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Genetic diversity and population structure of the marbled rockfish, *Sebastiscus marmoratus*, revealed by SSR markers

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Introduction

The marbled rockfish, *Sebastiscus marmoratus* (Scorpaeniformes, Scorpaenidae, Sebastinae), is commercially important near-shore species, which is widely distributed in the East Sea and the South Sea of China, and from southern Japan to eastern Korea (Shen 1993). Generally, the rockfish attains a length of 15–20 cm, while some individuals can grow up to 30–40 cm. *S. marmoratus* inhabits littoral rocky bottoms and migrates within oceans typically between different spawning and feeding areas, as tunas do. The rockfish is viviparous wherein eggs are fertilized internally, retained, and undergo development in the maternal reproductive system (Wourms *et al.* 1988).

Sebastiscus marmoratus is an important commercial species in Japan and China (Kita *et al.* 1996). Considerable research has been carried out on *S. marmoratus* in Japan, but most of these studies are related to physiological and ecological aspects (Mizue 1959; Shiokawa 1962; Watanabe 2003; Yoko *et al.* 2006; Wang *et al.* 2005, 2009). Till date, not much has been reported about genetic variation in the marbled rockfish. Lack of appropriate polymorphic markers have limited the phylogenetic and population genetic work on this species. Among the various molecular makers, microsatellite DNA markers have been successfully used in revealing population genetic diversity, because they are co-dominant and highly polymorphic (Sekino and Hara 2001; Selkoe and Toonen 2006). To investigate the genetic characteristic of *S. marmoratus*, we developed 11 polymorphic microsatellite loci using the fast isolation by amplified fragment length polymorphism of sequences containing repeats (FIASCO) method (Xu *et al.* 2010).

For successful conservation and effective management of a species, including develop strategies for maintaining genetic diversity, it is important to determine the levels of genetic variation within and among populations. The marbled rockfish is a high-value worldwide marine food fish species; however, the wild resource of the marbled rockfish has been sharply decreased because of overfishing and water pollution. The only genetic work on the rockfishes was undertaken by Dong *et al.* (2008) in Zhejiang, People's Republic of China. This study used eight enzyme markers and reported a low level of polymorphism of only 27.78%. The objective of the present study was to assess the genetic diversity within populations and differentiation between populations of the rockfish in the South Sea and the East Sea by the use of 11 microsatellites. This information will in turn provide a theoretical basis for future marker-assisted breeding and conservation of this species.

Materials and methods

Sample collection and Genomic DNA extraction

A total of 120 samples of *S. marmoratus* were collected from four geographic locations, Zhoushan, Putian, Xiamen and Zhuhai (figure 1). Thirty wild individuals were selected per population, and tissue samples were obtained from fin clips and preserved in 95% ethanol, finally stored at −20°C. Total genomic DNA was isolated from the fin clips using the standard phenol–chloroform method with some modification, and subsequently dissolved in 100 μL of TE buffer.

Data collection

The 11 microsatellite loci used in this study are from Xu *et al.* (2010). PCR amplifications were carried out in 25 μL volumes, which contained 2.5 μL of 10× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μM of the forward and reverse primers, and 1.5 unit of *Taq* polymerase (Tiangen, Beijing, P. R. China). Thermal cycling conditions for each locus

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Figure 1. Sample sites for the marbled rockfish.

were: 5 min at 94°C, followed by 30 cycles of 94°C for 40 s, annealing temperature for 30 s (table 1), and 72°C for 40 s, followed by 1 cycle of 72°C for 5 min and then holding at 4°C. PCR amplification was performed on ABI 9700. The amplified products were denatured for 8 min at 96°C. Following the denatured products were 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 size standard of the lengths of PCR products (Xu *et al.* 2009).

