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Genetic Diversity and Population Structure of *Kalopanax pictus* (Araliaceae)

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***Kalopanax pictus* (Araliaceae) is a long-lived woody species primarily distributed throughout East Asia. This species is regarded as medically and ecologically important in Korea. We used starch gel electrophoresis to investigate its allozyme variation and genetic structure in samples from Korean populations (both natural and cultivated). Our analysis of 10 enzymes revealed 18 loci, of which 10 were polymorphic (55.6%). Genetic diversity at the species and population levels were 0.200 and 0.149, respectively, with the mean for cultivated populations (0.124) being lower than for natural (wild) populations (0.181). Asexual and sexual reproduction modes, perennial habitat, and longevity all were possible factors contributing to such high diversity. An indirect estimate of the number of migrants per generation (N_m = 1.08) indicated that gene flow was not extensive among these Korean populations. Therefore, we suggest that geographical distance as well as reproductive isolation between wild and cultivated plants may play roles in shaping the population structure of this species.**

Keywords: allozyme variation, genetic structure, *Kalopanax pictus*

Information on genetic variation and population structure is critical to the conservation of threatened taxa (Holsinger and Gottlieb, 1991; Allnutt et al., 2003). Genetic analyses can provide valuable insights into the process influencing extinction (Clarke and Young, 2000), 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).

The genus *Kalopanax* (Araliaceae) comprises one species, *K. pictus*, which is distributed in the temperate regions of East Asia (Lee, 1997). Typical populations are small and distributed in patches. *K. pictus* can be classified as a narrow habitat species because it is usually found on subsites of several Korean mountains, at elevations of 300 to 400 m. This long-lived perennial has yellow-green flowers (Sun et al., 1988), and is a polygamo-monoecious diploid species (2n=48), being predominantly out-crossed via wind-pollination (Kim, 1996; Lee, 1997). Although plants grow high in the mountains on fertile soil, they are also extensively cultivated as a medicinal muscle relaxant. *K. pictus* is also economically important for its stems, which historically were used in Korea for wooden shoes with clogs. Household goods are also valued because of the figured heartwood.

Until recently, much of the Korean forest has been disturbed by the cutting of trees and shrubs for firewood in rural areas (Huh, 1999; Huh et al., 2001). Most sites are now being revegetated both naturally and artificially (Huh et al., 2002). 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 and Huh, 2001). Therefore, the objectives of this study were 1) to estimate how much allozyme diversity is maintained in *K. pictus* and to describe how genetic variation is distributed within and among populations; and 2) to figure out how the domestication process has eroded such levels of genetic variation in cultivated populations, as has been seen with many other species (Doebley, 1989). In addition, we compared the genetic diversity and structure of wild and cultivated *K. pictus* populations with other woody species having similar life-history characteristics.

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MATERIALS AND METHODS

Sampling Procedure and Enzyme Electrophoresis

Leaf tissues were collected from four natural (wild) and five cultivated populations of *Kalopanax pictus* in Korea (Fig. 1). At least 26 plants were sampled from each population (one leaf per plant). In this country, the species tends to occur in small, isolated patches, on a limited number of sites. We found only four natural populations that had maintained effective sizes over a five-year period (1999-2003). Because of the sensitive nature of these wild populations, site information for scientific study is available from the authors only upon request.

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. Ten enzyme systems (per Soltis et al., 1983) were assayed -- glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1) and shikimate dehydrogenase (SKD, EC 1.1.1.25) were resolved on System 7; fluorescent esterase (EST, EC 3.1.1.2), acid phosphatase (ACP, EC 3.1.3.2), leucine aminopeptidase (LAP, EC 3.4.11.1), and peroxidase (PER, EC 1.11.1.7), on System 9; and glucose phosphate isomerase (PGI, EC 5.3.1.9), malate dehydrogenase (MDH, EC 1.1.1.37), 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.43), and phosphoglucomutase (PGM, EC 2.7.5.1), on System 10.

