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# Genetic Variation and Structure within Populations of Japanese Beech (*Fagus crenata*)*

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## Chapter I General introduction

### 1. Population genetics approach to reveal structure and dynamics in populations

Understanding the genetic structure and dynamics of populations in a species is important in terms of their evolution and conservation. Thus, to be able to understand the genetic structure and dynamics of a species is one of the ultimate goals for evolutionists, geneticists, ecologists, and conservationists. A population genetics approach provides a basic framework to reveal the genetic structure and dynamics, which have been responsible for evolution for a given species (Hartl and Clark 1997; Nei 1987; Weir 1996).

The Hardy-Weinberg equilibrium is a well-known principle in population genetics, which holds that allele frequencies in a population are expected to equal those in the previous generation. The principle assumes an ideal hypothetical population that satisfies the following conditions: 1) the organism is diploid; 2) reproduction is sexual; 3) generations are non-overlapping; 4) the gene under consideration has two alleles; 5) the allele frequencies are identical in males and females; 6) mating is random; 7) population size is very large; 8) migration is negligible; 9) mutation can be ignored; and 10) natural selection does not affect the alleles under consideration (Hartl and Clark 1997). Although the genetic structure and dynamics of population genetics in real populations are affected by an assemblage of various genetic processes, such as mating, gene flow (by pollen and seed in plants), inbreeding depression, random genetic drift, mutation, and selection, the principle provides a good framework within which researchers can deal with population genetics data. Some of these assumptions are often violated in natural populations. In such populations, we can infer the genetic processes that contribute to the violation of the assumptions using genetic data that are collected systematically and are carefully analyzed. Natural populations offer good opportunities for population and conservation genetics studies because their genetic data can reveal a population's evolutionary history. Thus, we can infer the genetic processes underlying the population structure and dynamics by using various combinations of population genetics indices (Sokal *et al.* 1989, 1997).

Spatial perspective is particularly important to characterize the population structure and dynamics in plant populations. Plants are immobile after their establishment and can usually disperse their own genetic material through pollen and seed. The characteristics of a plant population in a generation are expected not to be independent of those of the previous generation, and they are restricted by the spatial arrangement and the degree of pollen flow and seed dispersal of the previous generation (Sokal and Wartenberg 1983; Epperson 1990).

A demographic perspective is also an important characteristic in the population structure and dynamics of plant species. Usually, perennial plant populations, especially forest tree populations, vary in size with respect to age. An investigation of those size classes at a fixed time point gives us a good opportunity to estimate the dynamics of populations that have overlapping generations.

Forests are important, not only for providing ubiquitous raw materials, such as timber and pulp, but also for forming an environmental backbone vital for human survival. Therefore, the appropriate management and utilization of genetic resources of forest trees are essential. An understanding of how

forest tree species survive, reproduce, and regenerate is essential for implementing a valid conservation scheme. A better knowledge of a species increases the likelihood of managing it appropriately. Thus, a population genetics approach with spatial and demographic perspectives can give us excellent basic information about these plant species (Epperson 1992; Alvarez-Buylla *et al.* 1996b).

## 2. Literature review relevant to the present study

After Hunter and Markert (1957) discovered isozymes, they have been widely used to study plants. Isozymes are polypeptides whose amino acid sequence is transcribed directly from the nucleotide sequence of a gene. They differ significantly in amino acid composition, net ionic charge, molecular size, and configuration, and are therefore distinguished as bands that differ in relative mobility by gel electrophoresis. Isozymes were originally defined as variants of an enzyme that have identical or similar functions, and are present in the same individual (Markert and Møller 1959). There is a huge amount of data on isozyme variation in plant species, and several authors have reviewed the resulting population genetics studies (Loveless and Hamrick 1984; Hamrick and Godt 1989, 1995; Hamrick *et al.* 1992; Muona 1989; Tsumura 2001).

In forest tree species, especially economically important conifers, population genetic techniques using isozymes have been applied to study the degree of genetic variation within-species, within-populations, and among-populations. Forest tree species are genetically more diverse than most plant species, and they have greater nuclear genetic variation within their populations. Life history and ecological characteristics, such as geographical range, mating system, and seed dispersal, should influence the genetic variability found in a species (Hamrick *et al.* 1992).

Since Sokal and Oden (1978a,b) first introduced indices for spatial autocorrelation analysis to investigate the spatial pattern of genetic variation found within populations, they have become important to the study of genetic structure in forest tree species. Many simulation studies have provided basic guidelines for studying variation distributed within populations (Sokal and Wartenberg 1983; Sokal *et al.* 1989, 1997; Epperson 1990, 1995; Sokal and Jacquez 1991) and have revealed that mating system and gene flow have considerable influence on within-population genetic structure. Epperson and Allard (1989) studied within-population genetic structure in lodgepole pine (*Pinus contorta* ssp. *latifolia*), and found that single-locus genetic variation was randomly distributed within the population. Random or only weakly autocorrelated distributions of genetic variation were also found in several coniferous species (in which pollination and seed dispersal occur mainly by wind, and outcrossing rates are high), such as *Picea mariana* (Knowles 1991), *P. abies* (Leonardi *et al.* 1996), and *Pinus banksiana* (Xie and Knowles 1991). However, obvious positive autocorrelations were frequently found in tree species in which seed dispersal is limited, such as *Camellia japonica* (Ueno *et al.* 2000), *Quercus laevis* (Berg and Hamrick 1995), *Q. macrocarpa* (Geburek and Tripp-Knowles 1994), and *Q. petraea*, and *Q. robur* (Bacilieri *et al.* 1994; Streiff *et al.* 1998). These results indicate that seed dispersal influences how a population is genetically structured (Hamrick *et al.* 1993).

Natural and anthropological disturbances, such as forest fire (Boyle *et al.* 1990), forest cutting (Knowles

*et al.* 1992; Takahashi *et al.* 2000), and fragmentation (Young and Merriam 1994), can also influence the genetic structure of a population. Therefore, it is important to consider forest history when analyzing the population genetic structure.

The development of molecular markers, also applied to a range of biological studies, has provided new, powerful tools that have several features that are superior to isozymes. However, there are advantages to using isozymes for within-population genetic studies: 1) most molecular markers are dominant markers, while isozymes are usually codominant, which enables analyses on the allelic and genotypic levels; 2) there is no cost for developing markers for new species; 3) a well-established inheritance basis exists; 4) banding patterns are stable; 5) there are abundant data stocks available for other relevant species; and 6) it is financially feasible to handle huge numbers of samples (more than a thousand).

### 3. Species characteristics of *Fagus crenata*

The genus *Fagus* includes ten species worldwide (Maeda 1991): *Fagus grandifolia* (North America), *F. sylvatica* (Europe), *F. orientalis* (the southern Balkans to the Caucasuses), *F. longipetiolata*, *F. engleriana*, and *F. lucia* (China), *F. hayatae* (Taiwan), *F. multinervis* (Ulleung Island, Korea), and *F. crenata* and *F. japonica* (Japan).

*Fagus crenata* Blume is a common canopy tree species that grows in cool temperate broad-leaved deciduous forests in Japan, ranging from the Kuromatsunai lowland in Hokkaido Island in the north to Mt. Takakuma in Kagoshima Prefecture, Kyushu Island in the south. *F. crenata* forests are widely distributed in cool temperate climate zones as climax forests, especially in the mountainous areas along the Japan Sea side of Japan, where heavy snowfall occurs.

*F. crenata* is a monoecious species, and shows considerable fluctuation in seed production due to its irregular flowering interval (Maeda 1988). *F. crenata* is thought to be a species that outcrosses predominantly, because no self-pollinated individuals were found among six open-pollinated progenies, in a total of 88 saplings (Takahashi *et al.* unpublished data). Outcrossing rates were estimated to be 0.930 - 1.000 in *F. grandifolia* (Kitamura *et al.* 1998) and 0.94 - 0.98 in *F. sylvatica* (Rossi *et al.* 1996). Self-crosses rarely produce sound seeds, as observed in a controlled pollination experiment where the proportions of sound seeds in six self-crosses were less than 4% (Takahashi *et al.* unpublished data). *F. crenata* has wind pollination with gravity- and animal-assisted seed dispersal mechanisms (Yanagiya *et al.* 1969; Maeda 1988; Watanabe 1990). Most seeds are dispersed within 10 m of the mother tree, and few are dispersed beyond 30 m (Maeda 1988; Yanagiya *et al.* 1969). It is known that *F. crenata* seeds are scattered and hoarded by animals, such as wood mice (*Apodemus speciosus* and *A. argenteus*; Miguchi 1994), Japanese nutcrackers (*Nucifraga caryocatactes japonicus*), and jays (*Garrulus gladnarius pallidifrons*; Watanabe 1990). Although there are no discrete data on *F. crenata* seed dispersal, Johnson and Adkisson (1985) described seed dispersal of *F. grandifolia* by blue jays (*Cyanocitta cristata*). They found that about 75 blue jays transported and cached approximately 100,000 beechnuts over distances that ranged from tens of meters up to 4 km from a wood lot. *F. crenata* seeds also risk attacks by rodents and fungi during winter (Tomita *et al.* 2002).

The mortality of *F. crenata* seedlings is high, especially in the first year (Maeda 1988; Nakashizuka 1988), and is related to biotic and abiotic factors, such as light deficit and fungal attack (Nakashizuka 1988; Sahashi *et al.* 1994, 1995). Regeneration of beech trees is associated with gap formation within stands (Hara 1985; Nakashizuka 1987; Yamamoto 1989), but can suffer from the presence of *Sasa* species (Nakashizuka 1988).

The distributional shift of *Fagus* species after the last glacial maximum is relatively well known from palynological records. According to Tsukada (1982, 1983), *Fagus* forests were restricted to areas south of 37－38° N, and were abundant in the coastal regions of southwestern Japan during the peak of the last glacial period (ca. 20,000 yr. B.P.). About 12,000 yr. B.P., *Fagus* began to extend its distribution range both northwards and inland to higher elevations, and arrived at the northern end of Honshu Island ca. 9,000 yr. B.P. (Tsukada 1982). The forest subsequently reached the southern end of the Oshima Peninsula, Hokkaido Island ca. 6,000 yr. B.P. and its current northernmost location between 350 and 680 yr. B.P. (Fig. Ⅳ-1; Igarashi and Yasumura 1989; Sakaguchi 1989). Alternatively, as proposed by Takiya and Hagiwara (1997), Mt. Yokotsudake, in the south of the Oshima Peninsula in southwest Hokkaido, may have been a *F. crenata* refuge during the last glacial period.

Geographic genetic variation in *F. crenata* across its entire distribution has been characterized using isozyme (Tomaru *et al.* 1997) and mitochondrial (mtDNA) markers (Tomaru *et al.* 1998). Levels of genetic variation in 23 populations of *F. crenata* were high within species (mean expected heterozygosity, $H_e$ = 0.194) and populations ($H_e$ = 0.187), whereas the level of genetic diversity among populations was low (the coefficient of gene differentiation, $G_{ST}$ = 0.038; Tomaru *et al.* 1997) as observed in various long-lived woody plants (Hamrick and Godt 1989). Despite the low overall differentiation among populations, populations in southwestern Japan tend to have greater within-population variation and to be more differentiated than those in northeastern Japan. Allele frequencies observed at eight loci were significantly related to latitudinal and longitudinal gradients and showed clinal variation across the species range. Principal component analysis revealed that populations tended to cluster according to their geographical locations. This nonrandom pattern of geographic variation was more evident when maternally inherited organelle DNA, mtDNA, was used as a genetic marker (Tomaru *et al.* 1998). The authors studied the mtDNA variation in 17 *F. crenata* populations distributed throughout the species' range. Haplotype diversity within the populations was very low ($H_S$ = 0.031), while population differentiation was much higher ($G_{ST}$ = 0.963). Similar tendencies were also found in *F. sylvatica* (Demesure *et al.* 1996). These contrasting results between isozyme and mtDNA data reflect the different modes of inheritance between the two markers. Gene flow for maternally inherited mtDNA should be restricted to seed dispersal, while nuclear gene flow occurs by both seed and pollen dispersal. Therefore, the difference in the variation between mtDNA and isozymes may be largely a result of the much greater rate of gene flow associated with pollen dispersal than with seed dispersal. The strong geographic structure found in the mtDNA variation may reflect the distribution of the species in the last glacial maximum and subsequent colonization.

### 4. Objectives of the present study

A fine picture of the structure and dynamics in populations of a species is necessary to achieve appropriate management and conservation objectives and to understand the evolution of the species. Japanese beech (*Fagus crenata* Blume) is an important canopy tree species that is common in cool temperate deciduous forests in Japan. Therefore, it is important from ecological and population genetic viewpoints to understand the population structure and dynamics of the species. The genetic structure and dynamics in populations are affected by various genetic processes, such as mating, gene flow, inbreeding depression, random genetic drift, mutation, and selection. In order to obtain new insight into the population structure and dynamics of *F. crenata*, I plan to integrate spatial and demographic data with population genetics data obtained by isozyme analysis.

Geographic variation of *F. crenata* populations located in northern Honshu and Hokkaido is described in Chapter Ⅱ. Differences in the within-population spatial genetic structure between two *F. crenata* populations with contrasting forest histories are examined in Chapter Ⅲ to determine how forest cutting affects genetic variation and structure. In Chapter Ⅳ, the influence of generation time on genetic structure is examined using the northernmost marginal population, which has regenerated since it was founded. In Chapter Ⅴ, the genetic and demographic processes in a current-year seedling population are described to determine the effect of genetic and demographic processes. There is a brief summary of the software developed for this study (PSAwinD version 1.1.1.) in Chapter Ⅵ. Finally, Chapter Ⅶ contains comprehensive discussion of the results obtained in these studies.

