphic sites, 15 were singleton variable sites and 10 were parsimony informative sites. Overall, a total of 15 haplotypes were defined in the 80 individuals. There were 10 haplotypes in the Qingdao population and 7 haplotypes in the Zhoushan population, respectively. The distribution of haplotypes was nonrandom, and some samples had private haplotypes. It was observed that HPL01 was the most common haplotype in the two populations. There were 13 private haplotypes (86.67%) in the populations, with 8 private haplotypes in the Qingdao population and 5 private haplotypes in the Zhoushan population (Table 4).

The haplotype diversity (h) and nucleotide diversity (π) values were 0.746 ± 0.0728 and 0.00334 ± 0.00103 in the Qingdao population, and the corresponding values in the Zhoushan population were 0.712 ± 0.0470 and 0.00318 ± 0.00049. These results suggest that the

Table 4. Nucleotide substitutions and distribution of the 15 haplotypes of the mtDNA *COI* sequences of Japanese flounder.

Haplotype	Variable site position																								Locality		Total
						1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3	4	4	4	4	QD	ZS	
	3	4	6	7	8	5	7	7	7	0	2	2	4	5	5	6	8	0	1	6	7	0	3	4	6			
	0	6	5	0	2	2	2	6	8	5	4	7	6	0	9	5	4	3	3	4	4	0	5	8	6			
HPL01	C	G	A	T	T	G	A	G	A	T	T	G	A	T	A	A	T	T	G	A	G	G	T	G	A	14	17	31
HPL02		C				A																				6		6
HPL03		C																								8		8
HPL04				C																						4		4
HPL05											C															1		1
HPL06					C																			C		2		2
HPL07																								C		2		2
HPL08		C	G				G		G	C										G	T					1		1
HPL09	G	C				A						T		G			G	G	A				G	C		1		1
HPL10																				G							13	13
HPL11																G					A					1	1	2
HPL12																									G		5	5
HPL13		C						C	G												T						1	1
HPL14															G					G							2	2
HPL15													G							G				C			1	1
Total																										40	40	80

Each value is the number of 528-bp nucleotide sequences characterized by 25 variable nucleotide sites according to the reduced sequence data set in each area considering the decision rule. The letter "QD" and "ZS" indicated Qingdao population and Zhoushan population, respectively.

genetic diversity in the Qingdao population was higher than that in the Zhoushan population (Table 5). The gene flow (*Nm*) between the two populations was 1.70. The pairwise F_{ST} value was 0.128, which indicated a significant genetic differentiation between the two populations ($P < 0.001$). The AMOVA analysis revealed that 87.16% of the *COI* sequence variation was distributed within samples and 12.84% of the sequence variation was distributed among populations (Table 6).

DISCUSSION

The genetic diversity of the two wild populations from the Yellow and East China Seas were analyzed based on AFLP and *COI* sequencing analysis in the present study. As revealed by AFLP analysis, the percentage of polymorphic loci and Nei's genetic diversity values were 72.85% and 0.243, respectively in the Qingdao population, and the corresponding values were 56.35% and 0.189 in the Zhoushan population. Based on the *COI* gene sequence analysis, the haplotype diversity and nucleotide diversity values were 0.746 ± 0.0728 and 0.00334 ± 0.00103 in the Qingdao population, and the corresponding values in the Zhoushan population were 0.712 ± 0.0470 and 0.00318 ± 0.00049. The levels of genetic diversity of Japanese flounder in this study were comparable to the levels of genetic diversity of other marine fishery species around China as follows: Japanese Spanish mackerel (*Scomberomorus niphonius*) with genetic diversity values of $P = 38.54 \sim 45.70\%$ and $H = 0.0808 \sim 0.0984$ as measured by AFLP (Shui et al., 2008); small yellow croaker (*Larimichthys polyactis*) with genetic diversity values of $P = 55.34 \sim 60.09\%$ and $H = 0.124 \sim 0.138$ as measured by AFLP (Han et al., 2009); white croaker (*Pennahia argentata*) with genetic diversity values of $P = 47.13 \sim 57.63\%$ as measured by AFLP (Lin et al., 2009); and silver pomfret (*Pampus argenteus*) with genetic diversity values of $\pi = 0.880$ and $h = 0.006$ as measured by *COI* sequencing (Peng et al., 2009). The large population size is thought to be responsible for the great levels of genetic diversity in many marine fishes (Avise, 1998).

