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Population Genetic Structure of *Octopus minor* Sasaki from Korea and China Based on a Partial Sequencing of Mitochondrial 16S rRNA

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We determined a portion of mitochondrial 16S rRNA gene sequences (416 bp) to investigate the genetic structure of the octopus (*Octopus minor* Ssaki) population in Korea and China. Samples were obtained from Korea (Yeosu, Namhae, Jindo, Muan, Geomundo and Seosan) and China (Sandong) during the period of August 2006 to September 2007. Sequence analyses of 28 individual specimens collected from 7 localities revealed 11 haplotypes, ranging in a sequence divergence of 0.2% - 1.2%. Phylogenetic analyses using PHYLIP and networks subdivided the octopus into two clades (termed clade A and B) and the nucleotide divergence between them was 0.4%. This haplotype subdivision was in accordance with geographic separation: one at Yeosu, Namhae, Muan and Jindo, and the other at Seosan, Geomundo and Sandong. On the basis of hierarchial genetic analysis, genetic distance between localities in Korea and China were also found, but a significant population differentiation was not shown in this study (p>0.05). Consequently, most of the octopus populations in Korea had considerable distribution due to the mitochondrial gene flow that resulted in a formation of a genetically homogenous structure, whereas some of the Korean and Chinese populations had different genetic structures. Gene flow among populations may be restricted due to impassable geographic barriers that promote genetic differentiation.

Key words : Gene flow, mitochondrial DNA, octopus, population structure

Introduction

A major concern in the field of population genetics is the challenges to understand the causes of differentiation between populations across ranges of geographic distribution. With the advanced techniques at the level of DNA, genetic data now serve an important role in guiding the management of marine fishes [6], marine animals [7] and marine invertebrates [23]. Data on the genetic structure of populations are of particular interest because they may reveal evidence of restricted gene flow or genetic isolation that is undetectable through traditional demographic studies.

The octopus *Octopus minor* Sasaki, which is located around Korean coast is one of the most important fisheries resources in Korea. In particular, since southern waters are well developed by mud flats based, the region supports good habits for octopus continuing a number of capture rates [11]. However, annual production of octopus has been declined. Moreover, octopus has been imported in great numbers from China to the Korean fisheries market. However, the octopus is poorly understood in studies of management and conservation genetics. In this study, we used the mitochondrial 16S gene sequences to analyze the inter-population and intra-population genetic structure of this species in Korean and Chinese waters.

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Materials and Methods

Specimen

The octopuses were sampled from 6 localities on Korea coasts and 1 locality on a Chinese coast during the period of October 2006 - October 2007 (Fig. 1). We used a total of 28 individual specimens collected at 7 localities [Korea: Namhae (locality 2), Muan (locality 6), Yeosu (locality 3), Jindo (locality 5), Seosan (locality 7) and Geomundo (locality 4); China: Sangdong (locality 1)]. Samples were frozen at -70^{o}C until required.

Molecular works

Total genomic DNA was extracted from 0.05g of an eyeball using a DNA Isolation kit (Roche Co.) after homogenizing. The extracted genomic DNA was frozen at -20^{o}C until required. The partial region of the mtDNA 16S gene was amplified using L2510-16S (5′-CGCCTGTTTAACA

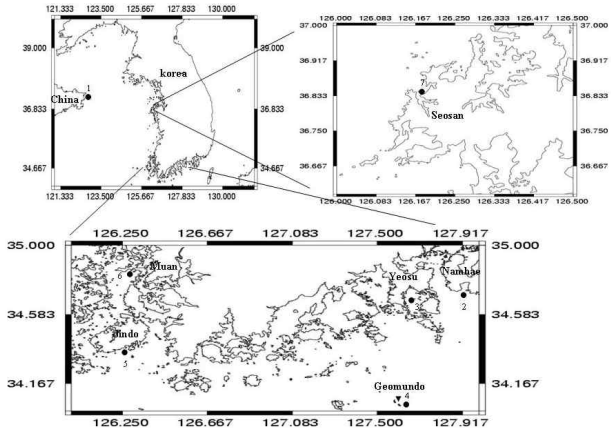

Fig. 1. Sampling locations of the octopus, *Octopus minor*, in Korea and China collected from August 2006 to August 2007.

AAGACAT-3′) and H3058-16S (5′-TCCGGTCTGAACTCAG ATCACGTA-3′) primers [10]. PCR reactions were performed under the following conditions in 25 µl mixture: 1.25 unit *Taq* DNA polymerase (FastStar *Taq* DNA polymerase, Roche Co.); 1× PCR reaction buffer (Roche Co.); 0.1 mM dNTPs; 20 pmol of each primer; and 5-30 ng genomic DNA. Amplifications were performed with the MyCycler thermal cycler (Bio-Rad). The thermocycling profile included an initial denaturation step of 95°C for 5 min, followed by 40 cycles of 15 sec at 94°C, primer annealing for 15 sec at 50°C, and extension for 30 sec at 72°C. The final extension step was increased up to 5 min. PCR product was separated by running at 50 V for 50 min in 2% agarose containing 0.5 µg ml^{-1} ethidium bromide and then checked for molecular size. The PCR product was purified using PCR Purification kit (NucleoSpin® Extract) by the following manufacture's instructions. Purified DNA fragment was stored at -20°C until used. The purified DNA was ligated with the vector, pUC 18 DNA (Roche Co.), using a One Shot® Machi1™-T1® cloning kit (Invitorgen).

