Genetic diversity and structure of *Carpinus laxiflora* populations in South Korea based on AFLP markers

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**ABSTRACT**

We applied eight primer-restriction enzyme combinations to investigate genetic diversity, genetic differentiation, and genetic structure of *Carpinus laxiflora* populations with AFLP markers. The average of effective alleles (\(A_e\)), the proportion of polymorphic loci (\(Pp\)), Shannon’s diversity index (\(I\)), and the expected heterozygosity (\(H_e\)) were 1.4, 82.2%, 0.371, and 0.241, respectively. The expected heterozygosity (\(H_j\)) from Bayesian method was 0.270. The level of genetic diversity was high compared to those of *Carpinus* species and other species with a similar life history. The inbreeding coefficient (\(F_{IS}\)) from approximated Bayesian method was 0.618, which was smaller than that for *Acer pseudosieboldianum* (\(F_{IS}=0.712\)). Genetic differentiation was 0.060 from AMOVA (\(\Phi_{SC}\)) and 0.056 from Bayesian method (\(\theta_B\)). The level of genetic differentiation was very small compared to that of *Carpinus* species and other species with a similar life history. According to UPGMA and Bayesian clustering, 10 populations were divided into two genetic groups. Except Mt. Chilgap and Minjuji, most of the populations were detected as weak genetic structures according to the geographical distribution such as mountain ranges. We might consider that demographic disturbance, local specific vegetation change history, and forest succession interrupted the genetic structure of *C. laxiflora* in South Korea.

**Introduction**

The two most important components of biodiversity, species diversity and genetic diversity, have significant impact on ecosystem stability and resilience (Wehenkel et al. 2006). Forest succession is a fundamental ecological process in which the type of forest gradually changes over time and becomes stable (Byeon and Yun 2018). As the secondary succession species settle in relatively poor environments such as low nutrients and light in the stand compared to the early succession species, genetic diversity is an essential factor to consider for the adaptability of the secondary species and the stability of the ecosystem after the succession (Wehenkel et al. 2011).

*Carpinus laxiflora* Blume belongs to Betulaceae family *Carpinus* genus and is a broad-leaved tree species (Korea Forest Service 2019). The distribution range is whole mountain region including Gangwon Province. This species is Monoecious, wind-pollinated, reproduce by seedlings. Its winged seeds are dispersed by wind. Flowers bloom in late April to early May and seeds are ripe in mid-October. The wood of high quality is easily used for manufacture of equipment and furniture.

*Carpinus laxiflora* have been evaluated as representative species of the climax forest in the process of secondary succession. However, recent studies suggest that some areas are expected to maintain *C. laxiflora* forests, but it is predicted that the succession will proceed to another deciduous broad-leaved forests such as *Carpinus cordata* or *Quercus acuta*. Therefore, it should be noted that *C. laxiflora* is considered as a representative species of climax forest (Hong et al. 2012; Byeon and Yun 2018).

Amplified fragment length polymorphism (AFLP) analysis is used fingerprint information obtained directly from genomic DNA (Vos et al. 1995). AFLP markers are not need prior sequence information of the species genome and Prior genome sequence information of the target species is not required for using AFLP and even the polyploid genome can be easily analyzed (Mba and Tohme 2005). AFLP markers are applied to various studies such as population genetics, genome mapping, DNA fingerprinting, phylogeny, etc. (Reisch and Bernhardt-Römermann 2014). It is possible to generate a large number of polymorphic loci and is a highly reproducible fingerprinting technique (Kumar et al. 2013). AFLP markers are dominant and able to overcome statistical bias in genetic analysis (Lynch and Milligan 1994). Because allele frequency can be indirectly estimated by the presence or absence of observed amplicons instead of estimating alleles frequency from genotype. However, several statistical
packages have been recently developed, which can analyze dominant marker data which can be estimated using various genetic parameters such as genetic diversity, F-statistics, etc. (Excoffier and Heckel 2006). We were able to investigate genetic differentiation, genetic structure, variance of genetic variation, gene flow, and more.

