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Genetic diversity and population structure of the ark shell *Scapharca broughtonii* along the coast of China based on microsatellites



Hong Yu, Sen Gao, Ailu Chen, Lingfeng Kong, Qi Li*

Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China

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ABSTRACT

As a commercially important species in East Asia, the natural resources of *Scapharca broughtonii* have been suffering from severe population decline across its main habitats. In China, recovery efforts for *S. broughtonii* are in progress. To provide scientific bases for fisheries management and conservation program, genetic diversity and population structure of seven wild populations of *S. broughtonii* from the northern China coast was assessed using seven microsatellite loci in this study. High genetic diversity was present in all the seven populations, as observed in mean allelic richness per locus (11.3–12.5), and average expected heterozygosity (0.835–0.867). No significant difference in allelic richness or expected heterozygosity was observed among the seven populations. Pairwise F_{ST} estimates and NJ tree topologies based on D_C distances indicated that the seven populations fell into two groups, showing a clear division between the populations from the south and north of the Shandong Peninsula. Genetic differentiation was further analyzed using AMOVA and assignment tests. Genetic barrier analysis using Monmonier algorithm also confirmed that the Shandong Peninsula was the putative barrier separating the northern and southern populations. In addition, marine currents probably play an important role in high gene flow among three populations from the same marine gyre.

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1. Introduction

The ark shell *Scapharca broughtonii*, a cold-tolerant species inhabiting along the northwestern Pacific coast, is an important commercially marine shellfish in Asian countries (Li and Li, 2008). In the past two decades, the natural resources of *S. broughtonii* have precipitously declined across its main habitats, including Japanese, Korea and Chinese coasts (Cho et al., 2007; Li and Li, 2008; Sekino et al., 2010) because of many factors (eg.: over-exploitation, the deterioration of environmental conditions). There is a growing concern about the resource status of the ark shell. In China, *S. broughtonii* is mainly distributed in the north Yellow Sea, and was one of the dominant species in the coasts of Shandong and Liaoning provinces in the past (Wang and Wang, 2008). In 1980s, the average density of *S. broughtonii* in Rongcheng waters (Shandong province) was 0.476/m², while it decreased to 0.01/m² in 2010 (Song and Guo, 2010). The decline of *S. broughtonii* has prompted several restoration strategies including artificial breeding program, establishing marine protected area, construction of artificial reefs,

* Corresponding author. Tel.: +86 532 82031622; fax: +86 532 82032773.

E-mail address: qili66@ouc.edu.cn (Q. Li).

and so on (Li and Li, 2008; Li et al., 2013). Although a well understanding of the genetic structure and gene flow among populations is needed for developing appropriate policies for management and conservation of *S. broughtonii* resources, there is few fully understanding of the genetic variation and population structure of the wild *S. broughtonii* populations along the coast of China to date.

Among the molecular markers available for analysis of population genetics, microsatellite markers are considered as an essential tool for examining genetic structure of populations, with characteristics of abundance, neutrality, codominance, unambiguous scoring of alleles, and easily amplified by PCR with small tissues (Tautz, 1989; Weber and May, 1989). Microsatellites have been widely used to assess genetic variation in many marine bivalves (Li et al., 2006; Yu et al., 2008; Brown and Stepien, 2010). In recent years, more than one hundred microsatellite markers have been developed for *S. broughtonii* (Sekino et al., 2010; Tian et al., 2012; Li et al., 2012), which provides enough candidate markers for investigation of population structure and genetic diversity in *S. broughtonii*.

In this study, seven polymorphic microsatellite markers were used to evaluate the genetic structure of seven wild *S. broughtonii* populations along the northern coast of China and estimate levels of gene flow among the populations, in order to provide useful information for designing suitable management guidelines and artificial breeding programs for *S. broughtonii*.

2. Materials and methods

2.1. Sampling and DNA extraction

Seven wild populations of *S. broughtonii* were collected from the northern coast of China in 2011, with 60 individuals per population. Five populations were obtained from the Yellow Sea, including Jimo, Lianyungang, Rizhao, Weihai and Zhuanghe; the other two populations were sampled from the Bohai Sea (Fig. 1). Sample sites were chosen to cover a wide portion of the species, based on the accessibility, biogeography of the ark shell and oceanography of sampling sites. Specimens were dissected and the adductor muscle was preserved in 95% ethanol until DNA extraction.

Genomic DNA was extracted from adductor muscle according to the protocol described by Li et al. (2002). The concentration of DNA was measured with an Ultraspec 2100 pro UV/visible spectrophotometer (Amersham Biosciences) for absorption at 260 nm.

