Low levels of allozyme variation in the southern populations of the Korean endemic shrub *Sophora koreensis* (Fabaceae): Implications for conservation

M. Y. CHUNG (정미윤) & M. G. CHUNG (정명기)

Division of Life Science and the Research Institute of Natural Science, Gyeongsang National University,
KR-52828 Jinju, Republic of Korea

ORCID iD. M. Y. CHUNG (정미윤): http://orcid.org/0000-0002-8756-5367
M. G. CHUNG (정명기): http://orcid.org/0000-0002-1283-3574

Author for correspondence: M. G. Chung (mgchung@gnu.ac.kr)

Editor: J. López-Pujol

Abstract

Low levels of allozyme variation in the southern populations of the Korean endemic shrub *Sophora koreensis* (Fabaceae): Implications for conservation.— We investigated allozyme variation of the rare shrub *Sophora koreensis* in Yanggu County (South Korea), at the southern margin of the species range. To gain insights into ecological processes at the landscape level, we studied two or three populations from four localities (in total, 10 populations) in a range of *ca*. 6 km. We found low levels of within-population genetic variation ($\%P = 13.6$, $A = 1.14$, and $H_e = 0.026$) and a moderate degree of among-population genetic differentiation ($F_{ST} = 0.203$). The analysis of molecular variance (AMOVA) revealed a substantially higher percentage of variation among populations within localities (17%) than among localities (5%). There was no significant relationship between pairwise genetic divergence and logarithm of pairwise geographic distance ($r = 0.032$, $P = 0.842$). These results suggest limited gene flow between populations within localities, while indicating that the optimal strategy for the preservation of the genetic variation of *S. koreensis* is to conserve as many populations as possible.

Key words: allozymes; conservation; genetic diversity; Korea; *Sophora koreensis*.

Resumen

Bajos niveles de variación isoenzimática en las poblaciones sureñas del arbusto endémico de Corea *Sophora koreensis (Fabaceae)*: implicaciones para su conservación.— Se investigó la variación isoenzimática del arbusto raro *Sophora koreensis* en el condado de Yanggu (Corea del Sur), en el extremo sur de su área de distribución. Para obtener información sobre los procesos ecológicos a nivel del paisaje, se estudiaron dos o tres poblaciones para cada una de las cuatro localidades (en total, 10 poblaciones) en un radio de unos 6 km. Se encontraron bajos niveles de variación genética intra-poblacional ($\%P = 13,6$, $A = 1,14$ y $H_e = 0,026$) y un grado moderado de diferenciación genética entre poblaciones ($F_{ST} = 0,203$). El análisis de la varianza molecular (AMOVA) reveló un porcentaje sustancialmente mayor de variación entre poblaciones dentro cada una de las localidades (17%) que entre localidades (5%). No hubo una relación significativa entre la divergencia genética y el logaritmo de la distancia geográfica entre pares de poblaciones ($r = 0,032$, $P = 0,842$). Estos resultados sugieren un flujo genético limitado entre poblaciones dentro de las localidades, además de indicar que la mejor estrategia para la preservación de la diversidad genética de *S. koreensis* es la conservación del máximo número de poblaciones posible.

Palabras clave: conservación; Corea; diversidad genética; isoenzimas; *Sophora koreensis*.

적요

한국특산식물인 개느삼(콩과식물)의 남쪽 분포역에서 낮은 수준의 알로자임 변이: 보전에 대한 암시.— 저자들은 한국 양구군의 희귀한 관목인 개느삼의 알로자임 변이를 조사하였다. 경관 수준에서 생태학적 과정에 대한 통찰을 얻기 위해, 저자들은 약 6km의 범위에서 4 곳의 리(시골 마을 지역) 각각에 2 ~ 3 집단을(총 10 개의 집단) 조사하였다. 자자들은 낮은 수준의 집단 내 유전적변이 수준을 파악하였으며 (%P = 13.6, A = 1.14, H_e = 0.026) 중간 수준의 집단 간 유전적 분화 정도를 구하였다(F_{ST} = 0.203). 분자분산분석(AMOVA)은 4 곳 지역 간 변이(5 %)보다 지역 내 매우 인접된 집단 간 변이(17%)가 훨씬 높았다. 집단 간 유전적 분기 쌍과 로그 지리적 거리 쌍 간에는 통계학적으로 유의한 상관관계가 없었다 (r = 0.032, P = 0.842). 이러한 결과는 지역 내 개체군간에 제한된 유전자 흐름을 암시하며 개느삼의 유전적 변이를 보존하기 위한 최적의 전략은 최대한 많은 집단 수를 보존하는 것이라고 지적하고 있다.

주요어: 알로자임; 보전; 유전적 다양성; 한국; 개느삼.

INTRODUCTION

Sophora koreensis Nakai (Fabaceae), endemic to the Korean Peninsula, is a deciduous broad-leaved small shrub (less than 1 m tall) narrowly distributed on lower mountain hillsides (altitude *ca.* 150–650 m above sea level). The species reproduces both sexually and vegetatively via rhizomes. Until recently it was included in its own genus, *Echinosophora* Nakai (Lee, 1996; Lee *et al*., 2004). In North Korea (the Democratic People's Republic of Korea, hereafter the "DRPK"), about 11 localities are known in one city and four counties (Ra *et al*., 2005; Chang *et al*., 2016; Fig. 1), whereas in South Korea (the Republic of Korea, hereafter the "ROK"), up to 16 localities have been found to date in one city and four counties, all in Kangwon Province (Cheon *et al*., 2009, and the present study; Fig. 1). In 1992 the ROK government designated two of the localities (HAN, Hanjeon Rural village, and YIM, Imdang Rural village; Fig. 1) as National Monument of ROK No. 372, in order to preserve and protect those areas (Yim, 1993).

