

Population genetic structure of chub mackerel *Scomber japonicus* in the Northwestern Pacific inferred from microsatellite analysis

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Abstract Marine pelagic fishes are usually characterized by subtle but complex patterns of genetic differentiation, which are influenced by both historical process and contemporary gene flow. Genetic population differentiation of chub mackerel, *Scomber japonicus*, was examined across most of its range in the Northwestern Pacific by screening variation of eight microsatellite loci. Our genetic analysis detected a weak but significant genetic structure of chub mackerel, which was characterized by areas of gene flow and isolation by distance. Consistent with previous estimates of stock structure, we found genetic discontinuity between Japan and China samples. Local-scale pattern of genetic differentiation was observed between samples from the Bohai Sea and North Yellow Sea and those from the East China Sea, which we ascribed to differences in spawning time and migratory behavior. Furthermore, the observed homogeneity among collections of chub mackerel from the East and South China Seas could be the result of an interaction between biological characteristics and marine currents. The present study underlies the importance of

understanding the biological significance of genetic differentiation to establish management strategies for exploited fish populations.

Keywords *Scomber japonicus* · Gene flow · Population structure · Microsatellite DNA · Northwestern Pacific

Introduction

Marine pelagic fishes generally exhibit large effective population sizes, high dispersal capability, prolific fecundity as well as long planktonic larval phases that are subject to passive transport by marine currents. The biological peculiarities of these fishes combined with the apparent lack of physical barriers in the marine realm, are thought to facilitate extensive gene flow and low genetic differentiation among populations [1–4]. However, this long held-view was challenged by recent studies that cryptic population structuring has been increasingly detected in highly abundant and widely distributed migratory marine fishes [5–9]. The complicated interaction between marine environment and biological characteristics of marine fishes could sufficiently account for the differentiation processing. Specifically, these complicated factors include historical vicariance events [10, 11], ocean fronts and currents [12], environmental gradients (e.g., salinity and temperature) in water bodies [13, 14], larval retention [15], natural homing as well as local habitat dependence [16, 17]. Hence, to understand the complex population structuring and dynamics of marine migratory fish requires discerning the relative significance of different mechanisms in shaping and maintaining the pattern of population structure.

Considering the complexity of the physical peculiarities of the marine environment and life history traits of marine

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pelagic fishes, it is difficult to detect distinct populations in marine ecosystems. Lack of detectable differentiation could be attributed to extensive gene flow, and it does not necessarily imply the absence of structuring [18, 19]. Information on population structure is essential for the development of rational and effective management strategies for vulnerable fishery resources [20, 21]. Failure to detect population units could lead to local overexploitation and subsequently to resource declines [22]. An effective way to obtain further insights to the factors that form and maintain genetic divergence is to combine genetic data with information on the physical characteristics of habitats as well as biological peculiarities of marine fishes, to study how these factors impact population structure patterns [7, 13, 16, 23].

Chub mackerel, *Scomber japonicus* (Houttuyn 1782) is a broadly exploited pelagic fish species and has a cosmopolitan distribution along warm and temperate waters of Indo-Pacific Ocean [24–26]. In the Northwestern Pacific, it is distributed from Japan along the Chinese coast south to the Malay Archipelago [27], and it is commercially exploited in waters of the East China Sea from Japan to northern Taiwan as important fishery resources for China, Korea, and Japan. However, increasing fishing effort has led to a reduction in abundance of *S. japonicus*, and collapse of chub mackerel fisheries was reported in the East China Sea [28, 29]. The need to establish appropriate management strategy for chub mackerel has led to several genetic studies using a range of molecular markers. A preliminary mitochondrial DNA study conducted by Tzeng et al. [30] suggested that chub mackerel off Taiwan belonged to a single gene pool. Shao and Chen [31] undertook further studies on genetic variations of chub mackerel based on random amplified polymorphic DNA (RAPD) in the Yellow Sea and East China Sea. Their results revealed the existence of two stocks of chub mackerel in the Yellow Sea and East China Sea. Zhang et al. [32] employed amplified fragment length polymorphism (AFLP) analysis on collection of chub mackerel from the Taiwan Strait and its adjacent waters, and found no significant genetic heterogeneity among samples. A more recent microsatellite analysis detected the presence of population structure in the East and South China Seas [33]. These studies have resulted in different conclusions regarding the genetic structure and population identities of chub mackerel at fine geographical scales. There has been no comprehensive study on the Northwestern Pacific-wide population structure of chub mackerel.

Microsatellite DNA has proven to be a useful marker in uncovering population structure that was not apparent using less variable markers [13, 34–36]. We estimated genetic variation and population structure of chub mackerel in the Northwestern Pacific using eight microsatellite loci

with the purpose to infer the relative role of biological characteristics and environmental factors involved in shaping contemporary population genetic structure of this species.

