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Comparison of Population Genetic Structure of Two Seashore-Dwelling Animal Species, Periwinkle *Littorina brevicula* and Acorn Barnacle *Fistulobalanus albicostatus* from Korea

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ABSTRACT

The genetic structure of marine animals that inhabit the seashore is affected by numerous factors. Of these, gene flow and natural selection during recruitment have strong influences on the genetic structure of seashore-dwelling species that have larval periods. Relative contributions of these two factors to the genetic structure of marine species would be determined mainly by the duration of larval stage. The relationship between larval period and genetic structure of population has been rarely studied in Korea. In this study, genetic variations of cytochrome oxidase subunit I (COI) were analyzed in two dominant species on rocky shore habitats in the Korean peninsula: periwinkle *Littorina brevicula* and acorn barnacle *Fistulobalanus albicostatus*. Both species are not strongly structured and may have experienced recent population expansion. Unlike periwinkle, however, barnacle populations have considerable genetic variation, and show a bimodal pattern of mismatch distribution. These results suggest that barnacle populations are more affected by local adaptation rather than gene flow via larval migration. The bimodal patterns of barnacle populations observed in mismatch distribution plots imply that they may have experienced secondary contact. Further studies on seashore-dwelling species are expected to be useful in understanding the evolution of the coastal ecosystem around Korean waters.

Keywords: adaptation, larvae, recent population expansion, recurrent process, mismatch distribution analysis

INTRODUCTION

The seashore where the land and sea meet is a very unique environment. It is challenging for marine animals to survive in these habitats because of harsh conditions such as strong waves and exposure to atmosphere. Nonetheless, a variety of organisms are present and they form seashore ecosystems.

Various factors are involved in the evolution of marine life dwelling in these habitats. In case of animal species without the larval stage such as sea slaters (*Ligia* spp.), the distribution of genetic variation may have been affected mostly by historical factors such as past fragmentation and secondary contact which may have been caused by geological changes during the Pleistocene (Jung et al., 2008). On the other hand, gene flow is likely to have a more strong influence on the genetic structure of seashore animals with the larval stage (Sotka et al., 2004). In addition, adaptation may also have a strong impact on survival of these species during recruitment to local habitats whose physical, chemical, and biological conditions are greatly diverse (Hedgecock, 1986). Duration of larval period may play a key role in shaping the genetic structure of marine animal species (Burton, 1983; Doherty et al., 1995; Bohonak, 1999). Of the marine animals, in seashore-dwelling species, a longer larval period prevents local adaptation and strengthens the gene flow, but a shorter larval stage or direct development prefer local adaptation rather than gene flow (Johannesson, 2003).

Representing a large variation between species, cytochrome oxidase subunit I (COI) sequences in the mitochondrial genome are popular as DNA barcoding markers in the identifi-

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Table 1. Summary statistics of genetic variation of *Littorina brevicula* and *Fistulobalanus albicostatus* in this study

Species	Location	No. of individuals	No. of haplotypes	Haplotype diversity	Nucleotide diversity	SSD	Tajima's D
Littorina brevicula	ANIN	8	2	0.5714±0.0945	0.0009±0.0009	0.0427	1.4442
	NAMHAE	6	3	0.7333±0.1552	0.0014±0.0013	0.0310	0.3106
	YONGYUDO	9	3	0.5556±0.1653	0.0013±0.0011	0.0001	−0.9361
	BORYEONG	8	3	0.6071±0.1640	0.0010±0.0010	0.0376	−0.4479
	SEOSAN	8	1	0.0000±0.0000	0.0000±0.0000	–	0.0000
	AYAJIN	7	5	0.9048±0.1033	0.0026±0.0020	0.0136	0.2390
	Total	46	8	0.5961±0.0646	0.0012±0.0010	0.0034	**−1.5263**
Fistulobalanus albicostatus	SINAN	7	7	1.0000±0.0764	0.0061±0.0040	0.0659	−1.2497
	JEBU	10	8	0.9333±0.0773	0.0072±0.0044	0.0118	−0.3784
	JEJU	8	7	0.9463±0.0772	0.0079±0.0048	0.0202	−0.1073
	SEOCHEON	12	9	0.9394±0.0577	0.0046±0.0029	0.0082	−1.3940
	Total	37	28	0.9625±0.0224	0.0070±0.0039	0.0118	**−1.5352**

Significant value ($p < 0.05$) of SSD and Tajima's D is presented in boldface.
SSD, sum of squared deviation.