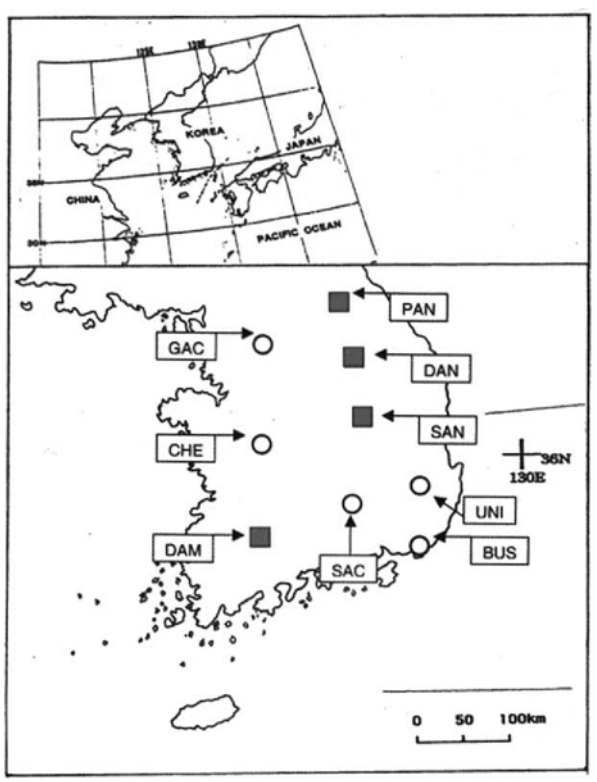

Figure 1. Collection sites for populations of *K. pictus* for allozyme analysis. ▒: Wild populations; ○: Cultivated populations. PAN: Paengchang, Kwon-do, DAN: Dnayang, Cheongchungbuk-do, SAN: Sangju, Gyeongsangbuk-do, MAM: Damyang, Ceonlanam-do, GAC: Gacheon, Gyeongki-do, CHE: Cheongyang-gun, Chungcheongnam-do, SAC: Sacheon, Gyeongsangnam-do, UNI: Unionyang-up, Ulsan, BUS: Kumjeong-gu, Busan.

Data Analysis

Enzymatic data were based on allele and genotype frequencies in each population. The following genetic parameters were calculated using a POPGENE computer program (ver. 1.31) developed by Yeh et al. (1999): the percentage of polymorphic loci (P_p for population level and P_s for species level); mean numbers of alleles per locus (A) and per polymorphic locus (A_p); effective number of alleles per locus (A_e); and gene diversity (H_e) (Hamrick et al., 1992). Diversity at the level of species (indicated with the subscript s) and mean population (subscript $_P$) was calculated as described by Hartl and Clark (1989). The observed heterozygosity (H_o) was compared with Hardy-Weinberg-expected values, using Wrightís fixation index (F) for inbreeding coefficients (Wright, 1965). Deviations from genotype frequencies expected under the Hardy-Weinberg equilibrium were tested by the GENEPOP program (ver. 3.1; Raymond and Rousset, 1995). Multiple tests to search for unique alleles were performed according to the sequential Bonferroni procedure (Lessios, 1992). A Wilcoxon paired-sample test was conducted for the comparison of genetic parameters to infer whether differences existed between the two groups (i.e., natural vs. cultivated).

To elucidate the organization of variability within *K. pictus*, we examined the genetic variation by partitioning the total genetic diversity (H_t) to within- (H_s) and among- (D_{st}) population components, using the genetic diversity statistics of Nei (1973). A measure of the differentiation among populations, relative to the total diversity, was calculated at each locus as $G_{st} = D_{st} / H_t$. Weir and Cockerham (1984) estimates of Wrightís F_{st} (G_{st}) were computed for variable loci with FSTAT (ver. 1.2; Goudet, 1995).

To determine the extent of genetic departure, we

calculated the Nei genetic identity (I) and genetic distance (D) for each pairwise combination of populations (Nei, 1972). A genetic distance matrix was used to construct a dendrogram, using UPGMA (unweighted pair group method with arithmetic average) in the neighbor algorithm of the Phylogeny Inference Package (PHYLIP ver. 3.57; Felsenstein, 1993).

