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- Note -

The Population Genetic Structure of the Oyster *Crassostera gigas* (Bivalvia: Ostreidae) from Gamak Bay in Korea

Eun Seob Cho* and Hee Dong Jeong

South Sea Fisheries Research Institute, NFRDI, Yeosu 556-823, Republic of Korea

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To analyze the population genetic structure of the oyster *Crassostrea gigas* Thunberg, 34 specimens werecollected from Gamak bay in March, 2007. Total genomic DNA was extracted from each sample and PCR was performed to identify haplotypes of oyster by using HCO2918 and LCO1491 primers. Four kinds of haplotypes (CR1, CR2, CR3, and CR4) were identified. Among these group, CR3 showed the highest relative frequency at 73% than any other of haplotypes. On the basis of hierarchical genetic structure, the population of Gamak showed a higher genetic relationship with Namhae, but the genetic distance between southern and western coasts was negative and no statistical significance was found (p>0.05). Consequently, the oyster from Korea coast is determined to be both homogenous and large.

Key words : Gamak Bay, genetic structure, haplotype, oyster, population

Introduction

Korea has a long history of artificial sea-farming of invertebrates by line-hanging method. The oyster *Crassostera gigas* Thunberg in Korea has been prevalent along the western and southern coasts [13]. It had long been considered as the only species cultured in the Korean oyster industry [18]. In recent years, the amount of the oysters in the South Sea has significantly decreased. Adult overexploitation, unstable seed production, disease, and deteriorated water quality all play important roles in this decline in oyster production [12]. Some researchers suggested that the accumulation of recessive traits and indiscreet cross breeding of local oyster populations could also be associated with the decline [10]. However, the main reasons are not yet clear and this area requires further investigation.

Recently, molecular methods with advanced DNA amplification and sequencing have been applied to these types of studies. Indeed, some researchers have already studied the genetic characteristics of oyster using new molecular techniques [3,4,6,8,9,16]. In Korea, genetic variations and analysis of *C. gigas* inferred from mitochondrial DNA COI gene were also examined [1,10,12,13]. In Gamak Bay, where there is a relatively narrow water channel, shellfish has long been cultured. The oyster *C. gigas*, in particular, has served as,one of the important fisheries resources of shellfish cultivated in Gamak Bay. The purpose of this study is to analyze the haplotypes of oyster *C. gigas* from Gamak Bay using molecular methods. In this study, a partial sequencing of mtDNA COI gene was carried out to determine distribution of haplotypes, degree of gene flow, and the population gene structure of the oyster *C. gigas*.

*Corresponding author
Tel : +82-61-690-8959, Fax : +82-61-686-1588
E-mail : escho@momaf.go.kr

Materials and Methods

This study used a total of 34 specimens of the oyster *C. gigas* collected from Gamak Bay, Yeosu, in March, 2007. Samples were frozen at -70°C until required. Total DNA was extracted from their musculature by the method described in Asahida [2]. PCR segments were produced by the primers HCO2918 and LCO1491 toward the mtDNA COI gene. The primer sequences were as follows: HCO2918, 5'-TAAACTTC AGGGTGACCAAAAAATCA-3' and LCO1491, 5'-GGTCAA CAAATCATAAAGATATTGG-3'. PCR reactions were performed under the following conditions in 25 μl containing 1.25 units of *Taq* DNA polymerase (FastStar *Taq* DNA polymerase, Roche Co., USA), 0.5 mM dNTPs, 1× PCR reaction buffer (Roche Co., USA), 5-20 ng total genomic DNA and 20 pmol of each primer. The thermocycling profile included an initial denaturation step of 95°C for 5 min, followed by 35 cycles of 1 min at 94°C, 1 min at 55-60°C, and 90 sec at 72°C. The final extension step was increased to 5 min. The PCR was carried out by MyCycler (Bio-Rad, USA).