## Chapter Ⅱ Genetic variation of Japanese beech populations both in northern Honshu and Hokkaido

### 1. Introduction

*Fagus crenata* Blume, Japanese beech, is currently distributed in discrete populations from Mt. Takakuma in Kyushu to Kuromatsunai lowland in Hokkaido (Fig. Ⅱ-1). Though *F. crenata* forests covered extensive areas in northeastern Japan in the early 20th century, they have decreased dramatically owing to logging prompted by increased demand for beech lumber after the 1950s. However, relatively large areas of beech forest remain in the mountainous areas of northern Honshu and Hokkaido on the Japan Sea side where there is heavy snowfall.

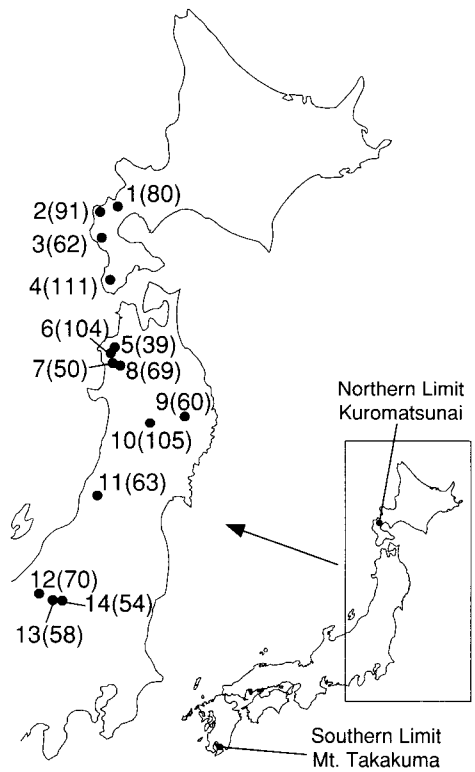


Fig. Ⅱ-1 The location of 14 sampled populations (with sample size given in parenthesis) and both northern and southern limits of the natural distribution of *Fagus crenata*.

Knowledge concerning genetic variation at allozyme loci has been considerably accumulated. Hamrick and Godt (1989) and Hamrick *et al.* (1992) reviewed the published studies on various plant species having different life history and ecological characteristics, and found long-lived woody species to be genetically more diverse than plant species with other life forms. The extent of genetic variation both within species and within populations should be influenced by factors such as geographic range, mating system and seed dispersal (Hamrick and God 1995). The main objectives of a series of my studies are to elucidate within-population genetic structure and its relationship with forest history for *F. crenata*. Studying the levels of both genetic variation within populations and genetic differentiation among populations is very important for the studies on genetic structure because it provides basic information on patterns of genetic variation within species.

As described in the previous chapter, the northernmost marginal population of *Fagus crenata* in Hokkaido Island colonized between 350 and 680 yr. B.P. (Igarashi and Yasumura 1989; Sakaguchi 1989). The dissemination of the species must be completed by the immigrant nuts from Honshu Island to Hokkaido Island, probably by some carriers, such as Japanese nutcracker (*Nucifraga caryocatactes japonicus*) and jays (*Garrulus gladnarius pallidifrons*; Watanabe 1990). Population genetics theory predicts that the extent of genetic variation within a population decreases, when the population went through the so-called bottleneck, i.e. the drastic decrease of effective population size (Hartl and Clark 1997). The colonization process of *F. crenata* onto Hokkaido Island may played a role of the bottleneck. If the number of immigrants from Honshu Island to Hokkaido Island was limited, the genetic variation in the populations in Hokkaido Island is expected to be smaller than that in Honshu Island.

The objective of my study in this chapter was to estimate the amounts of genetic diversity within and among *F. crenata* populations in northern Honshu and Hokkaido, with special attention to the difference in genetic variability between populations of northern Honshu and Hokkaido.

## 2. Materials and methods

Winter buds were collected from 39 to 111 individuals for each of 14 populations between October and April (Fig. Ⅱ-1). Samples were stored at －25℃ until isozyme analysis was conducted.

Isozyme analysis was conducted following the procedures of Tsumura *et al.* (1990). Polyacrylamide vertical slab gel electrophoresis was carried out according to the procedures reported by Davis (1964) and Ornstein (1964). Eleven enzyme systems were stained: alcohol dehydrogenase (*Adh-3*; E. C. 1.1.1.1), malate dehydrogenase (*Mdh-2* and *Mdh-3*; E. C. 1.1.1.37), 6-phosphogluconate dehydrogenase (*6Pg-2*; E. C. 1.1.1.44), diaphorase (*Dia-1*; E. C. 1.8.1.4). glutamate oxaloacetate transaminase (*Got*; E. C. 2.6.1.1), glucokinase (*Gk-1* and *Gk-2*; E. C. 2.7.1.2), acid phosphatase (*Acp*; 3.1.3.2), amylase (*Amy-3*; E. C. 3.2.1.1), alanine aminopeptidase (*Aap-1* and *Aap-2*; E. C. 3.4.11.2), fumarase (*Fm*; E. C. 4.2.1.2) and phosphoglucoisomerase (*Pgi-1*; E. C. 5.3.1.9). From these, 14 putative loci with a total of 50 alleles were inferred.

The following statistics were used to estimate genetic diversity within each population: (*i*) the proportion of polymorphic loci (*Pl*; 95% criterion); (*ii*)

the average number of alleles per locus (*Na*), (*iii*) the effective number of alleles per locus (*Ne*), calculated by $Ne = 1 / \Sigma p_i^2$, where $p_i$ is the allele frequency; and (*iv*) the observed heterozygosity ($H_o$) and the expected heterozygosity ($H_e$) where $H_e = 1 - \Sigma p_i^2$ (Nei and Roychoudhury 1974). The following two statistics were used to estimate the degree of genetic differentiation among populations: (*i*) Nei's genetic distance (*D*; Nei 1972, 1987) and (*ii*) the genetic diversity $H_T = H_S + D_{ST}$ and $G_{ST} = D_{ST}/H_T$ (Nei 1973) where $H_T$ is the total population gene diversity, $H_S$ is the average gene diversity within populations, and $G_{ST}$ is the relative magnitude of gene differentiation among populations.

To investigate the inbreeding in the species, the inbreeding coefficient (Wright 1965), was calculated as $F_{IS} = 1 - H / 2pq\ (1 + 1 / (2N-1))$, where $H$ is the number of heterozygotes observed and $2pq\ (1 + 1 / (2N-1))$ is the number of heterozygotes expected (Kirby 1975). The $\chi^2$-tests of $F_{IS}$ was carried out to test whether $F_{IS}$ values were significantly different from zero.

The geographic distribution of rare alleles (defined as alleles with average frequency lower than 0.05) was also investigated to understand the dissemination patterns of *F. crenata*.

### 3. Results

The *Mdh-2* and *Gk-1* loci showed no variation and the other 12 loci were polymorphic (95% criterion). The $\chi^2$-test was carried out at every polymorphic loci to investigate the deviation from the Hardy-Weinberg equilibrium. The results of the tests were not significant at every surveyed loci. The common alleles at all loci were identical throughout all populations. The values of *Pl*, *Na*, *Ne*, $H_e$ and $H_o$ were calculated (Table II - 1).

The inbreeding coefficients ($F_{IS}$) were calculated for the seven loci where no null allele was detected and where $H_e$ exceeded 0.1. The range of $F_{IS}$ for seven loci was from -0.076 (*6Pg-2*) to 0.142 (*Gk-2*) and their average was 0.036 ± 0.181 (mean ± SE). The value of *Gk-2* (0.142) was significant at the 5% level, but the other values of $F_{IS}$ were not statistically significant. These results suggested that the genotype frequencies at most loci were concordant with the Hardy-Weinberg expectation in *F. crenata* populations.

The average total gene diversity in all the populations ($H_T$) and the average gene diversity within each population ($H_S$) were 0.198 and 0.195, respectively (Table II-2). Thus, 98.5% of gene diversity ($H_S/H_T$) was distributed within populations and 1.5% of gene diversity ($G_{ST}$) was distributed among

Table II-1 The average of *Pl*, *Na*, *Ne*, $H_e$, and $H_o$ in 14 natural populations of *Fagus crenata*.

| | Hokkaido Island | | | | northern Honshu Island | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Population | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | Mean | SE |
| *Pl* (%) | 57.1 | 64.3 | 57.1 | 57.1 | 64.3 | 42.9 | 50.0 | 64.3 | 64.3 | 57.1 | 57.1 | 57.1 | 57.1 | 64.3 | 58.20 | 5.95 |
| *Na* | 2.4 | 2.4 | 2.6 | 2.5 | 2.4 | 2.5 | 2.8 | 2.6 | 2.6 | 2.9 | 2.9 | 2.9 | 2.4 | 2.9 | 2.63 | 0.20 |
| *Ne* | 1.35 | 1.36 | 1.34 | 1.29 | 1.33 | 1.27 | 1.30 | 1.38 | 1.38 | 1.34 | 1.35 | 1.39 | 1.36 | 1.36 | 1.34 | 0.03 |
| $H_e$ (%) | 19.90 | 21.40 | 20.10 | 18.00 | 20.20 | 16.00 | 18.10 | 20.40 | 21.30 | 19.30 | 20.00 | 21.30 | 19.90 | 20.20 | 19.70 | 1.43 |
| $H_o$ (%) | 16.20 | 16.00 | 17.50 | 13.50 | 16.80 | 11.90 | 13.40 | 15.40 | 18.40 | 16.10 | 19.50 | 18.80 | 14.50 | 16.20 | 16.00 | 2.09 |

Symbols : *Pl*, the proportion of polymorphic loci (95% criterion); *Na*, the average number of alleles per locus ; *Ne*, the effective number of alleles per locus; $H_e$, the expected heterozygosity; and $H_o$, the observed heterozygosity.

populations; the genetic distances ($D$) range from 0.000 to 0.006.

In total, 24 rare alleles were found. Five rare alleles ($Dia^c$, $Got^d$, $Acp^a$, $Amy\text{-}3^d$, and $Aap\text{-}1^a$), commonly detected in Honshu populations, were not found in Hokkaido populations. All other 19 rare alleles were detected in both Honshu and Hokkaido. No rare allele unique to Hokkaido populations was found. Thus, Hokkaido populations lacked the less frequently occurring rare alleles (Fig. II-2).

Table II-2 Gene diversity among 14 natural populations of *Fagus crenata*.

| Locus | $H_T$ | $H_S$ | $D_{ST}$ | $G_{ST}$ |
|---|---|---|---|---|
| *Adh-3* | 0.219 | 0.217 | 0.002 | 0.009 |
| *Mdh-2* | 0.000 | 0.000 | 0.000 | 0.000 |
| *Mdh-3* | 0.317 | 0.314 | 0.003 | 0.009 |
| *6Pg-2* | 0.034 | 0.033 | 0.001 | 0.024 |
| *Dia* | 0.145 | 0.143 | 0.002 | 0.015 |
| *Got* | 0.104 | 0.100 | 0.004 | 0.036 |
| *Gk-1* | 0.000 | 0.000 | 0.000 | 0.000 |
| *Gk-2* | 0.208 | 0.206 | 0.002 | 0.008 |
| *Acp* | 0.594 | 0.582 | 0.012 | 0.021 |
| *Amy-3* | 0.533 | 0.526 | 0.006 | 0.012 |
| *Aap-1* | 0.102 | 0.100 | 0.002 | 0.019 |
| *Aap-2* | 0.025 | 0.015 | 0.000 | 0.016 |
| *Fm* | 0.397 | 0.390 | 0.007 | 0.017 |
| *Pgi-1* | 0.110 | 0.109 | 0.002 | 0.015 |
| *Mean* | 0.198 | 0.195 | 0.003 | 0.015 |
| *SE* | 0.052 | 0.051 | 0.001 | 0.002 |

Symbols : $H_T$, the total population gene diversity; $H_S$, the average gene diversity within populations; $D_{ST}$, the average gene diversity among populations; and $G_{ST}$, the relative magnitude of gene differentiation among populations.

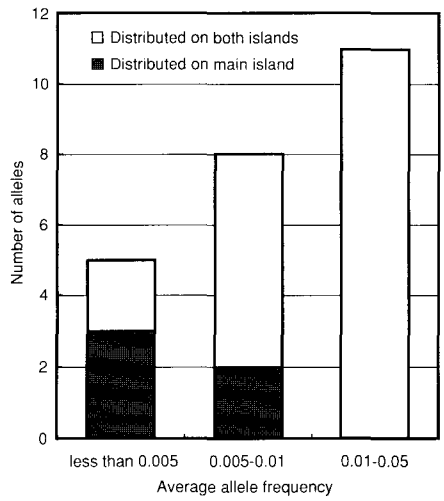


Fig. II-2 Distribution of rare alleles between Honshu (main land) and Hokkaido Island.