Finally, the genetic structure within and among populations was evaluated using Wright (1965) *F* statistics; F_{IT}, F_{IS}, and F_{ST}. F_{IT} and F_{IS} measure excesses of homozygotes or heterozygotes relative to panmictic expectations within samples and within populations, respectively. Deviations of F_{IT} and F_{IS} from zero were tested using χ^2-statistics (Li and Horvitz, 1953). Two indirect estimates of gene flow were then calculated. Estimates of the number of migrants per generation (N_m) were based on G_{ST} or the average frequency of private alleles found in only one population (Slatkin, 1985). The correlation between geographical and genetic distances was tested by a modified Mantel's test (Smouse et al., 1986).

RESULTS

The level of genetic variation was high in our nine *Kalopanax pictus* populations. Ten of 18 loci (55.6%) showed polymorphism in at least one population, while the remaining eight (*Acp-1*, *Acp-3*, *Lap-2*, *Got*, *Mdh-3*, *Per-1*, *Pgd*, and *Pgm*) were monomorphic in all populations. An average of 57.5% of the loci was polymorphic within populations, with individual-population values ranging from 33.3 to 50.0% (Table 1). The majority of the polymorphic loci expressed two (*Acp-2*, *Per-2*, *Per-3*, and *Pgi*) or three alleles (*Est-2*, *Est-3*, *Lap-1*, *Mdh-2*, and *Skd*), whereas the remaining one (*Mdh-1*) expressed four.

Across populations, the average number of alleles per locus (*A*) was 1.57, ranging from 1.44 to 1.72 (Table 1). The effective numbers of alleles per locus at the species (A_{es}) and the population levels (A_{ep}) were 1.36 and 1.29, respectively. Numbers of alleles per polymorphic locus (A_p) were 2.41 across populations, varying from 2.29 to 2.57. Mean genetic diversity within populations was 0.149. In particular, the population PAN had the highest expected diversity (0.202); population SAC, the lowest (0.096).

F_{IS}, a measure of the deviation from random mating within the nine populations, was 0.391, ranging from 0.270 for *Pgi* to 0.529 for *Per-3* (Table 2). The observed significant and positive F_{IS} value (0.391) indicated a significant deficit of heterozygotes in the populations.

Total genetic diversity values (H_T) varied between 0.058 (*Pgi*) and 0.589 (*Mdh-1*), for an average over all polymorphic loci of 0.360 (Table 2). Interlocus variation in the within-population genetic diversity (H_S) was high (0.285). On a per-locus basis, the proportion of

Table 1. Allozyme variation within nine populations of *K. pictus*. Population sizes (PS), percentage of polymorphic loci (*P*), mean number of alleles per polymorphic population (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 Fig. 1.

Population	PS	P_p	*A*	A_p	A_e	H_{op} (SD)	H_{ep} (SD)
Wild							
PAN[1]	67	44.4	1.67	2.50	1.45	0.127 (0.014)	0.202 (0.058)
DAN	55	44.4	1.61	2.38	1.35	0.095 (0.012)	0.173 (0.054)
SAN	56	38.9	1.56	2.43	1.38	0.134 (0.014)	0.172 (0.060)
DAM	60	50.0	1.72	2.44	1.36	0.103 (0.012)	0.177 (0.055)
Mean	59.5	44.4	1.64	2.44	1.39	0.115	0.181
Cultivated							
GAC	35	38.9	1.61	2.57	1.29	0.088 (0.012)	0.155 (0.055)
CHE	32	38.9	1.56	2.43	1.21	0.066 (0.010)	0.120 (0.046)
SAC	26	33.3	1.44	2.33	1.15	0.054 (0.010)	0.096 (0.036)
UNI	37	38.9	1.50	2.29	1.24	0.075 (0.011)	0.135 (0.047)
BUS	30	33.3	1.44	2.33	1.20	0.061 (0.010)	0.116 (0.046)
Mean	32.0	36.7	1.51	2.39	1.22	0.069	0.124
t-Test		*	*	ns	**	**	**
Total[2]		40.2	1.57	2.41	1.29	0.089	0.149
Species		55.6	1.94	2.70	1.36	–	0.200

[1]Acronyms as in Figure 1.
[2]Population level.
*p<0.05; **p<0.01; ns: not significant (p>0.05).

