Genetic Diversity and Population Structure of Glehnia littoralis (Umbelliferae) in Korea

Man Kyu Huh*, Joo Soo Choi, Hong Wook Huh[1], Yung Hyun Choi[2], Byung Tae Choi[2]

Department of Molecular Biology, Dongeui University, 1:Department of Biology Education, Pusan National University, 2:College of Oriental Medicine, Dongeui University

Glehnia littoralis Fr. Schmidt (Umbelliferae) is a short-lived herbaceous species that are mostly distributed throughout East Asia. Although G. littoralis has been regarded as ecologically important one, there is no report on population structure in Korea. Starch gel electrophoresis was used to investigate the allozyme variation and genetic structure of Korean populations of this species. A high level of genetic variation was found in G. littoralis populations. Nine enzymes revealed 18 loci, of which 12 were polymorphic (66.7%). Genetic diversity at the species and population levels were 0.159 and 0.129, respectively. The sexual and asexual reproduction, high fecundity, and colonization process are proposed as possible factors contributing to genetic diversity. An indirect estimate of the number of migrants per generation (Nm = 1.45) indicated that gene flow was not extensive among Korean populations of this species. It is suggested that the ability of vegetation and artificial selection may have played roles in shaping the population structure of this species. we recommend that a desirable conservation population should be included at least 30 plants per population and especially those with high variation

Key words : *Glehnia littoralis*, population structure, allozyme variation, conservation population

Introdution

The genus *Glehnia* consists of only two species distributed throughout the world and finds only one species in Korea[1]. Genus *Glehnia* are distributed in temperate regions with centers of diversity in East Asia and West-America. East Asian natural *Glehnia littoralis* Fr. Schmidt (Umbelliferae) grows primarily on sand dunes or on sandy cliffs near the sea in East Asia. In Korea, the species usually grows on sea coastal or waste places near beach. Typical populations of *G. littoralis* in Korea are small and distributed in patches.

The species is diploid (2n = 22) with white flowers. G. littoralis can reproduce either clonally or sexually via flowers. Rhizomes generally are horizontal, with shallow elongations or prostrate stem rooting at the nodes. The fibrous root systems form extensive networks in the sand, which makes this species an economically important tool for preventing sand erosion. This particular property is exploited during the development of effective watersheds, stabilizing the sand along fragile field embankments, deforested areas, and in places prone to mud slides.

* To whom correspondence should be addressed at : Man Kyu Huh, Department of Molecular Biology, Dongeui University, Busan, 614-714 Korea.
· E-mail : mkhuh@dongeui.ac.kr · Tel : 051-890-1529
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It mainly enriches yin and down beares fire, resolves toxin and softens hardness as a medicine[2]. According to modern research, *G. littoralisis* both hypotensive and hypoglycemic (i.e., it lowers both blood pressure and blood sugar). It has a relatively strong inhibitory effect against *Pseudomonas pyocyanea*[2]. In addition, until the past several years, most parts of Korean beach had been disturbed by house and road constructions[3].

Although this species has been considered as an important species in ecology and medicine in Korea, population structure of this species has not been studied. The objectives of our study were to estimate how much allozyme diversity is maintained in the species, and to describe how genetic variation is distributed within and among populations. In addition, we compared the genetic diversity and population structure of *G. littoralis* with plant species having similar life-history characteristics.

Materials and Methods

1. Sampling procedure and enzyme electrophoresis

Leaf tissues were collected from nine natural populations of *G. littoralis* in Korea (Fig. 1). More than 30 plants (one leaf per plant) for eight populations except population JUN were

sampled from each population. The population JUN has totaling approximately 22 reproductive individuals in a 1.0 km^2. To avoid including individuals from the same rhizome, the distance between the selected individuals was about 2.0 m. The procedures for homogenization, starch gel electrophoresis, and enzyme assay were those described by Soltis et al.[4]. Leaves were homogenized by mechanical grinding to release enzymes from cell and organellar membranes, using a Tris-HCl grinding buffer-PVP solution. Electrophoresis was performed with an 11.0% starch gel. Ten enzyme systems were assayed: fluorescent esterase (FE, EC 3.1.1.-), superoxide dismutase (SOD, EC 1.15.1.1), and peroxidase (PER, EC 1.11.1.7) were resolved on System 9 of Soltis et al.[4]; glucose phosphate isomerase (PGI, EC 5.3.1.9), isocitrate dehydrogenase (IDH, EC 1.1.1.42), malic enzyme (ME, EC 1.1.1.40), 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.43), phosphoglucomutase (PGM, EC 2.7.5.1), and shikimate dehydrogenase (SKD, EC 1.1.1.25) on Soltis et al.'s System 10[4].

