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Small-scale spatial genetic structure of *Asarum sieboldii* metapopulation in a valley

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Abstract

Background: *Asarum sieboldii* Miq., a species of forest understory vegetation, is an herbaceous perennial belonging to the family Aristolochiaceae. The metapopulation of *A. sieboldii* is distributed sparsely and has a short seed dispersal distance by ants as their seed distributor. It is known that many flowers of *A. sieboldii* depend on self-fertilization. Because these characteristics can affect negatively in genetic structure, investigating habitat structure and assessment of genetic structure is needed. A total of 27 individuals in a valley were sampled for measuring genetic diversity, genetic distance, and genetic differentiation by RAPD-PCR.

Results: The habitat areas of *A. sieboldii* metapopulation were relatively small (3.78~33.60 m^2) and population density was very low (five to seven individuals in 20×20 m quadrat). The habitat of *A. sieboldii* was a very shady (relative light intensity = 0.9%) and mature forest with a high evenness value (J = 0.81~0.99) and a low dominance value (D = 0.19~0.28). The total genetic diversity of *A. sieboldii* was quite high (h = 0.338, I = 0.506). A total of 33 band loci were observed in five selected primers, and 31 band loci (94%) were polymorphic. However, genetic differentiation along the valley was highly progressed (G_{st} = 0.548, N_m = 0.412). The average genetic distance between subpopulations was 0.387. The results of AMOVA showed 52.77% of variance occurs among populations, which is evidence of population structuring.

Conclusions: It is expected that a small-scale founder effect had occurred, an individual spread far from the original subpopulation formed a new subpopulation. However, geographical distance between individuals would have been far and genetic flow occurred only within each subpopulation because of the low density of population. This made significant genetic distance between the original and new population by distance. Although genetic diversity of *A. sieboldii* metapopulation is not as low as concerned, the subpopulation of *A. sieboldii* can disappear by stochastic events due to small subpopulation size and low density of population. To prevent genetic isolation and to enhance the stable population size, conservative efforts such as increasing the size of each subpopulation or the connection between subpopulations are needed.

Keywords: Founder effect, Genetic differentiation, Genetic diversity, Genetic fragmentation, RAPD, Self-compatibility, Understory herb

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Background

Forest understory vegetation is an important component of the forest ecosystem. It functions as a key factor in controlling microbial, chemical, and physical mechanisms during soil organic matter formation and helps decomposing leaves to increase the accumulation of carbon and nitrogen (Qiao et al. 2014). In addition, forest understory vegetation is composed of various plant species and contributes to the enhancement of forest biodiversity by providing habitats to numerous animals. These forest understory herbs are vulnerable to environmental change because their population densities can increase slowly due to slow growth and reproduction rates. Therefore, they are slow to reoccupy suitable habitats once extirpated or greatly reduced in population numbers (Meier et al. 1995).

Asarum sieboldii Miq., a species of forest understory vegetation, is an herbaceous perennial belonging to the family Aristolochiaceae. It grows in a mountain valley and a shaded slope of the forest in Republic of Korea, Prismorsky Krai of Russia, Japan, and eastern China (GBIF Secretariat 2021). *Asarum sieboldii* is famous for its medicinal properties in East Asia. It has been widely used in traditional medical practices for curing inflammation and ache in China, Korea, and Japan (Yamaki et al. 1996). Particularly, the dried root of *A. sieboldii* was called "Seshin" in Korea. It contains asarinin and aristolochic acid.

The flower of *A. sieboldii* is protogynous which the pistil matures first and then the stamen matures. At the first stage of flowering, pistils mature before stamens mature, and cross-pollination with pollen of other individuals occurs by ants or flies. During the second stage of flowering, stamens with the opened anther fold upwards and directly contact the pistils to enable self-pollination (Nesterova and Nakonechnaya 2018). Wang and Gao (2012) estimated that *A. sieboldii* is mainly pollinated by natural self-pollination and cross-pollination is less likely to occur than selfing. If the plant propagates mainly by self-pollination for a long time, it is expected to have a negative impact on the genetic diversity and environmental adaptation of plants. According to the "dead-end hypothesis" of Stebbins (1957), a population that reproduces through selfing can accumulate deleterious mutations (Lynch et al. 1995) and may result in the loss of genetic diversity to adapt to changing environmental conditions (Awad and Roze 2018). Short seed dispersal distance of *A. sieboldii* can also negatively affect genetic diversity. The seed of *A. sieboldii* has elaiosomes, which attract ants as seed dispersal mediators. Ants move the seed of *A. sieboldii* to their nest to eat elaiosome. However, the seed dispersal distance of ants is shorter than other insect dispersal mediators with less than 1 m (Palfi et al. 2017). Besides, the population of *Asarum* species are known to have scattered and discontinuous distribution pattern with small population size and have low population density (So and Kim 2013). These population inhabitation and reproduction characteristics are known to increase genetic drift and affect genetic structure and diversity (Llorens et al. 2017), which could lead to the risk of an extinction. To conserve *A. sieboldii* population as a component of understory vegetation, assessment of the genetic structure is needs to proceed.