Table 2. Estimates of genetic diversity statistics and 10 polymorphic loci in *K. pictus*. 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}
Mdh-1	0.589	0.571	0.007	0.340	0.359	0.029
Mdh-2	0.260	0.222	0.038	0.305	0.407	0.146
Est-1	0.493	0.328	0.165	0.508	0.673	0.334**
Est-2	0.478	0.254	0.224	0.313	0.635	0.469**
Skd	0.305	0.256	0.048	0.340	0.444	0.158
Per-2	0.242	0.210	0.032	0.452	0.525	0.132
Per-3	0.444	0.336	0.108	0.529	0.644	0.243*
Lap-1	0.456	0.350	0.107	0.498	0.615	0.234*
Pgi	0.058	0.051	0.007	0.270	0.360	0.118
Acp-2	0.267	0.270	0.006	0.351	0.366	0.023
Mean	0.360	0.285	0.075	0.391	0.502	0.189

*p<0.05; **p<0.01.

Table 3. Wright's fixation indices for nine populations of *K. pictus*. Population locations depicted in Fig. 1.

Population	*Mdh-1*	*Mdh-2*	*Est-1*	*Est-2*	*Skd*	*Per-2*	*Per-3*	*Lap-1*	*Pgi*	*Acp-2*
Wild										
PAN	0.315*	0.357	0.254	0.361	–	–	0.548**	0.288	–	0.339
DAN	0.156	–	0.819***	–0.017	0.636***	0.266	0.478**	0.691***	–	0.388*
SAN	0.140	0.238	–	–	0.187	0.239	0.231	0.400*	–	0.300
DAM	0.233	0.292	0.454*	–0.038	0.523**	–	0.537**	0.677***	–	0.356
% of significant	25.0	0	50.0	0	66.7	0	75.0	75.0	–	25.0
Cultivated										
GAC	0.221	0.379*	–	–	0.241	0.717***	0.646**	0.440**	–	0.525**
CHE	0.561***	0.385*	0.518***	0.568*	0.432*	–	–	–	–	0.357
SAC	0.471**	–	0.621***	0.464*	0.258	–	–	0.357	–	0.427*
UNI	0.375*	–	0.579***	0.524*	–	–	0.703***	0.205	0.354	0.258
BUS	0.744***	–	0.664***	0.132	0.635***	–	-	0.342	0.195	0.252
% of significant	80.0	100.0	100.0	75.0	66.7	–	100.0	25.0	0	40.0

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

total genetic variation due to differences among populations (G_{ST}) ranged from 0.023 for *Acp-2* to 0.489 for *Est-2*, with a mean of 0.189. This indicated that about 18.9% of the total allozyme variation was among populations. The estimate of gene flow, based on G_{ST}, was slightly high among Korean populations of *K. pictus* (N_m = 1.08). Values of genetic distance (D) were <0.159. Genetic identity values among pairs of populations ranged from 0.853 to 0.994.

Our analysis of fixation indices, calculated for all polymorphic loci in each population, showed a slight deficiency of heterozygotes relative to Hardy-Weinberg expectations (Table 3). For example, most fixation indices were positive (62/63), of which 30 indices (48.4%) departed significantly from zero ($p < 0.05$). Fixation *F* values for cultivated populations differed more significantly from zero than did wild populations.

Clustering of *K. pictus* populations, using UPGMA, was performed based on genetic distances (Fig. 2). The dendrogram showed two distinct groups, although cultivated and natural populations were not well separated from each other. This tree also did not reveal any genetic differentiation among local populations for either cultivated or natural *K. pictus*. Geographically close populations were situated in close positions in the dendrogram.

