Material Selection for Genetic Restoration of Abies koreana on Mt. Jirisan in the Republic of Korea

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Abstract

A strategy is required for selecting appropriate materials for the restoration of *Abies koreana* on Mt. Jirisan, where the habitat of *A. koreana* is continuously shrinking. The current study aimed to analyze the genetic characteristics of *A. koreana* in three subpopulations (Banyabong, Byeoksoryeng, and Cheonwangbong) on Mt. Jirisan using 10 nuclear simple sequence repeat (nSSR) markers and calculate the sampling distance for each subpopulation for avoiding genetically similar samples. Based on the calculated sampling distance, we proposed the size of a sample containing more than 95% of the alleles at a frequency greater than 0.05. AMOVA showed that the difference in genetic variation across subpopulations of *A. koreana* on Mt. Jirisan was small, approximately 3% of the total. Spatial genetic structure analysis results suggested that it would be appropriate to collect samples of the Banyabong subpopulation at intervals of 10 m or more, when sampling *A. koreana*, whereas for the Byeoksoryeong and Cheonwangbong subpopulations, samples should be collected at intervals of 20 m or more. Results of random sampling of 5 to 30 individuals indicated that, by applying a 10 m distance within the Banyabong subpopulation, more than 95% of the total alleles with a frequency ≥ 0.05 were secured when more than 25 individuals were extracted. Therefore, as a restoration strategy for *A. koreana* on Mt. Jirisan, we proposed the collection of more than 25 samples, keeping 10 m distance within the Banyabong subpopulation, which has a relatively high genetic diversity.

1. Introduction

In the 20th century, global surface temperature increased by 0.7°C (IPCC 2007), resulting in altered weather variables, such as precipitation (Bukhari and Bajwa 2011). The sub-alpine forest, located between timberline and treeline, is vulnerable to changed weather variables because it has poor environmental conditions, such as low temperatures, strong winds, and dry soil (Lim et al. 2006; Nagy and Grabherr 2009; Bukhari and Bajwa 2011; Kim et al. 2019). To conserve the sub-alpine forest, the Korea Forest Service has designated seven endangered sub-alpine coniferous species (*A. nephrolepis, Picea jezoensis, Thuja koraiensis, Juniperus chinensis, Picea pumila*, and *Taxus cuspidate*) as conservation targets since 2016. The study titled 'Investigation of the Current Status of Endangered Sub-Alpine Coniferous Species (2017–2018)' was carried out on these species, followed by the 'The first Monitoring (2019–2020)'. Mean temperature of the sub-alpine coniferous area, consisting of the dominant species of sub-alpine forest in Korea, increased by 0.8°C over the past 30 years (1971–2000; 6.4–7.2°C); this is higher than the average temperature increase rate in Korea (Kim and Lee 2013; Lim et al. 2019). The coniferous species in sub-alpine forest experienced physiological stresses, such as water stress due to increased evapotranspiration and imbalance in photosynthesis and respiration due to global warming, leading to a decrease in growth and an increase in dead trees (Park and Seo 1999; Koo et al. 2001; Kong 2002; Lim et al. 2006; Kim et al. 2019; Lim et al. 2019; Jiao et al. 2020; Kim et al. 2021). The sub-alpine coniferous area in Korea decreased by approximately 25% in 20 years (1990–2010) (Kim et al. 2019; Lim et al. 2019). The populations gradually decreased in size and increased in spatial isolation, resulting in a decrease in genetic diversity due to genetic drift or inbreeding (Young et al. 1996; Tang et al. 2008; Ahn et al. 2017). Low levels of genetic variation within a population limit their ability to adapt to environmental changes, and reduce seed

production and seedling emergence, resulting in population decline and ultimately extinction (Booy et al. 2000; Hamrick 2004; Tang et al. 2008; Broadhurst and Boshier 2014). Therefore, preserving genetic diversity is important for the maintenance and conservation of sub-alpine coniferous forest in preparation about changing environmental conditions due to climate change (Kim et al. 2019). Accordingly, the Korea Forest Service announced the ' The second conservation and restoration strategies for endangered sub-alpine conifer species' in 2021. This measure outlined policy objectives for a 5-year period (2022–2026) and encompasses research into restoration strategies considering genetic diversity.

