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Conservation Ecology and Genetics of Two Dioecious Plant Species in South Korea

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Abstract

Dioecious plants may pose special conservation problems because the population dynamics can be affected by sex-specific trade-offs between growth and reproduction, and differences of adaptability for environmental conditions. Moreover, if the population size is the same, dioecious plants only about half the individuals produce seeds compared with cosexual plants. Therefore, for conservation of the endangered dioecious species needs to be well-integrated knowledge derived from ecological, demographic and genetic attributes related with the sexual system. This study was conducted to propose a long term conservation plan through analysis of the ecological traits - sex ratio, size distribution and spatial pattern - and genetic aspects - genetic diversity and spatial genetic structure - in two dioecious plant species of South Korea.

Ilex cornuta 호랑가시나무 (Aquifoliaceae) is a dioecious evergreen shrub, and an endemic species of East Asia. Inland populations of *I. cornuta* in South Korea are known to almost have been reduced or destroyed due to excessive exploitation and habitat destruction. However, in Jeju Island, the ecological information such as distribution and structure of *I. cornuta* is almost unknown. Thus, I analyzed sex ratios, sex-related ecological traits and spatial distribution patterns for *I. cornuta* populations of Jeju Island. In Yongsu-ri population (YS population

hereafter), an overall sex ratio of reproductive trees was male-biased (0.66), while Gotjawal Provincial Park population (GP population hereafter) did not differ significantly from 1:1. Females were slightly larger and taller than males in two populations, but these were no statistically significant intersexual differences. *I. cornuta* genets usually formed a clump with several basal sprouting stems (ramets), but the differences between sexes were not significant. Vitality was significantly associated with sex in YS population, and males had more defense against infection than females. The significant spatial segregation was not observed between males and females in YS and GP populations. Unlike GP population designated as a protected area, YS population is surrounded by the farmland and exposed to risk factors such as biased sex ratio, high infection probability and anthropogenic damage. For YS population, suitable protective devices are needed to prevent *I. cornuta* trees being disappeared due to habitat disturbance by anthropogenic activities. GP population has a problem of small population size. Therefore, the conservation program for assisted natural regeneration of *I. cornuta* trees is needed.

Torreya nucifera 비자나무 (Taxaceae) is a dioecious gymnosperm currently distributed in southern parts of South Korea and Japan. *Torreya* population in Jeju Island is known as the largest (N = 2,861) and oldest (mostly 200-400 years old, max. ~900 years old) in the world. In this study, I examined sex- and size-related spatial distributions in three

quadrats of old-growth *T. nucifera* forest in extremely heterogeneous Gotjawal terrain of Jeju Island. Univariate spatial analysis showed that spatial patterns of *T. nucifera* trees differed among the three quadrats. In quadrat A, individual trees showed random distribution at all scales regardless of sex and size groups. When assessing univariate patterns for sex by size groups in quadrat B, small males and small females were distributed randomly at all scales whereas large males and large females were clumped. All groups in quadrat C were clustered at short distances, but the pattern changed as distance was increased. Bivariate spatial analyses testing the association between sex and size groups showed that spatial segregation occurred only in quadrat C. Males and females were spatially independent at all scales. However, after controlling for size, small males and small females were spatially separated. Diverse spatial patterns of *T. nucifera* trees across the three sites within *Torreya* population is considered to reflect various factors such as the ecological characteristics of individuals as well as the environmental heterogeneity of Gotjawal terrain and artificial interference.

This study was performed to examine genetic diversity and spatial genetic structure using nine microsatellite markers in an old-growth *Torreya* population of Jeju Island. Overall genetic diversity (H_E = 0.513) of *Torreya* population was lower than other tree species with similar ecological characters. Also, *Torreya* population showed a significant bottleneck signature ($P < 0.001$), and a relatively small effective population size (N_e/N = 0.56). Spatial autocorrelation analysis using Moran's I and the kinship coefficient (F_{ij}) revealed individuals being genetically more similar at a distance of up to approximately 180 m and 150 m, respectively. The intensity of spatial genetic structure (S_p = 0.0130) was relatively strong to than the other species that share similar life history traits with *T. nucifera*. We found a genetic sub-structure with the north-south directions according to results of the genetical bandwidth mapping and the location of genetic barriers in *Torreya* population. GENELAND analysis based on a Bayesian model revealed that *Torreya* population was composed to four clusters (K = 4). The distribution of four clusters also showed the north-south directions, especially the southern area included all four clusters of *Torreya* population. According to the results of the spatial genetic analysis, this population was divided into three genetic groups (Group I, Group II and Group III), and 4% of total genetic variation was attributed to differences among groups (P = 0.010). The present study, for the first time, displayed low genetic diversity, significantly genetic sub-structure with three genetically distinct groups and strong spatial genetic structure in *Torreya* population. This results are emphasize that the urgency of conservation activities to maintain or improve the level of genetic variations. And the spatial genetic sub-structure within *Torreya* population should be considered for future conservation and management practices. Specially, we propose Group III need to be conserved with top priority as the major source because it shows both large heterogeneity and all four genetic clusters. For *ex situ* conservation of *T. nucifera* the sampling strategy is efficient at least above 150 m between individual trees to reduce genetic relatedness.

In South Korea forests, dioecious woody species is known to be about 24%, which is relatively higher compared to 8% in the world. In South Korea, however, few ecological studies with dioecious sexual system have been conducted. In this study, I have provide conservation strategies for the dioecious *I. counuta* and *T. nucifera* based on informations such as sex-related ecological traits, spatial structure and genetic assesment. These results will help to determine the future direction of the research for the conservation of the endangered dioecious plants in South Korea.

Keyword: Conservation of dioecious plants, Genetic diversity and spatial genetic structure, *Ilex cornuta*, Sex ratio and sex-related distribution pattern, *Torreya nucifera*

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Chapter 1

General Introduction

Dioecy, characterized by the separation of male and female functions in different individuals, is rarely found in only 9-10% among land plants, approximately 4-6% of angiosperms and 36% of gymnosperms (Renner and Ricklefs 1995; Ming et al. 2011; Renner 2014). Although the incidence of dioecy in plants globally is relatively uncommon, it occurs in close to half of all families and the majority of orders including both primitive and advanced groups (Renner and Ricklefs 1995; Heilbuth 2000; Käfer et al. 2017).

Dioecious plants have a reproductive handicap such as reduced mate assurance and seed-shadow handicap compared to non-dioecious species (Pannell and Barrett 1998; Heilbuth et al. 2001; Ohya et al. 2017). That is, for a new population to be established, at least one individual in each sex to be present, and the dioecious population may be slows to spread because only females produce seeds. Also, the occurrence of dioecy is correlated with a number of ecological factors that which is geographical distribution (e.g. tropical climates and island habitats), woody growth form, small and inconspicuous flowers, fresh fruits, and pollination/dispersal modes (e.g. small and generalist insect pollinators/and animal-dispersed seeds) (Bawa 1980; Renner and Ricklefs 1995; Sakai et al. 1995; Vamosi et al. 2003). The complex of ecological factors associated with dioecy may susceptible to anthropogenic disturbances (Vamosi and Otto 2002). Indeed, the proportion of endangered plants in dioecious families was significantly higher than non-dioecious sister-groups in the IUCN Red List of Threatened Species (Vamosi and Vamosi 2005).

Conservation of dioecious plants may pose special problems due to sexual dimorphism caused in sex-specific trade-offs between growth and reproduction for resource allocation (Obeso 2002; Barrett et al. 2010; Queenborough et al. 2013). Sex-dependent life histories induce sex-related population structure such as sex ratios and sex-specific spatial distribution in natural populations (Nanami et al. 2005; Barrett et al. 2010; Munné-Bosch 2015). However, the sexual difference patterns are not observed consistently among all dioecious plants because the species and/or population fluctuate across temporal, spatial and morphological scale (i.e. population age, intra- and inter- specific interactions, habitat environment and disturbance) (Nanami et al. 2005; Queenborough et al. 2013). Also, the genetic status of plant species is constantly affected by factors such as ecological traits (i.e. life form, breeding system and seed dispersal), demographic history (genetic bottleneck and population size change), evolutionary processes (mutation and natural selection), and environmental heterogeneity (Hamrick et al. 1992; Frankham 2005; Hu et al. 2014).

Therefore, for conservation of the crisis dioecious plant species needs to be well-integrated knowledge derived from evolutionary processes, demographic, and ecological attributes related to sexual system. For conservation of dioecious plants in South Korea, however, ecological studies considering sexual system are very rare. Thus, the main objective in this study is to proposes the management and conservation plan through analysis for ecological traits, spatial distribution patterns, and spatial genetic structure considering the sexual system of the dioecious trees *Ilex cornuta* and *Torreya nucifera* in South Korea.

In chapter 2, I investigated the flowering sex ratios, sex-related ecological traits and spatial distribution patterns for two populations of *I. cornuta* in Jeju Island. I addressed the following questions: (1) Do the sex ratios deviate significantly from 1:1? (2) Differ between sexes in size distribution, clonal growth, and vitality? (3) Do spatial patterns differ between sexes?

In chapter 3, I examined sex- and size-related spatial distributions of old-growth forest of dioecious tree *T. nucifera* in extremely heterogeneous Gotjawal terrain of Jeju Island. The following aspects were examined: (1) The distribution pattern of *T. nucifera* trees according to sex and size at each site, (2) The site differences in those distribution patterns, and (3) The pattern of spatial segregation of sexes and sizes at each site.

In chapter 4, I analyzed the genetic diversity and spatial genetic structure using nine polymorphic microsatellite markers in an old-growth *Torreya* population of Jeju Island. I focused this study on the following questions: (1) What level of genetic diversity in *Torreya* population currently? (2) Did *Torreya* population experience demographic events such as population size change or genetic bottleneck in the past? (3) Is there spatial genetic structure in population? (4) If the genetic sub-structure appears within *Torreya* population, what level of genetic differences?

Chapter 2

Sex ratios and spatial structure of the dioecious tree *Ilex cornuta* (Aquifoliaceae) in Jeju Island, South Korea

2.1 Introduction

Understanding the complex ecological, demographic and genetic factors is essential for the conservation of endangered species (Oostermeijer et al. 2003). Especially, in dioecious species, characterized by the separation of male and female functions in different individuals, ecological differences between sexes has been considered a key factor in the reproductive success and conservation status (Nanami et al. 2005; Queenborough et al. 2013). Sexual dimorphism of dioecious plants usually relates to the differences in morphology, physiology and phenology, caused in sex-specific trade-offs between growth and reproduction for resource allocation (Delph 1999; Obeso 2002). In general, female plants tend to contribute a greater effort in sexual reproductive than males because reproducing of males is limited to pollen production while females must produce seeds and fruits (Lloyd and Webb 1977; Obeso 2002; Barrett and Hough 2013). Many studies of perennial dioecious plants show that small trunk size, lower growth rates, vulnerability to stress and higher mortality in females as a direct consequence of high reproduction cost of females (Allen and Antos 1993; Obeso 2002; Gao et al. 2009; Iszkuło et al. 2011).

Sex-dependent life histories induce sex-related population structure such as sex ratios and sex-specific spatial distribution in natural populations (Nanami et al. 2005; Barrett et al. 2010; Munné-Bosch

2015). The sex ratios, defined as the proportion of males to females, expected 1:1 primary sex ratio by the theoretically frequency-dependent selection (Fisher 1930), but commonly significant equality deviations (Barrett et al. 2010; Sinclair et al. 2012). Male-biased sex ratios predominate in natural populations that associated with sexual difference in flowering and mortality (Barrett et al. 2010; Sinclair et al. 2012; Barrett and Hough 2013), although female-biased has also been widely documented among *Salix* spp. (Ueno et al. 2007). Adaptations to different costs associated with reproduction also can result in spatial segregation of the sexes (SSS), the non-random distribution of the sexes, in partitioning of the environment along a resource gradient (Bierzychudek and Eckhart 1988; Nuñez et al. 2008; Matsushita et al. 2016). Particularly spatial distribution of mature males and females is crucial to population survival because they can only reproduce by outcrossing (Bawa 1980; Osunkoya 1999; Hultine et al. 2007). Generally, females with resource limitations due to high reproductive costs will occupy more favorable or less stressful conditions regarding elevation (Garbarino et al. 2015; Vessella et al. 2015), water availability (Nuñez et al. 2008), and/or soil fertility (Lawton and Cothran 2000). For example, female trees of *Salix glauca* (Dudley 2006), and *Austrocedrus chilensis* (Nuñez et al. 2008) were relatively more abundant in shadier and moister habitats.

Ilex cornuta (Aquifoliaceae) is a broad-leaved evergreen shrub with the sexual system of dioecy. It is an endemic species of East Asia

(Galle 1997), and currently distributes in eastern China and South Korea. In South Korea, *I. cornuta* is known to be occurring within limited range below 35 ° 37 ' N (Uyeki 1941), with Jeolla-do and Jeju Island. *I. cornuta* is valued horticulturally for its attractive and distinctive rectangular foliage and for its large red berries so that more than 123 cultivar are developed (Ko 2004). This species is listed as Least Concern in the Korea Plant Red List (www.korearedlist.go.kr) and vulnerable by Korea National Arboretum (2008). Previous studies of *I. cornuta* focused on distribution (Lee 1983; Koh 2004), taxonomy (Hwang et al. 2007; Son 2008), morphology (Yim 1979; Koh 2004; Son 2008), propagation (Lee and Jo 1982), demography (Park et al. 2000), vegetation (Ko 2004; Kwon et al. 2011), genetics (Son et al. 2007; Hong et al. 2015). Recently several studies pointed out the crisis of *I. cornuta* in South Korea. Ko (2004) investigated the changes of nine natural habitats of *I. cornuta* in inland by ecological processes and artificial interferences compare with the past research data (Yim 1979; Lee 1983). As a result, the wild populations were almost destroyed in seven habitats (Haenam, Hampyeong, Wando and Yeonggwang in Jeollanam-do) due to excessive exploitation and habitat destruction, except for Buan (in Jeollabuk-do; designated the Natural Monument No. 122 as the northern range limit in South Korea) and Muan (in Jeollanam-do; more than 100 individuals of males and females). However, since then, the population in Muan has been destroyed, and only a few individuals are known to remain (unpublished data with field survey in 2014). Hong et al. (2015) found that genetic monomorphism of the natural *I. cornuta* population in Buan, based on AFLP marker. Indeed, this population consists of only male trees without female trees. Although there are some studies on the crisis status of *I. cornuta* trees in inland, the ecological information such as distribution and structure of individuals and/or populations in Jeju Island is almost unknown.

Considering the sharply reduction of the distribution range of the natural populations in the inland, *I. cornuta* populations in Jeju Island, which are experiencing recent development pressure and climate change (Lee et al. 2013), may also be at risk. Son et al. (2007) reported that *I. cornuta* in South Korea was derived from Jeju Island to the inland, based on the high genetic variation of ITS (internal transcribed spacer) found in Jeju Island populations rather than inland. The source population is essential for future conservation and restoration efforts (Ruciñska and Puchalski 2011). However, nothing is known about the sex-related population ecology of *I. cornuta* in Jeju Island. Therefore, effective conservation actions for this species urgent knowledge of contemporary population demography and structure related to sexual system. Perhaps the population size, age structure and environmental factors will result in population-specific sex ratios and/or spatial distribution patterns. Also, if *I. cornuta* populations are affected by sex-specific trade-offs between growth and reproduction, then there will be difference in ecological traits characteristics between sexes such as stem size, vegetative growth (e.g. clonality) and vitality. Here, we address the following questions: (1) Do the sex ratios deviate significantly from 1:1? (2) Differ between sexes in size distribution, clonal growth, and vitality? (3) Do spatial patterns differ between sexes?

2.2 Materials and Methods

2.2.1 Study species and sites

Ilex cornuta Lindl. & Paxton (Aquifoliaceae) is a densely foliaged evergreen shrub that can grow up to 3 m in height (Lee 2003). The leaves are thickly leathery and usually 6-spined on the oblong shape, but the number of spines varies due to highly mutated. The flowering occurs between April and May that dull white flowers clumped in the axils of branches produced the previous year and pollinated by insects. The red fruits are ripened between September and October and contain four seeds, which are typically bird dispersed. *I. cornuta* combines sexual reproduction with a form of vegetative propagation by suckers arising from shallow lateral roots.

This study was conducted at two localities (Yongsu-ri and Jeju Gotjawal Provincial Park) in Jeju Island, South Korea, where the remaining natural population of *I. cornuta* present (Fig. 2.1). Yongsu-ri population (YS population hereafter) is located near Yongsu Reservoir in Hangyeong-myeon, Jeju-si (33°19′ N, 126°10′ E) which is an artificial reservoir built in 1957. The surrounding areas are all used as agricultural land, and *I. cornuta* trees remains in empty spaces between farmlands. According to 2008-2017 data from Gosan Regional Meteorological Office close to the YS population, the mean monthly temperature ranges from 5.8°C in January to 26.5°C in August (mean annual temperature:

15.7°C). Its mean annual precipitation is 1151.6 mm with a peak in August (324.1 mm) with mean annual wind speed of 6.6 m/s (Korea Meteorological Administration 2018). YS population belongs to lower part of lava terraces and to evergreen broad-leaf forest zone with a total of 66 plants taxa, including *Pinus thunbergii*, *Sageretia thea* (Kwon et al. 2011).

Jeju Gotjawal Provincial Park (GP population hereafter) is located in Daejeong-eup, Seogwipo-si (33°12′ N, 126°10′ E). Jeju Gotjawal Provincial Park (area = 154.7 ha) was designated as a protected area in 2011 by the Natural Parks Act for the systematic preservation and management of Gotjawal as a unique topography that can be seen only in Jeju Island (http://jejugotjawal.or.kr/default/index.php?cid=15). *I. cornuta* trees form a population around the observatory in the Provincial Park. According to 2008-2017 data from Daejeong Regional Meteorological Office close to GP population, the mean monthly temperature ranges from 5.9°C in January to 27.2°C in August (mean annual temperature: 16.2°C). Its mean annual precipitation is 1456.9 mm with a peak in August (466.5 mm) with mean annual wind speed of 3.7 m/s (Korea Meteorological Administration 2018). This site serves as a habitat for various animals and plants, and species diversity including endangered species such as *Quercus gilva*, *Copris tripartitus* and *Accipiter gularis* is very high (http://jejugotjawal.or.kr/default/index.php?cid=15). The terrain is belongs to Hangyeong-Andeok Gotjawal located in the eastern part of

Jeju Island (Song 2000). Figure 2.2 demonstrates characteristic features of *I. cornuta* trees in natural YS and GP populations of Jeju Island, South Korea.

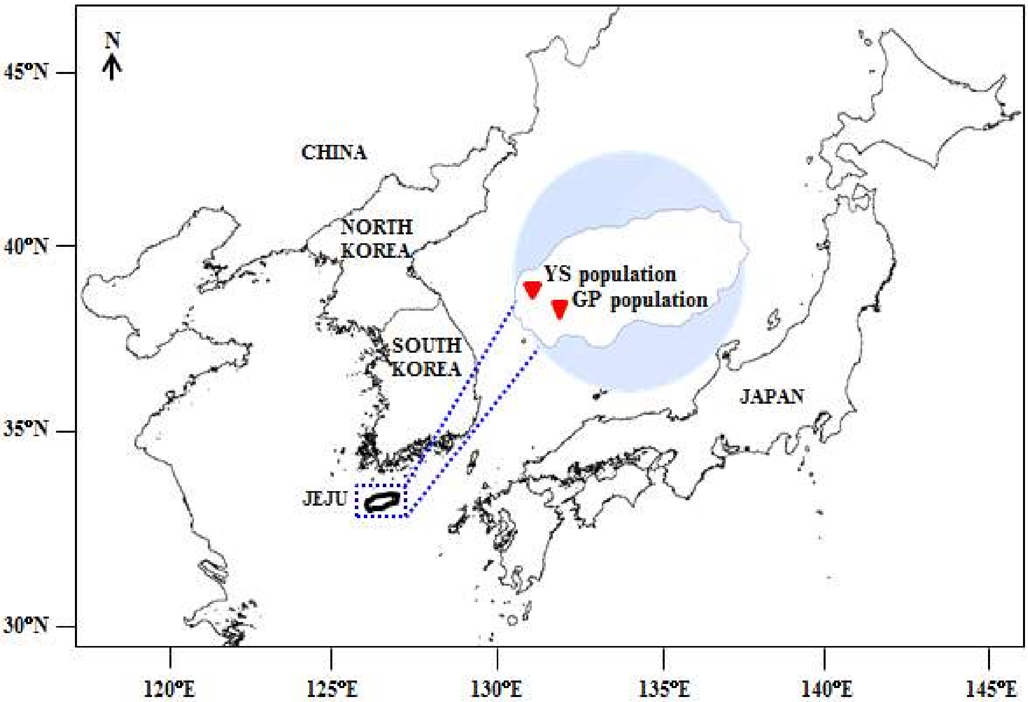


Fig. 2.1 Location of *Ilex cornuta* populations in Jeju Island, South Korea.