In ROK, the number of shoots in each population is on the order of tens to hundreds (Kim *et al*., 2016; J. M. Chung, pers. comm.). Owing to the species' limited distribution and endemic nature, *S. koreensis* has been assessed by several authors and authorities in ROK following the criteria of the IUCN (International Union for Conservation of Nature) Red List Categories (IUCN, 2001). However, the survey results were inconsistent: "Vulnerable" [VU B2ab (Chang *et al*., 2005); VU B2ab(iii, iv) (MOE, 2012)] or "Endangered" [EN B1ab(iii)+2ab(iii) (Chang *et al*., 2016; Kim *et al*., 2016)]. *Sophora koreensis* had been included in ROK's official listing of special protection of endangered wild plants (the 2nd grade) until 2012 (MOE, 2012), when it was delisted without providing any scientific justification (Kim *et al*., 2016). For further information on ecology and conservation status, we suggest referring Kim *et al*. (2016).

Given its threatened status and the lack of current conservation measures (Kim *et al*., 2016), it is necessary to implement appropriate conservation and management strategies to ensure the long-term maintenance of genetic diversity in *S. koreensis*. It has been generally agreed that demographic and genetic information of endangered plant species is essential for formulating comprehensive conservation strategies (Falk & Holsinger, 1991; Hamrick & Godt, 1996). Although several short-term ecological and demographic surveys have been conducted for *S. koreensis* in ROK (Cheon *et al*., 2009; Oh *et al*., 2009), levels of genetic diversity at regional (e.g. ROK) or species levels are currently not well understood. For the latter case, it might be nearly impossible because of practical unavailability of samples from DPRK. The only genetic study available to date, apart from the isolation and characterization of 13 microsatellite loci for the species (Lee *et al*., 2012), is a fine-scale genetic structure (FSGS) survey using ISSR in two populations (that were nearly

the same as HAN-2 and YIM-1; Fig. 1) in Yanggu County; a strong genet-based FSGS was found (most clones' aggregation at less than 6-m interplant distance), presumably due to limited seed and pollen dispersal (Chung *et al*., 2006), together with a moderate degree of genetic differentiation between the two populations ($\Phi_{ST} = 0.184$).

In this study, we expand the previous study of Chung *et al*. (2006) to investigate levels and distribution of genetic variation at the landscape level (in a range of *ca*. 6 km) in a part of Yanggu County (Fig. 1). With this spatial scale, we are especially interested in getting insights into the degree of genetic differentiation between adjacent populations within each locality (e.g. Williams, 1994; Williams & Guries, 1994; Chung, *et al*., 2002). In general, marginal populations of plant species exhibit significantly reduced within-population genetic variability and increased among-population differentiation relative to geographically central populations (reviewed in Eckert *et al*., 2008). This might be the consequence of lower gene flow and higher random genetic drift in small peripheral populations (Schnabel & Hamrick, 1990; Gibson & Hamrick, 1991). Given the fact that populations in ROK are within the southernmost limit of the species distribution, we would expect low levels of within-population genetic variation and a moderate degree of genetic differentiation. With the genetic data obtained we are providing suggestions for in situ and ex situ conservation strategies.

MATERIALS AND METHODS

Study species

Sophora koreensis is a pioneer species that can potentially invade open, sunny forest gaps (Yim, 1993). The study species is rapidly spreading by the underground rhizomes in disturbed areas (e.g. burnt areas). For example, we identified a 180-cm long rhizome in HAN-2 (Fig. 1) in May 2004,
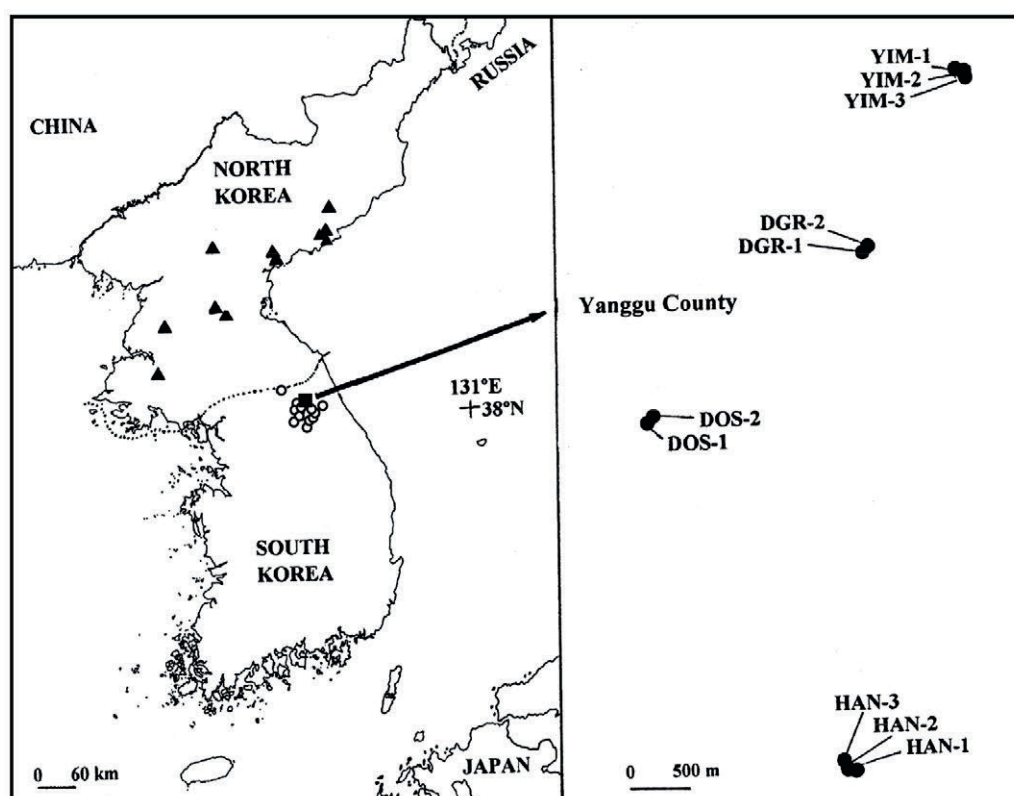
Figure 1. Current and past distribution of *Sophora koreensis* in Democratic People's Republic of Korea (11 closed triangles) (Chang *et al*., 2016) and in Republic of Korea (12 open circles and four localities in a closed square) (Cheon *et al*., 2009; the present study), and the 10 sampled populations (closed circles) from four localities (a "locality" is a group of adjacent populations within an administrative boundary) in Yanggu County, Kangwon Province, Republic of Korea. HAN: Hanjeon Rural village; DOS: Dosa Rural village; DGR: Deokgok Rural village; YIM: Yimdang Rural village.

about three years and half after a fire affected the whole locality. Density of individuals in HAN locality was relatively high until 2009 (Cheon *et al*., 2009), when it started to decrease due to more shade conditions produced by the growth of broad-leaved deciduous trees such as oaks and ashes (J. M. Chung, pers. comm.). In May, 10 to 20 yellow flowers (*ca*. 3–5 cm long) per inflorescence bloom and are frequently visited by the bumblebee *Bombus diversus diversus* (Chung *et al*., 2006). The species is self-compatible under greenhouse conditions (Chung *et al*., 2006). Fruits (legumes, *ca*. 7 cm long) ripen from July to September and contain two or three seeds. The drying of the pods makes them shrink and seeds fall around the parent plant ("shattering"; Quick *et al*., 2017). There are apparently no specialized mechanisms of seed dispersal.

