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Levels and Partitioning of Genetic Diversity of *Camellia japonica* (Theaceae) in Korea and Japan

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Abstract

We analyzed allozymes from 620 trees to estimate the levels of allozyme diversity within and genetic differentiation among populations of *Camellia japonica* in Korea and Japan. Both at the population and species levels, *C. japonica* (mean expected heterozygosity, H_e = 0.169 and 0.190) maintains high levels of genetic diversity. Although Korean populations of the species are located in edge of its distribution, they (0.209) harbor higher levels of genetic diversity than that found within populations in Japan (0.145). The mean G_{ST} value among 16 populations (0.091) of *C. japonica* was similar to those among six Korean (0.055) and 10 Japanese populations (0.086). The results indicate that a low degree of allozyme differentiation between populations in Japan and Korea, though the land connection between the southern Korean peninsula and southern Japanese archipelagos no longer existed after the middle Pleistocene. There was no significant correlation between genetic distance and geographic distance, indicating that isolation by distance may not be a primary factor for shaping genetic structure among populations. Instead, genetic diversity and structure in populations of *C. japonica* may result from the balance between occasional gene flow and genetic drift.

Key words: Allozyme, *Camellia japonica,* east Asia, gene glow, genetic differentiation.

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Introduction

The coastal forests of northeast Asia possess a unique suite of broad-leaved evergreen woody species such as *Camellia japonica* L., *Castanopsis* spp., *Eurya* spp., *Ficus* spp., *Ligustrum* spp., *Litsea* spp., *Neolitsea* spp., *Persea* spp., *Quercus* spp., etc. Since these species are important members of coastal forest vegetation in Japan and Korea (NUMATA, 1974), population studies such as population dynamics (e.g., YAMAMOTO, 1992; SATO *et al.,* 1994; TANOUCHI *et al.,* 1994), pollination ecology (e.g., YUMOTO, 1987), allozyme variation in local populations (e.g., WENDEL and PARKS, 1985; CHUNG and KANG, 1994, 1996), and population genetic structure (e.g., CHUNG and EPPERSON, 2000) have been conducted.

During the Ice Age (the glacial Würm), the Sea of Japan (East Sea) and Yellow Sea were about 100 m lower than at present and a land connection existed between Korea and Japan (KIM and HONG, 1991). However, little is known about the degree of genetic differentiation among plant populations extending over Korea and the Islands of Japan (e.g., M. G. CHUNG and M. Y. CHUNG, 2000a). The distribution of genetic variation among populations is of primary importance to the conservation of genetic diversity of a plant species (HAMRICK *et al.,* 1991).

Camellia japonica is widely distributed in Japan (Honshu, Shikoku, and Kyushu) and the southern Korean peninsula. *Camellia japonica* usually occurs in old-growth forests on several islands near the southern coast of the regions and coexists *Eurya japonica, Persea thunbergii, Neolitsea sericea,* and *Cinnamonum insularimontanum,* etc. *Camellia japonica*

