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Original Research Articles

Genetic population structure of the pen shell *Atrina pectinata* along the coastlines of China revealed by microsatellites

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Background

An ecologically and economically important species in East Asia, the natural resources of pen shell *Atrina pectinate* have suffered severe population declines due to habitat destruction, pollution, and overfishing. Assessing genetic diversity and population structure is the basis for establishing conservation programs for *A. pectinate*.

Results

Our data indicated that high genetic diversity was found in all six populations, with mean allelic richness (*Ar*) ranging from 8.255 to 9.734, mean observed heterozygosity (*Ho*) from 0.574 to 0.680, and mean expected heterozygosity (*He*) from 0.620 to 0.691. The five *A. pectinate* populations were divided into two clusters. This clustering result was partly consistent with their geographical origin; the RZ population did not cluster with the northern populations (DL, CD), suggesting that there is no genetic divergence and geographical differentiation between the North China Sea (CD, DL, and RZ) and the Southeast China Sea (HK and ST).

Conclusion

Our results show no significant genetic differentiation between samples from the North China Sea and the Southeast China Sea. High dispersal potential of larvae by passive drift with ocean currents may explain the lack of genetic differentiation between samples. The results suggest a weak level of genetic structure in *A. pectinate* with a long planktonic larval stage.

INTRODUCTION

The pen shell, *A. pectinate*, of the family Pinnidae, is a large wedge-shaped bivalve mollusc naturally distributed along the Indo-West Pacific coast from southeast Africa to Melanesia and New Zealand.[1] As a benthic bivalve with a life span of up to 7 years, the pen shell, living in muddy to sandy substrates, plays an important role in maintaining the ecological stability of seagrass beds, lagoons and coral reefs[1] The family Pinnidae in the Indo-Pacific. Indo-Pacific Moll. 1:175–226. It is also an economically important mollusc in many countries, including China, Korea and Japan. In China, as one of the Eight treasures of seafood, its large, nutritious adductor muscle is traditionally dried and preserved as 'conpoy'. As a lucrative commercial fishery, *A. pectinate* has suffered continuous population declines due to over-fishing, habitat loss, pollution, and other factors.[2] Therefore, knowledge of the population genetic structure of *A. pectinate* is essential for the sustainable management of natural resources. Using mtCOI and nrITS genes, Liu et al investigated the species diversity and evolutionary history of five *A. pectinata* from different geographical groups, supporting that these five morphological forms corresponded to five divergent genetic lineages.[3] A genetic structure analysis of three *A. pectinata* populations along the west coast of South Korea was performed using 13 SSRs and found no significant genetic differentiation.[2] The mitochondrial cytochrome c oxidase subunit I (COI) gene and seven microsatellite loci were used to assessed the population genetic structure of eight *A. pectinate* populations, including seven from northern coast of China and one from the north coast of Korea.[4] The results showed that there was no genetic differentiation between China and North

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Table 1. Collection detail of *A. pectinate* samples

Sample name	Geographic locations	Sea region	Sample size	Collection date
DL	Liaoning (38°95′ N, 121°53′ E)	BHS	28	2021.10-12
CD	Shandong (37° 55′ N, 120° 47′ E)	BHS	30	2021.10-12
RZ	Shandong (35°08′N, 119°53′E)	YS	29	2021.10-12
ST	Guangdong (23°26′N, 113°41′E)	ESC	28	2021.10-12
HK	Hainan (20°03′N, 110°10′E)	SCS	33	2021.10-12
BH	Guangxi (21°27′N, 109°04′E)	SCS	36	2021.10-12

Korean intraspecific populations, and low but significant genetic differentiation between the Northern China and North Korean populations. These results provided important clues for the genetic conservation and management of *A. pectinate* along the Bohai Sea and Yellow Sea. However, the pen shell populations, especially those with variable shells, are mainly distributed in the South China Sea,[5] and until now, the comparative analysis of genetic diversity and population structure of *A. pectinate* between the North China Sea (NCS) and South China Sea (SCS) has never been carried out.

To accurately analyze the level of genetic diversity and gene flow within and among populations of *A. pectinata* in the north and south east coast of China, genic SSR (expressed sequence tag SSR, EST-SSRs) were developed to analyze the genetic structure of *A. pectinata* collected from five natural habitats along the coast of Bohai Sea (BHS), Yellow Sea (YS), East China Sea (ECS) and South China Sea (SCS). Compared with genomic SSRs, genic SSRs are more conservative and have relatively higher transferability, especially in cryptic species.[6] Meanwhile, with high divergence and hybridization, six pen shell populations along China coast have been revealed as cryptic species by molecular and morphological data,[7] so the EST-SSRs are more suitable for the genetic structure analysis of *A. pectinate*. To date, EST-SSRs have been widely used to study the population genetic structure of barley (*Hordeum vulgare*), sugar beet (*Beta vukgaris*), Pacific white shrimp (*Litopenaeus vannamei*) and pearl oyster (*Pinctada maxima*).[8-11] Although many EST-SSRs have been developed for *A. pectinate*the identification and polymorphism validation of EST-SSRs has mainly been carried out.[12] To our knowledge, no EST-SSRs have been used to analyse the population genetic structure of *A. pectinate* from different geographical locations along the Chinese coast.

In the present study, five populations of *A. pectinate* and its genus, *Atrina vexillum*, as an outgroup were collected from 6 different habitats along the Chinese coast. Nine polymorphic EST-SSR markers were used to assess the genetic diversity within and between populations and to estimate the level of gene flow between them, which will help in the conservation, genetic management, and utilization of *A. pectinate*.