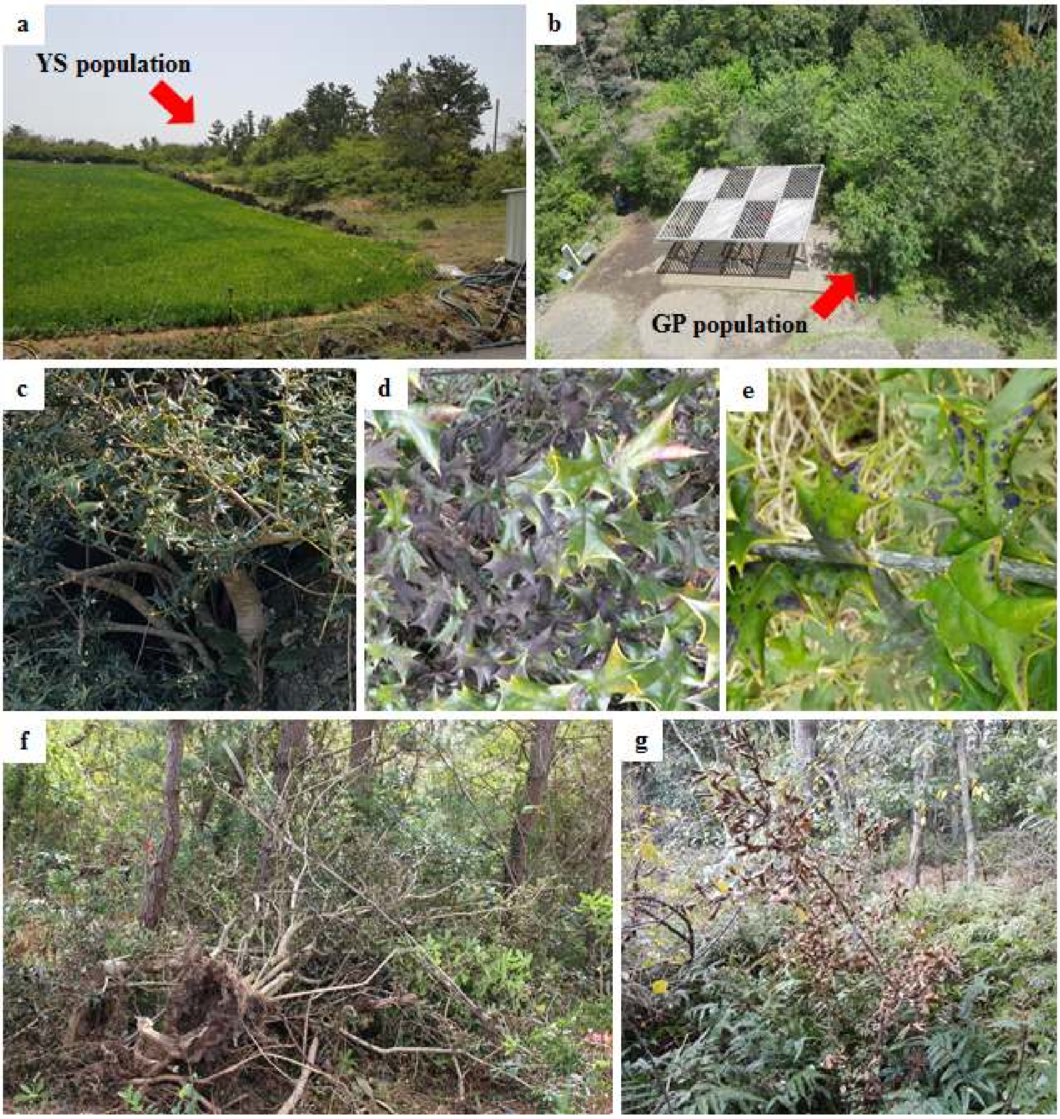


Fig. 2.2 Wild *Ilex cornuta* trees in the two populations of Jeju Island, South Korea. **a** Full view of YS population. **b** Full view of GP population. **c** *I. cornuta* genet usually formed a clump with several ramets. **d-e** *I. cornuta* is experiencing disease problems such as powdery mildew and tar spot in YS population. **f** *I. cornuta* tree was picked in YS population. **g** *I. cornuta* tree died in GP population during the survey period (2016-2017).

2.2.2 Data collection

This study was conducted during 2016-2017. All individuals of *I. cornuta* with a basal diameter ≥ 1.5 cm within two sites were tagged and recorded ecological characters. For each of *I. cornuta* tree, we recorded sex, basal diameter and height. The sex was determined based on flower morphology, especially the presence or absence of pollen and pistil development, and fruiting. And individuals whose flowering and fruiting were not observed during the study period were recorded as ' Unsexed'. Each clump of trunks was considered a genet, and each individual trunk a ramet. Assigning trunks to genets was not difficult due to trunks from the same genet normally grow in a circle and genets are far from each other. The basal diameter was measured up to 0.1 cm for main trunk within genet with vernier calipers, which is the widest trunk was considered the main trunk, and the height was measured up to 0.1 m. We also counted the number of ramets consisted per genet. All individuals were observed for disease problems include leaf spot, tar spot and powdery mildew. For each individual, the proportion of damaged area was recorded.

To spatial distribution analysis, all individuals of *I. cornuta* (≥ 1.5 cm basal diameter) within two sites were mapped based on geo-referenced using a Garmin 76cx GPS unit (Garmin International, Olathe, Kansas, USA). Trees near to each other were difficult to be distinguished by coordinate values, so the distance between individuals was measured and mapping in the field.

2.2.3 Data analyses

Sex determination was used to split the population in three classes: male (M), female (F), and unsexed (U; i.e. trees with no sex expression). Sex ratios were expressed as the proportion of males divided by all flowering individuals [(males/(males+females)]. The significant of deviations from a sex ratio of 1:1 was tested using the chi-square test.

Differences of basal diameter and height have been analyzed between sexes (and/or reproductive vs. non-reproductive trees) and two populations using Mann-Whitney test. Size distribution of *I. cornuta* trees in populations was divided three size classes according to basal diameter: small, < 3.0 cm; middle, 3.0-6.0 cm; large, ≥ 6.0 cm. Pearson correlation analysis was performed to examine whether the relationships between basal diameter and height. One-way ANOVA was used to examine the difference in the clonal growth (the number of ramets per genet) between sexes or three size groups. The trees' vitality based on infection prevalence was classified into three categories: healthy, < 10% damaged area by infection; medium, 10-60%; weak, ≥ 60%. To examine the differences in vitality between sexes or sizes, one-way ANOVA was used. The associations between vigor and each of the ecological traits (sex and size) were tested two-way contingency table analyses. All statistical analyses were conducted with SPSS v.16.0 (SPSS, Chicago, Illinois, USA).

To investigate for spatial distribution patterns of *I. cornuta* trees in two population using O-ring statistic O(*r*), which is employed to describe the average density of points at a distance of *r* (Wiegand and Moloney 2004; Law et al. 2009). Value of O(*r*) was calculated as follows. Around each individual data point, numerous circles with radius *r* were drawn and the correlation between the average number of individuals within numerous circles and radius *r* was deduced to determine the O value. O-ring statistic analyse included univariate and bivariate point patterns. First, univariate statistical analysis of $O_{11}(r)$ was used to analyze spatial distribution patterns between individuals along distance scale *r* (0-50 m in YS pop.; 0-40 m in GP pop.). To conduct significance test for O(*r*) value at each distance *r*, null hypothesis was formed using complete spatial randomness (CSR). If the observed $O_{11}(r)$ values were grater than similar to and less than 95% confidence intervals obtained 199 Monte Carlo simulations, the distribution patterns were assumed to be clumped, random or regular respectively. Second, to examine spatial association/ segregation between males and females, bivariate point pattern analysis of O(*r*). Random labeling approach was used to assess the significance of spatial segregation of sexes. If the observed $O_{12}(r)$ values were greater than or less than 95% confidence intervals obtained 199 Monte Carlo simulations, these indicate significant spatial association or segregation between sexes, respectively. The overlapping of the confidence intervals indicates spatial independency in the distribution pattern of the sexes. All calculations and simulations were performed using PROGRAMITA software (Wiegand and Moloney 2004).

2.3 Results

2.3.1 Sex ratio of *I. cornuta* populations

In YS population, we found 94 individuals (≥ 1.5 cm in basal diameter), which are 49 males, 25 females and 20 non-reproductive (Unsexed; U) trees (Table 2.1). An overall sex ratio of reproductive trees was male-biased (0.66).

In GP population, we found 33 individuals (≥ 1.5 cm in basal diameter), which are 13 males, 13 females and 7 non-reproductive trees. The sex ratio of flowering trees did not differ significantly from the expected 1:1 sex ratio (*P* = 1.000).

2.3.2 Size distribution between male and female trees

In YS population, the mean main-trunk basal diameter of the all trees was 4.6 ± 3.3 cm (mean ± SD), and the mean height was 1.5 ± 0.6 m (Table 2.1). Females were slightly larger (5.1 cm vs. 4.7 cm basal diameter) and taller (1.6 m vs. 1.5 m height) than males, but these were no statistically significant intersexual differences (*P* = 0.681 for basal diameter and *P* = 0.365 for height; data not shown). Non-reproductive individuals of *I. cornuta*, unsexed (U), were significantly smaller (3.5 cm vs. 4.8 cm mean basal diameter; *P* = 0.018) and shorter (1.2 m vs. 1.6 m mean height; *P* = 0.015) than reproductive trees (females and males). According to the three size classes based on basal diameter, 33 (35.1%)

in the small group, 39 (41.5%) in the middle group, and large group 22 (23.4%). And males were more numerous than female in all size classes (Fig. 2.3). As a result of analyzing the correlation between basal diameter and height, the correlation coefficient (*r*) was 0.590, showing a high correlation between the two factors ($P < 0.001$).

In GP population, the mean basal diameter is 4.3 ± 2.0 cm and mean height is 1.9 ± 0.6 m. Females were slightly larger (4.9 cm vs. 4.1 cm basal diameter) and taller (2.2 m vs. 1.9 m height) than males, but these differences were not significant ($P = 0.418$ for basal diameter and $P = 0.390$ for height). Non-flowering individuals of *I. cornuta* were smaller (3.4 cm vs. 4.5 cm basal diameter; $P = 0.156$), and significantly shorter (1.3 m vs. 2.1 m height; $P = 0.002$) than flowering individuals. Most (57.6%) of the trees were concentrated in the middle group (3.0-6.0 cm basal diameter). The correlation coefficient between basal diameter and height was 0.700, also showing a high correlation between the two factors ($P < 0.001$).

Table 2.1 Number of individuals, sex ratio and size distributions of *Ilex cornuta* in YS and GP populations in Jeju Island, South Korea

	Study site	
Characteristic	YS population	GP population
Number of individuals		
Males	49	13
Females	25	13
Unsexed	20	7
Total	94	33
Sex ratios (M/M+F)	0.66 **	0.50 ^{NS}
Basal diameter (cm) ± SD		
Males	4.7 ± 3.2	4.1 ± 1.8
Females	5.1 ± 4.0	4.9 ± 2.4
Unsexed	3.5 ± 2.5	3.4 ± 1.2
Overall mean	4.6 ± 3.3	4.3 ± 2.0
Height (m) ± SD		
Males	1.5 ± 0.7	1.9 ± 0.4
Females	1.6 ± 0.7	2.2 ± 0.7
Unsexed	1.2 ± 0.5	1.3 ± 0.4
Overall mean	1.5 ± 0.6	1.9 ± 0.6

For sex ratios, NS = not significant, ** $P < 0.01$, in chi-square tests.

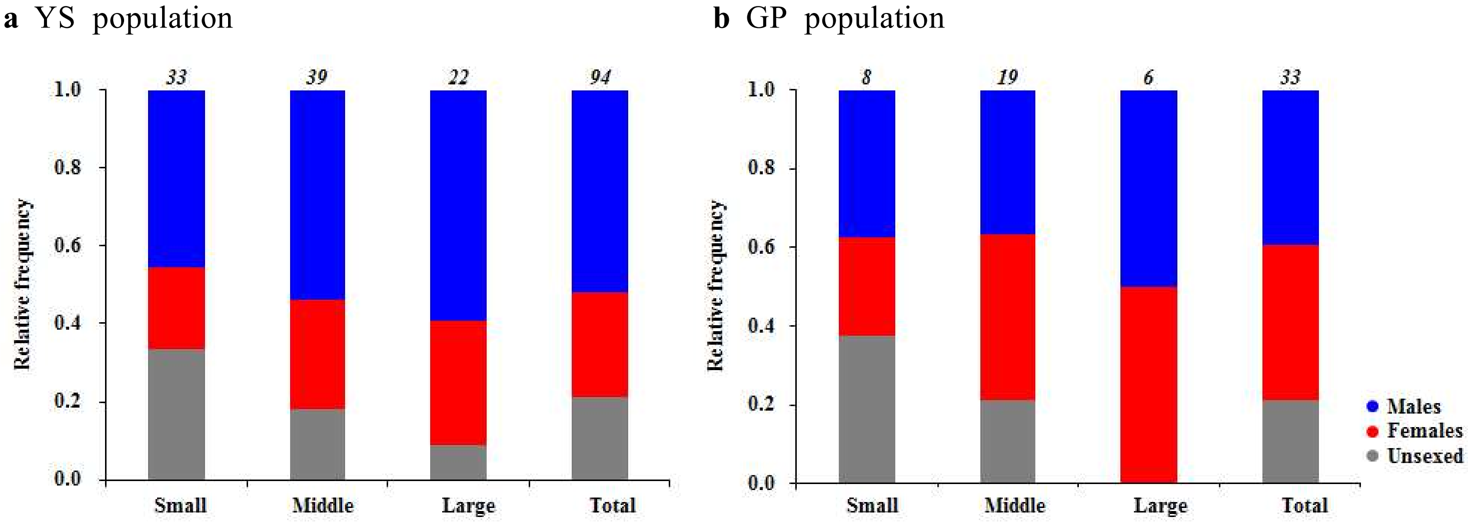


Fig. 2.3 Relative frequency of males, females, and unsexed *Ilex cornuta* trees for each size classes in the YS and GP populations of Jeju Island. Sample size is indicated above each bar.

Size: Small, < 3.0 cm basal diameter; Middle, 3.0-6.0 cm; Large, ≥ 6.0 cm.

2.3.3 Sexual differences in clonal growth and vitality

In YS population, the genets usually formed a clump with several basal sprouting stems (ramets) (Fig. 2.2c), and the mean number of ramets per genet was 1.7 ± 1.5 (range 1-10). One-way ANOVA using either sex of size as independent variable showed that differences of male (1.6 ± 1.4) and female (2.0 ± 2.0) were not significant (Table 2.2). Among three size groups also not differed significantly in vegetative propagation (*P* = 0.984). In GP population, the mean number of ramets consisted per genet was 3.8 ± 2.7 (range 1-12), and more than YS population (Table 2.2). Males (3.2 ± 2.0) were fewer than females (4.6 ± 3.0), but these differences were not significant (*P* = 0.183). The large size trees' genet had more ramets (6.0 ± 4.6) than in the small (3.2 ± 0.8) and middle groups (3.3 ± 2.2), but these differences were not significant (*P* = 0.116).

Many individuals in YS population were experiencing disease problems such as leaf spot, tar spot and powdery mildew (Fig. 2.2d-e). Trees assigned to the healthy group comprised about one-half of the population (52.1%, 31.9% and 16.0% for healthy, medium and weak, respectively). In two-way contingency table analyses, vitality was significantly associated with sex, while not significantly associated with size (Table 2.3). In healthy group, proportion of males (healthy males/total males) was about two times higher than females (65% vs. 36%). In GP population, trees assigned to the healthy group comprised about two-thirds of the population (61.5%, 34.6% and 3.9% for healthy, medium and weak, respectively). In two-way contingency table analyses, vitality was not significantly associated with sex and size (Table 2.3).

Table 2.2 One-way ANOVA of the effect of sex and size on the clonal growth of reproductive *Ilex cornuta* trees in YS and GP populations of Jeju Island, South Korea

YS population							GP population						
Source of variation	df	F	*P*	Categories of sex and size	X ± SD	N	Source of variation	df	F	*P*	Categories of sex and size	X ± SD	N
Sex	1	1.303	0.258	Male	1.6 ± 1.4	49	Sex	1	1.894	0.183	Male	3.2 ± 2.0	13
				Female	2.0 ± 2.0	25					Female	4.6 ± 3.0	13
Size	2	0.016	0.984	Small	1.8 ± 2.0	22	Size	2	2.377	0.116	Small	3.2 ± 0.8	5
				Middle	1.7 ± 1.5	32					Middle	3.3 ± 2.2	6
				Large	1.7 ± 1.6	20					Large	6.0 ± 4.6	15

Size: Small, < 3.0 cm basal diameter; Middle, 3.0-6.0 cm; Large, ≥ 6.0 cm.

Table 2.3 Two-way contingency table of vitality vs. sex and size among reproductive *Ilex cornuta* trees in YS and GP populations of Jeju Island, South Korea

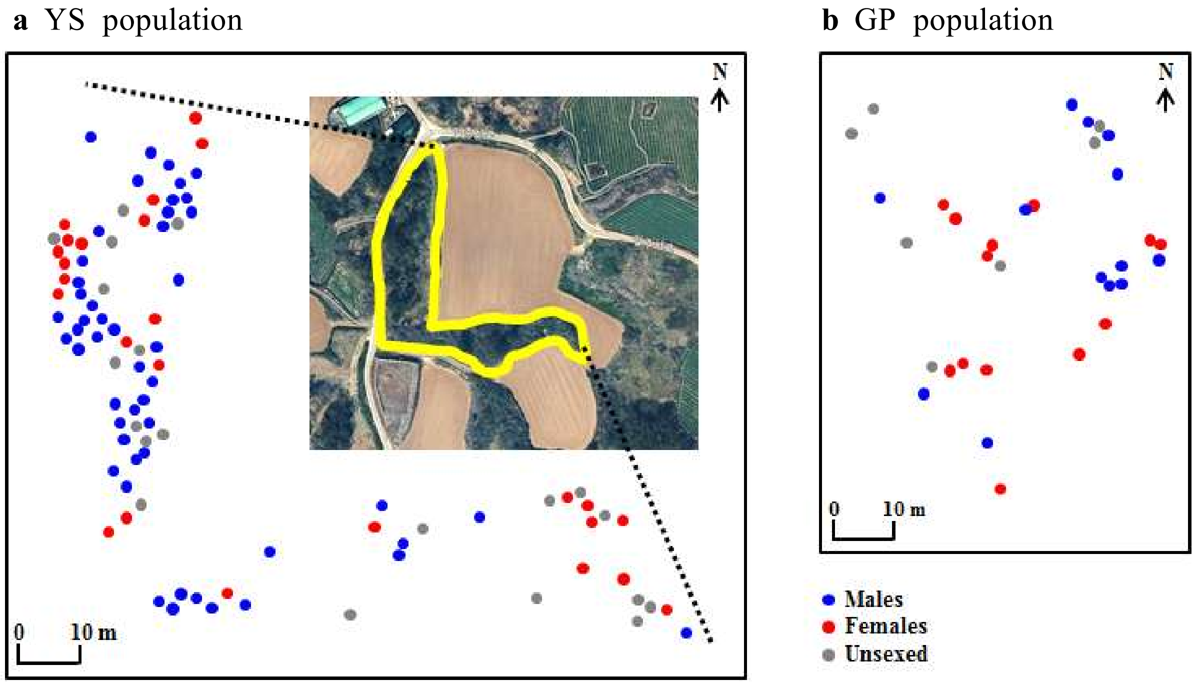
	YS population			GP population		
	Healthy	Medium	Weak	Healthy	Medium	Weak
Sex						
Male	32	11	6	9	4	0
Female	9	12	4	7	5	1
	G = 6.20, *P* = 0.057			G = 1.75, *P* = 0.688		
Size						
Small	14	5	3	2	3	0
Middle	15	11	6	12	2	1
Large	12	7	1	2	4	0
	G = 3.52, *P* = 0.510			G = 7.95, *P* = 0.117		

The likelihood ratio chi-square (G) is reported for each table.
Healthy, < 10% damaged area by infection; Medium, 10-60%; Weak, ≥ 60%. Size: Small, <3.0 cm basal diameter; Middle, 3.0-6.0 cm; Large, ≥ 6.0 cm.

2.3.4 Spatial distribution patterns with sex-related

In YS population, *I. cornuta* trees were distributed in the patch space surrounding the farmland (Fig. 2.4a). Univariate analyses showed that all trees were clumped (0-18 m, 20-25 m, and 28-32 m) or regular (41-50 m) distributed at most scale (Fig. 2.5). When sex was considered, spatial pattern changed. Males were clumped (0-17 m, 31-32 m) or regularly (42-50 m) distributed at some distances, while females were randomly distributed at all scales, except for 0-8 m with clumped. Bivariate analyses for spatial association between sexes showed that spatial segregation was not occurred (Fig. 2.7a). Rather, males and females were weakly grouped at 4-16 m, 25-27 m and 30-36 m scales.

Figure 2.4b shows the spatial distribution of female, male and unsexed trees in GP population. *I. cornuta* trees were randomly distribution at most scale, except for 0-5 m and 39-40 m scales (Fig. 2.6). Based on univariate point pattern analysis, males were clumped at 2-4 m scales, and females were randomly distributed at all scales, except for 31-34 m (weakly clumped). The bivariate spatial association between sexes showed a random pattern (Fig. 2.7b).


a YS population

b GP population

Fig. 2.4 Spatial distribution of *Ilex cornuta* males, females and unsexed trees (basal diameter ≥ 1.5 cm) in the two study sites in Jeju Island, South Korea. **a** YS population; the upper right is the aerial photo, and the yellow line represents the boundary on which individuals are distributed. **b** GP population.

