ISSN (Print) 1225-9918
ISSN (Online) 2287-3406
Journal of Life Science 2015 Vol. 25. No. 12. 1347~1353 DOI : http://dx.doi.org/10.5352/JLS.2015.25.12.1347

The Study of Genetic Diversity and Population Structure of the Korean Fleshy Shrimp, *Fenneropenaeus chinensis*, Using Newly Developed Microsatellite Markers

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Received September 21, 2015 /Revised November 3, 2015 /Accepted November 27, 2015

The fleshy shrimp, *Fenneropenaeus chinensis*, is the family of Penaeidae and one of the most economically important marine culture species in Korea. However, its genetic characteristics have never been studied. In this study, a total of 240 wild *F. chinensis* individuals were collected from four locations as follows: Narodo (NRD, n = 60), Beopseongpo (BSP, n = 60), Chaesukpo (CSP, n = 60), and Cheonsuman (CSM, n = 60). Genetic variability and the relationships among four wild *F. chinensis* populations were analyzed using 13 newly developed microsatellite loci. Relatively high levels of genetic variability (mean allelic richness = 16.87; mean heterozygosity = 0.845) were found among localities. Among the 52 population loci, 13 showed significant deviation from the Hardy–Weinberg equilibrium. Neighbor-joining, principal coordinate, and molecular variance analyses revealed the presence of three subpopulations (NRD, CSM, BSP and CSP), which was consistent with clustering based on genetic distance. The mean observed heterozygosity values of the NRD, CSM, BSP, and CSP populations were 0.724, 0.821, 0.814, and 0.785 over all loci, respectively. These genetic variability and differentiation results of the four wild populations can be applied for future genetic improvement using selective breeding and to design suitable management guidelines for Korean *F. chinensis* culture.

Key words : Fleshy shrimp, *Fenneropenaeus chinensis*, genetic variability, microsatellite loci, population structure

Introduction

The fleshy shrimp, *Fenneropenaeus chinensis*, is an economically important species in the family Penaeidae. *F. chinensis* is distributed from the west coast of the Korean Peninsula to the east coast of northern China [3] and is characterized by long-distance migrations of up to 2,000 km between spawning and feeding sites [18]. The *F. chinensis* South Korean aquaculture industry started in 1960, and production increased very quickly to reach 1,533 metric tons in 1997. However, a white spot syndrome virus (WSSV) disease outbreak decreased production dramatically to about 998 metric tons in 1998 [13]. Disease outbreaks cause mass mortality among cultured *F. chinensis* worldwide, particularly in Asian countries. To solve this problem, *F. chinensis* was replaced with *Litopenaeus vannamei* as the major cultured species in South Korea in 2003 [11], but damage to shrimp farming associated with WSSV has been increasing in recent years. The West Sea Mariculture Research Center (Taean, Korea) conducts a breeding program to produce shrimp strains that are more resistant to WSSV. If a large number of cultured shrimp are released from aquaculture facilities, they could alter the genetic composition of wild populations by either displacing them or interbreeding. This could reduce the population's ability to adapt to new environments. Accordingly, basic knowledge about the geographic distribution, genetic diversity, and population differences of *F. chinensis* is important. It is now widely recognized that this information can be obtained through recently developed molecular genetics techniques. However, few reports have been published about *F. chinensis* population genetics in South Korea.

Because microsatellite markers and simple sequence repeats have high levels of polymorphism, co-dominant inheritance, genome-wide distributions, and high reproducibility, they have been applied widely in population genetics,

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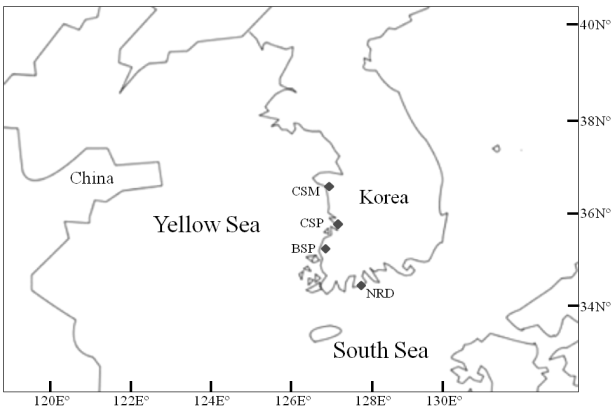