About 35 species of the Carpinus genus have been reported worldwide: one species in North America, two species in Europe, and most species in Asia (Jeon et al. 2007). They are diploid species with $2n = 16$ except Carpinus betulus ($2n = 64$) in Europe. The distribution of C. laxiflora is known in Korea, Japan and China. This species is widely distributed from Hokkaido to Kyushu in Japan (Kitamura et al. 1992). However, it was not recorded as C. laxiflora but as Carpinus viminea var. viminea in Flora of China (Flora of China 2019).

There are commonly reported studies of community structure and vegetation succession in forest stands (Park et al., 2009; Hong et al. 2012; Byeon and Yun 2018). In phylogeny studies, C. laxiflora was estimated evolved species than Carpinus viminea var. viminea in China (Jeon and Chang 2000; Jeon et al. 2007). Fossil evidence has indicated that the genus Carpinus was the most differentiated in terms of the variety of species and prospered on the Korean Peninsula in the Miocene period (Kim and Nam 2017). Wood anatomy research was also carried out to gather information about species identification from timber (Chun 1992a, 1992b). There has been no reported study on the genetic diversity of C. laxiflora in Korea. Only three species (C. betulus, C. orientalis, C. laxiflora) have been studied abroad (Coart et al. 2005; Cárabas et al. 2015; Tono et al. 2016). In the present study, we conducted AFLP analysis to investigate genetic diversity, genetic differentiation, and genetic structure of C. laxiflora.

**Materials and methods**

**Sample collection and DNA extraction**

Through the literature review, we collected data on the native habitats and search tree species for sampling. A total of 296 fresh leaf samples were collected from 10 populations (Figure 1). Individuals within each population were separated from each other by at least 50 m to minimize the collection of genetically close samples. Location information individual tree is collected using GPS (Garmin, GPSmap 60CSx) and the height and diameter at breast height (DBH) of each individual were recorded when sampling. Sampled leaves were grinded by FastPrep120 homogenizer (MPBIO, USA). Total genomic DNA was extracted from each sample.
using GeneAll Plant SV Mini Kit (Seoul, South Korea) according to the manufacturer’s instructions. Total DNA concentration was measured using NanoDrop 2000 spectrophotometer (Thermo Scientific, USA).

**AFLP analysis**

The AFLP analysis was carried out as described by Vos et al. (1995) with changes. Total genomic DNA (500 ng) was digested using 10 U EcoRI and MseI endonuclease mixture (New England Biolabs, Ipswich, USA) in a total volume of 25 μL. About 15 μL ligation solution containing 5 pmol EcoRI adaptor, 50 pmol MseI adaptor (Applied Biosystems, Foster City, USA) and 100 U T4 DNA ligase (New England Biolabs, Ipswich, USA) was added to the in a total volume of 5 μL. The resulting DNA (template DNA) was then diluted 1:10 in deionized water. PCR pre-amplification was performed in 50 μL solution containing 10× reaction buffer (2 mM MgCl2), 0.2 mM dNTPs, 10 pmol AFLP primers, 0.5 pmol EcoRI and MseI selective amplification primer, fluorescent labeled 0.5 pmol EcoRI and 3 nucleotide selective amplification primer, fluorescent labeled 0.5 pmol EcoRI + 3 nucleotide selective amplification primer (Applied Biosystems, Foster City, USA) and 1 U Taq DNA polymerase (RBC Bioscience, New Taipei, Taiwan) and 15 μL template DNA. For selective PCR amplification, 10-fold diluted pre-amplification product was used as template. Diluted pre-amplification template DNA (3 μL) was added to 9 μL solution containing 10× reaction buffer (2 mM MgCl2), 0.2 mM dNTP, 2.5 pmol MseI + 3 nucleotide selective amplification primer, fluorescent labeled 0.5 pmol EcoRI + 3 nucleotide selective amplification primer (Applied Biosystems, Foster City, USA) and 1 U Taq DNA polymerase (RBC Bioscience, New Taipei, Taiwan). The amplification products were fractionated by capillary electrophoresis using ABI Prism 3730xl Genetic Analyzer (Applied Biosystems, USA). The fragment size was identified using GeneMapper v5.0 software (Applied Biosystems, USA). The height of the peak for distinguishing the amplified products from the noise peak was set to a fluorescence value (rfu) of 100 or more, and AFLP fragments were scored within the size range of 100–450 bp. The fingerprints in each individual were gathered as presence (1) or absence (0) of fragment.