Abies koreana, a native species of Korea, is a representative sub-alpine conifer in Korea that grows at altitudes higher than 1,000 m in Mt. Jirisan, Mt. Hallasan, Mt. Deogyusan, and Mt. Gayasan (Kim and Choo 2000; Chun et al. 2021). The Korean fir, *A. koreana*, had been designated as “endangered” by the International Union for Conservation of Nature (IUCN) Red List in 2011 (Kim et al. 2011). Mt. Jirisan, the highest mountain in the southern part of Korean peninsula, covers the largest area, with 43% (5,198 ha) of the total area of sub-alpine coniferous in Korea, and *A. koreana* as the main species (Lim et al. 2019; Chun et al. 2021). Kim et al. (2021) reported that *A. koreana* of Mt. Jirisan has been declining in population, and dying every year since 2009. Research has shown that 49.9% of *A. koreana* in Mt. Jirisan has disappeared over the past 10 years (2009–2018) (Chun et al. 2021), and seedling emergence has decreased by 22.4% in the last 2 years (Kim et al. 2018). In order to mitigate the loss of *A. koreana* habitat, appropriate restoration strategies, considering genetic diversity, would be required (McKay et al. 2005).

Utilizing restoration materials that conserve the genetic characteristics of individuals in their natural habitat is an important factor in successfully leading restoration by increasing adaptability to future environmental conditions (McKay et al. 2005; Bischoff et al. 2008; FAO 2014; Chae et al. 2022; Seo et al. 2023). In order to use native plants as restoration materials, genetic characteristics of the populations need to be understood (McKay et al. 2005). The number of alleles in each population is a key indicator of optimal sampling (Marshall and Brown 1975). Although it is recommended to have as many alleles as possible to explain the population, an efficient sampling strategy would still be required, since manpower and costs are limited in the field. Our sampling strategy involved more than 95% of alleles composing in the target population with a frequency ≥ 0.05 (Marshall and Brown 1975).

In a previous study, applying the same sampling strategy to *A. koreana* populations on Mt. Hallasan, an appropriate strategy representing the genetic characteristics of *A. koreana* on Mt. Hallasan was proposed; more than 35 individuals were suggested to be collected at a distance of 5 m to 10 m each (Chae et al. 2022). Mt. Hallasan is located on an island and has one main peak, whereas Mt. Jirisan is located inland and has many peaks, and sub-alpine conifer species are continuously distributed along the ridge, forming the largest sub-alpine coniferous area in Korea. According to Hong et al. (2011), the two *A. koreana* populations differ in genetic characteristics. Therefore, it would be desirable to introduce different sampling strategies for both. *A. koreana* on Mt. Jirisan decreased by 14.6% over the past 20 years, requiring an appropriate restoration strategy (Lim et al. 2019).

This study aimed to analyze the genetic characteristics of *A. koreana* on Mt. Jirisan using nSSR markers and calculate the sampling distance for each subpopulation to reduce the number of genetically similar

samples. Finally, based on the calculated sampling distance, we proposed the number of effective sample size, including appropriate genetic diversity of *A. koreana* on Mt. Jirisan population.

2. Materials and Methods

2.1. Study site and plant materials

For three subpopulations (Banyabong, Byeoksoryeng, and Cheonwangbong) on Mt. Jirisan in the Republic of Korea, 99 *A. koreana* samples per subpopulation were selected for this study (Fig. 1). The height and diameter at breast height (DBH) of each individual were measured (Table 1), location of each individual was recorded using GPS (GPS map60CSx; Garmin, Schaffhausen, Switzerland), and the location information was used for spatial structure analysis.

Table 1
Summary of sampled *Abies koreana* subpopulations on Mt. Jirisan in the Republic of Korea

Subpopulation	Number of samples	Height (m)	DBH[a] (cm)	Latitude (N)	Longitude (E)	Altitude (m)
Byeoksoryeng	99	21.60 ± 7.82	13.96 ± 2.63	35.31641	127.56964	1517–1537
Banyabong	99	11.31 ± 8.83	7.67 ± 1.84	35.32602	127.64328	1390–1420
Cheonwangbong	99	14.63 ± 11.98	6.03 ± 4.06	35.33692	127.73066	1410–1440

[a] DBH : diameter at breast height

2.2. DNA extraction and nSSR marker analysis

Genomic DNA was extracted from the leaves of *A. koreana* using the Plasmid SV mini kit (GeneAll Biotechnology, Seoul, Korea). Its concentration was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and was stored at -60°C.

The 10 nSSR markers, used in the present study, were developed using other species of *Abies* (Table 2). Polymerase chain reaction (PCR) amplification of NFF7, NFH15, SF83, Aag02, Aat04, Aat05, Aat15, and Aat12 was performed with 25 μL of reaction mixtures containing 20 ng of template DNA, 1× reaction buffer, 2.5 mM $MgCl_2$, 0.5 mM dNTPs, 0.5 μM primer mix, and 0.5 U Taq DNA polymerase (BioFact, Daejeon, Korea). The PCR conditions included denaturation at 94 ℃ for 5 min; 10 cycles at 94 ℃ for 30 s, 58–63 ℃ for 30 s, temperature decreased by 1 ℃ in every cycle until a touchdown at 48–53 ℃, and 72 ℃ for 30 s; 25 cycles at 94 ℃ for 30 s, 52 ℃ for 30–60 s, and 72 ℃ for 40–60 s; and a final extension at 72 ℃ for 10 min. PCR amplification of C28104 and C49 was performed with 25 μL reaction mixtures containing 30 ng of template DNA, 1× reaction buffer, 2.5 mM $MgCl_2$, 0.5 mM dNTPs, 1.00 μM FAM-labeled M13 (–19) sequencing primer, 0.5 μM primer mix, and 0.5 U Taq DNA polymerase (BioFact, Daejeon, Korea). The PCR conditions included denaturation at 94 ℃ for 5 min; 10 cycles at 94 ℃ for 60 s, 58 ℃ for 60 s, and 72 ℃

