Zoological Studies **51**(3): 406-414 (2012)

Zoological Studies

Significant Genetic Differentiation among Ten Populations of the Razor Clam *Sinonovacula constricta* along the Coast of China Revealed by a Microsatellite Analysis

Dong-Hong Niu[1], Bing-Bing Feng[1], Da-Bo Liu[1], Yu-Min Zhong[1], He-Ding Shen[1], and Jia-Le Li[1,2,*]

[1]*Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Shanghai Ocean University, Ministry of Education, 999 Hucheng Huan Road, Shanghai 201306, China*

[2]*Aquaculture Division, E-Institute of Shanghai Universities, Shanghai Ocean University, 999 Hucheng Huan Road, Shanghai 201306, China*

(Accepted November 9, 2011)

Dong-Hong Niu, Bing-Bing Feng, Da-Bo Liu, Yu-Min Zhong, He-Ding Shen, and Jia-Le Li (2012) Significant genetic differentiation among ten populations of the razor clam *Sinonovacula constricta* along the coast of China revealed by a microsatellite analysis. *Zoological Studies* **51**(3): 406-414. The razor clam *Sinonovacula constricta* is a common shellfish in human seafood diets. Little is known about the genetic structure of the species on the coast of China. In this study, the genetic diversity and differentiation of *S. constricta* from the northern, middle, and southern coasts of China were estimated using 8 microsatellites. The 10 populations possessed high allelic (*Ar* = 6.0-7.0) and genetic diversities (*Ho* = 0.737-0.909 and *He* = 0.836-0.882) across the 8 microsatellite loci. The F_{ST} analysis indicated significant genetic differentiation (F_{ST} = 0.044, $p < 0.05$) among the 10 populations. Two populations in the middle region significantly differed from the remaining populations (F_{ST} = 0.039-0.125, $p < 0.05$). Moreover, the phylogenetic Neighbor-joining tree analysis showed that the 10 populations were divided into 2 groups: 1 group included populations in the middle region and 2 populations of the northern region, and the other group consisted of populations in the southern region and the remaining ones from the northern region. Potential reasons for the high genetic diversity and significant population differentiation are discussed. Perhaps, there is a cryptic species according to the significantly high genetic divergence among the 10 populations of *S. constricta*. http://zoolstud.sinica.edu.tw/Journals/51.3/406.pdf

Key words: *Sinonovacula constricta*, Microsatellite, Genetic diversity, Genetic differentiation.

Marine organisms with a planktonic larval stage often exhibit lower levels of genetic structure than those with direct development because of high dispersal and the absence of obvious geographical barriers (Kyle and Boulding 2000, Collin 2001, Pavesi et al. 2011). For instance, between 2 European species of *Hydrobia*, the species with a 1-3-d planktonic stage had a significantly lower population structure than the directly developing species (Wilke and Davis 2000). However, this issue is still controversial. Mitochondrial cytochrome *b* sequence data of 4 species of the marine gastropod *Littorina*, 2 with planktonic larvae and 2 direct developers, showed a high level of genetic structuring in one with planktonic larvae and a lower level of structure in one of the direct developers (Kyle and Boulding 2000). *Macoma balthica* showed significant differentiation between the Baltic Sea and other populations from Europe with a 2-5-wk pelagic larval phase according to data from mitochondrial (mt)DNA cytochrome *c* oxidase subunit I (COI) (Luttikhuizen et al. 2003). *Coelomactra antiquate*, a large benthic clam, has a 9-14-d planktonic life stage. An allozyme analysis

*To whom correspondence and reprint requests should be addressed. Tel/Fax: 86-021-61900401. E-mail:jlli@shou.edu.cn

revealed a high level of genetic differentiation between 3 northern populations and 1 southern population (Kong et al. 2007). Populations of the surf clam *Paphia undulata* (which has a 12-d planktonic stage) showed genetic differences within a small geographical range in the Gulf of Thailand by ISSR analysis (Donrung et al. 2011). Thus, factors other than planktonic larval dispersal may affect the genetic structure. In spite of the migration potential due to planktonic larval dispersal, other factors such as geographical isolation (Mariani et al. 2002), temperature, salinity (Collin 2001), and habitat fragmentation (Hummel et al. 1994) also significantly contribute to the genetic structure.

Sinonovacula constricta, of the Mollusca (Bivalvia: Veneroida: Solecurtidae), lives in lower-to-mid intertidal zones along coasts of the western Pacific Ocean (Qi 1984). Similar to most marine bivalves, *S. constricta* has a 6-9-d planktonic larval stage and is a benthic razor clam as an adult. Despite the attention paid to several biological aspects of this species during the last few decades, including studies of biochemistry (Tamari 1986), developmental biology (Chung 2008), and immunology (Song et al. 2009, Feng et al. 2010), the genetic characteristics of local populations are not well understood. In addition, the species has a high market value because of its short culture cycle, rapid growth rate, and delicious taste. Indeed, it is one of 4 major aquacultured clams along with *Crassostrea gigas*, *Ruditapes philippinarum*, and *Tegillarca granosa* in China (Xie 2003). Although this clam was introduced to the Liaoning, Hebei, and Shandong coasts of China hundreds of years ago (Xie 2003) and is widely distributed along the coast of China, its farming in China began in coastal regions of Fujian and Zhejiang Provinces. Long-term anthropogenic activities may have led to exotic seedlings being released into the local environment and gene flow occurring between introduced and native populations.

