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Full Length Research Paper

Genetic analysis of farmed and wild stocks of large yellow croaker *Larimichthys crocea* by using microsatellite markers

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The large yellow croaker (*Pseudosciaena crocea*) is one of the most economically important mariculture fish species in China. In this study, the genetic diversity and relationship among a wild stock, four farmed stocks and a selectively bred strain of large yellow croaker were assessed by 14 microsatellite markers. A total of 108 different alleles were detected over all loci. The average number of allele per locus ranged from 5.57 to 7.93, with an average of 6.75; the observed and expected heterozygosity ranged from 0.572 to 0.665 and from 0.649 to 0.751, with an average of 0.621 and 0.694, respectively; the Shannon's diversity index ranged from 1.34 to 1.64, with an average of 1.48. The selectively bred strain had the lowest genetic diversity; all farmed stocks showed a slight reduction of genetic variability contrasted with wild stock. All stocks suffered severe bottleneck. The pair-wise F_{ST}, the phylogenetic tree, the factor correspondence analysis and the model based clustering analysis revealed that, the Ningbo stock, which was from Zhejiang province, was different from the remaining stocks from Fujian province. This study suggested that (1) the farmed stocks were at relatively low level of genetic diversity compared with the wild stock; (2) samples from Ningbo investigated in this study have a distinct divergence with those from Fujian province; (3) there had emerged significant differentiation among farmed stocks.

Key words: *Pseudosciaena crocea*, large yellow croaker, genetic structure, microsatellite markers.

INTRODUCTION

The large yellow croaker is one of the most economically important mariculture fish species in China. It is mainly distributed in coastal regions of East Asia, from Yellow Sea to South China Sea, especially in the coasts of Zhejiang and Fujian provinces of China. At early 1970s, the annual captured amount of wild large yellow croaker was more than 100,000 tons. However, because of overfishing, the nature resource had severely declined since 1970s, and it had nearly exhausted from the middle of 1980s to the end of 1990s; therefore, the researches on artificial hatchery technique were launched in 1985 and brought a breakthough in 1987. By 2006, the annual cultured production of large yellow croaker reached 69,600 tons, up to 2 billion fingerlings were produced (Wang et al., 2007). However, with the rapid development of large yellow croaker mariculture industry, a series of problems including small size broodstocks for breeding, over-density of mariculture and deterioration of environment had been emerged. All of those problems raised the erosion of the commercial characters, such as growth decreasing, frequent diseases and poor taste.

The molecular markers (for example, allozyme, RFLP, RAPD, AFLP, SSR, SNP and EST) have reproducibility and high polymorphism contrasted with the traditional marker techniques (phenotypic markers). They have made aquaculture studies more rapid, efficient and stable (Liu and Cordes, 2004). In previous studies, allozymes, RAPD and AFLP had been used in the researches on large yellow croaker (Quan et al., 1999; Wang et al.,

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Table 1. The stock information of large yellow croaker used in this study.

Stock	Region	Location	Number	Sampling time
NB	Zhejiang province	Ningbo	48	November, 2007
FD	Fujian province	Fuding	48	December, 2007
ND	Fujian province	Ningde	48	December, 2007
LJ	Fujian province	Lianjiang	48	December, 2007
MY	Fujian province	Experimental station	48	December, 2007
Wd	Fujian province	GuanJingYang sea	48	October, 2009

2002; Ding et al., 2006). However, so far no information about genetic characteristics of the farmed, selectively bred and wild of large yellow croaker are reported, despite their importance for fishery industries in China.

The microsatellite technique is a powerful tool and it has been widely used in many researech areas of fish genetics and breeding (O'Really and Wright, 1995; DeWoody and Avise, 2000; Alarcón et al., 2004; Liu and Cordes, 2004). In this study, we presented the status of genetic diversity and relationship for six large yellow croaker stocks by using 14 polymorphic microsatellite markers (Guo et al., 2005). This information could be useful to understand the current genetic characteristics of large yellow croaker and give sights to the preservation and management of the large yellow croaker resources in China.

MATERIALS AND METHODS

Samples collection and DNA extraction

Six samples including one farmed stock from Ningbo (NB) of Zhejiang province, three farmed stocks from Fuding (FD), Lianjiang (LJ) and Ningde (ND) of Fujian province, one wild stock (Wd) captured from Guanjingyang sea in Fujian province where is the most important mariculture area in China and one selectively bred strain Minyou no.1 (MY) were collected (Table 1; Figure 1). Each sample consisted of 48 individuals. Fin clip of each fish was collected and preserved in 75% ethanol. Genomic DNA of each individual was extracted from fin clip by using the standard phenol-chloroform technique (Wang et al., 2000) and then its concentration was fitted to 30 ng/µl.

Microsatellite genotyping

14 polymorphic microsatellite markers which developed by our laboratory were chosen for this study. Polymerase chain reaction (PCR) were performed in 10 µl volume containing 60 ng DNA, 1 × PCR buffer, 0.1 µM each primer set, 0.25 mM dNTP mix, 1.5 mM $MgCl_2$, 0.5 unit of Taq DNA polymerase(Takara, Dalian, China). The amplification was carried out by thermal cycler (MJ PTC200, MJ Research, USA). The PCR condition involved denaturing at 95°C for 5 min, and then followed by 30 cycles of 30 s at 95°C, 30 s at annealing temperature (Table 2), 30 s at 72°C and final extending for 10 min at 72°C. The PCR products were separated via electrophoresis on 6% denatured polyacrylamide gels. The fragments were visualized by silver staining (Wang et al., 2004) and alleles were sized by a 10 bp DNA ladder (Invitrogen, USA).