Products from specific PCR amplification were analyzed using a 2% agarose run at 50V for 50 min and visualized after staining in 0.5 g ml^{-1}ethidium bromide. The PCR product was directly sequenced using the PCR Purification kit (NucleoSpin Extract, Korea) by following manufacturer's instruction. The DNA was purified using an Applied Biosystem model ABI 3730XL automated sequencer and a Big Dye terminator cycle sequencing kit (Perkin-Elmer Applied Biosystems, UK). Sequence data were aligned using the multiple alignment program Clustal W [17]. When homologous sequences differed by one nucleotide, the sequences were considered as different haplotypes. Haplotype designations (CR1, CR2, CR3, and so forth) were applied to new sequences as they were discovered. This study was used for the oyster *C. gigas* derived from Boryeong, Hwaseong, Namhae, Goseong, Haenam, and Gadeok [1]. Hierarchical genetic relationships among populations and sets of populations were assessed by the Holsinger and Mason-Gamer (H-MG) method [7]. Statistical significance of the difference between pairs of localities was tested by permutations (1,000 bootstrap) [5]. This study tested the degree of hierarchical subdivision between localities with the AMOVA (Analysis of Molecular Variance) program [5] incorporated in Arlequin ver 1.1 [14].

Results and Discussion

The PCR product using HCO2198 and LCO1491 primers was successfully attained on the gel and estimated at 658 bp (Fig. 1). Four kinds of haplotypes (CR1, CR2, CR3, and CR4) were found from a partial sequence of the mtDNA COI gene from 34 individuals of the oyster *C. gigas* collected from Gamak Bay, compared with Boryung, Hwasung, Namhae, Gousung, and Gaduck (Table 1) [1]. The most frequent CR3 haplotype was found in 25 individuals and accounted for 73%. The CR1, CR2, and CR4 haplotypes occurred to two, three, and four individuals, respectively. However, the haplotypes II, V, VI, and VIII [1] did not show in the present study. The hierarchical relationship among the populations analyzed by H-MG method [7] is shown in Fig. 2. The population of Gamak used in this study belonged to southern coasts rather than

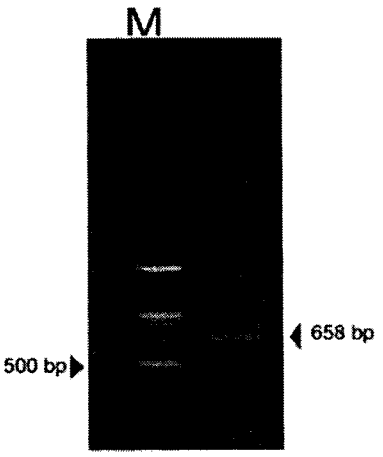

Fig. 1. The amplified PCR product using HCO2918 and LCO1491 primers for mitochondrial DNA COI gene. M. 100 bp DNA ladder. PCR sample was used to CR1 haplotype.

Table 1. Variable nucleotide positions in mitochondiral DNA COI gene of eight haplotypes for the oyster *Crassostera gigas* and numbers of individuals of each haplotype collected from each loaclity

Haplotype	Nucleotide position							Number of individuals						
								Western coast		Southern coast				
	52	215	250	493	513	562	619	Boryung	Hwasung	Namhae	Haenam	Gousung	Gaduck	**Gamak**
I (CR3)	T	C	A	A	T	T	G	4	4	5	4	3	2	**25**
II	.	.	.	.	.	.	A	1	0	0	0	0	1	**0**
III (CR1)	.	A	.	.	.	.	.	0	0	0	1	0	0	**2**
IV (CR2)	.	.	G	.	.	.	.	0	1	0	0	0	0	**3**
V	.	.	.	G	.	.	.	0	0	0	0	1	0	**0**
VI	.	.	.	.	G	.	.	0	0	0	0	1	0	**0**
VII (CR4)	C	.	.	.	.	.	.	0	0	0	0	0	1	**4**
VIII	.	.	.	.	.	C	A	0	0	0	0	0	1	**0**

Bold letters mean to carry out the present study. Boryung, Hwasung, Namhae, Haenam, Gousung, and Gaduck were obtained from An et al.[1].