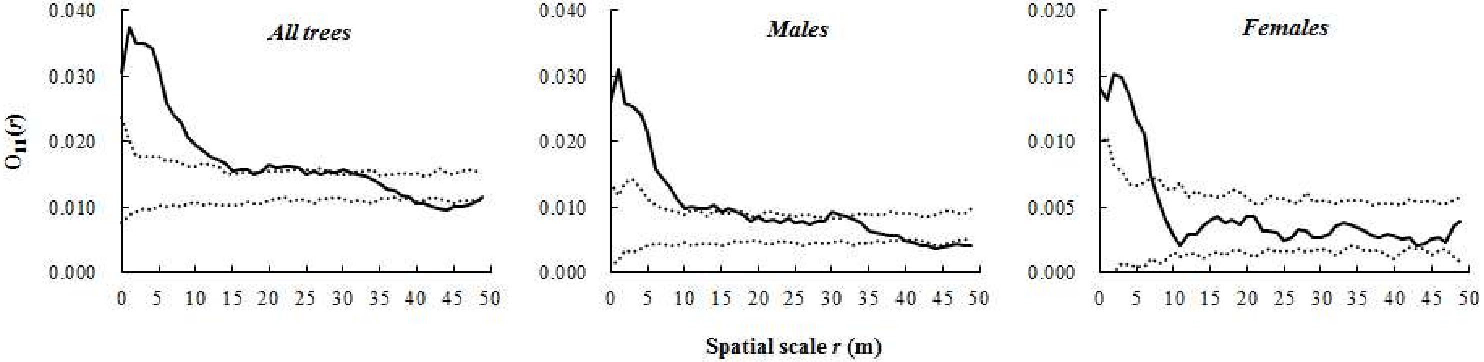


Fig. 2.5 Univariate analysis of the spatial pattern of *Ilex cornuta* trees, considering sex (males and females) using the O-ring statistics $O_{11}(r)$ in YS population. Solid lines indicate $O_{11}(r)$, and dashed lines indicate 95% confidence envelopes under the complete spatial randomness null model.

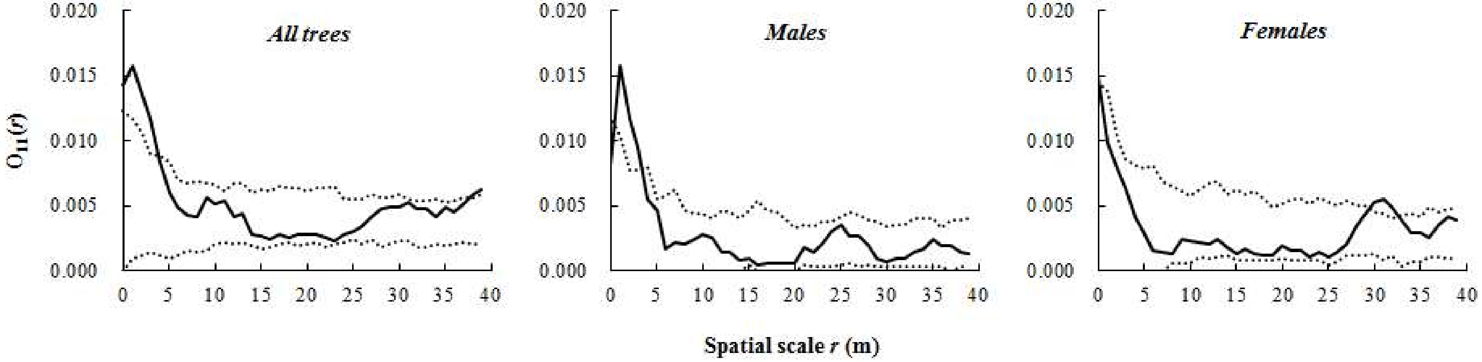
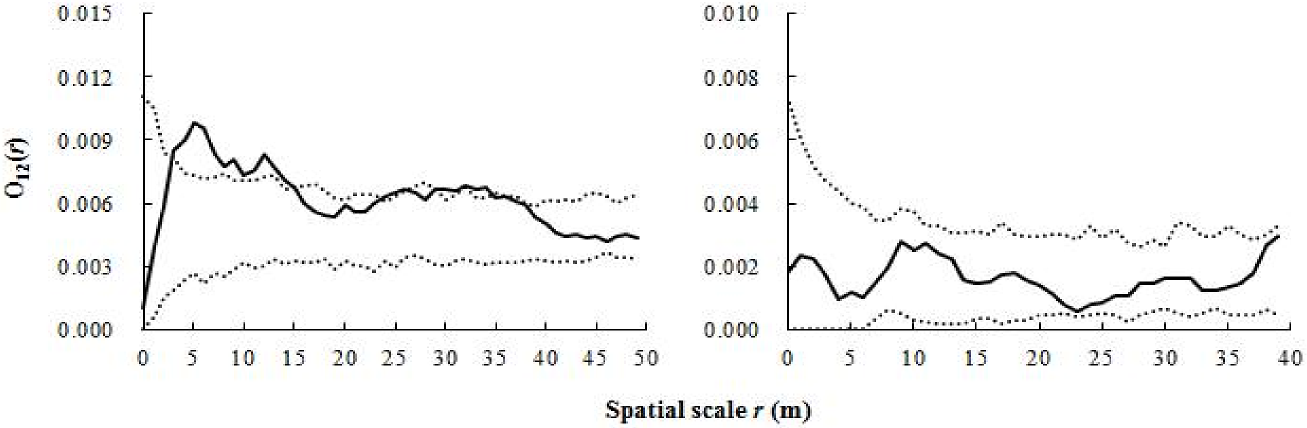


Fig. 2.6 Univariate analysis of the spatial pattern of *Ilex cornuta* trees, considering sex (males and females) using the O-ring statistics $O_{11}(r)$ in GP population. Solid lines indicate $O_{11}(r)$, and dashed lines indicate 95% confidence envelopes under the complete spatial randomness null model.



a YS population

b GP population

Fig. 2.7 Bivariate analysis of the spatial pattern of *Ilex cornuta* trees, considering sex (males and females) using the O-ring statistics $O_{12}(r)$ in YS and GP populations. Solid lines indicate $O_{12}(r)$, and dashed lines indicate 95% confidence envelopes under the random labeling null model.

2.4 Discussion

2.4.1 Male-biased sex ratio of *I. cornuta* in YS population

The sex ratio parameter is fundamentally applied to describe the population structure, due to an important factor in the reproductive success, and show adaptability to particular environmental conditions (Renner and Ricklefs 1995; Vessella et al. 2015). In this study, we found that YS population has a flowering sex ratio of 0.66 (25 females and 49 males) that is significantly male-biased (Table 2.1). This finding consistent with the observation that male-biased sex ratios are twice as frequent as female-biased sex ratios in dioecious plant species, which is usually explained by the increased need for resources in females to produce ovaries, seeds and fruits (Barrett et al. 2010; Munné-Bosch 2015). Sex ratios may also be age- or stage-specific in populations, especially male-biased sex ratio in relatively small (young) or/and large (older) group are likely to be a result of delay flowering and higher mortality rates in females (Queenborough et al. 2007; Zhang et al. 2010; Kang and Shin 2012). For example, *Salix sachalinensis* (Ueno et al. 2007) was strongly biased towards females at population level, but males outnumbered females in small size classes (diameter ≤ 3 cm). In YS population, however, proportion of males was higher than females in all size classes (Fig. 2.3), so it was difficult to expect a change in sex ratios according to the size. There were 20 unsexed individuals that their was unable to identify flowering and fruiting, although their basal diameter was sufficient for the reproductive activity. Precocious reproduction in males making it possible to identify their sex earlier (Thomas and LaFrankie 1993; Osunkoya 1999; Queenborough et al. 2007). If most of the individuals who have not confirmed flowering are female, 1:1 sex ratio can be expected in YS population.

However, the part of unsexed *I. cornuta* trees were observed to be on the verge of death which are the branches were damaged by human trimming and were almost laid down or almost no leaves were left. Also, 80% of the unsexed trees were found to be moderate or weak vitalities due to pest infection (data not shown). Reproductive status of some dioecious tree species is a stronger indicator of environmental stress (Hultine et al. 2007). Rowland and Johnson (2001) reported that in the most stressed population of *Populus deltoides* var. *wislizenii*, over half of the mature trees remained non-reproductive and a substantial number switched from reproductive to non-reproductive status in response to environmental stress. It is guessed that part of unsexed *I. cornuta* were inhibited by human induced disturbance rather than physiologically immature stage. This is supported by the fact that unsexed *I. cornuta* trees occurred not only in small size class but also in meddle and large size classes (≥ 3.0 cm in basal diameter). Males prevail under stressful circumstance in many species, because of the higher energetic costs for females (Obeso 2002; Nunez et al. 2008). In YS population also the anthropogenic disturbance seems to have induced the male dominance. The increased extinction risk is often linked to an insufficient number of individuals of the opposite sex, particularly in small and isolated populations (Pannell and Barrett 1998). Further monitoring is needed to reproductive status of *I. cornuta* trees in YS population.

2.4.2 Small population size of *I. cornuta* in GP population

The sex ratio did not differ significantly from 1:1 in GP population (13 females and 13 males). However, this population has a problem of small population size. Schmidt and Jensen (2000) suggested that low population size leads to reduced reproductive rates, and thus population size itself is the best predictor of population viability. Also, previous studies have supported a positive correlation between population size and neutral genetic variation within populations (e.g. Frankham 1996; Leimu et al. 2006). There have been no studies on the genetic variation in GP population, but it may be difficult to expect a high level of genetic variation considering the population size. Also, Ashman (2000) reported that pollinator visitation rates can decrease as patch size decreases. Decrease of the pollinator in *I. cornuta* can be a fatal cause to the population survival because *I. cornuta* have obligate outcrossing mating system. GP population is located in a protected area, there is less concern of habitats destruction by anthropogenic activities in the future. However, since small population is vulnerable to demographic variability, *I. cornuta* trees of this population may be further reduced or extinct due to factors such as competition with other species and climate change.

2.4.3 Sexual differences in clonal growth and vitality

I. cornuta combines sexual reproduction with a form of vegetative propagation. The trade-offs between investment in vegetative propagation and sexual reproduction, vegetative growth may limit allocation to flowering and fruit production (Rocheleau and Houle 2001; Van Drunen and Dorken 2010; Barrett 2015). So, males often exceed females in their capacity for clonal propagation (Dorken and Van Drunen 2010). As an example, Matsushita and Tomaru (2012) found in *Lindera triloba*, a significant increase of ramets in male genets than the female. The genets of *I. cornuta* formed a clump with several ramets, and were composed of a large main ramet and many small sprouted ramets. However, in two populations, there were no significant sexual differences in the clonal growth (Table 2.2). Clonality can be the adaptive strategy of plants in harsh environments (Bond and Midgley 2001; Barrett 2015). For example, *Salix* individuals often exhibit clonal growth particularly in frequently poor sites (Douhovnikoff et al. 2005; Ueno et al. 2007). *I. cornuta* trees of GP population formed more ramets on average than the trees of YS population (1.7 ± 1.5 in GP pop. vs. 3.8 ± 2.7 in YS pop.). GP population is located Gotjawal terrain, which represents poor soil development and oligotrophic and stressful environment to plants. And the species diversity is high, and the inter-specific competition is high. Actually *I. cornuta* trees are limited to relatively open spaces near the observatory to avoid competition with hardwoods in Jeju Gotjawal Provincial Park. Perhaps it is thought that these environmental factors promoted the clonal propagation of *I. cornuta* trees in GP population.

Females more susceptibility to abiotic and biotic stresses (Munné-Bosch 2015), really female-biased fungal infections appear to be more common (Kaltz and Shykoff 2001; Zhang et al. 2009; Moritz et al. 2016). For example, female *Salix viminalis* experience more severe fungal infections both in field experiment and literature survey (Moritz et al. 2016). The proportion of infected *I. cornuta* trees above the medium in YS population were as high as about 50%. Also, for healthy group in YS population, proportion of males was about two times higher than females (65% vs. 36%). In other words, male *I. cornuta* were more defense against infection than the females. In GP population, however, the vitality by infection were not significantly associated with sex. Compared to YS population, the proportion of healthy trees was 1.2× higher, and the weak trees were 4.0 times lower in GP population. It is guessed that *I. cornuta* trees are more vulnerable to disease in the disturbed YS population by human induced.

2.4.4 Spatial distribution patterns with sex-related

Spatial segregation of the sexes (SSS) has been reported in > 30 dioecious species from 20 families (Barrett and Hough 2013), and in the vast majority of cases male-biased sex ratios are reported in more stressful sites (Bierzychudek and Eckhart 1988; Mercer and Eppley 2010). Our bivariate point pattern analysis not detected SSS (Fig. 2.7). SSS has been observed mostly in herbaceous plants (Bierzychudek and Eckhart 1988; Eppley 2005), but rarely in shrubs (Schmidt 2008; Zhang et al. 2010)) or in trees (Nicotra 1998; Garbarino et al. 2015). A few studies reported SSS for dioecious woody plants (Nuñez et al. 2008; Zhang et al. 2010; Matsushita et al. 2016). Van Drunen and Dorken (2014) also detected that a positive association between SSS and wind pollination. *I. cornuta* is woody growth form and pollinated by insect. SSS associated with microhabitat differences such as resource gradient (Eppley et al. 1998). but, in highly diverse microhabitats at small scales created in Gotjawal terrain, located YS and GP populations, it may be difficult to expect a formation of resource gradient such as moisture or soil nutrients. That means, SSS would be difficult to expect in the two population. Also, *I. cornuta* trees in YS population are undergoing very serious artificial interferences. In YS population, the large numbers of individuals with more than 70% damage by cutting and disease were observed. For example, as part of the habitats was destroyed with the removal of pine trees due to Pine Wilt Disease, *I. cornuta* trees inhabited there were completely disappeared. There was a limit in confirming the spatial distribution patterns of *I. cornuta* due to human disturbance activities.

2.4.5 Conservation implications

This is the first study to reveal the sex-related populations structure of dioecious *I. cornuta* in Jeju Island. In summary, we found not only intersexual ecological traits of *I. cornuta* but also the differences between populations, so emphasize the need for appropriate conservation and management of each population.

For YS population, suitable protective devices are needed to prevent *I. cornuta* trees being disappeared due to habitat disturbance by anthropogenic activities. This population has problems with little females, many non-reproductive trees, high infection probability and artificial damage (e.g. pruning and logging). Population persistence almost certainly depends on the number of females fertilized and the number of seeds produced (Groom 2001). Further monitoring is needed to reproductive status of *I. cornuta* trees in YS population. Monitoring of the flowering and fruiting process, and surveying of seedling and sapling are necessary. Also, the protected fence should be installed so that habitat is not destroyed by cultivated lands extension or human activities.

GP population has a problem of small population size. Therefore, the conservation program for assisted natural regeneration of *I. cornuta* trees is needed. During the survey a number of seedling were observed around the mature *I. cornuta* trees. However, because this population is located near the observatory, so the seedling and/or sapling around the trail is a risk of damaged by the visitors. First of all, because *I. cornuta* grows only in limited areas of Gotjawal Provincial Park, intensive protection based on current population location is required. For example, by removing some of the trees that compete with *I. cornuta*, management is needed to ensure that *I. cornuta* seedlings and saplings receive enough light, and grow into mature trees. However, removal should only be possible from relatively abundant competing trees distributed throughout Gotjawal Provincial Park.

For this study, we explored the *I. cornuta* populations in the western Jeju Island based on the previous distribution data (Lee 1983; Son et al. 2007; Kwon et al. 2011), and found only YS and GP population in which enough individuals are inhabited. In addition, we confirmed sporadic distribution of *I. cornuta* trees in Daejeong-ri, Hyeopjae-ri and Wollyeong-ri, the western Jeju Island. In interviews with local residents, they used to say that *I. cornuta* trees were distributed all over the place in the past, but recently it is hard to find due to human land use (e.g. farmland, roads and tourism) and damage by agricultural chemicals. During our field survey, it was observed that the number of *I. cornuta* trees in Yongsu-ri region was declining due to anthropogenic activities. If such human pressures continues without sustainable management of the resource, natural abundance of this species will be at risk. Therefore, in order to conservation of *I. cornuta*, it is first necessary to search and investigate the populations distributed in Jeju Island.

Chapter 3

Spatial distribution patterns of old-growth forest of dioecious tree *Torreya nucifera* in rocky Gotjawal terrain of Jeju Island, South Korea

3.1 Introduction

The spatial structure of plants in a population reflects complex interactions of ecological and evolutionary processes (Epperson 2005). Processes that can generate plant spatial patterns include intra- and inter-specific interactions (Stoll and Bergius 2005; Nanami et al. 2011), environmental heterogeneity (Zuo et al. 2008), breeding systems (Bleher et al. 2002), and disturbances (Wolf 2005; Rayburn and Monaco 2011). By analyzing spatial patterns of individuals, it might be possible to identify physical conditions and competitive factors contributing to the spatial structure of a population (Bell et al. 1993; Law et al. 2009; Rayburn et al. 2011). For example, clumped pattern most frequently observed in natural populations has been interpreted as evidence of positive interactions among individuals (Callaway 1995), patch distributions of resources (Schenk et al. 2003; Perry et al. 2009), and seed dispersion by gravity (Bleher et al. 2002; Gao et al. 2009).

Previous studies on spatial patterns of plants have focused on hermaphrodite or monoecious species whose parent plants can produce seeds (Nanami et al. 1999; Rayburn et al. 2011; Benot et al. 2013; Cheng et al. 2014). On the other hand, studies on the influences of dioecy consisting of separate male and female plants on spatial structure of tree populations are relatively limited (Gibson and Menges 1994; Hultine et al. 2007; Garbarino et al. 2015). For dioecious plants, spatial distribution of mature males and females is crucial to population survival because they can only reproduce by outcrossing (Bawa 1980; Thomson and Barrett 1981; Osunkoya 1999). Furthermore, females tend to allocate a greater proportion of resources to sexual reproduction than to growth and maintenance compared to males (Lloyd and Webb 1977; Opler and Bawa 1978; Delph 1999; Obeso 2002). Differences in reproductive cost between sexes may result in differential fitness between sexes across environmental gradients. Such differences can subsequently generate spatial segregation of sexes (SSS) (Bierzychudek and Eckhart 1988; Nuñez et al. 2008). In other words, females with resource limitations due to high reproductive costs will occupy more favorable or less stressful conditions regarding elevation (Garbarino et al. 2015), water availability (Ortiz et al. 2002), and/or soil fertility (Lawton and Cothran 2000). For example, female *Acer negundo* (Dawson and Ehleringer 1993), *Juniperus virginiana* (Lawton and Cothran 2000), and *Salix glauca* (Dudley 2006) are found on sites with relatively greater amounts of moisture. However, conflicting results relevant to SSS have recently been documented in several plant species (Ueno et al. 2007, Schmidt 2008, Gao et al. 2009, Forero-Montaña et al. 2010; Garbarino et al. 2015).

Some studies on sex-related size (or age) structures of dioecious species have been published (Goto et al. 2006; Zhang et al. 2010; Garbarino et al. 2015). Reproductive costs are age-specific and reproductive investment is variable during lifespan of plants (Silvertown and Dodd 1999; Montesinos et al. 2006). Nanami et al. (2005) have reported that dioecious individuals tend to shift from clumped distribution to random distribution as tree size increases duo to density-dependent mortality caused by intra-specific competition. Therefore, interactions between sex and size in dioecious species should be considered in spatial structure study of dioecious population.

Torreya nucifera (Taxaceae) is a dioecious gymnosperm currently distributed in southern parts of South Korea and Japan. The largest (n = 2,861) and oldest (mostly 200-400 years old, max. ~900 years old) population of *T. nucifera* in the world (*Torreya* Forest hereafter) is located in Jeju Island, South Korea. In a previous study by Kang and Shin (2010), the *Torreya* population in Jeju Island could be separated into three sites depending on sex ratio and DBH (diameter at breast height). Abiotic heterogeneity influences adaptation activities such as competition, growth, and mortality of individuals, resulting in a unique spatial structure of the population (Scarano 2002; Zuo et al. 2008; Perry et al. 2009). Gotjawal terrain where *Torreya* Forest exists is a unique volcanic area with lava blocks scattered extremely disorderly (Jeon et al. 2012). Thus, Gotjawal terrain with severe topographic heterogeneity generates highly diverse microclimate (Choi and Lee 2015). Furthermore, Gotjawal terrain represents poor soil development, oligotrophic and stressful environment to plants. Therefore, current spatial structure of the *Torreya* Forest is likely to reflect the long history of its survival and mortality in such harsh environment. However, the spatial structure of *Torreya* Forest has not been examined so far.

As plants are sessile, their survival is mostly determined at quite a local scale, even at a scale of a few centimeters, by both abiotic and biotic factors rather than by spatial average of its overall population (Stoll and Prati 2001; Benot et al. 2013). If so, dioecious sexual system of *T. nucifera* trees, size difference among their three sites, and complexity of the terrain should all be considered in the study of spatial structure of *Torreya* Forest in Jeju Island. In this study, the following aspects were examined: (1) the distribution pattern of *T. nucifera* trees according to sex and size at each site, (2) the site differences in those distribution patterns, and (3) the pattern of spatial segregation of sexes and sizes at each site.

3.2 Materials and Methods

3.2.1 Study species and site

Torreya nucifera (Taxaceae) is a dioecious evergreen gymnosperm that can grow up to 25 m in height. Its pollination occurs in April and green fleshy aril-covered seeds mature in the fall one year after pol-lination. Various parts of this tree have traditionally been used. For example, its wood has been used for furniture and its seeds have been used for anthelmintic and oriental medicines and oil.

Torreya Forest (Natural monument No. 374 in South Korea) is located in Jeju Island (33°29′ N, 126°48′ E) (Fig. 3.1a). This forest (44.8 ha in area, 143 m mean a.s.l.) extends 1.4 km in north-south direction with a width of 0.6 km. It is located between two small volcanoes: Darangshioreum (382.4 m a.s.l.) and Dotoreum (a volcano near the southern end of the population; 284.2 m a.s.l.). In 1999, all *T. nucifera* trees with DBH $\geq$ 6 cm were tagged and tending was started for 11 plots along trails within the forest (Fig. 3.1b).