Statistical analysis

The observed and expected heterozygosity (H_O and H_E), Nei's unbiased genetic distance (Nei, 1978) (D_S) and Shannon's diversity index (I) were calculated by PopGene 1.32 (Yeh et al., 2000). The convert program (Glaubitz, 2004) was used to identify the diagnostic alleles per stock. The diagnostic allele was present in one stock and absent from other stocks. The null alleles in each stock were presented by Micro-Checker 2.2.3 (Van Oosterhout et al., 2004).

FSTAT 2.9.3.2 (Goudent, 1995) was used to analyze the number of alleles per locus and assessed Wright's F-statistics parameters, F_{ST} and F_{IS}. Arlequin 3.1 (Excoffier et al., 2005) was used to calculate the pair-wise F_{ST} of the six stocks and conducted an exact test of deviation from Hardy-Weinberg (H-W) equilibrium per locus based on 5,000 Markov chain iterations.

The Bottleneck 1.2.02 program (Piry et al., 1999) was used to investigate the evidence of recent bottlenecks. Generally, at selectively neutral loci the allele number and heterozygosity were at equilibrium between mutation and genetic drift. Therefore, in an equilibrium population, the expected heterozygosity (H_{eq}) which was calculated based on the observed number of alleles and the number of individuals equals to the heterozygosity (H_e) which was measured by Hardy-Weinberg equilibrium (Luikart and Cornuet, 1998). In contrast, when a recent bottleneck occurred, the mutation-drift equilibrium is transiently disrupted and the H_e will exceed the H_{eq}, because alleles lose faster than heterozygosity (Cornuet and Luikart, 1996; Luikart and Cornuet, 1998). As a result, one recent bottlenecked population is expected to have lots of loci with excess of heterozygosity (Luikart and Cornuet, 1998). In addition, three models (SMM, IAM and TPM) were used to perform the analysis by repeating 20,000 replications.

According to the Nei's genetic distance, phylogenetic tree was constructed by MEGA 4.0 program (Tamura et al., 2007) by Neighor-Joining algorithm. In addition, in order to gain an alternate method for identifying stock structure and to provide further insight into the genetic diversity, factor correspondence analysis (FCA) was applied by Genetix procedure (Belkhir et al., 2004) based on multi-locus genotypes. Also, individuals were clustered by the model based clustering method as applied in Structure 2.3.1 (Pritchard et al., 2000). This program could identify subgroups that own distinctive allele frequencies and assign individuals into K clusters (K was set beforehand, but could be varied across all runs.) according to their 'membership coefficients' which was interpreted as a probability of membership to each cluster. Analysis was performed under two conditions. One was the 'admixture' model, an approach that can tolerate mixed ancestry of individuals and the other one was correlated allele frequencies that were quite similar because of the shared ancestry or migration in different stocks (Pritchard et al., 2000). Twenty (20) runs for K value were performed from K = 1 to 8 using 100, 000 bure-in steps before 100, 000 Markov Chain Monte Carlo repeats. True number of clusters (K) was often identified by the maximal log probabilities Pr(X | K) called 'Ln P (D)' in structure output (Zeisset and Beebee, 2001;

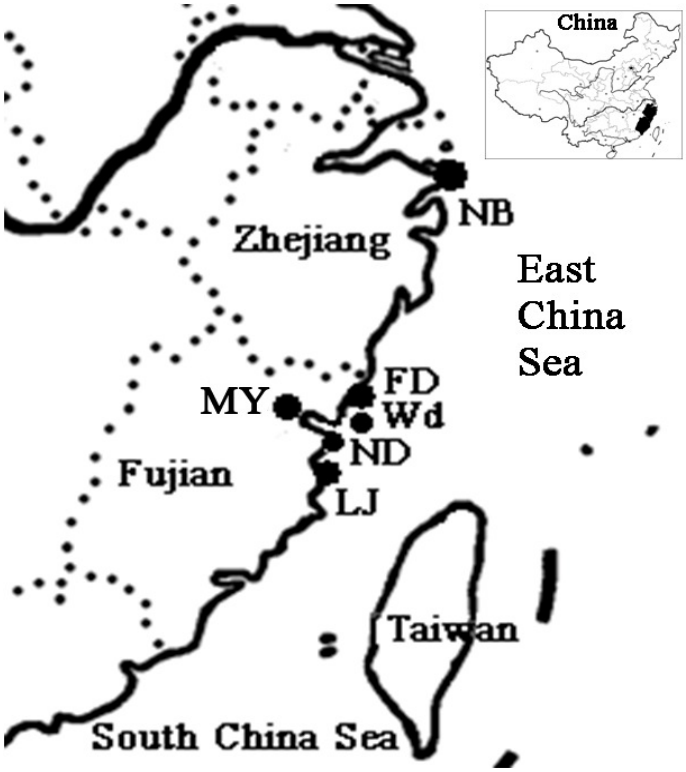

Figure 1. Distribution of six stocks of large yellow croaker collected from South China.