Fig. 1. Sampling sites (◆) for the four wild *Fenneropenaeus chinensis* populations: Narodo (NRD); Beopseongpo (BSP); Chaesukpo (CSP); and Cheonsuman (CSM).

genetic linkage map, genetic diversity, and phylogenetic studies [10, 16]. However, only a few microsatellite markers can be used for each shrimp species because of poor conservation of the microsatellite flanking sequences among different crustacean species, particularly in *F. chinensis* [15, 27]. Several microsatellite markers have been isolated from *F. chinensis* [5, 9, 24]. Unfortunately, despite the commercial importance of this shrimp species in Korea, studies describing its genetic background are scarce. In the present study, we assessed genetic diversity within and among wild Korean *F. chinensis* populations and examined the genetic structure among these populations using new microsatellite DNA markers.

Materials and Methods

Sample collection and DNA preparation

A total of 240 wild *F. chinensis* individuals were collected from four locations as follows: Narodo (NRD, n=60), Beopseongpo (BSP, n=60), Chaesukpo (CSP, n=60), and Cheonsuman (CSM, n=60) (Fig. 1 and Table 1). All muscle tissue samples were stored in 100% ethanol prior to DNA extraction. The tissue was homogenized in lysis buffer (MFX-2000; Toyobo, Osaka, Japan) containing 20 mg/ml proteinase K. Total DNA was isolated using the MagExtractor MFX-6100 automated DNA extraction system (Toyobo Co., Tokyo, Japan). The extracted genomic DNA was quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Barrington, IL, USA) and stored at -20°C until use.

Polymerase chain reaction (PCR) and microsatellite genotyping

Thirteen microsatellite loci that were amplified in *F. chinensis* were used in this study. Detailed information about the primers is presented in Table 2. Each PCR contained three primer sets that were labeled at the 5′ end of the forward primer with 6-FAM, HEX, or NED dyes (Applied Biosystems, Foster City, CA, USA). PCR amplification was carried out in a 10-μL reaction mixture containing 0.25 U *Ex-Taq* DNA polymerase (TaKaRa Biomedical Inc., Shiga, Japan), 1× PCR buffer, 0.2 mM dNTP mix, 10 pM of each primer, and 100 ng template DNA, using the PTC 200 DNA Engine (MJ Research, Waltham, MA, USA). The PCR conditions were: 5 min at 95°C, followed by 35 cycles of 30 sec at 95°C, 45 sec at 58°C, and 45 sec at 72°C, with a final extension of 10 min at 72°C. Microsatellite polymorphisms were screened using an ABI PRISM 3130 XL automated DNA sequencer (Applied Biosystems), and alleles were designated according to PCR product size relative to molecular size markers (GENESCAN 400 HD [ROX]; Applied Biosystems).

Statistical analysis

The number of samples (*n*), expected heterozygosity (*He*), and observed heterozygosity (*Ho*) were calculated using the Arlequin software package (ver. 3.0; [8]). Tests for allelic richness (A_R), number of alleles per locus (*Na*), and deviations from Hardy–Weinberg equilibrium (HWE) were estimated using GENEPOP ver. 4.0 (http://kimura.univ-montp2. fr/~rousset/Genepop.htm), and the adjusted P-values for both analyses were obtained using a sequential Bonferroni test for multiple comparisons. MICRO-CHECKER 2.2.3 was used to test for the presence of null alleles. F_{ST}

Table 1. Geographic area, location, abbreviation, locality, collection date, and sample size collected

Sampling area (abbreviation)	Date of collection	Latitude	Longitude	Sample size
NRD	May 2006	34°27′58.4"N	127°27′12.6"E	60
BSP	July 2006	35°21′52.0"N	126°26′21.1"E	60
CSP	May 2006	35°37′35.4"N	126°27′58.5"E	60
CSM)	Sep 2006	36°36′17.4"N	126°25′14.9"E	60

