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Original article

Genetic variation in natural populations of *Abies nephrolepis* Max. in South Korea

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Abstract – *Abies nephrolepis* Max. is a fir species occurring in Northeast China, the extreme southeast of Russia and Korea. In Korea, *A. nephrolepis* is one of only three native fir species growing in high, mountainous areas with a disjunct distribution. We investigated genetic variation in *A. nephrolepis* by examining 24 ISSR polymorphic loci in 248 individuals representing eight natural populations in Korea. The level of intra-population genetic diversity (H_e = 0.240) was similar to or slightly lower than that of plants with similar ecological traits and life history. Most of the genetic diversity was allocated among individuals within populations (G_{ST} = 0.082, Φ_{ST} = 0.041) and the number of migrants per generation (N_m) was 5.56. Nei's genetic distances were small ($\overline{D}$ = 0.027) and unrelated to geographic distances between populations. Implications for the conservation of genetic variation in *A. nephrolepis* in South Korea were discussed.

***Abies nephrolepis* Max. / ISSR / genetic diversity / genetic differentiation**

Résumé – Variabilité génétique dans des populations naturelles de *Abies nephrolepis* Max. en Coréee du Sud. *Abies nephrolepis* Max. est un sapin présent dans le Nord-Est de la Chine, l'extrême Sud-Est de la Russie et en Corée. En Corée, *A. nephrolepis* est une des trois espèces autochtones de sapin poussant dans des zones disjointes en haute montagne. Nous avons étudié la variabilité génétique chez *A. nephrolepis* grâce à 24 locus polymorphes ISSR sur un échantillon de 248 individus représentant huit populations naturelles en Corée. Le niveau de diversité génétique intra-population obtenu (H_e = 0,240) était semblable ou légèrement plus faible que celui obtenu pour des plantes avec des caractères écologiques ou une histoire semblables. La plus grande part de la diversité génétique était représentée par la variabilité entre individus dans populations (G_{ST} = 0,082, Φ_{ST} = 0,041) et le nombre de migrants par génération (N_m) était de 5,56. Les distances génétiques de Nei étaient faibles ($\overline{D}$ = 0,027) et sans relation avec les distances géographiques entre populations. Les implications pour la conservation de la diversité génétique de *A. nephrolepis* en Corée du Sud sont discutées.

***Abies nephrolepis* Max. / ISSR / diversité génétique / différenciation génétique**

1. INTRODUCTION

Abies is the second largest genus of the family Pinaceae (second to *Pinus*), consisting of 50 species more or less. Eastern Siberian fir or Khinghan fir (*Abies nephrolepis* Max.) is a fir species occurring in Northeast China, the extreme southeast of Russia and Korea [22] and it is one of the members of the section *Elate* by Liu's classification [23] and the section *Balsamea* by Farjon and Rushforth's classification [8]. It grows in mountain regions in the North and subalpine regions in the South. In Korea, *A. nephrolepis* is one of only three native fir species (*Abies holophylla*, *A. koreana* and *A. nephrolepis*) occurring in high, mountainous areas at altitudes of 750–1500 m [22]. Within its present range, *A. nephrolepis* occurs in relatively small disjunct populations.

A. nephrolepis is an evergreen coniferous tree growing to 20–25 m tall with a trunk diameter of up to 0.75–1 m. The bark is smooth with resin blisters and grey-brown in colour. The leaves are needle-like, flattened, 1.5–3 cm long and 1.5–1.8 mm wide, glossy dark green above, and with two white bands of stomata below. The young leaves are slightly notched at the tip. The cones are 4–5.5 cm long, 2–2.5 cm wide and greenish brown in colour. The scale bracts emerging between the scales in the closed cones are 6 mm long and bend backwards as the cones become mature. The winged seeds are released when the cones disintegrate at maturity at about 4–5 months after pollination [4, 22].

A. nephrolepis is an economically important species used for the production of pulp wood and timber. In addition, it is a very popular ornamental tree in gardens as well as a Christmas tree. Although *A. nephrolepis* is not a rare or an endangered tree species [13], it faces an uncertain future in its natural stands in Korea. Changing climate conditions such as global warming would threaten to render *A. nephrolepis*' current habitat on mountaintops unsuitable. Given the significant commercial role and the potential threat to natural habitats of *A. nephrolepis*, the maintenance of genetic variation, i.e. gene conservation of the species, is an urgent task. In order to facilitate various programmes in conservation and sustainable utilisation of *A. nephrolepis*, information on population genetics is needed.