## 4. Discussion

The *Pl*, *Na*, *Ne*, and $H_e$ values of *Fagus crenata* populations were 58.2%, 2.63, 1.34, and 19.7%, respectively (Table II-1). Müller-Starck *et al.* (1992) reviewed the studies of allozyme variation of *Fagus sylvatica* L. The values of observed heterozygosity reported were 28.9% using six loci (Comps *et al.* 1990), 25.1% using 16 loci (Müller-Starck 1985, 1989), and 22.2% using 13 loci (Müller-Starck and Ziehe 1991). The genetic variation of *F. crenata* found in this study was somewhat less than those of *F. sylvatica*. Hamrick and Godt (1989) summarized the allozyme diversity of 115 long-lived perennial woody plant species, reporting *Pl*, *Na*, *Ne*, and $H_e$ values of 50.0 ± 2.5%, 1.79 ± 0.06, 1.21 ± 0.02, and 14.9 ± 0.9%, respectively Thus, *F. crenata* populations showed still larger genetic variation than the average of other long-lived, perennial woody plants. *F. crenata* is a long-lived wood plant species, with a wide range of geographic distribution, and predominantly outcrossing mating system. These species characteristics may have influenced the level of genetic variation within populations.

*Fagus crenata*'s $G_{ST}$ and $D$ values indicated no differentiation among the 14 populations. The possible reason is the mode of seed dispersal of the species. It is known that the Japanese nutcracker (*Nucifraga caryocatactes japonicus*) and the Japanese jay

(*Garrulus gladnarius pallidifrons*) habitually store food stocks of *F. crenata* seed (Watanabe 1990). Genetic differentiation caused by genetic drift might not occur even when the migration size per generation is small (Hartl and Clark 1997). So, long distance gene flow by birds could contribute to the absence of genetic differentiation among populations of *F. crenata*.

Of the 24 rare alleles, five were not detected in Hokkaido populations. According to the palynological record (Tsukada 1982; Sakaguchi 1989), after the last glacial period, *F. crenata* gradually migrated northwards along the Japan Sea side coast of northern Honshu and reached the northern end of Honshu about 9,000 years B. P. Then somewhat before 4,210 yr. B. P., the species arrived on Hokkaido. Therefore, Hokkaido's *F. crenata* forests appear marginal from evidence of their present distribution and the pollen data. Five rare alleles might have been lost, as *F. crenata* was affected by a genetic bottleneck while crossing the Tsugaru Strait from mainland Honshu to the marginal island Hokkaido. Except for lacking only five rare alleles, Hokkaido populations were not highly differentiated from Honshu populations. Even though the Tsugaru Strait restricted gene flow, it appears that during each generation sufficient *F. crenata* seeds were transported from Honshu to Hokkaido, perhaps by such carriers as birds, enough to prevent genetic differentiation between the two islands.

## Chapter Ⅲ Differences in genetic structure between two Japanese beech populations with contrasting histories in terms of forest-cutting

### 1. Introduction

Forest tree species are genetically more diverse than plant species representing other life forms. Generally, the nuclear genetic variation of such species is largely found in the within-population component, and factors such as range of geographical distribution, mating system and seed dispersal all influence the genetic variability retained in the species (Hamrick *et al.* 1992). Spatial autocorrelation techniques (Sokal and Oden 1978a,b) are effective for identifying the major genetic processes involved in the generation of genetic structures in specific populations (Sokal and Jacquez 1991; Sokal *et al.* 1997). Simulation studies and forest tree population studies have revealed that mating system and seed dispersal have considerable influence on within-population genetic structure (Sokal and Wartenberg 1983). Coniferous species (in which pollination and seed dispersal occurs mainly by wind, and outcrossing rates are high) have often shown random or only weakly autocorrelated distributions of genotypes (Epperson and Allard 1989; Knowles 1991; Xie and Knowles 1991). In contrast, populations of species in which seed dispersal is limited have often shown genetic clustering. *Quercus* species, in which seeds are dispersed by gravity, are typical. For example, in a continuous old-growth population of *Q. laevis* studied by Berg and Hamrick (1995), the population showed one of the highest proportions recorded of positively significant autocorrelation, over scales of 10 m or less, presumably because of limited seed dispersal. However, the degree of autocorrelation observed was not as strong as that predicted by the authors' simulations. The authors suggested that pollen flow and bird-cached seed may have had important effects on the genetic structure of the population, which was less pronounced than expected given the isolation by distance.

Anthropological disturbances such as forest cutting and fragmentation also influence genetic structure. Knowles *et al.* (1992) studied two anthropologically disturbed *Larix laricina* populations: one regenerated from a site with scattered remnant trees, but not surrounded by an off-site seed source, and the other regenerated from a site lacking remnant trees but surrounded by an abundant off-site seed source. The former showed autocorrelation whereas the latter did not. Two out of six correlograms in the former population were significant according to the Bonferroni criterion. The cited authors considered that the limited number of remnant trees may have acted as point seed sources, so the progenies were less mixed in this population. Young and Merriam (1994) studied four fragmented and four continuous *Acer saccharum* populations, and found effects of fragmentation on the spatial genetic structure. Positive autocorrelations, suggesting a reduced degree of mixing of progenies, were found in the shortest distance class in fragmented populations, and negative autocorrelations were observed in the longest distance class, suggesting that incorporation of immigrant pollen pools occurred in mating events at forest patch edges.

*Fagus crenata* is also genetically diverse and almost all of the nuclear genetic variation of the species is maintained in the within-population component ($G_{ST}$ = 0.038; Tomaru *et al.* 1997). Understanding the genetic processes operating in natural populations is one of the most important goals in population and conservation genetics, because such knowledge is essential for enhancing the quality of conservation, for management of genetic resources, and for controlling the potential risk of genetic deterioration. I therefore studied the genetic structure and variability of two *Fagus crenata* (Japanese beech) populations that have had strongly contrasting histories in terms of cutting, using spatial autocorrelation and other relevant statistics, to clarify the influence of forest cutting on the genetic structure of the populations. The following questions were addressed in this chapter. Does forest cutting influence the genetic structure in the two populations? If so, how do the genetic structures differ between the two populations, and what differences in the genetic structure are likely to influence succeeding generations?

## 2. Materials and methods

### 1) Studied populations, sampling and isozyme analysis

Two populations of *Fagus crenata* forest were studied. One is located at the northern foot of Mt. Akitakomagatake (840m; 39° 47' 30"N, 140° 48' 0"E; designated AK) in Akita Prefecture, and the other is located at the southern foot of Mt. Kurikoma (870m; 38° 55' 30"N, 140° 47' 50"E; designated KU) in Miyagi Prefecture in northern Honshu, Japan (Fig. Ⅲ-1). Both

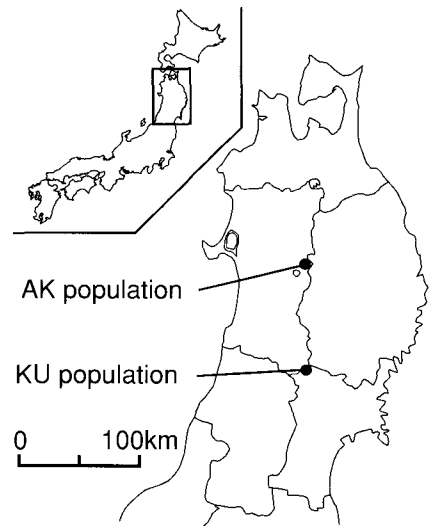


Fig.Ⅲ-1　Locations of the two studied *Fagus crenata* populations.

populations are located in the Ohu Mountains, where *F. crenata* forest is predominant. KU is located ca. 90 km south of AK.

A 0.77 ha survey plot was delineated in AK, and all 486 trees taller than 3 m in the plot were mapped, and a 1.23 ha survey plot was delineated in KU, in which 174 trees taller than 5 m were mapped. The height and diameter at breast height (DBH) of the 660 mapped trees were then measured, and histograms of DBH distribution are shown in Fig. Ⅲ-2. The ranges of height and DBH were 3 - 27 m and 2 - 92 cm in AK, and 8 - 25 m and 13 - 109 cm in KU, respectively. The average and standard deviations of the height and DBH values were 16.0 ± 5.0 m and 18.3 ± 11.4 cm in AK, and 19.6 ± 3.0 m and 46.7 ± 18.2 cm in KU, respectively. The tree density and basal area of *F. crenata* were 665.0 trees/ha and 23.9 $m^2$/ha in AK, and 141.5 trees/ha and 27.8 $m^2$/ha in KU, respectively. The two populations have contrasting histories in relation to forest cutting. KU is an old-growth beech forest, but AK was cut during the 1920s. A few beech trees were left as mother trees at that time. The remnant trees in the AK population could be easily recognized because they had much larger diameters (greater than 70 cm) than newly regenerated trees.

Experimental samples (winter buds) were collected from all mapped trees, and the following nine loci, encoding eight enzyme systems, were analyzed: *Mdh-3* (E.C.1.1.1.37), *6Pg-2* (E.C.1.1.1.44), *Dia-1* and *Dia-2* (E.C.1.8.1.4), *Got* (E.C.2.6.1.1), *Amy-3* (E.C.3.2.1.1), *Aap-1* (E. C. 3.4.11.2), *Fm* (E.C.4.2.1.2) and *Pgi-1* (E.C.5.3.1.9). The details of the procedures for isozyme analysis have been described previously (Tsumura *et al.* 1990; Tomaru *et al.* 1997)

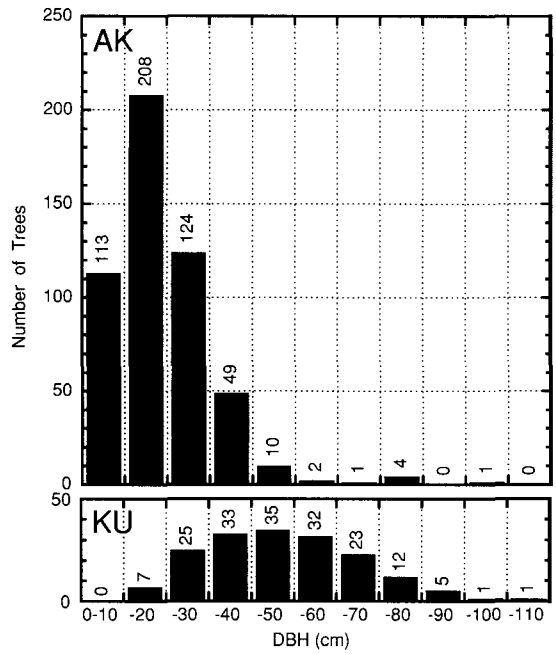


Fig.Ⅲ-2 Distribution of diameter at breast height (DBH) in (a) the AK population and (b) the KU population of *Fagus crenata*.

### 2) Data analysis

The proportion of polymorphic loci (*Pl*; I regarded the loci where frequency of the most frequent allele is less than 0.95 as polymorphic loci), the average number of alleles per locus (*Na*), the effective number of alleles per locus (*Ne*; Kimura and Crow 1964), the observed heterozygosity ($H_o$) and the expected heterozygosity ($H_e$) were calculated to determine the degree of genetic variability within the two populations. The $H_e$ values were derived by averaging $h_e$ over nine loci, where $h_e = 2n(1-\Sigma p_i^2)/(2n-1)$, $p_i$ is the allele frequency of the $i$ th allele at a given locus, and $n$ = sample size (Nei 1987).

I also calculated two types of coefficients of spatial autocorrelation: SND for nominal data and Moran's $I$ for interval data. In the nominal case, the number of joins of every genotype combination was counted at a given locus in a given distance class. The nominal autocorrelation test statistic is the standard normal

deviate (SND) of the observed number of joins from the expected number of joins (Sokal and Oden 1978a): SND= (Observed joins－Expected joins) / Variance$^{1/2}$. The SNDs are assumed to be normally distributed, and therefore they have critical values of ± 1.96 at the 5% level of significance. Genotypes whose frequencies lie outside the range 0.05 to 0.95, and which expected numbers of joins are less than unity, are excluded from calculation of the SND. Joins between individuals having identical genotypes are referred to as like joins, and joins between individuals having different genotypes are referred to as unlike joins. Sixteen like joins and 11 unlike joins in AK and 14 like joins and 11 unlike joins in KU satisfied the criteria, and were used for the calculation.

In the interval case, genotypes of each individual were transformed to 0.0, 0.5 or 1.0 according to the number of the copies of a given allele they possessed. Then Moran's $I$ coefficients were calculated using the following formula (Sokal and Oden 1978a): $I = n\Sigma\Sigma w_{ij} z_i z_j / (W\Sigma z_i^2)$, where $n$ = sample number; $w_{ij}$ = join matrix, being set to unity if the $i$ th and $j$ th individuals are in the distance class, and zero otherwise, $W$= the sum of join matrices $w_{ij}$; $z_i = x_i - x$, and $z_j = x_j - x$. The variables $x_i$ and $x_j$ are the genotypic scores for the $i$ th and $j$ th individuals, respectively, and $x$ is the mean score for all trees of the studied population. The expected value of $I = -1/(n-1)$. The variance of $I$ was calculated following the formulae of Sokal and Oden (1978a), and significance levels were calculated from SNDs on the assumption that $I$ is normally distributed. I divided the AK plot into 19 subplots of 20 m x 20 m and the KU plot into 18 subplots of 30 m x 30 m, and calculated allele frequencies in all the subplots and Pearson's correlation coefficients among them. If a pair of alleles in a certain locus was significantly negatively correlated, one allele was excluded from the following analyses to avoid nonrandom sampling of alleles because it is well established that similar gene frequency surfaces yield similar correlograms (Sokal and Oden 1978b; Sokal and Wartenberg 1983). Furthermore, alleles with frequencies lying outside the range 0.05 to 0.95 are also excluded. The following seven alleles satisfied the two criteria for inclusion in each of the two populations: *Mdh-3*$^b$, *Dia-1*$^b$, *Dia-2*$^b$, *Amy-3*$^b$, *Aap-1*$^b$, *Fm*$^b$ and *Pgi-1*$^c$.