MATERIALS AND METHODS

SAMPLE COLLECTIONS AND GENOMIC DNA PREPARATION

Five wild *A. pectinate* populations comprising 148 individuals were collected from five coastal locations in China between 2021 and 2022. Three populations were obtained from the North China Sea (NCS) including the Dalian population (DL), the Changdao population (CD) from the Bohai Sea (BHS) and the Rizhao population (RZ) from the Yellow Sea (YS); the other two populations were sampled in the South East China Sea (SECS), including Shantou (ST) and Haikou (HK). In addition, 36 individuals of *Atrina vexillum* from the Beihai Sea (BH) were included as an outgroup were included, giving a total of 184 samples (**Table 1**, **Figure 1**). The number of individuals in each population ranged from 28 to 36. Adductor muscle tissue was dissected from each sample and immediately preserved in liquid nitrogen, and then stored in a freezer at -80℃. Total genomic DNA was extracted from the adductor muscle using the TIANamp Marine Animals DNA Kit (Tiangen Biotech Co. Ltd., Beijing, China). DNA quality was assessed by agarose gel electrophoresis (1%) and quantity was determined using a Qubit fluorometer (Life Technologies), then the DNA was diluted to 20 ng/μl for further polymerase chain reactions (PCRs).

MICROSATELLITE ANALYSIS

Ten EST-SSR markers with high levels of heterozygosity for *A. pectinate* were analyzed, including AP-28, AP-29, AP-31, AP-41, AP-43, AP-1511, AP-0245 (developed by our library), c55835, c67433 and c69026[12] (**Table 2**). The forward primers of each pair were labeled with a fluorescent dye (6-FAM or HEX; Applied Biosystems, Foster City, CA, United States) at their 5'end. PCR was performed with 5μl Premix Taq (TaKaRa Taq version 2.0 plus dye), 0.1μl of each primer (10μmol/L), and 1μl template DNA in a final reaction volume of 10μl. Thermocycling conditions were as follows: 94℃ for 3 min, 30 cycles of 94℃ for 30 s, 50-60℃ for 20s, 72℃ for 3 min, and an extension at 72℃ for 5 min. PCR products were electrophoresed and genotyped on an ABI Prism 3730XL automated DNA sequencer (Applied Biosystems), and allele range was assessed using DNA sequencing analysis software v5.3.0 (Applied Biosystems) with a ROX 500 size standard.

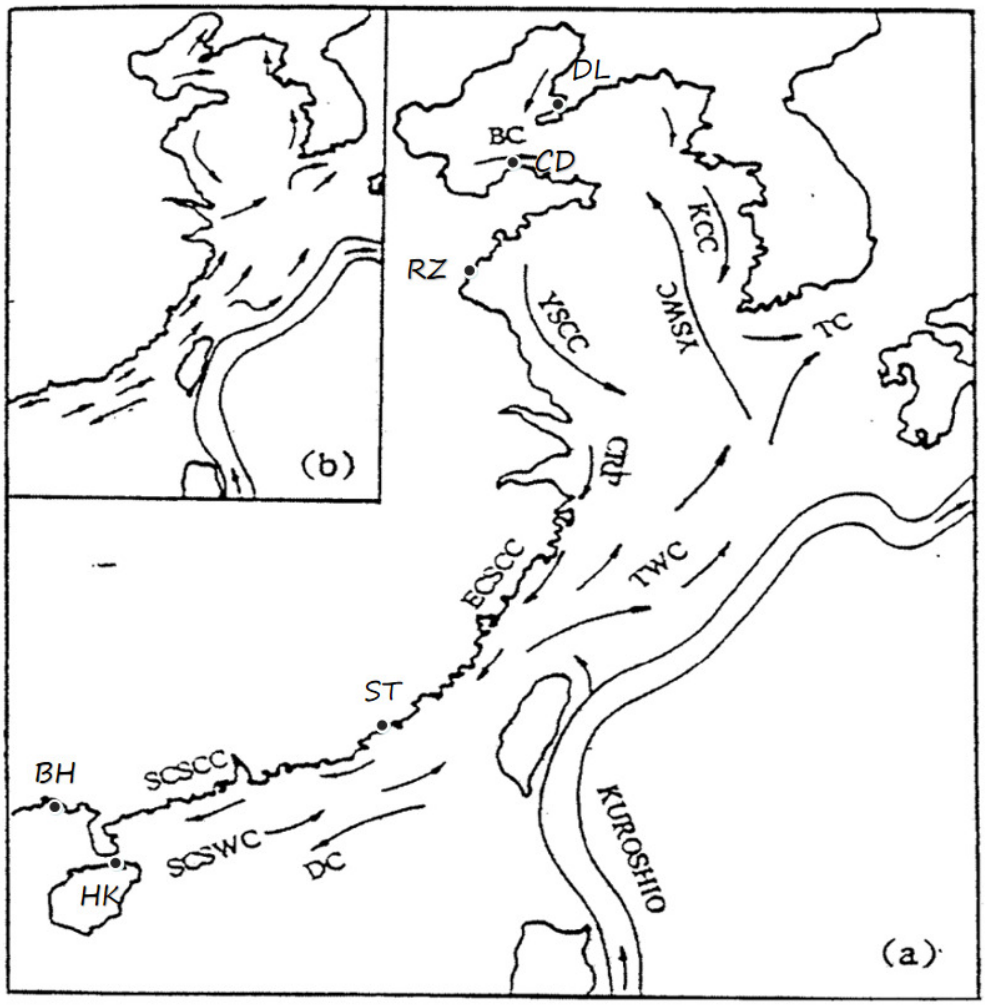

Figure 1. Collection sites and environmental conditions. (a) winter; (b) summer. Local currents are: Bohai Coastal Current (BCC), Yellow Sea Coastal Current (YSCC), Korean Coastal Current (KCC), Yellow Sea Warm Current (YSWC), Tsushima Current (TC), Yangtze River Estuary Plume (CRP), East China Sea Coastal Current (ECSCC), Taiwan Warm Current (TWC), South China Sea Coastal Current (SCSCC), South China Sea Warm Current (SCSWC), East Sand Current (DC).[13]