Since 1994, a new molecular marker technique of inter-simple sequence repeat (ISSR) has been available. The early

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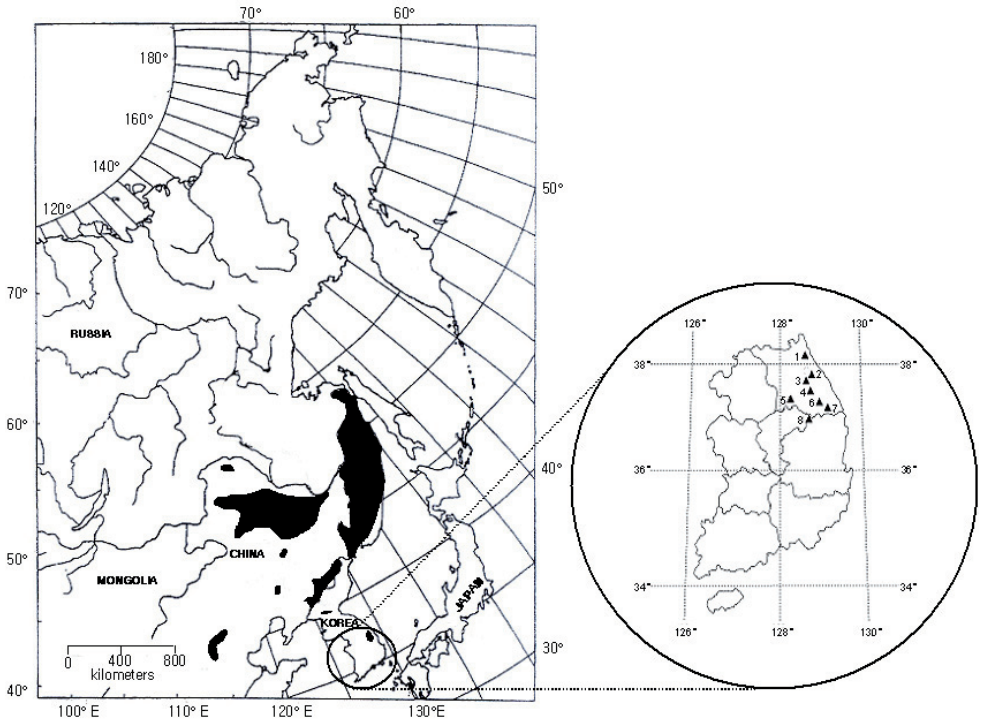

Figure 1. The range of *A. nephrolepis* after Farjon [9] and the locations of populations (closed triangle) sampled for ISSR analysis. Population numbers correspond to those of Table I.

studies focused on cultivated species and demonstrated the hypervariable nature of ISSR markers [37]. Since then, ISSR markers have become a popular tool in plant population genetic studies [10, 36]. Like RAPDs (Random Amplified Polymorphic DNAs), ISSR markers are quick and easy to handle, but they seem to have more reproducibility than RAPD markers because of high stringency conditions for PCR, i.e., the high annealing temperature granted by longer primers. However, similar to other dominant marker systems, the main problem with ISSRs is that heterozygous genotypes are undistinguishable from homozygous genotypes, which may result in loss of genetic information and statistical precision. Nevertheless, ISSRs may be used to estimate population genetic parameters by adopting appropriate statistical methods [24, 31].

To our best knowledge, no DNA marker studies have been conducted for *A. nephrolepis*. The objectives of this study were (1) to provide a first insight into the levels and distribution of ISSR variation in *A. nephrolepis* in South Korea, (2) to compare genetic variation in *A. nephrolepis* with previous reports for that of other firs in particular, as well as that of conifers in general, and (3) to determine implications for the gene conservation of *A. nephrolepis* in South Korea.

