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Genetic diversity and population genetic structure of the miiuy croaker, *Miichthys miiuy*, in the East China Sea by microsatellite markers

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ABSTRACT. Genetic diversity and patterns of population structure of the miiuy croaker were investigated using SSR markers. A set of 10 microsatellite loci revealed 40 alleles; the number of alleles varied from 2 to 10 for each marker. A relatively high level of genetic variability was observed between miiuy croaker individuals. Genetic diversity was relatively high within populations with corresponding high average gene flow. There were genealogical branches or clusters corresponding to sampling localities according to the UPGMA tree and principal component analysis. Knowledge of the genetic diversity and population structure will be crucial for establishing appropriate fishery management stocks for this species.

Key words: Miiuy croaker; Microsatellite; Genetic diversity; Population structure

INTRODUCTION

The miiuy croaker, *Miichthys miiuy*, is distributed throughout eastern China (Zhang and Hong, 2000; Shan et al., 2008a,b). It is a very important food fish species due to its good taste and high nutritive value (Sun et al., 2005; Peng et al., 2010). This species inhabits coastal waters and spawns in autumn from September to November (Li et al., 2005). However, the resources of the miiuy croaker have declined due to overfishing, pollution, coastal construction, and other factors (An et al., 2012). At the same time, high mortality and poor growth have been frequently encountered during artificial larval rearing, which hinders the mass production of this fish (Shan et al., 2009).

In recent years, most studies on the miiuy croaker were about morphological and physiological characteristics and molecular immunology (Liu et al., 2007; Cheng et al., 2011a,b; Xu et al., 2011a,b; Meng et al., 2012; Sun et al., 2005, 2012). However, little is known about the genetics of the miiuy croaker. Patterns of genetic diversity of individuals and populations could provide an important basis for ecological restoration measures (Väli et al., 2008), as well as the protection of germplasm resources. The identification of fish populations is an important step towards establishing conservation policies that can protect locally adapted populations by regulating fishing activities (Carvalho and Hauser, 1994).

As we all know, molecular genetic techniques can offer the ability to identify and delineate fish stock structure. Microsatellite markers have been considered some of the most efficient molecular markers, providing abundant genetic information due to their co-dominance, high mutation rate, abundance throughout the genome and ease of scoring. They could be used to analyze the genetic diversity and population structure of the miiuy croaker. In this study, about thirty individuals per population were collected from six regions of the East China Sea. We then used 10 microsatellite markers to investigate genetic diversity and population structure. This information could provide an important theoretical basis for the protection and sustainable utilization of this species.

MATERIAL AND METHODS

Sample collection, DNA extraction and SSR analysis

An average of thirty wild miiuy croaker individuals per population were collected from the following six geographic regions along the coast of the East China Sea: Zhoushan, Xiangshan, Wenling, Wenzhou, Yueqing, and Ruian (Figure 1). Fin tissues were dissected from each individual and immediately preserved in pure alcohol and stored at -20°C. Genomic DNA was extracted from the fin clips using the traditional phenol-chloroform method with some modifications. The quality and quantity of DNA were examined on agarose gel electrophoresis. DNA was finally adjusted to 100 ng/μL and stored at 4°C for later use.

Ten polymorphic SSR loci from our library were used in this study (Wang et al., 2010; Xu et al., 2011c). PCR was carried out in a 15-μL reaction mixture containing 9.9 μL sterile water, 1.5 μL 10X PCR buffer (containing 1.5 mM Mg^{2+}), 1.2 μL 2.5 mM dNTPs, 0.6 μL forward primer, 0.6 μL reverse primer, 1 μL diluted DNA template, and 0.1 μL 5 U/μL *Taq* DNA polymerase (Tiangen). PCR amplification was performed on an ABI 9700 according to the following profile: 95°C for 5 min; 30 cycles of 95°C for 30 s, annealing temperature for 30 s and 72°C for 30 s; and 72°C for 5 min. The amplified products were denatured for 8 min at 96°C. The denatured products were separated on 6% denaturing polyacrylamide (19:1

acrylamide:bis-acrylamide) gels and visualized by silver staining. Denatured pBR322 DNA/*Msp*I molecular weight marker (Tiangen) was used as the size standard to determine size of PCR products.