Materials and methods

Sample collection

A total of 213 individuals were collected at 10 localities along Chinese and Japanese coastal waters during 2008–2009 (Table 1; Fig. 1). Taxonomic status of the fishes was identified morphologically [37]. A piece of skeletal muscle was excised from each individual and preserved in 95 % ethanol for DNA extraction.

Microsatellite genotyping

Genomic DNA was isolated from the muscle tissue by proteinase K digestion followed by the standard phenol/chloroform method [38]. Eight microsatellite loci were PCR amplified using primers Sja3, Sja4, Sja5, Sja6, Sja8, Sja9 developed from chub mackerel [39] and Sa2599, Sa2770 from its congener, spotted mackerel *Scomber australasicus* [40]. PCR reactions were carried out in 25 μ l reaction mixture containing 20 ng template DNA, 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 0.5 μ M of each primer, 1U Taq polymerase (Takara). All PCRs were performed in an Eppendorf thermal cycler following optimized reaction conditions [39, 40]. PCR products were separated on 8 % non-denaturing vertical polyacrylamide gel electrophoresis and visualized with silver staining as described by Bassam et al. [41]. A sizing standard (100–300 base pairs) was run in the center and at both ends of each gel to calibrate allele size. Furthermore, a reference sample was run on each gel to ensure consistency in genotype scoring across runs.

Statistical analysis

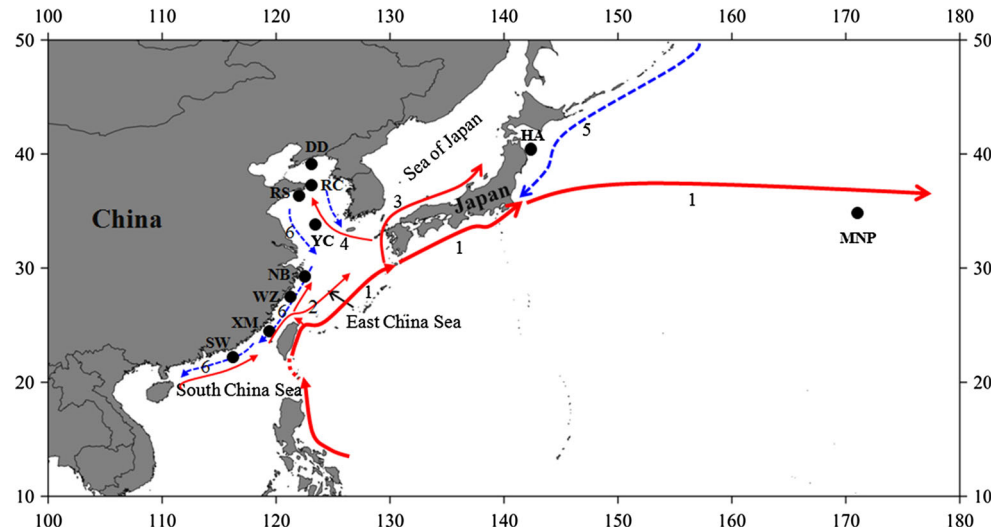
Microsatellite genetic diversity was quantified as number of alleles (A), observed heterozygosity (H_O) and expected heterozygosity (H_E) for each locus and sample site using POPGENE 1.32 [42]. Polymorphism information content (PIC) was calculated using allele frequencies according to the formula given by Botstein et al. [43]. The allelic richness (R_S), which is a standardized index of the mean number of alleles per locus irrespective of sample size, was calculated by Fstat 2.9.3 [44]. Deviations from Hardy–Weinberg equilibrium (HWE) expectation and linkage disequilibrium were tested for each site at each locus using GENEPOP 3.4 [45] with 10,000 burn-in steps, and 500 batches of 5,000 Monte Carlo Markov Chain (MCMC)