STATISTICAL ANALYSIS

The effect of null alleles, large allele dropout and allele scoring errors were checked using MICROCHECKER 2.2.3 software prior to population genetic analysis.[14]

The number of alleles (N_A), effective alleles (N_E), observed (H_O) and expected (H_E) heterozygosity, Hardy-Weinberg equilibrium (*HWE*) analysis, and inbreeding coefficient (F_{is}) were calculated using the GENALEX v6.5.[15] To investigate genetic differentiation between and among populations, analysis of molecular variance (AMOVA) and estimation of pairwise F-statistics (F_{ST}) values were performed using Arlequin 3.5 with 10,000 permutations.[16] Multiple simultaneous tests of F_{ST} values were adjusted using the sequential Bonferroni procedure.[17] The effective number of migrants per generation (the gene flow, *Nm*) was estimated using the equation: $Nm = (1-F_{ST})/4F_{ST}$. To standardize the number of alleles among different populations, a minimum of 26 samples per population were used to estimate allelic richness (*Ar*) using the program FSTAT v2.9.4.[18] Genetic distances (*Dc*) were estimated using the chord genetic distance method,[19] and a neighbor-joining tree (NJ tree) was constructed using the MEGA5.0 software based on *Dc*. The Mantel test was used to assess the correlation between genetic and geographic distance using GenAlEx6.5. Model-based Bayesian clustering in STRUCTURE version 2.2 was used to investigate the patterns of population structure further. The K-values were set between 1 and 3. For each value of K, the calculations were performed with a burn-in of 10,000 followed by 100000 iterations under an admixture model with correlated allele frequencies. Three mutation models of IAM, TPM, and SMM with 1000 replications were used to analyze the evidence for a genetic bottleneck in each population using the Bottleneck1.2.02.[20]

Table 2. Characterization of 9 polymorphic microsatellite loci for *A. pectinate*.

Locus	Primer sequence (5′-3′)	Repeat motif	T m(℃)	Accession No.
JY-28	F:CCACTTGGCAGTTGGAAGAT R:TGAATGGTTCTCAAAATGTGCT	(TG) 2	53	SRR24093206
JY-29	F:GAAATTTACTGTCCAAGTCCCG R:TCCAGGGTCATTTAGGTTCA	(ATG)6	54	SRR24093205
JY-31	F:TCGCATCGCTTTGAATACAC R:ATGTGTAAGGACGCGAAAGC	(CT)6	54	SRR24093204
JY-41	F:CACAGTAGGTGTCGGGACATAA R:GCTGACTGAGGCCAGCTAAC	(TG)6	58	SRR24093203
JY-43	F:GCCTATTGGTGTCCCATTGT R:TGGCGGACTTTCAAACAAAT	(TA)6	55	SRR24093202
JY-0245	F:CCCTGGTGGACAGTTCAGAT R:AGGTGATGAGAGAATGAGAGGG	(TC)7	55	SRR24093201
c55835	F:GACCATCCAAGACCAGCTCA R:CGGTTTGTGTGTTCAAGCCA	(AC)9	55.4	KX061857
c67433	F:GGCAGACCCTTGATGTACCA R:GGCAAAACAAGAAACAAAACGCA	(CA)9	60.8	KX061847
c69026	F:TCACAGTTGGACAGGTCTTTGT R:ATTCAGAGCAGGTGCCAGTC	(CA)11	57.4	KX061864

RESULTS

VARIATION OF MARKERS

According to the result of genotype analysis, these nine microsatellite loci were all highly polymorphic. A total of 174 alleles, including 115 and 59, were detected in five populations of *A. pectinate* and one outgroup of *A. vexillum*, respectively. The number of alleles (*Na*) per locus varied from 8 (JY-29) to 29 (c67433), and the average number of alleles at the locus level ranged from 4.667±0.333 (JY-41) to 18.833±1.327 (wc55853) (**Table 3**). The mean effective number of alleles (*Ne*) per locus in all samples ranged from 1.984±0.091 (JY-41) to 11.822±1.460 (wc55835), and the allelic richness (*Ar*) per locus ranged from 4.395±1.249 (JY-43) to 18.263±2.953 (wc55835). The mean allelic richness for each population was as follows: HK (8.255), CD (8.422), ST (8.556), DL (8.889), BH (9.921), and RZ (9.734). No significant difference in mean *Ar* was found between the six populations (P= 0.9995). The mean values of observed (*Ho*) and expected heterozygosity (*He*) ranged from 0.391±0.092 (JY-31) to 0.933±0.029 (JY43) and from 0.430±0.096 (JY-1511) to 0.908±0.012(wc55853), respectively. On average, the outgroup of the BH population had the lowest *He* value (0.620), and the DL population had the highest (0.691) (**Table 3**).

Linkage disequilibrium was assessed for all 216 pairwise combinations in 6 populations using Arlequin 3.5 software, the results showed that 25 pairwise linkage disequilibria were detected at the significant level p=0.05. The observed heterozygosity (*Ho*) were tested for concordance with the *HWE* (**Table 3**). Twelve cases of locus-population combinations (BH, DL and HK at c67433; CD at JY28; CD, DL, HK, RZ and ST at c5585; HK, RZ and ST at JY43;) out of 54 (six populations-nine loci) showed significant deviation from *HWE* (P<0.001, after sequential Bonferroni correction =0.05/54).[17] Of these, eight out of 12 deviations showed significant heterozygote deficiencies (*Ho*<*He*). The possibility of null alleles was detected using MICRO-CHECKER; the results suggested the presence of null alleles at wc55835 in all populations and c67433 in five populations (except BH). The genotypes of wc55835 and c67433 were therefore excluded from further analysis.