The SND and $I$ values were calculated using three sets of distance class in the two populations: 12 distance classes of 5 m intervals (0 - 5 m, 5 - 10 m and so on), six distance classes of 10 m intervals (0 - 10 m, 10 - 20 m and so on), and 20 distance classes, adjusted such that the values of $W$ were practically equal in each class, and so that there was statistically equivalent test power in each class. As analyses using these three types of distance classes showed very similar trends, I consider here only the results from the first type of distance class. The overall significance of the individual correlogram for each allele was assessed using the Bonferroni procedure. In this procedure, a modified Bonferroni probability level, $\alpha'$, for individual tests is calculated from $1-(1-\alpha)^{1/l}$ where $\alpha$ is set as the Type I error rate (here $\alpha$ = 0.05) and $l$ is taken as the number of distance classes (here $l$ = 12). The average correlograms, which are simply the arithmetic means of the individual correlograms, are calculated for the $I$ values. Their variances are calculated using the bootstrap procedure.

The NAC was calculated over 12 distance classes (i.e. the first type of distance class). The NAC value is the average number of alleles in common per

polymorphic locus between pairs of individuals in a given distance class. A mean is calculated for each distance class by averaging the pairwise NAC values following the counting rules of Surles *et al.* (1990). A grand mean is also calculated by averaging all possible pairwise NAC values without respect to distance. The grand mean represents the null hypothesis of spatial randomness of alleles. To assess the significance of excesses and deficits of NAC, I calculated variances of NAC values in each distance class using the bootstrap procedure. An excess of NAC at a certain distance suggests that individuals in that distance class share more alleles than would be expected in a random distribution. This excess must be offset by a deficit of NAC in other distance classes. The variance of grand mean NAC is also calculated, to examine whether the grand mean NAC values in the two populations differ significantly. The NAC can vary from 0 to 2, but it usually ranges from 1.25 to 1.75 in natural populations (Hamrick *et al.* 1993).

The inbreeding coefficient ($F_{IS}$) was derived from averaging $f_{is}$ over polymorphic loci, where $f_{is} = 1 - h_o/h_e$, $h_e$ is expected heterozygosity, and $h_o$ is observed heterozygosity. Heterogeneity in genotype frequencies at each of the nine loci and linkage disequilibrium between all pairs of nine loci in the two populations were examined using the chi-square test of the GDA version 1.0 program (Lewis and Zaykin 1999). Alleles with frequencies less than 0.05 were pooled together as one allele before the calculation. Some linkage-disequilibrium-retained locus pairs were observed in the AK population, but none was detected in KU. The distribution of two-locus genotypes of linkage-disequilibrium-retained locus pairs was examined by calculating the SND values of two-locus genotypes.

To assess whether the detected linkage disequilibrium is likely to diminish or not as tree density in AK decreases in the present generation, a simulation of the self-thinning process was carried out, using the DBH data collected for 486 trees in the population. The ECO-GENE version 1.0 program (Degen *et al.* 1996) was used for this simulation. In the simulation, the vitality of each tree decreases according to the degree its canopy overlaps with surrounding tree canopies every year. When the vitality of a tree falls to zero, the tree is regarded as dead. I used growth data of *Fagus sylvatica* provided by the software, and extended the simulation time to 35 years in five-year intervals. Because it is not certain that five-year growth of *F. sylvatica* is equivalent to five-year growth of *F. crenata*, I referred to the five-year growth period as a unit of simulation period (thus 35 simulated years are referred to as the 7 x simulation period). No mating was included in the simulation; I simply examined the process of self-thinning. As the results were identical among five repetitions, I attempted no more repetitions and regarded the results as one repetition. I then calculated two-locus genotypic SND of linkage-disequilibrium-retained locus pairs, standard SND, Moran's *I*, and linkage disequilibrium values for trees surviving in the simulation.

### 3. Results

#### 1) Genetic variability

In total, 33 alleles were detected among nine loci encoding eight enzyme systems in the two populations (Table Ⅲ-1), six of which were present in only one population. Parameters for evaluating genetic variability are presented in Table Ⅲ-2. The *Ne*, $H_e$, and $H_o$ values in the AK population are

slightly lower than those in KU, although the differences are not significant. The combined average values and standard errors of *Pl*, *Na*, *Ne*, $H_e$, and $H_o$ in the two populations were 78%, 3.30 ± 0.47, 1.30 ± 0.12, 0.202 ± 0.057 and 0.191 ± 0.053, respectively.

2) Spatial autocorrelation

The following seven alleles satisfied the criteria in this study for selecting alleles and were used for calculating Moran's *I* values: *Mdh-3*$^b$, *Dia-1*$^b$, *Dia-2*$^b$, *Amy-3*$^b$, *Aap-1*$^b$, *Fm*$^b$ and *Pgi-1*$^c$. As for the nominal case, 16 like joins and 11 unlike joins in AK and 14

Table Ⅲ-1 Detected alleles and their frequencies in the AK and KU populations of *Fagus crenata*.

| Locus | Allele | Population | |
|---|---|---|---|
| | | AK | KU |
| *Mdh-3* | *a* | 0.003 | - |
| | *b* | 0.801 | 0.873 |
| | *c* | 0.195 | 0.127 |
| *6Pg-2* | *c* | 0.998 | 0.997 |
| | *d* | 0.002 | 0.003 |
| *Dia-1* | *a* | 0.032 | 0.069 |
| | *b* | 0.947 | 0.925 |
| | *c* | 0.001 | 0.006 |
| | *d* | 0.020 | - |
| *Dia-2* | *a* | 0.020 | 0.012 |
| | *b* | 0.895 | 0.910 |
| | *c* | 0.085 | 0.071 |
| | *d* | - | 0.006 |
| *Got* | *a* | 0.005 | - |
| | *b* | 0.004 | 0.003 |
| | *c* | 0.982 | 0.975 |
| | *d* | 0.009 | 0.009 |
| | *e* | - | 0.012 |
| *Amy-3* | *a* | 0.013 | 0.032 |
| | *b* | 0.354 | 0.365 |
| | *c* | 0.013 | 0.010 |
| | *d* | 0.005 | 0.016 |
| | *e* | 0.612 | 0.558 |
| | *f* | 0.002 | 0.013 |
| | *g* | - | 0.006 |
| *Aap-1* | *a* | 0.002 | 0.016 |
| | *b* | 0.944 | 0.913 |
| | *c* | 0.053 | 0.072 |
| *Fm* | *a* | 0.277 | 0.267 |
| | *b* | 0.723 | 0.733 |
| *Pgi-1* | *b* | 0.006 | 0.012 |
| | *c* | 0.928 | 0.932 |
| | *d* | 0.066 | 0.056 |
| Total no.of detected alleles | | 30 | 30 |

Table Ⅲ-2 Parameters of genetic variability and inbreeding coefficients in the AK and KU populations of *Fagus crenata*.

| | Population | | Difference between two populations |
|---|---|---|---|
| | AK | KU | |
| *Pl* | 78 | 78 | |
| *Na* | 3.3 (0.4) | 3.3 (0.5) | NS |
| *Ne* | 1.31 (0.11) | 1.33 (0.13) | NS |
| $H_e$ | 0.200(0.057) | 0.203(0.057) | NS |
| $H_o$ | 0.189(0.054) | 0.193(0.052) | NS |
| $F_{IS}$ | 0.055(0.067) | 0.042(0.080) | NS |

Averages with standard errors in parentheses are shown.
Symbols : *Pl*, the proportion of polymorphic loci (95% criterion); *Na*, the average number of alleles per locus; *Ne*, the effective number of alleles per locus; $H_e$, the expected heterozygosity; $H_o$, the observed heterozygosity, and $F_{IS}$, inbreeding coefficient.

like joins and 11 unlike joins in KU satisfied the criteria and were used for calculating SND. As analyses of the three types of distance classes showed the same trends, only the results from the first type (i.e. 12 distance classes of 5 m intervals, from 0 - 60 m) are presented.

Seven individual allelic correlograms (of Moran's *I* values) calculated for the two populations are shown in Fig. Ⅲ-3. Six out of seven allelic correlograms (86 %) in AK showed positively significant *I*s in the shortest distance class (Fig.Ⅲ-4a), and one out of seven correlograms (14%) in KU showed positively significant *I*s in the first distance class (Fig.Ⅲ-4b). Even when the first two distance classes were pooled, the proportion of positively significant *I*s in KU was only 43%. The proportions of positively significant *I*s decreased as distance increased in both populations. Significantly negative *I*s were frequently observed in larger distance classes of AK. Six out of seven allelic correlograms (all but *Amy-3*) in AK, and two out of seven in KU were significant at the 5% Bonferroni criterion probability level. The average correlograms of the two populations and the variances for the two populations are also shown in Fig.Ⅲ-3. Among 14 individual correlograms, only the *I* value of the *Pgi-1*$^c$ allele in the first distance class significantly differed from the average *I* value ($P < 0.05$). The proportion of positively significant like joins in the first distance class was 0.38 in AK and 0.29 in KU (Fig.Ⅲ-4c,d). The proportion of positively significant like joins in KU decreased to zero by the fourth distance class, whereas that of AK only did so in the seventh distance class. Negatively significant unlike joins were thought of as the cumulative effect of positive associations of like joins. The proportion of negatively significant unlike joins in the first distance class was 0.27 in AK and 0.09 in KU. The proportion of negatively significant unlike joins in AK is higher than that of KU over the first three distance classes.

### 3) Number of alleles in common

The grand mean and the standard deviation of the NAC was 1.684 ± 0.001 in AK and 1.649 ± 0.002 in KU, and they differed significantly ($P < 0.001$). The

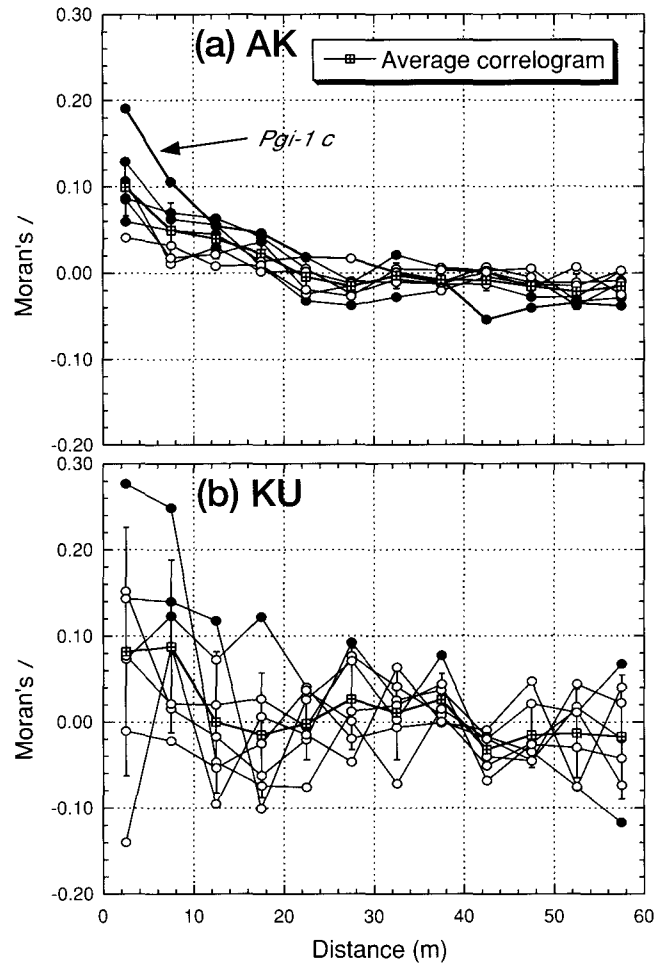


Fig. Ⅲ-3　Individual and average correlograms of Moran's $I$ in the AK and KU populations of *Fagus crenata*.

Variances of the Moran's $I$ values of the average correlogram were estimated using the bootstrap procedure. Standard deviations of the Moran's $I$ values of the average correlogram are presented with error bars. Solid circles denote significant Moran's $I$ values at the 5% probability level. The average correlogram and correlograms in which Moran's $I$ significantly differed from the $I$ value of the average correlogram in the same distance class at the 5% probability level are marked with bold lines.

NAC values of the two populations are shown in Fig. Ⅲ-5. The NACs were positively significant in the first three distance classes and negatively significant in the fifth and sixth distance classes in AK, showing the pattern expected according to isolation by distance. The NACs of KU were not significant, except in the second and eighth distance classes.