Torreya Forest terrain is defined as Gujwa-Seongsan Gotjawal, a transition lava zone distributing both pahoehoe and aa lava flows (Jeon et al. 2012). Although *T. nucifera* is a dominant species in this forest, the Forest consists of diverse evergreen trees with a total of 276 plant taxa, including *Mallotus japonicus*, *Machilus thunbergii*, *Orixa japonica*, and *Polystichum tripteron* [Korea Tree Health Association (KTHA) 1999;

Lee 2009; Shin et al. 2010; Choi and Lee 2015]. Figure 3.2 demonstrates characteristic features of wild *Torreya* Forest. According to 2007-2016 data from Gujwa close to the *Torreya* Forest, the mean monthly temperature ranges from 5.3°C in January to 26.7°C in August (mean annual temperature: 15.7°C). Its mean annual precipitation is 1774.2 mm with a peak in August (307.8 mm) with mean annual wind speed of 4.0 m/s (Korea Meteorological Administration 2017a).

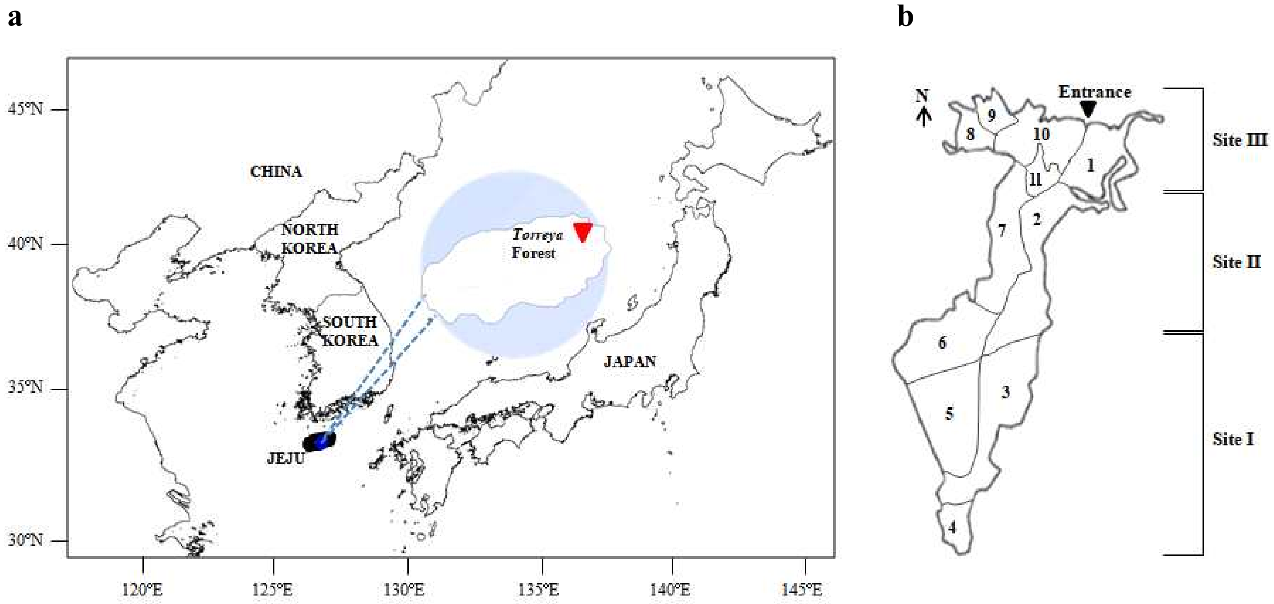


Fig. 3.1 Location and map of *Torreya* Forest in Jeju Island, South Korea. **a** Location of *Torreya* Forest in Jeju Island, South Korea. **b** The 11 plots divided by trails (Lee 2009) were categorized into three sites depending sex ratio and DBH gradient by Kang and Shin (2012): Site I in the southern part of the forest including plots 3-5, Site II in the middle part including plots 2-7, and Site III in the northern part including plots 1, 8, 9, and 10.

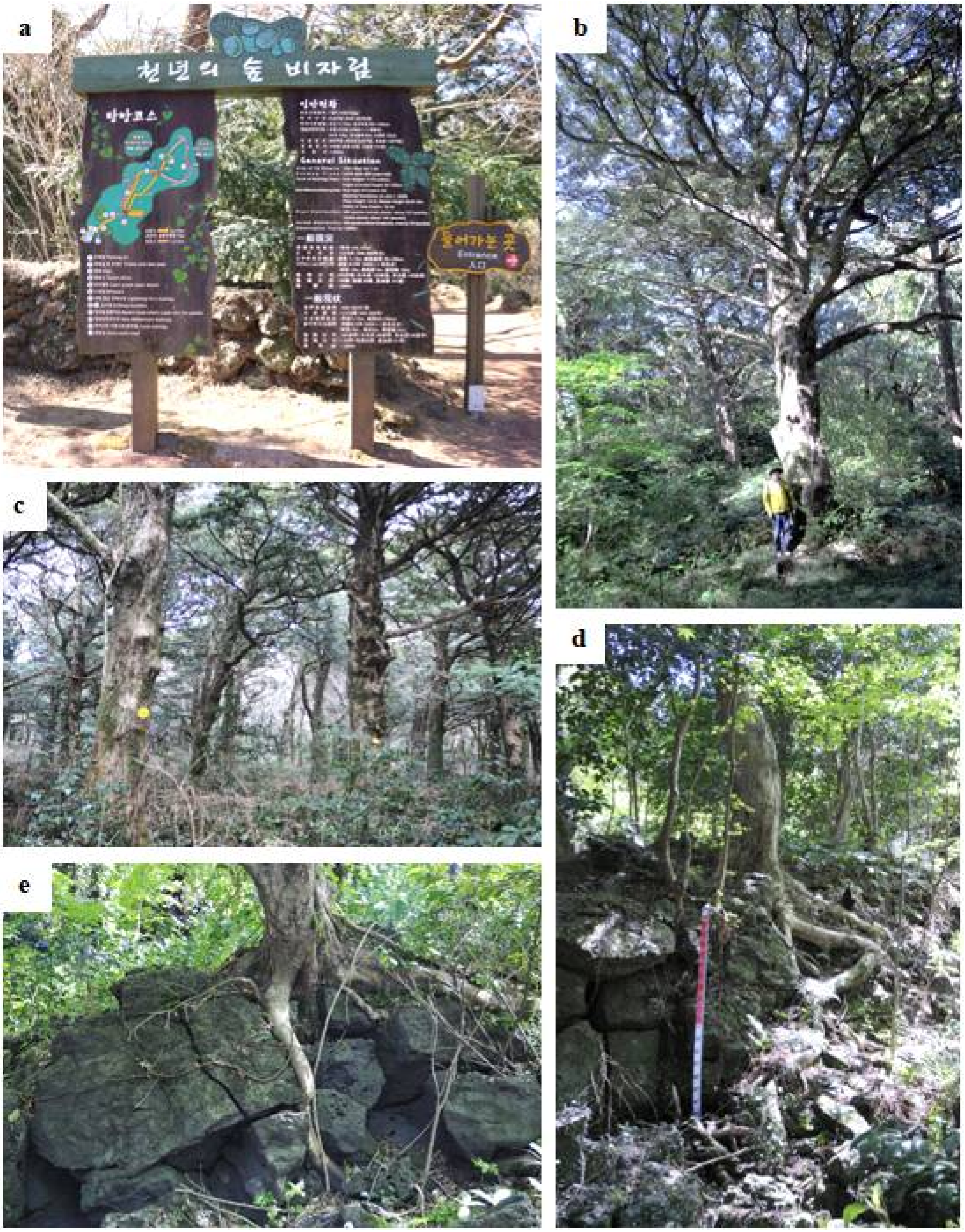


Fig. 3.2 Wild *Torreya* Forest in Jeju Island, South Korea. **a** A guideboard of *Torreya* Forest. **b** *T. nucifera* tree reaching 10 m in height. **c** Stand structure of *Torreya* Forest. **d** Rocky Gotjawal terrain of *Torreya* Forest. **e** *T. nucifera* tree rooted on lava blocks.

3.2.2 Data collection

The planimetric map was transformed into an image file through high-resolution scanning using a survey map of the location of *T. nucifera* trees (KTHA 1999). Warping was performed by assigning coordinates to each edge. Digitizing process was then used to obtain coordinates for 2,861 individuals (1,498 males and 1,363 females). By inputting attribute values such as sex, DBH, and sites for each individual, a database of location and ecological traits of *T. nucifera* trees was completed. Sex and DBH data were obtained from KTHA (1999) and Lee (2009). *Torreya* Forest exhibited a gradient of sex ratio and DBH from the southern end to the northern end of the forest. In this study, we analyzed spatial patterns of *T. nucifera* trees based on the three sites defined by Kang and Shin (2012). Numbers, sex ratio, and DBH of *T. nucifera* trees in the three sites are shown in Table 3.1. ArcGIS Ver. 9.3. (ESRI 2008) was used for all analyses described above.

In the *Torreya* Forest, there are somewhat unnatural spaces where *T. nucifera* trees are absent or scant due to the planting of *Pinus thunbergii* trees and various herbaceous plants, paved entrance, and trails. Therefore, a quadrat (160 x 300 m) was established in each of the three sites to represent the typical local condition (Fig. 3.3a): quadrat A for Site I, quadrat B for Site II, and quadrat C for Site III. The distribution of *T. nucifera* trees according to sex and size (DBH) in each quadrat is shown in Figure 3.3b.

Table 3.1 Numbers, sex ratio and DBH of *Torreya nucifera* trees in the three sites of *Torreya* Forest in Jeju Island, South Korea (adapted from Kang and Shin 2012)

Site	Plot ID	Altitude (m)	Area (ha)	Sex		Sex ratio	Density	Mean DBH (cm)
				Males	Females			
I	3	148	6.4	235	167	0.58 **	62.8	58.9 ± 20.6
	4	154	1.5	16	11	0.59 ^{NS}	18.0	40.5 ± 20.6
	5	145	7.1	268	165	0.62 ***	61.0	61.7 ± 19.4
	Mean					0.60 ***	57.5	59.7 ± 20.4
II	2	140	6.8	304	293	0.51 ^{NS}	87.8	54.2 ± 16.4
	6	144	5.3	194	200	0.49 ^{NS}	74.3	56.2 ± 15.9
	7	141	4.7	165	160	0.51 ^{NS}	69.2	45.0 ± 16.9
	Mean					0.50 ^{NS}	78.3	52.5 ± 17.0
III	1	130	4.0	64	47	0.58 ^{NS}	27.8	46.2 ± 21.7
	8	129	1.3	23	64	0.26 ***	66.9	57.0 ± 19.0
	9	128	1.3	18	21	0.46 ^{NS}	30.0	44.4 ± 17.1
	10	131	3.9	211	235	0.47 ^{NS}	114.7	39.4 ± 21.1
	Mean					0.46 $^{(*)}$	65.0	43.0 ± 21.5
	Overall mean					0.52 *	67.6	52.4 ± 20.1

Sex ratio = male/(male + female); Density = trees/ha; DBH = diameter at breast height.

(*) $P = 0.0510$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$; NS = not significant.

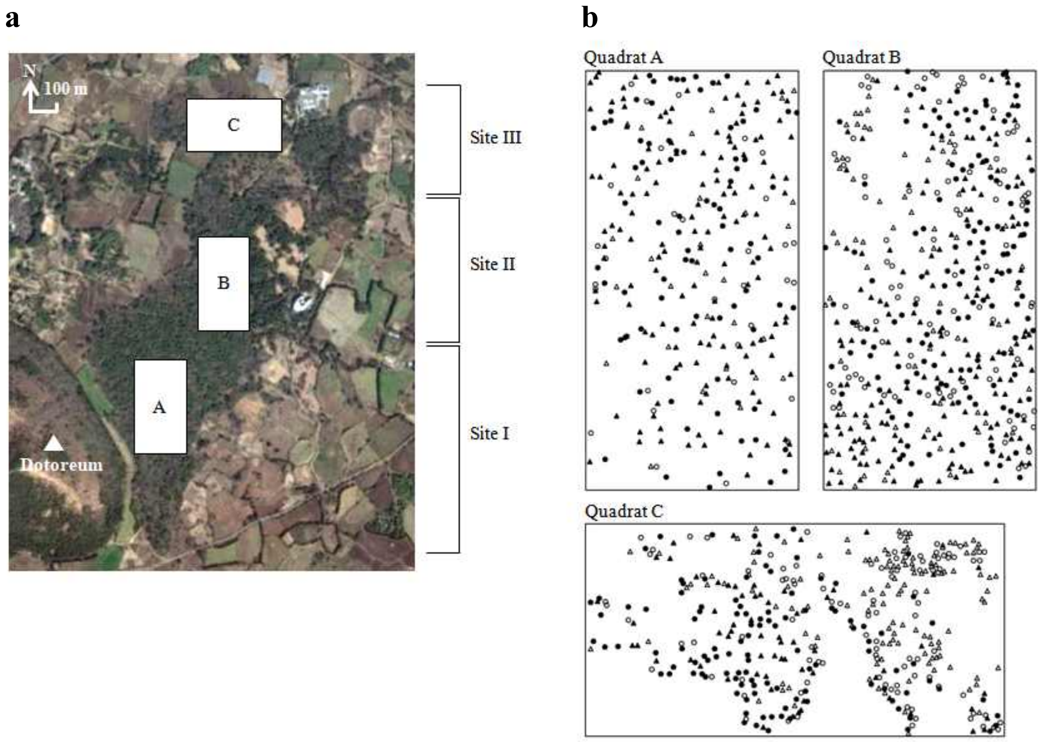


Fig. 3.3 Distribution of trees within quadrats in *Torreya* Forest of Jeju Island, South Korea. **a** Location of the three quadrats (160 x 300 m) in *Torreya* Forest. **b** Distribution of *T. nucifera* trees according to sex and size groups in each quadrat: small males (DBH < 50 cm, △), large males (DBH ≥ 50 cm, ▲), small females (DBH < 50 cm, ○), and large females (DBH ≥ 50 cm, ●).

3.2.3 Data analyses

T. nucifera trees were categorized into eight groups based on sex (males and females), size (small trees, DBH < 50 cm; and large trees, DBH $\geq$ 50 cm), and sex by size (small males, large males, small females and large females) for spatial point pattern analysis.

O-ring statistics $O(r)$ was employed to describe the average density of points at a distance of r (Wiegand and Moloney 2004; Law et al. 2009). Value of $O(r)$ was calculated as follows. Around each individual data point, numerous circles with radius r were drawn and the correlation between the average number of individuals within numerous circles and radius r was deduced to determine the O value. O-ring statistics included univariate and bivariate analyses (Zhang et al. 2010). Univariate statistical analysis of $O_{11}(r)$ was used to analyze spatial patterns between individuals in a group while bivariate statistical analysis of $O_{12}(r)$ was used to analyze spatial relationships between two different groups. $O_{11}(r)$ was applied to assess spatial patterns of *T. nucifera* trees within two sexes and/or two size groups (e.g. male, female, small, and large) in *Torreya* Forest. $O_{12}(r)$ was used to determine whether there was a spatial relationship between two sexes and/or two size groups (e.g. males vs. females and small vs. large trees). To conduct significance test for $O(r)$ value at each distance r, null hypothesis was formed using complete spatial randomness (CSR). Then 95% confidence intervals for both univariate and bivariate analyses were computed from 999 Monte

Carlo simulations. If O(*r*) value was above the CSR value, individuals were considered to be clumped. It O(*r*) value was within 95% confidence intervals, individuals were considered to be randomly distributed. If it was below the CSR value, individuals were considered to be regularly distributed. All calculations and simulations were performed using PROGRAMITA software (Wiegand and Moloney 2004).

3.3 Results

3.3.1 Ecological characteristics of the three sites

Ecological characteristics such as sex ratio, size, and density of *T. nucifera* trees in each quadrat are shown in Table 3.2. *T. nucifera* trees of quadrat A (at Site I) were male-biased (sex ratio = 0.62). Large trees were about four times more abundant than small trees (*n* = 241 vs. 62). The mean DBH was relatively large whereas its average density was the lowest among the three quadrats. On the other hand, *T. nucifera* trees in quadrat B (at Site II) and C (at Site III) showed a 1:1 sex ratio and somewhat higher density than quadrat A. In addition, in quadrat C, *T. nucifera* trees had the smallest mean DBH, and small trees were more abundant than large trees (*n* = 230 vs. 177).

3.3.2 Univariate spatial analyses

Univariate analyses showed that spatial patterns of *T. nucifera* trees differed between groups (e.g. male vs. female, small vs. large) of each quadrat (Figs. 3.4-3.6). In quadrat A, all trees and eight groups of *T. nucifera* showed random distribution at all scales (Fig. 3.4). In quadrat B, all trees demonstrated random distribution at all scales. However, when sex and size were considered, spatial pattern changed. Males were randomly distributed at all scales while females were weakly clumped at 0-17 m (except for 8-10 m). Small trees were randomly distributed at all scales while large trees were clumped (2-23 m) or regularly (54-58 m) distributed at some distances. By assessing univariate patterns of four groups of sex by size, small males and small females were distributed randomly at all scales. However, large males and large females were clumped at 0-16 m scales. Finally, all groups in quadrat C were clumped at short distances. They were distributed randomly or regularly as the distance was increased. When sex and size were combined, males (0-32 m) clumped more strongly than females (0-12 m) in the small tree group whereas females (0-24 m) clumped more strongly than males (0-17 m) in the large tree group. Thus, spatial patterns of *T. nucifera* trees varied among three quadrats. For example, large females had different distribution types for each quadrat that random (0-60 m) in quadrat A, clumped (5-16 m) in quadrat B and regular (48-60 m) in quadrat C (Figs. 3.4-3.6).

Table 3.2 Numbers, sex ratio and DBH of *Torreya nucifera* trees in the three quadrats (160 × 300 m) each established in each of the three sites of *Torreya* Forest.

			Sex		Sex ratio	Density	Mean DBH (cm)
			Males	Females			
Quadrat A							
	DBH	Small	31	31	0.62 ***	63.5	62.5 ± 18.3
		Large	158	85			
Quadrat B							
	DBH	Small	72	89	0.53 ^{NS}	99.4	54.1 ± 15.7
		Large	181	135			
Quadrat C							
	DBH	Small	129	101	0.48 ^{NS}	84.8	42.1 ± 21.6
		Large	65	112			

Sex ratio = male/(male + female); *** $P < 0.0001$; NS = not significant;
Density = trees/ha; Size: Small, DBH < 50 cm; Large, DBH ≥ 50 cm.

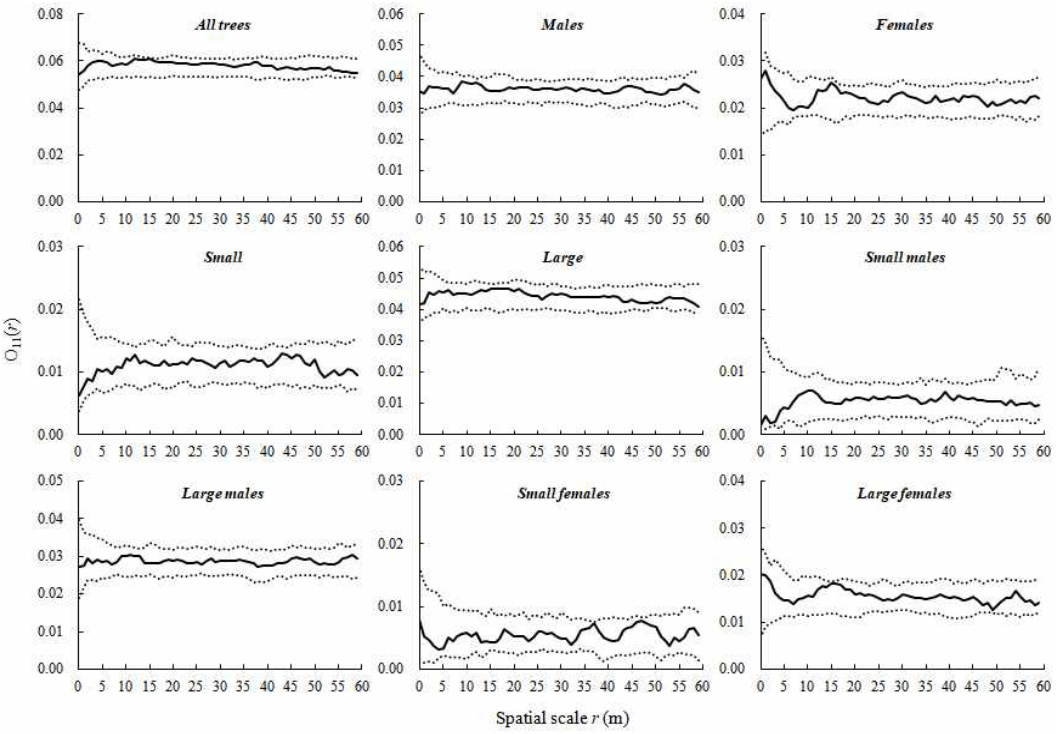


Fig. 3.4 Spatial patterns of *Torreya nucifera* trees in univariate analyses in quadrat A. Analyses were conducted for all trees pooled over sex and size groups, separately for each sex and size groups, and then for combination groups of sex and size (e.g. small males, large males, small females, and large females). Solid lines indicate $O_{11}(r)$ value from each analysis; dashed lines indicate 95% confidence envelopes regarding the random spatial structure null hypothesis.

