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Genetic Variation and Population Structure of *Crepidiastrum lanceolatum* (Compositae)

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Abstract *Crepidiastrum lanceolatum* (HOUTT.) NAKAI (Compositae) is distributed in East Asia including Korea. Genetic diversity and population structure of six *C. lanceolatum* populations in Korea and two populations in Japan were determined using genetic variation at 19 allozyme loci. The percent of polymorphic loci within the enzymes was 42.1%. Genetic diversity at the species level and at the population level was low (Hes = 0.077; Hep = 0.068, respectively), whereas the extent of the population divergence was relatively low (G_{ST} = 0.093). One of the most striking features of this study was the more significant difference within populations than among populations. An indirect estimate of the number of migrants per generation (*Nm* = 2.44) indicated that gene flow was moderate among eight populations of the species. In addition, analysis of fixation indices revealed a slight heterozygosity deficiency in some populations and at some loci. Narrow geographic ranges, short-lived perennial herbaceous, and small population sizes are mainly associated with the low level of genetic variation.

Key words: genetic diversity, population structure, narrow geographic range, *Crepidiastrum lanceolatum*

Introduction

Species rarely consist of a single panmictic population. Because of limited abilities for dispersal and habitat fragmentation, most species consist of subpopulations, among which genetic exchange is reduced. Because of random genetic drift, this may lead to genetic differentiation within a species. Sufficiently strong natural selection may produce similar patterns in the face of gene flow. When studying the geographic variability of a species, an accurate estimate of the level of gene flow is necessary for determinate of the relative importance of drift and selection as differentiation forces [6,7].

Endemic or narrow distributed species in an oceanic island has been considered as providing an excellent field for studying speciation and evolution. In oceanic islands, the endemic species should be evolved from ancestral species which migrated by long distance dispersal from adjacent continental areas [1]. Accordingly, ancestral stock would have maintained little genetic diversity and would have no genetic interconnection between the continental relatives [3,15,20]. Consequently, the mode of dispersal of ancestral groups would significantly affect their frequencies of establishment. For several flowering species, speciation patterns of endemic in oceanic islands have been intensively analyzed by enzyme electrophoresis [7,25]. As a general rule, geographically restricted or endemic species are known to contain fewer polymorphic loci and fewer alleles per polymorphic locus than widespread congeneric species [4,5]. However, there are few studies dealing with East Asian endemic or narrow distributed plants [8].

The genus *Crepidiastrum* consists of seven species to East Asia [10]. Only one species among this genus, *Crepidiastrum lanceolatum* (HOUTTO.) NAKAI, distributes in the southern part of islands in the Korean Peninsula. The distribution range of *C. lanceolatum* is restricted to small areas. The species is also found in the Southeast Asia such as Korea, Japan, Taiwan and the Republic of China [10]. A perennial herb with unbranched stems, *C. lanceolatum* occurs rocky slopes near seaside. *C. lanceolatum* is bisexual and outcrossing rates is not high [10]. Seed dispersal mechanisms of this species are unknown. Only two populations of *C. lanceolatum* on the Bonin (Ogasawara) Islands in Japan were studied by Ito and Ono [10]. However the genetic structure of natural populations of this species has not been studied enough. The purposes of this study were: 1) to estimate how much total genetic diversity is maintained in the species; 2) to describe how genetic variation is distributed within and among populations; 3) to assess genetic structure of *C. lanceolatum*, and 4) whether narrow distributed species is typically less polymorphic than the general

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widespread species with similar life characteristics.

Materials and Methods

Sampling procedure

Leaves of *Crepidastrum lanceolatum* (Compositae) were collected from six natural populations representing different habitats in Korea (populations 3-7, and 8) and two Japanese populations (populations 1 and 2) (Fig. 1). One leaf per plant was sampled during the period from May 1998 to June 1999. The distance between the selected individuals was about 1 m in order to avoid including individuals with common lineage. Twenty-eight to thirty-two plants were collected from each population. Leaves gathered from natural populations, placed in zip-lock bags, and transported to the laboratory on an ice.