**Data analysis**

We estimated genetic diversity parameters such as number of effective alleles (Ae), percentage polymorphic loci (%P), Shannon diversity index (I), and estimated heterozygosity (Hs). The genetic differentiation among populations (ΦST) was determined by analysis of molecular variance (AMOVA). Pairwise values of Nei’s genetic distance among populations were calculated and we performed principal coordinates analysis (PCA) using Nei’s genetic distance. We checked for significant correlation between pairwise value of Nei’s genetic distance and the logarithm of pairwise geographic distance using the Mantel test, which were predicted to have a linear relationship under a model of isolation by distance. All the above analysis was performed using GeneAlEx 6.41 (Peakall and Smouse 2006). AFLPsurv v1.0 software (Vekemans 2002) was used to estimate allele frequency at AFLP loci and expected heterozygote using the Bayesian approach. The f (analogous to FIS) and θII (analogous to FST) statistics were calculated using Bayesian inference for different models using HICKORY v1.1 software (Holsinger et al. 2002). Also, population-specific inbreeding coefficients (FI) were estimated by an approximate Bayesian computation using the software ABC4 (Foll et al. 2008). The genetic relationships among populations were evaluated by generating an unweighted pair-group method using arithmetic average (UPGMA) tree using Phylip v3.695 software (Felsenstein 2013). We used the Bayesian clustering method to elucidate the genetic structure among populations by using STRUCTURE v2.3 software (Pritchard et al. 2000). We assumed a pre-assigned number of genetic clusters (K) ranging from 1 to 11. It simulations were run 20 times for each value of K. All runs involved 30,000 Markov chain Monte Carlo (MCMC) sampling, after a burn-in period of 30,000 iterations. The optimum K value was evaluated by calculating ΔK. The value was calculated according to the method of Evanno et al (2005), which is based on mean log probability of data and standard deviation, using STRUCTURE HARVESTER program (Earl and vonHoldt 2012).

**Results**

**Genetic diversity within a population**

We observed a total 166 polymorphic bands from eight endonuclease-primer combinations (Table 1). The mean number of effective alleles (Ae), percentage polymorphic loci (%P), Shannon diversity index (I) and estimated heterozygosity (Hs) were 1.399, 82.2%, 0.371, 4.102.

**Table 1.** Number of AFLP amplified bands and polymorphic bands per primer-restriction enzyme combinations from *Carpinus laxiflora* populations.

| Primer-restriction enzyme combinations | Total number of amplified bands | Number of polymorphic bands |
|----------------------------------------|---------------------------------|----------------------------|
| E-AAG + M-CTG*                         | 22                              | 10                         |
| E-ACC + M-CAA                          | 38                              | 28                         |
| E-AGC + M-CTT                          | 38                              | 25                         |
| E-AAG + M-CTG                          | 34                              | 16                         |
| E-CTG + M-CTT                          | 25                              | 18                         |
| E-AAG + M-CAC                          | 40                              | 29                         |
| E-AGC + M-CAT                          | 32                              | 22                         |
| E-AGG + M-CTA                          | 25                              | 16                         |
| Total                                  | 254                             | 166                        |

*Restriction enzymes E- and M- are EcoRI I and Mse I, respectively.*
and 0.241, respectively (Table 2). The estimated heterozygosity ($H_j$) using Bayesian method was 0.270. Among 10 populations, the highest level of genetic diversity was observed for the Mt. Chilgap population ($A_e = 1.428$, $I = 0.397$, $H_e = 0.259$, $H_j = 0.282$). The Mt. Chik population had the lowest genetic diversity ($A_e = 1.373$, $I = 0.351$, $H_e = 0.228$, $H_j = 0.257$). The population-specific $F_{ST}$ obtained from the approximate Bayesian method varied from 0.503 (Mt. Sokri) to 0.736 (Mt. Chiak), with an average of 0.618.