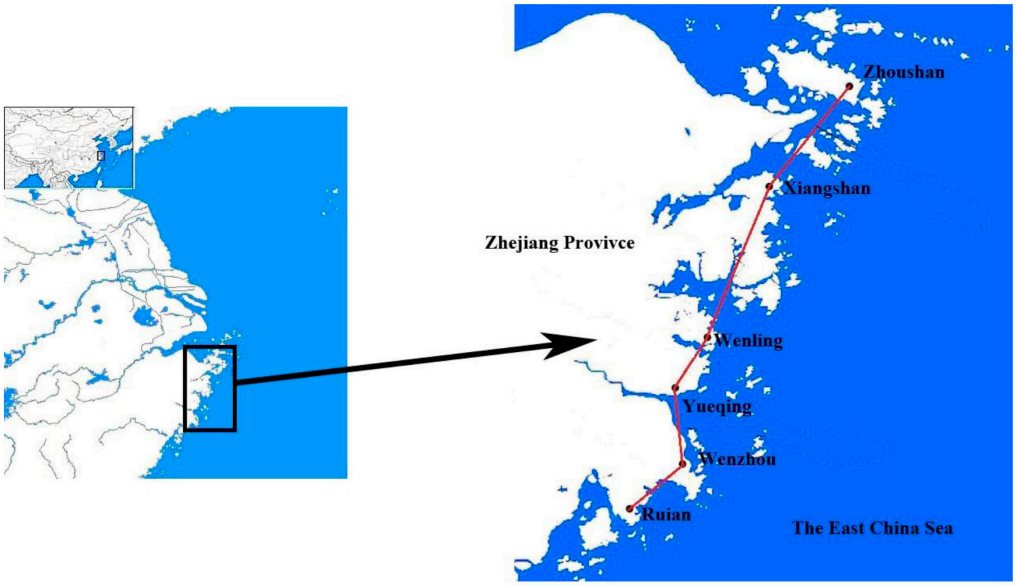

Figure 1. Sample locations for the miiuy croaker.

Data analysis

For the six populations, percentage of polymorphic loci, observed number of alleles (N_A), effective number of alleles (N_E), observed heterozygosity (H_O), expected heterozygosity (H_E), Shannon's information index (I), Nei's genetic diversity, and genetic distance were calculated by POPGENE version 1.32 (Yeh and Yang, 1997). Molecular variances within and between six populations were estimated by analysis of molecular variance (AMOVA) using ARLEQUIN 3.0 (Excoffier et al., 2005). The distribution of populations was presented using principal coordinate analysis (PCA) (Gower, 1966). MVSP (Multivariate Statistical Package ver. 3.1) software was used to construct PCA graphs the (Kovach, 1999).

RESULTS

SSR polymorphism and genetic variation

The 10 loci were all polymorphic in the populations studied. The detail of polymorphism is given in Table 1. The average H_O was 0.31 and average H_E was 0.43. The hierarchical F-statistics estimated according to Wright (1965), ranged from 0.03 to 0.14, with an average of 0.08. A total of 40 observed alleles were detected. N_E per locus ranged from 1.18 to 7.52.

Table 1. Genetic differentiation and the gene flow among populations of miiuy croaker.