Recently, the powerful tool of microsatellite markers has been extensively used in genetic studies (Goldstein and Schlotterer 1999). Studies on genetic diversity were conducted using microsatellite markers in many aquatic species, such as the Japanese scallop (*Mizuhopecten yessoensis*) (Sato et al. 2005), Pacific oyster (*Crassostrea gigas*) (Yamtich et al. 2005), Asian seabass (*Lates calcarifer*) (Yue et al. 2009), grass carp (*Ctenopharyngodon idella*) (Liu et al. 2009), Japanese eel (*Anguilla japonica*) (Han et al. 2010), and triangle pearl mussel (*Hyriopsis cumingii*) (Li et al. 2009).

In this work, 8 microsatellites were used to analyze 10 populations of *S. constricta* along the coast of China in order to determine genetic variations and the population structure. The results will potentially lead to better management and future selective breeding programs for *S. constricta.*

MATERIALS AND METHODS

Sample collection

In total, 400 wild adult individuals were randomly collected from 10 locations along the coast of China. These locations included Mingyang Town of Zhuanghe City (ZH) in Liaoning Province; Caijiabu Town of Hangu District (HG) in Tianjin City; Xingcun Town of Haiyang City (HY), and Qingdao City (QD) in Shandong Province; Sheyang County of Yancheng City (YC) in Jiangsu Province; the Dongtan Wetland of Chongming I. (CM) in Shanghai City; Xiangshan County of Ningbo City (NB), and Pengjie Town of Taizhou City (TZ) in Zhejiang Province; and Xiapu County of Ningde City (ND), and Yunxiao County of Zhangzhou City (ZZ) in Fujian Province (Fig. 1).

DNA extraction and microsatellite analysis

Mantle tissues from each specimen were collected and stored in 95% ethanol. Total genomic DNA was isolated from mantle tissues using the phenol-chloroform extraction method (Sambrook and Russell 2001).

Eight polymorphic microsatellites (*Sco-117*_EU272076, *Sco-146*_EU272060, *Sco-221*_EU272063, *Sco-222*_EU272066, *Sco-400*_EU285666, *Sco-431*_EU272072, *Sco-448*_EU272073, and *Sco-470*_EU272076) were selected from markers previously identified by our group (Niu et al. 2008). Polymerase chain reaction (PCR) amplification for each microsatellite locus was conducted on a PTC-100 PCR instrument (Eppendorf, Hamburg, Germany). Each PCR (25 μl in total volume) contained 20 ng of genomic DNA, 0.4 U of *Taq* polymerase (Takara, Otsu, Shiga, Japan), 1x Taq PCR buffer, 0.2 μM dNTPs, and 0.2 μM of each primer. Microsatellite loci were amplified under the following conditions: 3 min of denaturation at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at the optimal annealing temperature

(Niu et al. 2008), and 30 s at 72°C; with a final extension at 72°C for 10 min. Microsatellite alleles were identified by 8% polyacrylamide gel electrophoresis followed by staining with $AgNO_3$. Molecular weights were estimated with the PUC18 marker (Takara, Otsu, Shiga, Japan).

Data analysis

Each microsatellite was examined for genotyping errors using MicroChecker (Van Oosterhout et al. 2004). The observed (*Ho*) and expected (*He*) heterozygosities were analyzed using GDA (Lewis and Zaykin 2000). Allelic richness (*Ar*) per locus and the fixation index (F_{IS}) (Weir and Cockerham 1984) per population were calculated using FSTAT (Goudet 1995). Exact tests for deviations from Hardy-Weinberg equilibrium (HWE) were performed using Arlenquin 3.1 (Excoffier et al. 2005) with default parameters using a Markov chain method.

All estimates (F_{ST} and analysis of molecular variance (AMOVA)) of the population structure and their significance were calculated using Arlequin 3.1 (Excoffier et al. 2005). For the AMOVA, the total variance was divided into variances among populations, among individuals within populations, and within individuals. A D_A genetic distance matrix