**Genetic relationship and genetic structure**

The genetic relationship was found out from UPGMA dendrogram based on Nei’s genetic distance (Figure 2). Mt. Sokri and Mt. Chiak populations were genetically close. The UPGMA dendrogram showed two genetic clusters. Including Mt. Chiak and Mt. Sokri with the minimum genetic distance, we obtained one genetic group with 7 populations clustered (Mt. Sokri, Mt. Chiak, Mt. Seorak, Mt. Odae, Mt. Gariwang, Mt. Juwang, and Mt. Palgong) and another genetic group with three populations clustered including Mt. Chilgap, Mt. Minjuji, and Mt. Jiri. The first (PC1) and second (PC2) principal components accounted for 56.04% and 43.96% of genetic variation, respectively (Figure 3). We represented the PCA results in the form of a scatter diagram. The results of one cluster including Mt. Chilgap, Mt. Minjuji, and Mt. Jiri was similar with the UPGMA dendrogram. There was no significant correlation between genetic distance and geographic distance in the Mentel test ($r = 0.276$, $p = 0.05$). In Bayesian clustering analysis the value of $\Delta K$ was the highest at $K = 2$ based on the theory suggested by Evanno et al. (2005). Therefore, we have visualized the assignment probabilities from the two clusters for each individual (Figure 4). The proportion

**Genetic differentiation among populations**

AMOVA analysis revealed genetic differentiation between populations (Table 3). At the population level, 6% of the total molecular variation was attributed to inter-population differentiation, and 96% to individual differentiation within populations ($\Phi_{ST} = 0.060$, $p < 0.001$). From Bayesian analysis (Holsinger et al. 2002), the smallest deviance information criterion ($DIC = 6889.8$) was obtained from full model among four different models and the obtained $f$ (analogous to $\text{DIC}$ based on the theory suggested by Evanno et al. 2005). Therefore, we have visualized the assignment probabilities from the two clusters for each individual (Figure 4). The proportion

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### Table 2. Genetic diversity parameters estimated from *Carpinus laxiflora* populations with AFLP markers.

| Population | $N^a$ | $A_e$ | $%P$ | $I$ | $H_e$ | $H_j$ | $F_{ST}$ |
|------------|------|-------|------|-----|-------|-------|---------|
| Mt. Chiak  | 30   | 1.373 (0.026) | 78.3 | 0.351 (0.020) | 0.228 (0.014) | 0.257 (0.013) | 0.736 |
| Mt. Chilgap | 32   | 1.428 (0.027) | 67.4 | 0.397 (0.018) | 0.259 (0.014) | 0.282 (0.013) | 0.550 |
| Mt. Gariwang | 28  | 1.387 (0.027) | 77.1 | 0.356 (0.020) | 0.233 (0.014) | 0.267 (0.013) | 0.592 |
| Mt. Jiri    | 26   | 1.408 (0.026) | 83.1 | 0.382 (0.019) | 0.249 (0.014) | 0.277 (0.013) | 0.678 |
| Mt. Juwang  | 30   | 1.385 (0.027) | 81.3 | 0.360 (0.019) | 0.234 (0.014) | 0.262 (0.013) | 0.542 |
| Mt. Minjuji | 30   | 1.403 (0.027) | 83.7 | 0.375 (0.019) | 0.243 (0.014) | 0.272 (0.013) | 0.700 |
| Mt. Odae    | 30   | 1.402 (0.028) | 81.9 | 0.371 (0.019) | 0.241 (0.014) | 0.270 (0.013) | 0.658 |
| Mt. Palgong | 30   | 1.421 (0.028) | 84.3 | 0.384 (0.019) | 0.251 (0.014) | 0.282 (0.013) | 0.616 |
| Mt. Sokri   | 30   | 1.415 (0.028) | 83.7 | 0.381 (0.019) | 0.248 (0.014) | 0.276 (0.013) | 0.503 |
| Mt. Seorak  | 30   | 1.369 (0.026) | 81.3 | 0.352 (0.019) | 0.227 (0.014) | 0.257 (0.013) | 0.600 |
| Mean       |     | 1.399 (0.009) | 82.2 | 0.371 (0.006) | 0.241 (0.004) | 0.270 (0.003) | 0.618 |