Table 2. The summary information for 14 microsatellites of large yellow croaker used in this study.

Loci	Motif	Primer sequence (5′–3′)	*Ta* (℃)	Size of allele (bp)
LYC0002	$(TG)_2(AC)_2.(AC)_{10}.(AC)_5$	F:5'-ACCTCCAGTGGGATGTGA-3' R:5'-GGCTGTTTGTTATAATTTGTG-3'	50	60-110
LYC0004	$(TG)_9$	F:5'-CTCTTAGCCGTCATTCATCC-3' R:5'-CATTTAGCCAAGTTCACTTCC-3'	55	90-100
LYC0008	$(GT)_{11}$	F:5'-GAAACAATAGCTCGCTCCTG-3' R:5'-GACTCTGCCAGCACATTAGTG-3'	57-55	151-185
LYC0009	$(GT)_{10}TTA(TG)_4CTG$	F:5'-GTCAATCACGTCTGTCTCTGC-3' R:5'-TCAGCCATTGTCTGTGAGGT-3'	60	75-105
LYC0010	$(TG)_{19}$	F:5'-GTCTCAGCTGACTCCTGCTTC-3' R:5'-ATGGCTCTAAACATGGTAGG-3'	55	205-235
LYC0011	$(TG)_{11}$	F:5'-CTTTTATTGGCTCCGTATGA-3' R:5'-CACTCACACTAGCACGCAC-3'	55	82-133
LYC0012	$(AC)_7$	F:5'-CAGAACAAACAATGAATGGG-3' R:5'-GAGGAGCTCAACAGCAACA-3'	55	90-145
LYC0013	$(GT)_{28}$	F:5'-GCTGCGAGCTACTTTACTCAT-3' R:5'-AACTCACAAACATGCAC-3'	50	130-220
LYC0015	$(AC)_7…(AC)_2$	F:5'-ACAGTCTAAAGCTGCCAGCA-3' R:5'-TGAGACCAACCACATTTCTGT-3'	55	103-117
LYC0016	$(GT)_3…(GT)_{11}A(TG)_4$	F:5'-GAGCCTTGTGTGGTGAGCA-3' R:5'-GAAAACCCAGACCGTATTGT-3'	55-50	110-140
LYC0022	$(TG)_3C(GT)_2…(GT)_2CT(GT)_3…(GT)_{11}$	F:5'-AGAGATAACGTAGACATGATTG-3' R:5'-CAGCAAAAGTTCAAAATGGAG-3'	55-50	143-250

Table 2. continues.

LYC0027	$(AT)_2(GT)_9G(GA)_2…(GA)_2$ …$(TGA)_3(TG)_4$	F:5'-CACCCAATAATATCGCCATA-3' R:5'-GCACACACAATCATCATCATT-3'	50	74-95
LYC0033	$(CAGA)_2GG(GA)_3GG(GA)_7$	F:5'-GGATGGAGGAGTGATGATGG-3' R:5'-GCACTGAGACCTGAATGCTCC-3'	50	150-160
LYC0036	$(TA)_2CT(CA)_9$	F:5'-GCATTCATGGATTAGACTGC-3' R:5'-GGGTGAGTGTCGGAAGTTC-3'	50	203-225

Vernesi et al., 2003; Evanno et al., 2005) and it was also used in this study to determine the true number of clusters.

RESULTS

Null alleles, HWE, bottleneck effects and F_{IS}

Each stock presented the possible null alleles; accordingly, the data set was adjusted to be used in further analysis. The loci of deviation from H-W equilibrium were observed in each stock (Table 3). At *LYC0016* locus, all stocks were deviated from H-W equilibrium and five stocks were deviated from H-W equilibrium at *LYC0010* locus. The bottleneck analysis indicated IAM and SMM had more locus with H_e than that with H_{eq} except for three cases of FD, MY and Wd stocks in SMM; TPM was also detected heterozygosity excess by one tail or two tail test in all stocks (Table 4). The F_{IS} value of NB, ND, MY and Wd stocks was relatively higher than zero, while the value of FD and LJ stocks was approximately zero, which meant the heterozygotes were absent in FD and LJ stocks (Table 3).

Genetic diversity

The mean allele of ND stock was the highest (7.93) and the lowest was MY strain (6.00). The expected heterozygosity ranged from 0.649 (MY) to 0.751 (Wd) and the observed heterozygosity varied from 0.572 (MY) to 0.665 (FD). The Shannon's diversity index (*I*) ranged from 1.34 (MY) to 1.64 (Wd) on average. Through comparison of allele frequencies and distribution, 45 diagnostic alleles (41.7% of the total alleles) were detected in all stocks. The Wd stock had the most diagnostic alleles (U = 12), which was followed by the NB stock (U = 9); the Minyou no.1 strain only had 4 diagnostic alleles (Table 3).