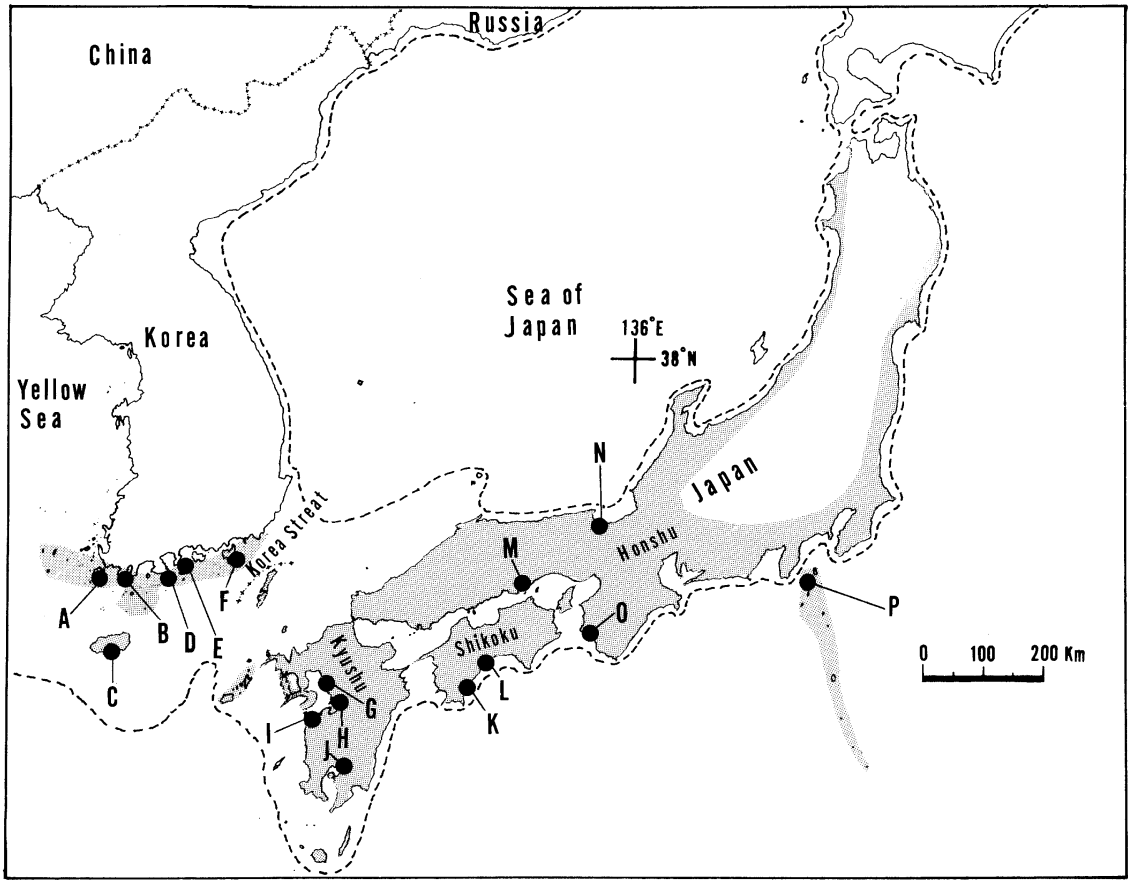

Fig. 1. – The geographical distribution (dotted area) and the location of 16 sampled populations of *Camellia japonica* (alphabetic codes as in *Table 1*) in Korea and Japan. Broken line indicates a coastal line or land connection in the middle Pleistocene.

flowers in late January through early March. The flowers have red, tough petals, elevated several whorl of fused stamens, and a dilute nectar, and are pollinated by birds and syrphid flies (YUMOTO, 1987; M. G. CHUNG, pers. obs.). Adults usually produce small number of fruits (on the order of tens), each of which contains one to three large seeds (ca. 1.3 cm long). It appears that most seeds simply fall right underneath maternal plants, and that there are no specialized mechanisms for primary or secondary seed dispersal (M. G. CHUNG, pers. obs.).

Although *C. japonica* is abundant in Japan in its range, some populations are relatively small and isolated. In Korea, *C. japonica* occurs on several islands near the southern coast of the southern sections (*Fig. 1*). The stumps of the species have been planted as one of the major ornamental trees in Korean and Japanese gardens. Unfortunately, it has been observed that the natural habitats of Korean *C. japonica* near the sea have been severely damaged by road construction. For these reasons, the Korean government has designated nine natural populations as natural monuments in Korea to preserve their natural habitats.

In general, marginal and isolated populations maintain less genetic diversity than continuously distributed populations (e.g., GODT and HAMRICK, 1996; GEMMILL *et al.,* 1998). Thus, it is predicted that more widely distributed populations in Japan would harbor higher levels of genetic diversity than the marginal, isolated island populations in Korea. Recently, allozymes of *C. japonica* have been studied within populations in Japan (WENDEL and PARKS, 1985) and Korea (CHUNG and KANG, 1996), separately, by two different research groups. For this reason, it is difficult to directly compare allozyme diversity in populations and allozyme differentiation among populations extending over Korea and Japan due to the differences in methods and sample size (KARRON, 1987).