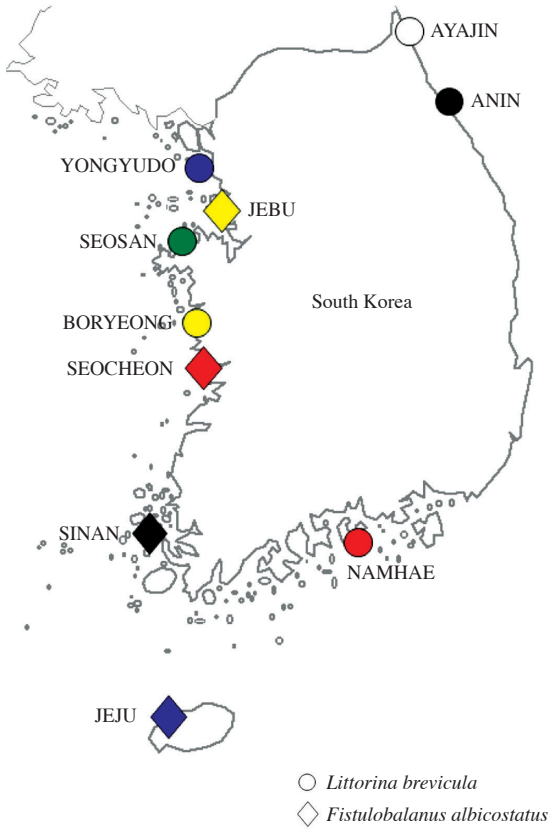

Fig. 1. Sampling locations in this study.

cation of animal species (Hebert et al., 2003). COI regions are also suitable for studying intraspecific genetic variation since COI sequences have considerable genetic variation within species (Marosi et al., 2013).

In this study, we compared the population genetic structure of two dominant seashore-dwelling species from Korea, differing in the length of the larval period: periwinkle *Littorina brevicula* and acorn barnacle *Fistulobalanus albicostatus*. Periwinkle is one of the most dominant species in the upper intertidal zone all around the Korean peninsula, and it is widely distributed from Hong Kong to northern Japan. Acorn barnacle that attaches itself to hard bottoms of the mid-littoral to sublittoral zones is present mostly on the west coast of Korea, and it is distributed along the western Pacific region from Sumatra to Korea and Japan (Lee and Kim, 1991). The larval period of periwinkle is 4–7 weeks, which is longer than that of acorn barnacle, 6–23 days (Lee and Kim, 1991; Desai et al., 2006; Chang et al., 2011). We, therefore, tried to investigate the effect of larval duration on the genetic structure of seashore-dwelling species found on the Korean peninsula by comparing these two species.

MATERIALS AND METHODS

Up to forty-six periwinkles and thirty-seven acorn barnacles were collected from seven and four locations in Korea, respectively (Table 1, Fig. 1). Specimens were fixed in 80% ethanol solution at the collection site, and were identified using a stereomicroscope in the laboratory. Parts of the body of each specimen were removed for DNA extraction. DNAs were extracted with DNeasy Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol.

Part of the COI region was amplified using primers LCO 1490 and HCO2198 (Folmer et al., 1994). Each polymerase chain reaction (PCR) mixture (total volume, 25 μL) was composed of 2.5 μL of PCR buffer (10×), 2.0 μL of dNTP mixture (10 mM), 1.5 μL of $MgCl_2$ solution (25 mM), 1 μL of each primer solution (10 μM), 0.5 μL of *Taq* DNA polymerase (Takara Bio Inc., Kusatsu, Shiga, Japan), 2 μL of genomic