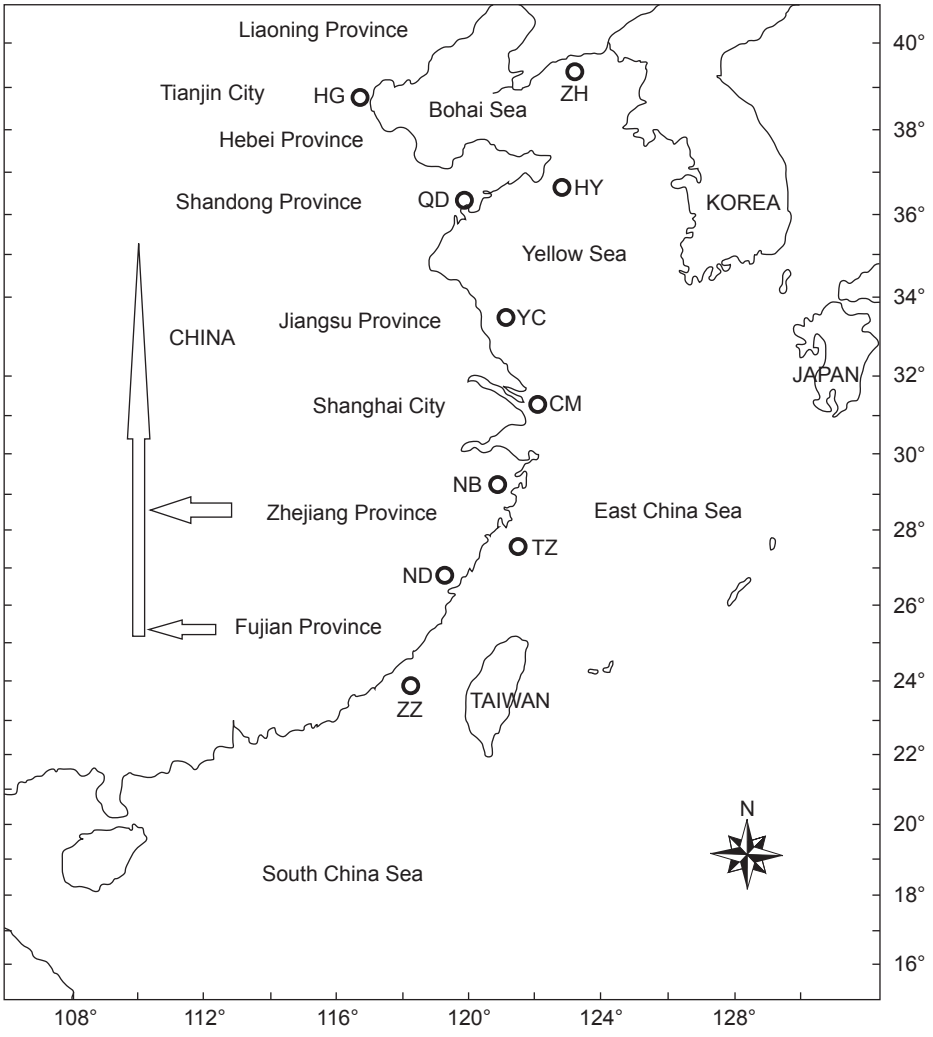

Fig. 1. Sampling sites of *Sinonovacula constricta* populations along the coast of China. Abbreviations are as follow: ZH (Zhuanghe City, Liaoning Province), HG (Hangu District, Tianjin City), HY (Haiyang City, Shandong Province), QD (Qingdao City, Shandong Province), YC (Yancheng City, Jiangsu Province), CM (Chongming I., Shanghai), NB (Ningbo City, Zhejiang Province), TZ (Taizhou City, Zhejiang Province), ND (Ningde City, Fujian Province), and ZZ (Zhangzhou City, Fujian Province). Arrows show the translocation of *S. constricta* populations for culture. The original picture was from Kong et al. (2007).

(Nei et al. 1983) was computed with Dispan (Ota 1993). The MEGA 4.0 software package (Tamura et al. 2007) was used to construct a Neighbor-joining (NJ) tree based on D_A distances.

RESULTS

Genetic variability

The allelic diversity (*Ar*), genetic diversities (*Ho* and *He*), and fixation index (F_{IS}) of each locus per population are summarized in table 1. In the 10 populations, the mean allelic richness ranged 6.0 (TZ)-7.0 (QD). The average observed heterozygosity varied 0.737 (ZH)-0.909 (CM), and the expected heterozygosities were high, ranging 0.836 (CM)-0.882 (QD), suggesting high genetic diversity among all populations. The mean fixation indices (F_{IS}) were low, varying -0.089-0.164. Of the 80 population-locus cases (10 populations × 8 loci), 62 tests showed significant deviation from HWE ($p < 0.05$). Null alleles were detected for 4 loci (*Sco*-221, *Sco*-222, *Sco*-400, and *Sco*-470).

Table 1. Allelic richness (*Ar*), observed (*Ho*) and expected (*He*) heterozygosities, and fixation indices (F_{IS}) at 8 loci in 10 populations of *Sinonovacula constricta*