Data analysis

SSR bands were scored in the form of single-individual genotypes. Number of alleles per locus (A), percentage of polymorphic loci (P) and heterozygosity (H) for every population were estimated using a POPGENE software package (Yeh *et al.* 1999). Gene flow (N_m) and Shannon's indices (I) were also calculated to characterize the gene diversity and the distribution of the variation using POPGENE program. An unbiased test of the exact test statistic was calculated using a Markov-chain method (the Markov-chain parameters used were: steps, 100 000). All results for multiple tests were corrected using Bonferroni correction (Rice 1989).

Genetic divergence between populations was quantified by estimating pairwise F_{ST} values and calculating their significance by bootstrapping analysis, using ARLEQUIN 2.0 (Schneider *et al.* 2000). In addition, a hierarchical analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) was performed using ARLEQUIN to estimate how variation was partitioned among populations. Based on Nei's (1972) standard genetic distance a neighbour joining (NJ) tree was constructed using program MEGA 4 to determine the genetic relationships between populations.

Results and discussions

All 11 loci were polymorphic in all of the studied populations of *S. marmoratus* and the details are summarized in table 1. For the four populations, the average polymorphism information content (PIC) per locus ranged from 0.306 to 0.841.

A total of 82 different alleles were detected, ranging in size from 101 bp to 255 bp. The number of the effective alleles per locus ranged from 1.762 to 10.058, with an average of 4.439. Observed heterozygosity (H_o) ranged from 0.389 to 0.935, and expected heterozygosity (H_e) ranged from 0.371 to 0.879 for per locus. The observed heterozygosities of the four populations: Zhoushan, Putian, Zhuhai and Xiamen were 0.641, 0.636, 0.600, and 0.691, respectively. The average observed heterozygosity (0.645) and the expected heterozygosity (0.650) were calculated at the population level. The Shannon's indices averaged 1.494 at the population level. All the indexes suggest that a high genetic diversity existed in these species which in turn suggests that there were low inbreeding in these four *S. marmoratus* populations. The genetic variability of Zhuhai population is relatively lower among the four populations, suggesting that this population may be of more concern from a conservation viewpoint.

All populations departed from Hardy–Weinberg Equilibrium (HWE) at some loci, which was revealed by F_{IS} and F_{IT} listed in table 2, and most of the deviations were significant. In the linkage disequilibrium test, eight significant pairwise tests were found among 11 loci ($P < 0.05$, adjusted P value = 0.0045) in Zhoushan and Zhuhai populations, and in Putian population it was five significant pairwise tests, while in the Xiamen popultion, the pairwise tests were all nonsignificant. The coefficient of hierarchical F_{ST} (Wright) was estimated, and ranged from 0.058 for Sema-23 to 0.378 for Sema-20, with an average value of 0.156. Generally, deviation from HWE was due to heterozygosity excess, which was followed in our study at most of the 11 loci in the four populations. However, in Zhuhai population, seven loci showed deviation from HWE without heterozygosity excess of the 11 loci used in our study, which could be explained by excess of certain genotypes. Selection, population mixing and nonrandom mating may be the factors driving deviations from HWE.

Significant genetic heterozygosity among the four *S. marmoratus* populations was indicated by AMOVA analysis. Fixation index (F_{ST}) is 0.031 ($P < 0.001$). The AMOVA analysis suggested that most genetic variation occurred within populations (96.87%), while that among populations was only 3.13% (table 3). Moreover, pairwise F_{ST} with majority of pairwise comparisons ranged from 0.044 to 0.006 (table 4). The pairwise F_{ST} analysis indicated that the significant difference existed between the Zhuhai population and other three populations ($P < 0.05$). The four populations were well distinguished from each other. The genetic distances among the four populations except for Zhuhai population were only a little more than 0.100, between Zhuhai

Table 1. Allelic variability at 11 microsatellite loci in rockfish *S. marmoratus*.