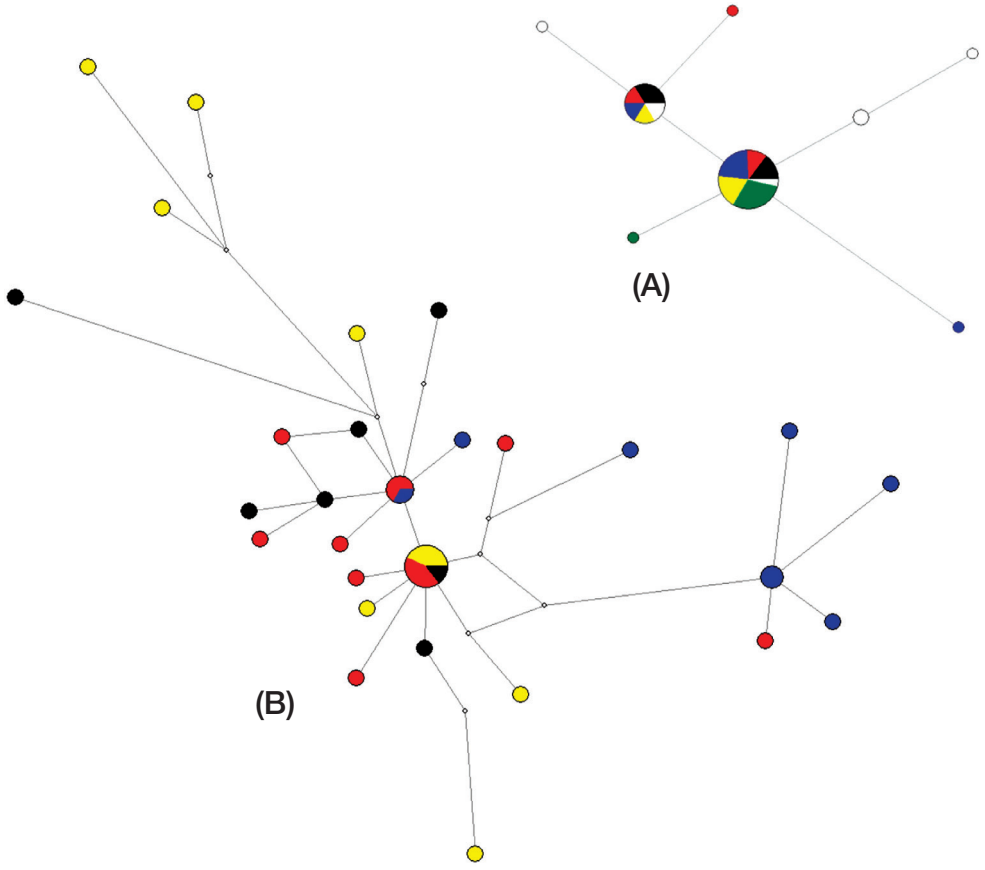

Fig. 2. Haplotype networks of *Littorina brevicula* (A) and *Fistulobalanus albicostatus* (B) drawn by NETWORK 5.0.0.0. Each color denotes sampling location of sequences represented in Fig. 1.

DNA solution, and 15.5 μL of distilled water. PCRs were performed by using the SimpliAmp Thermal Cycler (Applied Biosystems Ltd., Foster City, CA, USA). PCR machine settings were as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min and elongation at 72°C for 1 min, and final extension at 72°C for 7 min. PCR products were visually checked by using 1% agarose gel electrophoresis. Nucleotide sequences of amplified products were analyzed by Cosmo Genetech (Seoul, Korea).

Sequences were saved in FASTA file format. Then, multiple alignments were performed by using CLUSTAL_X (Thompson et al., 1997). The definition of haplotypes was carried out by using DnaSP 5.10.01 (Librado and Rozas, 2009). Genetic polymorphism was investigated with ARLEQUIN 3.11 (Excoffier et al., 2005) by calculating haplotype diversity (gene diversity) and nucleotide diversity per site. Haplotype networks were drawn with NETWORK 5.0.0.0 (Fluxus Technology, http://www.fluxus-engineering.com) by using the median joining algorithm (Bandelt et al., 1999). Recent demographic histories were inferred by performing mismatch distribution (MMD) analysis (Harpending, 1994) and Tajima's D test (Tajima, 1989) with ARLEQUIN software. Analysis of molecular variance (AMOVA) was performed to examine the hierarchical genetic structure by using ARLEQUIN.

RESULTS

The sequencing results for 46 individuals of periwinkle revealed 8 haplotypes (GenBank accession number: KU977411-KU977418) (658 bp). The two haplotypes with the highest frequencies appeared in almost all locations (Fig. 2A). When all individuals were pooled, haplotype diversity and nucleotide diversity were 0.5961 and 0.0012, respectively. SEOSAN was monomorphic, and therefore, it showed lowest genetic diversity, but AYAJIN showed the highest genetic polymorphism in terms of both haplotype diversity and nucleotide diversity (Table 1).

