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Genetic Diversity and Population Structure of *Codium fragile* (SURINGAR) HARIOT in Korea Using Allozymes

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The study of genetic diversity and population structure was carried out in the *Codium fragile* using allozyme analysis. Although this species has been regarded as a ecologically and economically important source, there is no report on population structure in Korea. Starch gel electrophoresis was used to investigate the allozyme variation and genetic structure of four Korean populations of this species. Of the 15 genetic loci surveyed, nine (60.0%) was polymorphic in at least one population. Genetic diversity was high at the species level (H_{ES}=0.144), and, that of the population level was relatively low (H_{EP}=0.128). Nearly 87% of the total genetic diversity in *C. fragile* was apportioned within populations. The predominant asexual reproduction, population fragmentation, low fecundity, geographic isolation, and colonization process are proposed as possible factors contributing to low genetic diversity in this species. The indirect estimated of gene flow based on G_{ST} was 1.69. The moderate level of gene flow in *C. fragile* populations is mainly caused by thallus developed from isolated utricles dispersal via sea current.

Key words – Allozyme, *Codium fragile*, genetic diversity

During the past 20 years, enzyme electrophoresis has been used to describe the population genetic structure of over 700 plant taxa[4,10]. This information has contributed greatly to an understanding of the evolutionary history of individual species and related group of species, and has provided insights into the relationships between allozyme diversity and life-history traits[5]. Despite the importance of knowledge on genetic variation for providing information for conservation purposes, detailed studies of genetic variation are not available for most native taxa in China and Korea, particularly marine species[6].

The algae are the most efficient of all plants in photosynthesis and conduction of food. *Codium fragile* (Suringar) Hariot is a wide spread species in all tropical to temperate seas throughout the world and also an abundant species in Korean seas[12,15]. The Korean populations of *C. fragile* are typically distributed in patches. In Geoje Island, it grows on sand-free rocks in tide pool as well as littoral to sublittoral zone. In this Island, various epiphytes and endophytes are commonly observed on the spongy body. *C. fragile* occurs also a perennial angiosperm inhabiting soft-bottom marine habitats, ranging from the intertidal to depths of approximately 3-8 m in temperate latitudes[8,13].

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C. fragile provides an interesting system for exploring patterns of population structure because it is found in near shore marine habitats panning intertidal and subtidal elevations[8]. Although molecular and biochemical approaches are now increasingly being applied to address the taxonomic and phylogenetic relationships within the animals, plants, and other algal species in Korea[6,7] and many studies have been carried out morphological and ecological characteristics of this species[12], no population genetic studies of this species have been conducted.

The objectives of this study are to estimate the level of genetic diversity in the species, and to describe its genetic variation pattern within and among populations. In addition, the results are compared with those of the other marine species in Koreas as well as plant species having similar life-history characteristics.

Materials and Methods

Plant materials

Materials were collected from four populations of *C. fragile* at Geoje Island in Korea (Fig. 1). Within the distinct distribution of the species in Korea we sampled four populations from Southern Geoje (GEO-S), Eastern Geoje (GEO-E), Western Geoje (GEO-W), and Northern Geoje (GEO-N), respectively (Table 1). One shoot per plant was

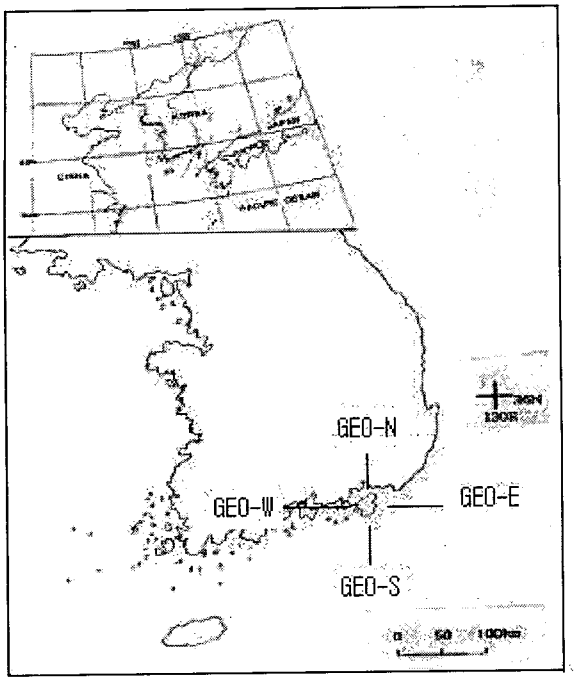

Fig. 1. Collection localities for populations of *Codium fragile* as source for allozyme analysis.