Table 1 Summary statistics for microsatellite DNA variability in chub mackerel

Location	Abbr.	Longitude/ latitude	Number	Locus Parameters	Sa2599	Sa2770	Sja3	Sja4	Sja5	Sja6	Sja8	Sja9	Average
Hachinohe	HA	40.47°N 141.41°E	24	A	7	9	11	10	12	12	8	4	9.1
				R_S	5.1	6.1	4.5	6.5	7.1	6.0	4.1	2.5	5.2
				H_O	0.739	0.435	0.583	0.250	0.87	0.667	0.333	0.292	0.521
				H_E	0.829	0.876	0.65	0.897	0.921	0.859	0.665	0.334	0.754
				PIC	0.784	0.842	0.620	0.862	0.892	0.824	0.617	0.311	0.719
Middle of the North Pacific	MNP	35°N 171°E	15	A	8	7	8	5	10	11	8	4	7.6
				R_S	4.8	5.1	5.4	4.3	6.6	6.8	4.3	2.0	4.9
				H_O	0.714	0.357	0.733	0.200	0.733	0.467	0.533	0.200	0.492
				H_E	0.773	0.828	0.818	0.779	0.899	0.91	0.683	0.193	0.735
				PIC	0.710	0.770	0.767	0.697	0.856	0.868	0.627	0.181	0.684
Dandong	DD	39.47°N 124.23°E	10	A	9	5	6	4	9	7	4	3	5.9
				R_S	6.4	3.3	4.5	4.0	5.5	5.1	2.8	2.4	4.2
				H_O	0.800	0.200	0.400	0.600	0.500	0.778	0.400	0.111	0.474
				H_E	0.895	0.442	0.779	0.711	0.8	0.817	0.363	0.307	0.639
				PIC	0.833	0.400	0.697	0.581	0.732	0.739	0.326	0.269	0.572
Rongcheng	RC	37.03°N 122.57°E	24	A	18	13	10	10	14	11	9	4	11.1
				R_S	7.6	6.9	4.9	5.3	7.2	6.2	4.4	2.7	5.7
				H_O	0.667	0.81	0.522	0.350	0.682	0.75	0.5	0.167	0.556
				H_E	0.934	0.913	0.728	0.786	0.922	0.876	0.72	0.395	0.784
				PIC	0.908	0.881	0.692	0.745	0.893	0.843	0.667	0.363	0.749
Rushan	RS	36.83°N 121.52°E	24	A	9	9	6	8	12	12	8	4	8.5
				R_S	5.5	5.3	2.8	5.5	6.8	6.4	4.0	2.3	4.8
				H_O	0.571	0.556	0.211	0.368	0.632	0.524	0.500	0.273	0.454
				H_E	0.839	0.821	0.376	0.848	0.9	0.891	0.651	0.349	0.709
				PIC	0.797	0.775	0.352	0.804	0.865	0.857	0.584	0.308	0.668
Yancheng	YC	34°N 124°E	24	A	14	10	7	9	11	10	7	5	9.1
				R_S	6.7	5.7	3.3	5.8	6.8	6.1	4.4	2.7	5.2
				H_O	0.619	0.762	0.182	0.368	0.625	0.889	0.400	0.273	0.515
				H_E	0.901	0.844	0.498	0.868	0.907	0.879	0.710	0.396	0.750
				PIC	0.869	0.804	0.469	0.826	0.867	0.838	0.662	0.369	0.713
Ningbo	NB	29°N 123.45°E	24	A	13	8	6	8	11	4	4	5	7.4
				R_S	6.3	4.7	2.8	5.7	6.4	3.0	2.1	2.7	4.2
				H_O	0.773	0.409	0.217	0.250	0.682	0.429	0.095	0.095	0.369
				H_E	0.878	0.737	0.352	0.866	0.891	0.479	0.224	0.340	0.596
				PIC	0.844	0.694	0.332	0.808	0.858	0.433	0.211	0.316	0.562
Wenzhou	WZ	27.97°N 121.37°E	24	A	13	11	8	8	13	9	6	5	9.1
				R_S	6.2	5.8	2.9	5.1	6.4	5.4	3.2	2.5	4.7
				H_O	0.739	0.714	0.304	0.150	0.739	0.500	0.400	0.227	0.472
				H_E	0.862	0.848	0.386	0.803	0.882	0.786	0.497	0.327	0.674
				PIC	0.828	0.808	0.364	0.757	0.850	0.742	0.460	0.304	0.639
Xiamen	XM	24.45°N 119.45°E	20	A	12	8	7	7	16	9	7	3	8.6
				R_S	6.5	5.4	3.3	4.8	7.8	5.0	3.7	1.8	4.8
				H_O	0.579	0.4	0.5	0.250	0.800	0.526	0.400	0.177	0.454
				H_E	0.896	0.851	0.437	0.797	0.944	0.765	0.537	0.169	0.675
				PIC	0.860	0.807	0.413	0.729	0.914	0.721	0.505	0.157	0.638
Shanwei	SW	22.12°N 116.43°E	24	A	13	9	4	9	13	9	7	4	8.5
				R_S	6.5	6.1	2.7	6.2	6.7	5.0	4.0	1.7	4.9
				H_O	0.696	0.813	0.292	0.438	0.565	0.500	0.542	0.091	0.492
				H_E	0.892	0.879	0.395	0.889	0.901	0.801	0.665	0.133	0.694
				PIC	0.860	0.835	0.363	0.846	0.871	0.756	0.617	0.127	0.659

A Allelic number, R_S allelic richness, H_O observed heterozygosity, H_E expected heterozygosity, PIC polymorphism information content

Fig. 1 Map showing sample locations of chub mackerel, samples are marked by abbreviations that correspond to Table 1. Ocean currents are shown: 1 Kuroshio Current; 2 Taiwan Warm Current; 3 Tsushima Current; 4 Yellow Sea Warm Current; 5 Oyashio Current; 6 China Coastal Current



steps per batch. Critical significance levels for multiple simultaneous tests were adjusted using the sequential Bonferroni correction [46].