POPULATION GENETIC STRUCTURE

The pairwise genetic distance (*Dc*) and genetic identity (*IN*) are shown in **Table 4**. Among five populations of *A. pectinate*, the pairwise *Dc* varied from 0.027 (DL-CD) to 0.191 (CD-RZ), and the genetic identity varied from 0.826 to 0.974 (**Table 4**). A neighbor-joining tree was constructed using Nei's genetic distance (D_C) values between five *A. pectinate* populations and *A. vexillum* as an outgroup (BH) based on seven microsatellite loci. The five *A. pectinate* populations were divided into two clusters, with the CD and DL populations from the Bohai seas (BHS) forming the first group, followed by the HK and ST populations from South East China Sea (SCES), and then with the RZ population from the Yellow Sea (YS) forming the second group (**Figure 2**).

Although this clustering result was partly consistent with their geographical origin, the RZ population did not cluster with the northern populations (DL, CD), suggesting that there is no significant genetic divergence and geographical differentiation between the North China Sea (CD, DL and RZ) and the Southeast China Sea (HK and ST) (**Figure 2**). According to the NJ tree topologies, AMOVA analyses were performed on *A. pectinate* for the levels of genetic differentiation between populations among groups, among populations within groups, between individuals within populations (**Table 5**). The results showed that most of the variation was within populations (93.389%), followed by among groups (5.829%) and among populations within

Table 3. Summary of the statics for nine microsatellite loci in pen shell *A. pectinate*.

Pop		JY-28	JY-29	JY-31	JY-41	JY-43	JY-0245	c69026	wc55835	c67433	Mean
BH	N	36	36	36	36	36	36	36	36	36	36
	Na	22	4	21	3	6	3	7	16	17	11
	Ne	8.416	1.088	9.565	1.617	2.359	1.774	2.318	7.579	9.159	4.875
	Ar	19.430	3.333	18.921	2.778	5.507	3.000	6.064	14.605	15.652	9.921
	I	2.583	0.219	2.603	0.616	1.072	0.771	1.042	2.344	2.453	1.522
	Ho	0.750	0.083	0.833	0.222	0.833	0.556	0.556	0.667	0.889	0.599
	He	0.881	0.081	0.895	0.382	0.576	0.436	0.569	0.868	0.891	0.620
	F	0.149	-0.029	0.069	0.418	-0.447	-0.273	0.023	0.232	0.002	0.016
CD	N	30	30	30	30	30	30	30	30	30	30
	Na	6	4	4	5	6	4	17	17	14	8.555
	Ne	4.186	2.211	1.317	2.209	2.695	3.061	8.955	12.766	5.056	4.717
	Ar	5.933	3.933	3.930	4.930	5.863	3.997	16.653	16.976	13.586	8.422
	I	1.540	0.914	0.513	1.054	1.183	1.186	2.476	2.695	2.011	1.508
	Ho	0.300	0.600	0.200	0.700	0.867	0.567	0.800	0.567	0.567	0.574
	He	0.761	0.548	0.241	0.547	0.629	0.673	0.888	0.922	0.802	0.668
	F	0.606	-0.095	0.169	-0.279	-0.378	0.158	0.099	0.385	0.294	0.107
DL	N	28	28	28	28	28	28	28	28	28	28
	Na	5	6	5	5	5	7	16	19	12	8.889
	Ne	3.329	3.099	1.524	1.922	2.685	3.881	10.182	11.362	5.744	4.859
	Ar	5.000	6.000	5.000	5.000	5.000	7.000	16.000	19.000	12.000	8.889
	I	1.359	1.332	0.759	0.952	1.217	1.515	2.507	2.671	2.075	1.599
	Ho	0.643	0.571	0.321	0.607	0.929	0.536	0.821	0.607	0.464	0.611
	He	0.700	0.677	0.344	0.480	0.628	0.742	0.902	0.912	0.826	0.691
	F	0.081	0.156	0.065	-0.266	-0.480	0.278	0.089	0.334	0.438	0.077
RZ	N	29	29	29	29	29	29	29	29	29	29
	Na	5	5	6	5	4	8	15	25	19	10.222
	Ne	2.486	2.453	1.703	2.176	2.877	3.377	9.229	16.754	8.313	5.485
	Ar	4.848	4.974	5.845	4.848	4.000	7.522	14.479	23.275	17.847	9.7338
	I	1.170	1.091	0.924	1.045	1.196	1.458	2.431	2.988	2.508	1.646

Pop		JY-28	JY-29	JY-31	JY-41	JY-43	JY-0245	c69026	wc55835	c67433	Mean
	Ho	0.485	0.697	0.394	0.727	0.970	0.515	0.879	0.697	0.758	0.680
	He	0.598	0.592	0.413	0.540	0.652	0.704	0.892	0.940	0.880	0.690
	F	0.189	-0.177	0.046	-0.346	-0.486	0.268	0.014	0.259	0.139	-0.010
HK	N	33	33	33	33	33	33	33	33	33	33
	Na	5	9	4	5	3	4	16	17	12	8.333
	Ne	3.305	3.109	1.536	1.890	2.499	2.441	10.012	8.087	5.840	4.302
	Ar	4.999	8.985	3.966	4.931	3.000	3.966	15.792	16.724	11.929	8.255
	I	1.354	1.445	0.680	0.885	0.988	1.031	2.482	2.408	2.054	1.481
	Ho	0.483	0.690	0.276	0.586	1.000	0.621	0.862	0.621	0.586	0.636
	He	0.697	0.678	0.349	0.471	0.600	0.590	0.900	0.876	0.829	0.666
	F	0.308	-0.017	0.210	-0.245	-0.667	-0.051	0.042	0.292	0.293	0018
ST	N	28	28	28	28	28	28	28	28	28	28
	Na	4	6	4	5	3	6	17	19	13	8.555
	Ne	3.446	3.161	1.512	2.093	2.618	3.174	9.924	14.385	4.723	5.004
	Ar	4.000	6.000	4.000	5.000	3.000	6.000	17.000	19.000	13.000	8.556
	I	1.305	1.341	0.691	1.046	1.018	1.281	2.511	2.815	2.016	1.558
	Ho	0.679	0.821	0.321	0.679	1.000	0.571	0.893	0.464	0.607	0.671
	He	0.710	0.684	0.339	0.522	0.618	0.685	0.899	0.930	0.788	0.686
	F	0.044	-0.201	0.051	-0.299	-0.618	0.166	0.007	0.501	0.230	-0.013
Pop		JY-28	JY-29	JY-31	JY-41	JY-43	JY-0245	c69026	Wc55835	c67433	
Total mean	N	30.667	30.667	30.667	30.667	30.667	30.667	30.667	30.667	30.667	
	Na	7.833	5.667	7.333	4.667	4.500	5.333	14.667	18.833	14.500	
	Ne	4.195	2.520	2.859	1.984	2.622	2.951	8.437	11.822	6.472	
	Ar	7.368	5.538	6.944	4.581	4.395	5.248	14.331	18.263	14.002	
	I	1.552	1.057	1.028	0.933	1.112	1.207	2.241	2.653	2.186	
	Ho	0.557	0.577	0.391	0.587	0.933	0.561	0.802	0.604	0.645	
	He	0.724	0.543	0.430	0.490	0.617	0.639	0.842	0.908	0.836	
	F	0.229	-0.060	0.101	-0.170	-0.513	0.091	0.046	0.334	0.232	