Locus	N_A	N_E	N_m	H_O	H_E	F_{IS}	F_{IT}	F_{ST}	PIC	I
Mimi-1	4	1.85	6.71	0.47	0.48	0.10	0.06	0.04	0.39	0.78
Mimi-19	3	1.25	3.38	0.15	0.20	0.20	0.26	0.07	0.19	0.42
Mimi-24	3	1.46	1.53	0.11	0.32	0.66	0.71	0.14	0.29	0.59
Mimi-28	3	1.38	1.68	0.19	0.28	0.08	0.20	0.13	0.25	0.52
Mimi-16-E10	3	1.66	7.43	0.38	0.40	0.04	0.01	0.03	0.33	0.64
Mimi-56-G06	6	2.84	7	0.48	0.65	0.19	0.28	0.12	0.61	1.32
Mimi-43-H04	10	7.52	2.98	0.54	0.87	0.29	0.35	0.08	0.85	2.13
Mimi-13-G10	3	2.36	9.52	0.46	0.58	0.13	0.16	0.03	0.49	0.94
Mimi-21-G10	2	1.18	2.07	0.07	0.15	0.40	0.46	0.11	0.14	0.289
Mimi-42-G06	3	1.49	2.92	0.23	0.33	0.22	0.28	0.08	0.29	0.60
Mean	4	2.30	4.52	0.31	0.43	0.23	0.27	0.08	0.38	0.82

N_A = observed number of alleles; N_E = effective number of alleles; N_m = gene flow; H_O = observed heterozygosity; H_E = expected heterozygosity; F_{IS} = within-population inbreeding coefficient; F_{IT} = among-population inbreeding coefficient; F_{ST} = F-statistic, $N_m = 0.25 (1 - F_{ST}) / F_{ST}$; PIC = polymorphism information content; I = the Shannon's information index.

For the six populations, the average polymorphism information content per locus ranged from 0.14 to 0.85. The average Shannon index (I) was 0.8230 for each locus. Values of gene flow at each locus varied with a range of 1.53 to 9.52. Analysis of genetic variation between populations of miiuy croaker based on 10 SSR loci is shown in Table 2. N_A was from 2.5 to 3.5 and N_E was from 1.81 to 2.37. AMOVA analysis suggested that genetic variation occurred mainly within individuals (71.97%), while that between populations was only 28.03% (Table 3).

Table 2. Details on effective number of alleles per population (N_E); observed number of alleles (N_A); and Shannon's information index (I) of genetic variation within populations of miiuy croaker.

Population	N_A	N_E	I
Zhoushan	3.5	2.37	0.85
Wenling	3.2	1.81	0.66
Wenzhou	3.4	2.18	0.73
Yueqing	3.0	2.04	0.83
Xiangshan	2.5	1.92	0.58
Ruian	2.6	1.98	0.64

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

Source of variation	d.f.	Sum of squares	Variation components	Percentage of variation	F statistic	P
Among populations	5	20.50	0.063	28.02	0.03	<0.01
Within populations	89	173.17	0.53	71.97		
Total	94	193.67	0.60			

Population structure

Data only from the polymorphic SSR loci were analyzed by MVSP. PCA results carried out with MVSP are shown in Figure 2. We constructed a two-dimensional scatter plot for the first two principal factors for the six miiuy croaker populations. We found that the Yueqing and Wenzhou populations could be in one cluster, and the other four populations could be in

another cluster. The genetic distance between the six populations showed remarkable differences, ranging from 0.03 to 0.10 (Table 4).