Stock structure

The value of the pair-wise F_{ST} of NB stock was bigger than any of the remaining ones, which indicated that it had higher differentiation from those of Fujian province; while the value of the pair-wise F_{ST} among stocks from Fujian province was relatively low. Nei's distances were ranged from 0.2326 between NB and Wd stocks to 1.2316 between FD and MY stocks (Table 5), which was in accord with the result of the pair-wise F_{ST} analysis. The neighbor-joining (NJ) dendrogram fell into two major clusters: MY, LJ, FD, ND, Wd stocks from Fujian province for one cluster and NB stock from Zhejinang for the other one. The former cluster was further segregated into four sub-clusters: ND, LJ and Wd stocks formed each cluster separately; FD and MY stocks belonged to another one (Figure 2). The clustering analysis by FCA revealed three major sets. The individuals of FD, ND, LJ and MY stocks were assigned to a bigger single set and the individuals of NB and Wd stocks were included in another two sets separately (Figure 3). Although, FD, ND, LJ and MY stocks are grouped into one set, they had clear borders. Further clustering analysis was performed by the model based clustering method under the condition of increasing number of inferred clusters (K = 1 to 8). The result suggested that six clusters were best fitted our data (Figure 4). The histogram output from Structure software (K = 6) clearly showed almost all individuals from one specific stock were consistently assigned to one cluster (Figure 5). In the histogram, each individual was represented by a vertical line which was partitioned into K different colored segments that represented the individual's estimated membership fractions. According to the fraction, the migrated or admixed individuals in each stock can be identified (Freeman et al., 2004; Corander and Marttinen, 2006). Two individuals in the yellow colored cluster (numbered 225 and 237 from LJ stock) were detected variable assignments and they had mixed genetic background with the pink and light-blue colored clusters, respectively.

DISCUSSION

Hardy-Weinberg equilibrium bottleneck effects

Deviation from H-W equilibrium could be traced to artificial or nature selection, inbreeding, stratification, existence of null alleles or mistyped heterozygote as homozygote (Balding, 2006). In breeding practice, kinship and small size broodstocks were commonly used so that inbreeding occurred easily and that might be the main reasons for the deviation from H-W equilibrium in this

Table 3. The genetic diversity of six stocks of large yellow croaker.

Parameter	NB	FD	ND	LJ	MY	Wd
LYC0002						
A	10	9	9	9	7	8
HE	0.87	0.83	0.82	0.83	0.82	0.83
HO	0.88	0.92	0.74	0.85	0.83	0.83
U	1	1	1	1	0	0
P		0.0217*				0.0446*
FIS	-0.011	-0.108	0.088	-0.018	-0.011	0.006
LYC0004						
A	4	5	6	5	5	6
HE	0.75	0.62	0.69	0.67	0.59	0.72
HO	0.86	0.62	0.79	0.81	0.49	0.67
U	0	0	0	0	0	0
P						
FIS	-0.145	0.01	-0.147	-0.218	0.178	0.075
LYC0008						
A	3	6	4	3	3	5
HE	0.53	0.68	0.61	0.46	0.53	0.67
HO	0.56	0.73	0.56	0.45	0.5	0.56
U	0	2	1	0	0	0
P						
FIS	-0.059	-0.074	0.083	0.022	0.058	0.169
LYC0009						
A	7	10	14	5	9	12
HE	0.74	0.8	0.9	0.7	0.76	0.84
HO	0.81	0.68	0.87	0.79	0.72	0.74
U	0	1	1	0	1	2
P					0.0334*	0.0480*
FIS	-0.103	0.155	0.028	-0.124	0.053	0.124
LYC0010						
A	7	7	10	10	7	8
HE	0.83	0.85	0.87	0.86	0.83	0.86
HO	0.54	0.63	0.52	0.71	0.52	0.68
U	2	0	0	1	0	3
P	0.0000**	0.0048**	0.0000**	0.0285*	0.0000**	
FIS	0.36	0.263	0.406	0.174	0.377	0.205
LYC0011						
A	7	8	10	5	10	17
HE	0.83	0.78	0.85	0.74	0.86	0.89
HO	0.67	0.72	0.79	0.56	0.81	0.73
U	1	0	1	0	2	2
P	0.0490*			0.0064**		
FIS	0.199	0.076	0.065	0.243	0.054	0.182
LYC0012						
A	7	12	13	10	9	10
HE	0.83	0.89	0.89	0.88	0.85	0.86

Table 3. conticue

HO	0.67	0.85	0.68	0.66	0.77	0.8
U	2	1	1	1	0	0
P			0.0083**	0.0000**		
FIS	0.191	0.049	0.238	0.253	0.01	0.076
LYC0013						
A	8	12	12	9	7	12
HE	0.83	0.84	0.88	0.85	0.74	0.86
HO	0.79	0.79	0.75	0.86	0.6	0.8
U	1	0	0	1	1	2
P			0.0000**		0.0000**	0.0287*
FIS	0.05	0.059	0.152	-0.004	0.194	0.074
LYC0015						
A	5	6	6	5	6	5
HE	0.62	0.79	0.75	0.77	0.76	0.76
HO	0.78	0.94	0.77	0.78	0.77	0.71
U	1	0	0	0	0	0
P	0.0415*					
FIS	-0.267	-0.182	-0.032	-0.018	-0.016	0.066
LYC0016						
A	4	7	6	5	5	5
HE	0.5	0.67	0.64	0.43	0.38	0.52
HO	0.28	0.83	0.19	0.25	0.24	0.15
U	1	1	0	1	0	1
P	0.0007**	0.0000**	0.0000**	0.0022**	0.0101*	0.0000**
FIS	0.441	-0.248	0.704	0.414	0.365	0.715
LYC0022						
A	5	7	8	7	6	6
HE	0.75	0.81	0.86	0.75	0.82	0.83
HO	0.62	0.85	0.66	0.66	0.65	0.71
U	0	0	1	0	0	0
P			0.0034**	0.0142*	0.0024**	
FIS	0.166	-0.058	0.237	0.125	0.203	0.145
LYC0027						
A	6	7	7	5	5	7
HE	0.71	0.65	0.66	0.71	0.36	0.72
HO	0.64	0.58	0.61	0.64	0.31	0.71
U	0	1	0	0	0	0
P						
FIS	0.102	0.102	0.079	0.101	0.141	0.018
LYC0033						
A	2	3	3	3	2	4
HE	0.21	0.12	0.06	0.13	0.16	0.48
HO	0.23	0.12	0.06	0.09	0.17	0.54
U	0	0	0	0	0	2
P						0.0006**
FIS	-0.118	-0.039	-0.015	0.306	-0.082	-0.133

