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# Evaluation of genetic diversity in *Pampus argenteus* using SSR markers

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**ABSTRACT.** In order to evaluate the germplasm resources of *Pampus argenteus* silver pomfret, the genetic diversity and population structure of 132 silver pomfret samples collected from the three regions (the East China Sea, the Yellow Sea and the Bohai Sea) were examined using 13 polymorphic microsatellite loci. Results indicated a high level of genetic diversity. The total number of observed alleles was 68, the mean allele number was 5.46 per locus, and the mean number of effective alleles was 4.91. The polymorphism information content ranged from 0.58 to 0.88. For the 13 polymorphic microsatellite loci, the results of analysis of molecular variance indicated that 92.45% of the genetic variation was contained within populations. Unweighted pair group method with arithmetic mean cluster analysis revealed significant genealogical branches or clusters corresponding to sampling localities. We concluded that there was high genetic diversity in these silver pomfret populations, and that this diversity was related to the complex environment. These results would contribute to important knowledge of genetic diversity and population structure, which would be crucial for establishing appropriate fishery management stocks for this species.

**Key words:** *Pampus argenteus*; Microsatellite marker; Genetic diversity; Population structure

 

## INTRODUCTION

The silver pomfret (*Pampus argenteus*), which belongs to the family Stromateoidei, is mainly distributed in the western regions of the Pacific Ocean (Kagwade, 1988), the eastern part of China, the western and southwestern Korean Peninsula and in western Asia all the way to the Gulf region (Cho et al., 1989). Silver pomfret is an inshore species and is usually found in schools over muddy bottoms, where it is generally associated with fish species of the genera *Nemipterus* and *Leiognathus*. Adults feed on ctenophores, salps, medusae and other zooplankton.

Silver pomfret is a highly valued marine food fish species and with worldwide market demand (Pati, 1982). The silver pomfret constitutes nearly 84% of the pomfret landings worldwide, and plays an important role in the fisheries of Kuwait, Iran, China, India, Korea, Malaysia, Thailand and Japan (FAO, 1995). However, in recent years, the silver pomfret fishery has been declining because of overfishing (Wen et al., 2006), especially in China. Currently, the fishery stocks in the China Sea are confirmed to be under environmental stress because of high fishing intensity and ecological change. Increased fishing behavior may prevent reproduction of a fish species and result in changes of its genetic diversity and population structure. With such a restrictive resource base, the germplasm of silver pomfret is declining at a rapid rate. Some surveys have indicated that the average body length of silver pomfret in coastal waters decreased from 19.8 cm in 1979 to 14.8 cm in 2003. Furthermore, the age composition and sexual maturity of silver pomfret are also both in decline (Zheng, 2003). Therefore, an association study for resource recovery of silver pomfret is urgently needed to help prevent further recession of these germplasm resources.

Population genetic analysis has been proven to be the best tool for evaluating genetic diversity and for obtaining information about the population structure of a species (Crandall et al., 1999). In general, marine fish species tend to show little intraspecific genetic differentiation among geographically separated populations (Ward et al., 1994). Ocean currents and the apparent lack of physical barriers seem to greatly facilitate extensive gene flow among marine fish populations (Palumbi, 1994). In silver pomfret, spawning occurs in the East China Sea from early April to late May, but occurs later (from May to June) in the Yellow Sea (Shi et al., 2005; Zhao et al., 2010). This phenomenon suggested that there are at least two clusters of silver pomfret; however, more accurate data are required to confirm this phenomenon.

Molecular genetic techniques offer the ability to identify and delineate fish stock structure. Several molecular markers have been currently used successfully to understand the structure of aquatic species (Peng et al., 2010; Zhao et al., 2011). Microsatellite simple sequence repeat (SSR) markers have been considered among the most efficient molecular markers, based on their ability to provide abundant genetic information because of their co-dominance, high mutation rates, abundance throughout the genome and ease of scoring. Therefore, such markers could be useful to determine the genetic diversity and population structure of the silver pomfret.

In this study, silver pomfret collected from seven geographic locations of the East China Sea, the Yellow Sea, and the Bohai Sea were analyzed using SSR markers to characterize genetic diversity and population structure. This baseline information will be critical for establishing proper management and harvesting regulations.

