- TEISSIER DU CROS, E. (2001): Forest Genetic Resources Management and Conservation. France as a case study, Ministry of Agriculture and Fisheries, Bureau of Genetic Resources, Commission of Forest Genetic Resources, INRA DIC, Paris.
- VANDEN BROECK, A. (2004): Potential gene flow from cultivated poplar into native European Black poplar (*Populus nigra* L.) in Belgium. PhD. Universiteit Gent, Gent.
- VAN DER BEN, D. (1997): Het Zoniënwoud. Een natuurmooient en zijn geschiedenis. Lannoo, Tielt, Belgium.
- VANDER MIJNSBRUGGE, K. (2003): Conservation of the heritage of autochthonous woody plants in Flanders (Belgium). *Scripta Botanica Belgica* **24**, 105–115.
- VANDER MIJNSBRUGGE, K., E. COART, H. BEECKMAN and J. VAN SLYCKEN (2003a): Conservation measures for autochthonous oaks in Flanders. *Forest Genetics* **10**, 207–217.
- VANDER MIJNSBRUGGE, K., A. VAN DEN BROECK, E. COART, R. DEVREESE and M. DUMORTIER (2003b): Bomen en struiken, pp. 37–41 in *Natuurrapport 2003* edited by M. DUMORTIER, L. DE BRUYN, H. PEYMEN, A. SCHNEIDERS, T. VAN DAELE, G. WEYEMBERGH, D. VAN STRAATEN and E. KUIJKEN, Instituut voor Natuurbehoud, Brussels.
- VANDER MIJNSBRUGGE, K., K. COX and J. VAN SLYCKEN, 2004: Afbakening van Vlaamse herkomstgebieden, rapport Ibw.Bb.R.2004.001, Instituut voor Bosbouw en Wildbeheer, Geraardsbergen.
- WAGNER, I. (1999): Schutz und Nutzen von Wildobst. Probleme bei der direkten Nutzung von Wildobstrelikten. *Forstarchiv* **70**: 23–27.
- WILSON, S. M., D. C. MALCOLM and D. A. ROOK (1999): Locating natural populations of Scottish native trees. *Scottish Forestry* **53**(4): 215–224.
- WILSON, S. M. and C. J. A. SAMUEL (2004): Genetic conservation of native trees, in *Forest Research Annual Report and Accounts for 2002–2003*, Forestry Commission, Edinburgh.

Classification of Genus *Acanthopanax* in Korea and Genetic Diversity Using Allozymes

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Abstract

Genus *Acanthopanax* is a long-lived woody species that is primarily distributed throughout Asia. Many species of this genus are regarded as medically and ecologically important. We evaluated a representative sample of the nine taxa with allozymes to estimate genetic relationships within the genus. As some Korean populations were isolated and patchily distributed, they exhibited a low level of genetic diversity. The narrow geographic ranges, artificial distribution of habitats, and small population sizes are proposed as factors contributing to low genetic diversity. *Acanthopanax seoulense* was similar to *A. sessiliflorus*, while a cluster of the *A. rufinerve* population is distant from any other species. *A. senticosus* is closely related to *A. seoulense* and *A. sessiliflorus*, whereas other species (*A. koreanum*) are more distinct from the Korean populations. Korean species are clustered together and clearly differentiated from the Chinese and Russian *Acanthopanax* taxa, genus *Acanthopanax*.

Key words: Allozyme; Genetic relationships; Genus *Acanthopanax*.

Introduction

The genus *Acanthopanax* or *Eleutherococcus* is a genus within the subfamily Araliaceae comprising of the

diploid species ($2n = 54$) and distributed mainly in northeastern Asia. *Acanthopanax* can be classified as narrowly adapted species because they are usually found on the subsites of several mountains, at elevations of 600 to 1,000 m. These long-lived perennials have purple, green, and white flowers and have a bisexual and mixed mating, reproductive system with selfing or out-crossing via insect-pollination (HUH et al., 2005). Although plants grow high in the mountains on fertile soil, they are also extensively cultivated as medicinal crops. Many species of this genus are economically important for their leaves, stems, and roots which historically were used in Korea for medicine, alcoholic liquor, and beverages.