Here, we report levels and partitioning of *C. japonica* in Korea and Japan, hoping that the results of this study could be used to provide baseline information for the development of conservation strategies for the species in the near future.

Materials and Methods

A total of 620 individuals from 16 populations of *C. japonica* were sampled from Korea and Japan (*Fig. 1*). Individuals were collected randomly, regardless of their size and age. On average, each 500 m^2 area was defined as a population. Leaves were kept on ice, transported to the laboratory, and stored at 4°C until protein extractions.

The leaf material was finely cut, and crushed with a mortar and pestle. A phosphate-polyvinylpyrrolidone extraction buffer (MITTON *et al.,* 1979) was added and the crushed extract was absorbed onto 4-mm X 6-mm wicks cut from Whatman 3MM chromatography paper, which were stored at –70°C until needed for analysis. Electrophoresis was performed using 10% starch gels. Fifteen putative loci for *C. japonica* from ten enzyme systems were resolved using a Poulik buffer system, a modification (HAUFLER, 1985) of SOLTIS *et al.* (1983) system 6. These were alcohol dehydrogenase (*Adh*), leucine aminopeptidase (*Lap-1, Lap-2*), and triosephosphate isomerase (*Tpi-1, Tpi-2, Tpi-3*). A discontinuous histidine citrate buffer system 1 (SOLTIS *et al.,* 1983) resolved fructose-1, 6-diphosphatase (*F1,6*). Buffer system 11 (SOLTIS *et al.,* 1983) resolved isocitrate dehy-

drogenase (*Idh*) and phosphoglucomutase (*Pgm-1, Pgm-2*). A morpholine citrate buffer system by CLAYTON and TRETIAK (1972) was used to resolve formate dehydrogenase (*Fdh*), phosphoglucoisomerase (*Pgi-1, Pgi-2*), and peroxidase (*Per-1, Per-2*). Stain recipes were taken from SOLTIS *et al.* (1983), except diaphorase (CHELIAK and PITEL, 1984) and formate dehydrogenase (WENDEL and WEEDEN, 1989). Putative loci were designated sequentially, with the most anodally migrating isozyme designated 1, the next 2, and so on. Similarly, alleles were designated sequentially with the most anodally migrating alleles designated superscript a. Although the genetic bases of the loci were not documented by controlled crosses, the isozymes expressed phenotypes that were consistent in subunit structure and genetic interpretation with other isozyme studies in plants, as documented by WEEDEN and WENDEL (1989).

For the analysis of allozyme diversity, a locus was considered polymorphic if two or more alleles were detected, regardless of their frequencies. Five standard genetic diversity parameters were estimated using a computer program developed by M. D. LOVELESS and A. SCHNABEL (pers. comm.): percent polymorphic loci (*%P*), mean number of alleles per polymorphic locus (*AP*), mean number of alleles per locus (*A*), effective number of alleles per locus (A_e), and gene diversity (H_e). The variation within population data is confounded by overall levels of variation in the species as well as how this variation is distributed within and among the populations (BERG and HAMRICK, 1997). Thus, it is informative to present summary data for the species as a whole as well as within population data. Subscripts refer to species (S) or population (P) level parameters.

Total genetic diversity (H_T), genetic diversity within populations (H_S), and the proportion of genetic diversity found among populations (G_{ST}) were calculated for all populations and for populations in Japan and Korea following NEI's (1973, 1977) genetic diversity formulae. A chi-square test was used to detect significant differences in allele frequencies among populations for each locus (WORKMAN and NISWANDER, 1970).

Genetic divergence among populations was also estimated by calculating NEI's (1972) genetic identity and distance for all pairs of populations. In addition, we used NTSYS (ROHLF, 1988) to conduct cluster analysis on genetic distances via the unweighted pairwise groups method using arithmetic average (UPGMA).

Two indirect estimates of historical gene flow were calculated. One estimate of *Nm* (the number of migrants per generation) was obtained using WEIGHT's (1951) equation: $Nm = (1\text{-}F_{ST})/4F_{ST}$, F_{ST} is equivalent to G_{ST} as calculated in this study. The second estimate was based on the average frequency of „private" alleles (SLATKIN, 1985; BARTON and SLATKIN, 1986).