## MATERIAL AND METHODS

### Sample collection, DNA extraction, and SSR analysis

Wild samples of silver pomfret were collected from the following seven geographic locations: Zhoushan, Xiangshan, Yueqing, and Wenling (in the East China Sea), Qingdao and Rizhao (in the Yellow Sea) and Laizhou (in the Bohai Sea) (Figure 1, Table 1). Fin clips were collected from wild silver pomfret individuals and were preserved in 95% ethanol. All individuals were 18-20 cm in length and weighed 290-320 g. Genomic DNA was isolated following the standard phenol-chloroform method with some modifications, which was subsequently dissolved in Tris-ethylenediaminetetraacetic acid buffer.


**Figure 1.** Maps of the silver pomfret (*Pampus argenteus*) sampling locations and regions.

Thirteen polymorphic SSR loci that were developed from a previously analyzed microsatellite-enriched library were used in this study (Qin et al., 2012). Polymerase chain reaction (PCR) was carried out for each individual in 15-μL reaction volumes, containing 10 μL sterilized water, 1.5 μL 10X PCR buffer with 1.5 mM $Mg^{2+}$, 1.2 μL 2.5 mM dNTPs, 0.6 μL forward primer, 0.6 μL 50 ng/mL reverse primer, 1 μL 0.1 μg/μL diluted DNA template, and 0.1 μL 5 U/μL *Taq* DNA polymerase (Tiangen). PCR amplification was performed on an ABI9700 thermal cycler according to the following profile: 95°C for 5 min; 95°C for 30 s, annealing temperature for 30 s, 72°C for 30 s, 30 cycles; and 72°C for 5 min. PCR products

were denatured for 8 min at 96°C, and were then separated on 6% denaturing polyacrylamide (19:1 acrylamide:bis-acrylamide) gels using silver staining. Denatured pBR322 DNA/*Msp*I molecular weight marker (Tiangen) was used as a size standard for determining allele sizes.

**Table 1.** Details about sample location.

| Population | Location | $N_A$ | $N_E$ | $I$ |
|---|---|---|---|---|
| Zhoushan | 30.05°N, 122.10°E | 5.38 | 4.51 | 1.51 |
| Wenling | 28.44°N, 121.34°E | 5.46 | 4.71 | 1.53 |
| Yueqing | 28.19°N, 121.02°E | 5.46 | 4.61 | 1.52 |
| Xiangshan | 29.53°N, 121.92°E | 5.31 | 4.28 | 1.47 |
| Rizhao | 35.37°N, 119.49°E | 5.46 | 4.62 | 1.53 |
| Qingdao | 36.11°N, 120.46°E | 5.31 | 4.36 | 1.49 |
| Laizhou | 37.24°N, 119.97°E | 5.46 | 4.85 | 1.56 |

$N_A$ = observed number of alleles; $N_E$ = effective number of alleles per population; $I$ = Shannon's information index of genetic variation within populations of *Pampus argenteus*.

## Data analysis

SSR bands were scored in the form of single individual genotypes, and arranged in a genotype matrix for analysis of allelic variation. For the seven populations, the 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 using Popgene Version 1.32. Genetic relationships among the seven populations were estimated by constructing a phylogenetic tree with MEGA 4.0 (Kumar et al., 2008). The partitioning of molecular variation within and among the seven populations was estimated with Arlequin 3.0 (Excoffier et al., 2005). On the basis of the inter-population genetic distance, an unweighted pair group method with arithmetic mean (UPGMA) dendrogram was constructed used the MEGA software. To assess the genetic structure of silver pomfret, we used a Bayesian Markov Chain Monte Carlo approach to estimate the number of genetic clusters. This model-based analysis was run using the STRUCTURE version 2.2.2 program (Pritchard et al., 2000). A pre-assigned number of genetic clusters (K) in the dataset was used, which was characterized by a set of allele frequencies at each locus under the assumption of Hardy-Weinberg equilibrium and linkage equilibrium. For each K value ranging from 2 to 7, we ran 40 replicates with a burn-in length of 150,000 followed by 1,000,000 iterations of each chain using the admixture model along with the assumption of correlated allele frequencies between groups (Falush et al., 2003). We also used K values from 2 to 7 for calculations in the cluster analysis. The seven populations were divided into three clusters when the K value was 3, which was the expected result.