for 60 s; 25 cycles at 94 ℃ for 30 s, 52 ℃ for 60 s, and 72 ℃ for 60 s; and a final extension at 72 ℃ for 10 min. PCR amplification was performed using the S1000™ Thermal Cycler (Bio-Rad, Hercules, CA, USA). The PCR products were separated on an ABI 3730xl DNA Analyzer (Applied Biosystems, Thermo Fisher Scientific, Foster City, CA, USA), and the genotypes were determined using GeneMapper v5.0 (Applied Biosystems, Thermo Fisher Scientific, USA). The Micro-Checker v2.2.3 software identified null alleles, large allele dropouts, and stutter bands in all loci (Van Oosterhout et al. 2004).

Table 2
Characteristics of 10 nuclear simple sequence repeat (nSSR) markers used in the study

Primer	Primer sequence (5'–3')	Product size	Repeat motif	Tm (°C)	Reference
NFF7	F: CCCAAACTGGAAGATTGGAC R: ATCGCCATCCATCATCAGA	121–163	$(GA)_{33}$	58	Hansen et al. 2005
NFH15	F: CGCCTCCCTCCATTACTTC R: TCGTCTAGAGAGGCGAAATTCT	91–125	(CA)	58	Hansen et al. 2005
SF83	F: AGCAGCATAACCAAGGGTCAA R: TCTGAATTTCTAAAGGCGGC	181–226	$(CTT)_3$... $(GCC)_5$	60 (touchdown)	Postolache et al. 2014
Aag02	F: TATTCCTCCACTTGGGTGCT R: GGTGGAGATCCGTATGCAAT	201–247	$(GA)_{13}$	61 (touchdown)	Postolache et al. 2014
Aat04	F: CCATGTATGGTGCTCCTCCT R: CCTTCATTGCAGAAAAGCAA	149–170	$(CAG)_{11}$	63 (touchdown)	Postolache et al. 2014
Aat05	F: AGCATCCACATTCCGTAACC R: AGTTGACCGTTGGAGAGCAG	170–197	$(GCA)_7$	63 (touchdown)	Postolache et al. 2014
Aat15	F: AGGAGGAGGTTCAGCATGTC R: CTTGCTCTCTGACCCAGTTG	352–362	(AGA)8	63 (touchdown)	Postolache et al. 2014
Aat12	F: ATCCATATCTCCTGCCTTGC R: CTTTCCAGGTGATCTGATTGC	309–335	(AG)12	63 (touchdown)	Postolache et al. 2014
C28104	F: CGAGGAAGAAGCCAAGTTATCAGG R: CACAGTTAAAAAGGCGGCCTACAG	162–225	(ATA)5	58	Uchiyama et al. 2013
C49	F: GACGAAGATCAGTACAAGGCACGA R: GCGATCCTTCAATTTGTCCTTCTC	271–295	(AGGAGA)7	58	Uchiyama et al. 2013

2.3. Summary statistics

Genetic diversity indices (*A*: number of alleles; A_e: number of effective alleles; H_o: observed heterozygosity; H_e: expected heterozygosity; *F*: fixation index) were calculated for each subpopulation. Genetic variation across subpopulations was confirmed using analysis of molecular variance (AMOVA). The Nei's genetic distance and pairwise F_{ST} value among the subpopulations were calculated. All analyses were performed using GenAlEx v.6.5. The significance level of F_{ST} was determined using the FSTAT v2.9.4 software with 3000 permutations (Goudet 1995).

2.4. Spatial genetic structure analysis

The spatial genetic structure was analyzed by evaluating the genetic relevance of each plant individual according to the spatial distance (Gelmi-Candusso et al. 2017). Spatial autocorrelation analysis was performed using location of the individual and GenAlEx v.6.5 (Smouse and Peakall 2012). Distance evaluation was performed by distance classes from 10 m to 100 m, and 999 permutations were performed for each evaluation with a 95% confidence interval (Smouse and Peakall 1999).