Population sampling

To determine levels of allozyme diversity in *S. koreensis*, in May 2004 we collected one leaf from 397 shoots belonging to 10 populations, that are distributed among four different localities (Fig. 1 and Table 1): three populations in HAN (*ca*. 80 × 100 m area) and YIM (*ca*. 80 × 150 m area) localities, and two populations in Dosa Rural village (DOS; 40 × 50 m area; Fig. 1) and in Deokgoek Rural village (DGR; 50 × 60 m area). Sample sizes ranged from 78 in HAN-2 to 12 in DGR-2 (Table 1). To prevent sampling of clonal ramets, we collected shoots at *ca*. 4 m intervals across each population. All sampled leaf material was kept on ice until it could be transported to the laboratory, where it was stored at 4°C until protein extraction.

Enzyme electrophoresis

Using a phosphate-polyvinylpyrrolidone buffer (Mitton *et al*., 1979), we extracted enzymes and conducted horizontal starch-gel electrophoresis using 13% gels. We resolved 14 putative loci from eight enzyme systems using two buffer systems (Table 2). Stain recipes were taken from Soltis *et al*. (1983), except for diaphorase (Cheliak & Pitel, 1984). We designated putative loci sequentially, with the most anodally migrating isozyme designated as *1*, the next *2*, and so on. We also designated different alleles per locus sequentially by alphabetical order (*a*, *b*).

Data analyses

To estimate genetic diversity and genetic structure, a locus was considered polymorphic if two or more alleles were observed, regardless of their frequencies. The following genetic diversity parameters were estimated using the programs POPGENE (Yeh *et al*., 1999) and FSTAT (Goudet, 2002): percent polymorphic loci (*%P*), mean number of alleles per locus (*A*), allelic richness (*AR*; El Mousadik & Petit, 1996) corrected by minimum sample size ($N = 12$ in DGR-2; Table 1); observed heterozygosity (H_o), and Nei's (1978) unbiased gene diversity (H_e).

We calculated mean fixation indices (*F*) across polymorphic loci for each population, and their levels of significance (or *P* values) by gene permutation tests (999 replicates) assuming the null hypothesis ($F = 0$) is true using the program SPAGeDi (Hardy & Vekemans, 2002). We estimated Wright's (1965) F_{IS} and F_{ST} following the method of Weir & Cockerham (1984) to measure the average level (grand mean) of inbreeding within populations and genetic differentiation among them, with the aid of FSTAT. We estimated the 95% confidence intervals (CI) by bootstrapping over loci (999 replicates) around mean of the *F*-statistics, and considered the observed *F*-statistics to be significant when 95% CI did not overlap zero. We tested differences between the two population groups (HAN and YIM) for observed statistics, *OSx* (*AR*, H_o, H_e, F_{IS}, and F_{ST}). We chose these two localities because they are national monuments; thus, any differences in genetic parameters might be informative for conservation plans. To do this, we used a permutation scheme (999 replicates) by randomly allocating whole samples to the different groups, keeping the number of samples in each group constant and calculating differences between the two groups for randomized statistics, *RSx*. We then obtained the *P* value of the test as the proportion of randomized data sets giving a larger *RSx* than *OSx*. These calculations were also performed using FSTAT (Goudet, 2002).

We conducted an analysis of molecular variance (AMOVA) using the program GenAlEx v6.5 (Peakall & Smouse, 2006) to test for the influence of individuals within populations, populations within localities, and localities on the observed genetic variation. In addition, we constructed an

Table 1. Levels of genetic diversity and fixation index in 10 populations of *Sophora koreensis*[1].

Species/population	N	Area (m)	NP	%P	AR	A	H_o (SE)	H_e (SE)	F
Sophora koreensis									
HAN-1	68	30 × 80		28.6	1.16	1.29	0.023 (0.019)	0.040 (0.031)	0.421
HAN-2	78	40 × 50		28.6	1.22	1.29	0.014 (0.006)	0.055 (0.036)	0.748
HAN-3	61	40 × 70		14.3	1.12	1.14	0.012 (0.009)	0.018 (0.012)	0.338
DOS-1	28	20 × 40		0.0	1.00	1.00	0.000 (0.000)	0.000 (0.000)	na
DOS-2	21	20 × 50		0.0	1.00	1.00	0.000 (0.000)	0.000 (0.000)	na
DGR-1	30	30 × 50		7.1	1.07	1.07	0.012 (0.012)	0.011 (0.011)	−0.070
DGR-2	12	30 × 40		7.1	1.07	1.07	0.024 (0.024)	0.038 (0.038)	0.371
YIM-1	31	40 × 50		21.4	1.21	1.21	0.055 (0.034)	0.072 (0.040)	0.227
YIM-2	24	40 × 60		21.4	1.11	1.21	0.009 (0.005)	0.009 (0.005)	0.000
YIM-3	44	50 × 60		7.1	1.07	1.07	0.010 (0.010)	0.015 (0.015)	0.333
Average	39.7			13.6	1.10	1.14	0.016 (0.012)	0.026 (0.006)	0.556[2]
Pooled samples	397		12	35.7		1.36	0.016 (0.004)	0.037 (0.023)	
S. fernandeziana[3]									
Population mean			5	19.0				0.022	
Pooled samples								0.038	
S. moorcroftiana[4]									
Population mean	*ca*. 40			27.5		1.50	0.100 (0.006)	0.122 (0.007)	0.146[2]
Pooled samples	398		10	33.3		1.90	0.101 (0.034)	0.150 (0.047)	

[1] Abbreviations: *N*: sample size; *NP*: number of populations surveyed; *%P*: percentage of polymorphic loci; *AR*: mean allelic richness (adjusted for a sample size of 12); *A*: mean number of alleles per locus; H_o: observed heterozygosity; H_e: H–W expected heterozygosity or Nei's gene diversity; SE: standard error; *F*: fixation index; *P*: level of significance (or *P*-value); na: not applicable.
[2] Grand mean (F_{IS}) following Weir & Cockerham (1984).
[3, 4] data from Liu *et al.* (2006) and Crawford *et al.* (2001), respectively.