# RESULTS

## SSR polymorphism and genetic variation

The 13 loci were all polymorphic in all of the studied populations and details of these polymorphisms are summarized in Table 2. The average $H_O$ was 0.71, and the average $H_E$ was 0.77. The *F*-statistics (Weir and Cockerham, 1984) which accounted for differences in sample sizes among populations, ranged from 0.01 to 0.08, with an average of 0.02. $N_A$ was detected

with a mean allele number of 5.46 per locus (ranging from 3 to 10). Considering all seven populations, the polymorphic information content (PIC) per locus ranged from 0.58 to 0.88, which indicated that all populations were highly polymorphic (PIC > 0.5; Shete et al., 2000). The average *I* was 1.07 for each locus. The estimated *N*m was variable, ranging from 2.89 to 21.50 after analyzing the populations in three clusters. Analysis of genetic variation among populations of silver pomfret based on the 13 SSR loci is shown in Table 2. In Table 1, $N_A$ ranged from 5.31 to 5.46 and $N_E$ ranged from 4.28 to 4.85.

**Table 2.** Relative measurements of genetic differentiation and the estimates of gene flow among populations of silver pomfret.

| Locus | $N_A$ | $N_E$ | $N_m$ | $H_O$ | $H_E$ | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ | PIC | $I$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Paar-Y13 | 4 | 3.39 | 12.57 | 0.56 | 0.71 | 0.20 | 0.21 | 0.02 | 0.65 | 1.293 |
| Paar-8 | 4 | 3.49 | 2.89 | 0.53 | 0.72 | 0.19 | 0.25 | 0.08 | 0.66 | 1.313 |
| Paar-12 | 4 | 3.75 | 20.64 | 0.74 | 0.74 | 0.01 | 0.01 | 0.01 | 0.68 | 1.353 |
| Paar-Y59 | 4 | 3.55 | 10.75 | 0.82 | 0.72 | 0.17 | 0.14 | 0.02 | 0.67 | 1.323 |
| Paar-Y99 | 3 | 2.89 | 10.10 | 0.59 | 0.66 | 0.07 | 0.09 | 0.02 | 0.58 | 1.08 |
| Paar-Y37 | 7 | 6.47 | 12.34 | 0.65 | 0.85 | 0.22 | 0.23 | 0.02 | 0.83 | 1.91 |
| Paar-Y33 | 10 | 9.30 | 19.95 | 0.76 | 0.90 | 0.14 | 0.12 | 0.01 | 0.88 | 2.261 |
| Paar-Y57 | 6 | 5.84 | 16.64 | 0.71 | 0.83 | 0.13 | 0.15 | 0.01 | 0.82 | 1.78 |
| Paar-133 | 5 | 4.07 | 10.47 | 0.71 | 0.76 | 0.04 | 0.06 | 0.02 | 0.713 | 1.498 |
| Paar-Y14 | 7 | 6.09 | 9.16 | 0.87 | 0.84 | 0.07 | 0.02 | 0.03 | 0.813 | 1.868 |
| Paar-14 | 4 | 3.61 | 21.50 | 0.81 | 0.73 | 0.13 | 0.12 | 0.01 | 0.673 | 1.338 |
| Paar-108 | 10 | 8.42 | 17.45 | 0.94 | 0.88 | 0.08 | 0.03 | 0.01 | 0.87 | 2.21 |
| Paar-Y34 | 3 | 2.99 | 16.78 | 0.56 | 0.67 | 0.15 | 0.16 | 0.01 | 0.59 | 1.10 |
| Mean | 5.46 | 4.91 | 10.96 | 0.71 | 0.77 | 0.12 | 0.12 | 0.02 | 0.73 | 1.56 |

$N_A$ = 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-statistics; PIC = polymorphism information content; $I$ = Shannon's information index.

## Population structure

Analyses of molecular variance (AMOVA) indicated that 92.45% of the genetic variance was contained within populations, and 7.55% was due to differences among populations (Table 3). The genetic distances among the seven populations were variable, ranging from 0.22 to 0.13 (Table 4). The UPGMA tree showed that there were significant genealogical branches or clusters corresponding to sampling localities (Figure 2).