Table 1. Codes and locations of the *Codium fragile* in this study

Code	Location	Direction
GEO-S	Geoje-Island, Gyeongsangnam-do	South
GEO-W	Geoje-Island, Gyeongsangnam-do	West
GEO-E	Geoje-Island, Gyeongsangnam-do	East
GEO-N	Geoje-Island, Gyeongsangnam-do	North

collected during the period from 2004 to 2005. Forty-five to 50 plants were sampled from each population.

Allozyme analysis

Approximately 1.0 of 1.2 g biomass shoot tissues were ground with a cold mortar and pestle in 500 to 550 ul of extraction buffer (0.05 ml of 0.1% 2-mercaptoethanol, 0.001 M EDTA, 0.01 M potassium chloride, 0.01 M magnesium chloride hexahydrate, 4% w/v 1 g PVP, 0.10 M Tris-HCl buffer, pH 8.0).

Electrophoresis was performed using 12.0% starch gels according to the methods by Soltis *et al.*[17]. Eight enzyme systems were assayed in this study. Glucose phosphate isomerase (PGI) and phosphoglucomutase (PGM) were resolved on system 9 of Soltis *et al.*[17]. Isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), and 6-phosphogluconate dehydrogenase (PGD) were resolved on system 10 of Soltis *et al.*[17], fluorescent esterase (EST), leucine aminopeptidase (LAP), and peroxidase (PER) were resolved on system Morpholine-citrate of Clayton and Tretiak[1].

The most anodally migrating isozyme was designated as '1' and other subsequent isozymes were sequentially numbered for the enzymes resolved in more than one zone of activity. The alleles of isozyme 1, 2, 3, and so on were designated sequentially as 'a', 'b', and so on, respectively.

Statistical analyses

Enzymatic data were based on allele and genotype frequencies in each accession. A locus was considered polymorphic if two or more alleles were detected, and the frequency of the most common allele was less than 0.99[19,20]. Several standard genetic parameters were estimated using a computer program developed by M.D. Loveless and A.F. Schnabel[2]. The percentage of polymorphic loci (P) (P_p for population level and P_s for species level), mean number of alleles per locus (A), effective number of alleles per locus (A_E), number of alleles per polymorphic locus (A_P), and gene diversity (H_E) were estimated from the data. Observed heterozygosity (H_O) was compared with the Hardy-Weinberg expected value using Wright's fixation index (F)[21]. These indices were tested for deviation from zero by χ^2-statistics following Li and Horvitz[9]. Nei's gene diversity formulae (H_T, H_S, D_{ST}, and G_{ST}) were used to evaluate genetic diversity within and among populations[11]. The G_{ST} coefficient corresponds to the relative amount of differentiation among populations[20]. Nei's genetic identity (I) was calculated for each pairwise combination of populations[11].

The genetic structure within and among populations was also evaluated using Wright's[21] F-statistics, F_{IT}, F_{IS} and F_{ST}. The F_{IT} and F_{IS} coefficients measure excesses of homozygotes relative to the panmictic expectations in the entire samples and within populations, respectively. In the context of multiallelic loci F_{ST} is denoted as G_{ST}[11]. The estimate of Nm (the number of migrants per generation) was based on G_{ST}[16].

A phylogenetic tree was constructed by the neighbor-joining (NJ) method [14] using the NEIGHBOR program in PHYLIP version 3.57[3].

Results

The level of genetic variation was high in four populations of *C. fragile* (Table 2). Nine of 15 loci (60.0%)

Table 2. Allozyme variation within four populations of *Codium fragile*

Population	P_P	A	A_P	A_E	H_{OP}(SD)	H_{EP}(SD)
GEO-S	46.7	1.87	2.86	1.32	0.077(0.012)	0.160(0.061)
GEO-W	40.0	1.67	2.67	1.22	0.068(0.011)	0.124(0.050)
GEO-E	33.3	1.60	2.80	1.23	0.062(0.010)	0.120(0.049)
GEO-N	26.7	1.53	3.00	1.14	0.061(0.011)	0.092(0.040
Mean	38.3	1.64	2.69	1.22	-	0.128

P_P, A, A_P, A_E, H_{OP}, and H_{EP} are given in text and population codes depicted in Table 1.

showed polymorphism in at least one population, while the remaining six (*Idh-2, Lap-1, Lap-2, Mdh-1, Per-3,* and *Pgi-2*) were monomorphic in all populations. The majority of the polymorphic loci expressed two (*Est-2, Idh-1, Pgi-1,* and *Pgm*) or three alleles (*Est-1, Mdh-2, Per-2* and *Pgd-2*). *Per-1* was three. An average of 38.3% of the loci was polymorphic within populations, with populations values ranging from 26.7 to 46.7%.