Strains	Microsatellite loci											
	Sema26	Sema32	Sema23	Sema27	Sema34	Sema19	Sema15	Sema20	Sema25	Sema37	Sema24	Mean
Zhoushan population												
A	14	6	5	6	10	3	2	3	9	5	5	6.184
A_e	7.273	4.324	3.374	4.189	6.481	1.386	1.342	2.089	5.298	2.867	2.260	3.717
PIC	0.850	0.733	0.651	0.726	0.828	0.261	0.222	0.409	0.789	0.608	0.516	0.599
H_o	0.900	0.900	0.684	0.850	0.750	0.316	0.300	0.400	0.700	0.600	0.650	0.640
H_e	0.885	0.788	0.723	0.781	0.873	0.286	0.262	0.535	0.832	0.668	0.572	0.655
F_{IS}	−0.018	−0.146	0.055	−0.091	0.145	−0.108	−0.152	0.257	0.162	0.104	−0.141	
P	0.241	0.009	0.228	0.001	0.334	1.000	1.000	0.251	0.0745	0.031	0.848	
Putian population												
A	9	7	4	5	6	2	2	2	8	6	5	5.000
A_e	4.765	5.120	2.766	2.524	4.378	1.301	1.992	1.899	5.400	4.613	1.592	3.305
PIC	0.770	0.777	0.589	0.536	0.736	0.204	0.374	0.366	0.791	0.750	0.345	0.567
H_o	1.000	1.000	0.632	0.789	0.737	0.235	0.389	0.667	0.650	0.500	0.400	0.636
H_e	0.819	0.840	0.637	0.654	0.785	0.214	0.500	0.460	0.884	0.784	0.350	0.628
F_{IS}	−0.228	−0.198	0.009	−0.214	0.063	−0.103	0.227	−0.474	0.238	0.381	−0.147	
P	0.244	0.000	0.122	0.018	0.001	1.000	0.379	0.112	0.005	0.000	1.000	
Zhuhai population												
A	12	7	5	8	8	3	2	3	9	6	5	6.182
A_e	9.139	6.494	2.684	3.380	4.592	2.057	1.424	1.459	7.049	4.188	2.181	4.059
PIC	0.881	0.827	0.584	0.669	0.761	0.425	0.253	0.288	0.842	0.724	0.507	0.653
H_o	1.000	0.824	0.474	0.941	0.733	0.579	0.364	0.158	0.588	0.471	0.471	0.600
H_e	0.815	0.872	0.644	0.725	0.809	0.528	0.312	0.323	0.884	0.784	0.558	0.669
F_{IS}	−0.096	0.057	0.270	−0.310	0.097	−0.100	−0.177	0.518	0.342	0.407	0.161	
P	0.331	0.195	0.068	0.024	0.335	0.200	1.000	0.020	0.001	0.001	0.081	
Xiamen population												
A	13	7	5	5	11	3	2	3	7	5	4	5.909
A_e	7.488	4.222	3.267	3.557	7.111	1.782	1.980	1.459	4.678	2.793	1.550	3.659
PIC	0.860	0.733	0.653	0.672	0.846	0.386	0.372	0.288	0.759	0.585	0.336	0.590
H_o	0.842	0.632	0.790	0.947	0.875	0.571	0.500	0.368	1.000	0.667	0.412	0.691
H_e	0.896	0.784	0.713	0.738	0.887	0.455	0.508	0.322	0.806	0.660	0.365	0.649
F_{IS}	0.062	0.199	−0.111	−0.293	0.014	−0.268	0.016	−0.146	−0.249	−0.010	−0.131	
P	0.004	0.000	0.590	0.0002	0.975	0.735	1.000	1.000	0.0001	0.050	1.000	
Mean of all populations												
A	12	6.75	4.75	6	8.75	2.75	2	2.75	8.25	5.5	4.5	5.818
A_e	10.058	6.143	3.121	4.164	6.581	1.649	1.762	2.288	7.367	3.802	1.890	4.4396
PIC	0.841	0.767	0.620	0.651	0.793	0.416	0.306	0.338	0.795	0.667	0.426	0.602
H_o	0.935	0.836	0.653	0.878	0.785	0.427	0.397	0.389	0.750	0.556	0.493	0.645
H_e	0.879	0.821	0.679	0.725	0.839	0.371	0.395	0.410	0.844	0.728	0.461	0.650

A, number of alleles per locus; A_e, effective number of alleles; PIC, polymorphism information content; H_e, expected heterozygosity; H_o, observed heterozygosity; F_{IS}, inbreeding coefficient; P, probability of significant deviation from Hardy–Weinberg equilibrium are given for each population and locus. Calculations assume that individuals with one microsatellite band are homozygous for the allele.