Also, *Asarum* species are known as a food source specific to the larvae of *Luehdorfia puziloi* (Papilionidae) (Shin 1991). *Luehdorifa puziloi* is an important pollinator of spring ephemeral herb *Erythronium japonicum* (Liliaceae) (Kawano and Nagai 1982). Adult *L. puziloi* individuals visit *E. japonicum* for pollination with nectar in the spring, and they lays eggs on the leaves of *Asarum* species when perianths of *E. japonicum* flower begin to fall. *Luehdorfia puziloi* is an important species as a "seasonal notification species," and adult emergence timing in early spring gets faster under the warmer soil temperature with global warming in the Republic of Korea (Kim and Nam 2018). Because phenological shift of each species is affected differently under altered climate condition (Kharouba and Vellend 2015), the interaction between *Asarum* species and *L. puziloi* also could be affected under climate change. Therefore, assessment of the population characteristics of *Asarum* species is important to understand the interaction with *L. puziloi*, which have the host specificity of larvae.

In previous preliminary studies, the genetic diversity of *A. sieboldii* population in eastern China was low and it was found that the differentiation by genetic drift was progressed to some extent (Liu et al. 2007). And also, a very low level of allozyme polymorphism was observed in the population of *A. sieboldii* in the Primorsky region of Russia (Nakonechnaya and Koren 2017). Because previous preliminary studies did not consider habitat characteristics of *A. sieboldii* and having problems with finding appropriate markers to measure the genetic diversity of *A. sieboldii*, estimating accurate genetic diversity and structure of *A. sieboldii* is needed. If the low genetic diversity of *A. sieboldii* is due to the self-pollination and the sparse population distribution, it is expected that *A. sieboldii* in South Korea also have low genetic diversity. However, the study on *A. sieboldii* in South Korea has focused on phylogenetics and medicine, and there is a lack of information on life history and genetic diversity. Therefore, this study aims to investigate the habitat characteristics of *A. sieboldii* metapopulation in Eobi valley, a habitat where *A. sieboldii* is relatively continuously distributed. Also, this study will identify genetic structure and measure genetic diversity of *A. sieboldii* metapopulation in the Eobi valley.

Table 1 The information of five studied *A. sieboldii* subpopulations (EB1~EB5) of the Eobi valley

Subpopulation	Location	Altitude (m)	Slope (°)	Relative light intensity (%)	Habitat area (m^2)	No. of individuals
EB1	37°34′32″N 127°30′47″E	613.6	16	1.01	3.78	5
EB2	37°34′35″N 127°30′52″E	571.1	16	1.15	5.20	5
EB3	37°34′38″N 127°30′54″E	549.1	11	1.21	33.60	6
EB4	37°34′44″N 127°30′56″E	500.6	19	0.65	14.08	5
EB5	37°34′50″N 127°31′00″E	450.0	20	0.34	9.46	6
Total						27