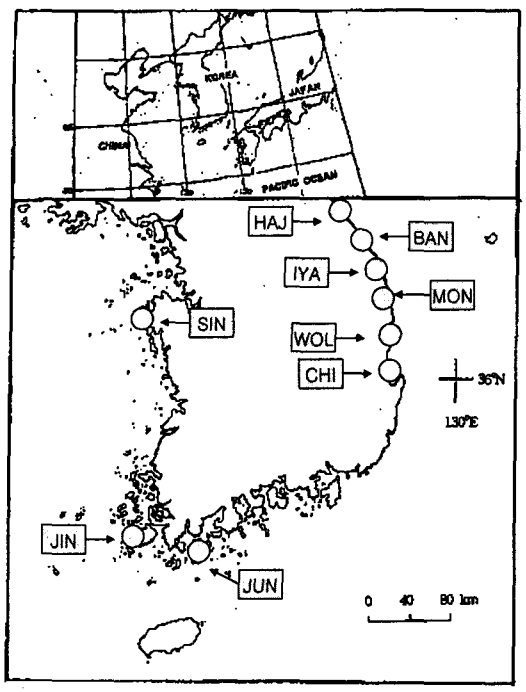

Fig. 1.Collection sites for populations of G. littoralis for isozyme analysis. HAJ: Hajin-po, Gosung-gun, Gangwoan-do BAN: Banam-ri, Geojin-up, Gangwoan-do IYA: Iyajin-ri, Gosung-gun, Gangwoan-do MON: Mongsang-dong, Donghae-ci, Gangwoan-do WOL: Wolsongjung, Uljin-gun, Gyeongsangbuk-do: CHI: Chil-po, Hunghae-up, Gyeongsangbuk-do; JUN: Jung-ri, Wondo-gun, Cheonlanam-do; JIN: Jindo, Jindo-gun, Cheonlanam-do SIN: Sindu-ri, Taean-gun, Chungcheongnam-do.

2. Data Analysis

Statistics of enzyme data was based on allele and genotype frequencies in each population. The following genetic parameters were calculated using a POPGENE computer program (version 1.31) developed by Yeh et al.[5]: the percentage polymorphic loci (Pp for population level and Ps for species level), mean number of alleles per locus (A), effective number of alleles per locus (Ae), and gene diversity (He)[6]. Species (indicated with the subscript s) and mean population (indicated with the subscript p) levels of genetic diversity were calculated as in Hartl and Clark[7]. Observed heterozygosity (Ho) was compared with Hardy-Weinberg expected values using Wright's fixation index (F) or inbreeding coefficients[8]. Deviations from genotype frequencies expected under the Hardy-Weinberg equilibrium were tested using the GENEPOP ver. 3.1 program[9]. Multiple tests were performed using the sequential Bonferroni procedure[10].

To elucidate the organization of the variation in G. littoralis, genetic variation was examined by partitioning of the total genetic diversity (Ht) to within (Hs) and among (Dst) population components using Nei's genetic diversity statistics[11]. A measure of differentiation among populations, relative to the total diversity was calculated at each locus as Gst = Dst / Ht. Weir and Cockerham's 12 estimates of Wright's Fst (Gst) were computed for variable loci with FSTAT ver. 1.2[13].

To elucidate the extent of genetic departure of populations from each other, Nei's genetic identity (I) and genetic distance (D) were calculated for each pairwise combination of populations[13].

The genetic structure within and among populations was also evaluated using Wright's F-statistics[8]: Fit, Fis, and Fst. Fit and Fis measure excesses of homozygotes or heterozygotes relative to panmictic expectations, within samples and within populations, respectively. Deviations of Fit and Fis from zero were tested using chi square-statistics[15]. Two indirect estimates of gene flow were calculated. Estimates of the number of migrants per generation (Nm) were based on Gst or the average frequency of private alleles (Nms), found in only one population[16]. Genetic diversity was tested against regions by Spearman rank to seek any correlation between genetic variation in the populations and environmental factors[17]. Correlation between geographical and genetic distances was tested using a modified Mantel's test[18].