4) Inbreeding coefficients and linkage disequilibrium

The $F_{IS}$ value of AK (0.055 ± 0.067) was slightly higher than that of KU (0.042 ± 0.080; Table Ⅲ-2), although the difference was not significant. No heterogeneity in genotype frequencies and no linkage disequilibrium were observed in KU, but both phenomena were detected in AK. The heterogeneity in genotype frequencies at the nine loci, and linkage disequilibria between the pairs of loci for the AK population, are shown in Table Ⅲ-3. Genotype frequencies at the *Aap-1* locus were significantly heterogeneous ($P < 0.001$). The $f_{is}$ value at this locus

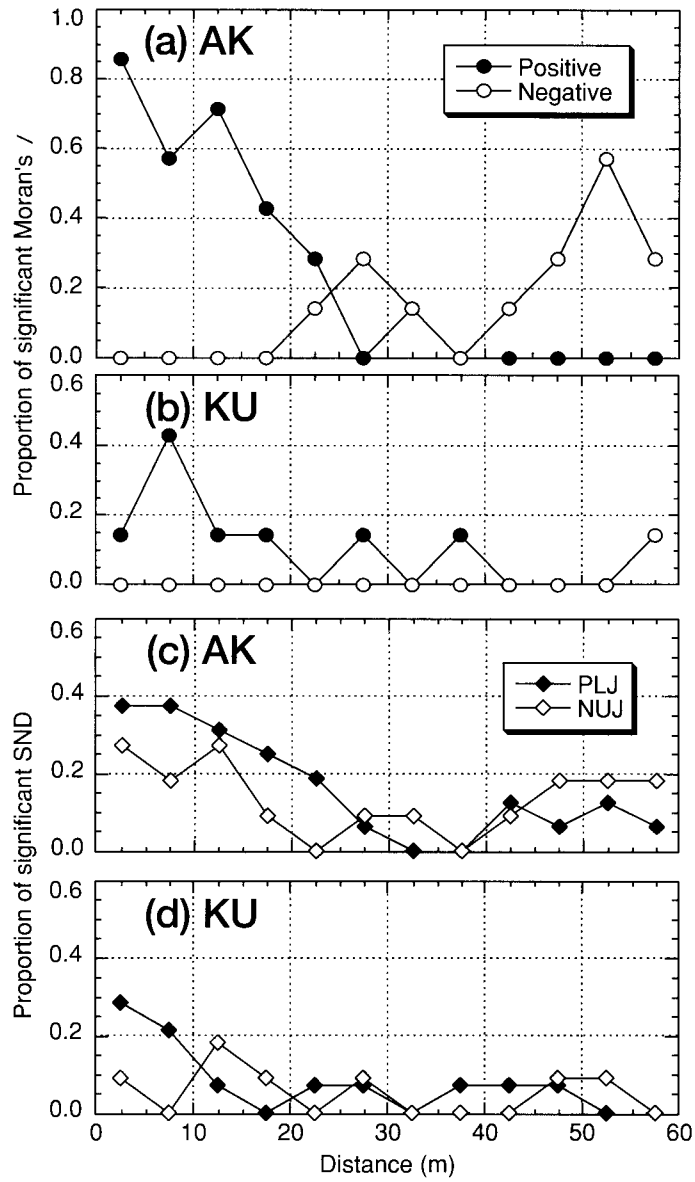


Fig. Ⅲ-4 (a) and (b) the proportions of significant Moran's *I* in AK and KU populations, respectively, of *Fagus crenata*. (c) and (d) the proportions of positively significant SNDs of like joins (PLJ) and negatively significant SNDs of unlike joins (NUJ) in AK and KU, respectively.

was 0.209, indicating more homozygotes than expected under Hardy-Weinberg equilibrium. Combined genotype frequencies between *Aap-1* and the other eight loci also deviated from Hardy-Weinberg equilibrium, at least at the 5% probability level. Even if the *Aap-1* locus was excluded from consideration, linkage disequilibrium still existed for four pairs of loci: *Mdh-3* - *Amy-3*, *Dia-1* - *Amy-3*, *Dia-2* - *Got*, and *Got* - *Fm*.

### 5) Simulation of the self-thinning process and its effect on genetic structure

The two-locus genotype distributions of four linkage-disequilibrium-retained locus pairs were

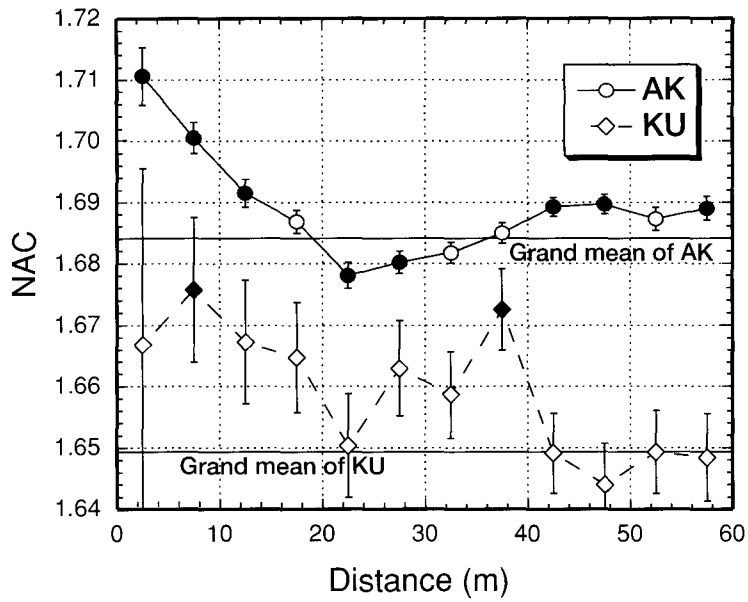


Fig. Ⅲ-5　NAC values over 12 distance classes in the AK and KU populations of *Fagus crenata*.

Grand means of NAC values in the AK and KU populations are shown by solid lines. Solid markers denote NAC values that significantly differed from the grand mean NAC value at the 5% probability level. Variances of the NAC values were estimated using the bootstrap procedure. Error bars show one standard deviation of the NAC values

Table Ⅲ-3　Chi-square heterogeneity test for the nine loci and linkage disequilibria among them in the AK population of *Fagus crenata*.

| No. | Locus | Heterogeneity test | Linkage disequilibrium | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 1 | *Mdh-3* | | | — | — | — | — | * | * | — | — |
| 2 | *6Pg-2* | | | | — | — | — | — | * | — | — |
| 3 | *Dia-1* | | | | | — | — | * | * | — | — |
| 4 | *Dia-2* | | | | | | * | — | * | — | — |
| 5 | *Got* | | | | | | | — | * | * | — |
| 6 | *Amy-3* | | | | | | | | * | — | — |
| 7 | *Aap-1* | *** | | | | | | | | * | * |
| 8 | *Fm* | | | | | | | | | | — |
| 9 | *Pgi-1* | | | | | | | | | | |

—, not significant.

*$P < 0.05$, ***$P < 0.001$.

examined by calculating SND values. Four out of 13 like joins genotypic correlograms (0.31) at four linkage-disequilibrium-retained locus pairs showed positively significant SNDs in the first distance class (Table Ⅲ-4). As the present tree density in the AK population is high (631.2 trees / ha), the observed linkage disequilibrium and the clustering of two-locus genotypes might be diminished during the process of self-thinning. Therefore, I attempted to simulate self-thinning, and to describe the genetic structure of the resultant population using the NAC, the parameters of spatial autocorrelation and linkage disequilibrium (Table Ⅲ -4). The heterogeneity of genotype frequencies at the *Aap-1* locus remained nonsignificant until the 3 x simulation period (data not shown). The positively significant SNDs of like joins at the four linkage-disequilibrium-retained locus pairs diminished during self-thinning (falling to zero by the 4 x simulation period), as expected. The proportion of significant *I*s and standard SNDs in the first distance class also decreased to zero. Although the linkage disequilibrium was diminished at the *Amy-3&Dia-1* locus-pair, the linkage disequilibrium at the other three pairs was reinforced. The grand mean of the NAC values, and the standard deviation after the 7 x simulation period was 1.665 ± 0.002. The NAC decreased from 1.684 (before simulation), but the value still significantly differed from the NAC of the KU ($P < 0.001$). The correlogram of NAC after the 7 x simulation period is presented in Fig. Ⅲ-6. The NAC in the second distance class is significant ($P < 0.05$). If the first two distance classes are pooled, the NAC correlogram suggests the genetic clustering of multilocus genotypes. It is interesting that the

Table Ⅲ-4 Changes in degrees of linkage disequilibria and proportions of positively significant SNDs of like joins in the first distance class during the self-thinning simulation using data from the AK population of *Fagus crenata*.

| Simulated period Present | 1 x | 2 x | 3 x | 4 x | 5 x | 6 x | 7 x |
|---|---|---|---|---|---|---|---|
| Density of surviving trees ( / ha) | | | | | | | |
| 631.2 | 513.0 | 402.6 | 319.5 | 245.5 | 203.9 | 172.7 | 141.6 |
| Linkage disequilibrium | | | | | | | |
| *Amy-3 & Mdh-3* | * | *** | *** | *** | *** | *** | *** | *** |
| *Amy-3 & Dia-1* | * | NS | * | * | * | * | * | NS |
| *Got & Dia-2* | * | * | * | * | * | * | ** | ** |
| *Got & Fm* | * | * | * | * | * | * | * | ** |
| Proportion of positively significant SND of like joins in the first distance class | | | | | | | | |
| *Amy-3 & Mdh-3* | 2/5 | 2/5 | 1/5 | 1/5 | 0/3 | 0/3 | 0/2 | 0/0 |
| *Amy-3 & Dia-1* | 1/3 | 0/3 | 0/3 | 0/3 | 0/2 | 0/2 | 0/2 | 0/0 |
| *Got & Dia-2* | 1/2 | 1/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/1 | 0/1 |
| *Got & Fm* | 0/3 | 1/3 | 0/3 | 0/3 | 0/2 | 0/2 | 0/2 | 0/1 |

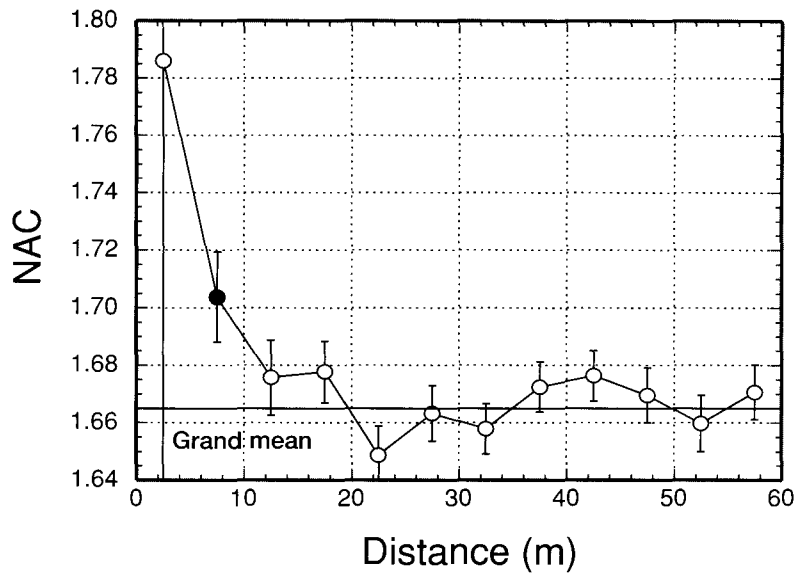


Fig Ⅲ-6　NAC values over 12 distance classes of the AK population of *Fagus crenata* after the 7x simulation period.

The grand mean NAC value is shown as a solid line. The solid marker denotes a NAC value that differed significantly from the grand mean NAC value at the 5% probability level. Variances of the NAC values were estimated using the bootstrap procedure. Error bars show one standard deviation of the NAC values.

decrease of the grand mean resulted from decreases of the NACs in longer distance classes. The NAC in the first distance class increased substantially ($\Delta$ = 0.075) compared to changes in other distance classes.

## 4 Discussion

### 1) Genetic variability

The *Ne*, $H_e$, and $H_o$ values of the AK population were slightly lower than those of KU, and the $F_{IS}$ value derived for AK was slightly larger than that of KU. The grand mean of NAC was significantly higher for AK than for KU, indicating there was less genetic variability and higher genetic similarity among individuals in AK. The NAC would be expected to be more sensitive to differences in genetic variability than other parameters used in this study, because it is based on pairs of individuals, whereas the other parameters are locus-based. The AK population was subjected to cutting during the 1920s, and tree density of the population was reduced to 6.5 trees/ha at that time. The slight, but significantly, lower genetic variability found in AK is attributable to the founder effect caused by the cutting, which left just a few trees standing. The degree to which the genetic variability was reduced by the cutting would have been weakened by pollen flow from surrounding populations, if present (see below).

### 2) Genetic structure

The proportion of positively significant *I* and positively significant like joins in short distance classes were higher than in longer distance classes (Fig. Ⅲ-4), indicating the existence of genetic clustering in both populations. However, the degree of the autocorrelation differed markedly between the populations. The proportion of positively significant *I*

(0.14) and SND values of like joins (0.29) in the first distance class in KU is more obvious than the genetic structure found for some conifer species. The proportion of positively significant *I*s found in previous studies include 8 to 14% in two *Picea mariana* populations (Knowles 1991) and 2 to 9% in three populations of *Pinus banksiana* (Xie and Knowles 1991). The significant SND was 7% in two populations of *P. contorta* ssp. *latifolia* (Epperson and Allard 1989). However, the genetic structure observed in KU is still in the range found for other Fagaceae tree species. Proportions of significant *I*s found in previous studies include 67% in a *Quercus laevis* population (Berg and Hamrick 1995), 44% in a *Q. macrocarpa* population (Geburek and Tripp-Knowles 1994), and 25% in a *F. crenata* population (Kawano and Kitamura 1997). Previously published SND values range from 10 to 14% in two French *F. sylvatica* populations (Merzeau *et al.* 1994) and from 0 to 25% in 14 Italian *F. sylvatica* populations (Leonardi and Menozzi 1996). Thus, the genetic structure found in KU is fairly high compared to estimations for related species. Limited seed dispersal is presumably related to the observed genetic structure in this population, as seeds of *F. crenata* are abundantly dispersed up to 10 m around a mother tree, but usually not dispersed beyond 30 m (Yanagiya *et al.* 1969; Maeda 1988). Kawano and Kitamura (1997) estimated genetic neighborhood area ($A$), which is defined as the area centered on individuals within which 86.5% of parents are to be detected, to be $A$ = 3,050 - 4,091 m$^2$, equivalent to a circle of 31.2 - 36.1 m radius. Similarly, Troggio *et al.* (1996) estimated mean pollen flow distance to be 31.7 m in a *F. sylvatica* population. Demesure *et al.* (1996) reported maternally inherited cpDNA to be more highly differentiated ($G_{ST}$ = 0.83) than nuclear DNA ($G_{ST}$ = 0.054), according to an isozyme analysis of 85 *Fagus sylvatica* populations. Tomaru *et al.* (1998) also found a striking difference between genetic differentiation of maternally inherited mtDNA ($G_{ST}$ = 0.963) and nuclear-coded isozyme markers ($G_{ST}$ = 0.039) among 17 *F. crenata* populations. These findings indicate that there is a much higher level of pollen flow than seed dispersal. Tomaru *et al.* (1997) estimated the average number of migrants exchanged per generation ($Nm$) to be 6.3, which is significantly higher than the level believed to be necessary to prevent population differentiation ($Nm > 1$).