NRD, Narodo; BSP, Beopseongpo; CSP, Chaesukpo; CSM, Cheonsuman

Table 2. The 13 *Fenneropenaeus chinensis* microsatellite loci used in this study, with primer sequences, repeat motifs, and annealing temperatures (*Ta*) for polymerase chain reaction amplification

Locus	Repeat motifs	Primer sequence (5′-3′)	T_a (°C)	GenBank accession no.
KFc37	$(AC)_{11}$	F: TCTGCATGCACGTTAGTAAAT R: TGTTCCTCCAAATATACATGTGT	58	KT695606
KFc41	$(GT)_6(GTCT)_{10}$	F: CAGGTAACGGTCGGGAGTGGAG R: ACCTTTGCGGACTGATAAAGTC	58	KT695607
KFc42	$(CT)_{10}$	F: GGCTCTTCCCTCTCCTTATTACAC R: TTAGGGGGAGAAAATGTGGC	58	KT695608
KFc253	$(AC)_{17}$	F: TAAACGGGCAGTGAGTGAGAATA R: AAATATGTGTTGTTTGCCAGTGTG	58	KT695609
KFc346	$(AC)_{16}$	F: GAACCATCAGGGAGCTGCTGCAAC R: TCCACGTCCTGTCCAACGGAGAG	58	KT695610
KFc438	$(AC)_{23}$	F: CTCTTAGTGAAACCCTTTTACAG R: GCGTGAGTGTGAGTGAAT	58	KT695611
KFc568	$(AG)_{26}$	F: GCCGTATGGATGTTACTCTATCAC R: GTTCAAGATCCACTGTCTCATTTC	58	KT695612
KFc593	$(GAGT)_{17}$	F: GAGTGGCTGGGTGAATTCAT R: ACCCTAACTCACCAAATTCACCT	58	KT695613
KFc614	$(AC)_9$	F: CACAAACGCACATAGAACAAACAC R: AGGGTTAGAATAACGCGTGGAG	58	KT695614
KFc657	$(GT)_{14}$	F: GGCCGTGTGTCTCATCTCCAC R: GTTCTTGGGAATACGGTGAGGTCA	58	KT695615
KFc658	$(GT)_{43}$	F: ACATACGAGGGCGAATCCAAGA R: CGTCCTGAACCAATTAGTTGAGGC	58	KT695616
KFc673	$(GT)_{11}$	F: GGGCGTGTCTTTTTGGTTTGCTTT R: CTGTTCTGGTCCCGCTTGTGTTC	58	KT695617
KFc959	$(GT)_{26}$	F: ATTAAGTTCAGGCTGTGTGCGTAT R: GAGACACGACCGAACTGTGATAG	58	KT695618

values (1,000 permutations; [25]) were calculated using Arlequin 3.0. The population structure patterns were further investigated using a model-based Bayesian clustering procedure in STRUCTURE (ver. 2.3; [21]), which assigns individuals to K populations based on their multilocus genotype. STRUCTURE was run for K = 2 using a burn-in length of 50,000 and a run of 50,000 steps. The overall inbreeding coefficient (F_{IS}; [25]) was also estimated using GENEPOP ver. 4.0. Analysis of molecular variance (AMOVA; [7]) was used to test for population structure with Arlequin 3.0. A principal coordinate analysis (PCoA) was performed using GENALEX (ver. 6.0; [19]). A neighbor-joining (NJ) tree was constructed based on the chord distances (D_{CE}; [2]) to evaluate the genetic population relationships using the POPULATION program (ver. 1.2.30). Bootstrap values were calculated using 1,000 replicates.

Results and Discussion

Genetic variability

Microsatellite markers have been used in various shrimp species, and the pedigree of mixed populations can be determined using a few microsatellite markers [14]. However, little information is available on the use of microsatellite markers in *F. chinensis* [27] for understanding population structure or genetic diversity [15]. In this study, *F. chinensis*, a commercially and recreationally valuable species, were collected from the west coast of the Korean Peninsula (four separate collections in 2006) to illuminate its population structure and genetic diversity.