A. senticosus native to northeastern China, Korea, and Japan and is presently prescribed for medicinal use in France, Germany, Korea, and China. *A. koreanum*, *A. chiisanensis* and *A. seoulense* are native to Korea and also a traditional medicine species.

A. senticosus is sold in the United States as “Siberian Ginseng”. It is known in China as ci wu Jia. It is also a more expensive medicine than other *Acanthopanax* species. Siberian Ginseng has been used for bronchitis, heart ailments, and rheumatism, and as a tonic to restore vigor, improve general health, restore memory, promote healthy appetite, and increase stamina (FOSTER, 1996).

East Asian regions such as China, Korea, Japan and Russia are well known for various *Acanthopanax* species (LEE, 1997). The genus *Acanthopanax* is comprised of

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about 5~7 species in Korea. The taxonomy of *Acanthopanax* is based mainly on morphological characteristics. However their characteristics sometimes have limited usefulness in resolving species identification.

Typical populations of many *Acanthopanax* species except *A. sessiliflorus* are small and distributed in patches. The main concern of persistence of *Acanthopanax* is continued habitat destruction and fragmentation. Consequently, wild *Acanthopanax* are in decline and populations are fragmented by human activities such as over-gathering medicinal plants. Thus Insights into the relative gene diversity among and within wild accessions of *Acanthopanax* would be useful in plant breeding and also for the development of strategies for ex situ conservation of plant genetic resources (BARTISH et al., 2000).

Information on genetic variation and population structure is critical to the conservation of threatened taxa (HOLSINGER and GOTTLIEB, 1991; THOMPSON, 1999). Genetic analyses can provide valuable insights into the process influencing extinction, while genetic data are used to define units for conservation management and for inferring changes in population structure and dynamics (MORITZ, 1995; NEWTON et al., 1999).

Although it is important to gain knowledge of the genetic variation for conservation purposes, detailed information on the levels and distribution of this variation, as well as population structure, are not available for most woody taxa in Korea (HUH et al., 2005). Therefore, the objectives of this study were to estimate how much genetic diversity is maintained in genus *Acanthopanax* and to describe how species-specific markers, which may be useful in germplasm classification are distributed among species.

Materials and Methods

Plant materials

All of the 17 populations were collected from nine taxa in Korea, China and Russia (Table 1). The plant materials consisted of various accessions belonging to eight species and one variety of the genus *Acanthopanax*. One young leaf per mature tree (≥ 5 yr) was sampled. Fifteen plants were randomly collected from each population. In addition, two species of the same family Araliaceae, *Kalopanax pictus* and *Panax ginseng* were provided for the outgroups and used to compare the phylogenetic relationship.

Allozyme analysis

The procedures used for homogenization, starch gel electrophoresis, and enzyme assay were those described by SOLTIS et al. (1983). Leaves were homogenized by mechanical grinding to release enzymes from the cellular and organellar membranes, using a Tris-HCl grinding buffer-PVP solution (WENDEL and WEEDEN, 1989). Electrophoresis was performed with an 11.5% starch gel. Enzyme systems were assayed glutamate oxaloacetate transaminase (GOT) and shikimate dehydrogenase (SKD) were resolved on System 7 (SOLTIS et al., 1983); fluorescent esterase (EST), acid phosphatase

Table 1. – Codes and locations of the genus *Acanthopanax* and the outgroups in this study.

Species	Code	Localities
<i>Acanthopanax</i>	SES101	Mt. Seolack, Kangwon Prov., Korea
<i>sessiliflorus</i>	SES111	Suwon, Gyeonggi Prov., Korea
<i>A. senticosus</i>	SEN204	Mt. Hack, Kangwon Prov., Korea
	SEN241	Gongju, Chungcheong Prov., Korea
<i>A. senticosus</i> for.	SEN301	Mt. Taebak, Kangwon Prov., Korea
<i>inermis</i>	SEN302	Mt. Soback, Kangwon Prov., Korea
<i>A. koreanum</i>	KOR415	Yangsan, Gyeongsangnam Prov.
	KOR406	Masan, Gyeongsangnam Prov.
<i>A. chiisanensis</i>	CHI511	Suwon, Gyeonggi Prov., Korea
	CHI502	Mt. Giri, Gyeongsangnam Prov., Korea
<i>A. rufinerve</i>	RUF601	Mt. Taebak, Kangwon Prov., Korea
	RUF602	Mt. Odae, Kangwon Prov., Korea
<i>A. seoulense</i>	SEO701	Mt. Bukhan, Seoul Prov., Korea
	SEO702	Wonju, Kangwon Prov., Korea
<i>A. siebolianum</i>	COR801	Changchun, China
	COR802	Fusun, China
<i>A. gracilistylus</i>	GRA901	Nerchinsk, Russia
<i>Kalopanax pictus</i>	KAL100	Mt. Giri, Gyeongsangnam Prov., Korea
<i>Panax ginseng</i>	PAN100	Suwon, Gyeonggi Prov., Korea