Sequencing

The purified DNA using an Applied Biosystem model ABI 3730XL automated sequencer and a Big Dye terminator cycle sequencing kit (Perkin-Elmer Applied Biosystems, UK). For the sequencing reaction, 30 ng of purified PCR products, 2.5 pmol of primer, and 1 µl of Big Dye terminator were mixed and adjusted to a final volume of 7 µl with dH_2O. The reaction was run with 5% DMSO for 30 cycles of 15 sec at 95°C, 5 s at 50°C, and 4 min at 60°C. Both strands were sequenced for crosscheck.

Haplotype

Sequence data were aligned using the multiple alignment program Clustal W [24]. When homologous sequences differed by ≥ one nucleotide, the sequences were considered as different haplotypes. Haplotype designations (O1, O2, O3, and so forth) were applied to new sequences as they were discovered.

Phylogenetic analysis

Sequences were determined by parsimony, distances and maximum likelihood (ML) methods. To understand the possible genetic relationships, PHYLIP (Phylogenetic Inference Package) ver. 3.573c [5] was used in this study. This search for parsimony analysis was repeated several times from different random staring points using the stepwise addition option to make certain the most parsimonious tree was found. For distance analysis, subprogram DNADIST in PHYLIP was used to obtain a matrix of Kimura's two-parameter distance [13]. Distance matrix was analyzed by subprogram NEIGHBOR in PHYLIP with algorithms based on Saitou and Nei's neighbor-joining (NJ) method [19]. All nucleotide substitution was equally weighted and unordered alignment gaps were treated as missing information. The data set was iterated 100 times using a subprogram SEQBOOT. Reliability of the tree was constructed using subprogram CONSENSE in PHYLIP after pairwise sequence distances were estimated by Kimura's two-parameter method, which attempts to correct observed dissimilarities for multiple substitution in sequences evolving with a transition bias.

Genetic diversity

To investigate the magnitude and pattern of genetic diversity within localities, the genetic diversity and mean number of pairwise differences among haplotypes, gene diversity, and nucleotide diversity were calculated using Arlequin ver 1.1 [20]. The mean number of differences between all pairs of haplotypes in the sample was obtained by considering the number of mutations having occurred since the divergence of any two haployptes, and the frequency of the ones involved in the calculation. Nucleotide diversity was calculated by estimating the probability that two randomly chosen homologous sequences would be different [15].

Genetic migration

Genetic distance (F_{ST}), coefficient of coancestry (D), and female migration rate (N_m) between pairs of populations

were computed based on pairwise F_{ST} indices following the approach described in Excoffier et al. [4]. Permutatations were performed to test the significance of the differentiation between pairs of localities. F_{ST} values do not increase linearly with divergence time, but they can lineralize (mutation rate is low and divergence time is relatively short) with the following as: D=-log(1-F_{ST}), where D is a coancestry coefficient that is approximately linear with divergence time [18]. Pairwise F_{ST} values were converted to estimates of pergeneration migration time using the equilibrium relationship: F_{ST}=1/(2Nm+1).

Hierarchical structure

Hierarchical genetic relationships among populations and sets of populations were assessed by the Holsinger and Mason-Gamer (H-MG) method [9]. This study tested the degree of hierarchical subdivision between specified sets of localities with the AMOVA (Analysis of Molecular Variance) program [4] incorporated in Arlequin ver 1.1 [20] Statistical significance of the difference between pairs of localities was tested by permutations (1,000 bootstrap) [4].

Results

DNA analysis

The primer pair L2510 (forward)-H3058 (reverse) was used successfully in amplifying the octopus genomic DNA, and a PCR product of predicted size (416 bp) was obtained (Fig. 2). The individual haplotype is listed in Table 1. A total of 11 haplotypes (O1-O11) was obtained by a partial sequencing 416 bp of the mtDNA 16S gene from 44 individual octopus specimens collected from 7 localities including

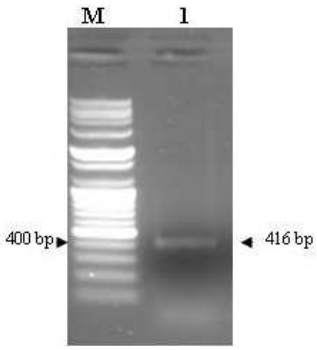

Fig. 2. Amplification PCR product using the primers L2510-16S (forward) and H3058-16S (reverse) for the octopus, *Octopus minor*. 100 bp DNA ladder was used as molecular size marker in this study.