The proportion of positively significant $I$ and SND values in AK (0.86 and 0.38, respectively) is much higher than the corresponding proportions in KU and in other Fagaceae species. The forest cutting is presumed to be tightly related to the strong genetic structure in AK (see below). Negatively significant *I*s were frequently observed in longer distance classes in AK (more than 40 m; Fig. Ⅲ-4a), indicating considerable genetic dissimilarity among trees separated by large distances. This is attributable to the limited number of reproductive trees with different genotypes responsible for establishing the present tree population. Thirteen out of 14 correlograms did not differ from the average correlogram, suggesting that no selection operates over these 13 alleles. Only the *Pgi-1*$^c$ correlogram (in the first distance class; $P < 0.05$) in the AK population was significantly different from the average correlogram (Fig. Ⅲ-3a). Two remnant trees with a DBH greater than 70 cm had a *c/d* genotype at the *Pgi-1* locus (Fig. Ⅲ-7), and around the more southerly one there was a cluster of trees sharing this genotype. The clustering is clearly responsible for the

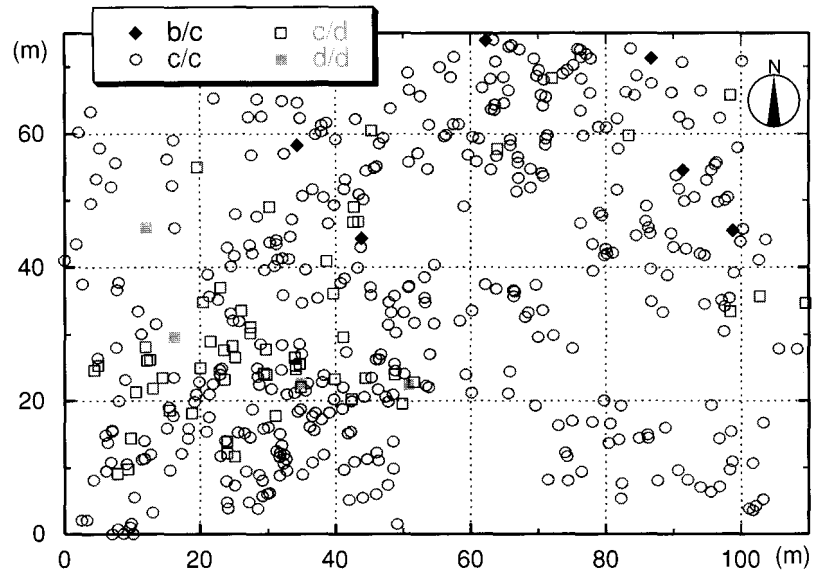


Fig. Ⅲ-7　Scattergram of four genotypes at the *Pgi-1* locus in the AK population of *Fagus crenata*.

The two larger squares show remnant trees (with DBHs greater than 70 cm) with the *c/d* genotype, the southerly one surrounded by a cluster of newly regenerated trees sharing the *d* allele.

particularly high *I* value in the first distance class at the locus. I did not regard the clustering as evidence of selection.

### 3) Differences in genetic structure and variability between populations

There were contrasting results between the AK and KU populations. First, no linkage disequilibrium was observed in KU. The genotype frequency of the *Aap-1* locus in AK, however, deviated from Hardy-Weinberg equilibrium (Table Ⅲ-3), and four locus pairs showed linkage disequilibrium. Secondly, the proportions of positively significant *I*s (0.86) and positively significant like joins (0.38) in the first distance class in AK were higher than the corresponding proportions in KU (0.14 and 0.29, respectively; Fig. Ⅲ-4). Thirdly, the grand mean of NAC in AK is significantly ($P < 0.001$) higher than that of KU. The founder effect caused by the forest cutting in AK during the 1920s is almost certainly responsible for these differences.

The linkage disequilibria of combined genotype frequencies between the *Aap-1* locus and the other eight loci are attributable to the heterogeneity of genotype frequency at the *Aap-1* locus itself. However, four other two-locus combinations also retained linkage disequilibrium (Table Ⅲ-3). A dramatically reduced density of reproductive trees would be expected to induce genetic drift in the genetic composition of remnant trees (Hartl and Clark 1997). A decrease in density of reproductive trees reduces the degree of overlap of seed shadows of different mother trees, and consequently reduces mixing of different progenies, which would increase the level of genetic clustering. Young and Merriam (1994) studied four fragmented and four continuous *Acer saccharum* natural populations, and found less mixing of genotypes in fragmented populations than

in continuous populations. Knowles *et al.* (1992) also reported that populations regenerated from limited numbers of reproductive trees showed more obvious genetic structure in *Larix laricina* populations.

#### 4) Effect of self-thinning on genetic structure and variability

The present tree density of AK is quite high (631.2 trees /ha). It is important to know whether the observed differences in genetic structure between two populations will be diminished during the self-thinning process. Simulation of the self-thinning process suggested that the genetic clustering of two-locus genotypes of four linkage-disequilibrium-retained locus pairs would tend to diminish, falling to zero in the 4 x simulation period. The proportion of positively significant *I*s and SNDs also decreased to zero in the simulation. The reason for the weakening of the genetic clustering to more than the level of the KU would be attributed to no regeneration in the simulation. The correlogram of NAC indicated that the genetic clustering would still exist after seven simulation periods. As the NAC is based on multiple loci, it can detect the cumulative effect of faint genetic clustering in individual loci. Therefore, the NAC would detect the underlying genetic structure more sensitively than Moran's *I* and SND. The grand mean of NAC decreased from 1.684 to 1.665 in the simulation but it remained significantly higher than the NAC of KU (1.649), indicating less genetic variability and higher genetic similarity of the AK. Even though the genetic clustering of two-locus genotypes at three linkage-disequilibrium-retained locus pairs were completely eliminated during the simulation, linkage disequilibria still remained. The disequilibrium at a given locus would usually be resolved in a single generation of random mating; however, linkage disequilibrium of combined genotype frequencies between two given loci caused by founder effects is retained over several generations (Hartl and Clark 1997).

#### 5) Implications for genetic conservation and management

It is clear that forest cutting slightly but significantly decreased the genetic variability and reinforced the genetic structure in AK by reducing the mixing of half-sib progenies derived from a limited number of reproductive trees. The changed genetic structure, however, is probably only a temporary effect, as the changes would be almost eliminated during the self-thinning process, according to the simulation. However, linkage disequilibrium and the reduced genetic variability were not eliminated in the self-thinning process simulation. These results suggest that the reduced genetic variability and linkage disequilibrium would have a significant influence over several generations. Reductions in variability imply a higher potential for inbreeding depression, and the existence of linkage disequilibrium means distortions in the composition of the gene set in the population. If the natural composition of the gene set is assumed to be the most highly adapted to a given environment, linkage disequilibrium also implies reductions in the adaptability of populations in succeeding generations, which could be detrimental for conservation of important genetic resources.

## Chapter IV Genetic structure in the northernmost marginal population of Japanese beech population: influence of the founding event on genetic structure

### 1. Introduction

Abundance and distribution patterns of genetic variation within and among plant populations are consequences of genetic processes such as mating system, gene flow, random genetic drift, mutation and selection (Loveless and Hamrick 1984; Hamrick *et al.* 1992). Similarly, spatial genetic structure within plant populations is a consequence of the same genetic processes (Sokal *et al.* 1997; Sokal and Jacquez 1991). Thus, spatial autocorrelation analysis (Sokal and Oden 1978a,b) can be valuable for both examining the genetic structure of a population and identifying the main genetic processes responsible for the structure. The genetic structure of a wide range of forest tree populations has been studied (Berg and Hamrick 1995; Epperson and Allard 1989; Epperson and Alvarez-Buylla 1997; Knowles *et al.* 1992; Young and Merriam 1994). Early studies on conifers found very little clustering of genetic variation, and even when detected, the degree of clustering found was slight (Epperson and Allard 1989; Knowles 1990; Xie and Knowles 1991). In contrast, genetic clustering was frequently found in species in which seed dispersal was limited, such as *Camellia japonica* (Ueno *et al.* 2000), *Quercus* spp. (Bacilieri *et al.* 1994; Berg and Hamrick 1995; Geburek and Knowles 1994; Ubukata *et al.* 1999) and *Fagus crenata* (Kawano and Kitamura 1997;Ohkawa *et al.* 1998; Takahashi *et al.* 2000). Thus, within-population genetic structure is obviously influenced by the mechanism of seed dispersal (Hamrick *et al.* 1993).

Founding events of populations have major effects on within-population genetic variation and structure. The effect generally reduces genetic variation, with respect to the source population, although clinal distributions of genetic variation along geographic ranges can arise from a series of successive founding events (Ledig 2000; Miyamoto *et al.* 2001; Suyama *et al.* 1997; Tomaru *et al.* 1997). Several studies have indicated that within-population genetic structure can also be influenced by re-founding processes after forest cutting (Dayanandan *et al.* 1999; Knowles *et al.* 1992; Takahashi *et al.* 2000).

Beech has been well studied with respect to genetic structure within populations (Kawano and Kitamura 1997; Kitamura *et al.* 1997a,b 1998; Leonardi and Menozzi 1996; Ohkawa *et al.* 1998; Takahashi *et al.* 2000) as well as phylogeography (Demesure *et al.* 1996; Merzeau *et al.* 1994; Ohkawa *et al.* 1998; Tomaru *et al.* 1997, 1998). Phylogeographic studies of *F. crenata* populations covering its whole geographic range have found little differentiation ($G_{ST}$ = 0.038) in the isozymes, but clear differentiation ($G_{ST}$ = 0.963) has been detected in maternally inherited mitochondrial DNA. These results suggest that a much higher rate of gene flow is associated with pollen flow than with seed dispersal (Tomaru *et al.* 1998). Similar results have also been obtained in *F. sylvatica* (Comps *et al.* 1990; Demesure *et al.* 1996). Thus, most of the nuclear genetic variation in *Fagus* has been retained within populations rather than among populations. Studies of the intra-population genetic structure have revealed that genetic clustering in *F. crenata* populations is probably due to limited seed dispersal (Takahashi *et al.* 2000).

As summarized in the Chapter I, palynological studies suggested that the current marginal population, i.e. the Utasai population, was established

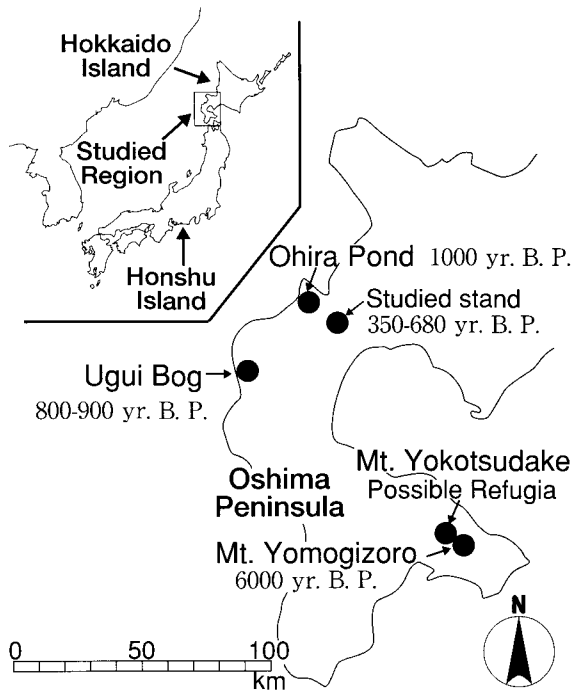


Fig. Ⅳ-1 Location of the studied *Fagus crenata* population, Utasai, and the historical periods when *F. crenata* forest was most likely to be established at five locations on Oshima Peninsula, Hokkaido Island according to palynological analysis by Igarashi (1994) and Takiya and Hagiwara (1997).

by immigrant nuts from populations located further south somewhere between 350 and 680 yr. B.P (Fig. Ⅳ-1). This implies that the Utasai population has undergone only a few generations since it was founded. Simulation studies by Sokal and Wartenberg (1983) and Epperson (1990) indicate that a considerable number of generation times (30 - 50 generations) are needed to reach quasi-stationary status. Therefore, the Utasai population may exhibit little or no within-population genetic structure because of the very few generation cycles that have passed since it was founded.

Here I present a study of genetic variation and structure in the most northerly, marginal population of *F. crenata* in this chapter. I discuss the relationship between its within-population genetic structure and founding process. The population is especially suitable for such analysis because of its well-recorded forest history. Acquiring greater knowledge of how genetic structure changes with time is important, since it will increase our overall understanding of the genetic structure of forest tree species.