(ACP), leucine aminopeptidase (LAP), and peroxidase (PER), on System 9; and isocitrate dehydrogenase (IDH), glucose phosphate isomerase (PGI), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (PGD), and phosphoglucomutase (PGM), on System 10.

Statistical analyses

Enzymatic data were based on allele and genotype frequencies in each accession. A locus was considered polymorphic if two or more alleles were detected, and the frequency of the most common allele was less than 0.99. Several standard genetic parameters were estimated using a computer program developed by M. D. LOVELESS and A. F. SCHNABEL (EDWARDS and SHARITZ, 2000). The percentage of polymorphic loci (P_p for population level and P_s for species level), mean number of alleles per locus (A), effective number of alleles per locus (A_E), number of alleles per polymorphic locus (A_p), and gene diversity (H_E) were estimated from the data. Observed heterozygosity (H_o) was compared with the Hardy-Weinberg expected value using Wright's fixation index (F) (Wright 1965). These indices were tested for deviation from zero by χ^2 -statistics following LI and HORVITZ (1953). NEI's gene diversity formulae (H_T , H_S , D_{ST} , and G_{ST}) were used to evaluate genetic diversity within and among populations (NEI, 1973). The G_{ST} coefficient corresponds to the relative amount of differentiation among populations. In addition, χ^2 -statistics were used to detect significant differences in allele frequencies among populations for each locus (WORKMAN and NISWANDER, 1970). NEI's genetic identity (I) (NEI, 1973) was calculated for each pairwise combination of populations. A phylogenetic tree was constructed by the neighborjoining (NJ) method (SAITOU and NEI, 1987) using the NEIGHBOR program in PHYLIP version 3.57 (FELSENSTEIN, 1993). The genetic structure within and among populations was also evaluated using Wright's (1965) F-statistics.

tics, F_{IT} , F_{IS} and F_{ST} . The F_{IT} and F_{IS} coefficients measure excesses of homozygotes relative to the panmictic expectations in the entire samples and within populations, respectively. Deviations of F_{IT} and F_{IS} from zero were tested using χ^2 -statistics (LI and HORVITZ, 1953). In the context of multiallelic loci F_{ST} is denoted as G_{ST} (NEI, 1973). The estimate of Nm (the number of migrants per generation) was based on G_{ST} (SLATKIN and BARTON, 1989).

Results

The level of genetic variation was high in 17 populations of *Acanthopanax* (Table 2). Eleven of 23 loci (47.8%) showed polymorphism in at least one species, while the remaining twelve (*Acp-1*, *Acp-3*, *Est-2*, *Got*, *Idh-1*, *Lap-1*, *Lap-2*, *Mdh-2*, *Per-3*, *Per-4*, *Pgd-2*, and *Pgi-1*) were monomorphic in all species. An average of 34.0% of the loci was polymorphic within species, with individual-accession values ranging from 13.0 to 47.8%. The majority of the polymorphic loci expressed two (*Est-1*, *Mdh-1*, *Skd*, *Acp-2*, *Per-1*, *Per-2*, *Pgi-2*, *Pgm-1*, and *Pgm-2*) or three alleles (*Idh-2* and *Pgd-1*).