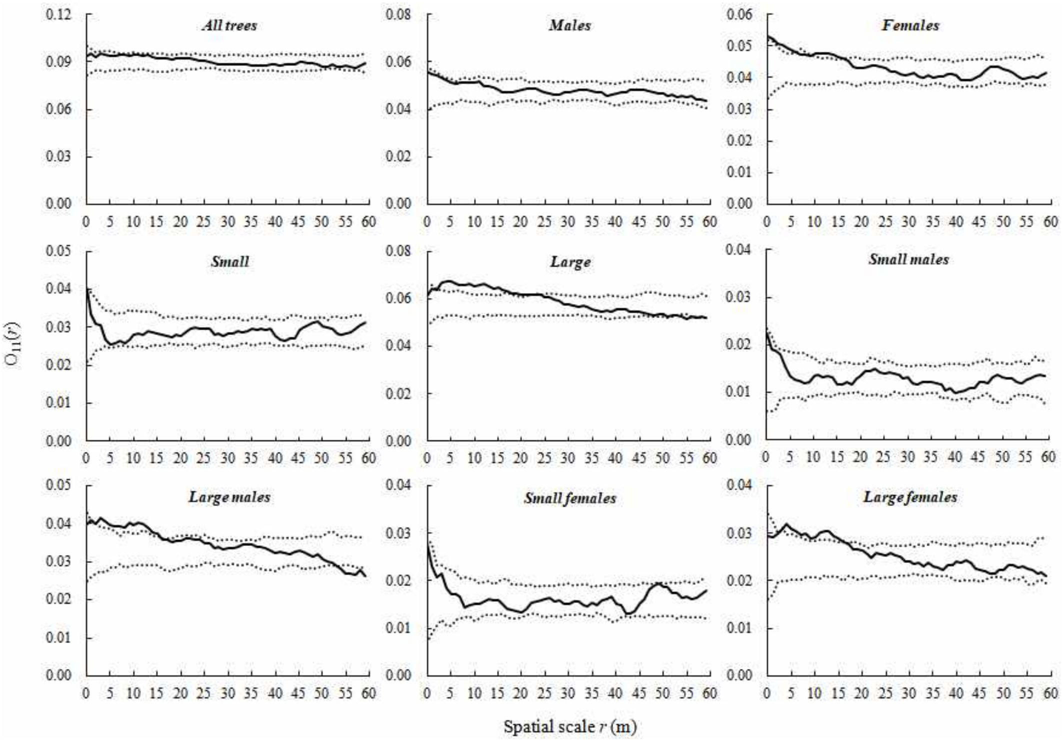


Fig. 3.5 Spatial patterns of *Torreya nucifera* trees in univariate analyses in quadrat B. Analyses were conducted for all trees pooled over sex and size groups, separately for each sex and size groups, and then for combination groups of sex and size (e.g. small males, large males, small females, and large females). Solid lines indicate $O_{11}(r)$ value from each analysis; dashed lines indicate 95% confidence envelopes regarding the random spatial structure null hypothesis.

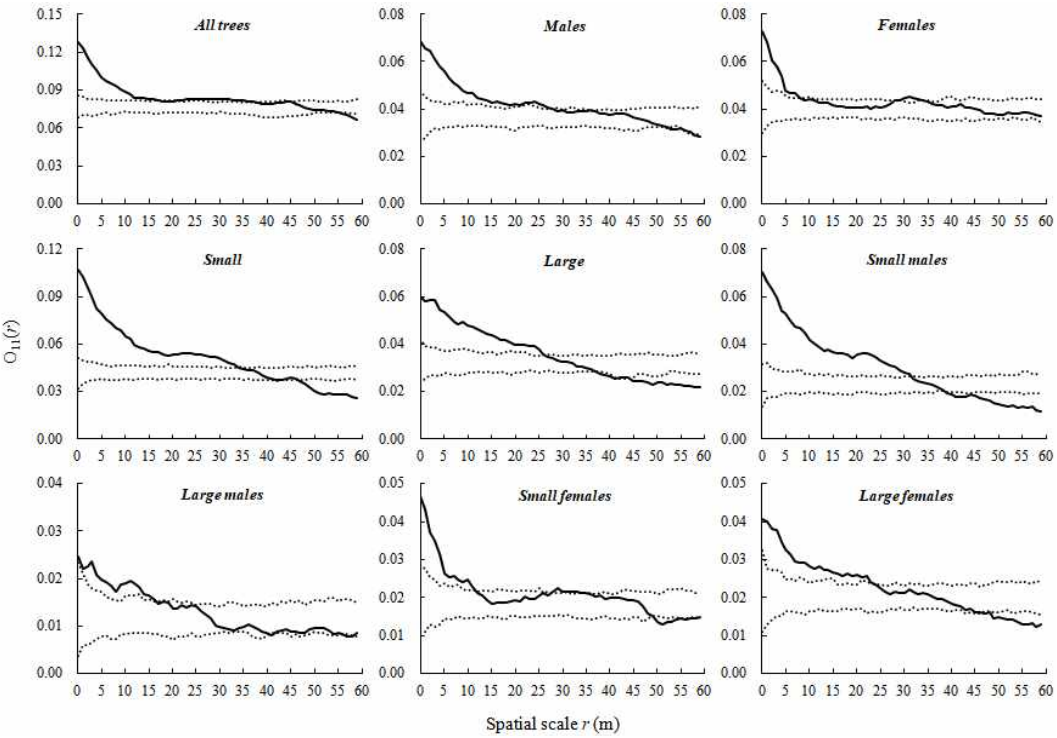


Fig. 3.6. Spatial patterns of *Torreya nucifera* trees in univariate analyses in quadrat C. Analyses were conducted for all trees pooled over sex and size groups, separately for each sex and size groups, and then for combination groups of sex and size (e.g. small males, large males, small females, and large females). Solid lines indicate $O_{11}(r)$ value from each analysis; dashed lines indicate 95% confidence envelopes regarding the random spatial structure null hypothesis.

3.3.3 Bivariate spatial analyses

Bivariate analyses for spatial associations between two sexes and/or two size groups showed that spatial segregation occurred only in quadrat C (Fig. 3.7). Males and females were spatially independent at all scales. Significant spatial segregation was observed between small and large trees (4-22 m). When combination groups of sex and size were examined, repulsion between groups was notable: small males vs. small females at 50-60 m scales; large males vs. small females at 12-17 m scales; small males vs. large females at 0-22 m scales; and large males vs. large females at 37-60 m scales.

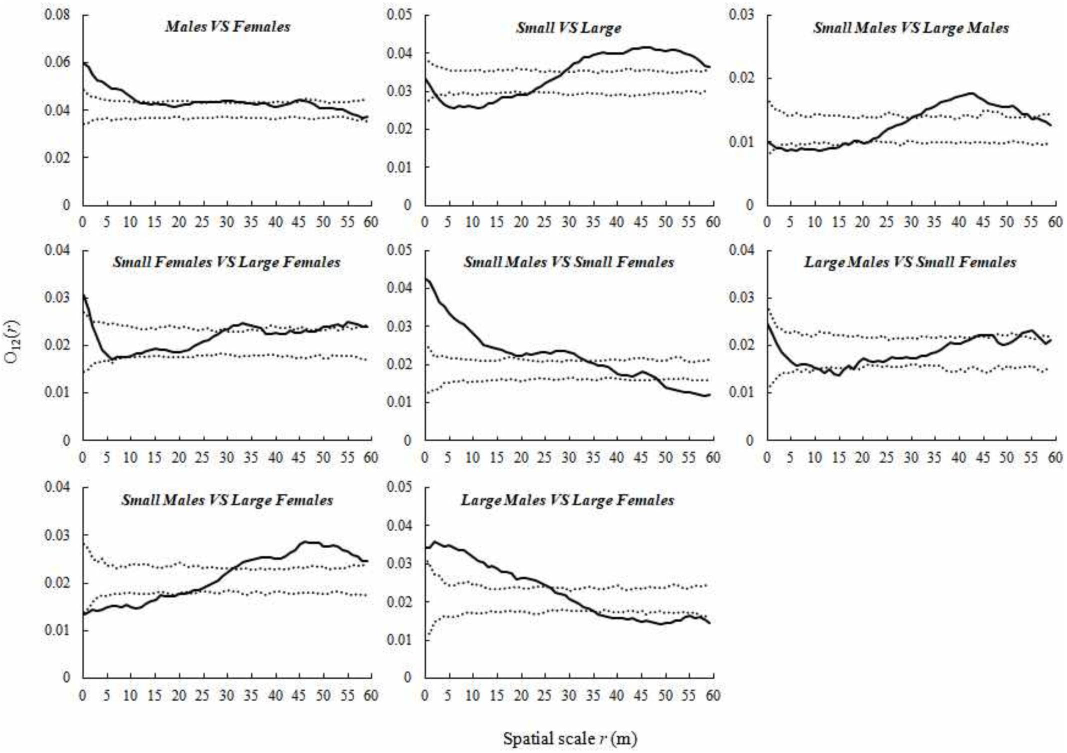


Fig. 3.7 Spatial patterns of *Torreya nucifera* trees in bivariate analyses in quadrat C. Analyses were conducted for all trees pooled over sex and size groups, separately for each sex and size groups, and then for combination groups of sex and size (e.g. small males, large males, small females, and large females). Solid lines indicate $O_{12}(r)$ value from each analysis; dashed lines indicate 95% confidence envelopes regarding the random spatial structure null hypothesis.

3.4 Discussion

3.4.1 Spatial patterns of individual *T. nucifera* trees within and among sites

This is the first study to reveal the spatial pattern of old trees that have survived for several hundreds of years in almost natural conditions in Korea. Individual trees of *T. nucifera* in *Torreya* Forest were distributed randomly, clumped or regularly with each other depending on the sites examined.

Site I at the southern end of *Torreya* Forest, adjacent to a small volcano called Dotoreum (284.2 m a.s.l.), is characterized by relatively large proportion of old age trees. Many studies on dioecious species have reported that there is a difference in distribution pattern of individuals according to sex (Zhang et al. 2010; Chen et al. 2014; Garbarino et al. 2015). However, both male and female trees of *T. nucifera* were randomly distributed in quadrat A, not supporting sex-specific segregation between male and female trees. Random distribution of both sexes has also been reported in the old-growth plot of *Acer barbinerve* (Pan et al. 2010). Several studies in recent years have also emphasized the effects of both abiotic and biotic conditions on plant spatial patterns. For example, old trees tend to be randomly distributed as a result of stochastic mortality and intra- or inter- specific competition for resources (Nanami et al. 2005). Dying seedlings under large trees are frequently found in *Torreya* Forest. Even if seedlings were initially clumped around female trees, only small gaps randomly scattered in Gotjawal terrain may allow seedlings to survive, and finally can generate random dispersion pattern.

Site II, corresponding to the middle area of the forest, showed intermediate values in sex ratio and size between Site I and Site III (Kang and Shin 2012). It has been reported that as the size of plant is increased, the clumped pattern is generally weakened (Wang et al. 2010; Cheng et al. 2013). Recently Garbarino et al. (2015) showed that large males and females of *Taxus baccata*, which is phylogenetically related to *T. nucifera*, were randomly distributed while small trees were mostly clumped. However, in quadrat B, regardless of sexes, small trees of both sexes were randomly distributed while large male and female trees were clumped at 0-16 m scales (Fig. 3.5). The clumped patterns of large trees are in contrast to the results as mentioned above. However, several authors suggest that the increase in the clumped pattern with age might be attributable to interspecific competition (Nanami et al. 2011), differential mortality of juveniles (Briggs and Gibson 1992), and clonal structure (Peterson and Squiers 1995). This forest is mixed with diverse broad-leaved plants and *Pinus thunbergii*. And, Lee (2005) recognized that inter-specific competition greatly reduced the vigor of *T. nucifera* trees. The fact that the vigor of trees, especially female trees, was significantly improved after removal of epiphytes from the canopy of *T.*

nucifera trees (Kang and Shin 2012) also supports the importance of inter-specific competition. The influence of competition on clumping of large trees in Site II remains to be tested because we did not examine the density or distance between neighboring trees in this site.

Site III in the northern part of the forest is characterized by relatively small trees and more females than males than in other sites (Kang and Shin 2012). Individual trees in quadrat C were clustered at short distances and distributed randomly or regularly as distance increased (Fig. 3.6). Even when combining sex and size, males (0-32 m) clumped more strongly than females (0-12 m) in small trees but females (0-24 m) clumped more strongly than males (0-17 m) in large trees. In other words, consistent spatial patterns across to sex and size groups do not exist at all in this site. A simple adaptive explanation for such complex patterns may not be possible at this stage.

In this forest, anthropogenic activities have been controlled for several hundreds of years (Kim 1985, Shin et al. 2010). However, we speculate that Site III might have been sporadically subjected to severe disturbances by human activities such as logging. This is particularly likely because this site (Site III) is located near the entrance to the forest which is very easy to access compared to Site I near a small volcano. Most convincing evidence for artificial planting in this site comes from aerial photos taken from 1967 to 2015 (Fig. 3.8). In 1967, it appeared that certain locations at Site III were largely free of vegetation. Since then, its vegetation coverage has increased significantly until 2015. Such drastic changes in vegetation coverage at those locations are not expected to occur by natural regeneration because it takes about 54 years for the DBH of *T. nucifera* to grow up to 6 cm DBH (Lee 2005). Thus, those photos are highly likely to reflect that artificial planting occurred intensively around 1970s when Korean government pushed a strong reforestation policy on a nationwide scale. Kang and Shin (2012) provided another piece of evidence for artificial planting in this site. In their study, some plots in Site III are deviated quite a bit from the site's sex ratio and mean size. For example, plot 10 (Fig. 3.1b) which was a relatively vacant area in aerial photo (Fig. 3.8a) consists of smaller trees than other plots and its density (114.7 tree/ha) being twice as high as the average population density of 67.6 trees/ha. If *T. nucifera* trees were planted during 1970s, planting would be concentrated in less rocky localities covered with some soils, perhaps being responsible for clumped spatial patterns at a fine scale as detected in this study. Unfortunately, written documents regarding the reforestation of *T. nucifera* trees are not available to confirm this inference.

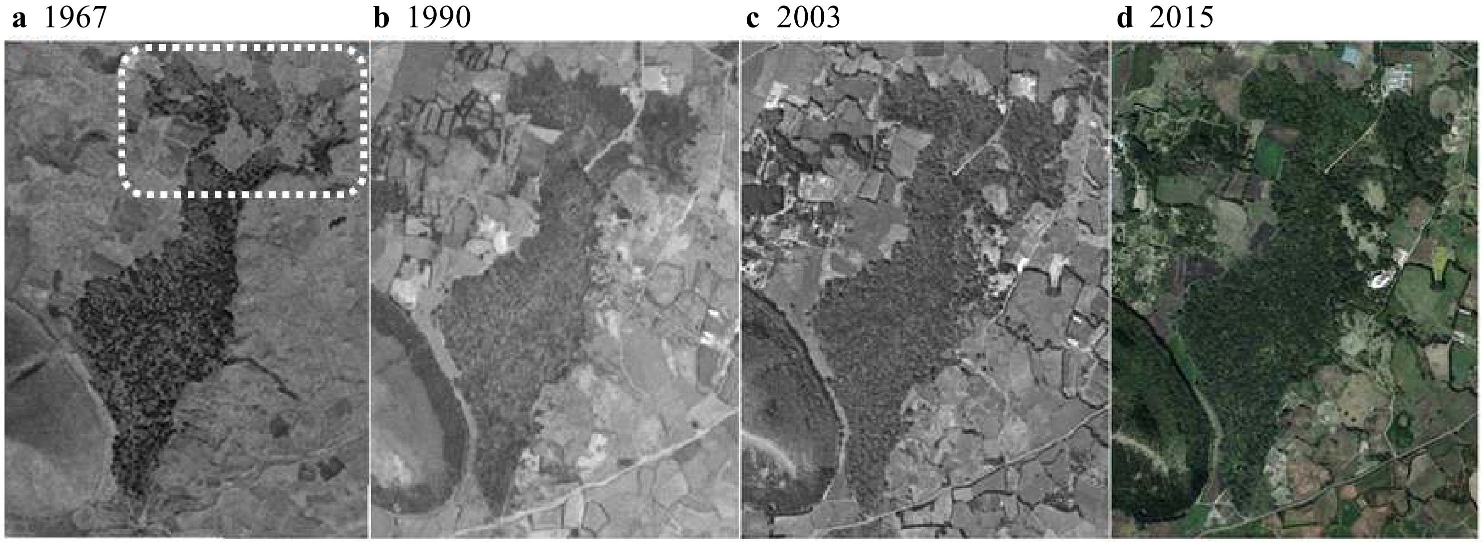


Fig. 3.8 Aerial photos of *Torreya* Forest in Jeju island from 1967 to 2015 (National Geographic Information Institute of Korea 2016).

3.4.2 Spatial segregation of sexes in *T. nucifera*

The SSS expected under the hypothesis of high reproductive cost of females has been reported in > 30 species from 24 families of seed plants (Barrett and Hough 2013). In this study, SSS occurred only in Site III, e.g. between small males vs. small females (50-60 m scales), large males vs. small females (12-17 m scales), small males vs. large females (0-22 m scales) and large males vs. large females (37-60 m scales) (Fig. 3.7). SSS detected only in the site with small trees with the highest density is analogous with the case of *Fraxinus mandshurica*. In dioecious species of *F. mandshurica*, spatial segregation between sexes was found in the secondary forests, but in the old-growth (Zhang et al. 2010). They ascribed a lack of SSS in old-growth forest of *F. mandshurica* to sex-specific responses to microenvironments. In this study, Site I trees which were larger than ones in other sites were randomly distributed regardless of sex and size groups. Currently, it is unknown whether Site I trees exhibit sex-differential responses to microenvironments.

3.4.3 Influence of rocky Gotjawal terrain

Diverse spatial patterns of *T. nucifera* trees across the three sites may imply both biotic and abiotic effects in this forest. Most of all, the role of Gotjawal terrain in terms of creating extremely diverse microhabitats, and subsequently stochastic processes of survival or mortality of trees needs to be emphasized. In stressful habitats such as the rocky coasts and alpine forests, spatial structure largely depends on constraining factors such as topography and disturbances (Camarero et al. 2000; Scarano 2002; Humphries et al. 2008). If so, we can draw a couple of inferences regarding the ecological role of Gotjawal terrain. Firstly, it seems that Gotjawal terrain is a major component affecting the distribution pattern of trees. In highly diverse microhabitats at small scales created in Gotjawal terrain, it may be difficult to expect a formation of resource gradient such as moisture or soil nutrients. That means, SSS would be difficult to expect. Furthermore, rocks and thin soils in Gotjawal terrain may cause stochastic processes over the survival and mortality, and consequently affecting spatial patterns of *T. nucifera* trees. Secondly, *T. nucifera* trees may have to respond to multiple stressors in circumstances with scattered lava blocks and little soil. Thus, diverse spatial patterns of *T. nucifera* trees may be an outcome of biotic as well as abiotic interactions that have occurred for hundreds of years.

Despite that this forest has been known to be a natural forest for such a long time, we found a new evidence for artificial interference in some plots especially near the entrance area. The rocky topography of Gotjawal terrain is certainly a component that has protected the historical and valuable *T. nucifera* trees in Jeju Island. Understanding of the spatial patterns of *T. nucifera* trees will help provide indispensable information on the interactions between trees on a stressful Gotjawal terrain. In order to figure out the biotic and abiotic factors affecting spatial patterns, it is also necessary to understand the tending and planting history in this forest.

3.4.4 Conclusions

Individual trees of dioecious *T. nucifera* were randomly distributed in an old-growth forest with over several hundreds of years of history. However, when the spatial relationship was assessed for sex and size groups, spatial patterns of trees tended to differ among the three sites within the forest, and spatial segregation was notable only in one site of which density was the highest among the three sites and artificial planting occurred several decades ago. Considering extremely heterogeneous topography and thin soils of Gotjawal terrain, it may not be possible to expect SSS shown in many other dioecious species. We did not directly examine the topographic features and its ecological effects in this study. Incorporation of topological traits in *Torreya* Forest in spatial pattern analyses would be rewarding to identify biotic and biotic effects affecting plant spatial distribution.

Chapter 4

Low genetic diversity and spatial genetic sub-structure in old-growth *Torreya nucifera* population in Jeju Island, South Korea

4.1 Introduction

Genetic information about rare and endangered species is increasingly needed for long-term appropriate conservation strategies (Frankham 2005; Hendricks et al. 2017). The genetic background of species is constantly affected by factors such as ecological traits (e.g. life form, breeding system and seed dispersal), demographic history (genetic bottleneck and population size change), and evolutionary processes (mutation and natural selection) (Hamrick et al. 1992; Frankham 2005; Hu et al. 2014). However, recent human-induced habitat fragmentation or degradation has had a severely adverse effect on genetic and demographic consequences (Leonardi et al. 2012; Piotti et al. 2013; Addisalem et al. 2016). Thus, in conservation biology, assessing the genetic impact of anthropogenic activity on natural populations has become more important issue in recent years.

Genetic diversity is the fundamental element for biological diversity that plays a critical role for survival and adaptive ability under changing environmental conditions, especially in trees with limited mobility, long life span and slow reproduction maturity (Frankham 2005; Pauls et al. 2013; Turchetto et al. 2016). Populations with low genetic diversity suffer from inbreeding depression in the short term, and fitness reduction in response to environmental changes in the longer term, resulting in population growth inhibition and high risk of extinction (Hughes et al.

2008; Hendricks et al. 2017; Ralls et al. 2017). Genetic diversity also appears to be spatially structured at different scales, such as population, subpopulation or among neighbouring individuals (Escudero et al. 2003). The structuration of genetic diversity within and between populations is referred to as spatial genetic structure, mainly determined by gene flow via seed and pollen (Ellstrand 1992; Sebbenn et al. 2011). It is also consistently influenced by various natural selection pressures, intrinsic plant traits (e.g. mating system, Duminil et al. 2009; Litkowiec et al. 2015) and environmental heterogeneity (Chung et al. 2003; Wang et al. 2014). Therefore, not only the amount of genetic variation but also the distribution patterns of genetic variation present in gene pool are important to understand population dynamics (Hou and Lou 2011; Hu et al. 2014; Hendricks et al. 2017).