Results

Habitat characteristics

At the survey site, the habitat area of each *A. sieboldii* subpopulation was relatively small (3.78~33.60 m^2) and population density was very low. In the 20×20 m quadrat of each study site, only five to seven *A. sieboldi*i individuals were observed (Table 1). The relative light intensity of *A. sieboldii* habitat was very low which ranged from 0.34 to 1.21%. According to the vegetation survey, from 22 to 34 species appeared in each subpopulation. The number of emerged plant species was lowest at EB4 with 22 species, whereas highest at EB5 with 34 species (Table 2). A total of 61 species appeared among five sites. Shannon-Wiener species diversity index (*H′*) ranged from 0.56 to 3.19 for each layer of the site. Pielou's evenness index (*J*) ranged from 0.81 to 0.99 for each layer of the site. Species richness index (*R*) ranged from 6.52 to 9.32 for each site. Dominance index (*D*) of each site ranged from 0.19 to 0.28. Dominant species of each site were [EB1: tree layer-*Acer pseudosieboldianum*, subtree layer-*A. pseudosieboldianum*, shrub layer-*A. pseudosieboldianum*, herb layer-*Actinidia arguta*], [EB2: tree layer-*Carpinus cordata*, subtree layer-*C. cordata*, shrub layer-*Weigela subsessilis*, herb layer-*A. arguta*], [EB3: tree layer-*Acer pictum* var. *mono*, subtree layer-*A. arguta* and *A. pseudosieboldianum*, shrub layer-*A. pseudosieboldianum*, *Staphylea bumalda*, *Stephanandra incisa* and *Lespedeza maximowiczii*, herb layer-*A. arguta*], [EB4: tree layer-*C. cordata*, subtree layer-*C. cordata* and *A. pseudosieboldianum*, shrub layer-*A. arguta* and *Deutzia grandiflora*, herb layer-*Pseudostellaria heterophylla* and *Polystichum tripteron*], and [EB5: tree layer-*Cornus controversa*, subtree layer-*A. pseudosieboldianum*, shrub layer-*W. subsessilis*, herb layer-*Hydrangea serrata* var. *acuminate* and *S. incisa*] (Table S1).

Table 2 Diversity parameters of studied sites of *A. sieboldii* habitat in Eobi valley

Site	Layer	# of species	*H′*	*J*	*R*	*D*
EB1	Tree	4	1.35	0.98	6.83	0.24
	Subtree	2	0.56	0.81		
	Shrub	4	1.33	0.96		
	Herb	19	2.69	0.91		
	Total	25				
EB2	Tree	4	1.37	0.99	7.02	0.19
	Subtree	3	1.04	0.95		
	Shrub	4	1.33	0.96		
	Herb	18	2.78	0.96		
	Total	24				
EB3	Tree	6	1.64	0.92	6.99	0.19
	Subtree	4	1.33	0.96		
	Shrub	5	1.58	0.98		
	Herb	15	2.58	0.95		
	Total	25				
EB4	Tree	4	1.29	0.93	6.52	0.28
	Subtree	3	1.05	0.96		
	Shrub	8	2.03	0.97		
	Herb	11	2.35	0.98		
	Total	22				
EB5	Tree	6	1.70	0.95	9.32	0.20
	Subtree	3	0.96	0.87		
	Shrub	4	1.21	0.88		
	Herb	25	3.19	0.99		
	Total	34				
Total		61			12.14	0.22

H′: Shannon-Wiener species diversity index (Pielou 1969), *J*: Pielou's evenness index (Pielou 1975), *R*: Richness index (Margalef 1958), *D*: Dominance index (Mcnaughton 1967)

Genetic diversity within subpopulations

A total of 33 band loci were observed in five selected primers, and 31 band loci (94%) were polymorphic (Table 3). Two band loci (6.5%) were only observed in the EB5 subpopulation. Average Nei's gene diversity (*h*) and Shannon's information index (*I*) of each subpopulation were 0.153 and 0.225, respectively (Fig. 1). Subpopulations of EB4 and EB1 showed the highest (EB4: h = 0.202; I = 0.296) and the lowest (EB1: h = 0.125; I = 0.176) intra-subpopulation genetic diversity, respectively. Nei's gene diversity and Shannon's information index of the total Eobi valley metapopulation were 0.338 and 0.506, respectively.

Table 3 The information about RAPD primers and amplified fragments

Primers	Annealing temp. (°C)	Sequence (5′→3′)	Number of bands		
			Polymorphic	Monomorphic	Total
N-8002	32	CAATCGCCGT	5	1	6
N-8004	32	TCGGCGATAG	8	0	8
N-8005	32	GAAACGGGTG	7	0	7
N-8045	32	CAAACGTCGG	5	0	5
N-8075	32	GAGGTCCACA	6	1	7
Total			31	2	33