Across accessions, the average number of alleles per locus (A) was 1.36, ranging from 1.13 to 1.57 (Table 2). The effective numbers of alleles per locus at the species (A_{es}) and the accession levels (A_{ep}) were 1.25 and 1.15, respectively. Numbers of alleles per polymorphic locus (A_p) were 2.05 across accessions, varying from 2.00 to 2.44. Mean genetic diversity within accessions was 0.096. In particular, the accession SES101 had the highest expected diversity (0.168); accession GRA901, the lowest (0.028).

Table 2. – Allozyme variation within 17 populations of *Acanthopanax*. Percentage of polymorphic loci (P_p), mean number of alleles per polymorphic locus (A_p), mean number of alleles per locus (A), effective number of alleles per locus (A_e), observed heterozygosity (H_{op}), and Hardy-Weinberg-expected heterozygosity or genetic diversity (H_{ep}). Population locations depicted in Table 1.

Population	P_p	A	A_p	A_e	H_{op} (SD)	H_{ep} (SD)
SES101	47.8	1.57	2.18	1.29	0.129 (0.013)	0.168 (0.041)
SES111	47.8	1.52	2.09	1.22	0.114 (0.012)	0.142 (0.036)
SEN204	47.8	1.52	2.09	1.18	0.097 (0.012)	0.123 (0.032)
SEN241	47.8	1.52	2.44	1.16	0.093 (0.011)	0.112 (0.030)
SEN301	39.1	1.43	2.11	1.19	0.097 (0.011)	0.120 (0.035)
SEN302	39.1	1.43	2.11	1.21	0.103 (0.012)	0.127 (0.036)
KOR415	39.1	1.43	2.11	1.19	0.098 (0.011)	0.127 (0.035)
KOR406	38.9	1.43	2.11	1.21	0.101 (0.012)	0.135 (0.036)
CHI511	34.8	1.35	2.00	1.16	0.089 (0.011)	0.107 (0.033)
CHI502	34.8	1.35	2.00	1.13	0.072 (0.010)	0.086 (0.030)
RUF601	34.8	1.35	2.00	1.12	0.072 (0.010)	0.085 (0.028)
RUF602	30.4	1.30	2.00	1.13	0.066 (0.009)	0.082 (0.030)
SEO701	21.7	1.22	2.00	1.08	0.041 (0.008)	0.052 (0.024)
SEO702	21.7	1.22	2.00	1.06	0.034 (0.007)	0.040 (0.020)
COR801	17.4	1.17	2.00	1.07	0.043 (0.008)	0.049 (0.024)
COR802	21.7	1.22	2.00	1.07	0.036 (0.007)	0.045 (0.023)
GRA901	13.0	1.13	2.00	1.04	0.019 (0.005)	0.028 (0.018)
Mean	34.0	1.36	2.05	1.15	0.077	0.096
SD	2.33	0.14	0.06	0.07	0.002	0.007

Table 3. – Estimates of genetic diversity statistics and 11 polymorphic loci in *Acanthopanax*. Total genetic diversity (H_T); genetic diversity within populations (H_S), and among populations (D_{ST}); deviations of genotype frequencies from Hardy-Weinberg expectations over all populations (F_{IT}), and within individual population (F_{IS}); and proportion of total genetic diversity partitioned among populations (G_{ST}).

Locus	H_T	H_S	D_{ST}	F_{IS}	F_{IT}	G_{ST}
<i>Est-1</i>	0.396	0.394	0.002	0.167	0.172	0.006
<i>Per-1</i>	0.065	0.055	0.009	0.291	0.391	0.142**
<i>Per-2</i>	0.301	0.270	0.030	0.208	0.288	0.101
<i>Pgd-1</i>	0.363	0.330	0.033	0.178	0.253	0.091
<i>Idh-2</i>	0.235	0.222	0.013	0.232	0.276	0.057
<i>Mdh-1</i>	0.045	0.042	0.003	0.046	0.113	0.071
<i>Skd</i>	0.244	0.227	0.017	0.247	0.301	0.071
<i>Acp-2</i>	0.226	0.249	0.007	0.233	0.255	0.029
<i>Pgi-2</i>	0.152	0.135	0.017	0.187	0.278	0.112*
<i>Pgm-1</i>	0.165	0.154	0.011	0.208	0.259	0.065
<i>Pgm-2</i>	0.233	0.229	0.004	0.151	0.167	0.019
Mean	0.223	0.210	0.014	0.195	0.250	0.069

* $p < 0.05$; ** $p < 0.01$.