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**Table 3. Distribution of genetic variations of 10 *Carpinus laxiflora* populations from analysis of molecular variance (AMOVA).**

| Source of variation | d.f. | Sum of squares | Variance components | Percentage of variance | $\Phi_{ST}$ |
|---------------------|------|---------------|---------------------|-----------------------|-------------|
| Among populations   | 9    | 563.065       | 1.381               | 6.0%                  | 0.060***    |
| Within populations  | 286  | 6202.952      | 21.689              | 94.0%                 | 1.000       |
| Total               | 295  | 6766.017      | 23.070              | 100.0%                | 1.000       |

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***$p < 0.001$.**

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**Table 4. Inbreeding coefficient ($f$) and population differentiation ($\theta_{IT}$) of *Carpinus laxiflora* using different models based on Bayesian method.**

| Model | Mean | SD ± | 2.5% | 97.5% | Mean | SD ± | 2.5% | 97.5% | DIC ± |
|-------|------|------|------|------|------|------|------|------|------|-------|
| $f$  | 0.865 | 0.106 | 0.586 | 0.995 | 0.056 | 0.004 | 0.049 | 0.063 | 6889.8 |
| $\theta = 0$ | 0.955 | 0.044 | 0.836 | 0.999 | 0.038 | 0.002 | 0.033 | 0.042 | 6943.7 |
| $f = \text{free}$ | 0.504 | 0.292 | 0.028 | 0.979 | 0.078 | 0.015 | 0.056 | 0.115 | 7490.8 |

$^a$Sample size, $N$: number of effective alleles, $A_e$: number of polymorphic loci, $I$: Shannon’s diversity index, $H_e$: expected heterozygosity, $H_j$: expected heterozygosity by Bayesian method with non-uniform prior distribution of allele frequency (Zhivotovsky, 1999), $F_{ST}$: inbreeding coefficient estimated by an approximate Bayesian computation.

$^b$Numbers in parentheses are standard deviations.

$^c$Numbers in parentheses are standard deviations.

$^d$DIC: deviance information criterion.

$^e$P: probability.

$^f$Model: statistics for different models. $f$: inbreeding coefficient estimated by an approximate Bayesian computation.

$^g$DIC: deviance information criterion.
Figure 2. UPGMA dendrogram based on Nei's genetic distance of each *Carpinus laxiflora* population.

Figure 3. Principal components analysis (PCA) for 10 *Carpinus laxiflora* populations. The axis 1 and axis 2 explained 56.04% and 43.96% of genetic variation, respectively.
of Mt. Chilgap, Mt. Minjuji, and Mt. Jiri was high in cluster I, whereas that of other populations was high in cluster II. Specially, the clustering pattern shown by the barplots indicated that cluster I was almost entirely dominated by the Mt. Chilgap and Mt. Minjuji populations.

Discussion

Genetic diversity within a population

The level of genetic diversity ($H_e = 0.241, H_I = 0.270$) was higher than those in Acer pseudosieboldianum ($H_e = 0.231, H_I = 0.253$; Ahn et al. 2016) and Carpinus orientalis ($H_I = 0.218$, Coart et al. 2005). The level of genetic diversity was lower than that of C. betulus ($H_I = 0.333$, Coart et al. 2005; $H_e = 0.309$, Carábus et al. 2015). In a microsatellite study, the estimated genetic diversity ($H_e$) of C. laxiflora in Japan was 0.780 (Tono et al. 2016). The level of genetic diversity was also higher than that of A. pseudosieboldianum ($H_e = 0.610$, Takayama et al. 2013). An AFLP metadata study revealed that genetic diversity depends on life history traits, as with some meta-analysis from isozyme, RAPD, and microsatellite studies (Reisch and Bernhardt-Römermann 2014). The level of genetic diversity ($H_e$) was higher than that of perennial ($H_e = 0.160$), common ($H_e = 0.200$), and wind pollinated ($H_e = 0.190$) species, which have similar life history traits with C. laxiflora. Also, this is a secondary succession species. Compared to microsatellite studies (Nybom 2004), the level of genetic diversity ($H_e = 0.780$, Tono et al. 2016) is also higher than perennial ($H_e = 0.650$), wind-dispersed ($H_e = 0.610$), and late-successional ($H_e = 0.700$) species. Consequently, we conclude that the level of genetic diversity in C. laxiflora is high compared with same genus species and species with similar life history traits.