Results

The *Lap-2* was being expressed, but it was not scored because of poor activity and/or resolution. The *F1,6* and *Pgi-1* were monomorphic in all populations sampled ($\%P_S$ = 86.7%). Mean values of A_S, AP_S, A_{eS}, and H_{eS} were 4.00, 3.60, 1.37, and 0.190, respectively (*Table 1*). High levels of allozyme variation were also observed within populations: $\%P_P$, A_P, AP_P, A_{eP}, and H_{eP} were 57.1%, 3.03, 2.16, 1.35, and 0.169, respectively (*Table 1*). Populations in Korea harbor significantly higher levels of genetic diversity (H_{ep}) than those in Japan (KRUSKAL-WALLIS, H = -2.440, P = 0.015). The lower levels of H_{eP} (less than 0.1) were found in populations I (0.091), N (0.098), and P (0.082) in Japan. Mean expected heterozygosity was not significantly correlated with sample size (SPEARMAN's rank-correlation coefficient, r_s = 0.097, P = 0.719).

Significant differences in allele frequencies among populations were found for all 13 loci ($P < 0.001$ in each case) (*Table 2*). The mean G_{ST} value 0.091 and overall, about 91% of the total variation in *C. japonica* is common to all populations (*Table 2*). Five private alleles (alleles detected in only one population, with a mean frequency of 0.031) were detected across all populations. It is worth noting that the mean G_{ST} value among 16 populations was similar to those among six Korean (0.055) and 10 Japanese (0.086) populations (*Table 2*). Indirect estimates of gene flow (*Nm*) based on the mean G_{ST} and private alleles were 2.51 and 3.02, respectively.

Average genetic identity for all pairs of populations was 0.941 ± 0.005[SE], well within the range of values expected for conspecific populations (CRAWFORD, 1989). The UPGMA phenogram gave few insights into the genetic structuring of the 16 populations of *C. japonica* (*Fig. 2*). The MANTEL-Z test showed no significant correlation between genetic distance and geographic distance (r = 0.321, $P >> 0.05$) and indicated that about 90% of the variation in the genetic distances was due to unknown factors other than distance. Data on allele frequencies are too lengthy to include here, but the data are available upon request from the senior author.

Discussion

At both population and species levels, *C. japonica* maintains higher levels of allozyme variation than most other long-lived, woody angiosperms (45% and 60% polymorphic loci, 1.68 and 2.10 alleles per locus, 1.20 and 1.26 effective alleles per locus, and 0.143 and 0.183 expected heterozygosity, respectively, HAMRICK *et al.,* 1992).

The broad-leaved evergreen woody species native to the southern Korean peninsula and Japan have been present in these regions from pre-Pleistocene times presumably from southern China (KONG and WATTS, 1993). As glaciation in these regions was restricted to the northernmost mountains of Korea and northern and central Japan (above 2,000 m above sea level), the broad-leaved evergreen trees were not directly affected by the glaciation (KONG and WATTS, 1993). This statement may be true of the fact that marginal populations in the southern Korea also harbor high levels of genetic diversity within populations. For *C. japonica*, levels of allozyme diversity within populations are not correlated with geographic boundaries. The paleoecology of this region would be one factor for maintaining allozyme diversity in populations of *C. japonica*. WENDEL and PARKS (1985) suggested that factors contributing to the high levels of genetic diversity found within populations of *C. japonica* would be large, abundant populations in many places, long distance pollen dispersal by birds, *Zosterops japonica* TEMMINCK & SCHLEGEL, self- incompatible breeding systems and high outcrossing rates (ca. 98%, WENDEL and PARKS, 1985), and long generation times (opportunities for the accumulation of mutations should be high, LEDIG, 1986). Finally, our recent study of genetic differentiation among seven age classes in a population (833 trees in a 60-m X 100-m area) of the species reveals that „reproductive gene pools" vary among reproductive episodes, thus, may lead to the differences in temporal genetic structure in populations. These genetically different cohorts established over time have played an important role in maintaining genetic diversity within populations of *C. japonica* (M. G. CHUNG and B. K. EPPERSON, unpubl. data). Coupled with the biological and ecological characteristics of *C. japonica* (e.g., long generation time, high outcrossing rates, and abundance), high levels of genetic diversity accumulated from generation to generation have been maintained in metapopulations of *C. japonica*. Why do Korean populations have more