Number of samples (*N*), No. of Different Alleles (*Na*), No. of Effective Alleles (*Ne*), allele richness (*Ar*), Shannon's Information Index (*I*), Observed Heterozygosity (*Ho*), Expected Heterozygosity (*He*), Fixation Index (*F*). Bold type indicates significant deviations from Hardy-Weinberg equilibrium after Bonferroni correction ($P<0.001$, after Bonferroni correction =0.05/54).

Table 4. Nei's genetic distance and Nei genetic identity of pen shell *A. pectinate*

	CD	DL	RZ	HK	ST	BH
CD	-	0.974	0.826	0.834	0.850	0.344
DL	0.027	-	0.863	0.853	0.872	0.338
RZ	0.191	0.147	-	0.950	0.942	0.240
ST	0.181	0.159	0.051	-	0.960	0.272
HK	0.163	0.137	0.060	0.041	-	0.247
BH	1.084	1.066	1.425	1.303	1.396	-

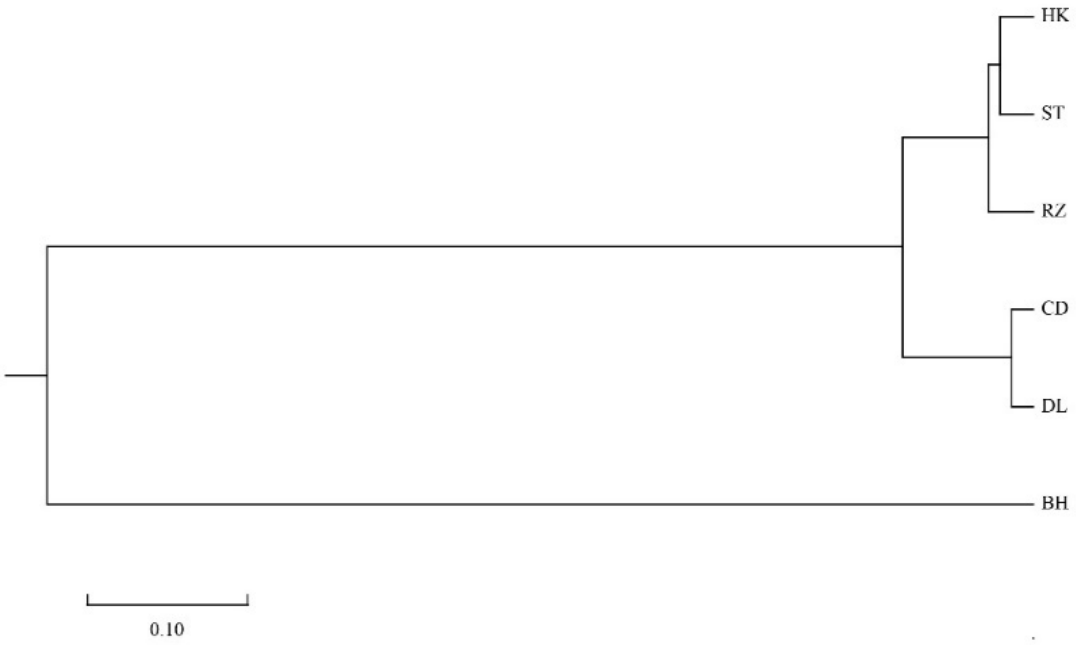

Figure 2. A neighbor-joining tree based on *Dc* distances for five *A. pectinate* populations based on seven microsatellites. The BH population of *A. vexillum* was used as the outgroup.

Table 5. Analysis of molecular variance (AMOVA) based on microsatellite datasets.

Source of variation	df	Variance components	Percentage of variation%	F-statistic
Among groups	1	0.139	5.829	F_{CT}=0.058
Among populations Within groups	3	0.019	0.782	F_{SC}=0.008*
Within population	291	2.228	93.389	F_{ST}=0.066**
Total variation	295	2.385	100	

*Significant at P<0.05, **significant at P<0.01

groups (0.782%), with among populations within groups and within populations showing significant variation (P<0.05).