To test whether mutation or genetic drift contributed to genetic differentiation of chub mackerel populations, we performed the allele size permutation test to compare allele identity-based statistic F_{ST} (IAM) [47] and allele size-based statistic R_{ST} (SMM) [48]. The procedure was implemented in the program SPAGeDi 1.3 [49]. R_{ST} were calculated for each locus and compared with the distribution of ρR_{ST} values generated by 1000 permutations. A significant one-side test establishes the alternative hypothesis that genetic differentiation is caused mainly by SMM-like mutation ($R_{ST} > \rho R_{ST}$), otherwise no contribution of SMM to genetic differentiation. In this study, a non-significant result showed that stepwise mutations were not informative ($P = 0.0866$). Therefore, F_{ST} rather than R_{ST} estimates were used for the subsequent genetic analysis, as estimated by the program Fstat 2.9.3 [44] and significance assessed with permutation tests (1,000 replicates). To test for isolation by distance in chub mackerel, correlation between genetic differentiation and geographical distance between sampling sites was assessed using a Mantel procedure. The logarithm of geographical distance in kilometers was regressed against $F_{ST}/(1 - F_{ST})$ as computed in IBDWS (<http://ibdws.sdsu.edu/~ibdws/>) [50, 51]. Significance of the regression slopes was tested with 1,000 permutations.

Three dimensional factorial correspondence analysis (3D-FCA) was performed in GENETIX 4.05 [52] to explore population divisions and relationships of chub mackerel, independent from a prior knowledge of their relationships. A model-based Bayesian clustering algorithm was undertaken using STRUCTURE 2.1 [53] to determine the number of genetic discrete populations

(K) with the highest posterior probability. The simulated K values ranged from 1 to 10 (total sites). Ten independent runs were implemented for each specific K -value in order to verify the consistency of the results. The simulations were conducted assuming an admixture model with correlated allele frequencies, which is considered as the superior model for detecting structure among closely related populations [54]. MCMC consisted of 100,000 burn-in iterations followed by 1,000,000 iterations. Finally, we conducted a hierarchical analysis of molecular variance (AMOVA) in ARLEQUIN 2.000 [55] to test population structure across the entire geographic sample range and among putative regional grouping of samples. For all calculations, significance was assessed by 1,000 random permutations and P values from multiple comparisons were Bonferroni adjusted [46].

Results

Genetic variability

Genotyping of 213 individuals of chub mackerel from 10 locations enabled scoring of 113 alleles at eight microsatellite loci. The amount of genetic variability in terms of average number of alleles, observed and expected heterozygosities was similar among sampling sites for the same microsatellite locus (Table 1). However, large difference was detected among microsatellite loci. The average number of alleles per locus (A) over samples ranged from 4.1 alleles for Sja9 to 12.1 for Sja5 and the average number of alleles per population ranged from 5.9 (DD) to 11.1 (RC). The allelic richness varied between 4.2 (DD) and 5.7 (RC). The observed and expected heterozygosities ranged from 0.369 to 0.556 and 0.596 to 0.784 per population,

Table 2 Summary statistics of the allele size permutation test for each locus and the 95 % confidence for permuted R_{ST} values

Locus name	F_{ST}	ρR_{ST} (95 % C.I.)	R_{ST}
Sa2559	0.0586	0.0597 (−0.0088 to 0.01969)	0.0799
Sa2770	0.0418	0.0403 (−0.0121 to 0.1144)	0.1329
Sja4	0.0205	0.0201 (−0.0339 to 0.1003)	0.0185
Sja5	0.0271	0.0275 (−0.0143 to 0.0916)	0.1052
Sja6	0.0719	0.0692 (−0.0058 to 0.1841)	0.0779
Sja8	0.0984	0.0826 (0.0023 to 0.1872)	0.0926
Sja9	−0.0068	−0.0597 (−0.0088 to 0.01969)	−0.0177
Multilocus	0.0477	0.0506 (0.0146 to 0.1012)	0.0866

Bold R_{ST} values indicate a significant test ($R_{ST} > \rho R_{ST}$) after 1,000 random permutation

respectively. The population with the highest polymorphism information content (0.749) was the population NB, whereas that with the lowest value (0.562) was the population RC.