F_{IS} , a measure of the deviation from random mating within the nine species, was 0.195, ranging from 0.046 for *Mdh-1* to 0.291 for *Per-1* (Table 3). The observed significant and positive F_{IS} value (0.195) indicated a significant deficit of heterozygotes in the species.

Total genetic diversity values (H_T) varied between 0.045 (*Mdh-1*) and 0.396 (*Est-1*), for an average over all polymorphic loci of 0.223 (Table 3). Interlocus variation in the within-accession genetic diversity (H_S) was high (0.210). On a per-locus basis, the proportion of total genetic variation due to differences among accessions (G_{ST}) ranged from 0.006 for *Est-1* to 0.142 for *Per-1*, with a mean of 0.069. This indicated that about 6.9% of the total allozyme variation was among species. The estimate of gene flow, based on G_{ST} , was slightly high among accessions of *Acanthopanax* ($N_m = 0.069$). Values of genetic distance (D) were < 3.159 . Genetic identity values among pairs of accessions ranged from 0.336 to 0.994.

Our analysis of fixation indices, calculated for all polymorphic loci in each accession, showed a slight deficiency of heterozygotes relative to Hardy-Weinberg expectations (Table 4). For example, most fixation indices were positive (110/130), of which 16 indices (14.5%) departed significantly from zero ($p < 0.05$). In contrast, of 20 negative indices, none was significant from zero ($P > 0.05$).

Adh-2-a allele can be recognized as unique allele of *A. sessiliflorus* and it is not found in any other species. Differences of allelic frequencies for other alleles are also existed among species (data not shown).

Clustering of *Acanthopanax* accessions, using NJ, was performed based on genetic distances (Fig. 1). The dendrogram showed several distinct groups. Although many accessions were well separated from each other, this tree also did not reveal any genetic differentiation among accessions for either *A. senticosus* or *A. senticosus* for. inermis.

Table 4. – Wright's fixation indices for 17 populations of *Acanthopanax* using allozyme analyses.

Accession	<i>Est-1</i>	<i>Per-1</i>	<i>Per-2</i>	<i>Pgd-1</i>	<i>Idh-2</i>	<i>Mdh-1</i>	<i>Skd</i>	<i>Acp-2</i>	<i>Pgi-2</i>	<i>Pgm-1</i>	<i>Pgm-2</i>
SES101	0.173	0.252	0.185	0.226	0.236	-0.051	0.346*	0.464**	0.163	0.283	0.216
SES111	0.083	0.364*	0.159	0.182	0.274	-0.100	0.363*	0.205	0.250	0.202	0.208
SEN204	0.020	0.269	0.324	0.137	0.331	-0.036	0.408**	0.292	0.250	0.234	0.131
SEN241	0.140	0.356*	0.260	0.050	-0.026	0.276	0.220	0.198	0.163	0.198	0.202
SEN301	0.105	-	0.253	0.146	-	-	0.216	0.228	0.331	0.149	0.292
SEN302	0.185	-	0.263	0.227	0.327	-	0.163	-0.047	0.271	0.181	0.100
KOR415	0.208	-	0.208	0.272	0.271	-	0.381*	0.307	0.036	0.272	0.181
KOR406	0.233	-	0.375*	0.270	0.324	-	0.243	0.244	0.084	0.357*	0.288
CHI511	0.208	-	0.065	-0.054	0.288	-	0.115	0.181	-	0.385*	0.113
CHI502	0.065	-	0.171	0.207	-0.038	-	0.369*	0.149	0.473**	-	-0.054
RUF601	0.171	-	0.075	0.229	0.267	-	-0.054	0.335	-	-0.017	0.100
RUF602	0.190	-	0.302	-	-0.056	-	-0.016	0.311	-	-0.035	0.105
SEO701	0.253	-	-	-	0.367*	-	0.163	-0.047	-	-	-0.036
SEO702	0.253	-	-	-	0.344*	-	-	-0.024	-	-0.035	-0.066
COR801	0.128	-	-	-	0.205	-	-	0.090	-	-	0.149
COR802	0.196	-	-	-	-0.024	-	-	0.283	-	-0.016	0.358*
GRA901	0.381*	-	-	-	-	-	-	-	-	-	0.272