Pairwise F_{ST} values ranged from 0.008 (between DL and CD) to 0.342 (between BH and HK) (**Table 6**). Eleven of the 15 pairwise F_{ST} values were significant after Bonferroni correction (P < 0.003, Bonferroni correction = 0.05/15), among which the pairwise F_{ST} values between *A. vexillum* (BH) and *A. pectinate* (DL, CD, RZ, HK and ST) showed relatively high genetic differentiation ranging from 0.302 (BH-DL) to 0.342 (BH-RZ), whereas the pairwise F_{ST} values between five *A. pectinate* populations were only 0.008 (DL-CD) to 0.042 (CD-HK, CD-RZ), indicating that *A. vexillum* is genetically distinct from *A. pectinate* (**Table 6**). Pairwise F_{ST} analysis indicated that only DL-CD, HK-ST, HK-RZ and RZ-ST showed no genetic differentiation (**Table 6**), consistent with the NJ tree clustering results (**Figure 2**). The effective number of migrants per generation (Nm) in *A. pectinate*

Table 6. Pairwise F_{ST} (below diagonal) and the estimated number of migrants per generation (*Nm*) based on seven EST-SSR loci (above the diagonal)

	BH	CD	DL	RZ	HK	ST
BH	-	0.545	0.578	0.490	0.482	0.494
CD	0.314*	-	30.450	5.637	5.651	6.654
DL	0.302*	0.008	-	7.894	6.906	8.624
RZ	0.338*	0.042*	0.031*	-	16.884	15.827
HK	0.342*	0.042*	0.035*	0.014	-	22.152
ST	0.336*	0.036*	0.028*	0.016	0.011	-

* Significant after Bonferroni correction ($P < 0.05/15$).

ranged from 5.651 (CD-HK) to 30.450 (CD-DL), suggesting considerable gene exchange especially between DL and CD, HK and ST, HK and RZ, ST and RZ (**Table 6**). For the bottleneck tests, no significant heterozygote excess was detected under three mutation models of IAM, TPM and SMM (**Table 7**), consistent with the normal L-shaped distribution of allele frequencies in each population. The result indicated that six populations had not experienced a genetic bottleneck in the recent past.

Genetic structure analyses used Structure 2.3 with the best-fit *K*=2 (**Figure 3A**). Although the results showed that the *A. pectinate* populations could be divided into two clusters, the five populations did not show any distinct structure (**Figure 3B**), suggesting that they were individual populations. Mantel test *R*-value for isolation by distance was 0.335 (*P*=0.150), indicating no significant relationship between genetic and geographic distance (*P*>0.05). This indicates no genetic divergence and geographic differentiation among *A. pectinate* populations along the Chinese coast (**Figure 4**).

DISCUSSION

GENETIC DIVERSITY AND DEPARTURES FROM HWE

Genetic diversity assessment is the basis for *A. pectinate* to establish breeding and conservation programs. In the present study, nine EST-SSR markers were used to study the genetic diversity of five *A. pectinate* populations including DL, CD, RZ, ST and HK, among which six EST-SSR markers were developed in our laboratory, the other three EST-SSR markers were identified by Sun et al.[12] Based on these nine SSR markers, 115 alleles were detected in 148 samples of *A. pectinate* with mean values of *Na*=9.067, *Ar*=8.978, *Ho*=0.634 and *He*=0.679, respectively, which were higher than those detected in the EST-SSR development of *A. pectinate* by Sun et al.[12] (with 3 out of 23 loci identical to those used in our study, i.e., c55835, c67433 and c69026). This result is due to the different genotyping method used by Sun et al.,[12] which used silver staining to visualize genotypes. Compared to the sequencing-based genotyping used in this study, the resolution of the silver staining method is much lower than the sequencing-based genotyping, which significantly affected the allele detection rate. The genetic diversity in the present study was lower than that in Korea[2,21]and China of *A. pectinate*.[4] The different SSR type may be the main cause of the low genetic diversity in our study. Some studies suggested that the average number of alleles for SSR primers derived from the non-coding DNA region (genomic SSRs) was higher than that of EST-SSR primers (genic SSRs).[22,23] Similarly, the mean allele (*Na*) per locus of EST-SSR markers in this study was lower (8.911) than that of genomic SSR markers (16.03) in the genetic structure of *A. pectinate* by An et al.,[21] which may be due to the location of EST-SSR markers in more conserved and expressed sequences compared to genomic SSR in relatively variable non-coding region, as shown in many studies.[23-27]

Most of the locus-population combination cases were in *HWE*, only 12 (BH, DL and HK at c67433; CD at JY28; CD, DL, HK, RZ and ST at c5585; HK, RZ and ST at JY43) of them showed significant deviation from *HWE* ($p<0.05$), and 8 out of these 12 cases (BH, DL and HK at c67433; CD, DL, HK, RZ and ST at c5585) showed significant heterozygote deficiencies (*Ho*<*He*), which was the main cause of deviation from *HWE*. Significant heterozygote deficiency has been frequently reported in marine invertebrates.[2,4,28,29] Studies have suggested that null alleles are a potential cause of this phenomenon.[28,30] In the present study, the null alleles at c55835 were detected in all six populations and c67433 in five populations (except BH), of which 8 were heterozygous deficient, consistent with the results of the *HWE* test (**Table 2**). This result indicates that null alleles play an important role in heterozygote deficiency and deviation from *HWE*, which was also found in *Argopecten irradians*,[31] *A. pectinate*,[4] *Meretrix meretrix*.[28,29] The occurrence of null alleles is associated with mutations within the primer sequence that can prevent primers binding and can be avoided by primer redesigning.[32] In addition, heterozygote excess was also detected at JY-43 in RZ, HK and ST (*Ho*>*He*).