Table 2. The allozyme loci assayed for *Sophora koreensis* and the buffer systems used to resolve them[1].

Buffer system	Enzyme and loci resolved (E.C. code)
6	Diaphorase (*Dia*) (E.C. 1.6.99.-)
	Fluorescent esterase (*Fe-1*, *Fe-2*, *Fe-3*) (E.C. 3.1.1.-)
	Phosphoglucoisomerase (*Pgi*) (E.C. 5.3.1.9)
	Phosphoglucomutase (*Pgm-1, Pgm-2*) (E.C. 5.4.2.2)
	Triosephosphate isomerase (*Tpi*) (E.C. 5.3.1.1)
MC	Isocitrate dehydrogenase (*Idh-1*, *Idh-2*) (E.C. 1.1.1.42)
	Malate dehydrogenase (*Mdh-1*, *Mdh-2*) (E.C. 1.1.1.37)
	6-Phosphogluconate dehydrogenase (*6Pgd-1*, *6Pgd-2*) (E.C. 1.1.1.44)

[1] Buffer systems 6 and MC (morpholine-citrate system, pH 6.1) were from Soltis *et al.* (1983) and Clayton & Tretiak (1972), respectively.

UPGMA (unweighted pair-group method using arithmetic averages) phenogram from Nei *et al.*'s (1983) genetic distance matrix with branch support produced by 1000 bootstrapping over loci, utilizing Populations v1.2.30 (Langella, 1999) and TreeView v1.6 (Page, 1996). We conducted a linear regression analysis between all pairwise $F_{ST}/(1 – F_{ST})$ (F_{ST} was calculated following Weir & Cockerham, 1984) and the corresponding logarithm (ln) of pairwise geographical distances (Rousset, 1997) to find the overall pattern of genetic structure at the regional scale (i.e. isolation-by-distance effects). Using FSTAT, we tested a linear regression model using a Mantel test (by making 999 replicates) under the null hypothesis of no spatial genetic structure (regression slope, $b = 0$).

RESULTS

Genetic diversity and fixation index

Of the 14 putative loci surveyed, only five (*Dia, Fe-1, Fe-2, 6Pgd-1*, and *Tpi*; Table 3) were polymorphic, resulting in low levels of allozyme variation (Table 1). At the population level, the mean percentage of polymorphic loci (*%P*) was 13.6, the mean number of alleles per locus (*A*) was 1.14, and the mean genetic diversity (H_e) was 0.026 (Table 1). Higher levels were found when the samples were pooled ($N = 397$, $\%P = 35.7$, $A = 1.36$, and $H_e = 0.037$; Table 1). YIM-1 exhibited the highest H_e estimate, whereas no variation was found in DOS-1 and DOS-2 (Table 1).

Average F values in four populations (HAN-1, HAN-2, HAN-3, and YIM-3; Table 1) indicated a significant deficiency of heterozygotes, with a grand mean of $F_{IS} = 0.556$ (Table 1; 95% CI was from 0.016 to 0.297). Permutation test (999 replicates) revealed no significant differences for *AR* ($P = 0.617$), H_o ($P = 0.568$), H_e ($P = 0.734$), and F_{IS} ($P = 0.340$) between HAN and YIM.

Genetic differentiation among populations

Significant differentiation among populations was found in *S. koreensis* at the landscape level (mean $F_{ST} = 0.203$, 95% CI was from 0.032 to 0.245). Permutation test indicated no significant difference for F_{ST} ($P = 0.813$) between HAN and YIM. We unexpectedly found high (F_{ST} among three populations

in HAN = 0.583; F_{ST} between two populations in DGR = 0.389) or moderate (F_{ST} among three populations in YIM = 0.233) degree of genetic differentiation among populations within the three localities (no allozyme variation in DOS). Consistent with this, the AMOVA analyses revealed a considerable genetic differentiation among populations within localities (17%; Table 4). This estimate was considerably higher than that of variation among the four localities (5%; Table 4).

Despite the non-statistical support of the UPGMA phenogram, it provides a general idea about the clustering of the studied populations (Fig. 2). Most groupings showed little relationship with populations' geographic location; a notable exception were the populations DOS-1 and DOS-2, that were placed together because of the same banding patterns (no variation). The correlation between pairwise genetic differentiation estimates and their corresponding natural ln of pairwise geographic distances was very low ($r = 0.032$, $P = 0.842$) and indicated that *ca.* 100% of the variation in genetic differentiation was due to unknown factors other than distance (Fig. 3).