Table 2. Summary of F-statistics and gene flow for all loci of the four populations.

Locus	F_{IS}	F_{IT}	F_{ST}	N_m	I
Sema26	−0.2123	−0.0829	0.1068	2.0912	2.4925
Sema32	−0.1826	−0.0374	0.1228	1.7864	1.8601
Sema23	−0.1363	−0.0708	0.0576	4.0895	1.3254
Sema27	−0.3696	−0.1305	0.1746	1.1822	1.6669
Sema34	0.0377	0.3057	0.2785	0.6477	2.1091
Sema19	−0.1851	−0.0488	0.1151	1.9224	0.7086
Sema15	−0.0360	0.1463	0.1759	1.1709	0.6240
Sema20	−0.0444	0.3498	0.3775	0.4123	0.9310
Sema25	−0.0489	0.0816	0.1244	1.7595	2.1208
Sema37	0.0488	0.1556	0.1123	1.9771	1.5703
Sema24	−00828	−0.0008	0.0757	3.0526	1.0191

N_m, gene flow; $N_m = 0.25\ (1-F_{ST})/F_{ST}$; I, Shannon's information index.

and the other three populations were 0.361, 0.348 and 0.336 respectively (table 5). On the basis of the inter population genetic distance, the NJ dendrogram was constructed (figure 2), which indicated that the samples from Zhuhai location were clustered in one group, while the Zhoushan,

Table 3. The analysis of molecular variance (AMOVA) among and within the four populations.

Source of variation	Variation components	Percentage of variation	F statistic	P
Among population	0.036	3.14	0.031	0.002
Among individuals	−0.006	−0.49	−0.005	0.618
Within populations				
Within individuals	1.119	97.34	0.027	0.301

Table 4. Pairwise F_{ST} values between four populations in the East Sea and the South Sea stocks of *S. marmoratus*.

	Zhoushan	Putian	Zhuhai	Xiamen
Zhoushan				
Putian	0.0398*			
Zhuhai	0.0216	0.0056		
Xiamen	0.0423*	0.0436*	0.0318*	

*Significant ($P < 0.05$).

Table 5. Genetic identity and genetic distance among four populations of the marbled rockfish.

Pop ID	Zhoushan	Putian	Zhuhai	Xiamen
Zhoushan	–	0.8989	0.6972	0.8807
Putian	0.1066	–	0.7060	0.8902
Zhuhai	0.3607	0.3481	–	0.7142
Xiamen	0.1270	0.1163	0.3366	–

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

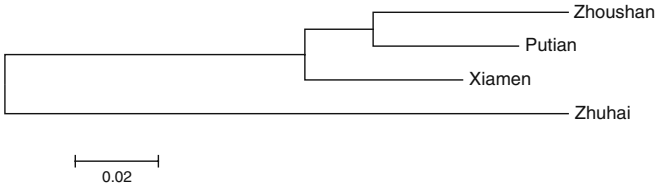

Figure 2. Neighbour joining cluster analysis based on Nei's genetic distance among four populations of *S. marmoratus*.

Putian and Xiamen populations were in another group. In conclusion, among different populations, the samples not only could be distinguished from each other but also could be easily separated between the two sea areas, the East Sea and the South Sea.

These results indicated that high genetic diversity existed in different geographic populations of *S. marmoratus*. All the four populations were found high genetic variation, which could be useful for further genetic breeding.

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