4.2. GENETIC STRUCTURE AND GENETIC DIFFERENTIATION

Knowledge of the population structure and gene flow is essential for effective management and conservation. In this study, the genetic relationships between the five populations were basically *consistent with* their natural geographic locations, except for RZ, which didn't cluster together with the north populations (DL, CD) from the Bohai Sea but with the southern populations (HK, ST). Genetic differences in

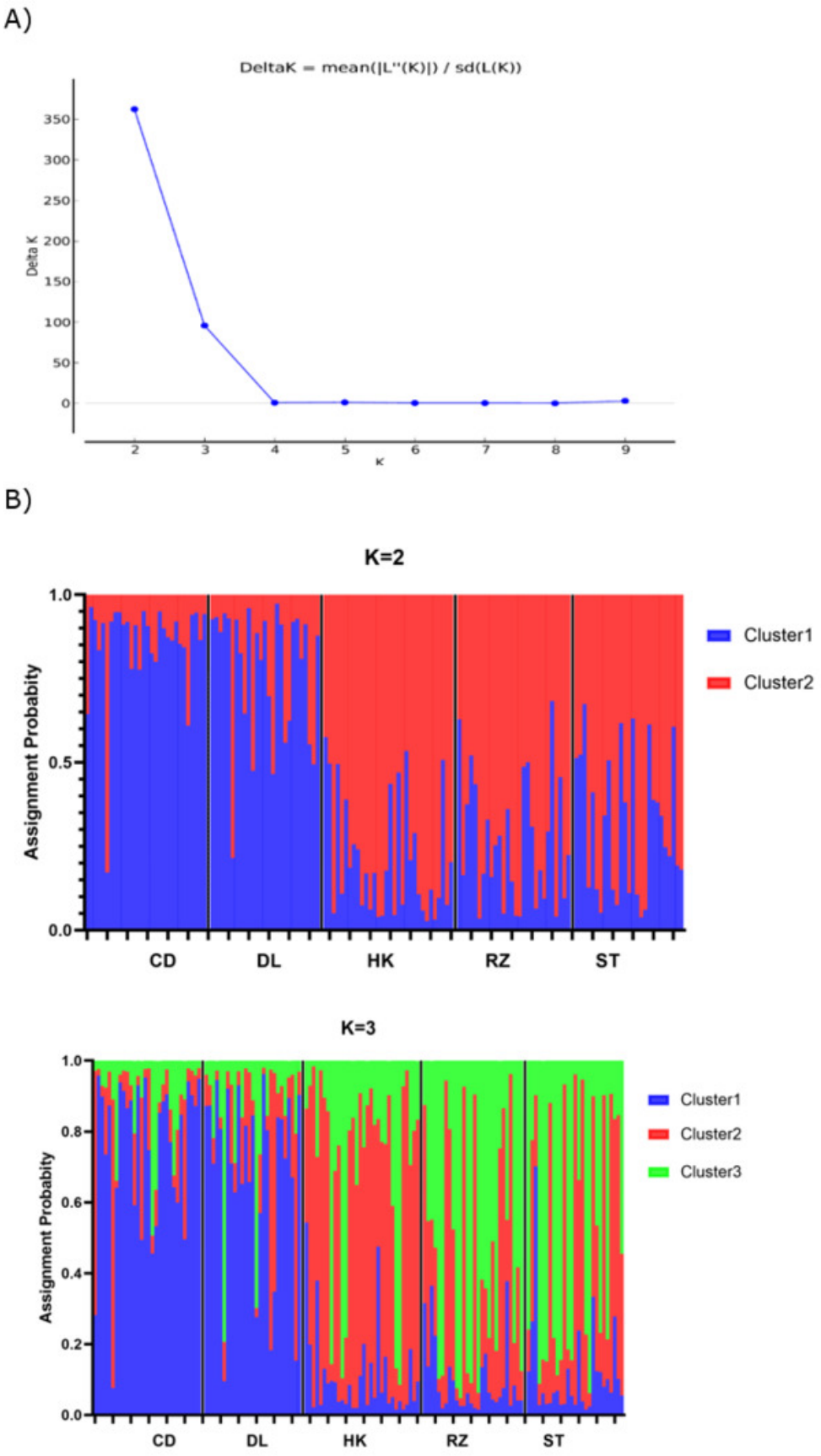

Figure 3. Cluster analysis by STRUCTURE for 5 populations of *A. pectinate* based on seven polymorphic microsatellite loci. (A) The distribution of isolate assignment for the number of clusters (*K*) ranging K from 2 to 10; (B) The estimated Delta K (Δ*K*) for the number of clusters ranging from 2 to 3.