DISCUSSION

Low levels of within-population variation and inbreeding

As expected, populations of *S. koreensis* exhibited low levels of genetic variation ($\%P = 13.6$, $A = 1.14$, $H_e = 0.026$). These estimates were clearly lower than the reference values for both endemic species and narrowly-distributed ones ($\%P = 26.3$, $A = 1.39$, $H_e = 0.063$, $N = 100$; $\%P = 30.6$, $A = 1.45$, $H_e = 0.105$, $N = 115$; Hamrick & Godt, 1989). The population-level estimates, however, are similar to those found in its congener *S. fernandeziana* (Phil.) Skottsb. ($\%P = 19$ and $H_e = 0.022$; Crawford *et al.*, 2001). *Sophora fernandeziana* is a hummingbird-pollinated species endemic to Robinson Crusoe Island (*ca.* 48 km², known also as Más a Tierra or Más Atierra until 1966), a part of the Juan Fernández Archipelago, located 667 km west of South America (Chile) at 33° S latitude, in the South Pacific Ocean. Crawford *et al.* (2001) attributed the low levels of allozyme diversity to random genetic drift operating in the small, scattered populations. Another studied congener is the
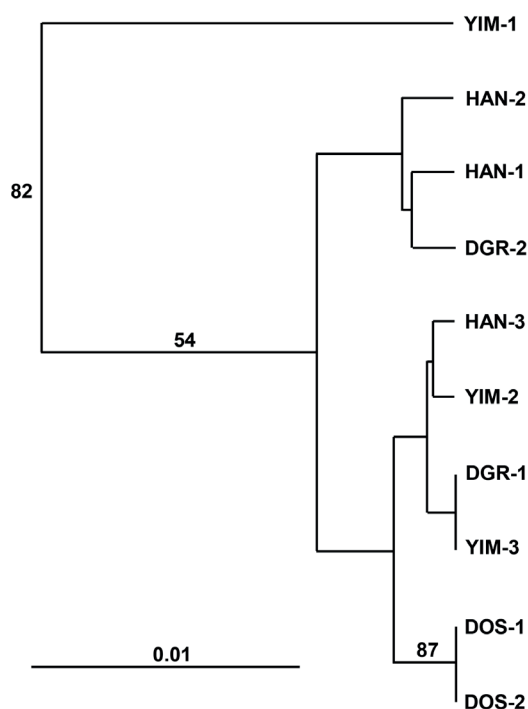
Figure 2. UPGMA phenogram based on Nei *et al.* (1983) genetic distances between populations of *Sophora koreensis*. Numbers above branches represent bootstrap support for 1000 replicates (only values greater than 50% are shown). See Table 1 for acronyms.

insect-pollinated *S. moorcroftiana* (Benth.) Benth. ex Baker from the Qinghai-Tibetan Plateau (QTP) in western China. Liu *et al*. (2006) examined allozyme diversity from 10 populations in the middle reaches (in a range of ~630 km) of the Yarlung Zangbo (Brahmaputra) River. As one may expect for a species with a wider geographic distribution, moderate levels of within-population genetic variation were found in *S. moorcroftiana* (%P = 27.5, *A* = 1.50, H_e = 0.122; Liu *et al*., 2006).

These three *Sophora* species would have followed their unique evolutionary trajectories. Past histories (e.g. uplift of the QTP for *S. moorcroftiana* and island colonization for *S. fernandeziana*) might have greatly contributed to levels of genetic diversity and genetic structure. The broad-leaved deciduous shrub *S. koreensis* is one of the elements of the mixed (boreal-temperate) forest on the Korean Peninsula. At the Last Glacial Maximum (LGM, 21,000 years before present, BP) *S. koreensis* might have occurred within a narrow mixed forest zone in the southern and western parts of the Peninsula (further south compared to its present distribution; see Fig. 2A in Chung *et al*., 2017). The onset of the Holocene (*ca.* 12,000 years BP) and the accompanying

climatic amelioration would had led to a post-glacial recolonization process for the mixed forests towards the north (i.e. to central and northern Korea) from putative refugial population(s) in southern Korea. Under this scenario, levels of genetic diversity in the current populations of *S. koreensis* in central and northern Korea would be low due to multiple founder effects (e.g. Kim *et al*., 2005; reviewed in Chung *et al*., 2017). Kim *et al*. (2015) revealed that within-population genetic diversity (H_e) of the draft bamboo *Sasa borealis* (Hack.) Makino & Shibata populations in South Korea was inversely proportional to the latitude (*r* = 0.719, *P* = 0.009). If this scenario is true for *S. koreensis*, we will expect that levels of within-population genetic diversity would decrease with increasing latitudes. However, a test of this hypothesis seems to be impossible because of political problems (at present it is not feasible to sample in DPRK by scientists from ROK).

The significant deficit of heterozygotes seen in the four populations (HAN-1, HAN-2, HAN-3, and YIM-3) can be attributed to the occurrence of selfing and/or biparental inbreeding, likely resulting from limited pollen and/or seed dispersal (Chung

Table 3. Allele frequencies at five polymorphic loci in 10 populations of *Sophora koreensis*.

Locus	Allele	Population									
		HAN-1	HAN-2	HAN-3	DOS-1	DOS-2	DGR-1	DGR-2	YIM-1	YIM-2	YIM-3
Fe-1	*a*	1.000	0.962	1.000	1.000	1.000	1.000	1.000	0.694	0.979	1.000
	b	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.306	0.021	0.000
Fe-2	*a*	0.985	0.936	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.015	0.064	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Tpi	*a*	0.971	0.968	0.943	1.000	1.000	1.000	1.000	0.871	0.979	1.000
	b	0.029	0.032	0.057	0.000	0.000	0.000	0.000	0.129	0.021	0.000
Dia	*a*	0.676	0.487	0.926	1.000	1.000	0.917	0.500	1.000	0.979	0.886
	b	0.324	0.513	0.074	0.000	0.000	0.083	0.500	0.000	0.021	0.114
6Pgd-1	*a*	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.210	0.000	0.000
	b	0.985	1.000	1.000	1.000	1.000	1.000	1.000	0.790	1.000	1.000

Table 4. Results of the analysis of molecular variance (AMOVA) for *Sophora koreensis*[1].

Source	df	SS	MS	Variance component	Percent variation
Among four localities	3	16.375	5.458	0.012	5
Among populations within localities	6	23.607	3.934	0.046	17
Within populations	784	164.269	0.210	0.210	78
Total	793	204.251		0.268	100

[1] Abbreviations: df, degrees of freedom; SS, sum of squares; MS, mean squares; percent variation, percentage of total variance contributed by each component.

et al., 2006). Even though we tried to prevent duplicate samples, unintentional inclusion of some samples would lead to over- or underestimation of fixation indices. In addition, as individuals in some populations are patchily distributed (J. M. Chung, pers. comm.), a Wahlund effect might be involved in some of the apparent inbreeding observed. Detailed field studies are needed to evaluate the relative contribution of selfing vs. biparental inbreeding to total inbreeding.