Locus		ZH	HG	HY	QD	YC	CM	NB	TZ	ND	ZZ
Sco-117	*Ar*	9.2	11.7	11.9	12.4	10.4	10.2	12.0	12.0	12.3	11.5
	Ho	0.900	0.975	0.947	0.975	1.000	1.000	1.000	1.000	1.000	1.000
	He	0.906	0.946	0.948	0.952	0.927	0.922	0.948	0.954	0.952	0.941
	F_{IS}	0.007*	-0.031*	0.001*	-0.025*	-0.080*	-0.085*	-0.059	-0.051	-0.052	-0.063
Sco-146	*Ar*	10.5	9.4	9.1	10.9	9.3	9.9	9.9	9.4	8.9	10.7
	Ho	0.654	1.000	0.967	0.975	0.875	0.950	1.000	1.000	0.967	1.000
	He	0.924	0.911	0.899	0.931	0.908	0.920	0.910	.907	0.898	0.930
	F_{IS}	0.296*	-0.100	-0.076	-0.048	0.037*	-0.033	-0.101*	-0.104	-0.078	-0.076*
Sco-221	*Ar*	7.4	4.6	4.3	6.3	5.7	5.5	7.8	5.3	6.2	5.4
	Ho	0.700	0.475	0.100	0.925	0.739	0.975	0.435	0.359	0.552	0.675
	He	0.867	0.755	0.709	0.828	0.811	0.809	0.884	0.794	0.795	0.789
	F_{IS}	0.195*	0.374*	0.861*	-0.118	0.090	-0.208*	0.514*	0.551*	0.310*	0.146
Sco-222	*Ar*	10.2	10.2	9.2	8.4	9.1	9.1	10.0	8.3	9.1	8.6
	Ho	0.588	0.816	0.850	0.925	0.800	0.946	0.875	0.875	0.743	0.900
	He	0.922	0.920	0.909	0.885	0.899	0.907	0.922	0.897	0.903	0.902
	F_{IS}	0.365*	0.115*	0.066*	-0.046*	0.111*	-0.043*	0.052*	0.024	0.180*	0.002
Sco-400	*Ar*	8.3	8.1	7.4	7.5	8.3	8.1	6.8	6.8	5.5	6.7
	Ho	0.579	0.769	0.825	0.500	0.925	0.975	0.656	0.650	0.613	0.525
	He	0.882	0.885	0.858	0.863	0.884	0.880	0.811	0.824	0.786	0.852
	F_{IS}	0.347*	0.133*	0.039*	0.424*	-0.046*	-0.109*	0.193*	0.213*	0.223*	0.387*
Sco-431	*Ar*	9.3	9.5	8.3	8.5	9.5	10.1	6.1	5.8	8.1	8.0
	Ho	1.000	0.975	0.900	0.950	1.000	1.000	1.000	0.975	0.975	1.000
	He	0.896	0.893	0.860	0.873	0.907	0.906	0.801	0.789	0.859	0.874
	F_{IS}	-0.117*	-0.093	-0.047	-0.089*	-0.104*	-0.105	-0.253*	-0.239*	-0.137*	-0.146*
Sco-448	*Ar*	6.1	6.1	7.7	6.6	6.6	3.0	5.4	6.0	5.2	6.2
	Ho	0.925	0.725	0.975	0.950	0.600	0.450	0.800	0.875	0.650	1.000
	He	0.791	0.684	0.861	0.825	0.722	0.420	0.711	0.789	0.624	0.803
	F_{IS}	-0.171*	-0.060*	-0.134*	-0.153*	0.171*	-0.073*	-0.126*	-0.110*	-0.041*	-0.250*
Sco-470	*Ar*	8.3	7.7	6.6	8.9	8.0	10.7	6.0	6.3	8.0	5.8
	Ho	0.550	0.625	0.871	0.975	0.750	0.974	0.739	0.850	0.778	0.900
	He	0.844	0.792	0.813	0.895	0.862	0.920	0.808	0.817	0.889	0.782
	F_{IS}	0.351*	0.213*	-0.072*	-0.090*	0.132*	-0.060*	0.087*	-0.041*	0.132*	-0.153*
Mean	*Ar*	6.9	6.7	6.5	7.0	6.7	6.6	6.4	6.0	6.3	6.3
	Ho	0.737	0.795	0.804	0.897	0.836	0.909	0.813	0.823	0.785	0.875
	He	0.879	0.848	0.857	0.882	0.865	0.836	0.849	0.846	0.838	0.859
	F_{IS}	0.164	0.064	0.063	-0.017	0.034	-0.089	0.044	0.028	0.066	-0.019

* Significant deviation from Hardy-Weinberg equilibrium ($p < 0.05$). Population abbreviations are explained in the legend to figure 1.

Genetic differentiation and population relationships

The AMOVA of microsatellites revealed that variations among populations, among individuals within populations, and within individuals were 4.400% ($p < 0.05$), 2.808% ($p < 0.05$) and 92.792% ($p < 0.05$), respectively (Table 2).

Values for F_{ST} estimates and D_A genetic distances are presented in table 3. The analysis of genetic differentiation revealed that F_{ST} values ranged 0.005 (between HY and QD)-0.125 (between HY and CM). Of all pairs of populations, the genetic differentiation was statistically significant ($p < 0.05$). It was interesting that F_{ST} values were rather high, ranging 0.039-0.125 between the YC + CM populations and the remaining populations. D_A genetic distances ranged 0.142 (between ZZ and QD)-0.399 (between CM and NB). The phylogenetic tree analysis showed that the 10 populations were divided into 2 groups: 1 group included the YC and CM populations in the middle region and the ZH and HG populations of the northern region, and another group comprised the ZZ, ND, TZ, and NB populations in the southern region and the HY and QD populations in the northern region (Fig. 2).

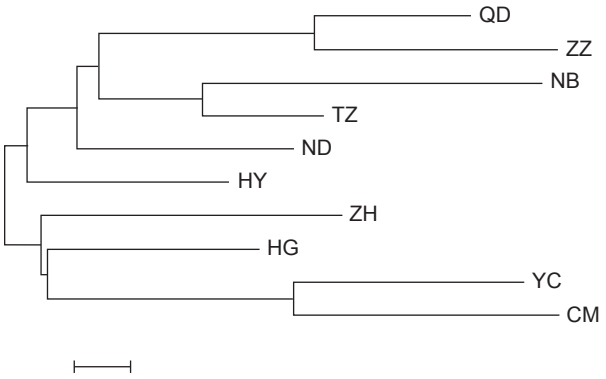

Fig. 2. A Neighbor-joining phylogenetic tree of 10 populations of *Sinonovacula constricta* based on Nei's minimum distance (D_A).

DISCUSSION

Genetic diversity

The maintenance of genetic variation is essential for the long-term survival of populations

Table 2. Analysis of molecular variance in 10 populations of *S. constricta*

Source of variation	Sum of squares	Variance component	Percentage of variance (%)
Among populations	135.971	0.158	4.400
Among individuals within populations	1224.002	0.101	2.808
Within individuals	1192.000	3.323	92.792
Total	2551.973	3.581	100.00

Table 3. Pairwise genetic differentiation (F_{ST}*) values (below the diagonal) and Nei's minimum distance (D_A) (above the diagonal) among 10 populations of *S. constricta* based on 8 microsatellite loci