The value of inbreeding coefficient ($f = 0.865$) estimated by the Bayesian procedure (Holsinger et al. 2002). It was higher than the value ($F_{IS} = 0.618$) estimated by an approximate Bayesian computation (ABC) (Foll et al. 2008). Bayesian inference method is widely used to estimate variety genetic parameters from dominant markers (Holsinger et al. 2002; Excoffier and Heckel 2006). But, there are statistical bias problems. Approximate Bayesian approach has been suggested to avoid bias of the Bayesian method (Foll et al. 2008). It would give more accurate estimates of $F_{IS}$. The value ($F_{IS} = 0.618$) in C. laxiflora estimated by approximate Bayesian method was lower than that in A. pseudosieboldianum ($F_{IS} = 0.712$, Ahn et al. 2016). It is known as a major component species of the C. laxiflora forest. There were no reports about mating system studies of Carpinus species. Most flowering plants are hermaphroditic which are known to have mixed mating (Whitehead et al. 2018). The ratio between outcrossing and selfing vary in accordance with species or environment of the stand. Plants have evolved to increase outcrossing in many ways such as self-incompatibility, sexual strategy, and pollen capture (Friedman and Barrett 2009). It was known wind pollination was an evolutionary strategy than that of animal or insect pollination. The results from mating system studies of 267 species revealed that wind-pollinated species was higher than those of animal- or insect-pollinated species (Goodwillie et al. 2005). Therefore, it is likely that outcrossing in wind-pollinated C. laxiflora at a higher rate than that in insect-pollinated A. pseudosieboldianum. However, some populations were observed to have high inbreeding than others. It will be necessary to carry out further mating system studies for an accurate interpretation.

Genetic differentiation among populations

The genetic differentiation ($\Phi_{ST}$) was 0.060. It was smaller than that in A. pseudosieboldianum ($\Phi_{ST} = 0.107$; Ahn et al. 2016), C. betulus ($F_{ST} = 0.074$, Coart et al. 2005), Carpinus orientalis ($F_{ST} = 0.086$, Coart et al. 2005). In microsatellite analysis, the level of genetic differentiation ($G_{ST} = 0.029$, Tono et al. 2016) was small compared with A. pseudosieboldianum ($\Phi_{ST} = 0.065$, Takayama et al. 2013), Acer mono ($\Phi_{ST} = 0.078$, Takayama et al. 2012; $F_{ST} = 0.073$, Liu et al. 2014). We also compared our results with ISSR analysis. Genetic differentiation was at a moderate level among deciduous broad-leaved species in Korea, such as Fraxinus chisianensis ($\Phi_{ST} = 0.041$, Cho et al., 2002).
Ulmus davidiana ($\Phi_{ST} = 0.042$, Ahn et al., 2013), Phellodendron amurense ($\Phi_{ST} = 0.076$, Lee et al. 2014), Stewartia koreana ($\Phi_{ST} = 0.118$, Yang et al. 2006). The level of genetic differentiation had also relevance to life history traits. In comparison to meta-data from AFLP analysis, the level of genetic differentiation ($\Phi_{ST} = 0.060$) was smaller than that of perennial ($\Phi_{ST} = 0.27$), common ($\Phi_{ST} = 0.20$), and wind-pollinated ($\Phi_{ST} = 0.26$) species. We concluded that the level of genetic differentiation of C. laxiflora ($GST = 0.029$) was small compared to that in species with similar life history traits. It is also known that the level of genetic differentiation is determined by gene flow, it means that migrate through both pollen and seeds (Tono et al. 2016). The genetic differentiation of C. laxiflora was smaller than that of Magnolia obovata ($GST = 0.044$). A prior cpDNA study reported that these species had similar migration histories through seeds (Iwasaki et al., 2012). The estimated pollen/seed migration ratio ($r$) of C. laxiflora was 31.608, higher than that ($r = 3.331$) of Magnolia obovata. They discussed the differences in the level of genetic differentiation between the two species. The values of the pollen/seed migration ratio ($r$) between two species likely reflect the differences in the amount of gene flow through pollen. They concluded that the wind-pollinated C. laxiflora had a higher amount of gene flow through pollen dispersal than in the insect-pollinated Magnolia obovata. A. pseudosieboldianum was reported to be a frequently appearing deciduous broad-leaved species in C. laxiflora forests (Byeon and Yun 2018). Acer species are also known to be insect-pollinated, including A. pseudosieboldianum (Matsui 1991). The level of genetic differentiation was smaller in the wind-pollinated C. laxiflora ($\Phi_{ST} = 0.060$) than the insect-pollinated A. pseudosieboldianum ($\Phi_{ST} = 0.107$, Ahn et al. 2016). It was due to the increased amount of geneflow owing to pollen dispersed by the wind. The level of genetic differentiation in C. laxiflora ($\Phi_{ST} = 0.060$) was small in comparison with other deciduous broad-leaved and insect-pollinated species, such as P. amurense ($\Phi_{ST} = 0.076$, Lee et al. 2014), and Stewartia koreana ($\Phi_{ST} = 0.118$, Yang et al. 2006). Therefore, we considered that the difference in the level of genetic differentiation among species was attributed to the different pollen dispersal modes.