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

Discussion

In allozyme analysis, nine species belonging to genus *Acanthopanax* maintain a lower than average level of genetic diversity compared with other plant species (HAMRICK and GODT, 1989). For example, its genetic

diversity of 0.096 is lower than that for temperate-zone species (0.146), dicots (0.136), species with a sexual reproduction mode (0.151), and those with a long-lived woody habit (0.177) (HAMRICK and GODT, 1989). The percentage of polymorphic loci at the species level for *Acanthopanax* is 34.0%, which is also lower than that for species with temperature-zone distributions (48.5%), dicots (44.8%), species with a sexual reproduction mode (51.6%), and long-lived and woody (64.7%) (HAMRICK and GODT, 1989).

A striking feature of this study is the lacking of intra-populations variation. Only 6.9% variation was found among populations and about 93.1% within populations. The Korean populations were less differentiated than other insect or wind-pollinated outcrossing species (HAMRICK et al., 1992).

Common life history traits, such as wind dispersal or animal dispersal of both pollen grains and seeds, reproductive capability, similar longevity and successional behavior, could more readily account for most of the homology in the population genetics of these species, and most likely for the low differentiation observed at the intraspecific (LOVELESS and HAMRICK, 1984).

Geographic range has been shown to be strongly associated with the level of variation maintained both within populations and at the species level (THOMPSON, 1999). Widely distributed species tend to maintain more variation than the more narrowly distributed species level (LOVELESS and HAMRICK, 1984; HAMRICK and GODT, 1989). For all Korean *Acanthopanax* taxa where the number of alleles per polymorphic loci was calculated, relatively widespread species (*A. sessiliflorus* and *A. senticosus*) had more alleles than restricted species (*A. koreanum*, *A. chiisanensis*, and *A. rufinerve*). In addition, effective population sizes also have an important role in maintaining genetic diversity. The periodical cutting of branches and stems has often been moved from hillside to nearby farmhouses for the purpose of medicine during the past several decades. Small populations tend to have

Figure 1. – A phylogenetic tree for *Acanthopanax* based on allozyme analysis.

fewer multilocus genotypes and genetic diversity than large populations. If *Acanthopanax* is self-compatible, smaller populations probably exhibit more selfing than larger populations by virtue of the limited opportunities for outcrossing during any flowering season. Considering the small and isolated populations observed in *Acanthopanax*, probable mating among relatives via localized pollinator behavior rather than self-pollinating occurs within these populations. Such structure can lead to biparental inbreeding or sib-mating, causing heterozygote deficiencies.

A substantial heterozygote deficiency in some populations and at some loci ($F_{IS} = 0.195$) (Table 3). The patch distribution of related individuals should generate a Wahlund effect. Our sampling included individuals from several patches per population, resulting in an overall deficiency of heterozygotes. It is probable that the combination of these factors may contribute to heterozygote deficiencies within these populations (Table 3).

A. senticosus and *A. senticosus* for. *inermis* were morphologically distinguished from each other. One of the most striking features between both taxa were the sharp and stiff spines (or thorns) growing along the stem. *A. senticosus* is covered with many spines, whereas *A. senticosus* for. *inermis* is thornless. Three species (*A. koreanum*, *A. seoulense* and *A. rufinerve*) from Korea formed another group in allozyme markers. *A. koreanum* was closely related to the populations from the southern part of the coast of the Korean Peninsula. *A. rufinerve* has many hairs on leaves. *A. chiisanensis* is most closely related to *A. senticosus*. Thus this species is considered a sister species of *A. senticosus*.

By current categories for threatened taxa in Korea, almost all species belonging to genus *Acanthopanax* should be considered threatened. The probability of extinction of any single population is high, since the populations of natural *A. senticosus* are so small. Ecological management of these populations will be necessary to preserve the species. Many other populations of *Acanthopanax* as well as *A. senticosus* are declining in population size because of habitat loss from harvesting for medicinal purposes. Populations showing the highest genetic diversity by allozymes could be recommended for *in-situ* conservation.