It is striking to note that there is a marked reduction in genetic diversity in the Zhoushan population compared with that in the Qingdao population. Genetic variation within populations can be lost through genetic drift or a bottleneck in the population size (You et al., 2001; Sekino et al., 2002; Kim et al., 2010). In fact, fishing is the main way to obtain aquatic products in the area of Zhoushan. Consequently, fishery resources have sharply declined in recent years (Xu et al., 2003; Yu et al., 2010). The

Japanese flounder population has also experienced serious overfishing and habitat loss in the coast of

Table 5. Descriptive statistics for the Japanese flounder populations based on *COI* sequencing.

Parameter	*n*	Number of haplotype	Number of polymorphic site	*h*	*π*
Qingdao	40	9	19	0.746 ± 0.0728	0.00334 ± 0.00103
Zhoushan	40	7	12	0.712 ± 0.0470	0.00318 ± 0.00049
Total	80	15	25	0.758 ± 0.0537	0.00329 ± 0.00058

The letter" *h*" and "*π*" indicated haplotype diversity and nucleotide diversity, respectively.

Table 6. Analysis of molecular variance of Japanese flounder *COI* sequences in the two populations.

Source of variation	Degree of freedom	Sum of squares	Variance component	Percentage variation (%)
Among Populations	1	5.238	0.112	12.84
Within populations	78	59.275	0.760	87.16
Total	79	64.513	0.872	

Zhoushan since the 1990s, which may result in low genetic diversity of Japanese flounder in this region.

The genetic differentiation coefficient (F_{ST}) is the most important index that can reflect genetic differentiation. Mickett et al. (2003) suggested that an F_{ST} value of 0.446 indicates a high genetic differentiation in channel catfish (*Ictalurus punctatus*) populations and that a value of 0.176 indicates a moderate genetic differentiation based on AFLP analysis. Yue et al. (2004) considered that an F_{ST} value of 0.0470 corresponds to a moderate genetic differentiation in Asian arowana (*Scleropages formosus*) populations. In the present study, the F_{ST}-value was 0.195 based on the AFLP data, which suggested a moderate genetic differentiation between the Qingdao and Zhoushan populations. The genetic differentiation was also confirmed by *COI* gene sequence analysis. The F_{ST} value of the two populations was 0.128 based on the *COI* gene sequencing analysis, which also suggested that the two populations were in the range for moderate genetic differentiation based on the guidelines of Wright (1978).

Marine organisms with planktonic larvae were assumed to lack population structuring in open ocean environments due to the lack of apparent barriers to gene flow (Beheregaray and Sunnucks, 2001). A genetic study using mtDNA and AFLP markers on *Nibea albiflora* revealed no significant genetic differences among fish from three locations along the coastal waters of China. The water currents in the Yellow and East China Seas facilitate the dispersal of *N. albiflora* eggs and larvae, and this dispersal is likely responsible for the lack of genetic differences among the geographically separate groups of *N.* albiflora (Han et al., 2006; Han et al., 2008). However, population genetic structuring in widely distributed marine species has been reported as well. Similar to our results, AFLP analysis of *S. niphonius* (Shui et al., 2008), *L. polyactis* (Han et al., 2009) and *P. argenteus* (Zhao et al., 2011) also revealed significant genetic differences among fish captured in the Yellow and East China Seas. Differences in life history dispersal, migrations, spawning and wintering sites are responsible for the genetic differences among species that have been observed.