DISCUSSION

Kalopanax pictus maintains a higher than average level of genetic diversity compared with other plant species. For example, its genetic diversity of 0.200 is greater than that for temperate-zone species (0.146), dicots

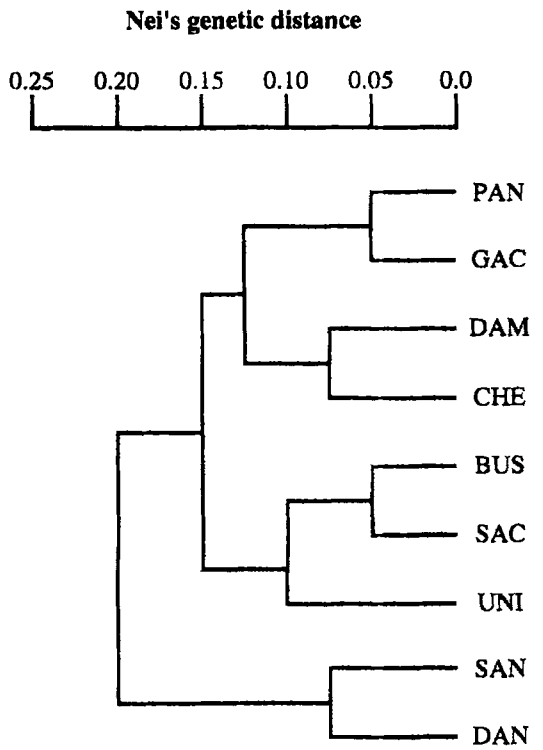

Figure 2. Phenogram of relationships among nine populations of *K. pictus*, based on data for genetic distance, as obtained by starch gel electrophoresis. See Fig. 1 for population locations.

(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 *K. pictus* is 55.6%, which is also higher than that for species with temperature-zone distributions (48.5%), dicots (44.8%), and species with a sexual reproduction mode (51.6%), but lower than for those that are long-lived and woody (64.7%) (Hamrick and Godt, 1989).

The relatively high level of genetic variation found in *K. pictus* is consistent with several aspects of its biology. First, the particular breeding system that a plant possesses is an important determinant of variability at both the species and population levels. Because *K. pictus* performs both asexual and sexual reproduction, its ability to regenerate by root or stump sprouting may explain the high level of genetic diversity found within populations (Huh, 1999). Whereas sexual reproduction might initially act to enhance genetic variation, asexual reproduction can maintain this enhanced variability (Bayer, 1990). Asexual reproduction assures the stabilization and persistence of a phenotype that is well adapted to the immediate environment (Huh, 1999).

Second, a perennial and/or long-lived species generally maintains relatively higher levels of variation than do annuals (Loveless and Hamrick, 1984). The rings observed in our sampling of wild *K. pictus* stems indicated that the plants are at least 20 to 30 years old. Because individuals of this species are so long-lived, opportunities for the accumulation of mutations should be high (Ledig, 1986).

One of the most striking features of our study results was the significant difference in diversity between natural (or wild) populations and cultivated populations. The average rate of polymorphic bands was 44.4% for the former and 36.7% for the latter (Table 1). Genetic diversity for our wild populations (mean $H_o = 0.115$ and $H_e = 1.181$) was significantly different from that of the cultivated populations (mean $H_o = 0.069$ and $H_e = 0.124$, $p < 0.05$). These comparisons suggest that diversity in the natural populations is higher than that for cultivated plants (per a one-tailed Wilcoxonís signed rank test; Table 1). Comparing the banding patterns from wild and cultivated populations revealed four unique bands for the former, but none for the latter. The most alleles found with the cultivated populations were a subset of wild populations. Therefore, these genetic diversity parameters indicate that cultivated populations are genetically depauperate relative to their presumptive progenitor, and that the domestication process has eroded the level of genetic variation in this species.

The life history characteristics of *K. pictus* would lead one to predict a change in its mating system. However, it should be noted that the number of age classes is quite different for our two groups of populations. Whereas the artificial populations comprised 5 to 15 different age classes, the natural populations consisted of 20- to 30-year-old plants. A few juveniles within these wild populations were being transferred either directly or indirectly (via botanical gardens) to artificial populations because it is difficult to relocate adult individuals from mountainous areas to an agricultural site. We also observed a pattern of greater genetic diversity as population sizes increased (Fig. 3).