Results

A high level of genetic variation was found in the nine G. littoralis populations. Tweleve of the 18 loci (66.7%) showed polymorphism in at least one population, while the remaining six loci (*Idh-1, Me-1, Per-1, Pgd-2, Pgi-1,* and *Pgm-1*) were monomorphic in all populations. An average of 42.0% of the loci was polymorphic within populations, with individual-

population values ranging from 16.7 to 61.1% (Table 1). The majority of the polymorphic loci expressed two(*Fe-2, Fe-3, Per-2, Skd, Sod-1,* and *Sod-2*) or three alleles (*Idh-2, Me-1, Pgd-1, Pgi2, and Pgm-2*), whereas the remaining one expressed four (*Fe-1*).

Table 1. Allozyme variation within nine populations of G. littoralis. Percentage of polymorphic loci (P), mean number of alleles per polymorphic population (Ap), mean number of alleles per locus (A), effective number of alleles per locus (Ae), observed heterozygosity (Hop), and Hardy-Weinberg expected heterozygosity or genetic diversity (Hep)

Pop.	PS	Pp	Ap	A	Ae	Hop (SD)	Hep (SD)
Eastern region							
HAJ[a]	34	50.0	2.44	1.72	1.31	0.083(0.012)	0.153(0.051)
BAN	30	50.0	2.56	1.78	1.30	0.095(0.013)	0.151(0.050)
IYA	30	33.3	2.17	1.39	1.15	0.045(0.009)	0.094(0.038)
MON	35	44.4	2.13	1.50	1.21	0.051(0.009)	0.122(0.041)
WOL	30	61.1	2.09	1.67	1.18	0.052(0.010)	0.118(0.035)
CHI	30	16.7	2.00	1.17	1.09	0.034(0.009)	0.057(0.028)
Southern region							
JUN	22	16.7	2.00	1.17	1.08	0.020(0.006)	0.050(0.029)
Western region							
JIN	30	50.0	2.56	1.78	1.35	0.074(0.011)	0.202(0.051)
SIN	30	55.6	2.60	1.89	1.42	0.107(0.013)	0.215(0.054)
Total mean		42.0	2.28	1.56	1.23	0.062	0.129
Species		66.7	2.58	2.06	1.26	-	0.159

[a]Abbreviation codes as in Figure 1. PS: Population sizes.

Across populations, the average number of alleles per locus (A) was 1.56, varying from 1.17 for the population with the lowest number of alleles to 1.89 for that with the highest number. The presence of fixed allele (Per-2-a) specific to population WOL allows for a molecular identification of the population based on allozymes. The effective numbers of alleles per locus at the species (Aes) and the population levels (Aep) were 1.26 and 1.23, respectively. Numbers of alleles per polymorphic locus (Ap) were 2.28 across populations, varying from 2.00 for the population with the lowest number of alleles to 2.56 for that with the highest number. Mean genetic diversity within populations was 0.129. Population SIN had the highest expected diversity (0.215), population JUN the lowest (0.050). In addition, genetic distance and geographic distance were highly correlated ($r = 0.58$, $p < 0.05$).

Fis, a measure of the deviation from random mating within the nine populations, was 0.515, ranging from 0.346 for Pgm-2 to 0.714 for Sod-1 (Table 2). The observed significant and positive Fis value (0.515) indicated a significant deficit of heterozygotes in the populations. Analysis of fixation indices, calculated for all polymorphic loci in each population, showed a slight deficiency of heterozygotes relative to Hardy-Weinberg expectations (Table 3). For example, 93.8% of fixation indices were positive (61/65), and 75.4% of those (49/61) departed significantly from zero ($p < 0.05$). Only four indices were negative and it was not significant.