2. MATERIALS AND METHODS

2.1. Plant materials

From late June to mid-July of 2006, foliage tissues were sampled from eight natural populations located throughout the native range of *A. nephrolepis* in South Korea (Tab. I, Fig. 1). Within each population, 30–34 trees were selected for foliage collection with a minimum distance of 30 m between them in order to decrease the risk of relatedness. The needle leaves were placed in ice chests, transported to the laboratory within 48 h, and stored at −80 °C until DNA was isolated.

Table I. Location of the sampled populations of *A. nephrolepis* in South Korea.

Population	Latitude (N)	Longitude (E)	Elevation (m)
1. Mt. Sorak	38° 06′	128° 28′	1 685
2. Mt. Ohdae	37° 48′	128° 33′	827
3. Mt. Gyebang	37° 43′	128° 28′	1 407
4. Mt. Gariwang	37° 27′	128° 33′	1 424
5. Mt. Chiak	37° 21′	128° 03′	1 115
6. Mt. Jang	37° 07′	128° 51′	1 407
7. Mt. Taebaek	37° 06′	128° 54′	1 325
8. Mt. Sobaek	36° 57′	128° 29′	1 439

2.2. DNA isolation and ISSR amplification

Total genomic DNA was isolated from 25 mg of needle leaf tissue following the protocol described by QIAGEN (2006; DNeasy Plant Mini and DNeasy Plant Maxi Handbook). Amplifications of DNA were performed in reaction volumes of 15 μL mixture containing 3 μL of template DNA (2 ng/μL), 3.75 μL of primer (UBC), 1.5 μL of each dNTP (Fermentas Life Sciences), 1.5 μL of 10× buffer (ABgene Ltd.), 0.12 μL of Taq Polymerase (5 U/μL, ABgene Ltd.), 0.025% of BSA (Boeringer Manheim), 1.2 μL of 25 mM $MgCl_2$ and 2.43 μL of H_2O. PCR was carried out in a PTC-2500 thermocycler (MJ Research

Table II. ISSR primer information.

Primer name	Sequence	Annealing temperature (°C)
UBC811	5′-$(GA)_8$C-3′	52
UBC812	5′-$(GA)_8$A-3′	50
UBC813	5′-$(CT)_8$T-3′	50
UBC815	5′-$(CT)_8$G-3′	52
UBC841	5′-$(GA)_8$YC-3′	54
UBC843	5′-$(CT)_8$RA-3′	52
UBC850	5′-$(GT)_8$YC-3′	54

Nucleotide abbreviations: Y = T or C; R = A or G.

Inc., USA) which was programmed for one cycle of 5 min at 94 °C followed by 45 cycles of 30 s at 94 °C, 45 s at annealing temperature (see Tab. II) and 60 s at 72 °C with a final cycle of 10 min at 72 °C. DNA fragments were separated by electrophoresis in 2% agarose gels containing 20 μL of ethidium bromide with a Tris-Borate-EDTA (TBE) buffer system at 200 V for 100 min. DNA bands were then visualised by UV light and documented using a UV trans-illuminator (Mitsubishi). Gel pictures were analysed with the software Gel Documentation and Analysis System (Syngene) and double-checked with the naked eye; only bands in the range between 200 and 2 000 bp were scored. A total of 37 primers (UBC set 9) were screened using three representatives from each of the eight populations in order to test amplification profiles for polymorphism, readability and reproducibility. This resulted in the selection of 7 primers for use in the analysis of all the populations (Tab. II).

2.3. Statistical analyses

For each primer, amplified fragments with the same molecular weight (bp) were recorded as present (1) or absent (0), and the resulting binary matrix was used in the analyses. It was assumed that each band represented one Mendelian locus with two alleles, with the 'dominant' or visible alleles and the 'recessive' or null alleles. It was also assumed that alleles from different loci do not migrate at the same position in a gel.