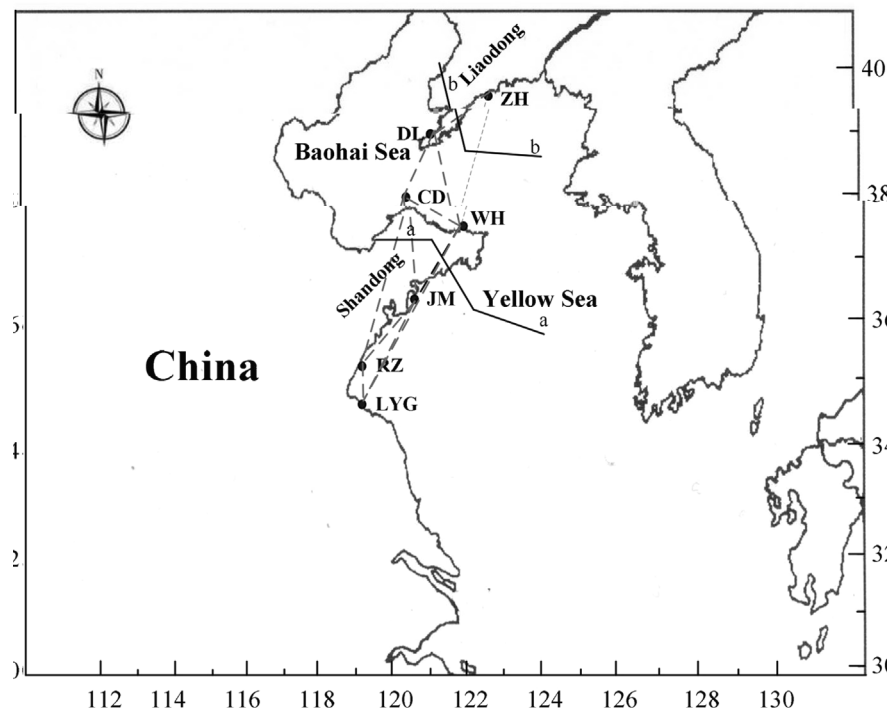


Fig. 1. Map showing locations and abbreviated names for seven *Scapharca broughtonii* populations (●). Areas of limited gene flow identified by barrier analysis were shown on the map (Full lines = main barriers to gene flow. Dotted lines = Delaunay triangulation).

2.2. Microsatellite analysis

Seven polymorphic microsatellite markers for *S. broughtonii* were analyzed: SB6 (Li and Li, 2008), ScBr03, ScBr20, ScBr14, ScBr04, ScBr05, ScBr09 (Sekino et al., 2010). PCRs were performed in a volume of 10 μ L containing 0.25 U *Taq* DNA polymerase (Takara), 1 \times PCR buffer, 0.2 mM dNTP mix, 1.5 mM MgCl₂, 1 μ M of each primer set, and 50 ng of genomic DNA. The PCR amplification conditions were as follows: 3 min at 94 $^{\circ}$ C, 35 cycles of 1 min at 94 $^{\circ}$ C, 1 min at the optimal annealing tem-

To evaluate the levels of genetic differentiation among populations, F_{ST} (Weir and Cockerham, 1984) was calculated using the software MSA. The Cavalli-Sforza and Edwards (1967) chord distance D_C was calculated, and an unrooted neighbor-joining tree (NJ tree) was constructed with the software POPULATIONS (<http://www.cnrs-gif.fr/pge>) based on D_C . Nodal support was assessed by bootstrapping with 1000 replicates. A multi-locus analysis of molecular variance (AMOVA), assessed with exact

Provided that populations JM, RZ and LYG are considered as one group, and ZH, CD, WH, and DL as one group, 92.5% and 90.4% of the individuals were assigned to their sampling groups.

3.3. Genetic barrier predication

Results revealed by the BARRIER analyses were corresponding to the analyses of genetic structure and the oceanography features of local sampling areas. A putative barrier, which directly separated seven populations into two groups, was detected when the number of barrier was set as only one using this program, indicating that the Shandong Peninsula might be a possible barrier hampering gene flow between the two groups (Fig. 1). Moreover, another barrier was shown up if change the number of barrier to two, isolating ZH population from the northern group, which suggested that the Liaodong Peninsula might act as another possible boundary for the gene flow.

4. Discussion

4.1. Genetic diversity and departures from HWE

In this study, seven microsatellite markers revealed a comparatively high level of genetic diversity in terms of allelic richness and expected heterozygosity in all the seven populations, indicating that the wild populations of *S. broughtonii* in China maintained considerable variations. Similar results were also detected in *S. broughtonii* populations from Korea (five populations), China (one population) and Russia (one population) by Cho et al. (2007) using COI sequences. Mass fecundity, external fertilization, and broad larval dispersal might be the cause of the high genetic diversity of *S. broughtonii*, which is a common phenomenon in many bivalves (Cassista and Hart, 2007; Lallias et al., 2010; Arias-Pérez et al., 2012). No significant difference in genetic diversity among the seven populations was detected in this study.

Heterozygote deficiencies related to HWE have been observed in many natural populations of marine mollusks using microsatellites (Prakoon et al., 2010; Arias-Pérez et al., 2012). In the present study, the population-locus cases deviated from HWE also showed heterozygote deficiencies (Table 1). As has been observed in previous microsatellite studies of bivalves (Li et al., 2007; Yu and Li, 2007), departure from HWE towards homozygote excess in this study could be largely caused by null alleles. In our study, some samples were failed to amplify at some loci, while easy to amplify at other loci, strongly suggesting the presence of null alleles. In addition, null alleles were also detected using MICRO-CHECKER program. In fact, microsatellites are known to suffer from extremely high levels of null alleles in marine invertebrate species (Li et al., 2003; Yu and Li, 2007).

such as *Chlamys farreri* and *Macra chinensis* (Zhan et al., 2009; Ni's et al., 2011). The Bohai Sea is characterized as having comparatively low rates of water exchange with the Yellow Sea (Wu et al., 2013). However, the populations CD and DL sampled from the Bohai Sea with weak divergence ($F_{ST} = 0.0019$) forming a cluster, did not showed significant differentiation from other two populations (ZH and WH) from the Yellow Sea. It is probably because the locations of CD and DL populations were near the boundary of the Bohai Sea and the Yellow Sea, and the currents flowing into the Bohai Sea may drive gene flow between the two populations and the other two populations (ZH and WH).

In conclusion, our results suggest that *S. broughtonii* populations along the northern China coast should be subdivided into two groups. There is no significant divergence within the groups, but significant population differentiations were detected between the two groups. Marine currents and the Shandong Peninsula may play important roles in population structuring of *S. broughtonii*. The results obtained from this study can provide useful information for fishery management as well as aquaculture industry and conservation activities.

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