Domestication processes that involve artificial selection may erode the level of genetic diversity in cultivated populations. Doebley (1989) has demonstrated that most crops show a reduced level of polymorphisms compared with their presumed progenitors. Likewise, Aldrich et al. (1992) have reported that wild species usually maintain a higher degree of polymorphy than do their cultivated counterparts. Nevertheless, in some species, such as barley and common buckwheat, cultivated populations have more genetic variability (Brown, 1978; Ohnishi and Asano, 1999) while for soybean,

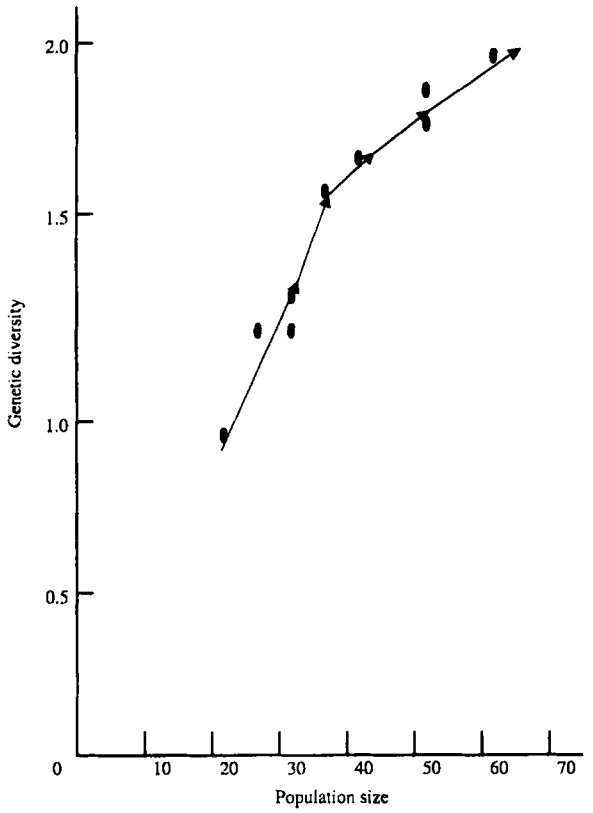

Figure 3. Relationship between population sizes and genetic diversity for nine populations of *K. pictus*.

domestication has not eroded its level of genetic variation (Brown, 1978; Kiang and Gorman, 1983). In general however, higher levels of variability are expected with wild species because they are not subject to selection pressures inherent with domestication and their maintenance of higher genetic variation favors survival under natural conditions (Chan and Sun, 1997).

Naturalized populations of cultivated species are, ultimately, a product of both their biological characteristics and historical cultivation practices (Hagen and Hamrick, 1998). Our cultivated populations of *K. pictus* may have been founded by a small sample of larger or moderate populations (i.e., the 'founder effect'). We identified a positive correlation between genetic distance and geographic distance ($r = 0.41$, $p < 0.05$). With an r value of 0.41, 16.5% of the variation in genetic distance value was explained by geographic distance. The northern populations clustered together, as expected, suggesting that population GAC might have originated from population PAN (Fig. 2). Two populations (CHE and DAM), from the coast of the Yellow Sea between China and Korea, formed another group.

The analysis of fixation indices, calculated for all polymorphic loci in our nine populations, showed a deficiency of heterozygotes relative to Hardy-Weinberg expectations (Table 3). Because most of the variation was spread within populations, a small founding sample from one population may have carried some proportions of the variation in the species. Fixation F values for the cultivated populations differed more significantly from zero than did the wild populations, thereby indicating nonrandom mating or a founder effect. In addition, the sign of those deviations was uniform (all significant positive values), but the deviations were restricted to particular populations or loci (see also Hagen and Hamrick, 1998).

Genetic differentiation among populations is principally a function of natural selection, genetic drift, and gene flow via pollen and seed dispersal (Loveless and Hamrick, 1984). We were most interested in the relatively high degree of differentiation recorded between populations, compared with those reported from studies with other woody species. For example, based on allozyme analyses, the genetic variation in predominantly outcrossed wind-pollinated species averages <10% between populations (Hamrick and Godt, 1989).In contrast, for *K. pictus*, about 18.9% of the total variation was due to differences among populations ($G_{ST} = 0.189$). That high level of differentiation also suggests gene flow among the populations is low ($N_m = 1.08$). Farmers in Korea historically have transplanted *K. pictus* from its natural habitat (mountains) to sites near their houses. Although we did not analyze the further subdivision of a local population, we may infer that genetic variation that resides mainly within wild populations is further maintained in subpopulations or demes that are distributed in patches. This can be explained either by random drifting of neutral alleles or by micro-environmental selection for adaptive alleles (Beebe et al., 2000).