average heterozygosity than Japanese ones? The sampling of the populations was done in a similar manner (i.e., a random collection was made, regardless trees' size and age). Estimates of the genetic diversity (e.g., expected heterozygosity) were very similar among seven age classes in a population (60-m X 100-m area) of *C. japonica* (M. G. CHUNG and B. K. EPPERSON, unpubl. data). The fruits have been collected to obtain oil from seeds for several hundred years in Japan (K. INOUE, pers. comm. of Shinshu University, Japan). Considering these, the populations in Korea and Japan would be different in some

Table 1. – Estimates of genetic variation over 13 loci within populations of *Camellia japonica*[a]).

Pop	*N*	*%P*	*AP*	*A*	A_e	H_o (SE)	H_e (SE)
Korean populations:							
A	32	60.00	3.00	2.20	1.43	0.138 (0.016)	0.214 (0.060)
B	50	80.00	2.83	2.47	1.49	0.151 (0.013)	0.235 (0.058)
C	25	46.67	3.00	1.93	1.35	0.062 (0.016)	0.151 (0.060)
D	23	60.00	2.89	2.13	1.43	0.099 (0.016)	0.204 (0.058)
E	49	73.33	3.36	2.73	1.48	0.143 (0.013)	0.227 (0.063)
F	50	66.67	3.20	2.47	1.52	0.163 (0.013)	0.222 (0.066)
Mean	38.2	64.44	3.02	2.32	1.45	0.126 (0.006)	0.209 (0.025)
Japanese populations:							
G	45	53.33	3.38	2.27	1.54	0.141 (0.013)	0.165 (0.065)
H	40	53.33	3.50	2.33	1.31	0.129 (0.014)	0.176 (0.055)
I	46	53.33	2.50	1.80	1.14	0.060 (0.009)	0.091 (0.039)
J	25	73.33	2.91	2.40	1.40	0.097 (0.017)	0.208 (0.057)
K	25	33.33	3.40	1.80	1.28	0.116 (0.021)	0.137 (0.058)
L	31	60.00	2.89	2.13	1.35	0.095 (0.014)	0.167 (0.060)
M	50	60.00	3.44	2.47	1.30	0.124 (0.012)	0.167 (0.054)
N	49	60.00	3.00	2.20	1.14	0.080 (0.010)	0.098 (0.036)
O	30	53.33	2.63	1.87	1.32	0.133 (0.016)	0.161 (0.059)
P	50	26.67	2.50	1.40	1.17	0.072 (0.009)	0.082 (0.049)
Mean	39.1	52.67	3.01	2.07	1.30	0.105 (0.004)	0.145 (0.017)
Population level mean:							
	38.8	57.08	3.03	2.16	1.35	0.113 (0.004)	0.169 (0.014)
Overall (species) level mean:							
	620	86.67	4.00	3.60	1.37		0.190

[a]) Abbreviations: Pop, population code; *N*, sample size; *%P*, percentage of polymorphic loci; *AP*, mean number of alleles per polymorphic locus; *A*, mean number of alleles per locus; A_e, effective number of alleles per locus; H_o, observed heterozygosity; H_e, HARDY-WEINBERG expected heterozygosity or genetic diversity; SE, standard error.

Table 2. – NEI's (1973, 1977) statistics of genetic diversity for *Camellia japonica.* Values presented are means over all polymorphic loci[a]).

Number of loci	H_T	H_S	G_{ST}	Significance	Number of private alleles (mean frequency)
All populations:					
13	0.220	0.194	0.091	13 < 0.001	5 (0.031)
Korean populations:					
12	0.295	0.274	0.055	9 < 0.01	4 (0.014)
Japanese populations:					
13	0.180	0.160	0.086	12 < 0.05	6 (0.026)

[a]) Abbreviations: H_T, total genetic diversity; H_S, genetic diversity within populations; G_{ST}, proportion of the total genetic diversity partitioned among populations.