The genetic variability indices for the four populations are summarized in Table 3. Allelic richness per locus (A_R) ranged from 3 to 31 across all populations. Mean A_R values were in the following order: NRD (16.2), CSM (17.5), BSP (16.8), and CSP (16.9). The total number of alleles per locus

Table 3. Allelic variability at 13 microsatellite loci surveyed in the four *Fenneropenaeus chinensis* populations

Population		Locus													
		KFc37	KFc41	KFc42	KFc253	KFc346	KFc438	KFc568	KFc593	KFc614	KFc657	KFc658	KFc673	KFc959	Mean
NRD	A_R	8	14	5	19	19	16	19	18	10	8	22	22	31	16.2
	N_a	8	14	5	19	19	16	19	18	10	8	22	22	31	16.2
	H_e	0.778	0.861	0.536	0.893	0.867	0.873	0.918	0.907	0.746	0.679	0.923	0.917	0.956	0.835
	H_o	0.300	0.650	0.400	0.783	0.733	0.833	0.900	0.800	0.610	0.650	0.833	0.950	0.967	0.724
	P	0.000**	0.005**	0.028*	0.004**	0.000**	0.435	0.537	0.035*	0.000**	0.692	0.000**	0.065	0.571	
	F_{IS}	0.617	0.246	0.256	0.124	0.156	0.046	0.020	0.119	0.175	0.043	0.098	-0.036	-0.012	
	(P)	0.000	0.006	0.030	0.005	0.000	0.446	0.565	0.047	0.000	0.687	0.000	0.068	0.565	
CSM	A_R	10	14	5	16	21	14	21	21	11	8	32	25	30	17.5
	N_a	10	14	5	16	21	14	21	21	11	8	32	25	30	17.5
	H_e	0.794	0.858	0.659	0.897	0.882	0.871	0.929	0.915	0.812	0.759	0.947	0.906	0.956	0.861
	H_o	0.750	0.700	1.000	0.767	0.867	0.900	0.883	0.817	0.400	0.783	0.933	0.900	0.967	0.821
	P	0.235	0.009**	0.000**	0.033*	0.184	0.154	0.016*	0.233	0.000**	0.559	0.620	0.864	0.078	
	F_{IS}	0.056	0.186	−0.523	0.146	0.017	−0.033	0.050	0.108	0.510	-0.033	0.015	0.007	-0.011	
	(P)	0.252	0.011	0.000	0.032	0.144	0.163	0.021	0.204	0.000	0.578	0.655	0.835	0.063	
BSP	A_R	8	11	3	17	23	17	20	19	13	7	28	22	30	16.8
	N_a	8	11	3	17	23	17	20	19	13	7	28	22	30	16.8
	H_e	0.785	0.845	0.561	0.904	0.905	0.885	0.936	0.910	0.809	0.644	0.936	0.900	0.943	0.843
	H_o	0.850	0.717	0.567	0.831	0.817	0.931	0.950	0.867	0.683	0.617	1.000	0.817	0.933	0.814
	P	0.653	0.116	0.774	0.130	0.009**	0.893	0.909	0.211	0.002**	0.755	0.389	0.029*	0.192	
	F_{IS}	−0.083	0.153	-0.011	0.078	0.098	−0.059	-0.015	0.048	0.157	0.042	-0.069	0.093	0.011	
	(P)	0.660	0.121	0.775	0.090	0.010	0.820	0.920	0.192	0.003	0.755	0.386	0.029	0.176	
CSP	A_R	8	11	3	16	25	15	20	22	11	9	32	22	26	16.9
	N_a	8	11	3	16	25	15	20	22	11	9	32	22	26	16.9
	H_e	0.774	0.808	0.560	0.886	0.891	0.887	0.922	0.922	0.790	0.676	0.950	0.892	0.947	0.839
	H_o	0.815	0.729	0.550	0.650	0.867	0.881	0.867	0.900	0.569	0.667	0.983	0.797	0.932	0.785
	P	0.563	0.152	0.909	0.000**	0.081	0.427	0.124	0.752	0.000**	0.248	0.853	0.165	0.367	
	F_{IS}	-0.035	0.093	0.017	0.268	0.027	0.004	0.060	0.024	0.261	0.014	-0.039	0.104	0.014	
	(P)	0.442	0.149	0.908	0.000	0.051	0.326	0.137	0.761	0.001	0.236	0.764	0.109	0.157	