Japanese flounder inhabit shallow shelf waters and utilize near-shore or estuarine habitats as nursery areas for larvae and juveniles. Li (1995) assumed that there are two stocks (Bohai - Yellow Sea stocks and East Sea stocks) throughout the range of Japanese flounder based on breeding migration routes and overwintering grounds (Figure 1). The overwintering grounds for the Bohai-Yellow Sea stock are found at the following locations: 33°30′ - 37°30′N and 122°30′ - 124°00′E (Figure 1, area I). In May, the adults migrate from the overwintering ground to the Yalu River (Figure 1, area A), Liaodong peninsula coast (Figure 1, area B) and south of the Shandong peninsula (Figure 1, area C) to spawn. For the East China Sea stocks, the overwintering grounds are found at the following locations: 27°00′ - 27°30′N and 121°30′ - 122°30′E (Figure 1, area II). The adults migrate to the coasts of Zhejiang (Figure 1, area D) and Fujian Province (Figure 1, area E) to spawn in late March. The migratory behavior, such as the different migration routes and overwintering grounds, and different mating periods in the Yellow Sea and East China Seas may be responsible for the genetic differentiation among populations of Japanese flounder (Shui et al., 2008; Han et al., 2009; Zhao et al., 2011).

The present study generated preliminary data on the genetic diversity and population differentiation of Japanese flounder in the Yellow Sea and East China Sea. More geographic stocks should be utilized for future detailed genetic studies to understand the population structure of Japanese flounder in the China Sea. The results of our study demonstrated significant genetic differentiation of Japanese flounder in the Yellow Sea and East China Sea. Genetically differentiated populations may show variation of traits, which is important for

aquaculture. Therefore, economically important traits, such as growth and disease resistance should be evaluated for these populations.

ACKNOWLEDGEMENT

This study was supported by grants from the Project of Zhejiang Province of China (No. 2008C12011, No. 2009C12078, No. 2010F20006 and No. 2010R411054).

REFERENCES

Avise J (1998). Phylogeography. Harvard University Press, Cambridge, MA.

Beheregaray LB, Sunnucks P (2001). Fine-scale genetic structure, estuarine colonization and incipient speciation in the marine silverside fish *Odontesthes argentinensis*. Mol. Ecol. 10: 2849-2866.

Excoffier L, Laval G, Schneider S (2005). ARLEQUIN, version 3.0: an integrated software package for population genetics data analysis. Evol. Bioinform. 1: 47-50.

Han ZQ, Gao TX, Wang ZY, Zhuang ZM, Su TF (2006). Analysis of genetic diversity of *Nibea albiflora* by AFLP markers. J. Fish. China, (In Chinese with English abstract). 30: 640–646.

Han ZQ, Gao T X, Yanagimoto T, Sakurai Y (2008). Genetic population structure of *Nibea albiflora* in Yellow Sea and East China Sea. Fish. Sci. 74: 544-552.

Han ZQ, Lin LS, Shui BN, Gao TX (2009). Genetic diversity of small yellow croaker *Larimichthys polyactis* revealed by AFLP markers. Afr. J. Agric. Res. 4: 605-610.

Hudson RR, Slatkin M, Maddison WP (1992). Estimation of levels of gene flow from DNA sequence data. Genetics, 132: 583-589.

Hulata G (2001). Genetic manipulations in aquaculture: a review of stock improvement by classical and modern technologies. Genetica, 111: 155-173.

Kim WJ, Kim KK, Han HS, Nam BH, Kim YO, Kong HJ, Noh JK, Yoon M (2010). Population structure of the olive flounder (*Paralichthys olivaceus*) in Korea inferred from microsatellite marker analysis. J. Fish. Biol. 76: 1958-1971.

Lewontin RC (1972). The apportionment of human diversity. Evolution. 6: 1-398.

Li SZ, Wang HM (1995). Fauna Sinica Ostichthyes Pleuronectiformes. Science Press, Beijing, China.

Lin LS, Ying YP, Han ZQ, XiaoYS, Gao TX (2009). AFLP analysis on genetic diversity and population structure of small yellow croaker *Larimichthys polyactis*. Afr. J. Biotechnol. 8: 2700-2706.

Liu YG, Chen SL, Li BF, Wang ZJ, Liu Z (2005). Analysis of genetic variation in selected stocks of hatchery flounder, *Paralichthys olivaceus*, using AFLP markers. Biochem. Syst. Ecol. 33: 993-1005.

Lynch M, Crease TJ (1990). The analysis of population survey data on DNA sequence variation. Mol. Biol. Evol. 7: 377-394.