Thirty of 80 locus-by-population tests exhibited significant departure from HWE with heterozygote excess, but none of which remained significant after correction for multiple tests. Significant linkage disequilibrium was detected between Sja3 and Sja9 over all populations. In addition, such case was also found between Sja3 and Sa2770 in the population RC and NB. Thus, locus Sja3 was excluded from subsequent analyses.

Population genetic differentiation

The allele size permutation test did not detect any R_{ST} value significantly $> \rho R_{ST}$ (Table 2), suggesting that F_{ST} should be preferred over R_{ST} for the measurement of genetic differentiation between chub mackerel populations. The global test for genetic differentiation ($F_{ST} = 0.049$, $P < 0.05$, 95 % C.I. 0.029–0.068) over all loci suggested population structuring of the analyzed samples. Pairwise F_{ST} values between 10 populations ranged from 0.0109 to 0.161, and most of them were significant after sequential Bonferroni correction except 7 comparisons (Table 3). F_{ST} estimates between population pairs that included RS samples were significant, whereas many non-significant outcomes were observed among samples collected from the East and South China Seas. The MNP population was significantly differentiated from other samples with pairwise F_{ST} values ranging from 0.0457 to 0.161, except from the geographically closest HA samples.

According to the Mantel test, a significant correlation ($r = 0.549$, $R^2 = 0.301$, $P < 0.05$) was observed between genetic distance determined as $F_{ST}/(1 - F_{ST})$ and geographical distance based on all loci, indicating isolation by distance among chub mackerel populations (Fig. 2). The linear regression explained 30.1 % of the variation in

genetic differentiation of chub mackerel populations. Three dimensional factorial correspondence analysis (3D-FCA) explaining 47.25 % of the overall variation, separated the three Bohai Sea and North Yellow Sea populations (DD, RC and RS) from the five populations (YC, NB, WZ, XM and SW), with the remaining two Japan populations (MNP and HA) in the top (Fig. 3). The Bayesian algorithm implemented in the program STRUCTURE indicated that all individuals could be assigned to four main genetic clusters (Fig. 4a). The four clusters showed continuous distributions, with neighboring populations assigning to the same cluster. As shown in Fig. 4b, most individuals from four populations (NB, WZ, XM and SW) were assigned into the first cluster regardless of geographic origin, whereas most individuals from three populations (DD, RC and RS) collected from the Bohai Sea and North Yellow Sea were assigned to the second cluster. Most individuals from the remaining two populations (HA and MNP) were assigned to a single cluster, respectively. The hypothetical grouping of populations was further explored by an analysis of molecular variance (AMOVA) tests (Table 4). Most of the total genetic variance was found within populations. Potential geographic structuring (organized as China versus Japan samples) was supported by the AMOVA ($P = 0.029$). The hierarchical AMOVA conducted with populations clustered according to the 3D-FCA analysis revealed that 1.37 % of genetic variance was distributed among groups ($P = 0.012$), whereas 4.48 % was partitioned among populations within group. An alternative AMOVA model, with populations clustered according to the Bayesian clustering analysis, showed comparatively more suitable variance partitioning with higher percentage of variance (1.89 %) among groups ($P = 0.038$). When the AMOVA was performed without considering population disjunction, 5.43 % of the genetic variability was apportioned among populations, and 94.57 % was detected among individuals within the population ($P = 0.000$).

Discussion

The eight microsatellite loci used in this study showed relatively moderate level of polymorphism in chub mackerel compared to other marine pelagic fishes [36, 56, 57]. No geographical trend appeared in the levels of microsatellite diversity. F_{ST} and R_{ST} are two commonly used estimators to assess the amount of genetic differentiation among populations. Comparisons of F_{ST} with R_{ST} values on microsatellite data have been suggested for checking the relative contribution of mutation versus migration rates to population differentiation [58, 59]. The allele size permutation test did not reveal any significant contribution of stepwise mutations to genetic differentiation of chub

Table 3 Matrix of pairwise F_{ST} values between ten chub mackerel populations based on seven microsatellite loci

Populations	HA	MNP	DD	RC	RS	YC	NB	WZ	XM	SW
HA										
MNP	0.046									
DD	0.064	0.131								
RC	0.032	0.046	0.050							
RS	0.037	0.061	0.081	0.037						
YC	0.015	0.068	0.053	0.022	0.034					
NB	0.063	0.161	0.070	0.088	0.091	0.049				
WZ	0.045	0.109	0.060	0.035	0.065	0.031	0.015			
XM	0.035	0.099	0.083	0.056	0.049	0.020	0.028	0.036		
SW	0.033	0.083	0.074	0.022	0.037	0.015	0.048	0.034	0.011	