Genetic distance between subpopulations

The average genetic distance between subpopulations calculated by Nei's genetic distance (Nei 1972) was 0.330. Distance between EB1 and EB5 showed the longest (0.680), whereas distance between EB2 and EB3 showed the shortest distance (0.084) (Table 4). In the UPGMA dendrogram based on Nei's genetic distance, EB5 established an outgroup as branched before the other 4 subpopulations, followed by EB4 (Fig. 2). EB2 and EB3 made a cluster. Genetic differentiation (G_{st}) (Nei 1973) between subpopulations was 0.548. Genetic flow (N_m) (McDermott and McDonald 1993) estimated by genetic differentiation was 0.412. The analysis of molecular variance (AMOVA; Excoffier et al. 1992) showed that 52.77% of variance was attributable to between-population differences. Within population variation was accounted for 47.23% of the genetic diversity (Table 5).

All population was assigned to one group (calculated after 10,000 permutations)

Discussion

In the habitat of *A. sieboldii* in the Eobi valley, diverse forest plant species were established. High Pielou's evenness index means that plant species in *A. sieboldii* habitat in the Eobi valley are evenly distributed and in a state of mutual competition (Table 2). According to Whittaker (1965), a dominance index below 0.3 indicates plant species are evenly distributed and in a state of mutual competition. It means *A. sieboldii* habitat is evenly dominated by many plant species (Table 2). In other words, the habitat of *A. sieboldii* in the Eobi valley is a very mature forest with a high evenness value and a low dominance value. The extremely low relative light intensity of the Eobi valley indicates that *A. sieboldii* habitat has a high canopy closure (Table 1). It means *A. sieboldii* is located in a very shady forest and has a high shade tolerance.

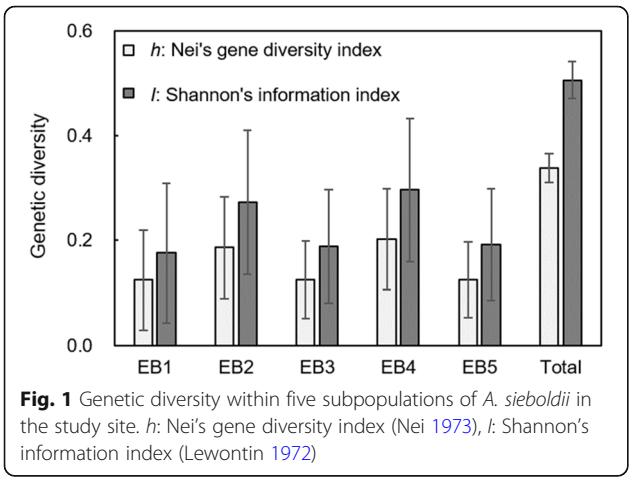

Fig. 1 Genetic diversity within five subpopulations of *A. sieboldii* in the study site. *h*: Nei's gene diversity index (Nei 1973), *I*: Shannon's information index (Lewontin 1972)

Genetic diversity of *A. sieboldii* was quite higher than expected. Nei's gene diversity of the total *A. sieboldii* metapopulation (h = 0.338) was higher than average genetic diversity of perennial herbs (h = 0.25; Nybom 2004) and annual *Persicaria thunbergii* (h = 0.297; Nam et al. 2016) (Fig. 1). It was higher than genetic diversity of herbs using both cross-fertilization and self-fertilization (0.18) (Nybom 2004). Also, it was much higher than the genetic diversity of *Aristolochia contorta*, the same member of Aristolochiaceae (h = 0.1552, I = 0.2370) (Nam et al. 2020). Genetic diversity of *A. sieboldii* was measured higher than previous studies of Liu et al. (2007) and Nakonechnaya and Koren (2017). In the study of Nakonechnaya and Koren (2017), which measured genetic diversity of *A. sieboldii* using allozyme marker, all loci were monomorphic, which showed that allozyme was not the proper marker for identifying genetic diversity of *A. sieboldii*. Liu et al. (2007) conducted study in a very wide range of eastern China and Qinling-

Table 4 Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among subpopulations (Nei 1972)

Subpopulation	EB1	EB2	EB3	EB4	EB5
EB1	–	0.850	0.820	0.666	0.507
EB2	0.163	–	0.919	0.792	0.678
EB3	0.199	0.084	–	0.754	0.646
EB4	0.406	0.233	0.282	–	0.654
EB5	0.680	0.389	0.437	0.425	–