Table 1. A list of sampling region, collection date, animal numbers and mitochondrial 16S region gene haplotypes

Collection locality (no. of individulas)	Collection date	Animal number	Haplotype
1. Sandong, China (4)	2006. 9. 4	OS1	O2
		OS2	O10
		OS3	O11
		OS4	O2
2. Namhae, Kyungsangnam Province (4)	2006. 9. 26	ON1	O1
		ON2	O2
		ON3	O2
		ON4	O4
3. Yeosu, Chunnam Province (4)	2006. 9. 27	OY1	O1
		OY2	O4
		OY3	O3
		OY4	O2
4. Geomundo, Chunnam Province (4)	2007. 8. 15	OG1	O6
		OG2	O7
		OG3	O1
		OG4	O4
5. Jindo, Chunnam Province (4)	2006. 9. 27	OJ1	O5
		OJ2	O6
		OJ3	O2
		OJ4	O2
6. Muan, Chunnam Province (4)	2006. 8. 31	OM1	O3
		OM2	O5
		OM3	O2
		OM4	O2
7. Seosan, Chungnam Province (4)	2007. 8. 15	OSE1	O8
		OSE2	O9
		OSE3	O2
		OSE4	O2

Korea and China (Table 1). Sequence alignment revealed 24 variable nucleotides, and all of them were transversional substitutions (5 G⇔C and 19 A⇔T). The transitional substitutions and paralled mutations did not show (Fig. 3A, 3B).

Sequence divergence

Pairwise comparison between pairs of haplotypes was carried out to understand the divergence and relationship among haplotypes (Table 2). The sequence divergence ranged from 0.2% to 1.2% (1-5 bp), and the largest divergence was found when O1, O3, O4, O5, and O6 were respectively compared with O10 and O11. The maximum divergence value always includes O10 and O11. Even excluding the haplotypes that showed the largest divergence value against O10 and O11, the remaining haplotypes also showed relatively large divergence against O10 and O11. The mean pairwise difference of O10 and O11 against others