	ZH	HG	HY	QD	YC	CM	NB	TZ	ND	ZZ
ZH		0.183	0.210	0.256	0.270	0.298	0.293	0.244	0.203	0.327
HG	0.014		0.160	0.297	0.254	0.244	0.266	0.185	0.196	0.343
HY	0.038	0.051		0.181	0.250	0.283	0.280	0.191	0.172	0.240
QD	0.047	0.051	0.005		0.308	0.334	0.312	0.244	0.225	0.142
YC	0.044	0.039	0.061	0.063		0.176	0.394	0.283	0.309	0.380
CM	0.082	0.052	0.125	0.112	0.020		0.399	0.306	0.290	0.393
NB	0.051	0.050	0.086	0.075	0.080	0.085		0.163	0.232	0.285
TZ	0.046	0.038	0.050	0.045	0.071	0.103	0.025		0.155	0.219
ND	0.033	0.011	0.065	0.059	0.050	0.061	0.059	0.056		0.253
ZZ	0.062	0.062	0.039	0.028	0.067	0.106	0.057	0.028	0.079	

* All pairwise F_{ST} values were statistically significant ($p < 0.05$). Population abbreviations are explained in the legend to figure 1.

because the extent of variation determines their potential to adapt to a changing environment. Genetic information about populations allows design of genetic management strategies for wild populations (Diz and Presa 2009). The mean expected heterozygosity (*He*) and the mean allelic richness (*Ar*) are useful for estimating population diversity (Diz and Presa 2009). The *Ar* ranged 6.0-7.0 and *He* ranged 0.836-0.882 in *S. constricta* populations, suggesting that high levels of genetic variations are present. These results are in agreement with our previous study (Niu et al. 2010) on the values of haplotype diversity of *S. constricta* populations using mtDNA-COI. Most studies of marine bivalves revealed high genetic diversity (Bohonak 1999), which may be related to biological characteristics. Most benthic marine invertebrates have external fertilization and extended dispersal of planktonic larvae that follow prevailing currents (Dame 1996). The larval stage can result in adaptations for dispersal and habitat selection, while the adult stage represents specialization in growth and reproduction (Dame 1996). High fecundity is commonly viewed as an evolutionary response to intense and unpredictable mortality in early life-history stages (Taris et al. 2006). *Sinonovacula constricta* can lay an average of 193,000 eggs per spawning event and has a 6-9-d pelagic larval phase. In addition, the high genetic diversity may be related to the wide distribution of the species (Dame 1996). Despite high fecundities, marine organisms show great variability in gamete quantity and quality, suggesting that the potential for variance in reproductive success exists prior to fertilization (Pujolar et al. 2011). *Sinonovacula constricta* is widely distributed along the west coast of the Pacific Ocean, from the Bohai Sea in the north to the South China Sea in the south in China. Therefore, the genetic diversity of this species is maintained at a high level.

HWE is analyzed by examining the significance of a permutation test for the null hypothesis (F_{IS} = 0), and low (F_{IS} = 0.05), moderate (F_{IS} = 0.10), and high (F_{IS} = 0.25) levels of inbreeding within populations (Hedgecock et al. 2007, Donrung et al. 2011). In this study, the majority of loci showed slight but significant deviations from HWE. However, for loci *Sco-221* and *Sco-400*, the northern and southern populations showed higher F_{IS} levels with lower heterozygosity than that predicted by HWE, suggesting deviation from HWE genotype frequencies (Stoner et al. 2002, Kang et al. 2011). A significant heterozygote deficiency was previously reported in marine invertebrate species, and this deficiency was likely explained by the presence of null alleles at these loci (Suck An et al. 2011). Another likely mechanism is the within-population genetic structure (Wahlund effect) due to spatial or temporal variations (Addison and Hart 2005), and more-substantial population substructuring in marine bivalves over either small or large geographical scales was found (Donrung et al. 2011), especially in species with free-spawning planktonic sperm (Addison and Hart 2005). In addition, microsatellites, developed from the CM population of the middle group, showed lower or negative inbreeding coefficients. But a high F_{IS} level existed in other populations indicating substructuring among those populations.

Genetic differentiation and population relationships

One major concern with population genetics of marine organisms is the observation of small but statistically significant genetic differences among populations (Pujolar et al. 2011). In this paper, *S. constricta* populations showed significant genetic divergence (F_{ST}: 0.005-0.125, $p < 0.05$) in pairwise comparison data. Significant genetic differentiation can be explained by environmental features including temperature and geographic barriers (Arruda et al. 2009, Zainudin et al. 2010). *Sinonovacula constricta* occupies marine bays along the coast of China, which form semi-closed environments that prevent gene flow between different bays. Therefore, geographical barriers can affect the genetic structure (Panithanarak et al. 2010, Kang et al. 2011, Stapley et al. 2011). On the other hand, different climates (a temperate climate in Liaoning, Hebei, and Shandong Provinces and Tianjin City, but a subtropical climate in southern Jiangsu, Zhejiang, and Fujian Provinces and Shanghai) have led to different spawning seasons (from June to Aug. in Liaoning Province, from Aug. to Oct. in Shandong Province, and from Sept. to Nov. in Zhejiang and Fujian Provinces) (Wang and Wang 2008) causing reproductive isolation and limiting gene flow. Therefore, despite the possibility of larval dispersal, local populations may be quite dynamically independent (Launey et al. 2002).