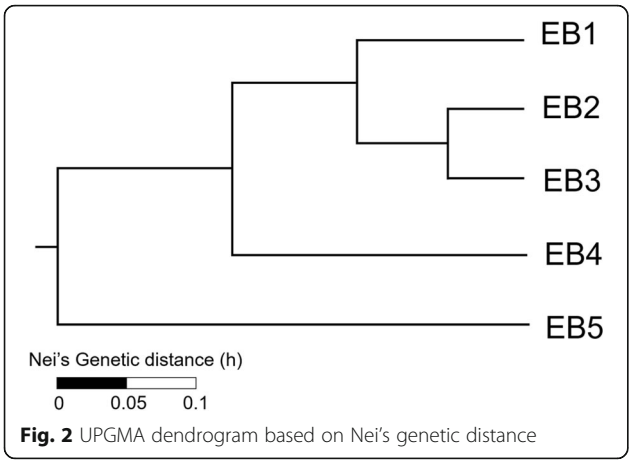

Fig. 2 UPGMA dendrogram based on Nei's genetic distance

Dabashan district. A large difference in the scale of the experiment seems to have made a difference in the level of genetic diversity. Although genetic diversity was studied in a narrow range of study sites, *A. sieboldii* showed a quite high level of genetic diversity. It is estimated that although *A. sieboldii* is self-compatible, it is more likely to be an outcrosser that relies on cross-fertilization in the present study. Sexual recombination through cross-fertilization can contribute to allelic richness. It seems necessary to explore the exact proportion of self-fertilization and cross-fertilization of *A. sieboldii* in the following study.

On the other hand, genetic differentiation was highly progressed (G_{st} = 0.548) compared to the genetic differentiation of perennial herb (0.19). It is higher than the G_{st} of herbaceous plants using both self and cross-fertilization (0.20) (Nybom 2004). The results of AMOVA showed 52.77% of variance occurs among populations (Table 5), which is evidence of population structuring. It means the *A. sieboldii* metapopulation in Eobi valley has genetically significantly differentiated despite the narrow range. It is estimated there was little genetic flow (N_m = 0.412) between subpopulations, which means that *A. sieboldii* metapopulation of the Eobi valley formed a spatial genetic structure (Doligez et al. 1998).

Asarum sieboldii showed a higher level of intra-subpopulation genetic diversity and inter-subpopulation genetic differentiation. At the scale of a valley in a mountain, these results suggest that the low density of population (So and Kim 2013) shaped the spatial genetic structure of *A. sieboldii*. It is expected that *A. sieboldii* population experienced small-scale founder effect. Seeds may have spread far from the original population occasionally and formed new populations along the valley. But because of the low density of population, geographical distance between individuals would have been far. Therefore, genetic flow occurred only within subpopulation level. It will make significant genetic distance between the original and new population by distance. Repetition of these situations over time and generations would have genetically structured *A. sieboldii* metapopulation by geographical distance. Subpopulations EB1~EB5 showed the isolated form by geographical distance along the valley (Fig. 2). The isolation by geographical distance seemed to make the lower-level genetic flow and a higher level of metapopulation-level genetic diversity in the present study.

This study is meaningful in that it revealed the pattern of *A. sieboldii* distributed along the valley although it was conducted within a narrow range. It has revealed how the genetic structure of *A. sieboldii*, which have scattered population and sparsely distributed individuals, is formed. Although the genetic diversity of *A. sieboldii* population is not as low as concerned, without subsequent genetic management, genetic isolation is worried due to high genetic differentiation and small subpopulation size. Small population size can impact the population negatively by genetic drift and diminish plant fitness. Also, it makes the population vulnerable to environmental and genetic stochastic events (Wallace 2002). Due to the small subpopulation size and low density of population, the subpopulation of *A. sieboldii* can disappear in an instant by stochastic events. Because *A. sieboldii* is an understory herb, it will take a long time to reoccupy its habitat again if it disappears (Meier et al. 1995). Also, because of the highly structured metapopulation, the population can be genetically fragmented. Population fragmentation decreases the evolutionary fitness by decreasing genetic diversity and outcrossing rate (Aguilar et al. 2008). To maintain *A. sieboldii* population, each subpopulation should have a stable population size to prevent stochastic disappearance. An effort for making genetic flow between subpopulations is required to maintain the genetic diversity and variation of *A. sieboldii* by preventing genetic drift. When planning a conservation strategy, it is necessary to consider the