Genetic differentiation among populations

Given the ~6 km study range, the F_{ST} value (0.203) is notably high. A similar result was found in ISSR-based genetic differentiation between HAN-2 and YIM-1 (Φ_{ST} = 0.184; Chung *et al*., 2006). These estimates are only slightly lower than those reported for endemic species [Nei's (1973) G_{ST} = 0.248] and narrowly-distributed species (G_{ST} = 0.242; Hamrick

& Godt, 1989). Our estimate of genetic differentiation for *S. koreensis* is even higher than that *S. moorcroftiana* populations (F_{ST} = 0.199; Liu *et al*., 2006), which is quite unexpected given the substantially larger sampling area of *S. moorcroftiana* (the distances between each locality range from 30 to 630 km; Liu *et al*., 2006). The authors attributed the moderate degree of genetic differentiation among 10 populations to the secondary seed movement by water streams (hydrochoric gene flow; note that the species occurs along relatively open river valleys). Furthermore, their findings of a significant correlation between genetic and geographic distance (r = 0.707, P = 0.002) suggest a balance between random genetic drift and gene flow. As insect movement would be limited, secondary seed dispersal via river water current might be possible. In contrast to these studies, Crawford *et al*. (2001) found high degree of genetic differentiation among five *S. fernandeziana* populations on Robinson Crusoe Island (*ca*. 48 km[2])
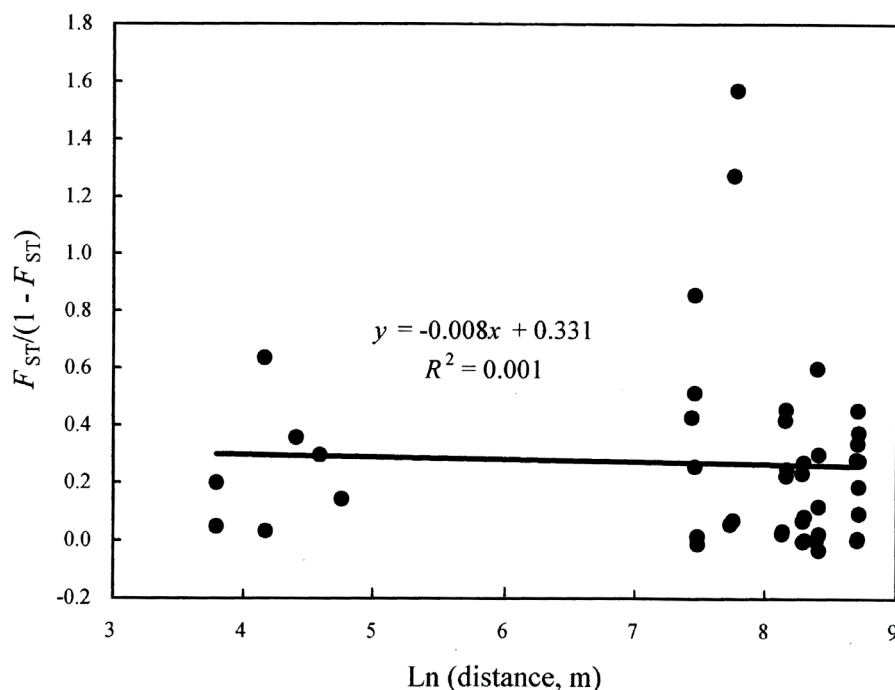
Figure 3. Differentiation between populations of *Sophora korennsis*. Multilocus estimates of pairwise differentiation of F_{ST}/ $(1-F_{ST})$ are plotted against natural logarithm (ln) of pairwise geographic distances in meters according to Rousset (1997). There was no significant relationship between pairwise F_{ST}/$(1-F_{ST})$ and ln of pairwise geographic distance ($r = 0.032$, $P = 0.842$).

($G_{ST} = 0.351$). The authors suggested that high genetic differentiation is due to the fact that its populations are small and have a scattered distribution along the island. Thus, random genetic drift would have operated in these small populations in the remote oceanic island.

Samples from the three populations (YIM-1, YIM-2, and YIM-3) in YIM were collected in an area of *ca.* 80 × 150 m and those from three populations (HAN-1, HAN-2, and HAN-3) in HAN were from an area of *ca.* 80 × 100 m. Even though the three populations are very closely located each other both in YIM and HAN, we found significant differences in allelic frequencies. For example, the allele *6Pgd-1*[a] was present in HAN-1 and YIM-1 with frequencies of 0.015 and 0.210, but it did not occur in the remaining populations (Table 3). Unexpectedly, this allele was not present in YIM-2 and YIM-3. Similarly, the allele *Fe-1*[b] was present in HAN-2, YIM-1, and YIM-2 with frequencies of 0.038, 0.306, and 0.021, respectively, but not in the remaining populations of both HAN and YIM localities. These findings strongly suggest limited gene dispersal within approximately 1 ha area, which is supported by the strong FSGS detected in HAN-2 and YIM-1 (*Sp*, a statistic that represents

the strength of FSGS, was 0.072 in HAN-2 and 0.093 in YIM-1; Chung *et al.*, 2006). Consistent with these results, we found little association between genetic and geographic distances (Figs. 2 and 3) and a considerable genetic differentiation among populations within localities (Table 4).

Implications for conservation

Before discussing on genetic aspects, and based on available ecological information, our own field observations, and personal communications, we provide two recommendations for in situ conservation in the study area. First, we suggest performing a selective removal of trees in the natural populations, as *S. koreensis* prefers sunny places, open forest gaps, or disturbed areas (e.g. burnt areas); when relatively fast-growing shrubs or trees (such as oaks and ashes) invade the populations producing shady conditions, many shoots are likely to disappear (J. M. Chung, pers. comm.). Indeed, removal of trees and have been conducted in the areas of Natural Monuments No. 372 (HAN and YIM) with some success (J. M. Chung, per. comm.). However, special care should be needed because the human-assisted clearance may also allow other native trees

that can grow in a similar environment to thrive. Second, for in situ protection it would be necessary to take preventive measures against damage caused by the human-mediated pressure. To do this, it seems to be preferable to install a wooden fence at the boundary of the natural populations in HAN and YIM.