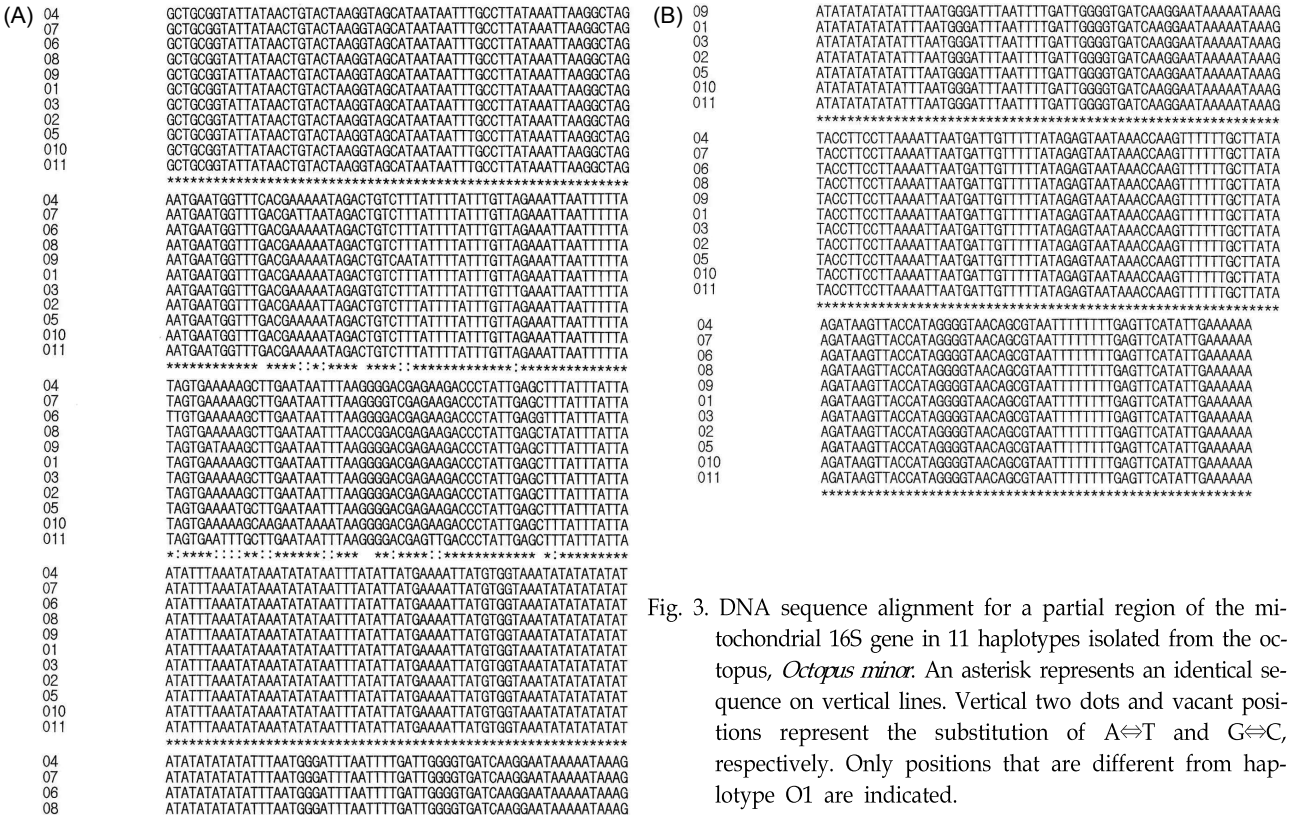

Fig. 3. DNA sequence alignment for a partial region of the mitochondrial 16S gene in 11 haplotypes isolated from the octopus, *Octopus minor*. An asterisk represents an identical sequence on vertical lines. Vertical two dots and vacant positions represent the substitution of A⇔T and G⇔C, respectively. Only positions that are different from haplotype O1 are indicated.

Table 2. Pairwise comparisons among 11 haplotypes obtained from the partial sequences of mitochondiral 16S region gene

	1	2	3	4	5	6	7	8	9	10	11
1	–	0.0024	0.0024	0.0024	0.0024	0.0048	0.0072	0.0072	0.0072	0.0096	0.0120
2	1	–	0.0048	0.0024	0.0024	0.0048	0.0072	0.0072	0.0096	0.0072	0.0096
3	1	2	–	0.0024	0.0048	0.0024	0.0048	0.0072	0.0048	0.0096	0.0120
4	1	1	1	–	0.0048	0.0048	0.0024	0.0048	0.0072	0.0120	0.0120
5	1	1	2	2	–	0.0048	0.0024	0.0048	0.0048	0.0120	0.0096
6	2	2	1	2	2	–	0.0024	0.0024	0.0048	0.0120	0.0072
7	3	3	2	1	1	1	–	0.0048	0.0048	0.0072	0.0072
8	3	3	3	2	2	1	2	–	0.0048	0.0072	0.0072
9	3	4	2	3	2	2	2	2	–	0.0048	0.0072
10	4	3	4	5	5	5	3	3	2	–	0.0048
11	5	4	5	5	4	3	3	3	3	2	–

Numbers above the diagonal are mean distance values and numbers below the diagonal are absolute distance values. 1, O1; 2, O2; 3, O3; 3, O4; 4, O4; 5, O5; 6, O6; 7, O7; 8, O8; 9, O9; 10, O10; 11, O11

is the largest (3.7 nucleotides; 0.8%), whereas others ranged from 1 (0.2%) to 2.5 (0.6%) nucleotides. Excluding this most divergent O10 and O11, haplotype divergence among others ranged from 0.2% to 0.9% (1-4 bp).

Haplotype distribution

Geographic distribution and frequency of haplotypes are listed in Table 3. The most frequent O2 haplotype (11 individual specimens in 28) was found largely at localities in Sandong (locality 1), Namhae (locality 2), Yeosu (locality 3), Jindo (locality 5), Muan (locality 6) and Seosan (locality 7), indicating a wide geographic distribution in Korea and China. Two haplotypes (O10 and O11) were found only in Sandong, but O7, O8 and O9 haplotypes also occurred in Geomundo (locality 4) and Seasan. It shows regional restriction and rarity in O7, O8, O9, O10 and O11 haplotypes. Sandong, Seosan and Geomundo showed no co-occurring haplotypes as to Namahe, Yeosu, Jindo and Muan localities.

Table 3. Relative frequencies of mitochondrial DNA 16S gene haplotypes through the populations

Haplotype	L1 (4)	L2 (4)	L3 (4)	L4 (4)	L5 (4)	L6 (4)	L7 (4)
O1	0	0.25	0.25	0.25	0	0	0
O2	0.50	0.50	0.25	0	0.50	0.50	0.50
O3	0	0	0.25	0	0	0.25	0
O4	0	0.25	0.25	0.25	0	0	0
O5	0	0	0	0	0.25	0.25	0
O6	0	0	0	0.25	0.25	0	0
O7	0	0	0	0.25	0	0	0
O8	0	0	0	0	0	0	0.25
O9	0	0	0	0	0	0	0.25
O10	0.25	0	0	0	0	0	0
O11	0.25	0	0	0	0	0	0

L1, locality 1: Sandong; L2, locality 2: Namhae; L3, locality 3: Yeosu; L4, locality 4: Geomundo; L5, locality 5: Jindo; L6, locality 6: Muan; L7, locality 7: Seosan. Numbers in parentheses indicate sample size of each population.