**Table 3.** Analysis of molecular variance among and within the seven populations.

| Source of variation | d.f. | Sum of squares | Variation components | Percentage of variation | *F* statistic | P |
|---|---|---|---|---|---|---|
| Among populations | 6 | 30.425 | 0.0083 | 7.55 | 0.0731 | P < 0.01 |
| Within populations | 133 | 710.000 | 4.6107 | 92.45 | | |
| Total | 139 | 740.425 | 4.6190 | | | |

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

| | Zhoushan | Laizhou | Xiangshan | Wenling | Rizhao | Yueqing | Qingdao |
|---|---|---|---|---|---|---|---|
| Zhoushan | | 0.00437* | 0.01819* | 0.00733* | 0.01207* | 0.01473* | 0.01198* |
| Laizhou | 0.1007 | | 0.00738 | 0.01381* | 0.00094* | 0.00511* | 0.00656* |
| Xiangshan | 0.0217 | 0.1108 | | 0.01397* | 0.00849* | 0.01831* | 0.00756* |
| Wenling | 0.0524 | 0.1267 | 0.0327 | | 0.01520* | 0.01571* | 0.00967* |
| Rizhao | 0.1265 | 0.0882 | 0.1129 | 0.1299 | | 0.00355* | 0.01472* |
| Yueqing | 0.0337 | 0.1000 | 0.0229 | 0.0285 | 0.0927 | | 0.00389* |
| Qingdao | 0.1216 | 0.1044 | 0.1057 | 0.1076 | 0.0327 | 0.0911 | |

*Indicates that the populations are significantly genetically different.

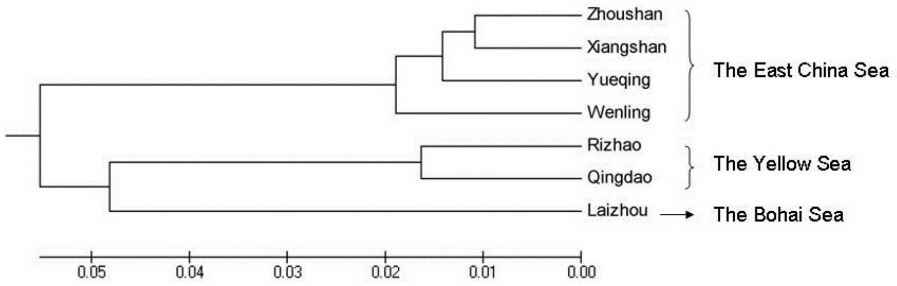


**Figure 2.** UPGMA dendrogram for seven populations of the silver pomfret based on Nei's genetic distance.

The population structure analysis showed that the seven populations could be divided into three clusters (Figure 3). STRUCTURE analysis suggested the presence of three genetic clusters as the most biologically reasonable solution, which was also indicated using the delta K method (Evanno et al., 2005). This result was consistent with the geographical distribution of the seven populations: samples from Zhoushan, Xiangshan, Wenling, and Yueqing belong to the East China Sea, samples from Qingdao and Rizhao belong to the Yellow Sea, and the last population belongs to the Bohai Sea.

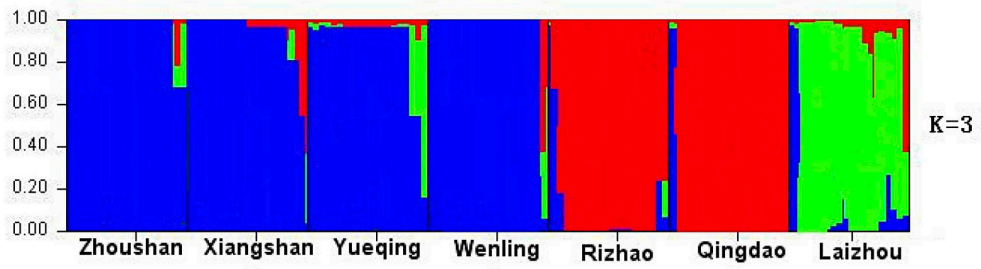


**Figure 3.** Genetic structure of seven silver pomfret populations. These populations were grouped in 3 ancestral clusters (K = 3) and reanalyzed to infer further structure.