Torreya nucifera S. et Z. (Taxaceae) is a dioecious woody gymnosperm, which is narrowly distributed in South Korea and Japan. The species is listed as “Least Concern” under the IUCN Red List (2008), but Katsuki and Luscombe (2013) reported that the risk of extinction of *T. nucifera* was not adequately assessed due to lack of ecological information. In South Korea, *T. nucifera* occurs below 35° 10′ N and five stands (Jangseong, Goheung, Haenam, and Hwasun in Jeollanam-do of inland area; Pyungdae-ri of Jeju Island) have been designated as Natural Monuments. In inland area, *T. nucifera* populations (*Torreya* population hereafter) are mainly located nearby temples and are known as artificial populations cultivated by planting seeds or seedlings. However, *Torreya* population in Jeju Island is regarded as a wild population that has been formed several hundreds of years ago and grown through natural regeneration (Kim 1985; Lee 2005; Shin et al. 2010). Especially, Jeju Island's *Torreya* population is known as the largest (*n* = 2,861) and oldest (mostly 200-400 years old, max. ~900 years old) in the world. In 1053 (King Munjong in the Goryeo Dynasty), *T. nucifera* fruits were payed as a tribute as a special product of Jeju Island, so it were preserved by the national law due to usefulness of this tree (Lee 2009). For example, its wood is fragrant and has been used for furniture, and its seeds have been used for anthelmintic and oriental medicines and oil.

Studies have been carried out on *Torreya* population in Jeju Island, including seed size variation (Kang 1993), demography [Korea Tree Health Association (KTHA) 1999; Lee 2005, 2009], vegetation structure (Shin et al. 2010; Lee and Oh 2013; Lee et al. 2014), reproductive ecology (Kang and Shin 2012), and spatial distribution pattern (Shin et al. 2017). The isozyme analysis was conducted for five *Torreya* populations including the one place in Jeju Island (Lee et al. 1999), revealed very low genetic diversity and relatively high differentiation among populations in South Korea. They suggest that artificial planting, unbalanced sex ratio, genetic drift and inbreeding may have contributed to such genetic patterns. On the other hand, Hong et al. (2000) reported that degree of genetic differentiation among *Torreya* populations was relatively low based on the inter-simple sequence repeat (I-SSR) markers. Previous genetic studies on *T. nucifera* are limited in establishing genetic resource management strategies for *T. nucifera* due to using molecular markers with low information content and low reproducibility. Slatkin and Arter (1991) point that if information contents from genetic markers are limited, the genetic status of populations may be ambiguously revealed. In particular, considering the local adaptability of an old-growth *Torreya* population in Jeju Island that is reflected in its long history in one place, current genetic status is valuable information to cope with future environmental changes. Therefore, it is necessary to develop more useful genetic markers (e.g. microsatellites) for evaluate of the genetic diversity and spatial genetic structure of extant population.

The genetic status in plants is not only related to internal genetic background but also affected by population age structure, spatial pattern and reproduction mode (Hilfiker et al. 2004; Hu et al. 2014). *Torreya* population in Jeju Island was overall significantly male-biased sex ratio, and it could be separated into three sites depending on the gradient of DBH (diameter at breast height) and sex ratio (Kang and Shin 2012). In a recent study, Shin et al. (2017) also reported that spatial distribution patterns of *T. nucifera* trees differed among the three sites, and a possibility of artificial planting in some part of the forest. Dioecious mating system, heterogeneous volcanic topography known as Gotjawal terrain, and management activities are considerable determinants of ecological features in *Torreya* population that persisted for several hundreds of years. Various evolutionary, ecological and anthropogenic factors are expected to have significant effects on the structuring of genetic diversity in *Torreya* population. However, the genetic structure in an old-growth *Torreya* population of Jeju Island has not been studied so far. In this study, we used nine microsatellite loci to solve the following questions: (1) What level of genetic diversity in *Torreya* population currently? (2) Did *Torreya* population experience demographic events such as population size change or genetic bottleneck in the past? (3) Is there spatial genetic structure in population? (4) If the genetic sub-structure appears within *Torreya* population, what level of genetic differences?

4.2 Materials and Methods

4.2.1 Study site and sampling

Torreya Forest (Natural Monument No. 374 in South Korea) is located in Pyungdae-ri, Jeju Island (33°29′ N, 126°48′ E) (Fig. 4.1a). This forest (44.8 ha in area and 143 m a.s.l.) extends 1.4 km in north-south direction with a width of 0.6 km and it is located between two small volcanoes: Darangshioreum (382.4 m a.s.l.) and Dotoreum (a volcano near the southern end of the population; 284.2 m a.s.l.). *Torreya* Forest terrain is defined as Gujwa-Seongsan Gotjawal, with lava blocks scattered and little soil available for growing trees (Jeon et al. 2012). According to 2007-2016 data from Gujwa-eup close to the *Torreya* Forest, the mean monthly temperature ranges from 5.3°C in January to 26.7°C in August (mean annual temperature: 15.75°C). Its mean annual precipitation is 1774.2 mm with a peak in August (307.8 mm) with mean annual wind speed of 4.0 m/s (Korea Meteorological Administration 2017b). *Torreya* Forest belongs to evergreen broad-leaf forest zone with a total of 276 plants taxa, including *Mallotus japonicus*, *Machilus thunbergii*, *Orixa japonica*, and *Polystichum tripteron* (KTHA 1999; Lee 2009; Shin et al. 2010; Choi and Lee 2015). In 1999, all *T. nucifera* trees with DBH $\geq$ 6 cm were tagged and tending was started for 11 plots along trails within the forest (KTHA 1999). Kang and Shin (2012) classified the population into three sites (the south-north axis of *Torreya*

Forest), groups of plots along the trails, according to their ecological traits such as sex ratio and DBH (Fig. 4.1b).

Leaf samples were randomly collected from 155 individuals in *Torreya* population of Jeju Island. The sampled individuals in this study are tagged with contain ecological traits such as sex, DBH, and geographic coordinates by KTHA (1999).

4.2.2 DNA extraction and microsatellite genotyping

Genomic DNA was extracted from leaves using the DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA). Each DNA samples was quantified using ND-Spectrophotometer (Nanodrop Technologies, DE, USA) and stored at -20°C. We have tested the cross-species transferability of *T. nucifera* with 28 microsatellite markers published for *T. jackii* (Li et al. 2011) and *T. grandis* (Yi et al. 2016). As a result, nine polymorphic loci were selected, and applied to 155 samples of *T. nucifera* (Table 4.1). PCR amplifications were carried out in a total 16 μL reaction containing 10 x reaction buffer, 1.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μM primermix, 0.3 ml of 10 mM each primer 0.04 μM M13(-29) FAM/HEX dye-labelled primer, 2.5 U *Taq* polymerase (Fermentas, Vilnius, Lithuania), and 20 ng of genomic DNA. Cycling conditions were as follows: an initial denaturing at 95°C for 5 min, followed by 35 cycles of 1 min at 94°C, 45 s at 56-60°C depending on the primer pair, and 1 min at 72°C, with a final extension step at 72°C

10 min. PCR products were visualized in an ABI 3130 *xl* Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Fragment sizes were determined using Gene Mapper v5.0 software (Applied Biosystems, Foster City, CA, USA).

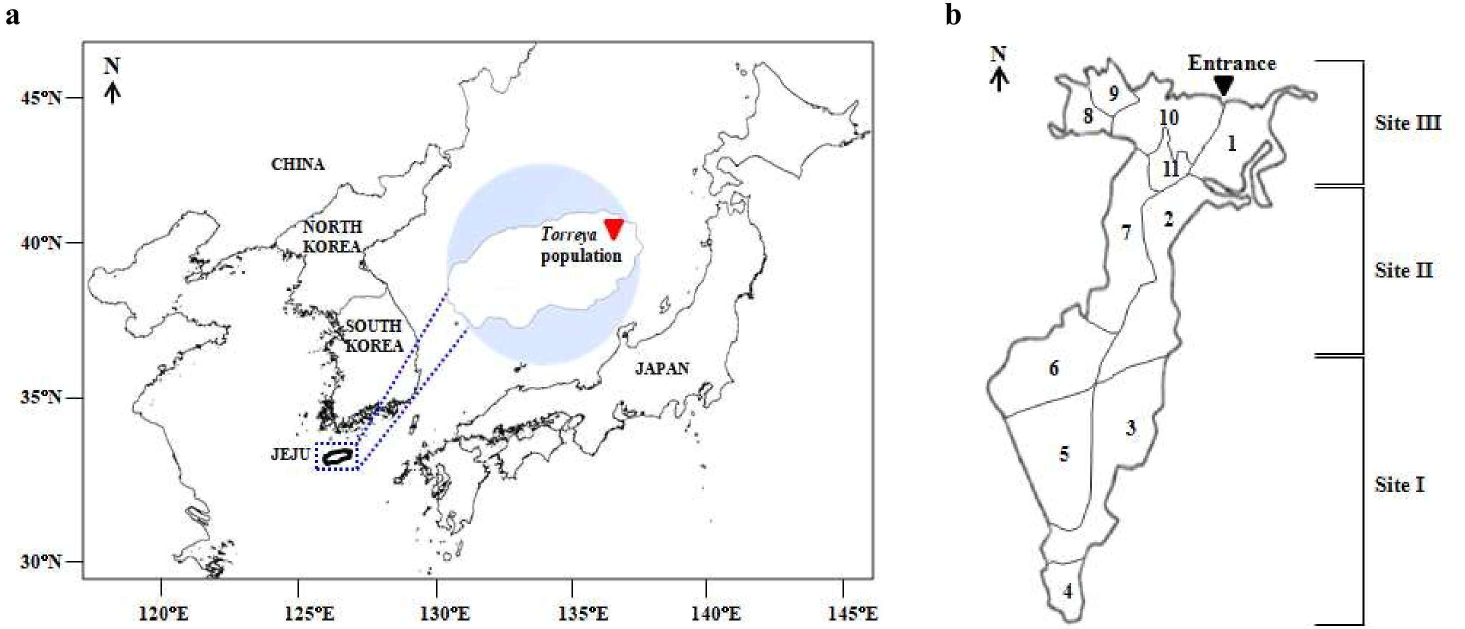


Fig. 4.1 **a** Location of *Torreya* population in Jeju Island, South Korea. **b** A map of *Torreya* population in Jeju Island. The 11 plots divided by trails (KTHA 1999) were categorized into three sites depending sex ratio and DBH gradient by Kang and Shin (2012): Site I in the southern part of the forest including plots 3-5, Site II in the middle part including plots 2, 6 and 7, and Site III in the northern part including plots 1, 8, 9, and 10.

Table 4.1 Characteristics of nine nuclear microsatellite markers used to evaluate genetic diversity of *Torreya nucifera* in Jeju Island, South Korea

Locus	Primer sequence (5 ′ – 3 ′)	Repeat motif	GenBank Accession No.	References
TG11	F: CTGGGTCATGCTGGTCTTTT R: GGGGTTCTGGTAGATGCAGA	$(GTT)_7$	KT894856	Yi et al. (2016)
TG12	F: ACCTGATGGTCTGGTGTGGT R: GTTGTTGGTAGGCCATTGCT	$(GCA)_5$ … $(GCA)_5$	KT894857	Yi et al. (2016)
TG19	F: GGACGTCTCAGCAATGTCAA R: GCAAAGAAAAGGATTGCCAC	$(CAT)_7$	KT894858	Yi et al. (2016)
TG32	F: GGCCGTGAGAGTAGCATAGC R: AGGTCCCTCACCATGAGCTT	$(GAA)_6GT(AGA)_6$	KT894859	Yi et al. (2016)
TG65	F: GCTTTCACTCGGGTTTGTCT R: AGCAGCAGCAGCAATAACAA	$(TTG)_8$	KT894863	Yi et al. (2016)
TG70	F: AGCCTCCGATGAATCCTCTT R: AACATCTGCTTTTCCATGCC	$(AAG)_7$	KT894864	Yi et al. (2016)
TG82	F: AACACCACACCACCTGATGA R: TACCGCTACAGCAACACCTG	$(CAG)_5(GAG)_5$	KT894867	Yi et al. (2016)
TJ55	F: CTTGCTCCCAGAATTTGACC R: TGAACTCTTATTCCCGATGC	$(CT)_7$	JF754932	Li et al. (2011)
TJ75	F: TAGGAATTTGCCATACTCTG R: CTGATTTACACTTGGTGACAC	$(GA)_7$	JF754934	Li et al. (2011)

4.2.3 Genetic diversity analysis

Genetic diversity in *Torreya* population was quantified using the following parameters: allele number averaged across loci (*N*a), the number of effective alleles averaged across loci (*N*e), Shannon's Information Index (*I*), observed heterozygosity (H_O), expected heterozygosity under Hardy-Weinberg equilibrium (H_E), and inbreeding coefficient (*F*) for each locus and for all loci together. These parameters were estimated using GenAlEx 6.41 program (Peakall and Smouse 2006).

4.2.4 Demographic history analysis

For estimate of effective population size (N_e) in *Torreya* population, LDNE program (Waples and Do 2008), which is a bias-corrected version of the linkage disequilibrium method was used. We also used BOTTLENECK v.1.2.02 software (Piry et al. 1999) to identify whether *Torreya* population has recently experienced a reduction in effective population size. "Recently" is defined as within approximately the past $2N_e$-$4N_e$ generations (Cornuet and Luikart 1996). Bottleneck test is based on difference between the expected heterozygosity (H_E) at Hardy-Weinberg equilibrium and the expected heterozygosity (H_{eq}) at mutation-drift equilibrium: the H_{eq}, calculated from the allele number, is reduced faster than the H_E which is calculated from the allele frequencies in populations experiencing a recent reduction of effective population size (Cornuet and Luikart 1996). That is, recent bottleneck population will exhibit a significant excess of heterozygosity ($H_E > H_{eq}$). To determine a significant of heterozyosity excess, three statistical tests, namely a 'sign test', a 'standardized differences test' and a 'Wilcoxon sign-rank test', were employed under different mutation models including infinite allele (IAM), step-wise mutation (SMM) and two-phased (TPM) models. Wilcoxon sign-rank test is generally the most useful of all the tests because it is the most powerful and robust when used with few polymorphic loci (Piry et al. 1999; Maudet et al. 2002). Qualitative graphical method, mode-shift analysis, suggested by Luikart and Cornuet (1998) was also used to visualize allele frequency distribution. As recommended by Piry et al. (1999), we ran 10,000 simulations with 95% single-step mutations, 5% multi-step mutations, and a variance of 12.

4.2.5 Spatial genetic structure analysis

Spatial genetic structure of *Torreya* population was investigated following several analytical approaches. First, we tested for spatial autocorrelation using Moran's Index (Moran 1948). Moran's I is a measure of spatial structure of genetic diversity at a single diallelic locus for sampled individuals within arbitrary distance classes because the level of spatial autocorrelation depends on the distance between sampling units. The expected values, $E(I)$, for the case of no autocorrelation, a spatially random distribution, are $-1/(n-1)$ (Sokal and Wartenberg 1983). Moran's I values higher than $E(I)$ indicate positive spatial autocorrelation, while smaller values indicate negative spatial autocorrelation (Degen et al. 2001). In this study, the sample individual coordinate data were divided into 10 consecutive distance classes of 60 m (0-600 m), and the 95% envelope for the correlogram is obtained after 1,000 permutations based on Monte-Carlo simulation to evaluate deviation from a spatially random distribution of each calculated measure. Spatial autocorrelation analysis was carried out using the program Spatial Genetic Software (SGS) ver. 1.0d (Degen 2000). Second, we estimated the spatial genetic structure of *Torreya* population using SPAGeDi 1.5 software (Hardy and Vekemans 2002). We divided the spatial distance between individuals into eight classes, and calculated the average pairwise kinship coefficient (F_{ij}; Loiselle et al. 1995). The F_{ij} at each distance class was tested by the 95% confidence interval derived from 10,000 permutations of the spatial positions of individuals. The intensity of spatial genetic structure in *Torreya* population was quantified using *Sp* statistics (Vekemans and Hardy 2004). *Sp* is computed as $Sp = -b_F/(1-F_1)$: where b_F is the regression slope of the kinship estimator F_{ij} (Loiselle et al. 1995) computed among all pairs of individuals against their geographical distances, and F_1 is the average kinship coefficient between individuals for the first distance class (0-50 m). The standard error (SE) of *Sp* value was calculated using the jacknife SE of b_F multiplied by $(1-F_1)$. Also, an indirect values of gene flow were jointly calculated for *Torreya* population relying on spatial genetic structure patterns estimates with assuming an equilibrium of isolation by distance. In such cases, the extent of gene flow can be expressed the neighbourhood size as $N_b \equiv 4\pi D_E \sigma_g^2$, where D_E is the effective population density of reproductive individuals, σ_g^2 is ½the mean-squared parent-offspring distance, and N_b can be estimated as the inverse of *Sp* yielding $N_b = (F_1 - 1)/b_F$. D_E value is product of the census density (*T. nucifera* 2,861 individuals in area 44.8 ha) and the N_e/N ratio (effective over census population size). In this study, we estimated N_b and σ_g using the effective population density of *Torreya* population(Hardy et al. 2006).

The following analysis were used to infer the genetic sub-structure within *Torreya* population. Third, we used the Genetical Bandwidth Mapping (GBM) as a visual tool for investigating spatial variation of allele frequencies. This is a nonparametric method, focused on a detailed visualization of the geographic areas associated with a considerable genetic change, called boundaries or barriers. GBM is based on the computation of Womble’s systemic function, and corresponds to the shortest distance to areas of significant variation of allele frequencies. The analysis was performed using the GenBMAP software (Cercueil et al. 2007). Fourth, BARRIER v2.2 software (Manni et al. 2004) was used in order to detect cryptic geographic barriers to gene flow within *Torreya* population. The barriers define zone of maximum genetic change either along a network connecting localities, and can be detected by using Monmonier's algorithm. Finally, we inferred the number of subtle genetic structures within a population using a Bayesian clustering method implemented in GENELAND 4.0.7 (Guillot et al. 2008) of the program R (R Development Core Team 2016). In GENELAND, spatially explicit information for all individuals is used along with genotypic data to deduce the optimal number of genetic clusters (*K*) and assign individuals to each subdivision. We ran 100,000 MCMC interactions with a thinning of 100 for K = 1-5. Each run assumed correlated alleles frequency model while allowed for null alleles within the data set. Post processing included calculation of posterior probabilities for each pixel of the 100 × 100 pixel domain after a burn in 200 stored iterations.

4.2.6 Comparative analyses among three genetic groups

To explore relationships among genetic groups, which are classified based on the structuration of genetic variation identified by GenBMAP, BARRIER, and GENELAND analysis in this study, we calculated pairwise Nei's (1978) genetic distances (*D*) and the population differentiation (F_{ST}). Furthermore, an analysis of molecular variance (AMOVA) was conducted to investigate the distribution of genetic variation within and among genetic groups. Theses analyzes were performed using GenAlEx 6.41 program (Peakall and Smouse 2006).

4.3 Results

4.3.1 Overall genetic diversity

All nine microsatellite loci generated a total 31 alleles across the 155 individuals from *Torreya* population in this study. The number of alleles per locus ranged from two for TG19, TG65, TG82 and TJ55 to seven for TJ75, with a mean of 3.4 ± 1.8 (Table 4.2). The observed heterozygosity (H_O) and the expected heterozygosity (H_E) were 0.528 and 0.513, respectively. The mean inbreeding coefficient (F_{IS}) was -0.031, and not significantly different from zero ($P = 0.702$), indicating absence of inbreeding.

4.3.2 Demographic history

The effective population size (N_e) was 87, with the bounds of CI varying between 82.8 and 110.3, and estimates of N_e/N being 0.56 (Table 4.2). That is, the 155 mature plants correspond to 87 unrelated and non-inbred individuals. Bottleneck test detected evidence of recent genetic bottleneck in *Torreya* population (Table 4.3). The excess heterozygosity tests showed significant excess under IAM, SMM and TPM models ($P < 0.05$).

Table 4.2 Genetic indices for *Torreya* population in Jeju Island, South Korea, based on nine polymorphic microsatellite loci and 155 individuals

Locus	N	N_A	N_E	I	H_O	H_E	F_{IS}
TG11	154	3	2.06	0.771	0.455	0.514	0.116
TG12	153	6	2.80	1.173	0.654	0.643	-0.017
TG19	155	2	1.72	0.608	0.413	0.417	0.011
TG32	155	3	2.05	0.849	0.445	0.512	0.131
TG65	154	2	1.65	0.583	0.383	0.394	0.027
TG70	154	3	2.67	1.040	1.000	0.625	-0.600
TG82	154	2	1.76	0.623	0.396	0.432	0.082
TJ55	153	2	1.33	0.412	0.288	0.246	-0.168
TJ75	155	7	5.99	1.865	0.716	0.833	0.140
	Mean	3.3	2.45	0.880	0.528	0.513	-0.031
	N_e			87 (82.8-110.3)			
	N_e/N			0.56			

N, number of samples; N_A, number of alleles averaged across loci; N_E, number of effective alleles averaged across loci; I, Shannon's information index; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; N_e, effective population size and their confidence interval in parenthesis and N_e/N, ratio of N_e to N.

Table 4.3 Bottleneck test with heterozygosity excess for *Torreya* population in Jeju Island, South Korea

Models	Sign test	Standardized differences test	Wilcoxon sign-rank test (one tail for H excess)
IAM	H_{ee} = 4.05 P = 0.00065	T2 = 4.140 P = 0.00002	P = 0.00098
SMM	H_{ee} = 4.68 P = 0.02398	T2 = 2.484 P = 0.00649	P = 0.00684
TPM	H_{ee} = 4.64 P = 0.02266	T2 = 2.702 P = 0.00345	P = 0.00293

H_{ee}, expected number of loci with heterozygosity excess; P, probability value; IAM, infinite allele model; SMM, step-wise mutation model; TPM, two-phased model.

4.3.3 Spatial genetic structure

In spatial autocorrelation analysis using Moran's Index, the expected values for the case of no autocorrelation are -0.0065 (Fig. 4.2). Correlogram using Moran's I showed significant positive spatial autocorrelation at distance classes of 0-180 m ($P < 0.05$). The significant positive Moran's I values decreased as distance class increased to intermediate distances, while significant negative values occurred at 360-420 m and 480-540 m.