2.5. Sample selection strategies

In order to establish an optimal sampling strategy, results of the spatial genetic structure analysis were used as the target distance. Within the representative subpopulation of Mt. Jirisan, 5 to 30 sample sizes were randomly extracted using Python (Chae et al. 2022). We calculated the number of alleles in each sample size and compared them with the number of alleles on Mt. Jirisan with a frequency ≥ 0.05; the mean comparison was performed using Duncan's test at $p \leq 0.05$ with R (De Mendiburu and Simon 2015).

3. Results

3.1. Genetic diversity and distances within subpopulations

Analysis of genetic diversity of *A. koreana* on Mt. Jirisan indicated the average of the number of alleles (*A*), the number of effective alleles (A_e), observed heterozygosity (H_o), expected heterozygosity (H_e), and fixation index (*F*) to be 11.1, 5.2, 0.570, 0.657, and 0.106, respectively (Table 3). Comparing genetic diversity across the subpopulations on Mt. Jirisan, we found the Byeoksoryoung subpopulation to show the highest genetic diversity (H_e = 0.663). The Banyabong subpopulation had the highest number of effective alleles among the three subpopulations and showed the lowest fixation index (F = 0.073). The Cheonwangbong subpopulation showed the lowest genetic diversity (H_e = 0.625).

Table 3
Genetic diversity of *Abies koreana* subpopulations on Mt. Jirisan

		N	*A*	A_e	H_o	H_e	*F*
Sub-population	Byeoksoryeng	99	9.4 ± 2.3	4.8 ± 1.2	0.574 ± 0.065	0.663 ± 0.062	0.115 ± 0.067
	Banyabong	99	8.9 ± 1.9	5.1 ± 1.4	0.589 ± 0.069	0.654 ± 0.071	0.073 ± 0.066
	Cheonwangbong	99	9.0 ± 2.2	4.8 ± 1.4	0.549 ± 0.067	0.625 ± 0.070	0.082 ± 0.081
Mt. Jirisan		298	11.1 ± 2.4[a]	5.2 ± 1.6	0.570 ± 0.066	0.657 ± 0.067	0.106 ± 0.069

[a] Mean ± SE

N: number of samples; *A*: number of alleles; A_e: number of effective alleles; H_o: observed heterozygosity; H_e: expected heterozygosity; *F*: fixation index

The AMOVA results showed that the genetic variance across subpopulations was 3% of the total, whereas that among individuals within each subpopulation was 97% of the total (Table 4). The Nei's genetic distance across subpopulations was 0.040–0.047, and the F_{ST} value between each subpopulation was 0.013–0.011 (Table 5).

Table 4
Result of genetic differentiation of subpopulations by analysis of molecular variance (AMOVA)

	Degrees of Freedom	Sum of Squares	Mean Squares	Estimated Variation	Percent of Variation (%)	*p*
Among populations	2	60.502	30.251	0.228	3	0.001
Within populations	294	2248.071	7.646	7.646	97	
Total	296	2308.572		7.875	100	

Table 5
Nei's genetic distance below the diagonal and pairwise F_{ST} values above the diagonal among the three subpopulations of *Abies koreana* on Mt. Jirisan

Subpopulation	Byeoksoryeng	Banyabong	Cheonwangbong
Byeoksoryeng	-	0.011***	0.013***
Banyabong	0.040	-	0.012***
Cheonwangbong	0.047	0.043	-
***: $p < 0.001$			

3.2. Spatial genetic structure

Patterns of spatial genetic structure of *A. koreana* subpopulations on Mt. Jirisan are shown in Fig. 2. If the observed mean pairwise genetic distance in each distance class was greater than or less than the 95% confidence interval (generated by 1000 permutations), the individual was considered to be randomly distributed. Individuals within the Byeoksoryeng subpopulation were genetically similar when distributed within a distance of 20 m, whereas those within a distance of 20 m–60 m were randomly distributed (Fig. 2a). Some genetically similar structures were found in individuals with distances in the range of 60 m–70 m, whereas those with distances over 80 m were randomly distributed. Within the Banyabong subpopulation, individuals over a distance of 10 m had the same genetic structure, and those with a distance of 10 m–30 m were randomly distributed (Fig. 2b). Although some genetically similar structures were found in individuals with distances between 30 m and 40 m, those with distances > 40 m were randomly distributed. Within the Cheonwangbong subpopulation, individuals distributed over 20 m distance had the same genetic structure, and those with a distance between 20 m and 30 m were randomly distributed (Fig. 2c). Although some genetically similar structures were found in individuals with distances between 30 m and 40 m, those with distances greater than 40 m were randomly distributed.

3.3. Sample selection strategies

For sample size selection of restoration materials containing alleles with a frequency ≥ 0.05, the sampling distance presented above was applied and the optimal sample size was determined. As seen from the previous genetic distance analysis across subpopulations, the difference between each subpopulation was small. Therefore, the appropriate number of samples was analyzed for the Banyabong subpopulation, which had the highest number of alleles among the three subpopulations. Random sampling of 5 to 30 individuals, by applying a 10 m distance, in the Banyabong subpopulation showed that more than 95% of total alleles with a frequency ≥ 0.05 were secured when more than 25 individuals were extracted (Fig. 3).