For each polymorphic locus, allele frequencies were estimated, assuming that the population is in Hardy-Weinberg equilibrium. The frequency of the null allele (q) was estimated by taking the square root of the frequency of the null homozygote (absence of a band) and then the frequency of the dominant allele (p) was estimated by $1-q$. On the other hand, the statistics for ISSR data can underestimate the genetic variation, because ISSR does not allow the recognition of heterozygotes. Accordingly, bands with frequencies higher than 1–(3/N), where N is the sample size of a population, were excluded in the further analyses [24, 31]: a polymorphic fragment is one in which the observed frequency of the bands is below 1–(3/N). Based on these data, the following levels of genetic diversity were estimated with the software POPGENE version 1.31 [41]: observed number of alleles (A), effective number of alleles (A_e), percentage of polymorphic loci (P), expected heterozygosity (H_e), Shannon's diversity index (I) [32] and Nei's gene diversity (H_T, H_S, and G_{ST}). Where there are two alleles at a locus, as in ISSR marker analyses, the G_{ST} is identical to Wright's F_{ST} [28] and seems to produce robust data that are relatively insensitive to assumptions about Hardy-Weinberg equilibrium, heterozygosity and levels of inbreeding [31]. An indirect estimate of gene flow among populations (N_m) was also calculated using the formula $N_m = (1 - G_{ST})/2\,G_{ST}$ [41].

A multi-locus approach, AMOVA (Analysis of Molecular Variance), originally developed for haploid data [6], was also applied to estimate genetic differentiation using Euclidean metric distances. For this, binary vectors based on the banding pattern of each individual (1/0 matrix) that do not require any a priori assumptions were used. For the dominant markers such as ISSRs, AMOVA-derived Φ_{ST} values are nowadays more widely used than G_{ST} values for the partitioning of genetic variability. Nybom and Bartish [30] reported that values for Nei's G_{ST} and for the AMOVA-derived Φ_{ST} were very similar when calculated on the same data set. We used the program ARLEQUIN version 3.0 [7] to perform the AMOVA analysis.

Pair-wise genetic distance coefficients for each pair of the populations were calculated following the methods of Nei [29]. Then, the matrix of genetic distances among populations was used to construct a UPGMA dendrogram using the POPGENE program version 1.31 [41]. The relationships between genetic distances and geographic distances were analysed using Isolation By Distance (IBD) software [3]. The IBD software program assesses the significance between a given distance matrix and the geographic distance through Mantel's test [25] and evaluates the strength of the relationship through regression of all pair-wise genetic distances with their corresponding geographic distances.

3. RESULTS

3.1. Genetic diversity within populations

Of the 38 ISSR fragments scored, 24 fragments (63%) were polymorphic. Using the polymorphic fragments, intra-population genetic diversity parameters were calculated.

The percentage of polymorphic loci ranged from 75% to 87.5% with a mean of 80.2%; the number of alleles per polymorphic locus (A) ranged from 1.75 to 1.88 with a mean of 1.80; the effective number of alleles per polymorphic locus (A_e) ranged from 1.350 to 1.425 with a mean of 1.394; expected heterozygosity (H_e) ranged from 0.217 to 0.257 with a mean of 0.240; and Shannon's diversity index (I) ranged from 0.336 to 0.396 with a mean of 0.369 (Tab. III).

3.2. Genetic diversity among populations

Total genetic diversity (H_T), averaged over 24 polymorphic loci, was 0.262 (± 0.018) and H_S, the genetic diversity within populations, was 0.240 (± 0.018). G_{ST} was 0.082, which can be interpreted to mean that 91.8% of the total genetic diversity was within populations and the rest of it (8.2%) was among populations. The hierarchical analysis conducted with AMOVA gave a similar pattern of population differentiation: 4.1% (ϕ_{ST} = 0.041) of the ISSR variation was distributed among populations and 95.9% was distributed within populations.

Indirect estimate of gene flow was relatively high. The overall rate of gene flow (N_m) among populations was 5.586, which means 5.6 migrants per generation.

Table III. ISSR genetic diversity in eight natural populations of *A. nephrolepis* in South Korea (standard deviations in parentheses).

Population	*A*	A_e	*P* (%)	H_e	*I*
Mt. Sorak	1.79 (0.41)	1.412 (0.338)	79.17	0.251 (0.183)	0.382 (0.257)
Mt. Ohdae	1.83 (0.38)	1.378 (0.332)	83.33	0.235 (0.175)	0.365 (0.242)
Mt. Gyebang	1.79 (0.41)	1.393 (0.340)	79.17	0.242 (0.176)	0.373 (0.246)
Mt. Gariwang	1.75 (0.44)	1.350 (0.333)	75.00	0.217 (0.182)	0.336 (0.257)
Mt. Chiak	1.79 (0.42)	1.424 (0.357)	79.17	0.253 (0.188)	0.383 (0.263)
Mt. Jang	1.83 (0.38)	1.363 (0.357)	83.33	0.222 (0.180)	0.348 (0.244)
Mt. Taebaek	1.75 (0.44)	1.408 (0.364)	75.00	0.244 (0.187)	0.371 (0.263)
Mt. Sobaek	1.88 (0.34)	1.425 (0.352)	87.50	0.257 (0.177)	0.396 (0.239)
Mean	1.80	1.394	80.21	0.240	0.369