In general, genetic differentiation of marine bivalves sampled over a large scale is consistent with a model of isolation by distance (IBD) (Arnaud et al. 2000, Launey et al. 2002), where populations are connected by continuous migration but have

the greatest gene flow between neighboring populations (Zhan et al. 2009). Moreover, genetic relationships of these *S. constricta* populations showed atypical patterns. The 2 populations of the middle region (YC and CM) were found to have relatively higher genetic differentiation (F_{ST}: 0.039-0.125) than populations of the northern and southern regions. In addition, consistent with our previous study using mtDNA-COI, high genetic differentiation (F_{ST}: 0.8262-0.8706) was found between the 2 groups along the coast of China (Niu et al. 2010). Examining genetic distances and the phylogenetic tree, it was interesting to note that the HY and QD populations in the northern region and the NB, TZ, ND, and ZZ populations in the southern region were clustered together, while the YC and CM populations in the middle coast were in another clade. This proximity could be due to natural larval exchange and/or human-mediated translocation (Arnaud-Haond et al. 2008, Diz and Presa 2009). It was reported that this species was first cultivated in Fujian Province, China approximately 800 yr ago, and then the clams were introduced to Zhejiang Province 500 yr ago (Qi 1984). They were finally introduced to the northern region, including Liaoning, Hebei, and Shandong Provinces (Xie 2003) (Fig. 1). Since the translocation from wild populations into other populations is an important cultural practice, the study of the potential genetic contribution of translocated individuals to native populations is important (Arnaud-Haond et al. 2004). For instance, genetic impacts of the cultural practice of spat collection and translocation between genetically distinct stocks of the black-lipped pearl oyster *Pinctada margaritifera cumingii* were studied by comparing samples collected in the 1980s and 2000s from 7 atolls in French Polynesia. The results showed that indices of genetic differentiation had distinctly decreased (Arnaud-Haond et al. 2004). This homogenization was attributable to the massive spat translocation to that area in the 1990s (Arnaud-Haond et al. 2008), demonstrating that impacts of translocations on the genetic composition can be great. In our study, because we lacked samples of this clam from the past, it was more difficult to distinguish between extensive natural gene flow and artificial gene flow linked to farming practices with experimental data. However, there are differences in propagation times for this clam because of the different climates from north to south along the coast of China. No natural gene flow exists between the northern and southern populations separated by large geographic distances because of the short planktonic larval phase and geographic isolation. Therefore, a possible cause of the closer genetic relationship among these populations in the northern and southern regions is the transfer of juvenile clams by farmers (Xie 2003). From this information, it was hypothesized that the seed of this species on the Zhejiang and Fujian coasts migrated into the northern region, which led to gene flow between the northern and southern regions (especially between the QD and ZZ populations) due to external fertilization and the planktonic larval stage. However, the culture of this clam developed in the Jiangsu and Shanghai areas in recent years. In addition, distant genetic relationships were observed between the 2 middle populations and populations of the northern and southern coasts in this study. Similarly, we found that COI divergence between the middle populations and the other populations reached 6.5% based on the nucleotide Kimura-two-parameter (K-2-P) model (Kimura 1980) (Niu et al. 2010). mtDNA-COI has become a molecular marker for identifying species (Hebert et al. 2003, Nalugwa et al. 2010, Dettai et al. 2011), and COI divergence might not exceed 3%-4% within a species (Hebert et al. 2003). When higher intraspecific divergence is observed, it suggests the occurrence of a cryptic species (Radulovici et al. 2010, Dettai et al. 2011). So, perhaps the middle populations has become a cryptic species because of long geographical and reproductive isolation (Hebert et al. 2003).

In conclusion, the results of the microsatellite analysis together with the mtDNA-COI analysis (Niu et al. 2010) indicate high genetic diversity and a significant population genetic structure of *S. constricta* along the coast of China. It is possible that anthropogenic activities in the aquaculture of *S. constricta* led to gene flow among different locations, causing the mixing of different genetic materials between the northern and southern regions. Accordingly, 2 strategies are possible. On one hand, separate management could be performed between the middle group and other populations along the coast of China. On the other hand, a common recommendation for translocation is that the genetic compositions of the translocated individuals be made similar to local populations in order to maintain the genetic pool of *S. constricta*.

Acknowledgments: This study was supported by the grants from the National Natural Science Foundation of China (31101897), the Shanghai

Leading Academic Discipline Project (Y1101), the Leading Academic Discipline Project of the Shanghai Municipal Education Commission (J50701) (Marine Biology), and the Shanghai University Scientific Selection and Cultivation Fund for Outstanding Young Teachers (SSC09016).

REFERENCES

Addison J, M Hart. 2005. Spawning, copulation and inbreeding coefficients in marine invertebrates. Biol. Lett. **1:** 450-453.

Arnaud S, M Monteforte, N Galtier, F Bonhomme, F Blanc. 2000. Population structure and genetic variability of pearl oyster *Pinctada mazatlanica* along Pacific coasts from Mexico to Panama. Conserv. Genet. **1:** 299-307.

Arnaud-Haond S, V Vonau, F Bonhomme, P Boudry, F Blanc, J Prou et al. 2004. Spatio-temporal variation in the genetic composition of wild populations of pearl oyster (*Pinctada margaritifera cumingii*) in French Polynesia following 10 years of juvenile translocation. Mol. Ecol. **13:** 2001-2007.

Arnaud-Haond S, V Vonau, C Rouxel, F Bonhomme, J Prou, E Goyard, P Boudry. 2008. Genetic structure at different spatial scales in the pearl oyster (*Pinctada margaritifera cumingii*) in French Polynesian lagoons: beware of sampling strategy and genetic patchiness. Mar. Biol. **155:** 147-157.