The population genetic structure of *S. koreensis* is summarized as: (1) low levels of genetic variation within populations, (2) moderate degree of differentiation among populations and considerable genetic variation among populations within localities, (3) little association between genetic and geographic distance, (4) lower genetic variation in smaller populations (e.g. DOS), and (5) a strong FSGS in populations in HAN-2 and YIM-1 (Chung *et al.*, 2006). In addition, as *S. koreensis* also reproduces vegetatively, effective population sizes will be especially small in highly clonally-formed populations. Thus, random genetic drift should have a more pronounced effect in those populations over generations (e.g. Chung *et al.*, 2004). Given our genetic results, we recommend that all extant populations in ROK should be conserved in situ. Preserving entire localities (i.e. packs of close populations) instead of single populations seems a reasonable policy, given the fact that populations within each locality are separated by a few meters (localities range from 0.2 to 1.2 ha); preserving together packs of close populations also allows to maintain gene flow between populations. To ensure long-term ex situ genetic variability of *S. koreensis*, and based on the results of the FSGS study of Chung *et al.* (2006), we suggest to collect seeds from shoots positioned at distances of *ca.* 10–12 m across entire localities. It is also highly advisable to carry out a genetic study across all known 16 populations in ROK with the microsatellite loci developed by Lee *et al.* (2012), that have been proven to be highly polymorphic. In parallel to this genetic survey, long-term ecological studies (e.g. minimum viable population size and demographic dynamics, seed dispersal, pollination biology, seed germination ecology) are also necessary to reach a comprehensive management of *S. koreensis*.

ACKNOWLEDGMENTS

REFERENCES

Chang, C. S., Kim, H., Son, S. & Kim, Y.-S. 2016. *The Red list of selected vascular plants in Korea*. Korea National Arboretum and Korean Plant Specialist Group, Pocheon.

Chang, C. S., Lee, H. S., Park, T. Y. & Kim, H. 2005. IUCN 적색목록 기준에 의한 환경부 멸종위기 야생식물종에 대한 평가 [Reconsideration of rare and endangered plant species in Korea based on the IUCN red list categories]. *Korean Journal of Ecology* 28: 305–320 [in Korean]. https://doi.org/10.5141/JEFB.2005.28.5.305

Cheliak, W. M. & Pitel, J. P. 1984. *Technique for starch gel electrophoresis of enzyme from forest tree species. Information report PI-X-42*. Petawawa National Forestry Institute, Chalk River.

Cheon, K.-S., Jang, S.-K., Lee, W.-T. & Yoo, K.-O. 2009. 개느삼의 분포와 자생지 환경특성 [The natural habitat and distribution of *Echinosophora koreensis* (Nakai) Nakai in Korea]. *Korean Journal of Plant Taxonomy* 39: 254–263 [in Korean].

Chung, J. M., Lee, B. C., Kim, J. S., Park, C.W., Chung, M. Y. & Chung, M. G. 2006. Fine-scale structure among genetic individuals of the clone-forming monotypic genus *Echinosophora koreensis* (Fabaceae). *Annals of Botany* 98: 165–173. https://doi.org/10.1093/aob/mcl083

Chung, M. Y., López-Pujol, J. & Chung, M. G. 2017. The role of the Baekdudaegan (Korean Peninsula) as a major glacial refugium for plant species: A priority for conservation. *Biological Conservation*, 206: 236–248. https://doi.org/10.1016/j.biocon.2016.11.040

Chung, M. Y., Nason, J. D. & Chung, M. G. 2004. Implications of clonal structure for effective population size and genetic drift in a rare terrestrial orchid, *Cremastra appendiculata* (Orchidaceae). *Conservation Biology* 18: 1515–1524. https://doi.org/10.1111/j.1523-1739.2004.00291.x

Chung, M. Y., Nason, J. D., Chung, M. G., Kim, K.-J., Park, C.-W., Sun, B.-Y. & Pak, J.-H. 2002. Landscape-level spatial genetic structure in *Quercus acutissima* (Fagaceae). *American Journal of Botany* 89: 1229–1236. https://doi.org/10.3732/ajb.89.8.1229

Clayton, J. W. & Tretiak, D. N. 1972. Amine citrate buffers for pH control in starch gel electrophoresis. *Journal of the Fisheries Research Board of Canada* 29: 1169–1172. https://doi.org/10.1139/f72-172

Crawford, D. J., Ruiz, E., Stuessy, T. F. *et al.* 2001. Allozyme diversity in endemic flowering plant species of the Juan Fernandez Archipelago, Chile: ecological and historical factors with implications for conservation. *American Journal of Botany* 88: 2195–2203. https://doi.org/10.2307/3558381

Eckert, C. G., Samis, K. E. & Lougheed, S. C. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molecular Ecology* 17: 1170–1188. https://doi.org/10.1111/j.1365-294X.2007.03659.x

El Mousadik, A. & Petit, R. J. 1996 . High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. *Theoretical and Applied Genetics* 92 : 832–839. https://doi.org/10.1007/BF00221895

Falk, D. A. & Holsinger, K. E. (Eds.) 1991. *Genetics and conservation of rare plants*. Oxford University Press, Oxford.

Gibson, J. P. & Hamrick, J. L. 1991. Genetic diversity and structure in *Pinus pungens* (table mountain pine) populations. *Canadian Journal of Forest Research* 21: 635–642. https://doi.org/10.1139/x91-087

Goudet, J. 2002. *FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2)*. Department of Ecology & Evolution, University de Lausanne, Lausanne. Retrieved May 13, 2011, from: http://www.unil.ch/izea/softwares/fstat.html

Hamrick, J. L. & Godt, M. J. W. 1989. Allozyme diversity in plant species. In: Brown, A. H. D., Clegg, M. T., Kahler, A. L. & Weir, B. S. (Eds.), *Plant population genetics, breeding and genetic resources*. Sinauer, Sunderland: 43–63.

Hamrick, J. L. & Godt, M. J. W. 1996. Conservation genetics of endemic plant species. In: Avise, J. C. & Hamrick, J. L. (Eds.), *Conservation genetics*. Chapman & Hall, New York: 281–304. https://doi.org/10.1007/978-1-4757-2504-9_9

Hardy, O. J. & Vekemans, X. 2002. SPAGeDi: a versatile computer program to analyze spatial genetic structure at the individual or population level. *Molecular Ecology Notes* 2: 618–620. https://doi.org/10.1046/j.1471-8286.2002.00305.x

IUCN (International Union for Conservation of Nature) 2001. *IUCN Red List Categories: Version 3.1*. IUCN, Gland & Cambridge.