Consequently, haplotype distribution can be explained as the co-existence of regional restriction in some haplotypes and far-reaching co-occurring in only one haplotype.

Phylogenetic relationship

Fig. 4 shows the results of phylogenetic analyses to investigate relationships among haplotypes using the NJ method incorporated in PHYLIP. Most haplotypes were weakly associated or unresolved. The genetic trees generated by NJ and ML methods had a similar topology and even analyses run through several transitions: transversion weightings of 1:0, 1:1, 1:10 and 1:20 did not affect them. 11 haplotypes obtained in this study were subdivided into two independent groups (clade A and clade B) although haplotype relationships within each clade were mostly not resolved. When members of haplotypes in each clade were imposed on their geographic location, they were divided into two geographic groups. Five haplotypes belonging to clade A were found exclusively in Namhae, Yeosu, Jindo and Muan, although overlapping in the distribution of the two clades occurred. Six haplotypes belonging to clade B were found exclusively in Geomundo, Seosan and Sandong. The bootstrap values at the nodes supporting each clade were somewhat higher in the NJ tree (66% in clade A and 49% in clade B) than in the ML tree (59% in clade A and 35% in clade B).

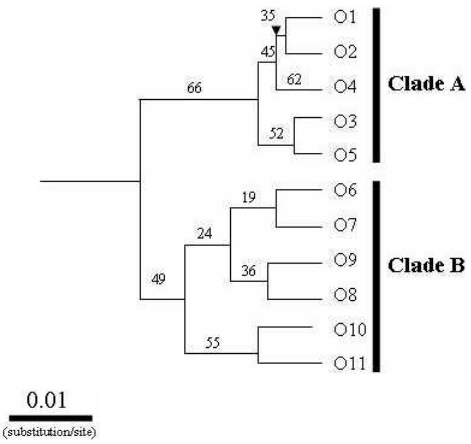

Fig. 4. PHYLIP analysis of mitochondrial 16S gene sequences using mtDNA sequences of 11 Octopus minor haplotypes. The tree was obtained using the subprogram NEIGHBOR incorporated in PHYLIP. The tree was rooted using *Octopus vulgaris*. The numbers shown on branches, which represent bootstrap values for 100 replications, were obtained from using the subprogram CONSENSE.

Networking

To further illustrate the genetic relationships among haplotypes, we used an unrooted one-step median network, which visualizes a possible evolutionary pathway among closely related haplotypes (Fig. 5). Although we expected more resolution in the closely related haplotypes, it provided us with limited information. Haplotypes belonging to clade A could have been derived from a single founder, haplotype O2 (labeled as 2). The network confirmed the result obtained from the PHYLIP analysis in that haplotypes were subdivided into two clades, separated by 0.4% of nucleotide divergence.

Genetic diversity

Within locality diversity was estimated in terms of haplotype diversity (*H*), maximum sequence divergence (MSD), mean number of pairwise differences (MPD) and nucleotide diversity (π) (Table 4). One of the biggest results is high *H*. In a range of 0.8-1, all but Namhae, Jindo and

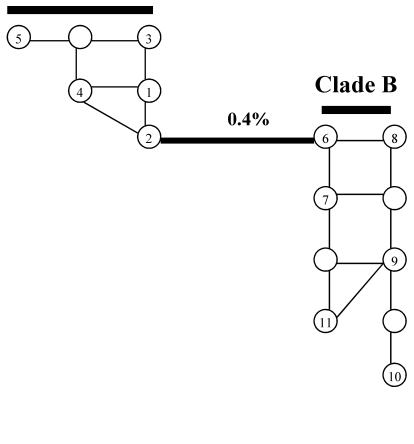

Fig. 5. Parsimonious one-step median networks analysis among 11 *Octopus minor* haplotypes. Each bar indicates one nucleotide difference from the neighboring haplotype. Numbers on each circle denote the haplotype name, omitting the antecedent alphabets, O. Note that haplotype 2 (O2) in clade A and haplotype 6 (O6) in clade B require two (0.4% of sequence divergence) to connect to each other.

Table 4. Within-locality diversity estimates

Locality	SS[a]	NH[b]	H[c]	NP[d]	MSD[e] (%)	MPD[f]	pπ[g]
1. Sandong	4	3	0.92	17	0.8	2.38	0.005
2. Namhae	4	3	0.85	5	0.5	1.50	0.003
3. Yeosu	4	4	1.00	9	1.8	4.68	0.015
4. Geomundo	4	4	1.00	15	1.2	4.44	0.012
5. Jindo	4	3	0.88	6	1.5	2.29	0.005
6. Muan	4	3	0.93	8	0.3	3.24	0.002
7. Seosan	4	3	0.86	12	1.7	3.95	0.008

[a]Sample size, [b]Number of haplotype, [c]Haplotype diversity, [d]Number of polymorphic sites, [e]Maxium sequence divergence [f]Mean number of pairwise differences, [g]Nucleotide diversity

Seosan (H=0.85, 0.88 and 0.86, respectively) were extremely high (H=0.9-1.0). Particularly, all individual specimens at Yeosu and Geomundo showed all and nearly all different haplotypes, respectively (both H=1.0). Yeosu and Geomundo showed highly results of MSD (1.8 and 1.2, respectively), MPD (4.68 and 4.44, respectively) and π (0.015 and 0.012, respectively) compared with other localities.