4. Discussion

Genetic diversity is influenced by ecological factors, such as the geographical distribution and breeding methods of species (Ueno et al. 2000; Shin et al. 2014). The *A. koreana* habitat on Mt. Jirisan is fragmented, and young trees are rapidly declining in number; therefore, genetic diversity is likely to decrease (Kim et al. 2018). Hence, it is necessary to establish an appropriate restoration strategy to maintain and conserve the genetic diversity of *A. koreana* on Mt. Jirisan.

In the current study, the H_e value of *A. koreana* on Mt. Jirisan was 0.657. The genetic diversity of *A. koreana* on Mt. Jirisan we observed (H_e = 0.672) was similar to that reported by Ahn et al. (2017) but was lower than that reported by Kwak et al. (2017) (H_e = 0.778). The H_e, an index representing the genetic diversity within a population, is estimated via the allele frequencies calculated by the marker (Hartl and Clark 1997). Therefore, differences in the results from various studies can be considered to be based on the type and number of markers used in the analysis.

Our results showed that genetic diversity of *A. koreana* on Mt. Jirisan was higher than that on Mt. Hallasan (H_e = 0.614) and Mt. Geumwonsan (H_e = 0.612) and was similar to that on Mt. Deokyusan (H_e = 0.655) (Chae et al. 2022). Mt. Hallasan is located on an island, and Mt. Geumwonsan has a small population of < 20 individuals with low genetic diversity; therefore, individual movement on Mt. Hallasan and Mt. Geumwonsan is limited (Chae et al. 2022). Mt. Jirisan and Mt. Deokyusan, located near the south of the Korean peninsula, are the main mountains with an area of more than 100 ha of sub-alpine coniferous forest (Lim et al. 2019). Therefore, *A. koreana* populations on Mt. Jirisan and Mt. Deokyusan showed similarly high levels of genetic diversity.

Genetic diversity of *A. koreana* on Mt. Jirisan was lower than that of *A. alba* (H_e = 0.724) (Belletti et al. 2017), *A. nordmanniana* (H_e = 0.822–0.961) (Hansen et al. 2005), *A. cilicica* (H_e = 0.689) (Sękiewicz et al. 2015), and *A. firma* (H_e = 0.747) (Iwaizumi et al. 2019), and higher than that of *A. pinsapo* (H_e = 0.596) (Cobo-Simón et al. 2020) and *A. fraseri* (H_e = 0.442) (Potter et al. 2008).

The AMOVA results showed the difference in genetic variation across subpopulations of *A. koreana* on Mt. Jirisan to be 3% of the total, indicating the genetic difference to be small across the subpopulations (Table 4). According to a previous study (Chae et al. 2022), the subpopulations of *A. koreana* on Mt. Hallasan showed a difference of 4% in genetic variation, similar to the subpopulations of *A. koreana* on Mt. Jirisan. In addition, the genetic distance (F_{ST}) across the subpopulations of *A. koreana* on Mt. Jirisan was 0.013–0.011 (Table 5). The results indicated little genetic difference across the subpopulations of *A. koreana* on Mt. Jirisan. According to a previous study, the F_{ST} across the three subpopulations on Mt. Jirisan was 0.014, and the optimal population on Mt. Jirisan, estimated by the Pritchard method, was reported to be one appropriate (Ahn et al. 2017). Therefore, it should not be difficult to secure most of the alleles, without considering the subpopulation, when selecting the restoration material of *A. koreana* on Mt. Jirisan.

An understanding of the spatial genetic structure of populations is required to maximize the genetic diversity or minimize consanguinity; knowledge of the spatial genetic structure can be used to develop

breeding programs and in-situ conservation of natural populations (Ueno et al. 2000). In this paper, while selecting materials for the restoration of *A. koreana* on Mt. Jirisan, spatial autocorrelation analysis was performed on the genetic variation of each subpopulation on Mt. Jirisan to avoid genetically similar samples. Analysis results showed that it would be appropriate to take samples of the Banyabong subpopulation at intervals of 10 m or more when sampling *A. koreana*, whereas for the Byeoksoryeong and Cheonwangbong subpopulations, it would be appropriate to collect samples at intervals of 20 m or more (Fig. 2). Chae et al. (2022) had proposed a strategy to sample *A. koreana* on Mt. Hallasan, at a distance of 5 m in the Bangaeoreum subpopulation and at 10 m in the Yeongsil and Jindalleabat subpopulations. The *A. koreana* subpopulations on Mt. Jirisan are characterized by lower density (number of *A. koreana* per 1 ha on Mt. Jirisan, 320.5; number of *A. koreana* per 1 ha on Mt. Hallasan, 647.9) (Lim et al. 2019) and higher height than *A. koreana* on Mt. Hallasan (average height of *A. koreana* of Mt. Jirisan, 15.85 m; average height of *A. koreana* on Mt. Hallasan, 4.17 m) (Chae et al. 2022). According to Epperson (1992), lower the density of the forest, farther can the pollen or seeds be dispersed, which might explain the results of the spatial autocorrelation analysis of population. In addition, higher plant heights can cause seed dispersion to occur farther away, which can change the results of spatial autocorrelation analysis (Thomson et al. 2011). Therefore, it is considered that the geographical distance of genetically similar individuals of *A. koreana* of Mt. Jirisan is farther than that of genetically similar individuals of *A. koreana* of Mt. Hallasan.