Enzyme electrophoresis

Homogenization, starch gel electrophoresis, and enzyme assay procedures were followed according to the methods of Soltis et al. [12]. Leaves were pulverized under liquid nitrogen using a mortar and pestle. An extraction buffer (Tris-HCl grinding buffer-PVP solution) was added to the leaf powder to soluble and stabilize the enzymes [12]. Electrophoresis was performed using 10% starch gels and total of ten enzymes were assayed for this study; acotinase (ACO), gerceroaldehyde-3-phosphate dehydrogenase (G3P), glucose phosphate isomerase (GPI), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), octanol dehydrogenase (ODH), 6-phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM), and shikimate dehydrogenase (SKD).

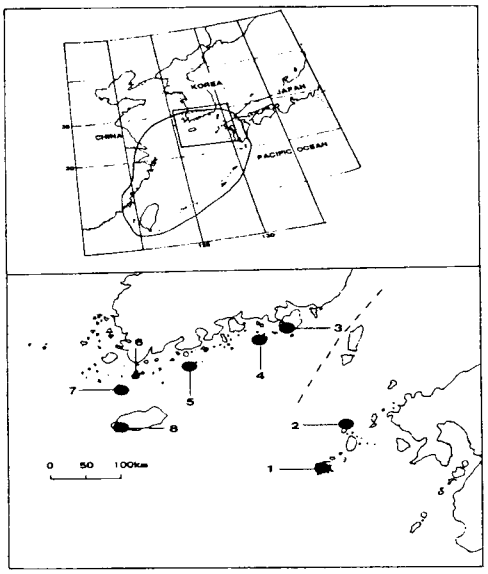

Fig. 1. Collection localities for populations of *C. lanceolatum* as source for allozyme analysis. Closed line (upper figure) is the geographical distributions of *C. lanceolatum*. 1: Fukue Isl., Nagasaki Pref.; 2: Goto Isl., Sasebo Pref.; 3: Kawng Isl., Kaeje Pref.; 4: Gal Isl., Younckgi Prof.; 5: Kaemoon Isl., Yecheon Prof.; 6: Pogil Isl., Yando Prof.; 7: Hachuja Isl., Cheju Prof.; 8: Daecheong, Cheju Prof.

For enzymes resolving in more than one zone of activity, the most anodal isozyme was arbitrarily designated '1' and subsequent isozymes were sequentially assigned higher numbers. Likewise, alleles were designated sequentially with the most anodally migrating allozyme designated 'a' and progressively slower forms 'b', 'c', and so on.

Analysis of data

Allozyme diversity was calculated for the species as a whole and on a population basis using five standard genetic parameters; percentage of polymorphic loci (P), mean number of alleles per locus (A), mean number of alleles per polymorphic locus (Ap), effective number of alleles per locus (Ae), and gene diversity (He) [9. Subscripts refer to species (s) or population (p) level parameters. Observed heterozygosity (Ho) was compared with Hardy-Weinberg expected value using Wright's fixation index (F) or inbreeding coefficients [27]. These indices were tested for deviation from zero by χ^2-statistics following Li and Horvitz [13]. Nei's gene diversity formulae (H_T, H_S, D_{ST}, and G_{ST}) were used to evaluate the distribution of genetic diversity within and among populations [17,18]. The G_{ST} coefficient, in particular, estimates relative population differentiation. In addition, χ^2-statistics were used to detect significant differences in allele frequencies among populations for each locus [26]. Nei's genetic identity (I) was calculated for each pairwise combination of populations [17]. We used the PC-SAS program [21] to conduct a cluster analysis on genetic distances via the unweighted pairwise group's method arithmetic average (UPGMA). The genetic structure within and among populations was also evaluated using Wright's (1965) F-statistics: F_{IT}, F_{IS}, and G_{ST}. The F_{IT} and F_{IS} coefficients measure excesses of homozygote or heterozygote relative to the panmictic expectations within the entire samples and within populations, respectively. Deviations of F_{IT} and F_{IS} from zero were tested using χ^2-statistics [13]. Indirect estimates of gene flow *Nm* (the number of migrants per generation) was based on G_{ST} [22,28].