## 2. Materials and methods

### 1) The studied population, sampling and isozyme analysis

I studied a beech (*Fagus crenata* Blume) population located in the Kuromatsunai lowland on Oshima Peninsula, Hokkaido Island, Japan (80 m; 42° 38' 56" N, 140° 20' 0" E; designated Utasai or UT). This represents the northernmost habitat of the species. A 100 m x 130 m survey plot was delineated, and all 119 trees in the plot taller than 3 m were mapped. The height and diameter at breast height (DBH) of the 119 trees were measured. Height ranged from 3 - 30 m (mean and standard deviation, 20.9 ± 7.6 m), while the DBH ranged from 4 - 98 cm (mean and standard deviation, 44.8 ± 23.4 cm). A histograms of DBH distribution is presented in Fig. Ⅳ-2. The tree density and basal area covered by *F. crenata* were 91.5 trees/ha and 18.4 $m^2$/ha, respectively. Winter buds were collected from all the mapped trees, and used for the analysis. The following 11 loci, encoding eight enzyme systems, were analyzed: *Mdh-2* and *Mdh-3* (E.C. 1.1.1.37), *6Pg-2* (E.C.1.1.1.44), *Dia-1* and *Dia-2* (E.C.1.8.1.4), *Got* (E.C.2.6.1.1), *Amy-3* (E.C.3.2.1.1), *Aap-1* and *Aap-2* (E.C.3.4.11.2), *Fm* (E.C.4.2.1.2) and *Pgi-1* (E.C.5.3.1.9). Details of the procedures for isozyme analysis have been described previously (Tomaru *et al.* 1997; Tsumura *et al.* 1990)

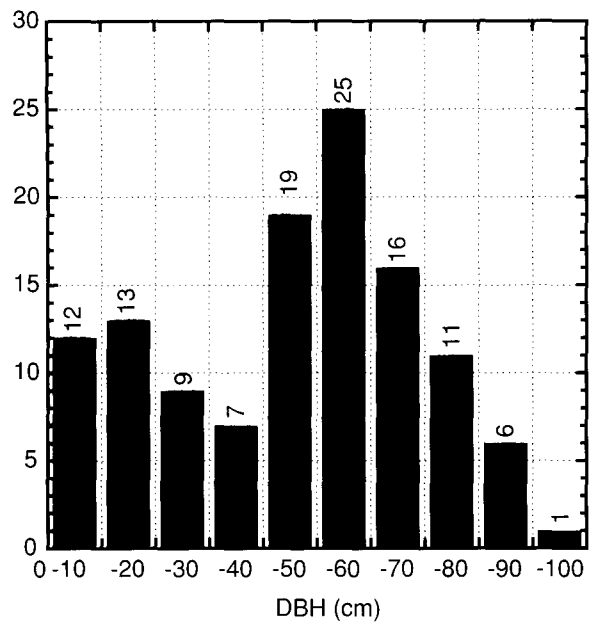


Fig. Ⅳ-2　Distribution of diameter at breast height (DBH) in the Utasai population of *Fagus crenata*.

2) Data analysis

Polymorphic loci were defined as loci where the frequency of the most prevalent allele was less than 0.95. The proportion of polymorphic loci (*Pl*), the average number of alleles per locus (*Na*), the effective number of alleles per locus (*Ne*; Kimura and Crow 1964), the average observed heterozygosity ($H_o$) and the expected heterozygosity ($H_e$; Nei 1987) were calculated and used to determine the degree of genetic variability.

The inbreeding coefficient ($F_{IS}$) was calculated as the average of $f_{is}$ weighted by the sample sizes for each locus, where $f_{is} = 1 - h_o/h_e$; $h_e$ being the expected heterozygosity; and $h_o$ the observed heterozygosity of the locus. Only variable loci with two or more alleles were included in the calculation of $F_{IS}$. The significance of the $f_{is}$ values was tested according Li and Horvitz (1953). Deviation in the genotype frequencies from Hardy-Weinberg expectations was examined at each of the variable loci, and the possibility of linkage disequilibrium between all pairs of the variable loci was examined. Both tests were performed using the chi-square test of the GDA version 1.0 program (Lewis and Zaykin 1999). Alleles with frequencies of less than 0.05 were pooled together as one allele for the two tests.

I examined the genetic structure of the population at three levels at the allelic level using Moran's *I* correlograms, at genotypic level using standard normal deviate (SND; Sokal and Oden 1978a) analysis and at the multilocus level by calculating the number of alleles in common (NAC; Berg and Hamrick 1995; Surles *et al.* 1990). The values, their expectations and variances were calculated over 12 distance classes of 5 m interval (0 - 5 m, 5 - 10 m and so on). For Moran's *I* analysis, I used only the most frequent allele at each polymorphic loci. An average correlogram of *I*, which was simply the arithmetic mean of the individual correlograms, was produced. The variances of average Moran's *I* values were calculated using a bootstrap procedure (sampling alleles with replacement one thousand times). For SND determinations, genotypes whose frequencies lay outside the range 0.05 to 0.95, and whose expected numbers of joins were less than unity, were excluded from the calculation. Eleven like joins and 15 unlike joins satisfied the criteria, and were used for the calculation of SND. For the detail procedures of data analyses, please see the Materials and methods in Chapter Ⅲ.

To evaluate the characteristics of genetic variability and within-population genetic structure at the Utasai population, I compared the results with two previously studied beech populations, that is, an old-growth beech population at Mt. Kurikoma (KU)

Table Ⅳ-1 Detected alleles and their frequencies at the 11 loci examined in the Utasai *Fagus crenata* population.

| | Locus | Allele | | | | |
|---|---|---|---|---|---|---|
| | | *a* | *b* | *c* | *d* | *e* |
| 1 | *Mdh-2* | 1.000 | | | | |
| 2 | *Mdh-3* | | 0.725 | 0.275 | | |
| 3 | *6Pg-2* | | 0.004 | 0.996 | | |
| 4 | *Dia-1* | 0.055 | 0.941 | 0.004 | | |
| 5 | *Dia-2* | 0.046 | 0.924 | 0.030 | | |
| 6 | *Got* | 0.008 | 0.013 | 0.958 | 0.021 | |
| 7 | *Amy-3* | | 0.378 | 0.013 | | 0.609 |
| 8 | *Aap-1* | 0.029 | 0.920 | 0.051 | | |
| 9 | *Aap-2* | | 1.000 | | | |
| 10 | *Fm* | 0.391 | 0.609 | | | |
| 11 | *Pgi-1* | | 0.013 | 0.924 | 0.063 | |

Table Ⅳ-2 Parameters of genetic variability and inbreeding coefficients at the 11 loci examined in the Utasai *Fagus crenata* population.

| No. | Locus | *Na* | *Ne* | $H_e$ | $H_o$ | $F_{IS}$ |
|---|---|---|---|---|---|---|
| 1 | *Mdh-2* | 1 | 1.00 | 0.000 | 0.000 | - |
| 2 | *Mdh-3* | 2 | 1.66 | 0.401 | 0.347 | 0.134 |
| 3 | *6Pg-2* | 2 | 1.01 | 0.008 | 0.008 | 0.000 |
| 4 | *Dia-1* | 3 | 1.13 | 0.112 | 0.084 | 0.248** |
| 5 | *Dia-2* | 3 | 1.17 | 0.143 | 0.126 | 0.120 |
| 6 | *Got* | 4 | 1.09 | 0.082 | 0.084 | -0.026 |
| 7 | *Amy-3* | 3 | 1.94 | 0.488 | 0.504 | -0.034 |
| 8 | *Aap-1* | 3 | 1.18 | 0.151 | 0.151 | -0.005 |
| 9 | *Aap-2* | 1 | 1.00 | 0.000 | 0.000 | - |
| 10 | *Fm* | 2 | 1.91 | 0.478 | 0.513 | -0.072 |
| 11 | *Pgi-1* | 3 | 1.16 | 0.142 | 0.134 | 0.053 |
| Average | | 2.5 | 1.30 | 0.182 | 0.178 | 0.046 |
| | | (0.3) | (0.11) | (0.056) | (0.057) | (0.031) |

Symbols : *Na*, the average number of alleles per locus ; *Ne*, the effective number of alleles per locus ; $H_e$ , the expected heterozygosity ; $H_o$, the observed heterozygosity and $F_{IS}$ , the inbreeding coefficient. Figures in parentheses show standard errors.

** $P < 0.01$

and a secondary beech population, at Mt. Akitakomagatake (AK), that were appeared in the previous chapter.

### 3. Results

#### 1) Genetic variability and linkage disequilibrium

In total, 27 alleles were detected among 11 loci in 119 mapped *Fagus crenata* trees of the Utasai population (Table Ⅳ-1). Two loci, the *Mdh-2* and *Aap-2*, were invariant. The average values and standard errors of the *Pl*, *Na*, *Ne*, $H_e$, and $H_o$ parameters describing the genetic variability at the 11 loci in the population were 64%, 2.5 ± 0.3, 1.30 ± 0.11, 0.182 ± 0.056 and 0.178 ± 0.057, respectively (Table Ⅳ-2). The $f_{is}$ ranged from -0.072 to 0.248. Most of $f_{is}$ values were not significant although the $f_{is}$ at the *Dia-1* locus was significant ($P < 0.01$), indicating an excess of homozygotes at the locus. The $F_{IS}$ value for the Utasai population was 0.046 ± 0.031 (mean and standard error, not significant). No heterogeneity in genotype frequencies was observed at the nine variable loci. Linkage disequilibrium was examined at all possible locus pairs among nine variable loci (36 pairs), and was found to be significant ($P < 0.05$) for two locus pairs (*Mdh-3* & *Amy-3* and *Mdh-3* & *Fm*).

#### 2) Genetic structure

As mentioned in Materials and methods, I used only the most frequent allele at each polymorphic locus for calculating Moran's *I* values. The following seven alleles were used for the calculation: *Mdh-3*$^b$, *Dia-1*$^b$, *Dia-2*$^b$, *Amy-3*$^e$, *Aap-1*$^b$, *Fm*$^b$, and *Pgi-1*$^c$. The average Moran's *I* value for the first distance class was 0.143 (not significant) and the value decreased as distance increased, indicating that genetic similarity decreases as distance increases (Fig. Ⅳ-3). The correlograms of *Mdh-3*$^b$ and *Dia-2*$^b$ showed positively significant *I*s in the shortest distance class, although the two correlograms were not significant with respect to the Bonferroni criteria. The proportion of positively significant *I*s in the shortest distance class was 0.29. The number of significant alleles in the 12 distance classes is presented in Fig. Ⅳ-4. No negatively significant *I*s were detected at distance classes less than 25 m. None of the seven individual correlograms showed an *I* value which differed significantly from that of the average correlogram, suggesting no selection was operating on the seven studied loci. Of 11 like joins considered, only the *b/c* genotype at the *Mdh-3* (SND = 2.735) was positively significant ($P < 0.01$) in the first distance class. Among the 15 unlike joins, two pairs were significantly negative in the first distance class (SND (*Mdh-3*, *b/b - c/c*) = 2.434; SND (*Fm*, *a/b - b/b*) = -2.212). Negatively significant unlike joins are thought to reflect the cumulative effects of the positive association of like joins. None of the positively and negatively significant correlograms were significant according to the Bonferroni criteria. The grand mean and the standard deviation of the NAC was 1.629 ± 0.002. The correlogram of the NAC values also showed a decrease in genetic similarity with increasing distance, although no NAC significantly differed from the grand mean NAC value (Fig. Ⅳ-5). Thus, this multilocus analysis indicated that there was no significant genetic clustering in the plot.

### 4. Discussion

#### 1) Genetic variability and linkage disequilibrium

I compared the genetic variability of UT with that of the KU population, an old-growth population, using information on isozyme frequencies at nine common

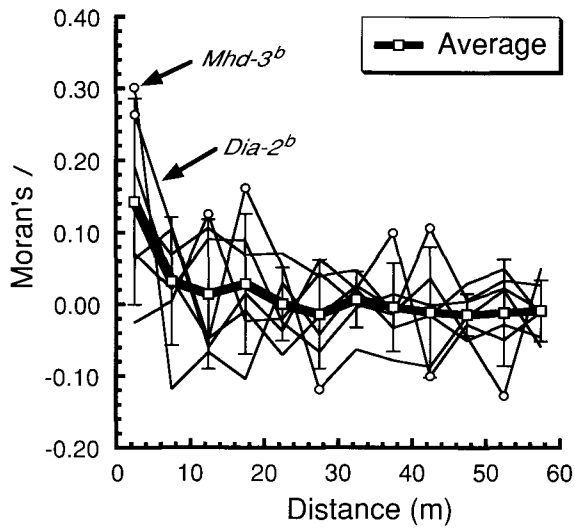


Fig.Ⅳ-3 Individual and average Moran's *I* correlograms of the Utasai *Fagus crenata* population, Oshima Peninsula, Hokkaido Island.

The bold line shows average values. Error bars denote standard deviations. Open circles denote significant values at the 5% probability level. Alleles with positively significant values in the first distance class are shown by name. Variances of average correlogram were estimated using the bootstrap procedure.