Table 3. continues

LYC0036						
A	3	2	3	4	3	3
HE	0.65	0.21	0.56	0.67	0.63	0.67
HO	0.17	0.05	0.46	0.81	0.62	0.4
U	0	0	0	1	0	0
P	0.0000**	0.0001**	0.0003**			0.0001**
FIS	0.745	0.778	0.179	-0.212	0.009	0.405
Mean						
A	5.57	7.21	7.93	6.07	6	7.71
HE	0.688	0.681	0.717	0.677	0.649	0.751
HO	0.599	0.665	0.604	0.639	0.572	0.644
I	1.39	1.51	1.62	1.41	1.34	1.64
All loci						
U	9	7	6	7	4	12
FIS	0.12	0.025	0.158	0.057	0.12	0.144

A, Average number of alleles; *U*, number of the diagnostic alleles; H_E, expected heterozygosities; H_O, observed heterozygosities; *I*, Shannon's diversity index; F_{IS}, inbreeding coefficient; *P*, the value for deviation from H-W equilibrium using exact test, and the value of P > 0.05 was not shown in the table. *, ** Indicates significant deviation from H-W equilibrium at P < 0.05 and P < 0.01 level respectively.

Table 4. The Sing test and Wilcoxon test for heterozygosity excess and deficiency under different mutation models in six stocks.

Stock	Sign test		Wilcoxon test
	IAM	**SMM**	**TPM**
NB	H_{eq} = 7.94	H_{eq} = 8.16	P (one tail for H excess): 0.00003
	H_e = 14	H_e = 9	P (one tail for H deficiency): 1.00000
	H_d = 0	H_d = 5	P (two tail for H excess and deficiency): 0.00006
FD	H_{eq} = 8.05	H_{eq} = 8.12	P (one tail for H excess): 0.01013
	H_e = 13	H_e = 5	P (one tail for H deficiency): 0.99170
	H_d = 1	H_d = 9	P (two tail for H excess and deficiency): 0.02026
ND	H_{eq} = 8.14	H_{eq} = 8.26	P (one tail for H excess): 0.00830
	H_e = 13	H_e = 9	P (one tail for H deficiency): 0.99329
	H_d = 1	H_d = 5	P (two tail for H excess and deficiency): 0.01660
MY	H_{eq} = 7.99	H_{eq} = 8.16	P (one tail for H excess): 0.02094
	H_e = 11	H_e = 8	P (one tail for H deficiency): 0.98236
	H_d = 3	H_d = 6	P (two tail for H excess and deficiency): 0.04187
LJ	H_{eq} = 8.03	H_{eq} = 8.30	P (one tail for H excess): 0.00671
	H_e = 12	H_e = 9	P (one tail for H deficiency): 0.99731
	H_d = 2	H_d = 5	P (two tail for H excess and deficiency): 0.01343

Table 4. continue

Wd	H_{eq} = 8.21	H_{eq} = 8.20	P (one tail for H excess): 0.00021
	H_e = 13	H_e = 6	P (one tail for H deficiency): 0.99985
	H_d = 1	H_d = 8	P (two tail for H excess and deficiency): 0.00043

H_{eq}, Heterozygosity excess expected; H_e, heterozygosity excess; H_d, heterozygosity deficiency; IAM, infinite allele model; SMM, step-wise model; TPM, two-phase model; P, probability.

Table 5. The pairwise F_{ST} (above diagonal) and Nei's genetic distance (below diagonal) of all populations.