Results

Eight of the 19 loci (42.1%) showed detectable polymorphism in at least one population (Table 1). The remaining eleven loci (*Aco-2*, *G3p*, *Gpi-1*, *Gpi-2*, *Odh*, *Idh-1*, *Mdh-1*, *Mdh-2*, *Lap*, *Pgm-1*, and *Skd-1*) were monomorphic in all populations. An average of 25.7% of the loci was polymorphic within populations, with individual population values ranging from 10.5% to 42.1%. The majority of the polymorphic loci expressed two (*Aco-1*, *Pgd-1*, *Pgd-2*, *Pgm-2*, and *Skd-2*) and only one did three alleles (*Idh-2*). The average number of alleles per locus (A) was 1.30 across populations, varying from 1.16 for the population with the lowest number

Table 1. Allozyme variation within eight populations of *C. lanceolatum*

Pop[a].	N[b]	P	Ap	A	Ae	Hop	Hep
1	29	31.58	2.17	1.37	1.22	0.058	0.122
2	28	36.84	2.14	1.42	1.21	0.056	0.122
3	28	21.05	2.00	1.21	1.07	0.027	0.049
4	29	26.32	2.00	1.26	1.10	0.030	0.067
5	29	42.11	2.25	1.53	1.15	0.054	0.097
6	29	21.05	2.25	1.26	1.04	0.023	0.031
7	32	15.79	2.00	1.16	1.05	0.021	0.036
8	28	10.53	2.50	1.16	1.02	0.013	0.018
Mean		25.66	2.16	1.30	1.11	0.035	0.068
SD		3.45	0.17	0.13	0.08	0.003	0.011
Species		42.11	2.25	1.53	1.11	-	0.077

Percentage of polymorphic loci (P), mean number of alleles per polymorphic locus (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). a: Abbreviation codes as in Fig. 1. b: Number of individuals in the sample.

of alleles and 1.53 for the population with the highest number of alleles. The effective number of alleles per locus (Ae) was same at the species and the population level (Aes = 1.11; Aep = 1.11). The mean genetic diversity within populations was 0.068. Genetic diversity at the species level is 0.077. In addition, the correlation between genetic distance and geographic distance was high (r = 0.61), and indicated that geographically close populations trended to be genetically similar and about 63% ($1 - r^2$) of the variation in genetic distance was caused by unknown other factors than distance.

F_{IS}, a measure of the deviation from random mating within the eight populations, was 0.478, and ranged from 0.125 for *Pgd-2* to 1.000 for *Skd-2* (Table 2). The observed significant and positive F_{IS} value (0.478) indicates that there was 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, 74.4% of fixation indices were positive (29/39), and 27 of those departed significantly from zero ($p < 0.05$). Only three of indices were negative, indicating an excess of heterozygotes at *Pgd-2* (populations 2 and 6) and *Gpi-3* (population 6), no one was departed significant from zero. Total genetic diversity values (H_T) varied between 0.087 (*Aco-1*) and 0.236 (*Gpi-3*), giving an average overall polymorphic loci of 0.184. The interlocus variation of genetic diversity in within populations (H_S) was low (0.164). On a per locus basis, the proportion of total genetic variation due to differences among populations (G_{ST}) ranged from 0.037 for *Pgd-2* to 0.153 for *Pgd-1* with a mean of 0.093, indicating that about 9.3% of the total allozyme variation was among populations. The mean estimate of gene flow based on G_{ST} was 2.44 among Korean populations of *C. lanceolatum* (*Nm* = 2.44). Values of genetic distance (*D*) were below 0.009 (Table 3). Genetic identity value among pairs of population was 0.991. The similarity among *C. lanceolatum* populations can be seen in the UPGMA dendrogram, where total populations cluster at below a genetic distance of 0.034 (Fig. 2).