Table 5 Analysis of molecular variance (AMOVA) for 27 individuals of *A. sieboldii* using 5 polymorphic RAPD markers (Excoffier et al. 2005)

Source of variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation	Fixation indices (*Fst*)	*P* value
Among populations	4	88.478	3.5201	52.77	0.52774	< 0.0001
Within populations	22	69.300	3.1500	47.23		
Total	26	157.778	6.6701			

fertilization and seed dispersion mechanism of *A. sieboldii* for increasing genetic flow between subpopulations. To prevent genetic isolation and to enhance stable population size, the habitat of *A. sieboldii* must be preserved. The valley area should be free from forest destruction, and minimizing damage from neighborhood resident or visitors is needed. Also, monitoring changes in genetic variation of the subpopulations, particularly the loss of alleles and effective population size is required. Further analysis determining the rate of cross-fertilization and seed dispersal pattern of *A. sieboldii* will be helpful.

Conclusions

Although this study was conducted within a narrow range, it is meaningful that it revealed how the genetic structure of *A. sieboldii*, which have scattered population and sparsely distributed individuals, is formed. By the result of this study, we can see that *A. sieboldii* in the Eobi valley have quite high genetic diversity, but genetic differentiation has progressed significantly and spatial genetic structure formed depending on the geographical distance. This means that although genetic diversity is high at the overall metapopulation level, there is little genetic flow between subpopulations, and genetically very different subpopulations were formed. If the present situation continues without an increase in the connection between subpopulations, genetic fragmentation of *A. sieboldii* subpopulation is concerned. Due to the small population size, the subpopulation can disappear in an instant by the genetic and environmental stochastic event. To stabilize *A. sieboldii* population, conservative efforts are needed, such as increasing the size of each subpopulation or the connection between subpopulations. Monitoring of population size and genetic variation, and protecting the habitat of *A. sieboldii* from forest destruction is needed. It should be accompanied with understanding the fertilization and seed dispersion mechanism of *A. sieboldii*.

Materials and methods

Sampling sites and field survey

A field survey was conducted in Eobi valley at Mt. Eobi, Seorak-myeon, Gapyeong-gun, Gyeonggi-do (37°34′N, 127°30~31′E) in July 2020 to investigate the community and population genetic structure of *A. sieboldii* along the valley (Fig. 3). Among the *A. sieboldii* subpopulations distributed along the valley, five sites (EB1~5; EB, Eobi valley) were selected. Because *A. sieboldii* individuals were distributed sparsely, the sites were randomly selected at intervals of 50 m altitude. At the sampling site, latitude, longitude, altitude, slope, and relative light intensity were measured. Relative light intensity was calculated by the proportion of the light intensity of the herb layer in sampling sites in neighboring open spaces

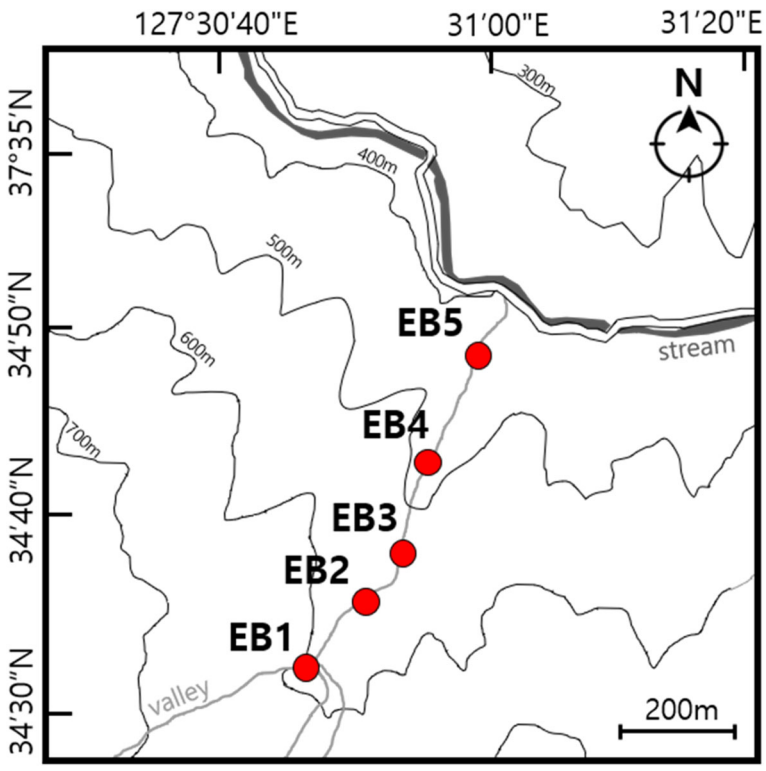

Fig. 3 Location of study sites in the Eobi valley. A total of five *A. sieboldii* subpopulations (EB1~EB5) were surveyed