For the purpose of sampling 95% alleles (with frequencies greater than 0.05) of *A. koreana* on Mt. Jirisan, we calculated the appropriate number of restoration materials. Results showed that if more than 25 samples are collected at a distance of 10 m for the Banyabong subpopulation, with relatively high genetic diversity, the latter of *A. koreana* on Mt. Jirisan would be stably secured. In the sub-alpine forest, where manpower and costs are limited due to difficulty in access, efficient selection of restorative materials can be made if sampling strategies such as the results of this study are introduced.

The Korea Forest Service reported that restoration efforts are urgently required for the endangered sub-alpine coniferous species *A. nephrolepis*, *Picea jezoensis*, *Thuja koraiensis*, *Juniperus chinensis*, *Picea pumila*, and *Taxus cuspidate*, in addition to *A. koreana* (Lim et al. 2019). Our present approach can be applied to develop conservation and restoration strategies for coniferous species other than *A. koreana* that are endangered given the changing and hostile environmental conditions.

5. Conclusions

A. koreana subpopulations on Mt. Jirisan need appropriate maintenance and conservation strategy to survive the changing environmental conditions resulting from climate change. Restoration using native species with genetic diversity adapted to the environmental conditions of their native habitat would enable long-term restoration success. We established restoration strategies via the analysis of genetic diversity and spatial genetic structure of *A. koreana* on Mt. Jirisan. In this study, genetic diversity of the three subpopulations on Mt. Jirisan was found to be relatively high, and genetic differentiation across subpopulations was rarely seen. Therefore, the restoration strategy for *A. koreana* on Mt. Jirisan would involve the collection of more than 25 samples at a distance of 10 m each within the Banyabong subpopulation.