Table 2. Estimates of genetic diversity statistics and 13 polymorphic loci in *C. lanceolatum*

Locus	H_T	H_S	D_{ST}	F_{IS}	F_{IT}	G_{ST}
Aco-1	0.087	0.075	0.012	0.478	0.551	0.140
Pgd-1	0.290	0.245	0.044	0.449	0.533	0.153
Pgd-2	0.026	0.025	0.001	0.125	0.157	0.037
Gpi-3	0.336	0.288	0.048	0.378	0.467	0.143
Pgm-2	0.112	0.105	0.007	0.454	0.488	0.063
Mdh-3	0.201	0.183	0.018	0.477	0.523	0.088
Idh-2	0.330	0.305	0.025	0.463	0.503	0.076
Skd-2	0.091	0.087	0.004	1.000	1.000	0.044
Mean	0.184	0.164	0.020	0.478	0.528	0.093

Total genetic diversity (H_T), genetic diversity within populations (H_S), among populations (D_{ST}), deviations of genotype frequencies from Hardy-Weinberg expectations over all populations (F_{IT}), within individual populations (F_{IS}), and proportion of total genetic diversity partitioned among populations (G_{ST}).

Discussion

Genetic diversity trends to be low in narrow distributed plant species [24] and *Crepidiastrum lanceolatum* is no exception. Namely, genetic diversity of *C. lanceolatum* (Hes = 0.077) is slightly lower than that of temperate-tropical species (0.170), sexual reproduction mode (0.151), outcrossing-animal (0.167), and short-lived perennial herbaceous (0.116) [5]. The percentage of polymorphic loci at species level was 42.1%. The value is lower than species with a reproduction mode that is sexual (51.6%) and temperature- tropical species (58.8%), but it is similar to species with endemic geographic ranges (40.0%) and short-lived perennial herbaceous (41.3%) [5]. The average number of alleles per locus was 1.30; this value is lower than that of species with a reproduction mode that is sexual (2.00) and short-lived perennial herbaceous species (1.70), temperate- tropical species (2.53), outcrossing-animal (1.99), and endemic geographic ranges (1.80) [5]. The same trend is observed at the population levels.

Several studies have been done on the genetic diversity of narrow distributed plants in some oceanic islands [10,24]. The common remarkable point throughout these studies was that little genetic diversity was found both within and between species in spite of their morphological diversity [2,6,10,15,25]. Above all things, geographic range has been shown to be strongly associated with the level of variation maintained within populations and at the species level [5]. The low genetic variation found with in *C. lanceolatum* as a species and low level of population differentiation suggest that dispersal into its present range may have occurred following a genetic bottleneck, since reductions in population

Table 3. Wright's fixation indices for eight populations of *C. lanceolatum*

Pop.	*Aco-1*	*Pgd-1*	*Pgd-2*	*Gpi-3*	*Pgm-2*	*Mdh-2*	*Idh-2*	*Skd-2*
1	0.429*	0.418*	-	0.319	-	0.611**	0.694**	1.000***
2	0.417*	0.533**	-0.021	0.448*	-	0.570**	0.613**	1.000***
3	-	0.439*	-	-	0.512*	0.272	0.615**	-
4	-	0.491**	-	0.375*	-	0.616***	0.472*	1.000***
5	1.000***	0.417	-0.054	0.435*	0.205	0.433*	0.504**	1.000***
6	-	-	-	-0.073	0.636***	-	-0.105	1.000***
7	-	-	-	0.532**	-	-0.036	0.356	-
8	-	-	-	-	0.523	-	-0.051	-

*= P<0.05; **= P<0.01; ***= P<0.001.