Significant F_{ST} values after Bonferroni correction for multiple tests are in bold. Refer to Table 1 for abbreviations of sampling sites

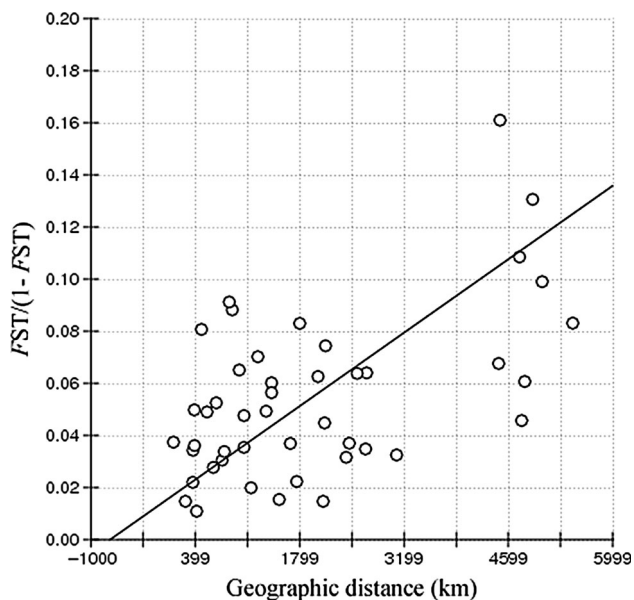


Fig. 2 Genetic isolation by distance in chub mackerel populations inferred from pairwise estimates of $F_{ST}/(1 - F_{ST})$ based on seven microsatellite loci and geographic distance ($r = 0.549$, $R^2 = 0.301$, $P = 0.020$)

mackerel populations, indicating that migration rates and drift become important relative to mutation in contributing to population differentiation of chub mackerel.

Overall F_{ST} detected weak but significant genetic differentiation of chub mackerel populations. Further evidence for population structuring was obtained by plotting pairwise estimates of $F_{ST}/(1 - F_{ST})$ against geographic distances. The Mantel test demonstrated that chub mackerel exhibited a clear isolation-by-distance pattern, which implied restricted gene flow among chub mackerel from different sampled locations. It should be taken into account that when long-distance samplings have been made, a significant IBD model can be obtained even if geographic distance is not the main factor causing the genetic

differences [60, 61]. Thus, the present geographical pattern of genetic differentiation was further tested using 3D-FCA and Bayesian clustering analysis. Both tests suggested a grouping of samples according to geographical positions, with the Bohai Sea and North Yellow Sea populations (DD, RC and RS) forming one group, and the East and South China Seas populations (NB, WZ, XM and SW) forming another group, with the remaining two Japan populations (HA and MNP) being distinct from these collected from China coastal waters. This pattern was further confirmed by the hierarchical AMOVA analysis. What was unexpected was the assignment of individuals from the South Yellow Sea population (YC), indicating a complicated genetic composition of this population. However, inclusion of mitochondrial DNA analysis and more comprehensive sampling in the South Yellow Sea and East China Sea is essential to investigate this problem further in the future.

Marine pelagic fishes generally exhibit low levels of genetic subdivision, eventually leading to panmictic populations [62]. Nevertheless, numerically small but significant genetic differentiation has been detected in many marine pelagic fishes despite high dispersal capabilities and large effective population sizes [5, 6, 9, 63]. In this study, multiple analyses showed that a weak but significant genetic structure existed in chub mackerel populations. The pertinent features of species biology and marine environment, coupled with their interactions, need be considered to explain the genetic patterns observed in chub mackerel. According to differences in the patterns of seasonal migration and spawning grounds, chub mackerel in Japan is managed as two separate stocks: the Pacific stock and the Tsushima Current stock [64]. The Tsushima Current stock of chub mackerel is distributed from the southern East China Sea to the northern Japan Sea, the Yellow Sea and Bohai Gulf. Multiple analyses showed genetic discontinuity between Japan and China samples of *S. japonicus* in our study, adding additional evidence for supporting this management policy. In the case

Fig. 3 Three dimensional factorial correspondence analysis (3D-FCA) showing relationships among chub mackerel populations based on seven microsatellite loci. Refer to Table 1 for abbreviations of sampling sites