Population	NB	FD	ND	MY	LJ	Wd
NB	—	0.2425***	0.2070***	0.2536***	0.2357***	0.2000***
FD	1.145	—	0.0669***	0.0361***	0.1094***	0.0835***
ND	0.6991	0.3657	—	0.0742***	0.0868***	0.0818***
MY	1.0416	0.2326	0.3956	—	0.1392***	0.1103***
LJ	0.975	0.4487	0.3754	0.6021	—	0.0967***
Wd	1.2316	0.4712	0.5586	0.4119	0.6027	—

*** Significant (P < 0.001) for pair-wise F_{ST}.

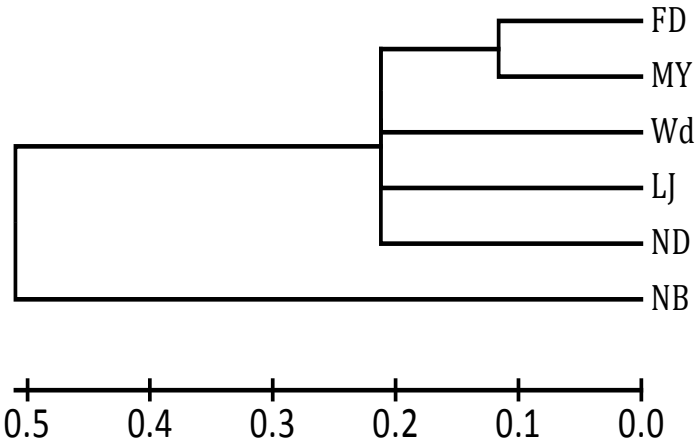

Figure 2. Neighbor-joining dendrogram based on Nei's genetic distance (*D*s).

study. Artificial selection for economical traits might be the second reason for the samples from cultured population, especially for the MY (Nielsen et al., 1998; Wittke-Thompso et al., 2005). The final reason could attribute to the presence of null alleles which was detected in all the samples in this study. Wilcoxon test under TPM for detection of bottleneck was the most powerful method when using less than 20 polymorphic loci (Piry et al., 1999) and all stocks suffered bottleneck by this algorithm. In Wd stock, loss of the rare alleles was the main reason for bottleneck, which could be explained by the average alleles per locus in Wd stock with only 7.71. In farmed and selectively breed stocks, the inbreeding was responsible for their bottleneck.

Genetic diversity

In recent years, the artificial selection of aquatic animals had greatly increased the probability of inbreeding and led to reduction of genetic variation (Norris et al., 1999). This tendency had also been observed in our studies and others (Wang et al., 2001, 2002). In practice, small size individuals characterized by the most weight and disease-resistant are often selected from different lines as broodstocks, which causes the Wahlund effect and decreases the genetic diversity (Pampoulie et al., 2006). In addition, bottleneck effect led to genetic drift and loss of rare alleles, both of which directly resulted in erosion of genetic diversity.

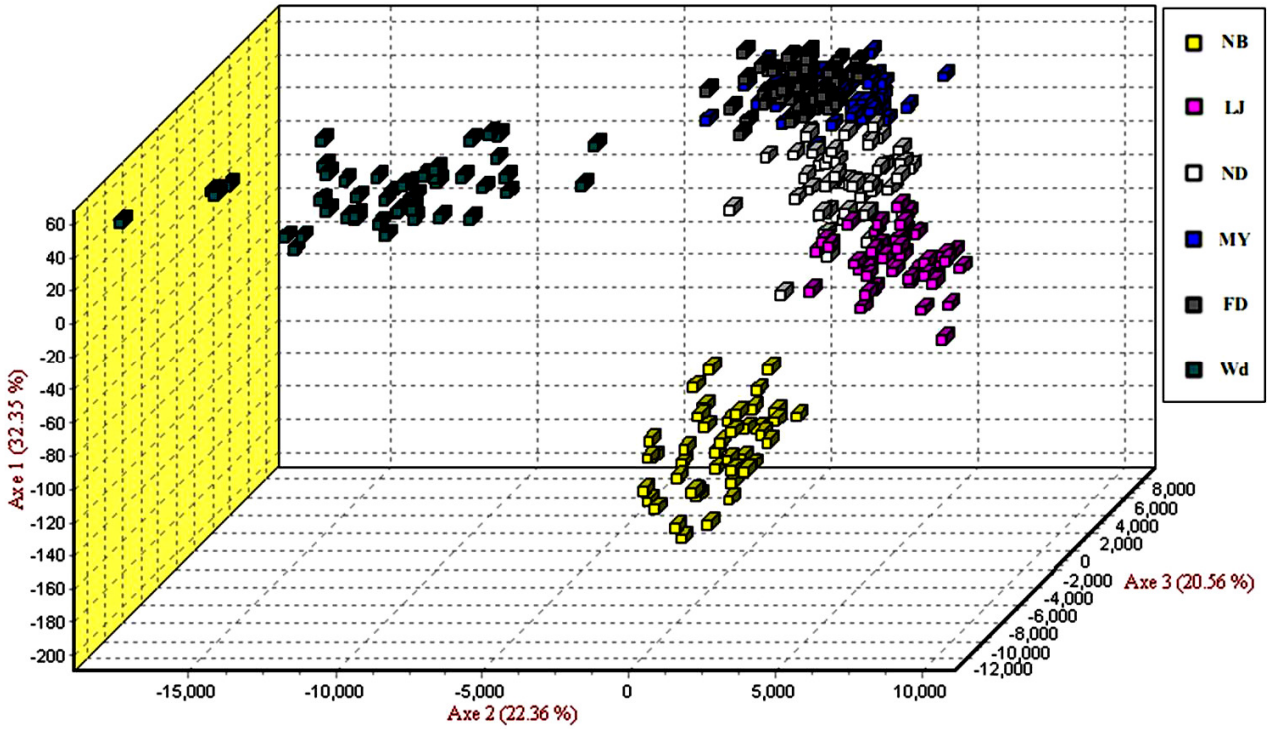

Figure 3. Three-dimensional scatter plots for the first three principal factors for 288 individuals of large yellow croaker. Axis 1 (32.35%), Axis 2 (22.36%) and Axis 3 (20.56%) represent the first, second and third principal factor, respectively.