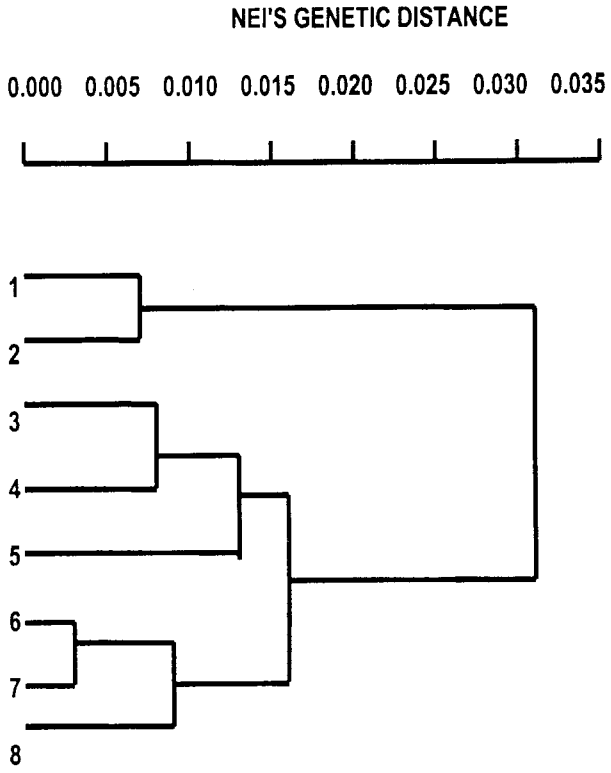

Fig. 2. A dendrogram showing the genetic relationships among the eight populations of *C. lanceolatum*, based on genetic distance data.

size and numbers across its range would be expected to fix different alleles in different population from a richer gene pool [4]. Witter and Carr [25] as well as Lowley and Crawford [4] have been also explained that the bottleneck effect is responsible for the low values of heterozygosity found in insular plants. However, the low genetic diversity of *C. lanceolatum* will be caused by small population size at the present time as well as by bottleneck event in the past [10]. In the Japanese populations of *Crepidiastrum*, total number of individuals of each population is small and their habitats are restricted due mainly to the grazing pressure by naturalized goats. Most Korean populations of *C. lanceolatum* are also small and distributed with tractors used for fishing. Under the neutral theory, estimate of effective population size (*Ne*) × mutation rate (*v*) using estimated total heterozygosity (*H*) is as followed: $Ne \times v = H/4(1-H)$. If *v* is an estimate of electrophoretically detectable neutral mutation rate, it is 1.3×10^{-7} [11,12,14]. The estimate values of *Ne* ranged from 2.5×10^4 individuals for population 7 to from 1.2×10^5 individuals for population 1. Total numbers of individuals for each population are estimated being lower than 2.0×10^4 for population 7 and 1.1×10^4 for population 1. The effective population size is usually smaller than the actual population size. Thus the low genetic diversity of this species can be explained by their small number of whole populations in the present time without postulating the bottleneck event in the past. Thus one should be careful when discussing the bottleneck effect of island species [10].

One of the most striking features of this study was the more significant difference within populations than among populations. When the sexual reproduction and outcrossed mating systems of *C. lanceolatum* are taken into account, the mean identity value of 0.991 among ten populations is higher than that expected for cogeneric species [4]. This high value is not especially surprising when viewed on the narrow geographic area over which the *C. lanceolatum* collections were made. Genetic differentiation among populations is principally a function of gene flow among population via pollen and seed dispersal [14]. Of the total variation observed in *C. lanceolatum* is due to differences among populations (G_{ST}=0.093). This low level of genetic differentiation also suggests that gene flow among population is moderate (*Nm*=2.44). Mean genetic identity between populations is somewhat low above (I=0.983). It is unclear how the populations are genetically homogeneous. It is highly probable that directional toward genetic uniformity in a relatively homogeneous habitat (i.e. rocky slope near sea, islands, and a little swampy land) is thought to be operated among populations of *C. lanceolatum*. In addition, there is another possibility that *C. lanceolatum* may be evolved relatively recent from an ancestor species.

The phylogenetic tree shown in Fig. 2 clearly distinguishes two clades, the Japanese and Korean clades. The positions of the populations except population 5 in the tree almost completely match the corresponding geographical positions. The correlation between genetic distance and geographical distance is relatively high in Korea and Japan.

Judging from the present distribution of the populations, the diffusion of *C. lanceolatum* seems to be by sea current. However, the hypothesis of the diffusion of this species by sea currents needs to be tested by future work.

Heterozygote deficiency becomes an eyesore (F_{IS}=0.478). If the number of individuals is a few due to limited numbers of founders, genetic drift after colonization, or the differential survival and spread of genotypes, populations of endemic species could consist of a few genotypes [16]. In addition, this 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 probably that the combination of these factors may contribute to heterozygote deficiencies within populations.

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