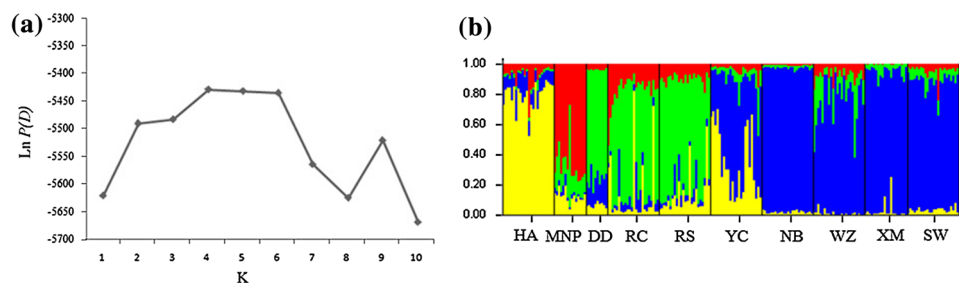
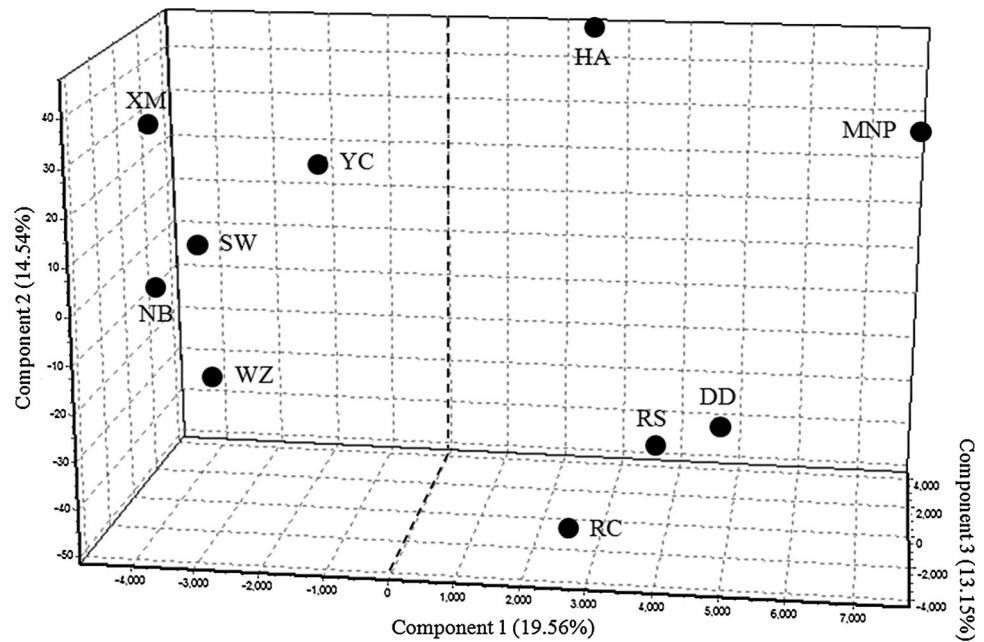


Fig. 4 **a** Number of chub mackerel populations with the highest posterior probability expressed as the mean likelihood $\ln P(D)$ for each assumed population (K); **b** Graphical results of the STRUCTURE analysis of seven microsatellite loci in chub mackerel

populations. Vertical lines are proportional to the probability of individual membership in simulated cluster. Refer to Table 1 for abbreviations of sampling sites. (Color figure online)

of chub mackerel off Japan's Pacific coast, genetic differentiation between the two Japan populations (MNP and HA) was detected from the Bayesian clustering analysis (Fig. 4b). Ocean currents are expected to be the predominant environmental factor influencing contemporary levels of gene flow between populations, especially in species with pelagic eggs or larvae, or in species with highly migratory adults [12, 65, 66]. There are two main ocean currents off the eastern coast of Japan, the Kuroshio-extension current and the Oyashio-extension current (Fig. 1). The former originates in the tropics further south and warm (25–27 °C at the surface layer in summer), while the latter originates in the boreal seas to the north and cold (6–10 °C in summer). Such currents might have the potential to facilitate larval retention relatively close to spawning ground [67]. Accordingly, genetic differentiation may arise, at least partly, by the combined adaptation of eggs and early larval stages to local environmental conditions on the spawning and nursery

grounds due to hydrological features. However, a more refined insight into this assumption requires additional evidence.

For chub mackerel off China coast, all tests based on microsatellites, including pairwise F_{ST} , 3D-FCA and Bayesian clustering analysis, illustrated that samples from the Bohai Sea and North Yellow Sea (DD, RC and RS) were divergent from those from the East China Sea, a pattern consistent with previous estimates of population structure derived from RAPD analysis [31]. Chub mackerel in Bohai Sea and Yellow Sea are generally from the wintering grounds off the waters of western Kyushu. There is a geographic variation in the spawning season, with chub mackerel in the southern East China Sea spawning approximately 4 months earlier than those in the western Japan Sea [68]. This difference in spawning time and natal homing to different spawning grounds might be responsible for the observed genetic differentiation between these areas.