Arruda CCB, CR Beasley, M Vallinoto, NS Marques-Silva, CH Tagliaro. 2009. Significant genetic differentiation among populations of *Anomalocardia brasiliana* (Gmelin, 1791): a bivalve with planktonic larval dispersion. Genet. Mol. Biol. **32:** 423-430.

Bohonak AJ. 1999. Dispersal, gene flow, and population structure. Q. Rev. Biol. **74:** 21-45.

Chung EY, CH Ko, HW Kang, KH Choi, JC Jun. 2008. Ultra-structure of oocytes during oogenesis and oocyte degeneration associated with follicle cells in female *Sinonovacula constricta* (BIVALVIA: PHARIDAE) in western Korea. Anim. Cells Syst. **12:** 313-319.

Collin R. 2001. The effects of mode of development on phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae). Mol. Ecol. **10:** 2249-2262.

Dame RF. 1996. Ecology of marine bivalves: an ecosystem approach. Boca Raton, FL: CRC Press.

Dettai A, SJ Adamowizc, L Allcock, CP Arango, DKA Barnes, I Barratt et al. 2011. DNA barcoding and molecular systematics of the benthic and demersal organisms of the CEAMARC survey. Polar Sci. **5:** 298-312.

Diz AP, P Presa. 2009. The genetic diversity pattern of *Mytilus galloprovincialis* in Galician Rías (NW Iberian estuaries). Aquaculture **287:** 278-285.

Donrung P, S Tunkijjanukij, P Jarayabhand, S Poompuang. 2011. Spatial genetic structure of the surf clam *Paphia undulata* in Thailand waters. Zool. Stud. **50:** 211-219.

Excoffier L, G Laval, S Schneider. 2005. Arlequin vers. 3.0: an integrated software package for population genetics data analysis. Evol. Bioinform. **1:** 47-50.

Feng B, L Dong, D Niu, S Meng, B Zhang, D Liu et al. 2010. Identification of immune genes of the Agamaki clam (*Sinonovacula constricta*) by sequencing and bioinformatic analysis of ESTs. Mar. Biol. **12:** 282-291.

Goldstein DB, C Schlotterer. 1999. Microsatellites: evolution and applications. Oxford, UK: Oxford Univ. Press.

Goudet J. 1995. FSATA (vers. 1.2): a computer program to calculate F-statistics. J. Hered. **85:** 485-486.

Han Y, Y Iizuka, WN Tzeng. 2010. Does variable habitat usage by the Japanese eel lead to population genetic differentiation? Zool. Stud. **49:** 392-397.

Hebert PDN, S Ratnasingham, JR de Waard. 2003. Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. Proc. R. Soc. Lond. B **270:** S96-S98.

Hedgecock D, S Launey, A Pudovkin, Y Naciri, S Lapegue, F Bonhomme. 2007. Small effective number of parents (Nb) inferred for a naturally spawned cohort of juvenile European flat oysters *Ostrea edulis*. Mar. Biol. **150:** 1173-1182.

Hummel H, M Wolowicz, R Bogaards. 1994. Genetic variability and relationships for populations of *Cerastoderma edule* and of the *C. glaucum* complex. Netherl. J. Sea. Res. **33:** 81-89.

Kang JH, YK Kim, MJ Kim, JY Park, CM An, BS Kim et al. 2011. Genetic differentiation among populations and color variants of sea cucumbers (*Stichopus japonicus*) from Korea and China. Int. J. Biol. Sci. **7:** 323-332.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through the comparative studies of sequence evolution. J. Mol. Evol. **16:** 111-120.

Kong L, Q Li, Z Qiu. 2007. Genetic and morphological differentiation in the clam *Coelomactra antiquata* (Bivalvia: Veneroida) along the coast of China. J. Exp. Mar. Biol. Ecol. **343:** 110-117.

Kyle C, E Boulding. 2000. Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. Mar. Biol. **137:** 835-845.

Launey S, C Ledu, P Boudry, F Bonhomme, Y Naciri-Graven. 2002. Geographic structure in the European flat oyster (*Ostrea edulis* L.) as revealed by microsatellite polymorphism. J. Hered. **93:** 331-351.

Lewis PO, D Zaykin. 2000. Genetic data analysis. Available at http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php Accessed at 30 May 2010.

Li J, G Wang, Z Bai. 2009. Genetic variability in four wild and two farmed stocks of the Chinese freshwater pearl mussel (*Hyriopsis cumingii*) estimated by microsatellite DNA markers. Aquaculture **287:** 286-291.

Liu F, JH Xia, ZY Bai, JJ Fu, JL Li, GH Yue. 2009. High genetic diversity and substantial population differentiation in grass carp (*Ctenopharyngodon idella*) revealed by microsatellite analysis. Aquaculture **297:** 51-56.

Luttikhuizen P, J Drent, A Baker. 2003. Disjunct distribution of highly diverged mitochondrial lineage clade and population subdivision in a marine bivalve with pelagic larval dispersal. Mol. Ecol. **12:** 2215-2229.

Mariani S, V Ketmaier, E De Matthaeis. 2002. Genetic structuring and gene flow in *Cerastoderma glaucum* (Bivalvia: Cardiidae): evidence from allozyme variation at different geographic scales. Mar. Biol. **140:** 687-697.

Nalugwa A, TK Kristensen, S Nyakaana, A Jørgensen. 2010. Mitochondrial DNA variations in sibling species of the *Bulinus truncatus*/*tropicus* complex in Lake Albert, Western Uganda. Zool. Stud. **49:** 515-522.