Gene flow

Genetic distance (F_{ST}), coancestry coefficient (D) and migration rates (Nm) are shown in Table 5. Analysis of F_{ST} between populations ranged from 0.09-0.39 (maximum: comparison between Sandong and Jindo. However, pairwise comparisons between Yeosu and Namhae/Jindo and Muan had negative values of F_{ST} (-0.10 and -0.15, respectively), indicating to compose homogeneous populations. In particular, the estimate obtained in a comparison between Sandong and all of the Korean populations showed relatively higher F_{ST} (0.23-0.39). However, a test of statistical significance of pairwise F_{ST} estimates showed that the Sandong population is not significantly differentiated from Korean populations (p>0.05). Likewise, no population pair was differentiated with statistical significance from Korean populations (p>0.05). Pairwise cmparisons of coefficients of coancestry (0-1, where D=0 is identical, shared ancestry) ranged from 0-0.4, which were also consistent with the F_{ST} estimates. The populations of Yeosu and Namhae/Jindo and Muan indicate nearly similar estimates and existence of ancestry, whereas Sandong, Geomundo and Seosan each have different ancestry. Furthermore, per generation migration rate (Nm) was extremely high between Yeosu and Namhae/Jindo and Musan. In the relationships between geographic distance and genetic distance, there was a somewhat consistent trend. The greatest genetic distance (F_{ST}=0.39) was found in a comparison between geographically no closer localities. Yeosu and Namhae/Jindo and Muan located in a distance of below 100 km showed essentially zero genetic distance. The increase in genetic isolation appears to understand in proportion to the increase in geographic distance. Consequently, it seems that gene flow does occur among localities, but local populations possess their own sets of haplotypes.

Genetic structure

Hierarchical analysis to test geographic relationships among populations is presented in Fig. 6. The six Korean populations were structured into two groups: Namahe, Yeosu, Muan, Jindo, and Geomundo, Seosan, but these two groups were not separated based on statistical significance (p=0.092). No statistically significant structuring was found between Korean populations. Moreover, there was no significant structuring between the Korea and China populations. Genetic distance in some nodes was negative (e.g., -0.157 between Namhae and Yeosu, -0.192 between Muan and Jindo) this is effectively zero, suggesting that the octopus in those regions appear to form one large genetic group.

Table 5. Mitochondrial 16S gene sequence of genetic distance (F_{ST}), coancestry coefficient (D) and per generation female migration rate (Nm) of each locality

Locality	1	2	3	4	5	6	7
1	–						
2	F_{ST} = 0.3421 D = 0.3685 Nm = 2.54	–					
3	F_{ST} = 0.3568 D = 0.4129 Nm = 2.57	F_{ST} = -0.1059 D = 0.0000 Nm = infinite	–				
4	F_{ST} = 0.2856 D = 0.2688 Nm = 7.46	F_{ST} = 0.0872 D = 0.0765 Nm = 5.55	F_{ST} = 0.1468 D = 0.1345 Nm = 1.78	–			
5	F_{ST} = 0.3952 D = 0.3389 Nm = 2.64	F_{ST} = 0.0391 D = 0.0344 Nm = 7.52	F_{ST} = 0.0275 D = 0.0297 Nm = 6.35	F_{ST} = 0.0954 D = 0.09253 Nm = 4.33	–		
6	F_{ST} = 0.3367 D = 0.2961 Nm = 3.65	F_{ST} = 0.0489 D = 0.0427 Nm = 8.26	F_{ST} = 0.0256 D = 0.0233 Nm = 7.34	F_{ST} = 0.1543 D = 0.1349 Nm = 1.92	F_{ST} = -0.1572 D = 0.0000 Nm = infinite	–	
7	F_{ST} = 0.2344 D = 0.1634 Nm = 8.42	F_{ST} = 0.0953 D = 0.0922 Nm = 6.08	F_{ST} = 0.1072 D = 0.1234 Nm = 6.92	F_{ST} = 0.1649 D = 0.1644 Nm = 2.36	F_{ST} = 0.1649 D = 0.1652 Nm = 2.22	F_{ST} = 0.2183 D = 0.2155 Nm = 1.18	–