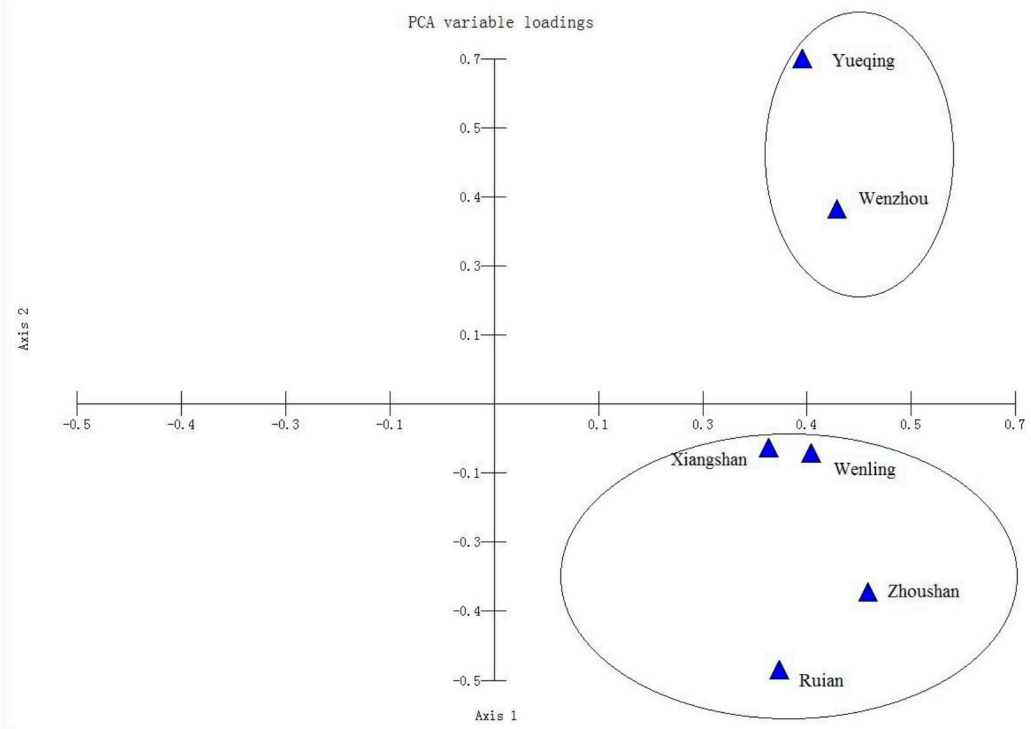

Figure 2. Two-dimensional scatter plot for the first two principal factors for six miiuy croaker populations.

Table 4. Nei's genetic distance of the six populations.

	Zhoushan	Wenling	Wenzhou	Yueqing	Xiangshan	Ruian
Zhoushan	-					
Wenling	0.0517	-				
Wenzhou	0.0415	0.0276	-			
Yueqing	0.0827	0.0888	0.0376	-		
Xiangshan	0.0563	0.0650	0.0557	0.0685	-	
Ruian	0.0780	0.0764	0.0687	0.1014	0.0488	-

DISCUSSION

Population genetic structure of a species can provide critical information for developing conservation and management strategies. In this study, we provided new evidence of genetic diversity for the miiuy croaker. Marine migratory fishes with high dispersal capabilities and large effective population sizes are anticipated to show high levels of gene flow and a low degree of differentiation (Beheregaray and Sunnucks, 2001). AMOVA showed that the

molecular variations observed in the miiuy croaker mainly occurred within the populations, which may suggest a weak and unstable regional genetic structure in this species. Such population structuring could indicate that gene flow is reduced between these regions.

Heterozygosity is an important index for population variation at the genetic level; H_O could be easily influenced by sample size, so H_E can reflect the genetic diversity better. The average H_E in this study at the population level was 0.43. It showed that genetic diversity in the species was relatively high. In addition, the average PIC (0.38) also proved the same result. The species with a relatively high average N_E suggests that it has good genetic variation and could be amenable to protection of germplasm resources, while there should be concern for the population with a lower genetic differentiation with regard to enhancing protection. In this research, the average N_E was 2.30, so we can conclude that this species has a relatively steady genetic diversity. Genetic diversity is closely related to adaptive power, viability and evolutionary potential. Finally, it also showed that this species has the genetic potential for breeding.

Genetic diversity and molecular systematic data can contribute to the development of effective conservation strategies. The genetic data obtained here for the miiuy croaker based on microsatellite markers demonstrated indirectly the adaptive genetic diversity. These data provide genetic information for each population, so that a great amount of genetic variation in the miiuy croaker can be preserved.

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