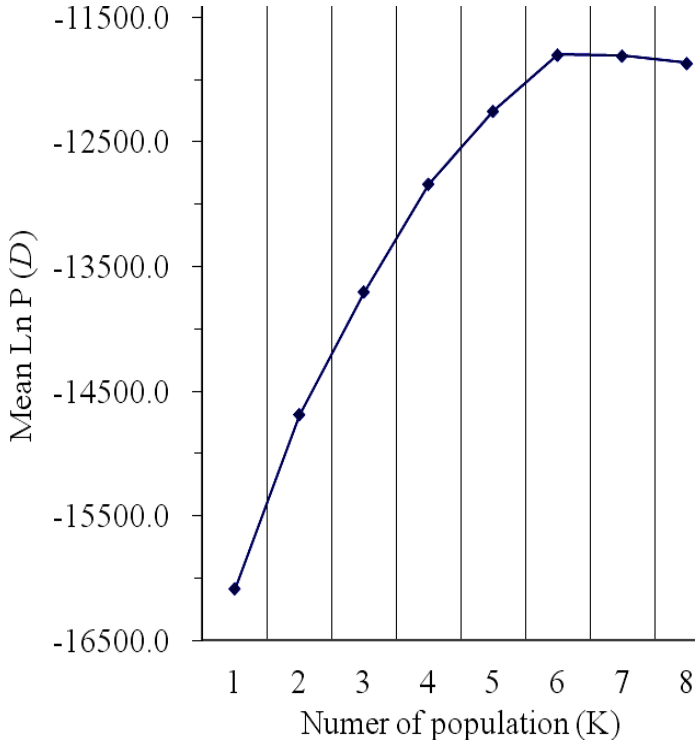

Figure 4. The estimated log likelihood for the different K value as average of 20 runs.