Across accessions, the average number of alleles per locus (A) was 1.64, ranging from 1.53 to 1.87 (Table 2). The effective numbers of alleles per locus at the species (A_{ES}) and the population levels (A_{EP}) were 1.23 and 1.22, respectively. Number of alleles per polymorphic locus (A_P) was 2.69 across populations, varying from 2.67 to 3.00. Mean genetic diversity within populations was 0.128. In particular, the population GEO-S had the highest expected diversity (0.160); population GEO-N, the lowest (0.092).

F_{IS}, a measure of the deviation from random mating within the four populations, was 0.410, ranging from 0.322 for *Pgd* to 0.589 for *Pgm* (Table 3). The observed significant and positive F_{IS} value (0.410) indicated a significant deficit of heterozygotes in the populations.

Total genetic diversity values (H_T) varied between 0.018 (*Est-2*) and 0.565 (*Pgm*), for an average over all polymorphic loci of 0.239 (Table 3). Interlocus variation in the within-population genetic diversity (H_S) was high (0.215). On a per-locus basis, the proportion of total genetic variation due to differences among populations (G_{ST}) ranged from 0.038 for *Est-1* to 0.380 for *Per-2*, with a mean of 0.129. This indicated that about 12.9% of the total allozyme variation was among populations. The estimate of gene flow, based on G_{ST}, was slightly high among populations of *C. fragile* (Nm=1.69). Values of genetic distance (D) were <0.093. Genetic identity values among pairs of populations ranged from 0.911 to 0.990.

Our analysis of fixation indices, calculated for all polymorphic loci in each population, showed a slight deficiency of heterozygotes relative to Hardy-Weinberg expectations (Table 4). For example, all fixation indices were positive (25/25), of which 21 indices (84.0%) departed significantly from zero ($p<0.05$). In contrast, none was negative index.

Clustering of populations, using the NJ algorithm, was performed based on the matrix of calculated distances (Fig. 2). The genetic distances among *C. fragile* populations can be seen in the dendrogram, where total populations cluster at a below genetic distance 0.015. The phylogenic tree showed two distinct groups GEO-E and GEO-N, GEO-W and GEO-S.

Table 3. Estimates of genetic diversity statistics and nine polymorphic loci in *Codium fragile*

Locus	H_T	H_S	D_{ST}	F_{IS}	F_{IT}	G_{ST}
Est-1	0.296	0.284	0.011	0.400	0.423	0.038
Est-2	0.018	0.017	0.002	0.339	0.394	0.084
Idh-1	0.319	0.307	0.012	0.422	0.444	0.037
Mdh-2	0.304	0.283	0.021	0.330	0.376	0.069
Per-1	0.102	0.069	0.033	0.569	0.642	0.326**
Per-2	0.209	0.130	0.079	0.457	0.664	0.380**
Pgd	0.275	0.264	0.011	0.322	0.350	0.040
Pgi-1	0.066	0.060	0.007	0.361	0.425	0.099*
Pgm	0.565	0.518	0.047	0.589	0.623	0.084
Mean	0.239	0.215	0.025	0.410	0.482	0.129

H_T, H_S, D_{ST}, F_{IS}, F_{IT}, and G_{ST} are given in text.
*$p<0.05$; **$p<0.01$.

Table 4. Wright's fixation indices for four populations of *Codium fragile* using allozyme analyses

Population	*Est-1*	*Est-2*	*Pgm*	*Idh-1*	*Mdh-2*	*Per-1*	*Per-2*	*Pgd-1*	*Pgi-2*
GEO-S	0.433*	-	0.753***	0.286*	0.450*	0.479**	0.650***	0.466**	-
GEO-W	0.311**	-	0.567***	0.372*	0.465**	-	0.525*	0.367*	0.444*
GEO-E	0.363*	-	0.549***	0.694***	0.272	-	-	0.328*	-
GEO-N	0.333**	0.351	0.452***	0.337**	-	-	-	0.253	0.310

Note: A dash indicates fixed loci. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

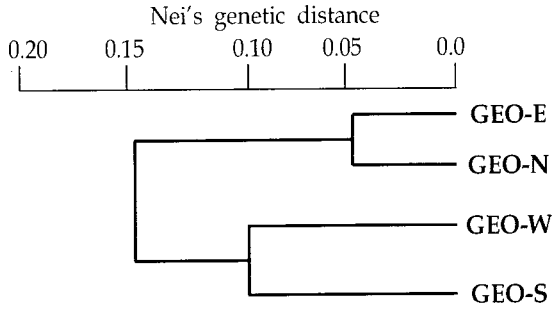

Fig. 2. A dendrogram showing the phylogenic relationships among the four populations of *Codium fragile*, based on data of genetic distance obtained by allozyme analysis.