Our study found four kinds of haplotypes (CR1, CR2, CR3, CR4). Position number of the variable sites was described by An et al. [1].

Nucleotide idenity to reference sequence of haplotype I is indicated with a dot.

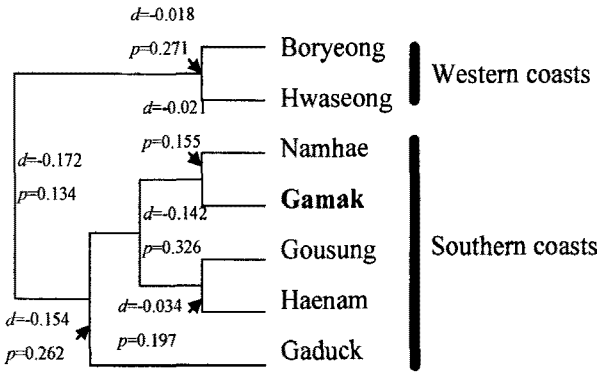

Fig. 2. Hierarchical genetic relationships among localities analyzed using the Holsinger and Mason-Gamer method [7]. The value at each node is the genetic distance (d) between its two daughter nodes and the p value is the significance of differentiation based on 1,000 random re-sampling.

western coasts and were joined of the Namhae population within southern coasts. The genetic distance (d) between Gamak and Namhae was negative value of -0.02, but the two populations did not show statistical significance ($p>0.05$). Although this study distinguished between groups on western and southern coasts, the genetic distance between all groups were negative and no statistically significant genetic structures were shown. It is suggested that the oyster *C. gigas* found in those waters form one large population and share a close genetic structure regardless of their geographic barrier.

It is assumed that the planktonic larval stage can serve as a significant factor for the dispersal capabilities compared with nonplanktonic marine organisms. Developing larvae can be passively carried by water currents, often over considerable distances [15]. Much of the southern coasts consist of a semi-closed water channel, but Namhae, Haenam, Gousung, Gaduck, and Gamak are close enough to be strongly affected by water movements based on geographic scale. Due to increased potential for larval dispersal [11], it is possible to attain a high genetic relationship within and among the populations in the southern coasts. Interestingly, western and southern coasts have different environmental characteristics, but spatial population genetic homogeneity is caused by passive drifting over a long distances. As a result of the mtDNA analysis in this study, CR3 haplotype was identified as being significant in western and southern coasts based on frequency and distribution. The other haplotypes were found in a single locality, indicating geographic restriction in their distribution. Consequently, haplotype I (CR3) can drift passively by water currents over a wide geographic distance and overcome environmental differences to become a successfully matured oyster. On the basis of genetic structure, a little genetic variability among populations in terms of their genotypes may result in a rapidly decreasing oyster production. Additional genetic studies offering a firmer assessment of genetic population structure of the oyster in Korea are urgently needed.

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초록 : 가막산 참굴의 집단 구조 분석

조은섭* · 정희동
(국립수산과학원 남해수산연구소)

본 연구는 2007년 3월 가막만에서 양식하고 있는 참굴 34마리를 대상으로 미토콘드리아COI gene 염기서열분석을 통하여 유전자 집단을 조사했다. PCR 증폭에 사용된 primer는 HCO2918과 LCO1491로 product는 658 bp였다. 유전자 배열 결과 가막만 참굴 집단에서 CR1, CR2, CR3, CR4 4개의 haplotype이 나타났다. 그 중 CR3 haplotype이 73% 발생되어 가장 높은 빈도율을 보였다. 계층구조에서도 가막산 참굴은 남해안산과 유전적 유연관계를 보이나, 유전적 거리는 남해안 및 서해안과도 마이너스 값을 보이며 통계적으로도 유전적으로 하나의 집단을 형성하고 있음을 알 수 있다.