Declarations

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Figures

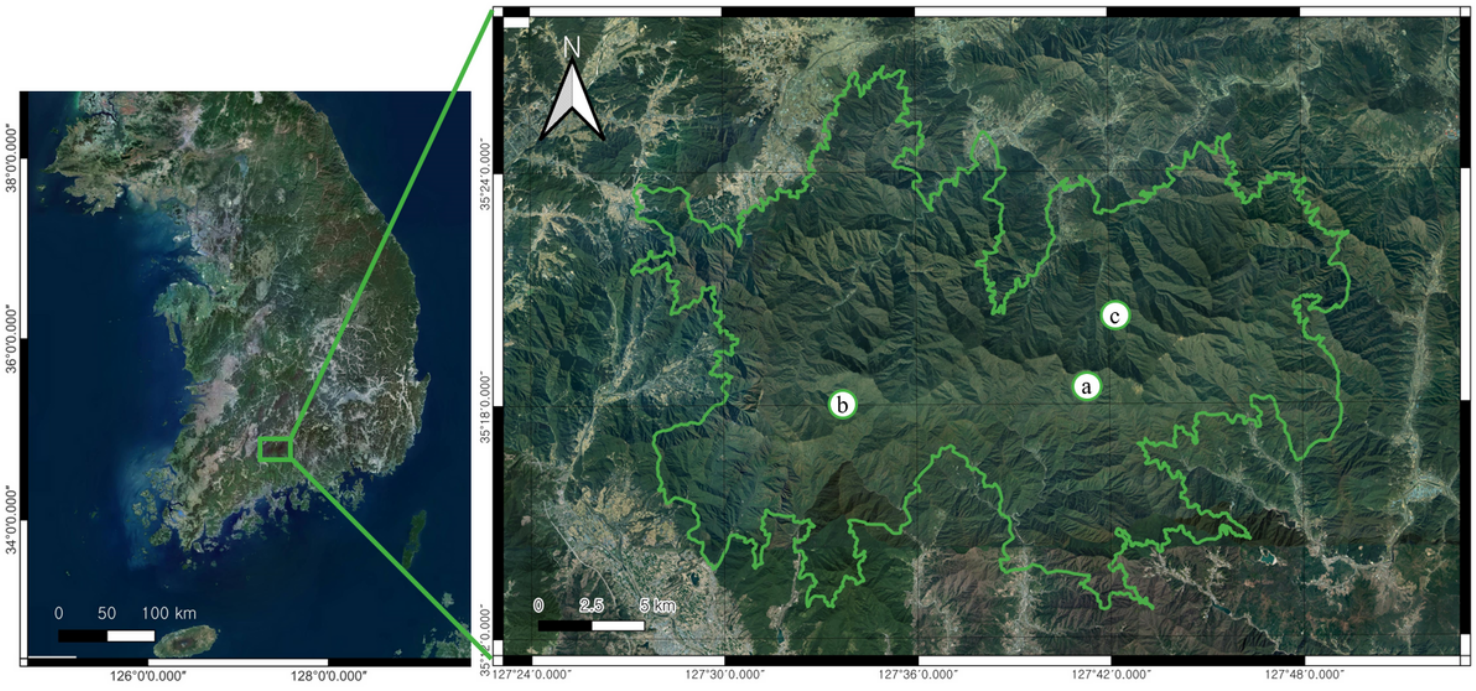

Figure 1

Locations of three subpopulations of *Abies koreana* on Mt. Jirisan in the Republic of Korea. (a) Byeoksoryeng; (b) Banyabong; (c) Cheonwangbong

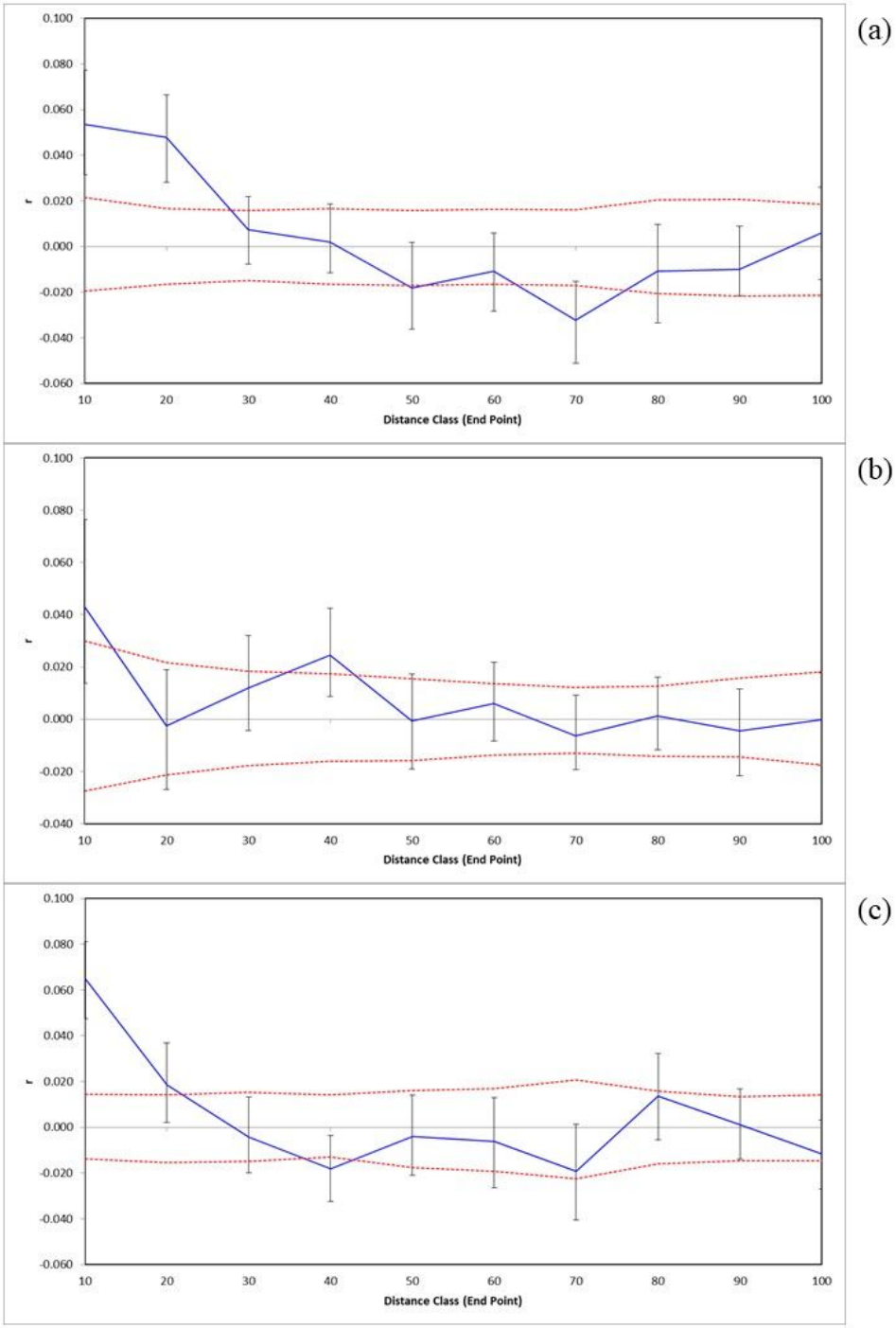

Figure 2

Results of spatial structure analysis for *Abies koreana* using 10 nuclear simple sequence repeat (nSSR) markers. The *r*value is an autocorrelation coefficient. The solid line represents the correlation between genetic distance and geographic distance. The dotted lines represent 95% confidence intervals. (a) Byeoksoryeng; (b) Banyabong; (c) Cheonwangbong