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References

- BARTISH, J. V., N. JEPSON, G. I. BARTISH, R. LU and H. NYBOM (2000): Inter- and intraspecific genetic variation in Hippophae (Elaeagnaceae) investigated by RAPD markers. *Plant Syst. Evol.* **225**: 85–101.
- EDWARDS, A. L. and R. R. SHARITZ (2000): Population genetics of two rare perennials in isolated wetlands: *Sagittaria isoetiformis* and *S. teris* (Alismataceae). *Am. J. Bot.* **87**: 1147–1158.
- FELSENSTEIN, J. (1993): PHYLIP (Phylogeny Inference Package) version 3.5s. Distributed by the author. Department of Genetics, Univ. Washington, Seattle.
- FOSTER, S. (1996): Siberian Ginseng-*Eleutherococcus senticosus*. Botanical Booklet Series, No 302. Austin: American Botanical Council.
- HAMRICK, J. L. and M. J. W. GODT (1989): Allozyme diversity in plant species, pp. 304–319 in *Plant population genetics, breeding and genetic resources*, edited by A. H. D. BROWN, M. T. CLEGG, A. L. KAHLE, B. S. WEIR, Sinauer Associates, Sunderland/MA.
- HAMRICK, J. L., M. J. W. GODT and S. L. SHERMAN-BROYLES (1992): Factors influencing levels of genetic diversity in woody plant species. *New Forests* **6**: 95–124.
- HOLSINGER, K. E. and L. D. GOTTLIEB (1991): Conservation of rare and endangered plants: Principles and prospects, pp. 195–223 in *Genetics and conservation of rare plants*, edited by D. A. FALK, K. E. HOLSINGER, Oxford University Press.
- HUH, M. K. (1999): Genetic diversity and population structure of Korean Alder (*Alnus japonica* : Betulaceae). *Can. J. For. Res.* **29**: 1311–1316.
- HUH, M. K., H. W. HUH and K. BACK (2005): Genetic diversity and population structure of Korean *Acanthopanax sessiliflorus* (Araliaceae) using AFLP. *Korean J. Genet.* **27**: 71–79.
- LEE, Y. N. (1997): *Flora of Korea*. Kyo-Hak Publishing Co, Seoul, Korea.
- LI, C. C. and D. G. HORVITZ (1953): Some methods of estimating the inbreeding coefficient. *Am. J. Hum. Genet.* **5**: 107–117.
- LOVELESS, M. D. and J. L. HAMRICK (1984): Ecological determinants of genetic structure in plant populations. *Ann. Rev. Ecol. System.* **15**: 65–95.
- MORITZ, C. (1995): Uses of molecular phylogenies for conservation. *Philosophical Transactions of the Royal Society of London Series B: Biol. Sci.* **349**: 113–118.
- NEI, M. (1973): Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA* **70**: 3321–3323.
- NEWTON, A. C., T. ALLNUTT, A. C. M. GILLIES, A. LOWE and R. A. ENNOS (1999): Molecular phylogeography, interspecific variation and the conservation of tree species. *Trends Ecol. Evol.* **14**: 140–145.
- SAITOU, N. and M. NEI (1987): The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425.
- SLATKIN, M. and N. H. BARTON (1989): A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* **43**: 1349–1368.
- SOLTIS, D. E., C. H. HAUFLE, D. C. DARROW and G. J. GASTONY (1983): Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Am. Fern J.* **73**: 9–27.
- THOMPSON, J. D. (1999): Population differentiation in Mediterranean plants: insight into colonization history and the evolution and conservation of endemic species. *Heredity* **82**: 229–236.
- WENDEL, J. F. and N. F. WEEDEN (1989): Visualization and interpretation of plant isozymes, pp. 5–45 in *Isozymes in plant biology*, edited by D. E. SOLTIS and P. S. SOLTIS, Chapman and Hall, London.
- WORKMAN, P. L. and J. D. NISWANDER (1970): Population studies on southern Indian tribes. II. local genetic differentiation in the Papago. *Am. J. Hum. Genet.* **22**: 24–49.
- WRIGHT, S. (1965): The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution* **19**: 395–420.