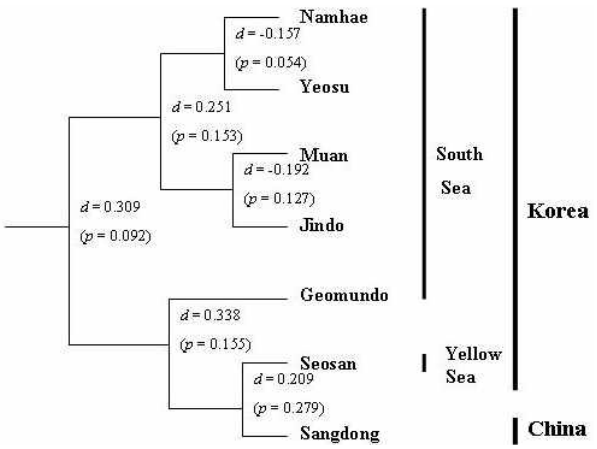

Fig. 6. Hierarchical relationships among localities using the Holsinger and Mason-Gamer method [9]. The value at each node is the distance between its two daughter nodes and the p value is the significance of differentiation based on 1,000 random re-samplings.

Discussion

A broad study of biology of the octopus concerning aspects of its physiology, behavior, growth and reproduction has made much progress over the decades [8,14,16,17,21,22]. The specimen for these studies was *O. vulgaris* occurring in European waters instead of *O. minor* in Korean coasts. Consequently, the biological magnitude in *O. minor* compared with *O. vulgaris* was poorly understood, but this species certainly produced the eggs for reproduction. After spawning and external fertilization, developing lavae spend a variable period of time as a part of the plankton. Possession of this planktonic life-history strategy is associated with a significant factor for the dispersal by water currents. Consequently, it is assumed that planktonic larval stage makes it difficult to predict genetic population structure and level of gene flow [2].

As a result of mtDNA analysis in this study, one of the interesting features is the finding of high haplotype numbers within local populations. On the other hand, a O2 haplotype was represented as a major one on Korea and China coasts in terms of frequency (a total of 39.4%) and distribution (several localities throughout Korea and China). It is understood that a strong movement of this haplotype is possibly associated with a high genetic relatedness, resulting in a homogenous population between Yeosu/Namhae and Muan/Jindo based on the F_{ST} analysis. Avise et al. [3] proposed five different distribution patterns of mtDNA clones. Among them category I is one that possesses distinct clones geographically separated. The octopus obtained from Korea and China revealed two distinct mtDNA clades (clades A and B) with the minimum sequence divergence of 0.4% (Fig. 5). Clade A is distributed in the south-central and western part of Korea such as Yeosu, Namhae, Muan and Jindo. Clade B showed a somewhat lengthy distribution occupying the

southeastern part of Korea including Geomundo and the east-central part of China. It clearly showed a discontinuous distribution between two clades with a substantial sequence divergence. Our genetic data appear to show the category I of the phylogeographic pattern described by Avise et al. [1,3]: phylogenetically discontinuous clones, geographically separated. Avise et al. [3] explained the occurrence of class I category to the presence of a long-term external barrier against gene flow or extinction of the intermediate genotypes connecting each clade in a species with a limited dispersal. We previously studied to differentiate Korean from Chinese octopus inferred from mtDNA COI gene using the PCR method and suggested that Korean populations (Yeosu, Namhae, Muan and Jindo) had a relatively high genetic similarity [12]. These results assure us that the geographical distance between populations is associated with increasing gene flow for larval dispersal and have a high genetic relatedness. On a more local scale, if the larvae passively drift over a long distance, they should overcome environmental differences to become successfully matur the octopus. The long dispersed larvae may undergo the process of competitive exclusion. Our current genetic data support low genetic distance and high gene flow among localities (Yeosu, Namhae, Muan and Jindo), whereas Seosan, Geomundo and Sandong are differentiated from these populations (α=0.309), although statistically significant genetic differentiation was not observed.

In conclusion, the analysis of mtDNA 16S gene sequences of the octopus to understand population genetic structure of the species showed that the octopus populations in Yeosu, Namhae, Muan and Jindo were highly interconnected. It is suggested that adjacent populations contribute to easily exchange migrants and planktonic dispersal between localities is associated with geographic distance.