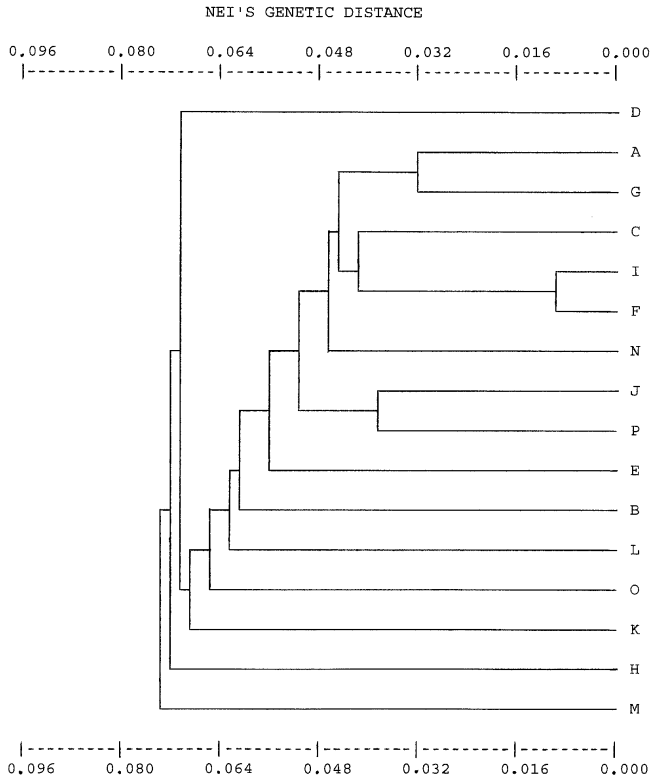

Fig. 2. – Dendrogram from UPGMA cluster analysis based on NEI's (1972) genetic distances between the 16 populations of *Camellia japonica*.

unknown factors (e. g., the history of populations, different cohorts within a local population, different fine- scale genetic mosaics or a different interaction between gene flow and genetic drift in local populations). It is worth noting that, unlike *C. japonica*, there was no significant difference in average heterozygosity between Japanese (H_{ep} = 0.258, 15 populations) and Korean populations (H_{ep} = 0.279, five populations) for *Eurya japonica* THUNB. (Theaceae) (M. G. CHUNG and M. Y. CHUNG, 2000a).

Based on a recent review of allozyme literature on long-lived woody species (HAMRICK *et al.,* 1992), patterns of geographic range and regional distribution significantly affect population differentiation. For example, long-lived woody species with continuously regional and widespread geographic range should have less genetic divergence among populations than species with narrow and endemic distributions. The mean G_{ST} value based on 73 woody angiosperms was 0.102 (HAMRICK *et al.,* 1992). The mean value was higher than those of *C. japonica* in Korea and Japan. This level of genetic differentiation also suggests that gene flow among populations has been high. Indirect gene flow estimates of *Nm* based on the mean G_{ST} value (2.51) and mean frequencies of private alleles (3.02) were high. For neutral genes, below *Nm* = 1 genetic drift is the predominant factor affecting population structure, whereas above *Nm* = 4 gene flow replaces genetic drift (HARTL and CLARK, 1989). The UPGMA phenogram and the MANTEL-Z test showed no significant correlation between genetic distance and geographic distance, indicating that isolation by distance may not a primary factor for shaping genetic structure among populations of *C. japonica*. The considerable low levels of genetic diversity found in three Japanese populations I, N, and, P may indicate that these populations have been affected more importantly by genetic drift. Thus, it is highly probable that genetic diversity and structure in populations of *C. japonica* may result from the balance between occasional gene flow and genetic drift. The northeast Asia has been frequently hit by typhoons, which can carry a lot of things between southern Korea and Japan. In addition, a probable long distance pollen dispersal by birds may in part be factors for maintaining low allozyme differentiation between populations in the southern Korea and Japan.