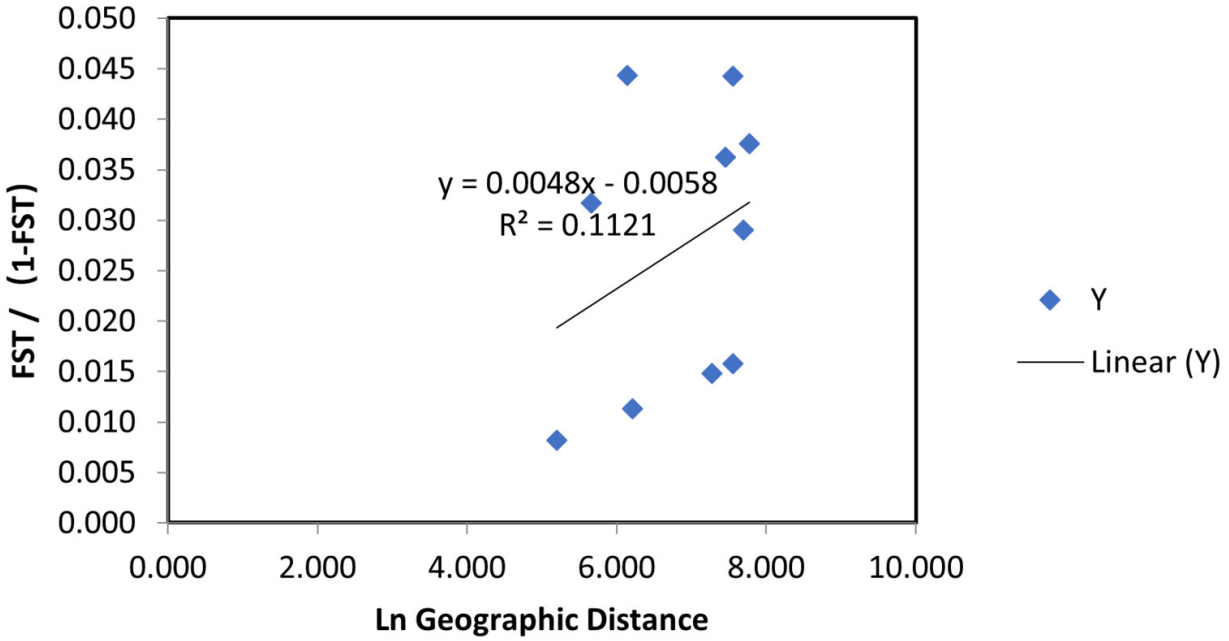

Figure 4. Mantel test of correction of genetic distance with geographic distance for populations of *A. pectinate*.

marine organisms between different geographical populations are often influenced by ocean currents,[28,33] dispersal capacity,[4] environmental tolerance and substrate and the interaction between these factors.[34,35] It is well known that the Bohai Sea, a semi-enclosed sea, has a relatively low water exchange rate with the Yellow Sea,[36,37] which to some extent hinders the migration of *A. pectinate* larvae from the Bohai Sea to the Yellow Sea, which may be the main reason for the separation of the RZ population from the CD and DL populations. Similar results have also been reported in other marine bivalves, including *Scapharca broughtonii*[5] and *Chlamys farreri*.[37]

For marine organisms, the dispersal ability of larvae plays an important role in the formation of the genetic structure.[38,39] Many marine organisms have pelagic larvae that can potentially link distant populations by dispersal on ocean currents.[40,41] The planktonic larval duration (*PLD*) of *A. pectinate* is approximately 30 days with main reproductive season from April to September.[4,42] In spring and summer, a warm, salty current known as the Taiwan Warm Current flows offshore of the Kuroshio, mainly through the Tsushima Strait into the Sea of Japan, but some of it flows intermittently north along the Korean coast into the Yellow Sea and merges with the Yellow Sea warm current (**Figure 1**).[43] This means that for the long-lived planktonic larvae of the pen shell, the Taiwan Warm Current could transport the planktonic larvae of the ST population from the East China Sea to the Yellow Sea, although the migration may be affected by the summer Yangtze River outflow, which reduces the salinity,[28,33] the wide range of salinity tolerated by *A. pectinate* may facilitate gene flow via long-distance dispersal of *A. pectinate* larvae.[44]

In addition, the geographical barriers caused by primary production, freshwater influence and turbidity are all present on continental margins and around high islands, which has implications for the effectiveness of barriers to larval dispersal.[34,45] However, *A. pectinate* could live down to depths of 100m,[4] so its larval dispersal might be less affected by the ecological or hydrographical barriers. Therefore, the larvae of *A. pectinate* from the ESC could overcome the barriers caused by the freshwater discharge of the Yangtze River and migrate to the Yellow Sea. These could be the reasons for the clustering of the RZ population from YS with ST from ESC and HK from SCS.

Despite the geographical barriers between the Yellow Sea and Bohai Sea, East China Sea and Yellow Sea, respectively, there was genetic exchange between the five populations of *A. pectinate*. According to this study, the pairwise F_{ST} values ranged from -0.003 to 0.059. Wright suggested that $0.05 < F_{ST} < 0.15$ represented a moderate degree of genetic differentiation; whereas $0.15 < F_{ST} < 0.25$ and $F_{ST} > 0.25$ indicate a high degree of genetic differentiation.[42] A lower F_{ST} value indicates a higher level of gene flow and lower genetic differentiation between populations.[22] Thus, in our study, low F_{ST} values were obtained within and between clusters (CD, DL, and RZ, ST, HK), indicating a high level of gene flow, and a relatively low genetic differentiation was detected among *A. pectinate* populations. The AMOVA test indicated no significant genetic differentiation within groups ($p<0.05$). In addition, the Mantel test ($P>0.05$) also suggested that the genetic structure of *A. pectinate* populations was not significantly influenced by geographical distances along the Chinese coasts (**Figure 4**). The same results were found in the population structure analyses of *A. pectinate* populations by An et al.,[21] which show no genetic differentiation between populations from different locations, likely due to the high dispersal potential. Marine organisms with high dispersal potential gener-

ally show low levels of genetic differentiation over large geographical distances.[3,46,47]

CONCLUSION

The present study suggests that although the five populations were divided into two sub-clusters, strong gene flow exists between them, especially within each sub-cluster. No genetic divergence and geographic differentiation were found among *A. pectinate* populations. The high connectivity level and low genetic structure of A. pectinate along the Chinese coast were probably caused by gene flow through larval dispersal.

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AUTHORS' CONTRIBUTION

Writing – original draft: Peican Zhu (Equal), Fukai Wang (Equal). Writing – review & editing: Peican Zhu (Equal), Fukai Wang (Equal), Bo Liu (Equal). Conceptualization: Biao Wu (Equal), Chunde Wang (Equal). Resources: Biao Wu (Equal), Bo Liu (Equal). Formal Analysis: Feng Wang (Equal), Xiaotong Zhang (Equal), Kai Yu (Equal). Investigation: Feng Wang (Equal), Xiaotong Zhang (Equal), Kai Yu (Equal). Methodology: Chunde Wang (Equal), Bo Liu (Equal). Funding acquisition: Bo Liu (Lead). Data curation: Bo Liu (Equal). Validation: Bo Liu (Equal).

Peican Zhu and Fukai Wang contributed equally to this work

CONFLICTS OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

ETHICAL APPROVAL

This article contains no studies with human participants or animals performed by authors.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

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All are available upon reasonable request.

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