Table 2. Estimates of genetic diversity statistics and 12 polymorphic loci in G. littoralis. Total genetic diversity (Ht), genetic diversity within populations (Hs), among populations (Dst), deviations of genotype frequencies from Hardy-Weinberg expectations over all populations (Fit), within individual population (Fis), and proportion of total genetic diversity partitioned among population (Gst)

Locus	Ht	Hs	Dst	Fis	Fit	Gst
Fe-1	0.508	0.402	0.106	0.512	0.613	0.208
Fe-2	0.015	0.014	0.001	0.464	0.496	0.060
Fe-3	0.131	0.122	0.009	0.656	0.680	0.070
Idh-2	0.119	0.103	0.016	0.557	0.615	0.131
Me-1	0.340	0.370	0.025	0.529	0.559	0.064
Per-2	0.056	0.050	0.006	0.466	0.520	0.100
Pgd-1	0.543	0.412	0.131	0.558	0.665	0.242
Pgi-2	0.295	0.284	0.014	0.408	0.431	0.039
Pgm-2	0.142	0.132	0.010	0.347	0.393	0.073
Skd	0.242	0.230	0.013	0.525	0.550	0.053
Sod-1	0.307	0.120	0.186	0.714	0.888	0.608
Sod-2	0.115	0.101	0.014	0.450	0.516	0.119
Mean	0.239	0.195	0.044	0.515	0.577	0.147

Table 3. Wright's fixation indices for nine populations of G. littoralis

Pop.	HAJ	BAN	IYA	MON	WOL
Fe-1	0.322	0.137	-	0.612***	0.691***
Fe-2	-	-	-	-	0.473*
Fe-3	0.716***	0.716***	-	-	0.636***
Idh-2	0.665***	-	-	-0.017	0.614***
Me-1	0.484**	0.357	0.467**	0.764***	0.523**
Per-2	0.523**	0.433*	-	-	-
Pgd-1	0.541***	0.336	0.759***	0.832***	-
Pgi-2	0.523**	0.841***	0.433*	0.357	0.292
Pgm-2	-	0.283	0.356	-0.017	1.000***
Skd	0.369*	0.523**	0.614***	0.453*	0.672***
Sod-1	-	-	-	0.636**	0.473*
Sod-2	-0.017	-	0.356	-	-

Pop.	CHI	JUN	JIN	SIN
Fe-1	-	-	0.597***	0.813***
Fe-2	-	-	-	-
Fe-3	-	-	0.473*	0.672***
Idh-2	-	-	0.641***	-
Me-1	-	0.716***	0.577***	0.606***
Per-2	-	-	-	-
Pgd-1	0.705***	0.621***	0.286	0.647***
Pgi-2	0.339	0.433*	0.243	0.486**
Pgm-2	-0.103	-	0.636***	0.517**
Skd	-	-	0.517*	0.563**
Sod-1	-	-	0.672***	0.853***
Sod-2	-	-	0.590**	0.517**

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

Total genetic diversity values (Ht) varied between 0.015 (*Fe-1*) and 0.543 (*Pgd-1*), for an average over all polymorphic loci of 0.239 (Table 2). Interlocus variation in the within-population genetic diversity (Hs) was low (0.195). On a per-locus basis, the proportion of total genetic variation due to differences among populations (Gst) ranged from 0.039 for Pgi-2 to 0.608 for Sod-1, with a mean of 0.145. This indicated that about 15% of the total allozyme variation was among populations. The estimate of gene flow, based on Gst, was slightly low among Korean populations of G. littoralis (Nm =

1.45). The other estimate based on the average frequency of 'rare' alleles found in only one population was also low (Nms = 1.06). Values of genetic distance (D) were below 0.084. Genetic-identity values among pairs of populations rangedfrom 0.942 to 0.996.

The designated hierarchical consisted of regions and populations as indicated in Table 4. In the hierarchy analysis, the greatest variance was exhibited among regions with respect to the total samples (Gst = 0.201). A large component of this value was explained by variation among regions with respect to the total (Gxy = 0.207), and this result consisted with the strong geographic effect indicated by phenogram.

Table 4. Hierarchical genetic differentiation in G. littoralis. Gst value combined cross loci

X	Y	Gst
Population	- Region	0.017
Population	- Total	0.145
Region	- Total	0.207

Discussion

G. littoralis maintain high levels of genetic diversity in populations than does the average plant species. For example, their genetic diversity of 0.159 (*G. littoralis*) are higher than that for species with a sexual and asexual reproduction mode (0.138), temperate-zone species (0.146), and short-lived herbaceous perennials (0.116)[19]. The percentages of polymorphic loci at the species level were 66.7% for G. littoralis. These values are also higher than average for species with a reproduction mode that is sexual and asexual (43.8%), short-lived herbaceous perennials (41.3%), and temperature-zone species (48.5%)[19]. These comparisons suggest that genetic diversity levels of *G. littoralis* are higher than those of plant species having similar life-history characteristics.