McDermott JM, McDonald BA (1993). Gene flow in plant pathosystems. Annu. Rev. Phytopathol. 31: 353-373.

Mickett K, Morton C, Feng J, Li P, Simmons M, Cao D, Dunham RA, Liu Z (2003). Assessing genetic diversity of domestic populations of channel catfish (*Ictalurus punctatus*) in Alabama using AFLP markers. Aquaculture, 228: 91-105.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89: 583-590.

Nei M (1987). Molecular Evolutionary Genetics. Columbia University Press, New York, USA.

Peng S, Shi Z, Hou J, Wang W, Zhao F, Zhang H (2009). Genetic diversity of silver pomfret (*Pampus argenteus*) populations from the China Sea based on mitochondrial DNA control region sequences. Biochem. Syst. Ecol. 37: 626-632.

Rozas J, Sanchez-Delbarrio JC, Messeguer X, Rozas R (2003). DnaSP, DNA polymorphism analyses by coalescent and other methods. Bioinformatics, 19: 2496-2497.

Sekino M, Hara M (2001). Application of microsatellite markers to population genetics studies of Japanese flounder *Paralichthys olivaceus*. Mar. Biotechnol. 3: 572-589.

Sekino M, Hara M, Taniguchi N (2002). Loss of microsatellite and mitochondrial DNA variation in hatchery strains of Japanese flounder *Paralichthys olivaceus*. Aquaculture, 213: 101-122.

Shui BN, Han ZQ, Gao TX, Miao ZQ (2008). Genetic structure of Japanese Spanish mackerel (*Scomberomorus niphonius*) in the East China Sea and Yellow Sea inferred from AFLP data. Afr. J. Biotechnol. 7: 3860-3865.

Tamura K, Dudley Nei M, Kumar S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596-1599.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The clustal–windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25: 4876-4882.

Wright S (1978). Evolution and the genetics of populations: variability within and among natural populations. University of Chicago Press, Chicago, USA.

Xu D, You F, Wu Z, Li J, Ni J, Xiao Z, Zhang P, Xu Y (2009). Genetic characterization of asymmetric reciprocal hybridization between the flatfishes *Paralichthys olivaceus* and *Paralichthys dentatus*. Genetica, 137: 151-158.

Xu H, Liu Z, Zhou Y (2003). Variation of *Trichiurus haumela* productivity and recruitment in the East China Sea. J. Fish. China. (In Chinese with English abstract). 4: 322-327.

Yeh FC, Yang RC, Boyle T (1997). Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belg. J. Bot. 129: 157.

You F, Wang K, Xiang J, Xu C (2001). Comparative analysis of biochemical genetic structure and variance between natural and cultured stocks on the left eyed flounder, *Paralichthys olivaceus* off Shandong coastal waters. Oceanol. Limnol. Sin. (In Chinese with English abstract). 32: 512-512.

You F, Zhang PJ, Wang KL, Xiang JH (2007). Genetic variation of natural and cultured stocks of *Paralichthys olivaceus* by allozyme and RAPD. Oceanol. Limnol. Sin. 25: 78-84.

Yu CG, Chen QZ, Chen XQ, Ning P, Zheng J (2010). Species composition and quantitative distribution of fish in the Zhoushan fishing ground and its adjacent waters. Oceanol. Limnol. Sin. (In Chinese with English abstract). 41: 410-417.

Yue GH, Li Y, Lim LC, Orban L (2004). Monitoring the genetic diversity of three Asian arowana (*Scleropages formosus*) captive stocks using AFLP and microsatellites. Aquaculture, 237: 89-102.

Zhao F, Dong Y, Zhuang P, Zhang T, Zhang LZ, Shi ZH (2011). Genetic diversity of silver pomfret (*Pampus argenteus*) in the Southern Yellow and East China Seas. Biochem. Syst. Ecol. 39: 145-150.

Zhang QQ, Xu XF, Qi J, Wang XL, Bao ZM (2004). The genetic diversity of wild and farmed Japanese flounder populations. J. Ocean. Univer. China. 34: 816-820. (In Chinese with English abstract).