Genetic relationship and genetic structure

Both UPGMA tree and Bayesian clustering separated all populations into two genetic groups. Based on Bayesian clustering, Mt. Chilgap, Mt. Minjuji, and Mt. Jiri populations occurred in higher in proportion in cluster I. The other populations occurred in a higher proportion in cluster II. The Korean peninsula and Japanese Archipelago had common flora in the Miocene and those were similar to the present, which was inferred from fossil studies (Kim et al. 2008). Specially, Carpinus kodaiarae-bracteata was estimated to be related essentially to the present C. laxiflora because of similar morphological characteristics (Huzioka 1942). Also, its fossils appeared in the Miocene strata of the Korean peninsula and the Japanese archipelago. After the Japanese Archipelago was separated, the plants in the Korean peninsula and Japanese Archipelago have undergone different evolutionary processes (Lim et al. 2010). The genetic structure in extant populations may be a result of migration through seeds as well as expansion and decline of the population due to repeated severe climate changes during the last glacial maximum (LGM) (Tono et al., 2016). In a phylogeographic study, the migration scenario of C. laxiflora in Japan was postulated (Iwasaki et al., 2012). During the LGM, separate populations occurred in few refugia regions. After LGM, the populations expanded from the refugia and differentiated. In a follow up study by using microsatellite markers, the genetic structure that had been shaped by migration was dissolved because of gene flow through seeds and pollen. But, the geographical distribution pattern was weakly observed in the genetic structure (Tono et al. 2016). The genetic structure of Acer mono in the northeast of China was estimated by microsatellite analysis (Liu et al. 2014). The survey populations were distributed along two main mountain ranges. However, weak genetic structure among mountain range were detected. They discussed that the relatively short geographic distances between populations and the continuous distribution of mountains provide a uniform landscape that facilitated gene exchange among populations. In addition, the weak genetic structure among populations suggests that they expanded from a single lineage. The C. laxiflora in South Korea is known to have populations that were distributed in mountain ranges such as Taebak mountains and Sobak mountains, and the vertical distribution range was from 300 to 750 m above sea level (Cho et al. 2004). The study populations were distributed in three mountain ranges, Taebak, Sobak, and Charyeong mountains. Mt. Chilgap and Mt. Chikak populations were distributed in the Charyeong mountain range. However, the genetic structures between two populations was different (Figure 5). The genetic structure of Mt. Minjuji was also different from other populations such as Mt. Sokri and Mt. Jiri which were distributed in the Sobak mountain range. Accordingly, most of the populations were observed to have weak genetic structures among different mountain ranges except the Mt. Chilgap and Mt. Minjuji populations. There is little conserved Carpinus forests on a large-scale in South Korea (Hong et al. 2012). In the Korean peninsula, there was a severe climate change during the LGM, which would have affected the decrease and expansion of the C. laxiflora populations. In order to interpret the results, we should consider that C. laxiflora is a secondary succession species and the demographic disturbances after the LGM. More specifically, Mt. Chilgap population had the lowest distributional height (561 m) among populations and was located in south Chungcheong province, which is located at the end of the Charyeong mountain range. According to a study of postglacial vegetation
history of this region, it was known to have been affected by climate change and demographic disturbances like cultivation at an earlier time than other regions (Jang et al. 2006). In particular, it has been reported that demographic disturbance is a major cause of destruction of temperate deciduous broad-leaved forests such as Quercus. Instead, some of the regions were dominated by Pinus densiflora forests (Park and Yi 2008). Currently, Mt. Chilgap is dominated by Quercus mongolica and P. densiflora forests, but it is predicted that succession would lead to C. laxiflora and Quercus forests (Kim et al. 2018). It was reported that temperate forest succession is strongly dependent on various local environmental factors such as seed rain, seed bank, availability of light, and accumulation of nitrogen in stands (Wehenkel et al. 2006). Therefore, we consider that the genetic structure of C. laxiflora had been influenced by demographic disturbance, local vegetation change history and forest succession after the postglacial period.