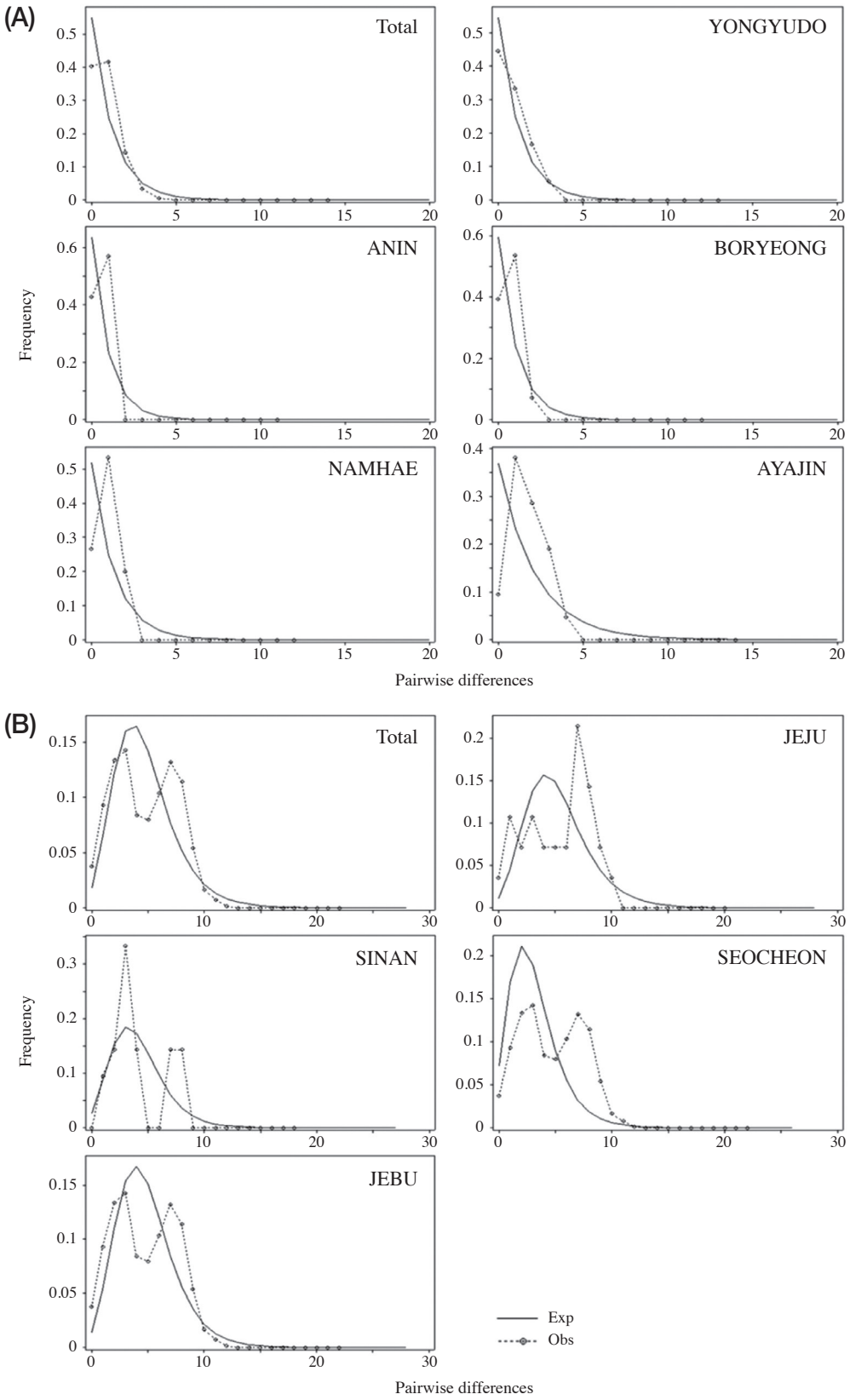

Fig. 3. Mismatch distribution plots of *Littorina brevicula* (A) and *Fistulobalanus albicostatus* (B). Exp and Obs denote an expected pattern of mismatch distribution under a sudden expansion model and an observed pattern of mismatch distribution, respectively.

Table 2. Results of analysis of molecular variance (AMOVA) for *Littorina brevicula*

Source of variation	d.f.	Sum of squares	Percentage of variation
Among populations	5	3.229	8.4
Within populations	40	15.185	91.6
Total	45	18.413	
F_{ST} = 0.0840		p = 0.5181	

Table 3. Results of analysis of molecular variance (AMOVA) for *Fistulobalanus albicostatus*

Source of variation	d.f.	Sum of squares	Percentage of variation
Among populations	3	15.364	13.47
Within populations	33	69.852	86.53
Total	36	85.216	
F_{ST} = 0.1347		p = 0.0010	

In case of acorn barnacle, 28 haplotypes were defined in 37 individuals (GeneBank accession number: KU977383-KU977410) (672 bp). The haplotype network of barnacle is more complicated than that of periwinkle (Fig. 2B). Although a few haplotypes with the highest frequencies appeared in most of the locations, 25 out of the 28 haplotypes were private, i.e., they appeared in only one location. It is not likely that the genetic relationship shows an association with geography. Acorn barnacle populations were genetically more diverse than populations of periwinkle. Haplotype diversity and nucleotide diversity of the pooled population of barnacle were 0.9625 and 0.0070, respectively (Table 1).