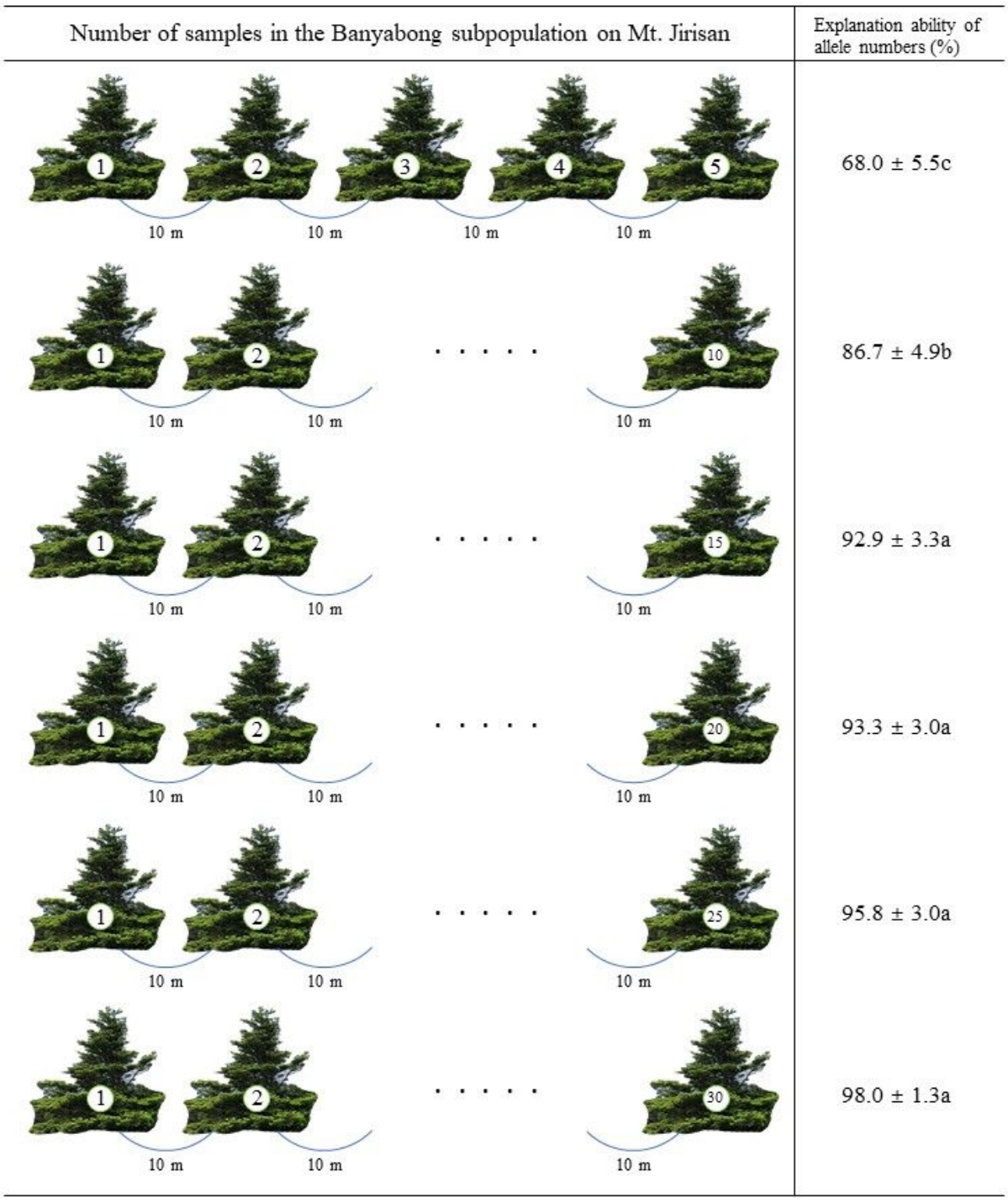

Figure 3

Comparison of allele numbers with a frequency ≥ 0.05 in *Abies koreana* on Mt. Jirisan according to the sample size extracted based on the sampling strategy. Values with different letters are significantly different according to Duncan's test at $p \leq 0.05$