A = observed number of alleles per locus, A_e = effective number of alleles per locus, *P* = percentage of polymorphic loci, H_e = expected heterozygosity, *I*= Shannon's diversity index.

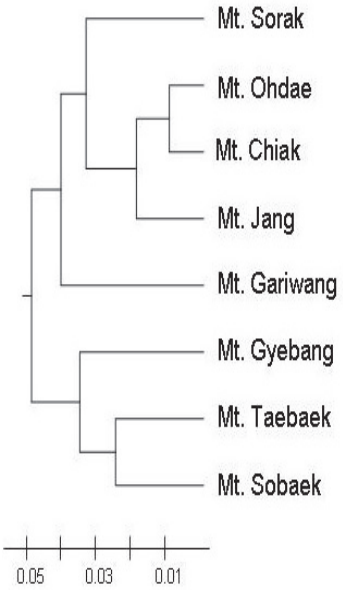

Figure 2. UPGMA dendrogram based on Nei's genetic distance [29].

3.3. Genetic relationships

The unbiased genetic distances (*D*) of Nei [29] ranged from 0.006 to 0.054 with an average value of 0.027. This small mean value reveals once again that the eight populations studied here are closely related and there is a free gene exchange among them. To visualise the results better, a dendrogram produced by the UPGMA clustering technique is presented in Figure 2. The dendrogram failed to show decisive geographic patterns: the first group consisted of the populations Mt. Ohdae, Mt. Chiak, Mt. Jang, Mt. Sorak and Mt. Gariwang, while the second group was composed of Mt. Taebaek, Mt. Sobaek and Mt. Gyebang. Nevertheless, the populations Mt. Sorak and Mt. Sobaek, the most geographically distant populations, were clustered into the most separate groups.

The Mantel test with 10 000 random permutations revealed no correlation between geographic distance and genetic distance measurements ($r = 0.1743$, $P = 0.1847$).

4. DISCUSSION

4.1. Genetic diversity

Nybom [31] reviewed population genetic studies (307 entries) in which nuclear DNA markers were used for assessment of among- and within-population diversity in wild plants. She showed that estimates derived by the dominant markers (RAPD, AFLP and ISSR) are very similar and may be directly comparable. When the mean expected heterozygosity (H_e = 0.240) of *A. nephrolepis* in South Korea was compared with that of Nybom's study [31], it exhibited a moderate level of genetic diversity since the grand mean of H_e in RAPD-based studies was 0.22. Additionally, the genetic diversity of *A. nephrolepis* was similar to or slightly lower than that of plants with similar ecological traits and life history (H_e for a long-lived perennial = 0.25, H_e for an outcrossing breeding system = 0.27 and H_e for seed dispersal by wind = 0.27) [31]. The mean expected heterozygosity (H_e) of 0.240 for *A. nephrolepis* in South Korea was also relatively low compared with that of other conifers (Tab. IV). Only a few conifers such as *A. ziyuanensis*, *Picea asperata* and *Pinus muricata*, which have extremely restricted distribution ranges, showed a lower estimate of H_e than *A. nephrolepis* (Tab. IV).

Direct comparison between ISSR and allozyme variation would be inaccurate, but some studies reported that the gross pattern of genetic variation for allozyme and dominant molecular markers are similar [1, 12, 21, 27, 34]. Lee et al.. [20] also showed a positive correlation between genetic diversity estimated from allozymes and RAPDs. Based on allozyme data, several firs seemed to have a low genetic diversity in comparison with other conifers [11, 18]. For example, H_e (0.130) for the genus *Abies* was lower than that of other gymnosperms (H_e for *Picea* = 0.218, H_e for *Pinus* = 0.136 and H_e for *Pseudotsuga* = 0.163) [11]. Particularly, firs of the section *Balsamea*, to which *A. nephrolepis* belongs, seemed to retain a low to moderate level of allozyme variation compared with other firs [18]: H_e for *A. fraseri* = 0.085; H_e for *A. lasiocarpa* = 0.124; H_e for *A. lasiocarpa* = 0.129; and H_e for *A. sachalinensis* = 0.157.