If we consider an N_m value of 1.08 to be similar to 1.0, then genetic drift should be a factor in determining populations of *K. pictus*. Thus, the levels of gene flow we calculated here are not of sufficient magnitude to counterbalance genetic drift, and may play a major role in shaping the genetic structure of the populations. Gene flow for this species may be partially explained by its modes of seed and pollen dispersal. For example, fruits mature from late October to early November, after which they are transported by birds and rodents (Huh, observation). Fruiting in *K. pictus* is an exceptionally low event, with only one or two seeds occurring in each drupe. This means that most populations are small and isolated from each other.

The transplanting of materials from the mountains to the lowlands does not greatly aid in the preservation of this narrowly distributed species, but rather it results in the destruction of habitat. Conservation of rare species requires that ecological and genetic factors be considered (Neel and Ellstrand, 2003). Specific environmental conditions, such as a mountain habitat (400 to 500 m above sea level) and very fertile soil, may be of primary importance in the preservation of most populations. Therefore, artificial transplants cannot play a crucial role in determining the conservation of rare or endemic species. Moreover, the degree to which genetic variation is distributed among populations is critical to preserving genetic diversity and the evolutionary potential of a species (Hamrick and Godt, 1989; Sin et al., 2002).

Based on the available data from this study, such as a relatively high G_{ST} value, we believe that several populations in each group should be preserved. This especially includes those with high variation, such as populations PAN and DAM. These two could be used as a source of genetic diversity in restoring genetically poor populations. In addition, we recommend that desirable conservation populations should each include at least 55 plants because greater genetic diversity is observed with increasing population sizes (Fig. 3).

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LITERATURE CITED

Aldrich PR, Doebley J, Schertz KF, Stee A (1992) Patterns of allozyme variation in cultivated and wild *Sorghum bicolor*. Theor Appl Genet 85: 451-460

Allnutt TR, Newton AC, Premoli A, Lara A (2003) Genetic variation in the threatened South American conifer *Pilgerodendron uviferum* (Cupressaceae), detected using RAPD markers. Biol Conserv 114: 245-253

Bayer RJ (1990) Patterns of clonal diversity in the *Antennaria rosea* (Asteraceae) polyploid agamic complex. Amer J Bot 77: 1313-1319

Beebe S, Skroch PW, Tohme J, Duque MC, Pedraza F, Nienhuis J (2000) Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. Crop Sci 40: 264-273

Brown AHD (1978) Isozymes, plant population genetic structure and genetic conservation. Theor Appl Genet 52: 145-157

Chan KF, Sun M (1997) Genetic diversity and relationships detected by isozyme and RAPD analysis of crop and wild species of *Amaranthus*. Theor Appl Genet 95: 965-973

Clarke GM, Young AG (2000) Introduction: Genetics, demography and the conservation of fragmented populations. *In* AG Young, GM Clarke, eds, Genetics, Demography and Viability of Fragmented Populations. Cambridge University Press, pp 1-6

Doebley J (1989) Isozymic evidence and the evolution of crop plants. *In* DE Soltis, PS Soltis, eds, Isozymes in Plant Biology. Dioscorides Press, Portland, OR, pp 46-72

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package) version 3.5s. Distributed by the author. Department of Genetics, University of Washington, Seattle

Goudet J (1995) FSTAT v-1.2: A computer program to calculate *F*-statistics. J Hered 86: 485-486

Hagen MJ, Hamrick JL (1998) Genetic variation and population genetic structure in *Trifolium pratense*. J Hered 89: 178-181

Hamrick JL, Godt MJW (1989) Allozyme diversity in plant species. *In* AHD Brown, MT Clegg, AL Kahler, BS Weir, eds, Plant Population Genetics, Breeding and Genetic Resources. Sinauer Sunderland, MA, pp 304-319