Allelic richness (A_R), number of alleles per locus (N_a), expected heterozygosity (H_e), observed heterozygosity (H_o), inbreeding coefficient (F_{IS}), and probability of significant deviation from Hardy–Weinberg equilibrium (P) are given for each population and locus. *P < 0.05; **P < 0.01. Calculations assume that individuals with one microsatellite band are homozygous for the allele. NRD, Narodo; BSP, Beopseongpo; CSP, Chaesukpo; CSM, Cheonsuman

(N_a) ranged from 3 to 31. KFcg959 had the highest A_R and N_a values in the NRD population. Mean N_a ranged from 16.2 to 17.5. The mean expected and observed heterozygosities ranged from 0.835 to 0.861 and from 0.724 to 0.821, respectively. Mean heterozygosity was highest in CSM (H_e = 0.861, H_o = 0.821), followed by BSP (H_e = 0.843, H_o = 0.814), CSP (H_e = 0.839, H_o = 0.785), and NRD (H_e = 0.835, H_o = 0.724). The mean H_e value was higher than the mean H_o value in all populations. Among the 52 population-locus cases (4 populations × 13 loci), 13 cases showed significant deviations (p<0.01). All populations departed from HWE at the KFcg614 locus. The NRD population departed from HWE at 6 of the 13 microsatellite loci, and the CSM, BSP, and CSP populations departed from HWE at three, two, and two microsatellite loci, respectively. The F_{IS} value estimated for all populations at the KFcg614 locus was significantly different from zero (p<0.05); all populations were in an excess heterozygosity condition for the KFc673 and KFc959 loci in NRD, the KFc42, KFc438, KFc657, and KFc959 loci in CSM; the KFc37, KFc42, KFc438, KFc568, and KFc658 loci in BSP; and the KFc37 and KFc658 loci in CSP.

Our results reveal that the newly developed microsatellite markers were a powerful approach to monitoring genetic diversity between the four geographically different wild Korean *F. chinensis* populations investigated. Heterozygosity is an important measure of population diversity at the genetic level. The mean observed heterozygosity values of the NRD, CSM, BSP, and CSP populations were 0.724, 0.821,

0.814, and 0.785 over all loci, respectively. These values were lower than the mean expected heterozygosity for the four populations. The overall heterozygosity in this study was high and differed from the reported mean heterozygosity, which is known to be low (7.3%), within crustacean populations as a whole [12].

In the present study, 18 of the 52 population-locus cases deviated significantly from HWE after applying the Bonferroni correction. However, the KFc37, KFc41, and KFc42 loci in the NRD; the KFc614 locus in the CSM; and the KFc614 locus in the CSP population also deviated from HWE without excess heterozygosity. These deviations may have been caused by selection, population mixing, nonrandom mating, presence of null alleles, or the limited sample size used in our analysis [20, 22]. The presence of null alleles, for example, is a classical source affecting the accuracy of microsatellite loci during parentage assignment [1] and null allele frequencies > 5% can compromise pedigree estimates [17].

Genetic differentiation between the four populations

F_{ST} is the proportion of total genetic diversity that separates groups, and values range from 0 to 1. If there is no population substructure (i.e., no stable groups), F_{ST} will approach 0. An F_{ST} range of 0.00-0.05 indicates little genetic differentiation [26]. Significant pairwise F_{ST} values (p<0.05) were observed between the NRD and CSM, NRD and BSP, NRD and CSP, CSM and BSP, and CSM and CSP populations (Table 4). In our study, pairwise F_{ST} tests detected low levels of genetic differentiation among the populations, particularly between the CSP and BSP populations, indicating that a geographical barrier was not effectively maintaining genetic integrity among the populations in the four areas.