From the results of MMD analysis, the observed patterns of MMD were not significantly different from the expected patterns under a sudden expansion model in both species (Table 1). This suggests that these two species may have experienced recent population expansion. One remarkable difference between the two species is that acorn barnacle populations showed a bimodal pattern of MMD unlike periwinkle populations which showed a unimodal pattern (Fig. 3). The results of Tajima's D test indicated a slightly different story. The D value for each local population of both species was not significantly negative. But the pooled population of these two species showed significant negative D values, which suggests they may have experienced expansion of population in view of the whole population. It is, therefore, likely that the results of the two population demographic analyses are consistent on the whole.

According to the results of AMOVA, the F_{ST} value among periwinkle populations was lower than that among barnacle populations, i.e., gene flow rate in the former was higher than that in the latter. This is also supported by the fact that the genetic variance component of periwinkle lying between populations (8.4%) (Table 2) was lower than that of acorn barnacle (13.46%) (Table 3). As the overall F_{ST} values for both species were low, there was considerable gene flow among populations.

DISCUSSION

Periwinkle *Littorina brevicula* and acorn barnacle *Fistulobalanus albicostatus* commonly live on rocky seashore habitats and have larval stages in their life cycles. The two seashore- dwelling species in this study showed common genetic properties. Firstly, a high gene flow rate (low F_{ST} values) was observed in both species, which is common in marine animal species with the larval period (Sotka et al., 2004). A previous study on Korean populations of *Littorina brevicula* using different genetic markers such as ND6 and cyt b genes reached similar conclusions to our study; a high level of gene flow (Kim et al., 2003). Secondly, two species may have experienced recent population expansion probably during the Pleistocene, as inferred from MMD analyses and Tajima's D tests. Although they were not observed in each local population, negative Tajima's D values were observed in the pooled populations of both species. Pooling of whole local populations can be supported by the high gene flow rate among populations, i.e., an almost panmictic state.

On the other hand, these two species were significantly different in terms of the other genetic properties. The most remarkable property was the difference in genetic diversity. Acorn barnacle populations showed much more diversity than periwinkle populations. Moreover, individuals constituting each local population of barnacle were not closely related. The next difference between these two species was detailed histories of populations. In MMD analyses, a bimodal pattern was observed in acorn barnacle populations, but a unimodal pattern was observed in periwinkle populations.

Such differences between these two species could be due to historical as well as recurrent processes. Bimodal patterns in MMD plots usually suggest that past fragmentation was followed by secondary contact or genetic admixture (Strasburg et al., 2007). Therefore, the present acorn barnacle populations may have been formed by secondary contact of genetically diverged populations that had been separated by geological changes probably during the Pleistocene.

The second but a more important factor shaping the different population genetic structure was disparity in gene flow rates between these two species. In general, the longer are the larval periods, the higher is the possibility of recruitment of individuals from the common gene pool to a variety of local seashore habitats. Resultantly, it may weaken local

adaptation and lower genetic divergence between local populations (Doherty et al., 1995; Johannesson, 2003). Actually, the larval period of periwinkles is 4−7 weeks, which is longer than the 6−23 day larval period of *Fistulobalanus albicostatus* (Lee and Kim, 1991; Zvyagintsev and Korn, 2003; Desai et al., 2006; Chang et al., 2011). Such a long duration of the larval stage may explain the genetic homogeneity in Korean population of periwinkles. In addition, it ensures continuous gene flow from the southern region of China to the Korean peninsula via strong currents such as the Kuroshio Current. In comparison, acorn barnacle populations with a shorter larval period may have been more affected by local adaptation, which may result in genetic divergence among local populations and guarantee high genetic polymorphism in local populations.

This study suggests that the genetic structure of coastal marine animal species could be affected by a variety of factors such as historical events or recurrent processes. Further studies on seashore-dwelling species are expected to be useful in understanding the evolution of the coastal ecosystem around Korean waters.

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