The relatively high level of genetic variation found in G. littoralis is consistent with several aspects of its biology. First, the breeding system of a species is an important determinant of variability at both the species and population levels. *G. littoralis* is bisexual and self-incompatible, apparently making it a species that is primarily animal-pollinated (e.g. Bombus spp.). Predominantly outcrossing species maintain higher levels of intrapopulation genetic variation than do predominantly inbreeding species [19]. Second, a perennial species such as *G. littoralis* generally maintains relatively higher levels of variation than do annuals[19]. Finally, the reproduction modes of *G. littoralis* play an important role in genetic variability. Vegetative reproduction and spread can affect the genetic structure of populations[20]. Cook contended that clonal growth could retard the loss of genetic diversity within populations[21]. *G. littoralis* can regenerate from fibrous roots when many plants are harvested by firewood or otherwise destroyed. Species with independent ramets could spread the risk of mortality, thereby reducing the probability of genet death and preserving genetic diversity[3]. Hartnett and Bazzaz have also argued that physiological independence among ramets may maintain genetic diversity by buffering clones against localized, patch-specific selection forces[22]. Sexual reproduction could enhance and maintain genetic variation[23]. *G. littoralis* reproduces by seed or by vegetative spread. Asexual reproduction assures the stabilization and persistence of a phenotype that is well adapted to the immediate environment[24].

The correlations between rainfall, altitude, latitude, and the shortest distance between populations were examined (data not shown). Genetic diversity versus other factors except latitude and the distances between populations did not show a significant correlation. A patterns of decreasing genetic diversity are observed with increasing latitude. From the results, we suppose that *G. littoralis* may be diffused from north regions into south regions.

Genetic differentiation among populations is principally a function of natural selection, genetic drift, and gene flow via pollen and seed dispersal[6]. For *G. littoralis*, about 14.5% of the total variation *G. littoralis* was due to differences among populations (Gst = 0.145). In contrast, the genetic variation in predominantly outcrossed wind-pollinated species averages <10% between populations[19].

The southern coastal populations of the Korean Peninsula are relatively small and maintained less genetic variation than the Eastern and Western populations. The uneven distribution of locality specific bands could be explained by isolation-by-distance and might reflect migration from populations to same region of the Korean Peninsula via sea current. It is unclear why the south coastal populations has not invaded regions of Gyungsangnam-do and why its distribution is ecologically restricted only in sandy dunes, sandy beaches or abandoned fields near the sea in East Asia.

Elavated extinctions risks can be clearly perceived in the population JUN of *G. littoralis*. Most populations have gradually destroyed a number of plants in the seaside population and eroded the site. The diminished genetic diversity found within these south populations is probably attribute to genetic drift. In subsequent generations, the loss of heterozygosity that results from genetic drift and inbreeding is likely to lead diminished fitness in these pitcher plants. Diminishing pitcher population sizes will inevitably lead to

elevated environmental, stochastic, and genetic extinction risks such as those facing the south populations. Every country in the world is playing more attention in selecting the best medicines from wild plants for treatment of cancer and cardiovascular diseases[25].

For a conservation perspective, transplant from regions to other regions is not a great help to conservation of the narrow distributed species but an action of destruction of habitats. The conservation of rare species requires consideration of ecological and genetic factor[26]. Ecological factors such as natural habitats may be of primary importance in the preservation of most populations, artificial transplants cannot play an important role in determining as rare or endemic species conservation. The level of distribution of genetic variation among populations is of primary importance to the conservation of genetic diversity and the evolutionary potential of species[19]. Based on the available data, such as relatively high Gst value, several populations of each group should be preserved, especially those with high variation, such as populations JIN and SIN. These populations could be used as a source of genetic diversity for the restoration of genetically poor populations. In addition, we recommend that a desirable conservation population should be included at least 30 plants per population because high genetic diversity is observed with increasing population sizes.

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