Discussion

In allozyme analysis, four populations belonging to *C. fragile* maintain a lower than average level of genetic diversity compared with other plant species[4]. For example, its genetic diversity of 0.128 is lower than that for temperate-zone species (0.146), dicots (0.136), species with a sexual reproduction mode (0.151), and those with a long-lived woody habit (0.177)[4]. The percentage of polymorphic loci at the species level for *C. fragile* is 38.3%, which is also lower than that for species with temperature-zone distributions (48.5%), dicots (44.8%), species with a sexual reproduction mode (51.6%), and long-lived and woody (64.7%)[4].

Effective population sizes also have an important role in maintaining genetic diversity. The periodical cutting of thallus has often been moved from habitats to nearby seaside the purpose of foods during the past several decades. Small populations tend to have fewer multilocus genotypes and genetic diversity than large populations. If *C. fragile* is self-compatible, smaller populations probably exhibit more selfing than larger populations by virtue of the limited opportunities for outcrossing during any flowering season[10]. Considering the small and isolated populations observed in *C. fragile*, probable mating among relatives rather than self-pollinating occurs within these populations. Such structure can lead to biparental inbreeding or sibmating, causing heterozygote deficiencies.

A substantial heterozygote deficiency in some populations and at some loci (F_{IS}=0.410) (Table 5). The patch distribution of related individuals should generate a Wahlund effect. Our sampling included individuals from several patches per population, resulting in an overall deficiency of heterozygotes. It is probable that the combination of these factors may contribute to heterozygote deficiencies within these populations (Table 5).

In clustering of populations, the phylogenic tree showed two distinct groups GEO-E and GEO-N, GEO-W and GEO-S (Fig. 2). The GEO-E population had been disturbed by artificial actions for port structure and ship-building yards. The GEO-N population had been also disturbed by artificial actions for fishing grounds. The sizes of both populations were smaller than those of GEO-W and GEO-S populations.

By current categories for threatened taxa in Korea, almost all species belonging to *C. fragile* populations should be considered threatened. The probability of extinction of any single population is high, since the populations of natural populations (GEO-E and GEO-N) are so small. Ecological management of these populations will be necessary to preserve the species[18]. Many other populations of *C. fragile* are declining in population size because of habitat loss from harvesting for several artificial actions such as sea-form, port structure, and reclamation of sea. Populations showing the highest genetic diversity by allozymes could be recommended for in-situ conservation.

Table 5. Nei's unbiased genetic identity values of among *Codium fragile* (above diagonal) and genetic distances among populations (below diagonal)

Population	GEO-S	GEO-W	GEO-E	GEO-N
GEO-S	-	0.951	0.938	0.911
GEO-W	0.050	-	0.920	0.976
GEO-E	0.064	0.084	-	0.990
GEO-N	0.093	0.024	0.010	-

Based on the available data, such as relatively high G_{ST} value, several populations of each group should be preserved, especially those with high variation, such as populations GEO-W and GEO-S. These populations could be used as a source of genetic diversity for the restoration of genetically poor populations.

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초록 : 알로자임을 이용한 청각의 유전적 다양성과 집단구조

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알로자임 분석을 이용하여 청각의 유전적 다양성과 집단구조를 분석하였다. 이 종은 한국내 생태적, 경제적 중요한 자원이지만 유전적 분석이 수행되지 않았다. 전분 젤 전기영동으로 이 종의 한국내 네 집단에 대해 알로자임 변이와 유전 구조를 조사하였다. 15개 대립유전자좌위에 대해 9개 좌위(60.0%)가 적어도 한 집단에 대해 다형현상을 나타내었다. 종수준에서 유전적 다양성은 매우 높았다(H_{ES}=0.144). 집단수준에서 유전적 다양성은 비교적 낮았다(H_{EP}=0.128). 청각에서 전체 유전적 다양도의 87%는 집단내에 내포되어 있었다. 청각의 번식방법은 유성생식보다는 무성생식이 우세하고, 집단의 단절, 낮은 자손의 생성, 지리적 격리, 그리고 정착과정이 낮은 유전적 다양성을 설명하는 요인으로 사료된다. 조사한 청각 집단에서 세대당 이주하는 개체수는 1.69로 평가되었다. 이 값은 보통 수준의 유전자 흐름으로 해류를 통한 이동이 주된 요인으로 보인다.