Table 4 Results of analysis of molecular variance (AMOVA) for different hierarchical analyses of chub mackerel populations

Structure tested	Observed partition			<i>P</i>
	Variance	% total	<i>F</i> statistics	
One gene pool				
Among populations	0.134	5.43	$F_{ST} = 0.054$	0.000
Within populations	2.337	94.57		
Two gene pools (HA, MNP) (DD, RC, RS, YC, NB, WZ, XM, SW)				
Among groups	0.009	0.36	$F_{CT} = 0.004$	0.029
Among populations/ within groups	0.132	5.31	$F_{SC} = 0.053$	0.000
Within populations	2.337	94.33	$F_{ST} = 0.057$	0.000
Three gene pools (HA, MNP) (DD, RS, RC) (YC, NB, WZ, XM, SW)				
Among groups	0.034	1.37	$F_{CT} = 0.014$	0.012
Among populations/ within groups	0.111	4.48	$F_{SC} = 0.045$	0.000
Within populations	2.337	94.15	$F_{ST} = 0.059$	0.000
Four gene pools (HA) (MNP) (DD, RS, RC) (YC, NB, WZ, XM, SW)				
Among groups	0.047	1.89	$F_{CT} = 0.019$	0.038
Among populations/ within groups	0.102	4.09	$F_{SC} = 0.042$	0.000
Within populations	2.337	94.02	$F_{ST} = 0.060$	0.000

Bold *P* numbers are significant values. Refer to Table 1 for abbreviations of sampling sites

Previous analysis of tagging data indicated that chub mackerel in Fujian offshore waters should be divided into two stocks, the East China Sea stock and South Fujian-East Guangdong stock [69]. However, our genetic analysis was inconsistent with this view. Samples collected from the East and South China Seas appeared to constitute a genetically homogenous group. This agreed with the absence of genetic structure reported for chub mackerel sampled around Taiwan waters inferred from AFLP analysis [32]. The similar genetic pattern was also assessed by Zeng et al. [33]. They observed that *S. japonicus* individuals were strongly admixed between the populations from the East China Sea extending to the eastern coast of Hainan Island in the South China Sea. However, the population off the southern coast of Hainan Island in the central South China Sea was genetically different from the other populations. Further sampling in the central South China Sea will be necessary to clarify the population structure of chub mackerel in these areas.

Like many marine pelagic fishes, chub mackerel exhibits highly migratory behavior, large population size and long high potential for dispersal during early life stage (about 29 days-period passive larval stage). Major currents along the China coast are the Kuroshio and the coastal currents (Fig. 1). The warm and highly saline Kuroshio

current, originating from east of Philippine Islands, flows northward along the east coast of Taiwan and reaches the steep east–west continental shelf break of the East China Sea. Then, it splits into two branches, the major one flowing northeast towards the southern coast of Kyushu, and the minor one flowing northward as a shallow surface current [70]. The path of the Kuroshio northeast of Taiwan shows large seasonal variations. It moves seaward in spring and summers and shoreward in fall and winter [71]. The cold and low-saline China Coastal Current flows southwards along the Chinese coast from the Bohai Sea to the Taiwan Strait [72]. At the mercy of these currents, individuals of chub mackerel from the East China Sea may migrate southward along the Taiwan coast to the northern South China Sea. Furthermore, pelagic larvae from the South China Sea could follow the northwards current to the East China Sea for recruitment into the local population [32]. The importance of ocean currents in shaping the genetic structure of spotted mackerel *S. australasicus* populations has recently been demonstrated [65]. It is likely that the same hydrographic features have shaped the genetic patterns in these two closely related species, which have similar biological characteristics. Consequently, the interaction between biological characteristics and marine currents likely facilitates genetic homogeneity of chub mackerel populations of the East and South China Seas.

In conclusion, microsatellite marker is proven to be an effective method to delineate genetic diversity and structure of highly migratory fish species. The results of the present study provided evidence that the biological characteristics of chub mackerel and the oceanographic properties of the Northwestern Pacific could be responsible for the observed population differentiation of chub mackerel. It is noteworthy that the investigation of temporal genetic differentiation in the analyzed locations is needed to test whether the observed population genetic patterns in chub mackerel are stable on a temporal scale. Although sample sizes for microsatellites are small, these sample sizes were still large enough and the microsatellite marker polymorphic enough to provide power to detect population structure of chub mackerel in the studied areas. However, the combined use of nuclear and mitochondrial markers, more comprehensive sampling integrated with local ecological investigations could help to shed more light on the exact ecological process in shaping genetic population structure of chub mackerel.

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