The relatively low level of genetic diversity in *A. nephrolepis* is not surprising. When populations remain small for any extended period, as in the case of

Table IV. Estimates of genetic diversity in dominant DNA markers of conifers with different geographic distribution, based on all loci including polymorphic and monomorphic loci.

Species	Marker	H_e	G_{ST} (F_{ST})	Reference
Broad distribution				
Picea abies	AFLP	0.378*	0.029	[2]
Picea mariana	RAPD	0.321	0.053	[12]
Pinus attenuata	RAPD	0.15 (0.17*)	0.24	[38]
Pinus densiflora	RAPD	0.392*	0.105	[19]
Pinus oocarpa	AFLP	0.342	0.073	[5]
Pinus oocarpa	RAPD	0.358	0.112	[5]
Pinus pinaster	AFLP	0.161*	0.061	[26]
Pinus sylvestris	RAPD	0.356 (0.482*)	0.019	[34]
Pseudotsuga menziesii	RAPD	0.19 (0.22*)	0.05	[1]
Mean		0.286 (0.301*)	0.058	
Narrow distribution				
Abies koreana	RAPD	0.418*	0.081	[14]
Abies nephrolepis	ISSR	0.240*	0.082	Current study
Abies ziyuanensis	AFLP	0.136	0.482	[35]
Picea aspearta	AFLP	0.156	0.340	[40]
Pinus longaeva	RAPD	0.130 (0.321*)	0.039	[20]
Pinus muricata	RAPD	0.13 (0.16*)	0.29	[38]
Pinus radiata	RAPD	0.17 (0.23*)	0.18	[38]
Taxus cuspidata	ISSR	0.316*	–	[17]
Mean		0.144 (0.281*)	0.213	

* Based on polymorphic loci, H_e = expected heterozygosity, G_{ST} (F_{ST}) = differentiation among populations.

A. nephrolepis, sampling effects may become cumulative. This gives rise to random changes in gene frequency due to the sampling of gametes from generation to generation, resulting in the reduction of genetic diversity. This may be reflected in the past migration patterns of *A. nephrolepis* in South Korea. According to the fossil record, *Abies* in Korea appeared in the Cretaceous and began to expand southward at the end of the Pleistocene, 12 500 to 9 000 y B.P. [15, 16]. Then *Abies* forests in Korea began to shrink northward and fragment during the Holocene warming (about 6 000 y B.P.) [16]. The Holocene warming also caused an upward elevation shift of *A. nephrolepis* populations to an altitude of over 750 m [16], where it formed relatively small and isolated populations. As a result, *A. nephrolepis* has been possibly subjected to increased levels of inbreeding, genetic drift and founder effects.

A. nephrolepis and *A. koreana* are very closely related species and belong to the same sections, *Elate* [23] or *Balsamea* [8]. Data from chloroplast DNA sequences [33] as well as those from the internal transcribed spacers (ITS) of nuclear ribosomal DNA [39] supported this very close phylogenetic relationship between the two species. It is reported that *A. koreana* is a daughter species diverged from *A. nephrolepis* and intergradation between the two species occurred during the Quaternary [4]. However, interestingly, within-population diversity in *A. koreana* was reported to be much higher than that in *A. nephrolepis* (Tab IV). This result is striking and somewhat puzzling because, generally, an ancestral species remains at a higher level of genetic diversity than its divergent daughter species. The differences in materials and molecular makers used for the two studies may give a partial explanation. The genetic diversity estimated in *A. koreana* was restricted to RAPD loci that were highly polymorphic and megagametophytes were used as materials, so allele frequencies for each locus were estimated in a direct and more accurate way [14]. Meanwhile, when we estimated genetic diversity of *A. koreana* with the same ISSR markers used as in the present study, H_e of 0.237 for 3 natural populations of *A. koreana* was slightly lower than that for *A. nephrolepis* (unpublished data).