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References

1. Avise, J. C. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution* **43**, 1192-1208.
2. Avise, J. C. 1994. pp. 511, *Molecular markers, natural history, and evolution*. Chapman and Hall. New York.
3. Avise, J. C., J. Anold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annu. Rev. Ecol. Syst.* **18**, 489-522.
4. Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479-491.
5. Felsenstein, J. 1993. *PHYLIP (Phylogeny Inference Package) ver. 3.5c*. Department of Genetics, University of Washington, Seattle.
6. Grant, W. S., A. M. Clark, and B. W. Bowen. 1998. Why restriction fragment length polymorphism analysis of mitochondrial DNA failed to resolve sardine (*Sardinops*) biogeography: insights from mitochondrial DNA cytochrome b sequences. *Can. J. Fish Aqua. Sci.* **55**, 2539-2547.
7. Harley, E. H., D. P. Ohland, and P. B. Best. 1995. Systematics of Cetaceans using restriction site mappling of mitochondrial DNA. *Mol. Phylo. Evol.* **4**, 10-19.
8. Hernadez-Garcia, V., J. L. Hernadez-Lopez, and J. J. Castro-Hdez. 2002. On the reproduction of *Octopus vulgris* off the coast of the Canary Islands. *Fish Res.* **57**, 197-203.
9. Holsinger, K. E. and R. J. Mason-Gamer. 1996. Hierarchical analysis of nucleotide diversity in geographically structured populations. *Genetics* **142,** 629-639.
10. Inoue, J. G., M. Masaki, K. Tsukamoto, and M. Nishida. 2000. Complete mitochondrial DNA sequence of the Japanese sardine *Sardinops melanostictus*. *Fish Sci.* **66**, 924-932.
11. Jung, J. M. and D. S. Kim. 2001. Influence of sea condition on catch fluctuation of long line for common octopus, *Octopus variddilis* in the coastal waters of Yeosu. *Bull. Kor. Soc. Fish Tech.* **37**, 321-325.
12. Kim, J. I., T. Y. Oh, W. S. Yang, and E. S. Cho. 2008. Development of molecular marker to distinguish *Octopus minor* Sasaki caught in Korea and that in China. *J. Life Sci.* **18**, 284-286.
13. Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.* **116**, 111-120.
14. McQuaid, C. D. 1994. Feeding behaviour and selection of bivalve prey by *Octopus vulgaris* Cuvier. *J. Exp. Mar. Biol. Ecol.* **177**, 187-202.
15. Nei, M. 1987. pp. 512, *Molecular Evolutionary Genetics*. Columbia Univ. Press, New York, NY.
16. Quetglas, A., M. Gonzalez, and I. Franco. 2005. Biology of the upper-slope cephalopod *Octopus salutii* from the western Mediterranean Sea. *Mar. Biol.* **146**, 1131-1138.
17. Quetglas, A., M. Gonzalez, A. Carbonell, and P. Sanchez. 2001. Biology of the deep-sea octopus *Bathypolypus sponsalis* (Cephalopoda: Octopodidae) from the western Mediterranean Sea. *Mar. Biol.* **138**, 785-792.
18. Reynolds, J., B. S. Weir, and C. C. Cockerham. 1983. Estimation for the coancestry coefficient: basis for a

short-term genetic distance. *Genetics* **105**, 767-779.
19. Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406-425.
20. Schneider, S., J. M. Kueffer, D. Roessli, and L. Excoffier. 1996. *Arlequin:* a software package for population genetics. Genetics and Biometry Lab., Department of Anthropology, University of Geneva.
21. Smale, M. J. and P. R. Buchan. 1981. Biology of *Octopus vulgaris* off the East coast of South Africa. *Mar. Biol.* **65**, 1-12.
22. Smith, C. D. 2003. Diet of *Octopus vulgaris* in False bay, South Africa. *Mar. Biol.* **143**, 1127-1133.
23. Sweijd, N., A. Lopata, R. Bowie, T. Marinaki, P. Cook, and E. Harley. 1998. A PCR technique for forensic species level identification of abalone tissue. *J. Shell. Res.* **17**, 889-896.
24. Thomson, J. D., D. G. Higgins, and T. J. Gibson. 1994. Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalities and weight matrix choice. *Nucleic Acids Res.* **22**, 4673-4680.

초록 : 미토콘드리아 16S rRNA 염기서열에 의한 한국, 중국 낙지의 유전자 집단 분석

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본 연구는 2006년 8월부터 2007년 9월까지 여수, 남해, 진도, 무안, 거문도, 서산 및 중국의 산동에서 포획한 낙지 유전자 집단을 분석하기 위하여 미토콘드리아 16S rRNA 염기서열로 조사했다. 유전자 분석은 총 28 개체로부터 11개의 haplotype이 발견되었다. 유전자 분화율은 0.2-1.2% 범위로 나타났다. Haplotype에 대한 PHYLIP 및 network 조사에 따르면 낙지는 두개의 clade (clade A/clade B)로 나뉘어지며, clade 사이의 분화율은 0.4%로 나타났다. 지역적 거리에 따라 haplotype이 다음과 같이 분화되었다. 하나는 여수, 남해, 무안, 진도 haplotype과 다른 하나는 서산, 거문도, 산동 haplotype으로 나뉘어졌다. 계층구조 분석에서도 한국 낙지집단 및 중국과의 유전적 차이를 볼 수 있으나, 현저한 지역적 차이는 나타나지 않았다. 따라서 한국연안에 서식하고 있는 일부 낙지집단은 gene flow에 의해서 유전적 동질성을 나타낼 수 있지만, 한국집단 간 뿐만 아니라 중국집단과의 유전적 분화는 지역적 거리 및 장벽으로 인하여 제한적인 gene flow로 설명될 수 있다.