using a portable photometer (LI-250, LI-COR, Lincoln, NE, USA). Vegetation survey was conducted by Braun-Blanquet's method (Braun-Blanquet 1964). Because *A. sieboldii* was distributed sparsely in a random pattern, a 20 m × 20 m size quadrat was set to fully cover the width of the valley. The vegetation is divided into four layers (tree, subtree, shrub, and herb). Dominance and abundance indices were recorded in six levels (+, 1, 2, 3, 4, 5). Shannon-Wiener diversity index (Pielou 1969), species richness (Margalef 1958), evenness (Pielou 1975), and dominance (Mcnaughton 1967) were calculated from the vegetation survey. One leaf per each *A. sieboldii* individual was collected at the site and used for DNA extraction. Five or six leaves per subpopulation were collected, therefore a total of 27 leaves were stored at -80°C before DNA extraction.

DNA extraction

Total genomic DNA of *A. sieboldii* was extracted from 27 leaf samples collected from five subpopulations. Leaf samples were ground with liquid nitrogen by mortar and pestle, and genomic DNA was extracted using a DNeasy Plant mini kit (Qiagen, Hilden, NRW, Germany). After extraction, the concentration of extracted gDNA was quantified by Nanodrop One^{c} (Thermo Fisher Scientific, Waltham, MA, USA). DNA products were diluted by triple distilled water to the concentration of 40 ng/μl and used for the subsequent amplification reaction.

RAPD analysis

Of the total 11 random primers, five primers which showed polymorphic bands consistently in repeated experiments were selected (Table 3). Random Amplified Polymorphic DNA (RAPD) analysis was conducted from PCR product in a final volume of 10 μl containing 2 μl of template DNA (40 ng/μl), 1 μl of 10x PCR buffer, 2 μl of dNTP mix (2.5 mM each), 2 μl of preselected primer (10 pmole/μl), 0.2 μl of BS eTaq DNA polymerase (5 U/μl; Biosesang, Seongnam, ROK) and 2.8 μl of triple distilled water. Amplification was performed in MiniAmp™ Plus Thermal cycler (Applied Biosystems, Foster City, CA, USA) with the following conditions: initial denaturation at 95°C for 5 min, followed by 40 cycles of 45 s of denaturation at 95°C, 45 s of primer annealing at 32°C, 1 min 35 s of extension at 72°C, and final extension step at 72°C for 2 min (Nam et al. 2016). Amplified products were detected by 1.5% agarose gel electrophoresis stained by GelRed™ (Biotium, Heyward, CA, USA).

Analysis of marker polymorphism

The clear presence and absence of a single fragment at each polymorphic loci were scored as 1 and 0 within the binary matrix, respectively. Scored data were used to calculate the genetic diversity of intra-and inter-population. Number of polymorphic loci, Nei's gene diversity (*h*; Nei 1973), Shannon's information index (*I*) (Lewontin 1972) were calculated as indices of intrapopulation genetic diversity. Also, UPGMA dendrogram from calculated Nei's genetic distance (Nei 1972) was analyzed genetic differentiation of interpopulation by using Popgene32 software (Yeh et al. 2000). The analysis of molecular variance (AMOVA; Excoffier et al. 1992) was conducted to detect population differentiation and structuring. We carried out AMOVA by treated RAPD phenotype as a haplotype (Huff et al. 1993), assigning all populations to one group. AMOVA analysis was undertaken by using the software ARLEQUIN ver. 3.5.2.2 (Excoffier et al. 2005).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41610-021-00186-x.

Additional file 1: Table S1. Constancy table of vegetation survey conducted by Braun-Blanquet's method (Braun-Blanquet 1964).

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Authors' contributions

HJJ performed the field survey, analyzed experimental data, and wrote the manuscript draft. JGK conceived the research idea, performed the field survey, and reviewed/edited the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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