Hamrick JL, Godt MJW, Sherman-Broyles SL (1992) Factors influencing levels of genetic diversity in woody plant species. New For 6: 95-124

Hartl DL, Clark AG (1989) Principles of Population Genetics. 2nd ed., Sinauer Associates, Inc, MA

Holsinger KE, Gottlieb LD (1991) Conservation of rare and endangered plants: Principles and prospects. *In* DA Falk, KE Holsinger, eds, Genetics and Conservation of Rare Plants. Oxford University Press, pp 195-223

Huh MK (1999) Genetic diversity and population structure of Korean alder (*Alnus japonica*; Betulaceae). Can J For Res 29: 1311-1316

Huh MK, Huh HW (2001) Genetic diversity and population structure of wild lentil tare. Crop Sci 41: 1940-1946

Huh MK, Lee HY, Mishra SH, Huh HW (2001) Genetic diversity and population structure of *Carex breviculmis* (Cyperaceae). J Plant Biol 43: 136-142

Huh MK, Choi JS, Moon SG, Huh HW (2002) Genetic diversity of natural and cultivated population of wild *Oenanthe javanica* in Korea. J Plant Biol 45: 83-89

Kiang YT, Gorman MB (1983) Soybean. *In* SD Tankley, TJ Orton, eds, Isozymes in Plant Genetics and Breeding, Part A. Elsevier, Amsterdam, pp 295-328

Kim TJ (1996) Korean Resources Plants. Seoul National University, pp 169

Ledig FT (1986) Heterozygosity, heterosis, and fitness in outbreeding plants. *In* ME Soule, ed, Conservation Biology. Sinauer Sunderland, MA, pp 77-104

Lee YN (1997) Flora of Korea. Kyo-Hak Publishing Co, Seoul

Lessios HA (1992) Testing electrophoretic data for agreement with Hardy-Weinberg expectations. Marine Biol 112: 517-523

Li, C.C., Horvitz, D.G. (1953). Some methods of estimating the inbreeding coefficient. Amer J Hum Genet 5: 107-117

Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. Annu Rev Ecol Syst 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

Neel MC, Ellstrand NC (2003) Conservation of genetic diversity in the endangered plant *Eriogonum ovalifolium* var. *vineum* (Polygonaceae). Conserv Gen 4: 337-352

Nei M (1972) Genetic distance between populations. Amer Nat 106: 282-292

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70: 3321-3323

Newton AC, Allnutt T, Gillies ACM, Lowe A, Ennos RA (1999) Molecular phylogeography, interspecific variation and the conservation of tree species. Trends Ecol Evol 14: 140-145

Ohnishi O, Asano N (1999) Genetic diversity of *Fagopyrum homotropicum*, a wild species related to common buckwheat. Genet Resour Crop Evol 46: 389-398

Raymond M, Rouset F (1995) GENEPOP version 1.2: A population genetics software for exact tests and ecumenicism. J Hered 86: 248-249

Shin KS, Shin YK, Kim JH, Tae KH (2002) Phylogeny of the genus *Goodyera* (Orchidaceae; Cranichideae) in Korea based on nuclear ribosomal DNA ITS region sequences. J Plant Biol 45: 182-187

Slatkin M (1985) Rare alleles as indicators of gene flow. Evolution 39: 53-65

Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst Zool 35: 627-632

Soltis DE, Haufler CH, Darrow DC, Gastony GJ (1983) Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer Fern J 73: 9-27

Sun BY, Kim CH, Soh WY (1988) Chromosome numbers of Araliaceae in Korea. Kor J Plant Tax 18: 291-296

Wendel JF, Weeden NF (1989) Visualization and interpretation of plant isozymes. *In* DE Soltis, PS Soltis, eds., Isozymes in Plant Biology. Chapman and Hall, London, pp 5-45

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38: 1358-1370

Wright S (1965) The interpretation of population structure by *F*-statistics with special regard to systems of mating. Evolution 19: 395-420

Yeh FC, Yang RC, Boyle T (1999) POPGENE version 1.31. Microsoft Windows-based Freeware for Population Genetic Analysis