Conclusions

Our study attempted to estimate genetic diversity, genetic differentiation and genetic structure of C. laxiflora in South Korea by using AFLP analysis. The level of genetic diversity of was high compared with same genus species and similar life history traits. The value of inbreeding coefficient was lower than A. pseudosieboldianum, which known as a major component species of the C. laxiflora forest. We assumed that outcrossing rate in wind-pollinated C. laxiflora was higher than that of insect-pollinated A. pseudosieboldianum. The estimated value of genetic differentiation was small in comparison with Carpinus species and those with similar life history traits because gene flow among populations was increased due to pollen dispersal by wind. The study populations were located in three mountain ranges. However, we observed weak genetic structure among populations on different mountain ranges except among the Mt. Chilgap and Mt. Minjuji populations. Postglacial vegetation history indicated that the Mt. Chilgap population had been influenced by demographic disturbance and forest succession. We suggest that we need a comprehensive approach considering demographic disturbance, local specific vegetation change history, and forest succession to interpret the estimated genetic structure of C. laxiflora in South Korea.

References

Ahn, JY, Hong, KN, Lee, JW, Yang, BH. 2013. Population genetic variation of Ulmus davidiana var. japonica in South Korea based on SSR markers. J Korean Soc. 102(4):560–565. Ahn JY, Hong KN, Baeck SH, Lee MW, Lim HY, Lee JW. 2016. Genetic diversity and genetic structure of Acer

Figure 5. Geographic distribution pattern of two clusters estimated by using Bayesian clustering. Red represents proportion in cluster I and green represents cluster II.
Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23(21):4407–4414.
Wehenkel C, Bergmann F, Gregorius HR. 2006. Is there a trade-off between species diversity and genetic diversity in forest tree communities? Plant Ecol. 185(1):151–161.
Wehenkel C, Corral-Rivas JJ, Hernández-Díaz JC. 2011. Genetic diversity in relation to secondary succession of forest tree communities. Pol J Ecol. 59(1):45–54.

Whitehead MR, Lanfear R, Mitchell RJ, Karron JD. 2018. Plant mating system often vary widely among populations. Front Ecol Evol. 6(38):1–9.
Yang BH, Han SD, Koo YB, Park YG. 2006. Genetic variation in the natural populations Korean stewartia (Stewartia koreana Nakai) based on I-SSR analysis. Korean J Plant Resour. 19(1):189–195.
Zhivotovsky, LA. 1999. Estimating population structure in diploids with multilocus dominant DNA markers. Mol Ecol. 8(6):907–913. doi:10.1046/j.1365-294x.1999.00620.x.