The kinship coefficient (F_{ij}) showed a similar spatial genetic structure pattern to Moran's I. Significant genetic relatedness was detected up to approximately 150 m in *Torreya* population (Fig. 4.3). And, for lager distance classes, negative values were observed. The kinship coefficient over the shortest distance (F_1) was 0.0335 (Table 4.4). The regression slope (b_F) of the mean kinship coefficients on the logarithm of spatial distance was negative and significant ($b_F = -0.0126 \pm 0.0060$; $P < 0.001$). And, the statistic for the extent of the spatial genetic structure (Sp) was 0.013 ± 0.0058. Estimated overall gene dispersal parameters (σ_g) was 41.12 m in *Torreya* population (Table 4.4).

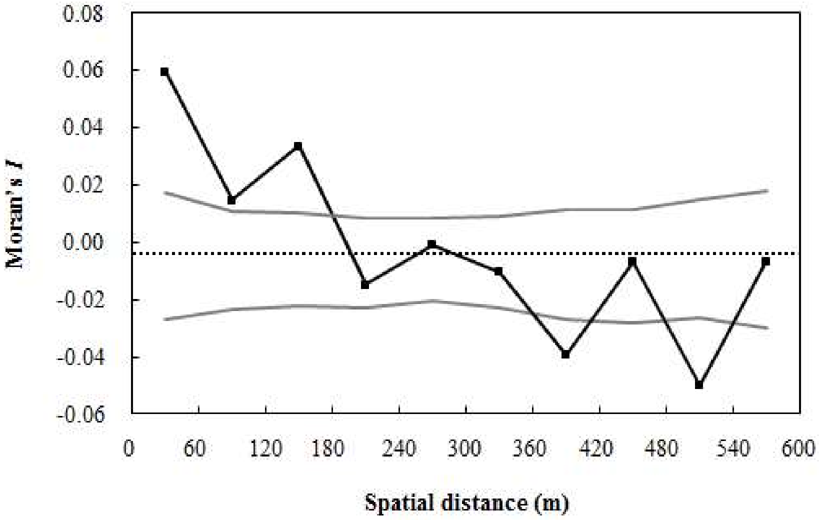


Fig. 4.2 Correlogram using Moran's *I* to depict genetic spatial autocorrelation in *Torreya* population. The black solid line represents the mean Moran's Index, and dashed line show the expected value for no correlation; E(*I*) = -0.0065. The gray solid lines (reference lines) show the 95% confidence intervals under the null hypothesis, which was calculated by 1,000 permutations of the multilocus genotypes.

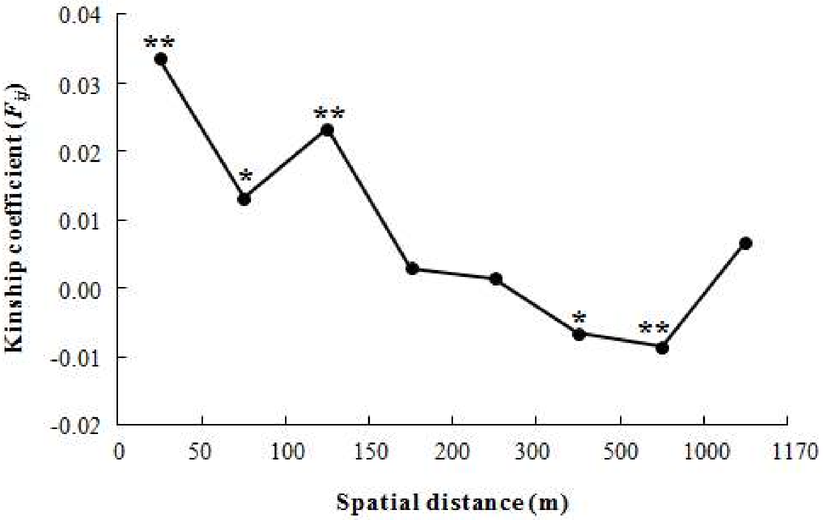


Fig. 4.3 Correlogram from spatial autocorrelation using the kinship coefficient (F_{ij}) over eight distance intervals in *Torreya* population. * $P < 0.05$; ** $P < 0.001$.

Table 4.4 Estimation of the fine-scale spatial genetic structure in *Torreya* population in Jeju Island, South Korea

Spatial genetic structure parameters			Gene dispersal parameters		
F_1	b_F	S_p	N_b	D_E	σ_g (D_E)
0.0335^*	-0.0126^*	0.0130	76.71	35.76	41.12

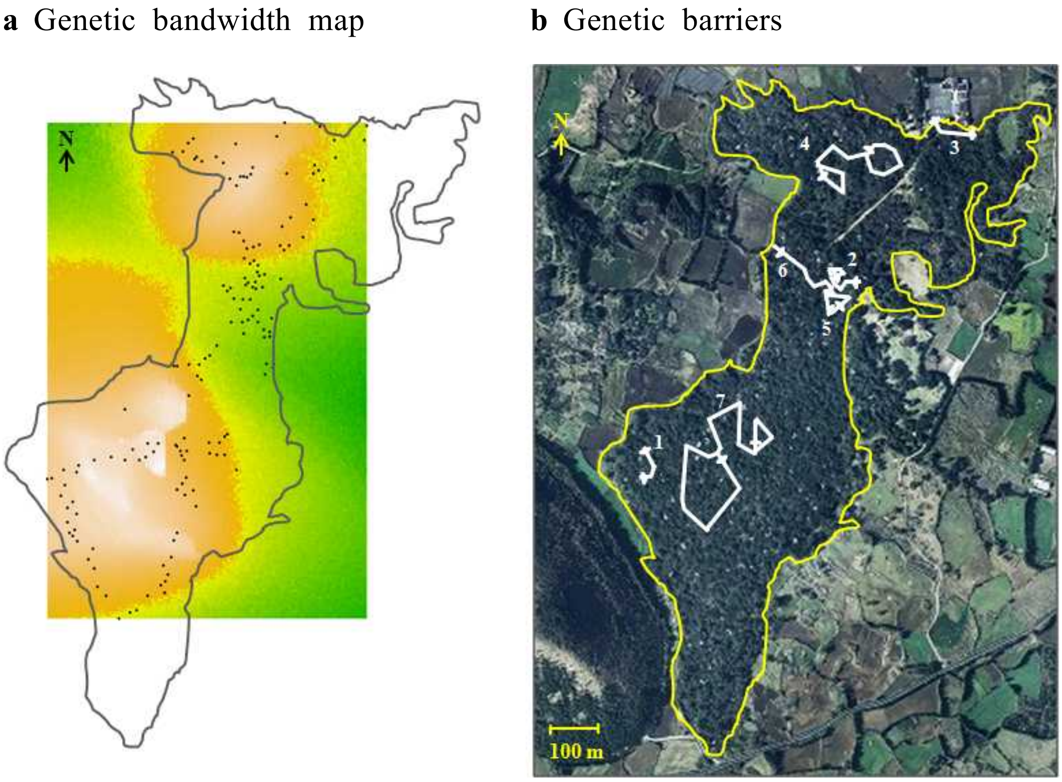
F_1, average kinship coefficient between individuals of the first class; b_F, regression slope of the kinship estimator F_{ij} computed among all pairs of individuals against geographical distances; S_p, quantification of the spatial genetic structure; N_b, neighbourhood size; D_E, effective population density determined as trees/ha and σ_g, gene dispersal distance (in meters) using estimates of effective densities (D_E). $^*P < 0.001$.

4.3.4 Within-population genetic structure

The GBM revealed that *Torreya* population had a distinctive genetic structure (Fig. 4.4a). Both the northern and southern areas of *Torreya* population were genetically heterogeneous with a sharp changes in allele frequency. Especially, the southern area showed sharp variation of allele frequencies or large heterogeneity. The intermediate area, on the other hand, was more homogeneous among individuals.

The analysis using BARRIER revealed seven barriers in *Torreya* population (Fig. 4.4b). Barriers 3 and 4 were formed in the northern area. Barriers 2, 5 and 6 were formed in either loop or linear in the intermediate area. And, Barriers 1 and 7 were formed in either short or loop around individuals in the southern area of *Torreya* population.

In GENELAND genetic clustering analysis, K values ranging from one to five were tested, and the optimal number of clusters was determined to be four (K = 4) (Fig. 4.5). Also, four genetic clusters have shown a specific pattern from the northern to the southern of *Torreya* population. The northern and intermediate areas included to one and/or two clusters. In contrast, in the southern area, all four genetic clusters were observed in *Torreya* population.



a Genetic bandwidth map **b** Genetic barriers

Fig. 4.4 Genetic structure within *Torreya* population in Jeju Island, South Korea. **a** Genetic bandwidth map: Green areas indicate regions of genetic homogeneity. Orange areas indicate intermediate homogeneity, and white areas indicate genetic heterogeneity. The solid gray line indicates the borders of *Torreya* Forest. **b** Figure represents the location of seven genetic barriers. Different numbers (1-7) are given to each barrier and marked in white color arrows.

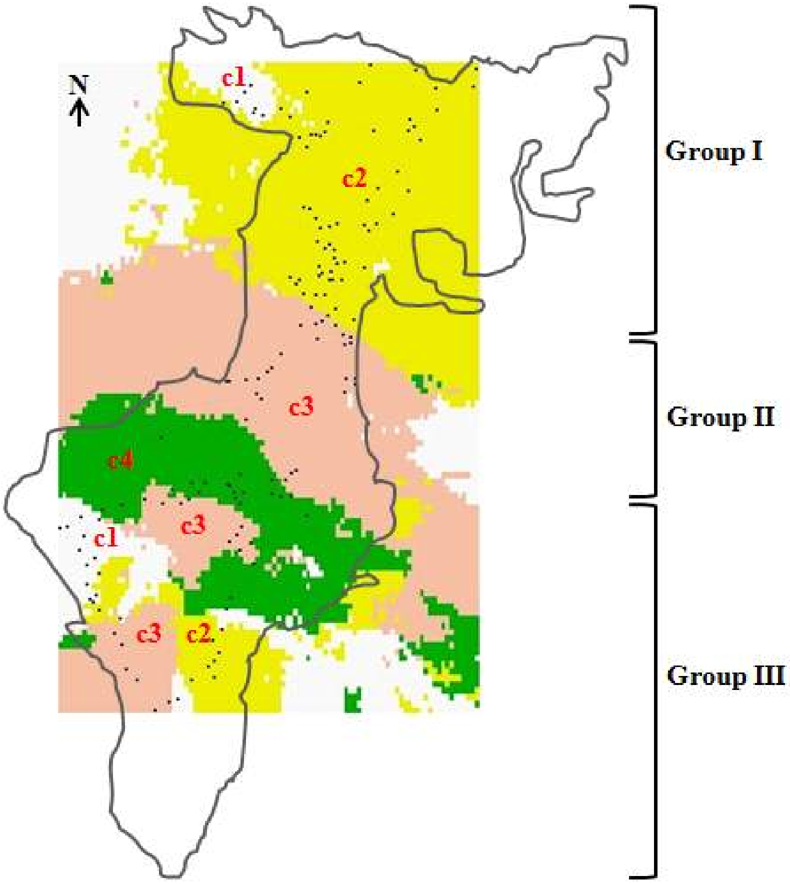


Fig. 4.5 A map of estimated population membership showing the range of the four inferred genetic clusters (c1-c4) within *Torreya* population. This population categorized into three genetic groups (Group I, Group II and Group III) depending composition of genetic clusters: Group I in the northern part of the forest including c1 and c2, Group II in the middle part including c3, and Group III in the southern part including c1-c4. The solid gray line indicates the borders of *Torreya* Forest.

4.3.5 Genetic differences among three genetic groups

Analyzes of spatial genetic structure performed in this study revealed three genetically distinct groups (Group I, Group II, and Group III) in *Torreya* population (Fig. 4.5). An analysis of genetic diversity for three genetic groups showed the genetic differences among three genetic groups. The expected heterozygosity of Group I (H_E = 0.487) was lower than Group II (0.525) and Group III (0.511). And, F values were negative only in Group I and Group II, while positive in Group III (0.014) (Table 4.4). But, F_{IS} value not significantly different from zero for three genetic groups, indicating absence of inbreeding.

The pairwise comparison (three genetic group and 3 pair) showed Group II and Group III are genetically closer than Group I (Table 4.5). The maximum genetic distance of Nei (1978) was 0.035 between Group I and Group III, and the minimum was 0.014 between Group II and Group III (Table 4.6). Also, the minimum F_{ST} value was 0.005 between Group II and Group III. The analysis of molecular variance (AMOVA) demonstrated 4% of total genetic variation was attributed to differences among groups, and 96% within three genetic group (Table 4.7).

Table 4.5 Genetic diversity estimates based on nine polymorphic microsatellite loci for thee genetic groups within *Torreya* population in Jeju Island, South Korea

Genetic group	N	I	H_o	H_E	F
Group I	66	0.840	0.504	0.487	-0.046
Group II	28	0.877	0.624	0.525	-0.184
Group III	61	0.872	0.509	0.511	0.014

N, the number of samples; I, Shannon's information index; H_o, observed heterozygosity; H_E, expected heterozygosity; F, inbreeding coefficient.

Table 4.6 Pairwise genetic comparison between genetic groups within *Torreya* population in Jeju Island, South Korea

Pair of genetic group	D	F_{ST}
Group I - Group II	0.034	0.018
Group I - Group III	0.035	0.018
Group II - Group III	0.014	0.005

D, Nei's genetic distance; F_{ST}, genetic differentiation.

Table 4.7 Analysis of molecular variance (AMOVA) among three genetic groups (Group I, Group II, and Group III) within *Torreya* population in Jeju Island, South Korea

Source of variance	d.f.	SS	MS	Variance component	Percentage total (%)
Among genetic groups	2	25.937	12.968	0.172	4
Within genetic groups	152	689.844	4.538	4.538	96

d.f., Degree of freedom; SS, sum of squares; MS, expected mean squares.

4.4 Discussion

4.4.1 Low genetic diversity of *Torreya* population

High genetic diversity in plants is believed to be typical for plants with long-lived perennial species, wide geographical ranges, wind pollination, and outcrossing (Hamrick and Godt 1996; Wright et al. 2008; Walisch et al. 2014). *T. nucifera* is a dioecious, long-lived, woody gymnosperm with an outcrossing breeding system involving extensive pollen dispersal by wind and restricted primary seed dispersal by gravity. The genetic variation in *Torreya* population (H_E = 0.513), however, was relatively low compared with other species of similar ecological characters. That is, the H_E value estimated here was lower than the mean values presented by Nybom (2004) for long-lived perennial (H_E = 0.68), narrow geographic range (H_E = 0.56), outcrossing (H_E = 0.65), and late successional status species (H_E = 0.70). Genetic variation of *Torreya* population was slightly lower than *T. jackii* (H_E = 0.555; Li et al. 2011) listed as Endangered in the IUCN (2008) criteria, which is congeneric species with *T. nucifera*. It was also very low compared with values reported for other conifers such as *Abies koreana* (H_E = 0.759; Kwak et al. 2017) and *Taxus cuspidata* (H_E = 0.714; Park et al. 2011), which are designated as the important endemic species of forest genetic resources in South Korea.

Generally, the biased sex ratio in dioecious plant populations may occur as a result of distinct growth rates and mortality of males and females related to different resource allocation patterns between sexes (Obeso 2002; Barrett et al. 2010). However, unbalanced sex ratio in populations experiencing habitat fragmentation and population size reduction may negatively affect genetic diversity for at least two reasons such as an effective population size reduction and a decrease in reproductive output (Vandepitte et al. 2010; Rosch et al. 2018). In English yew (*Taxus baccata* L.), low RAPD variation appeared only in small and female-biased populations (Hilfiker et al. 2004). Some studies have found the proportion of females positively linearly related to genetic diversity (Vandepitte et al. 2009; Rosch et al. 2018). In contrast, Litkowiec et al. (2015) reported there are no significant correlation between sex ratio and the extent of genetic variation in another *T. baccata* populations. In the case of *Torreya* population, although significantly male-biased sex ratio (0.52; Sex ratio is calculated as males/males+females), unbalanced sex ratio seems not to be a direct cause of low genetic diversity, because of sufficient number of individuals of the opposite sex (n = 1,363) that can produce reproductive outputs. Yet, it is necessary to understand the interaction between genetic diversity and ecological characteristics related to sex ratio that because the sex ratio in *Torreya* population was varied at temporal and spatial scale according to Kang and Shin (2012).

Historical factors such as the Quaternary glacial cycles, past demographic changes, and more recent human-induced habitat disturbance have contributed to contemporary elements of genetic diversity within and between populations of species (Oostermeijer et al. 2003; Kwak et al. 2017). Jeju Island was extensively exposed at the low sea-level stand during the Last Glacial Maximum and late-glacial period (Chung 2007), and conifers were prevail as relatively large continuous populations due to cold and dry conditions (Kong 1998; Anderson et al. 2017; Kwak et al. 2017). In the past, *T. nucifera* trees were probably distributed throughout Jeju Island. And, the distribution of *T. nucifera* trees may have experienced expansions and contractions caused by global cyclical cooling-warming events. Currently, *T. nucifera* is considered to be a restricted and isolated population because no other population is reported on Jeju Island except for this survey site. Isolated populations tend to exhibit loss of genetic variation because natural selection or demographic phenomena is more prominent (Hou and Lou 2011; Furches et al. 2013; Turchetto et al. 2016). Also, considering that the only extant *Torreya* population in Jeju island is surrounded by agricultural land, so it is believed that the distribution range of *T. nucifera* trees has been reduced by human development pressure, resulting in remains at the present minimum population size. Shin et al. (2017) suggested the possibility for strong artificial interference in this population based on aerial photographs (1967 to 2015) of *Torreya* Forest. This is because the drastic increase of vegetation coverage in some area was observed despite the slow growth of *T. nucifera* species which on average, DBH increases about 2 cm during 10 years (KTHA 1999). In the present study, *Torreya* population showed a significant bottleneck signature ($P < 0.05$), and a relatively small effective population size ($N_e/N = 0.56$). The significant population bottlenecks may be explained by a recent decline in population size due to habitat destruction. *Torreya* population appear to have undergone recent strong bottleneck with increasing human activity during the past century, including deforestation and agriculture. Genetic drift is strongly influenced genetic variation in population, which accelerated of loss of alleles when the population is small and isolated (Frankham 1996; Solórzano et al. 2016). Our results show that, *T. nucifera* had a broader distribution in the past, as the climate warmed and the anthropogenic disturbance increased, *Torreya* population is further smaller and isolated, with reduction of geographic ranges and effective population size, and eventually leading erosion and depletion of genetic diversity. Consequently, the rate of loss of genetic variation of *T. nucifera* may be more rapid by human impact.

4.4.2 Fine-scale spatial genetic structure

Pollen and seed dispersal (gene flow) are the principal determinants of spatial genetic structure in tree species (Dick et al. 2008), and can be studied using indirect methods using spatial autocorrelation analysis (Vekemans and Hardy 2004; Hardy et al. 2006). In this study, we performed spatial autocorrelation analysis using Moran's I and S_p statistics to assessment of fine-scale spatial genetic structure of *Torreya* population. Moran's I detected significant genetic relatedness of *T. nucifera* individuals within 180 m (Fig. 4.2). Also, significant positive autocorrelation using kinship coefficient (F_{ij}) was detected up to approximately 150 m (Fig. 4.3). In the case of wind-pollinated woody species, spatial genetic structure is generally weak and extends over relatively short distances within approximately 30-40 m (Jump and Peñuelas 2007). For example, autocorrelation was observed within 37 m for *Pinus koraiensis* (Hong et al. 2001), 20 m and 40 m for *Abies koreana* (Kang 2002), and 24 m for *Populus davidiana* (Lee et al. 2016). And, the intensity of spatial genetic structure in *Torreya* population (S_p = 0.0130) was relatively strong to than the mean values reported by Vekemans and Hardy (2004) for outcrossing species (0.0126), woody species (0.0102) and wind-dispersed species (0.0064). Additionally, this statistic was higher than the values observed for *Quercus petraea* (0.0083; Streiff et al. 1998), *Pinus storobus* (0.0108; Epperson and Chung 2001), and *Boswellia papyrifera* (0.002-0.014; Addisalem et al.

2016) which are these species that share similar life history traits with *T. nucifera*.

Our results consistent shows that the spatial genetic structure of *T. nucifera* trees was obviously strong despite extensive long-distance pollen dispersal. In plant populations, the effects of gene flow on spatial genetic structure are largely driven by pollen movement (Sokal et al. 1989), but can also be influenced by the complex of various ecological and environmental elements. For example, the genetic patch in wind-pollinated dioecious *Populus koreana* (Shin et al. 2014) was formed to a relatively large size within 400m as a result of influenced by regeneration, seed dispersal and water-stress on riparian environment. The strong spatial genetic structure of *T. nucifera* trees is considered to reflect the interplays of various factors such as the ecological characteristics of individuals (e.g. dioecy and old-growth) as well as the environmental heterogeneity of Gotjawal terrain and historical events (e.g. bottleneck and artificial interference).

4.4.3 Genetic sub-structure within *Torreya* population

This is the first study to reveal the genetic structure within *Torreya* population that have survived for several hundreds of years in almost natural conditions in South Korea. Adequate knowledge of spatial genetic structure is important for the conservation and management of natural populations that can inform decisions on the targeting of scarce conservation resources at particular populations and the sampling area necessary to represent the genetic diversity within them (Escudero et al. 2003; Jump and Peñuelas 2007). We found that the genetic sub-structure according to the south-north direction in the forest. These results were also somewhat consistent with the classification trends of three sites (Fig. 4.1b) according to the ecological traits revealed by Kang and Shin (2012).