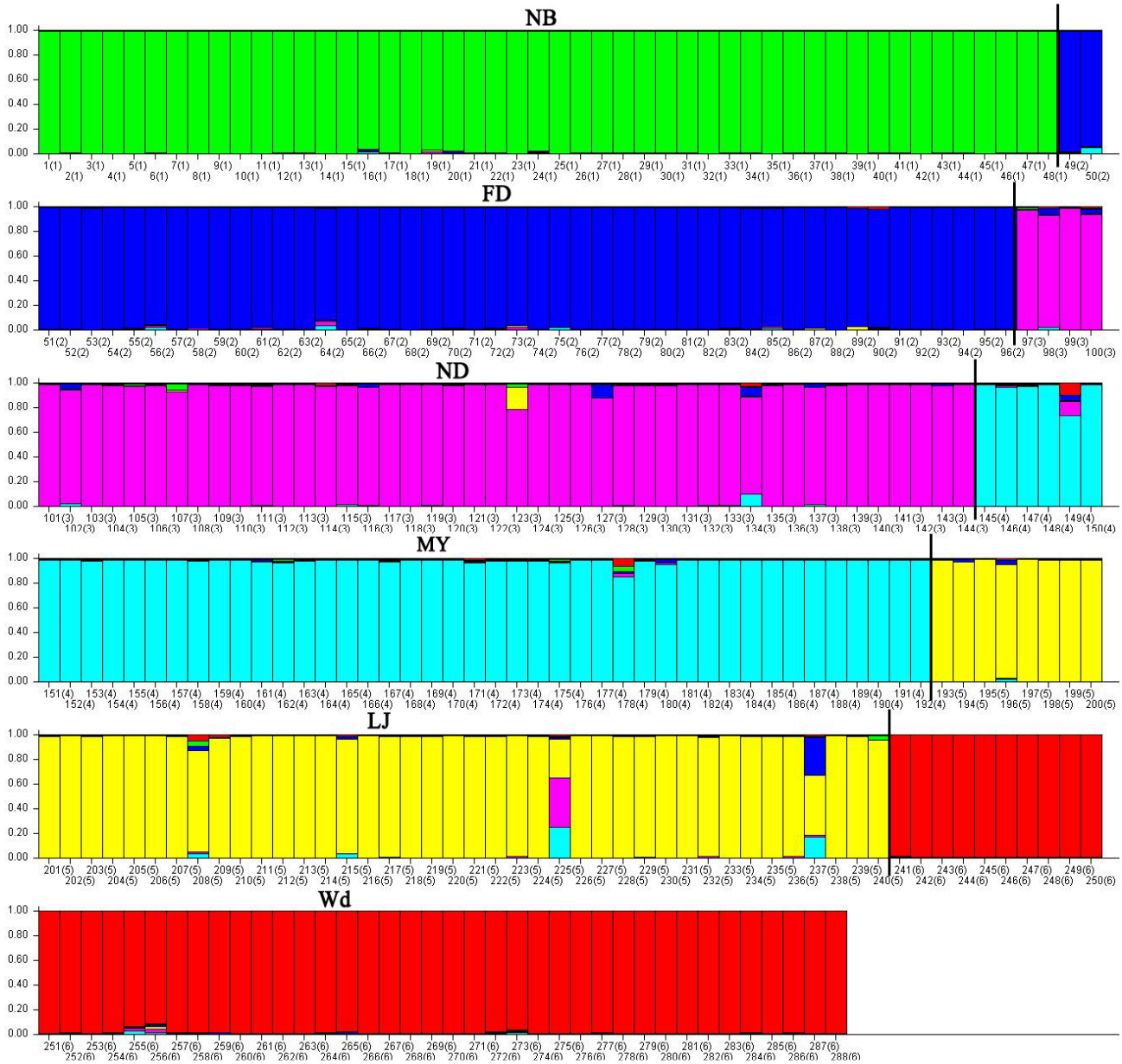

Figure 5. Estimated stock structure for large yellow croaker from structure analysis for K = 6. Each individual is represented by a thin vertical line, which is partitioned into K colored segments that represent the individual's estimated membership coefficients. Black lines separate individuals of different stocks, and abbreviations of different stocks are given in Table 1.

Among the farmed stocks, NB exhibited the lowest genetic diversity, which implied that the farmed stocks of Zhejiang province suffered more serious germplasm degradation than that in Fujian province. In addition, there was no significant decrease in genetic diversity level of wild stock when comparing with previous studies (Wang et al., 2002; Huang et al., 2006), whereas with regard to the cultured stocks, their genetic variation level slightly declined. Although, the NB stock had the least alleles, its heterozygosity and Shannon's diversity index were not the lowest among six stocks, which demonstrated that one stock lost alleles without losing heterozygosity because rare alleles did not contribute much to heterozygosity (Nei and Roychoudhury, 1974).

Loss of diagnostic alleles was considered to be more reliable to reflect genetic variability level than heterozygosity, as the former one was sensitive to genetic change and consequently was viewed as more harmful than loss of heterozygosity (Hedgecock and Sly, 1990; Evans et al., 2004; Pampoulie et al., 2006). The MY and Wd stocks had the least and the most diagnostic alleles separately, which agreed with their genetic diversity level. Similar findings had also been reported in other species (Was and Wenne, 2002; Horreo et al., 2008; Griffiths

et al., 2009).

Stock structure

Among the six stocks, only 15.9% of the total genetic variation was explained by difference among the population; the remaining 84.1% corresponded to difference within populations in this study. Commonly, the genetic divergence related to its original found stocks or to nature/artificial selection (Mjølnerød et al., 1997). NB stock originated from the wild resourse which were captured in Zhoushan of Zhejiang province of China and had come through five generations by artifical breeding. On the other hand, the original parents of the rested farmed and selective breeding stocks derived from Guanjingyang gulf in Fujian province in different years, from 1987 to 2001. In this study, NB stock exhibited the largest value of pair-wise F_{ST} among six stocks, demonstrating distinct difference from the remaining stocks. Those displayed results correspond to the fact that they might have various origins.

The stocks from Fujian province also displayed the relatively large value of pair-wise F_{ST} and Nei's gentic distance making them belong to different clusters in NJ dendrogram. Although, they had the unifrom origin that was the Guanjingyang gulf in Fujian province, it was difficulty to determine their definite originated parents because at the beginning, their detailed pedigree information was not recorded. Hence, it was accepted that those current farmed stocks eigher originated from the primitive parents who were collected in Guanjingyang gulf at different year from 1987 to 2001 or derived from the offsprings which were bred by the same founder population. Consequently, the farmed stocks must have big genetic differentiation from each other and from Wd stock through 3 to 4 generations' isolated breed. Our study also revealed those identical trends that stocks from Fujian province had emerged significant differentiation. FD and MY stocks displayed the lowest variation among six stocks. This stated that they still shared the common ancetry after multy-generation artifical breeding; although, their original parents were collected from Guanjingyang gulf in the years of 1998 and 1999. And this result also stated that FD and MY stocks only gone by less than three segregated generations.

Those analyses of difference in six stocks agreed with the NJ phylogenetic tree, in which all individuals were mainly assigned to two clusers. NB stocks formed one cluster with another five stocks grouping into the other cluster. These results could be explained by the different geographic distribution of six stocks and the multy-generation segregated breeding of farmed stocks. However, the situation of all stocks' differentiation could not be insight detailly. FCA and model based clustering method provide us more information on genetic structure.

FCA detected three sets by all individuals, however, the scatted dots of all individuals have rather clearly borders regardless of whether the places of those stocks are adjacent to one another or not. This stongly certifies, from the other aspect, that the high genetic variation had emerged among six stocks.

Six inferred clusters which were defined by the Ln P (*D*) fitted our study the best. Each stock was assigned to one of the six clusters with an estimated membership of over 95.5% (data not shown), which also revealing that those stocks had rather large difference because of founded effect and segregated artifical breeding. Two individuals (numbered 225 and 237 from LJ stock) were found to have inconsistent genetic background in LJ stock. Hence, contrasting with other clustering methods, such as NJ or FCA, the model based clustering method not only can identify the isolated population that was bred by multy generations but also can perform very well in the studies of identifying populations and assessing the affects of propagated population on wild resources. As well, the model based clustering method can recognize the individuals of admixture or migration based on the percent of shared ancestry and provided fine-number of clusters based on likelihood probabilities.

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