The mean G_{ST} value among 16 populations of *C. japonica* was similar to those among six Korean and 10 Japanese populations. Similar results were found in *Eurya japonica* (G_{ST} among five Korean and 15 Japanese populations was 0.072, M. G. CHUNG and M. Y. CHUNG, 2000a) and *Cymbidium goeringii* REICHB. fil. (Orchidaceae) (G_{ST} values between 17 populations in Korea and seven Japanese populations was 0.029, M. Y. CHUNG and M. G. CHUNG, 2000). The results indicate that a low degree of allozyme differentiation between populations in Japan and Korea, though the land connection between the southern Korean peninsula and southern Japanese archipelagos no longer existed after the middle Pleistocene.

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Genetic Variation of *Taxus cuspidata* SIEB. et ZUCC. in Korea

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Abstract

Japanese yew (*Taxus cuspidata* SIEB. et ZUCC.) is a shade-tolerant, dioecious gymnosperm native to Korea, Japan, Manchuria and eastern Siberia. Five natural populations of *T. cuspidata* in Korea were investigated using starch-gel electrophoresis in an attempt to determine the extent and distribution of genetic variation. The level of genetic diversity (A = 1.7, P_{95} = 45.7%, P_{99} = 60%, H_o = 0.172, and H_e = 0.168) and the degree of genetic differentiation (G_{ST} = 0.067) were comparable to those of other conifers with similar life-histories and ecological traits. Five natural Japanese yew populations seemed to be in equilibrium with the expectations of HARDY-WEINBERG. Isolation by distance was detected. NEI's genetic distance, D, was positively correlated with geographic distance (r = 0.794, p = 0.006). *Nm,* the number of migrants per generation, was 3.48 and 2.87, depending on estimation procedure, and is similar to values in wind-pollinated conifers. Individual trees widely scattered around natural populations appeared to be critical to the maintenance of genetic variation in Japanese yew. Implications for the conservation of genetic diversity of *T. cuspidata* are discussed.

Key words: Taxus cuspidata, genetic diversity, differentiation, allozymes.

Introduction

In Korea, there are two species and two varieties of the genus *Taxus; Taxus cuspidata* SIEB. et ZUCC., *T. cuspidata* var. *latifolia* NAKAI, *T. cuspidata* var. *nana* HORT., and *T. caespitosa* NAKAI which was introduced from Japan. Of these, *Taxus cuspidata* SIEB. et ZUCC., the most abundant native yew species, grows on high mountainous regions throughout Korea, Japan and China (LEE, 1987). This species is a long-lived, shade-tolerant, dioecious gymnosperm. In Korea, it is generally found in mixed forest stands together with other conifer species, such as *Abies nephrolepis, A. koreana, A. holophylla, Pinus koraiensis* and *Picea jezoensis,* and alpine broad-leaved tree species like *Quercus* spp. and *Betula* spp. In recent time, the distribution of yew species has been severely reduced due to natural disturbances and/or human activity. Additionally, the demands for yew species has rapidly increased since it was identified as the primary source of the compound Taxol® (paclitaxel), a promising new anticancer drug (see WHEELER, 1995 and references therein). So, the conservation and sustainable management of this species has gained much attention.

Allozyme variation in species and within and among populations has been extensively studied in forest trees, especially in conifers (HAMRICK et al., 1992; LEDIG, 1986, 1998). Comparatively, only a few studies have been made on yew species. Population genetic studies using DNA markers are also very sparse in yews. Recently, EL-KASSABY and YANCHUK (1994) used allozyme markers to study genetic diversity and were able to determine the inheritance of 21 allozyme loci in Pacific yew (*T. brevifolia*). WHEELER et al. (1995) studied genetic diversity and structure of Pacific yew sampled from North America and Canada using 22 isozyme loci. In addition, GÖÇMEN et al. (1996) constructed a genetic linkage map for Pacific yew based on RAPD markers. LEWANDOWSKII et al. (1992) and HERTEL (1996) studied inheritance of some isozyme markers in English yew (*T. baccata*) and HERTEL and KOHLSTOCK (1996) studied genetic variation and geographic structure of English yew in north eastern Germany, using 7 isozyme loci. However, to the best of our knowledge, there is a complete lack of information

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