Based on GBM and the location of genetic barriers, we found genetic sub-structure with the north-south direction in *Torreya* population (Fig. 4.4). The intermediate part was genetically homogeneity that allele frequencies very slow spatial variation. Genetic similarity is generally higher among neighboring than among more distant individuals as a result of limited pollen and seed transport (Vekeman and Hardy 2004). *T. nucifera*'s seed (mean weight = 703 ± 27 mg) is relatively heavy, so it usually dispersed close to the mother tree (Kang 1993). In present study, the mean parent-offspring distance σ_g (D_E) of *T. nucifera* was 41.12 m (Table 4.4). Considering the mean height of *T. nucifera* trees is as high as 10.4 m (Kang and Shin 2012), so this gene dispersal distance is relatively close between individuals, further indicating that the seedlings tend to grow around their mother tree. Maybe this area where genetic similarities between near individuals have appeared, seems to be formed by a natural regeneration process as a result of seed dispersal mechanism by gravity (adjacent to parents and offspring) with little human disturbance. In contrast, both the southern and northern parts in *Torreya* Forest were very heterogeneous among near trees. The characteristics of the individuals in these two locations are quite contrasting in the mean DBH, sex ratios, density and spatial distribution patterns (see Kang and Shin 2012; Shin et al. 2017). For example, the southern area of this forest, Site I, is characterized by relatively large proportion of old-aged trees (mean DBH = 59.7 ± 20.4 cm), male-biased sex ratio (0.60), low density (57.5 trees/ha), random distribution of *T. nucifera* trees. These ecological traits show that the southern area is relatively the old-growth area in *Torreya* Forest. So, it is considered that the genetic heterogeneity appears to exist among the surviving old trees due to stochastic mortality in the area. On the other hands, the northern area, Site III, is characterized by relatively small trees (43.0 ± 21.5 cm), female-biased (0.46), high density (65.0 trees/ha), clumped, random or regular distribution. Ecological traits such as high proportion of female trees and high density show the possibility of selective planting of female trees to obtain fruits over long years in this area which is characterized by lower elevation and surrounded by agricultural land easily accessible. Also, Shin et al. (2017) suggested the possibility for strong artificial interference in Site III, due to the drastic increase of vegetation coverage in the area despite the slow growth of *T. nucifera* trees based on aerial photographs (1967 to 2015). So, *T. nucifera* trees in the northern part are not entirely natural expansions, but rather some anthropogenic activities involved, resulted in a genetically heterogeneous structure at small-scales.

The GENELAND analysis based on a Bayesian model revealed that *Torreya* population was composed to four clusters (K = 4), and genetic clusters displayed an interesting pattern (Fig. 4.5). The distribution of four clusters also showed a direction toward north and south, especially the southern area included all four clusters of *Torreya* population. This area is presumed to be the oldest site of the present *Torreya* population by previous studies (Kang and Shin 2012; Shin et al. 2017), and displayed the highest genetic heterogeneity even in GBM. Not only the previous ecological studies but also the genetic results revealed in the present study consistently explains the structure that distinguishes the north-south direction within *Torreya* population.

4.4.4 Genetic differences among three genetic groups

We divided *Torreya* population into three genetic groups (Group I, Group II, and Group III) as depict Fig. 4.5, according to the composition of inferred genetic clusters by GENELAND analysis and the substructure of the south-north direction revealed by GBM and BARRIER analyses. The genetic indices were slightly different among three genetic groups, and 4% of total genetic variation was attributed to differences among groups (P = 0.010). Especially, Group I displayed low genetic diversity (below the overall mean H_E for *Torreya* population), and contained fewer genetic clusters (Table 4.5; Fig. 4.5). Gene flow was relatively high between Groups II and III, but Group I showed a low level of gene flow not only with Group III but also with geographically adjacent Group II. These results showed that Groups II and III are comparatively similar, while Group I is genetically distinct. According to analyses of ecological traits for *T. nucifera* trees in the three genetic groups, Group I, the northern part of *Torreya* population, display relatively small trees (mean DBH = 51.7 ± 20.6 cm) and female-biased sex ratio (0.39), while Group III, the southern part of this population, characterize by relatively large proportion of old-aged trees (mean DBH = 70.0 ± 21.8 cm) and male-biased sex ratio (0.61). And, the middle part of this population, Group II, show intermediate values in sex ratio (0.46) and mean size (57.1 ± 16.0 cm) between Groups I and III. We have identified three groups based on distribution of genetic variation in this study, and the ecological traits of genetic groups has been consistent with the distinction of three sites classified by Kang and Shin (2012) (Group I-Site III, Group II-Site II, and Group III-Site I). That is, Group I, characterized by low genetic diversity, simple genetic composition and relatively limited gene flow, includes a range of Site III reported to have been subjected to strong artificial interference.

Although *Torreya* Forest has been protected for a long time, no official records of forest practices such as artificial planting and logging of *T. nucifera* trees. Maybe, considering the long history of this forest and economic values of this species, it is difficult to believe that the forest is entirely natural. Forest management such as artificial regeneration with reproductive material of unknown origin can reduce the genetic diversity and change genetic structures in unpredictable ways within populations (Finkeldey and Ziehe 2004; İçgen et al. 2006). For example, the managed populations of *Metasequoia glyptostroboides* (Li et al. 2005) showed the loss of genetic variation and biased genetic composition compared to wild populations. In the case of Group I, the level and distribution of genetic variation are considered to be the results of a strong influence of anthropogenic activities. Also, although there are no physical barriers that restrict the dispersal of pollen and seeds in *Torreya* population, maybe it is suspected that disturbances caused by humans such as fire, selective logging, and planting may have acted as barriers to gene flow, resulting in caused to genetic differences among genetic groups within-population. Our results indicated that anthropogenic influence and management can alter both processes influencing the amount and distribution of genetic variation in *Torreya* population.

4.4.5 Conservation implications

Central to the effective conservation programme must be a clear understanding of the extant genetic diversity and spatial genetic structure in the species or populations (Hamrick and Godt 1997; Hendricks et al. 2017). The present study, for the first time, displayed low genetic diversity, strong spatial genetic structure, and significantly genetic sub-structure with three genetically distinct groups and in *Torreya* population. Based on the results of this study, we propose the following measures to conserve *T. nucifera*. First, since *Torreya* population currently possesses low genetic diversity and experienced genetic bottleneck, we emphasize the urgency of conservation activities to maintain or improve the level of genetic variations. Management related to planting to increase the number of individuals should be discontinued in the absence of a genetic information. Considering the difficulties of natural regeneration of *T. nucifera* due to rocky Gotjawal terrain of thin soils and competition with broad-leaved trees, seed collection from the old-aged trees that adapted and survived to environmental changes during the long-term period, is essential for future generations. It is also necessary to secure the genetic resources through the search of additional individual trees and/or other populations in Jeju Island except for this study area. Second, the spatial genetic sub-structure within *Torreya* population should be considered for future conservation and management practices. *Torreya* population should be managed separately according to the three genetic groups. Perhaps Groups II and III could be managed into integrates for the effectiveness of management, due to similar the expected heterozygosity and genetic distance between groups. However, Groups I should be managed differently from the other two groups. Third, special conservation consideration should be given to Group III.

Group III has genetically large heterogeneity and all four genetic clusters observed in this population. In addition, the southern part located this group is considered to be the oldest site of the present *Torreya* population, which includes the oldest *T. nucifera* tree and old-aged trees of relatively large proportion. Therefore, we propose Group III need to be conserved with top priority as the major source. Finally, other strategies such as periodic individuals translocation and *ex situ* conservation are available, because it is difficult to expand the population and to settle seedling due to *Torreya* Forest is surrounded by agricultural land, and located on Gotjawal topography. For *ex situ* conservation of *T. nucifera* the sampling strategy is efficient at least above 150 m between individual trees to reduce genetic relatedness. However, a more cautious approach is needed. In other words, it is needed well-integrated knowledge derived from genetic, demographic, and ecological data of this population before these strategies become viable alternatives.

Chapter 5

Final conclusion

5.1 *Ilex cornuta* 호랑가시나무

This is the first study to reveal the sex-related populations structure of dioecious *I. cornuta* in Jeju Island (Chapter 2). YS population was significantly males-biased sex ratio that males were about twice than females. This finding consistent with the observation that male bias predominant in natural populations due to males' earlier flowering, and the higher mortality of females (Barrett et al. 2010). Many *I. cornuta* trees in YS population also were experiencing disease problems, and the vigor was significantly associated with sex. *I. cornuta* males more belong to healthy group than female trees, reflecting greater susceptibility to infection due to high reproductive expenditure in female. GP population showed 1:1 sex ratio, and genets formed about two times more ramets on average compared with YS population in clonal growth. Gotjawal terrain, where located GP population, represents poor soil development and oligotrophic and stressful environment to plants. It seems to environmental factors related to topography promoted the clonal propagation of *I. cornuta* trees in GP population. Also, spatial segregation of the sexes (SSS) not found in two population because of the gradient of resources related to SSS seems not formed due to Gotjawal topography and human disturbance. These results emphasize that conservation of the two populations of *I. cornuta* should be approached from different perspectives based on the ecological characters of each population. For example, YS population is located beside agriculture land, so *I. cornuta* trees suffer from anthropogenic activities. GP population, in contrast, is located within Jeju Gotjawal Provincial Park designated as a protected area, so there is little risk of disturbance by human. However, here *I. cornuta* trees are limited to open canopies, and the number of individuals is very small. For conservation of YS population requires a protective device from habitat disturbance by anthropogenic activities. For GP population, the conservation programs such as natural regeneration are needed to resolve small population size problem.

We explored the *I. cornuta* populations in the western Jeju Island based on the previous distribution data, and found only YS and GP population in which enough individuals are inhabited. In addition, we confirmed sporadic distribution of *I. cornuta* trees in Daejeong-ri, Hyeopjae-ri and Wollyeong-ri of Jeju Island. In interviews with local residents, they used to say that *I. cornuta* trees were distributed all over the place in the past, but recently it is hard to find due to human land use (e.g. farmland, roads and tourism) and damage by agricultural chemicals. Therefore, in order to conservation of *I. cornuta*, it is first necessary to search and investigate the populations distributed in Jeju Island.

Ilex is the largest genus of woody dioecious plants (Galle 1997; Tsang 2005) and has the combination of woodiness, dioecy, entomophily and endozoochory, which is a unique in most temperate floras (Tsang

2005). So, the genus has received a disproportionate amount of scientific attention. Especially, northern European species, *I. aquifolium* has been studied as a model plant of dioecy (Obeso et al. 1998). In South Korea, eight taxa of *Ilex* are distributed including *I. cornuta*, *I. crenata*, *I. integra*, *I. macropoda*, *I. macropoda* for. *pseudomacropoda*, *I. rotunda* and *Ilex* × *wandoensis* (Yim 1979; Miler and Kim 2002). That is, genus *Ilex* is a very important taxon for understanding the ecology and evolution of Korean dioecious plants.

5.2 *Torreya nucifera* 비자나무

Torreya Forest in Jeju Island, the largest (N = 2,861) and oldest (mostly 200-400 years old, max. ~900 years old) population of *T. nucifera* in the world. This is the first studies to reveal the spatial distribution patterns and population genetic structure of old trees that have survived for several hundreds of years in almost natural conditions in Korea (Chapter 3 and Chapter 4). These results were consistent with previous study that *Torreya* population can be divided into three sites (Site I, Site II and Site III), according to ecological traits (Kang and Shin 2012). Individual trees of dioecious *T. nucifera* were randomly distributed in an old-growth forest with over several hundreds of years of history. However, when the spatial relationship was assessed for sex and size groups, spatial patterns of trees tended to differ among the three sites within the forest, and spatial segregation was notable only in one site of which density was the highest among the three sites and artificial planting occurred several decades ago (Chapter 3). Considering the extremely heterogeneous topography and thin soils of Gotjawal terrain, it may not be possible to expect SSS shown in many other dioecious species. We did not directly examine the topographic features and its ecological effects in this study. Incorporation of topological traits in *Torreya* Forest in spatial pattern analyses would be rewarding to identify biotic and abiotic effects affecting plant spatial distribution.

As discussed in Chapter 4, *Torreya* population was characterized by low genetic diversity and heterogeneous genetic sub-structure. Understanding the pattern and mechanisms responsible for patchy genetic structures would be valuable for the adaptive management and conservation of one-thousand years old population of *T. nucifera* especially vulnerable to climate change and anthropogenic activities. Especially, *T. nucifera* trees in Group III, the southern part of *Torreya* Forest, exhibited higher genetic diversity, large genetic heterogeneity and the composition of most various genetic clusters. We propose that *T. nucifera* trees on Group III need to be conserved with top priority as the major source, and those individuals with the most common alleles also should be concerned. The gene source population has all of the available genetic variation that could contribute to the species in future generations (İçgen et al. 2006). Considering the long history of *Torreya* population in Jeju Island, this population is presumed to be most similar to the origin and/or gene source of the *T. nucifera* trees in South Korea. If so, the low levels of heterozygosity of *Torreya* population in Jeju Island emphasizes the urgency of conservation activities to maintain or improve the level of genetic variations. It is necessary to secure the genetic resources through the search of additional individual trees and/or populations in Jeju Island except for this study area.

The knowledge derived from genetic, demographic, and ecological approaches need to be well-integrated for species conservation (Escudero et al. 2003; Ralls et al. 2017). Conservation strategies should be developed by integrating the studies conducted on the *Torreya* population, and research should continue to respond appropriately to changes in the future environment. In the quantitative survey in 1999, all trees ≥ 6 cm DBH were tagged and their ecological traits were measured. However, no further investigations have been conducted so far for about 20 years. In order to conserve and understand population dynamics, quantitative survey of growing trees should be constantly updated. For example, by analyzing and collecting data from dead trees over the last 30 years, it may be possible to understand the patterns of death and control external factors that have affected the death.

5.3 Suggestions for conservation of endangered dioecious plants in South Korea

In South Korea forest, dioecious woody species is known to be about 24% (Kim and Kim 2011) including *Ilex*, *Juniper*, *Rhus*, *Salix*, and *Torreya*, which is relatively higher compared to about 8% (Coder 2008) in the world. In South Korea, however, ecological researches considering dioecious sexual system have been very limited.

Species grow and multiply by adapting to different kinds of environmental changes that their respective habitats undergo, including diurnal and seasonal changes and those caused by universal physical disturbances (Joshi et al. 2001). Especially, the ecological differences between males and females in dioecious plants appear in all morphology, physiology, and life cycle, and are directly related to adaptability to environmental change (Vega-Frutis et al. 2013). Therefore, the population studies considering the sexual system is essential for the conservation of dioecious plants. In this study, I have provide conservation strategies for the dioecious *I. counuta* and *T. nucifera* based on informations such as sex-related ecological traits, spatial structure and genetic assesment. These results will help to determine the future direction of the research for the conservation of the endangered dioecious plants of Korea.

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Abstract in Korean

한국 자웅이주 식물 두 종의 보전생태와 유전

암·수의 기능이 서로 다른 개체로 분리된 자웅이주식물에서 성 특이적인 생장과 번식, 환경에 대한 적응성 차이는 개체군 고유의 시공간적 구조에 영향을 미친다. 게다가 개체군 크기가 동일하다면, 자웅이주식물은 양성화식물에 비해 절반의 개체만이 종자를 생산한다. 그러므로 위기에 처한 자웅이주식물의 보전을 위해 성체제를 고려한 생태특성, 개체군통계, 유전학적 측면 등 다방면의 통합된 연구가 필요하다. 본 연구는 국내 자웅이주 식물 두 종을 대상으로 성체제에 따른 생태특성 - 성비와 식물의 크기, 분포 양상 등 - 과 유전적 양상 - 유전다양성과 공간적 유전구조 - 의 분석을 통해 이들의 장기적 보전방안을 제시하고자 수행하였다.

호랑가시나무(*Ilex cornuta*)는 감탕나무과의 자웅이주식물로 동아시아 특산이다. 국내 육지 개체군 대다수는 크기가 급감하거나 소멸되었지만 제주도에 잔존하는 호랑가시나무의 생태연구는 수행된 바 없다. 본 연구에서는 제주도 호랑가시나무 두 개체군을 대상으로 성비, 생태특성, 분포유형을 분석하였다. 용수리 개체군(Yongsu-ri population, YS 개체군)의 성비는 유의하게 수나무로 편향(0.66)된 반면, 곶자왈도립공원 개체군(Gotjawal Provincial Park population, GP 개체군)은 1:1 평형성비(0.50)를 보였다. 두 개체군 모두 암나무가 수나무에 비해 평균 근원직경과 수고가 컸으나 통계적으로 유의하지는 않았다. 호랑가시나무 개체들은 맹아 형성으로 여러 개의 러밋을 구성한다. 그러나 두 개체군에서 클론 생장은 성과 크기(근원직경)에 따라 다르지 않았다. YS 개체군에서

수나무의 수세는 암나무보다 유의하게 높았다. O-ring 통계량을 이용한 공간분석 결과, YS와 GP 두 개체군에서 암나무는 수나무에 비해 임의분포하는 경향이 나타났다. 또한 두 개체군에서 호랑가시나무의 성 간 서식지분리현상(Spatial Segregation of Sexes, SSS)은 관찰되지 않았다. 경작지와 도로에 둘러싸인 YS 개체군은 성비편향과 병해충 피해가 현저하였다. 추후 인간활동으로 인한 서식지 파괴 및 개체의 감소가 우려되는 만큼 YS 개체군 주변에 보호시설 설치가 시급하다. 또한 보전지역에 위치한 GP 개체군은 인간교란의 위험은 적지만, 개체 수가 매우 적은만큼 치수의 발생 및 생장을 위한 관리방안이 필요하다.

비자나무(*Torreya nucifera*)는 한국과 일본의 일부지역에 제한적으로 분포하는 주목과의 자웅이주 나자식물이다. 국내 최남단에 존재하는 제주도 평대리 비자나무 개체군은 세계적으로 최대크기 (2,861개체), 최대수령(평균수령 320년)의 순림이다. 본 연구에서는 서식지 이질성이 높은 곶자왈 지형의 비자나무 노령림 내 A, B, C의 3개 방형구에서 성과 크기(DBH)와 관련된 공간분포유형을 분석하였다. O-ring 통계량을 이용한 단변량분석 결과, 평균 DBH가 가장 큰 방형구 A에서 비자나무는 성과 크기에 관계없이 모두 임의분포하였다. 반면, 평균 DBH가 두 방형구의 중간값인 방형구 B에서 크기가 작은 암나무와 수나무는 임의분포하고, 큰 암나무와 수나무는 군생분포하였다. 평균 DBH가 가장 작은 방형구 C의 모든 비자나무는 짧은 구간에서 군생분포하고, 거리가 증가할수록 임의 또는 균일분포하였다. SSS 현상은 관찰되지 않았지만 방형구 C에서 크기를 통제했을 때 즉, 작은 크기의 수나무와 암나무 간 서식지분리가 나타났다. 개체군 내 세 지역 간 분포유형 차이는 비자나무 생태특성 뿐 아니라 곶자왈의 극단적으로 이질적인 지형적 요소, 인위적 간섭 등 다양한 요인들이 반영된 결과로 사료된다.

비자나무 개체군의 유전다양성과 공간적유전구조를 조사하기 위해 microsatellite 표지자 분석을 수행하였다. 개체군 수준에서 비자나무의 유전다양성(H_E = 0.513)은 분포범위, 번식양상 등 생태특성이 유사한 타수종에 비해 낮은 경향을 보였다. 또한 비자나무 개체군에서 매우 유의한 유전적 병목현상이 확인되었고($P < 0.001$), 유효개체군의 크기는 실제 개체군의 절반 수준이였다(N_e/N = 0.56). Moran's I와 F_{ij}를 이용한 공간적자기상관성 분석결과, 각 180 m 와 150 m에서 유전적으로 유사한 구조가 확인되었다. 또한 공간적유전구조의 강도는 비자나무와 유사한 생활사를 보이는 수종들과 비교해 상대적으로 강했다(S_p = 0.0130). GBM과 BARRIER 분석 결과, 비자나무 개체군 내 남북방향으로 구별되는 유전적 하위구조가 나타났다. 또한 GENELAND 분석에서 개체군이 4개의 최적 군집수(K = 4)를 갖는 것으로 나타났고, 역시 남북방향으로 분포구성에 차이가 있었다. 공간유전분석 결과에 따라 비자나무 개체군을 세 유전그룹(Group I, Group II, Group III) 으로 나누어 유전변이량을 분석한 결과, 전체 유전변이량의 4%가 그룹 간 발생하였다($P < 0.001$). 본 연구에서 밝혀진 제주도 비자나무 개체군의 낮은 유전다양성과 유전적 하위구조는 비자나무의 보전 및 관리에 반드시 고려되어야 한다. 특히 유전다양성이 높고, 4개의 유전군집을 모두 포함하는 Group III는 보전에 있어 가장 우선순위가 되어야 한다. 또한 비자나무의 현지 외 유전자 보전을 위한 표본 추출 시, 개체 간 거리를 150 m 이상으로 두는 것이 효율적일 것으로 판단된다.

한국의 산림에서 자웅이주 목본은 약 24%로 알려져 있다. 전 세계 목본의 8%가 자웅이주인 것을 고려하면, 국내 자웅이주 목본의 비율은 상당히 높다. 하지만 국내에서 자웅이주 성체제를 고려한 생태연구는 매우 제한적으로 이루어졌다. 본 연구에서는 한국의 위기종이면서 자웅이주인

호랑가시나무와 비자나무를 대상으로 생태특성과 유전적양상의 분석을 통해 많은 종이 위기상태인 자웅이주식물의 보전전략 수립을 위한 정보를 제공하였다. 이 결과는 아직 거의 알려진 바 없는 국내 자웅이주 위기식물의 지속적 보전을 위한 생태적 대책과 관리방안 설정에 기여할 것이다.

주요어: 비자나무, 성비와 분포유형, 유전다양성과 공간적유전구조, 자웅이주 식물의 보전, 호랑가시나무

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