Nei M, F Tajima, Y Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evol. **19:**

153-170.
Niu DH, H Chen, SL Wang, GW Lin, JL Li. 2010. Population genetic structure of *Sinonovacula constricta* along the coast of China. Chin. J. Zool. **45:** 11-18. (in Chinese with English abstract)
Niu DH, JL Li, RL Zheng. 2008. Isolation and sequences characterization of microsatellite DNA in razor clam (*Sinonovacula constricta*). Period. Ocean Univ. China **38:** 733-738. (in Chinese with English abstract)
Ota T. 1993. Dispan: genetic distance and phylogenetic analysis. Available at http://www.bio.psu.edu/People/Faculty/Nei/Lab/Programs.html. Accessed at 30 May 2010.
Panithanarak T, R Karuwancharoen, U Na-Nakorn, TTT Nguyen. 2010. Population genetics of the spotted seahorse (*Hippocampus kuda*) in Thai waters: implications for conservation. Zool. Stud. **49:** 564-576.
Pavesi L, E De Matthaeis, R Tiedemann, V Ketmaier. 2011. Temporal population genetics and COI phylogeography of the sandhopper *Macarorchestia remyi* (Amphipoda: Talitridae). Zool. Stud. **50:** 220-229.
Pujolar JM, D Bevacqua, M Andrello, F Capoccioni, E Ciccotti, GA De Leo, L Zane. 2011. Genetic patchiness in European eel adults evidenced by molecular genetics and population dynamics modelling. Mol. Phylogenet. Evol. **58:** 198-206.
Qi QZ, MY Yang. 1984. The growth and development of the planktonic larvae, spats and young shells of *Sinonovacula constricta*. Taiwan Strait **1:** 90-96. (in Chinese with English abstract)
Radulovici AE, P Archambault, F Dufresne. 2010. DNA barcodes for marine niodiversity: moving fast forward? Diversity **2:** 450-472.
Sambrook J, DW Russell. 2001. Molecular cloning: a laboratory manual, 3rd ed. New York: Cold Spring Harbor Laboratory Press.
Sato M, K Kawamata, N Zaslavskaya, A Nakamura, T Ohta, T Nishikiori et al. 2005. Development of microsatellite markers for Japanese scallop (*Mizuhopecten yessoensis*) and their application to a population genetic study. Mar. Biotechnol. **7:** 713-728.
Song J, L Li, Z Liu, Q Li, P Ran. 2009. Sequence analysis and expression of a cDNA clone encoding tropomysin in *Sinonovacula constricta*. Mol. Biol. Rep. **36:** 315-321.
Stapley J, C Wordley, J Slate. 2011. No evidence of genetic differentiation between anoles with different dewlap color patterns. J. Hered. **102:** 118-124.
Stoner DS, R Ben-Shlomo, B Rinkevich, IL Weissman. 2002. Genetic variability of *Botryllus schlosseri* invasions to the east and west coasts of the USA. Mar. Ecol. Progr. Ser. **243:** 93-100.
Suck An H, BH Kim, JW Lee, CM Dong, SK Kim, YC Kim. 2011. Comparison between wild and hatchery populations of Korean pen shell (*Atrina pectinata*) using microsatellite DNA markers. Int. J. Mol. Sci. **12:** 6024-6039.
Tamari M, M Kandatsu. 1986. Occurrence of ceramide aminoethylphosphonate in edible shellfish, agemaki, *Sinonovacula constricta* (food & nutrition). Agric. Biol. Chem. **50:** 1495-1501.
Tamura K, J Dudley, M Nei, SP Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software vers. 4.0. Mol. Biol. Evol. **24:** 1596-1599.
Taris N, B Ernande, H McCombie, P Boudry. 2006. Phenotypic and genetic consequences of size selection at the larval stage in the Pacific oyster (*Crassostrea gigas*). J. Exp. Mar. Biol. **333:** 147-158.
Van Oosterhout C, WF Hutchinson, DPM Wills, P Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes **4:** 535-538.
Wang R, Z Wang. 2008. Science of marine shellfish culture. Qingdao, China: China Ocean Univ. Press. (in Chinese)
Weir B, CC Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution **38:** 1358-1370.
Wilke T, GM Davis. 2000. Infraspecific mitochondrial sequence diversity in *Hydrobia ulvae* and *Hydrobia ventrosa* (Hydrobiidae: Rissooidea: Gastropoda): Do their different life histories affect biogeographic patterns and gene flow? Biol. J. Linn. Soc. **70:** 89-105.
Xie ZM. 2003. Aquculture technology of marine economic shellfishes. Beijing: China Agriculture Press. (in Chinese)
Yamtich J, ML Voigt, G Li, D Hedgecock. 2005. Eight microsatellite loci for the Pacific oyster *Crassostrea gigas*. Anim. Genet. **36:** 524-526.
Yue GH, ZY Zhu, LC Lo, CM Wang, G Lin, F Feng et al. 2009. Genetic variation and population structure of Asian seabass (*Lates calcarifer*) in the Asia-Pacific region. Aquaculture **293:** 22-28.
Zainudin R, MNOR Shukor, N Ahmad, MMDZ Badrul, AR Mustafa. 2010. Genetic structure of *Hylarana erythraea* (Amphibia: Anura: Ranidae) from Malaysia. Zool. Stud. **49:** 688-702.
Zhan A, J Hu, X Hu, Z Zhou, M Hui, S Wang et al. 2009. Fine-scale population genetic structure of Zhikong scallop (*Chlamys farreri*): Do local marine currents drive geographical differentiation? Mar. Biotechnol. **11:** 223-235.