As previously verified with allozyme studies [11], RAPD-derived estimates of within-population genetic diversity proved to be closely associated with life history traits and ecological traits [30, 31]. On the other hand, the only major discrepancy between allozymes and RAPDs was found for geographical range. Within-population genetic diversity is strongly affected by geographic range when allozymes are used [11], but not so in the RAPD-based studies [31]. This tendency was also found in the previous studies for coniferous tree species (Tab. IV). For example, among the conifers occurring in Korea, *Abies koreana* has an extremely restricted geographic range with isolated populations, but it shows a higher level of RAPD-derived within-population diversity than *Pinus densiflora*, that has a widespread geographic range. However, the comparisons of genetic diversity for conifers reviewed in the present study (Tab. IV) showed that trees with wide and continuous distributions tended to retain a higher level of intra-population genetic diversity than those with limited and fragmented distributions. Lynch and Milligan [24] suggested that dominant marker analysis such as RAPD and ISSR be restricted to loci with a null homozygote frequency greater than $3/N$ (N = the number of individuals sampled in a population) in order to avoid biased estimates of genetic parameters. However, this restriction may be satisfied for only a few highly polymorphic loci, resulting in the exclusion of all the monomorphic loci. Therefore, the use of Lynch and Milligan's correction may cause substantial overestimates of genetic diversity measurements, especially for endemic and narrowly distributed plants, because such plants possibly retain more numerous monomorphic loci than widespread ones. This may give a partial explanation for the lack of relationship between dominant marker-based within-population genetic diversity and geographic range, which was reported in Nybom's study [31].

4.2. Genetic differentiation and relationships

The overall G_{ST} value of 0.082 and ϕ_{ST} value of 0.041 indicate that 4–8% of the genetic variation present in *A. nephrolepis* is partitioned among populations. This value is relatively low compared with 15 previous estimates of G_{ST} (or F_{ST}) for other conifers, which were as low as 0.019 for *Pinus sylvestris* and as high as 0.482 for *Abies ziyuanensis* (Tab. IV). This indicates that *A. nephrolepis* populations in South Korea are not highly differentiated. Continuous and more widespread distribution of *A. nephrolepis* populations during the Pleistocene glacial periods [4, 16] may be partially attributable to the low degree of population differentiation. On the other hand, conifers with broad and continuous distribution patterns

showed a lower degree of population differentiation than those with narrow and discontinuous distribution patterns (Tab. IV).

The lack of relationship between genetic distance and geographic distance, as shown in Mantel's test ($r = 0.174$, $P = 0.185$), might suggest that genetic drift was important in the structure of *A. nephrolepis*, as already mentioned. Of course, geographic distance alone may not adequately reflect the complexities of slope, aspect, wind direction and a large number of other factors. Nevertheless, no relationship between geographic distance and genetic distance suggests that near neighbours are not more likely to exchange genes than distant populations. In other words, there is no isolation by distance through a stepping stone model. The UPGMA dendrogram (Fig. 2), showing an indecisive geographic pattern, also supports this hypothesis.

4.3. Implications of gene conservation

Given the relatively low level of genetic diversity and the vulnerability to climate change, *A. nephrolepis* is a prime candidate for conservation. In addition to in situ conservation, *A. nephrolepis*' genetic resources should be preserved ex situ in a seed bank and/or field gene bank in case restoration is needed or to establish new populations in suitable habitats if climate change scenarios unfold as projected. The low degree of population differentiation implies that only a few numbers of populations throughout its range would be enough for in situ as well as ex situ conservation. However, common garden tests and investigations using other markers should be performed to confirm or reject this suggestion, because the lack of genetic differences among populations at DNA level does not rule out adaptive variation at other loci. Among the populations sampled, Mt. Sobaek is one of the best candidates for ex situ conservation and use as a seed source for restoration, because not only does it have the highest level of genetic diversity and the maximum number of alleles, but it is also the population in the southern extreme of the *A. nephrolepis* distribution range.

Finally, for a better understanding of evolution and population genetics of *A. nephrolepis*, we need to conduct further studies on the genetic variation in *A. nephrolepis* from the main distribution range (e.g., the extreme southeast of Russia and Northeast China) in the future. In this case, it is highly recommended that other codominant markers in addition to ISSRs be used.

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