Kim, I. R., Yu, D. & Choi, H.-K. 2015. AFLP 마커를 이용한 조릿대 개체군의 식물지리학적 연구 [A phytogeographical study of *Sasa borealis* populations based on AFLP analysis]. *Korean Journal of Plant Taxonomy* 45: 29–35 [in Korean]. https://doi.org/10.11110/kjpt.2015.45.1.29

Kim, Y.-S., Kim, H. & Son, S.-W. 2016. Sophora koreensis. *The IUCN Red List of Threatened Species 2016: e.T13188557A13189529*. Retrieved December 5, 2016, from https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T13188557A13189529.en

Kim, Z. S., Hwang, J. W., Lee, S. W., Yang, C. & Gorovoy, P. G. 2005. Genetic variation of Korean pine (*Pinus koraiensis* Sieb. et Zucc.) at allozyme and RAPD markers in Korea, China and Russia. *Silvae Genetica* 54: 235–246.

Langella, O. 1999. *Populations version 1.2.30*. Retrieved August 14, 2013, from http://bioinformatics.org/~tryphon/populations/

Lee, J.-Y., Lee, D.-H. & Choi, B.-H. 2012. Isolation and characterization of 13 microsatellite loci from a Korean endemic species, *Sophora koreensis* (Fabaceae). *International Journal of Molecular Sciences* 13: 10765–10770. https://doi.org/10.3390/ijms130910765

Lee, W. K., Toru, T. & Heo, K. 2004. Molecular evidence for inclusion of the Korean endemic genus "*Echinosophora*" in *Sophora* (Fabaceae), and embryological features of the genus. *Journal of Plant Research* 117: 209–219. https://doi.org/10.1007/s10265-004-0150-x

Lee, W. T. 1996. 한국식물명고 [*Standard illustrations of Korean plants*]. Academy Publishing Co., Seoul [in Korean].

Liu, Z.-M., Zhao, A.-M., Kang, X.-Y., Zhou, S.-L. & López-Pujol, J. 2006. Genetic diversity, population structure, and conservation of *Sophora moorcroftiana* (Fabaceae), a shrub endemic to the Tibetan Plateau. *Plant Biology* 8: 81–92. https://doi.org/10.1055/s-2005-872889

Mitton, J. B., Linhart, Y. B., Sturgeon, K. B. & Hamrick, J. L. 1979. Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. *Journal of Heredity* 70: 86–89.

MOE (Ministry of Environment, Republic of Korea) 2012. 한국의 멸종위기 야생동·식물 적색자료집 관속식물 [*Red data book of endangered vascular plants in Korea*]. National Institute of Biological Resources, Incheon [in Korean].

Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America* 70: 3321–3323. https://doi.org/10.1073/pnas.70.12.3321

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.

Nei, M., Tajima, F. & Tateno, Y. 1983. Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. *Journal of Molecular Evolution* 19:153–170. https://doi.org/10.1007/bf02300753

Oh, B. U., Han, J. W., Yang, S. K., Jang, E. S., Jang, C. G., Kim, Y. Y. & Kang, S. H. 2009. 강원도 양구군 특산식물 개느삼(콩과) 자생지 내의 식물상과 식생 – 양구군 남면 죽곡리, 황강리를 중심으로 [Flora and vegetation in a habitat of *Echinosophora koreensis* (Nakai) Nakai (Leguminosae), a Korean endemic plant in Yanggu-gun (Kangwon), Korea–Focused on Jukgok-ri and Hwanggang-ri]. *Journal of the Korea Society of Environmental Restoration Technology* 12: 19–28 [in Korean].

Page, R. D. M. 1996. TreeView: an application to display phylogenetic trees on personal computers. *Computer Applications in the Bioscience* 12: 357–358.

Peakall, R. & Smouse, P. E. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Quick, Z. I., Houseman, G. R. & Büyüktahtakin, I. E. 2017. Assessing wind and mammals as seed dispersal vectors in an invasive legume. *Weed Research* 57: 35–43. https://doi.org/10.1111/wre.12232

Ra, U. C., Park, H.-S., Ju, I.-Y. *et al*. 2005. *Red data book of DPR Korea (plant)*. MAB National Committee of DPR Korea, Botanical Institute, Biological Branch, Academy of Sciences, Pyongyang.

Rousset, F. 1997. Genetic differentiation within and between two habitats. *Genetics* 151: 397–407.

Schnabel, A. & Hamrick, J. L. 1990. Organization of genetic diversity within and among populations of *Gleditsia triacanthos* (Leguminosae). *American Journal of Botany* 77: 1060–1069. https://doi.org/10.2307/2444577

Soltis, D. E., Haufler, C. H., Darrow, D. C. & Gastony, G. J. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *American Fern Journal* 73: 9–27. https://doi.org/10.2307/1546611

Weir, B. S. & Cockerham, C. C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38: 1358–1370. https://doi.org/10.2307/2408641

Williams, C. F. 1994. Genetic consequences of seed dispersal in three sympatric forest herbs. II. Microspatial genetic structure within populations. *Evolution* 48: 1959–1972. https://doi.org/10.2307/2410520

Williams, C. F. & Guries, R. P. 1994. Genetic consequences of seed dispersal in three sympatric forest herbs. I. Hierarchical population genetic structure. *Evolution* 48: 791–805. https://doi.org/10.2307/2410487

Wright, S. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution* 19: 395–420. https://doi.org/10.2307/2406450

Yeh, F. C., Yang, R. C. & Boyle, T. B. J. 1999. *POPGENE. Version 1.31. Microsoft Windows-based free ware for population genetic analysis*. Department of Renewable Resources, University of Alberta, Edmonton. Retrieved March 12, 2011, from http://www.ualberta.ca/~fyeh/index/htm

Yim, K.-B. 1993. 천연기념물: 식물편 [*Botanical treasures (natural monuments) of Korea*]. Daewonsa, Seoul [in Korean].