Table 4. Pairwise F_{ST} estimates (below the diagonal) and Cavalli-Sforza and Edwards's chord distance (D_{CE}, above the diagonal) between the four *Fenneropenaeus chinensis* populations

	NRD	CSM	BSP	CSP
NRD	-	0.1864	0.3030	0.3582
CSM	0.0213*	-	0.0991	0.1216
BSP	0.0227*	0.0132*	-	0.0380
CSP	0.0270*	0.0169*	-0.0007	-

Significance was tested at the 5% level with the Bonferroni correction applied for multiple tests. *p<0.05. Pairwise F_{ST} (Weir and Cockerham, 1984) and D_{CE} distance (Cavalli-Sforza and Edwards, 1967) are measures of genetic differentiation and genetic distance between populations, respectively. NRD, Narodo; BSP, Beopseongpo; CSP, Chaesukpo; CSM, Cheonsuman

Genetic distances (D_{CE}) were also calculated for all possible population pairs. The D_{CE} measure ranged from 0.0380 to 0.3582. The smallest estimate for D_{CE} was between BSP and CSP (0.0380), whereas the highest estimate was between NRD and CSP (0.3582); the genetic distances between NRD and CSM, NRD and BSP, CSM and BSP, and CSM and CSP were 0.1864, 0.3030, 0.0991, and 0.1216, respectively (Table 4). The result of the genetic distances among the *F. chinensis* populations was further confirmed by the findings of PCoA (Fig. 2). The NJ tree constructed based on D_{CE}, indicated that the four populations were allocated into three major groups (Fig. 3); that is, one group included the CSM population, one group included the BSP and CSP populations, and one group included the NRD population. The PCoA produced a result similar to that of the cluster analysis (Fig. 3) and showed clear separation of the four populations into three clusters.

The results of hierarchical AMOVA tests to form putative groups estimated from each NJ tree topology provided additional evidence to support previous findings in *F. chinensis* populations. The NJ tree topology included a group for the

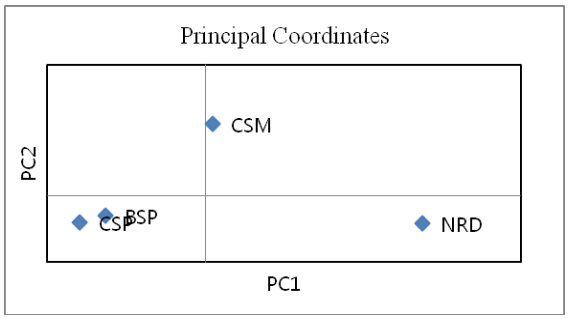

Fig. 2. Principal coordinates analysis to determine the relationships among the wild *Fenneropenaeus chinensis* population based on chord distance (Cavalli-Sforza and Edwards, 1967).

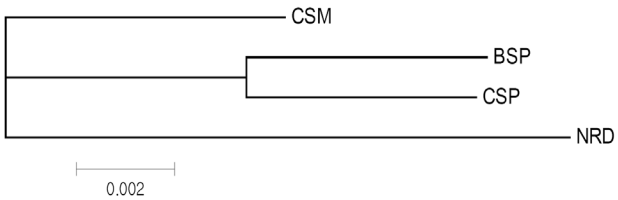

Fig. 3. Neighbor-joining tree illustrating the relationships between the four *Fenneropenaeus chinensis* populations based on the Cavalli-Sforza and Edwards chord distance.