## DISCUSSION

Since once abundant marine resources worldwide have now been vastly reduced due to unsustainable exploitation (Kenneth, 1991), many genetic studies have been initiated to investigate the impacts of fishing pressure on the germplasm resource of numerous species. In this study, we provide new evidence for high levels of genetic diversity in silver pomfret. The large population size that is characteristic of many marine fish species facilitates the maintenance of high levels of genetic diversity (Avise, 1998), and common life history traits, such as planktonic eggs and larvae, aid in the dispersal of genetic variation among populations (Palumbi, 1994).

In addition, our results provide evidence that silver pomfret in China have high genetic diversity and different populations have their own population structure. $F_{ST}$ values among the 13 loci were significantly different ($P < 0.001$), suggesting that the seven

populations originated from different geographic areas. AMOVA also supported significant differentiation among populations. The fixation index among populations in our study was 0.0731, which indicated a moderate level of genetic differentiation. Peng et al. (2009) found no significant genetic differentiation among silver pomfret from the Bohai area, the East China Sea area, and the South China Sea area using the cytochrome oxidase 1 (*COI*) gene of mitochondrial DNA sequences, which is in stark contrast to results of the present study. The main difference is likely because of the different methods and markers used. The *COI* gene is a mitochondrial DNA sequence, whereas SSRs are nuclear markers. The SSR markers that we used can resolve more genetic differentiation among populations than the mitochondrial DNA control region sequence.

Heterozygosity is an important index for assessing population variation at the genetic level; $H_O$ is easily influenced by sample sizes, while $H_E$ can better reflect genetic diversity (Bao et al., 2007). The average $H_E$ observed in the present study at the population level was 0.77, demonstrating that genetic diversity in the species is relatively high. In addition, the high average PIC value (0.73) confirmed this result. The populations also showed a high average effective number of alleles, which suggested that this species had high genetic variation and would be useful for germplasm resource protection, whereas populations with lower genetic differentiation should be paid more attention to and protection measures should be enhanced. In this study, the average $N_A$ was 5.46, further confirming that this species had high genetic diversity. Genetic diversity is closely related to adaptability, viability, and evolutionary potential. Ultimately, it could result in weakening of the species. In the present study, the Shannon diversity index for each locus ranged from 1.08 to 2.26, with an average of 1.48. This result showed that there is abundant genetic diversity in silver pomfret. Owing to the high genetic diversity maintained under high fishing intensity, this species has good genetic potential for breeding.

Population genetic structuring has been reported in several widely distributed marine species (Chapman et al., 1999; Aubert and Lightner, 2000; Rhodes et al., 2003). Based on Nei's genetic distance, the present cluster analysis indicated that samples from the Zhoushan, Yueqing, Xiangshan, and Wenling locations were obviously separated from the other three populations, and the Rizhao and Qingdao populations were separated from the Laizhou population. In other words, populations from the East China Sea, the Yellow Sea, and the Bohai Sea all showed unique evolutionary trajectories. In conclusion, samples of the seven populations could not only be distinguished from each other, but could also be easily separated among the three seas. According to the STRUCTURE program, when the K value was three, the seven populations were divided into three clusters. This result indicated that the three clusters had their own gene pools which differed from each other. In fact, the Yellow Sea and the Bohai Sea belong to the same sea area. However, because of the isolation of the Shandong Peninsula, the Laizhou population evolved independently from the Bohai Sea population. The structure analysis clearly indicated that geographic isolation directly led to evolution between populations. Nonetheless, some genetic infiltration among populations was also apparent. This was likely because geographic isolation was not very strict, facilitating partial crossing among populations. Overall, these results indicated that these silver pomfret populations have abundant evolutionary potential.

Genetic diversity and molecular systematic data can contribute to the development of effective conservation strategies. The genetic data obtained here from silver pomfret based on microsatellite markers reflected indirect adaptive genetic diversity. These results provided

useful data of unique genetic information of each population, which would aid in preserving a large amount of genetic variation in silver pomfret. Preserving such variation might help to inhibit the dissolution of locally well-adapted phenotypes (Godt et al., 1996; Ueno et al., 2005; Jones and Gibson, 2011). Although such information would help to maintain a species within its ecological community, more genetic diversity researches related to adaptive traits and knowledge about ecosystem functions and species interactions would be helpful for understanding how the species adapt to changing environmental conditions.

In summary, all of the indices calculated in the present study indicated high genetic diversity among geographically distinct silver pomfret populations, and this species showed clearly distinct differentiating potential. These results could prove to be useful for further protection of this species resources.

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