Table 5. Hierarchical analysis of molecular variance results for groups with several combinations of populations

No. of groups	Group definitions	Among groups (F_{CT})	Among populations within group (F_{SC})	Within populations (F_{ST})
2	NRD vs. (CSM-BSP-CSP)	0.0147 (0.2494)	0.0099* (0.0000)	0.0244* (0.0000)
2	(NRD-BSP-CSP) vs. CSM	0.0010 (0.4943)	0.0167* (0.0000)	0.0177* (0.0000)
3	NRD vs. CSM vs. (BSP-CSP)	0.0220 (0.1643)	-0.0012 (0.8603)	0.0208* (0.0000)

Probability values associated with *F* statistics are shown in parentheses. *F* statistic significantly greater than zero. * $p<0.05$. NRD, Narodo; BSP, Beopseongpo; CSP, Chaesukpo; CSM, Cheonsuman

NRD population and another group for the CSM, BSP, and CSP populations. In this case, the fixation index among groups (F_{CT}) was not significant (F_{CT}=0.0147, P=0.2494), whereas it was significant among populations within groups (F_{SC}=0.0099, P=0.0000). The NJ tree topology defined a group that included the NRD, BSP, and CSP populations and another group that included the CSM population. In this case, the fixation index among groups (F_{CT}) was not significant (F_{CT}=0.0010, P=0.4943), whereas it was significant among populations within groups (F_{SC}=0.0167, P=0.0000). These results suggest that the populations remained structured within at least one group in each test case, as the F_{SC} values estimated in both test cases were significant. Therefore, we separated them into three groups. The first group included the NRD population, the second included the CSM population, and the third included the BSP and CSP populations. These results show that the fixation index among groups (F_{CT}=0.0220, P=0.1643), and among populations within groups (F_{SC}=-0.0012, P=0.8603), were not significant (Table 5). The NJ tree topology and AMOVA results suggest that the four populations could be assigned to a group that included the NRD population, a group that included the CSM population, and a group that included the BSP and CSP populations (Table 5). An evaluation of the evolutionary relationships among the four wild populations showed that BSP and CSP had the highest degree of genetic identity, followed by CSM and BSP, CSM and CSP, NRD and CSM, and NRD and BSP, whereas NRD and CSP were most distantly related. A previous study demonstrated the most likely explanation for this result is that wild populations of Chinese *F. chinensis*, which are extensively distributed in the Yellow and Bohai Seas, are two independent populations [15]. These two populations share the same wintering ground that lies in the mid-depth waters of the Yellow Sea [4]. An independent population of *F. chinensis* has been found off the west and south coasts of the Korean Peninsula [18]; thus, further attempts are necessary to evaluate the genetic population relationships in this species. The goal of this study was to examine the importance of conservation and further genetic improvements in wild Korean *F. chinensis*. Information on genetic variation and differentiation in these four wild populations can be applied for future genetic improvement thorough selective breeding and to design suitable management guidelines for Korean *F. chinensis*.

Acknowledgments

The authors would like to acknowledge the help provided by Dr. Litticia Bryant at Queensland University of Technology in regard to constructive comments on the manuscript. This work was supported by a grant from the National Fisheries Research and Development Institute (R2015038).

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초록 : 새로 개발한 미세위성체 마커를 이용한 한국 대하의 유전다양성 및 집단구조

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대하(*Fenneropenaeus chinensis*)는 우리나라에서 경제적으로 가장 중요한 양식생물 중 하나이다. 그러나 대하의 유전적 특성에 대한 연구는 전무하다. 본 연구에서는 새로 개발된 13개 미세위성체 유전자좌를 이용하여 우리나라에 서식하는 4개 지역 대하의 유전 다양성 및 집단간 관련성을 분석하였다. 평균 대립유전자 richness =16.87, 평균 이형접합률 =0.845를 보여 유전 다양성은 비교적 높은 수준을 보였다. 52개 유전좌중에서 13개 유전자좌가 집단간 분석에서 Hardy – Weinberg 평형에서 유의적인 차이로 벗어났다. Neighbor-joining, principal coordinate 및 molecular variance 분석 결과로 우리나라 대하 집단은 3개 집단(나라도, 천수만, 법성포 및 채석포)으로 구성되어 있으며, 이 결과는 유전적 거리에 근거한 군집 결과와 일치하였다. 본 연구에서 조사된 유전 다양성 및 분화 결과는 앞으로 대하의 지속 가능한 자원관리 및 선발 육종을 통한 유전적 개량에 적용될 수 있을 것이다.