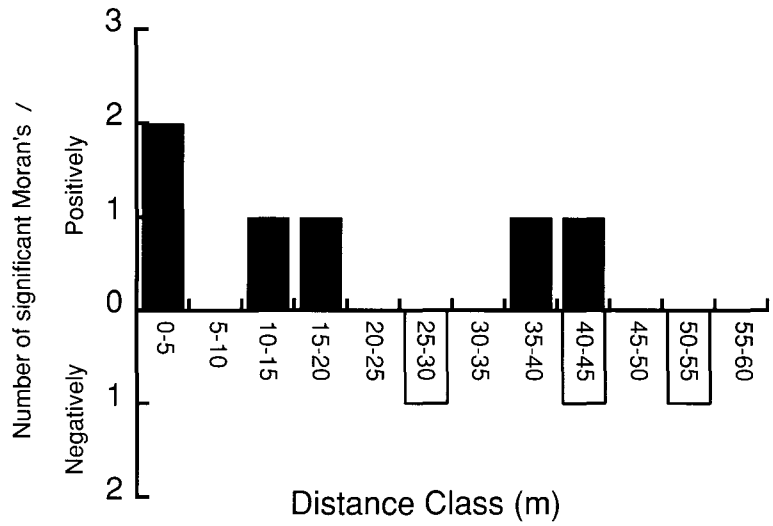


Fig. Ⅳ-4 Numbers of positively and negatively significant Moran's *I* values in the Utasai *Fagus crenata* population, Oshima Peninsula, Hokkaido Island.

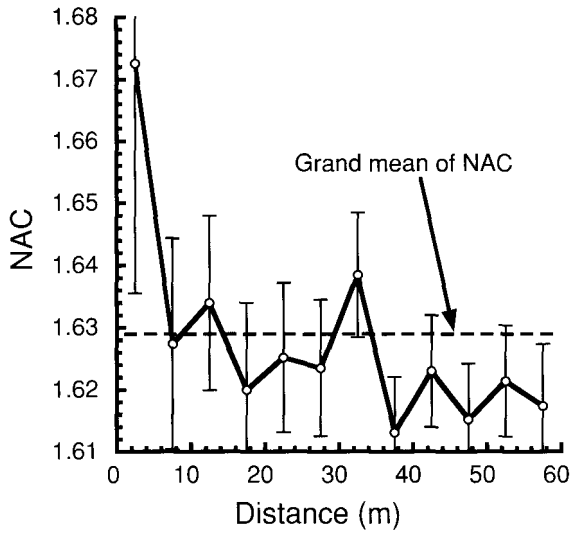


Fig. Ⅳ-5　NAC values over 12 distance classes in the Utasai *Fagus crenata* population, Oshima Peninsula, Hokkaido Island.

The grand NAC mean is shown by the dashed-line. No NAC values significantly differed from the grand mean at the 5% probability level. Variances of the NAC values were estimated using the bootstrap procedure. Error bars show standard deviations.

loci (except the *Mdh-2* and *Aap-2*). Most parameters were similar between the two populations, suggesting that UT had similar genetic variability to the KU populations. However, the number of alleles at the *Amy-3* was only three in the UT, while the corresponding value was seven in KU. The *Na* for UT was slightly (but not significantly) lower than those for KU, perhaps due to the putative founder effects that influenced the Utasai population. This tendency is consistent with the results from a previous study by Takahashi *et al.* (1994), who found that rare alleles at low frequencies in populations on Honshu Island were absent in populations on Hokkaido Island. The cited authors discussed that this tendency could be due to founder effects that occurred during the colonization process as *F. crenata* extended its range northwards after the last glacial period. Similar reductions in the number of alleles in shifts from putative refugia to current marginal regions, presumed to be due to a series of founding events, have been found in *Pinus coulteri* (Ledig 2000) and *Alnus trabeculosa* (Miyamoto *et al.* 2001).

Linkage disequilibrium was found at two pairs of loci in UT, but none were observed in the old-growth population, KU. Igarashi (1994) suggested that *F. crenata* extended its distribution northwards approximately 20 m / yr. on Hokkaido Island. Takiya and Hagiwara (1997) estimated the movement to be closer to 11 m / yr. However, it usually takes about 40 years for *F. crenata* to be mature enough to produce nuts (Hashizume 1981), which are then dispersed no further than 30 m from the parent tree (Hashizume *et al.* 1984; Maeda 1988) when they drop. Thus, these estimated migration rates are clearly impossible unless other dissemination mechanisms capable of accelerating migration were involved, for example, long distance seed dispersal by birds, such as Japanese nutcrackers (*Nucifraga caryocatactes*

*japonicus*) and jays (*Garrulus gladnarius pallidifrons*; Watanabe 1990). Many thousands of green *F. grandifolia* nuts were dispersed by blue jays (*Cyanocitta cristata*) in a woodlot was studied in southeastern Wisconsin, and the distances the jays carried the nuts ranged up to 4 km (Johnson and Adkisson 1985). The number of cached nuts per trip ranged from three to 14, averaging seven. The authors considered that carriage by blue jays may be the primary means by which beech seeds were dispersed to patches of forest lacking *F. grandifolia*. These considerations suggest that the Utasai population may have been established from small numbers of seeds collected by birds. Founder effects can cause not only reductions in the number of alleles but also linkage disequilibrium (Hartl and Clark 1997), so the disequilibrium observed in the Utasai population could be related to events in the founding process of the population. Once linkage disequilibrium has been established, several generations are needed to dissipate it, even if the loci are not actually linked on a chromosome (Hartl and Clark 1997). Since the Utasai population appears to have been founded some time around 350 - 680 yr. B. P., the population may not yet have had enough time to dissipate linkage disequilibrium generated during its foundation, but it would be expected to diminish in future generations.

### 2) Genetic structure

The Moran's *I* and NAC correlograms showed that values of these coefficients decreased as the distance increased. Two allelic and three genotypic (one like join and two unlike joins) correlograms were significant in the first distance class. These results suggest that genetic clustering was present in the Utasai population. However, none of the correlograms for UT was significant according to Bonferroni criteria, although the proportion of significant correlograms based on these criteria was 0.43 in KU (Takahashi *et al.* 2000). In UT, the proportions of positively significant *I*, positively significant like joins, and negatively significant unlike joins in the first distance class were 0.29, 0.09, and 0.13, respectively. The corresponding proportions in KU were 0.43 (when the first two distance classes were pooled), 0.29 and 0.09 (Takahashi *et al.* 2000). Thus, the genetic structure in UT is weaker and less clear than in KU. These findings can be explained by conclusions derived from simulation studies (Sokal and Wartenberg 1983; Epperson 1990). Sokal and Wartenberg (1983) examined temporal aspect of genetic structure in a series of Monte-Carlo simulations, and found that correlograms became significant within the first five generations of foundation, even when starting from a random distribution of genetic variation (Sokal and Wartenberg 1983). However, the *I* values in the first distance class and patch sizes, as defined by the X-intercepts of the correlograms, continued to increase thereafter. The simulations showed 30 - 50 generations were needed before the correlograms reach a quasi-stationary status (Epperson 1990). The UT population can be considered a very young population in terms of generations since its foundation. The number of generations that UT has undergone may not be sufficient for it reach the quasi-stationary status in genetic structure that the species would be expected to develop eventually, i.e. it could still be progressing towards its inherent genetic structure endpoint.

The UT population and the AK population (which was 're-founded' from a few remnant trees after

forest cutting during the 1920s) showed interesting, divergent, differences from the KU population. The AK population retained stronger linkage disequilibrium (12 out of 36 locus pairs) than UT (two out of 36 locus pairs). Furthermore, the proportions of positively significant like joins (0.09) and allelic correlograms (0.00; according to the Bonferroni criterion) in the first distance class in the UT population were smaller than those of KU (0.29 and 0.43), while the corresponding for AK (0.38 and 0.86) were larger than those of KU. No NAC values were significant in UT, while NAC values of the other two populations were positively significant at shorter distance classes. If we assume the genetic structure for KU, an old-growth forest, to be typical of old-growth populations of the species, the genetic structure in UT was weaker than old-growth forest, whereas that in AK was stronger.

The within-population pattern of genetic variation is not independent of previous generations, especially in species that have limited seed dispersal mechanisms (Hamrick *et al.* 1993). Instead, the genetic structure of current generations may be influenced by the cumulative effect of the genetic clustering of many preceding generations (Knowles *et al.* 1992). Knowles *et al.* (1992) studied two tamarack (*Larix laricina*) populations that have markedly different anthropological disturbance histories. One population had regenerated from a site with scattered remnant trees but with no other seed source nearby. The other had regenerated from a site that had no remnant trees, but it was surrounded by abundant sources of seed. The former showed spatial autocorrelation, whereas the latter did not. The cited authors also drew attention to the role that preceding generations could play in the genotypic arrays of the remnant trees. The Utasai population was likely to be founded from seeds randomly cached by birds from more southern populations that may be genetically unrelated to each other. In contrast, in AK, the genotypic array of the population should reflect the genetic clustering of the trees present prior to the forest cutting. Thus, the UT and AK populations resulted from very different founding processes (or, more precisely, 're-founding' in the case of AK) in terms of the influence of genetic structure before their foundation. One plausible explanation for the differences in genetic structure between the UT and AK populations is that UT may have been established at a place where *F. crenata* had not existed previously, so it was not influenced by a pre-existing genetic structure, while AK would be expected to reflect, at least in part, the genetic structure of the preceding generations.

## Chapter V　Demographic and genetic processes operating on a Japanese beech current-year seedling population

### 1. Introduction

When considering evolutionary processes within plant populations, demographic (Alvarez-Buylla *et al.* 1996b; Farris and Mitton 1984; Linhart *et al.* 1981; Tonsor *et al.* 1993) and population genetic (Hartl and Clark 1997) processes are important components. Most forest tree species, as compared to plant species with other life forms, retain an abundant amount of genetic variation that is structured within populations (Hamrick *et al.* 1992; Muona 1989). The within-population genetic variation is maintained and carried from generation via genetic processes, such as mating system, gene flow by pollen and seed, inbreeding depression, random genetic drift, mutation, and

selection (Hartl and Clark 1997). It is important to know how these processes maintain within-population genetic variation in species, and how they interact with each other to achieve optimal genetic variation that ensures population adaptation and survival (Wright 1982). Additionally, understanding of the microevolutionary mechanisms of plant species can help in planning conservation strategies (Alvarez-Buylla *et al.* 1996b; Epperson 1990, 1992).

Early studies used genetic markers and a demographic perspective in experimental populations of cereal plants to identify the viability and fertility components of selection (Clegg and Allard 1973; Clegg *et al.* 1978). Subsequently, this approach has been used to characterize the genetic processes within plant populations (Alvarez-Buylla *et al.* 1996b; Farris and Mitton 1984; Linhart *et al.* 1981; Tonsor *et al.* 1993). In a population of *Plantago lanceolata*, a weedy perennial herb, an excess of homozygote individuals in the seed bank, relative to the Hardy-Weinberg equilibrium, decreased over the course of the plant life cycle and no excess homozygote individuals were observed in the adult stage (Tonsor *et al.* 1993). Similarly, temporal changes occurred in the genetic variation from seed-rain seeds to adults, in a population of *Cecropia obtusifolia*, a tropical pioneer tree (Alvarez-Buylla *et al.* 1996a). Excess homozygote individuals were eliminated in a naturally regenerated *Pinus sylvestris* population at between 10 and 20 years of age (Yazdani *et al.* 1985), and at three years of age in an artificial population (Muona *et al.* 1987). Heterogeneity of genetic variation is evident among life-cycles-stages, as observed in individuals grouped by gaps, as in the *Cecropia obtusifolia* population (Alvarez-Buylla *et al.* 1996a), or by clumps, as in a *P. ponderosa* population (Linhart *et al.* 1981). Thus, it is important to integrate genetic and demographic data to obtain a finer picture of the temporal change in genetic variation within populations (Alvarez-Buylla *et al.* 1996a,b).

Forest tree species usually produce large numbers of seeds under selection pressure, and proportionately few survive until maturation. Selection processes are intense in the early stages(Epperson 1992),particularly in inbred individuals (Muona *et al.* 1987; Yazdani *et al.* 1985). Therefore, because of the drastic reduction in population, massive mortality during the seedling stage is important in life cycle fluctuation in genetic variation. In this study, I present data on temporal changes in genetic variation coupled with demographic data, within a *Fagus crenata* current-year seedling population.

## 2. Materials and methods

### 1) Studied beech population and field methods

Seedling and adult populations of *Fagus crenata* were studied. For adult tree studies, a survey plot (adult-tree plot) covering 2.1 ha was delineated in a natural *F. crenata* forest located at the southern foot of Mt. Kurikoma (870 m; 38° 55' 30" N, 140° 47' 50" E). The *F. crenata* population produced an abundant amount of nuts in the autumn of 1995. All adult trees that produced nuts in the autumn ($n$=220) were mapped within the adult-tree plot (Fig. V-1). The range of diameters at breast height (DBHs) of the trees was 20 - 109 cm (average: 51.2 ± 16.9 cm), while adult nut-producing trees had a DBH larger than 50 cm.

To investigate the genetic variation of the seedling population, I delineated a survey plot (50 m long and 1 m width), almost at the center of the adult-tree plot in late spring in 1996 (Figs. V-1,2). The plot was

divided into two 0.5-m-wide subplots: the observation subplot (OP) and the sampling subplot (SP). Moreover, each subplot was divided into five 10-m-long blocks. The OP was used to investigate the emergence and disappearance of *F. crenata* current-year seedlings, and only those that survived were sampled in the following autumn. All germinating seedlings, defined as seedlings whose cotyledons were fully expanded, were sampled in the SP during field observations, conducted seven times during the summer and autumn (5 June, 13 June, 20 June, 5 July, 30 July, 30 August, and 21 October.) Newly germinated seedlings were labeled and recorded, including the causes of mortality (*i.e.*, damping-off, herbivory, and unknown). were also recorded in the observation subplot. All the surviving seedlings in the OP were sampled on the 21 October. Winter buds were also collected from adult trees in the adult tree plot. All samples were subjected to isozyme analysis.

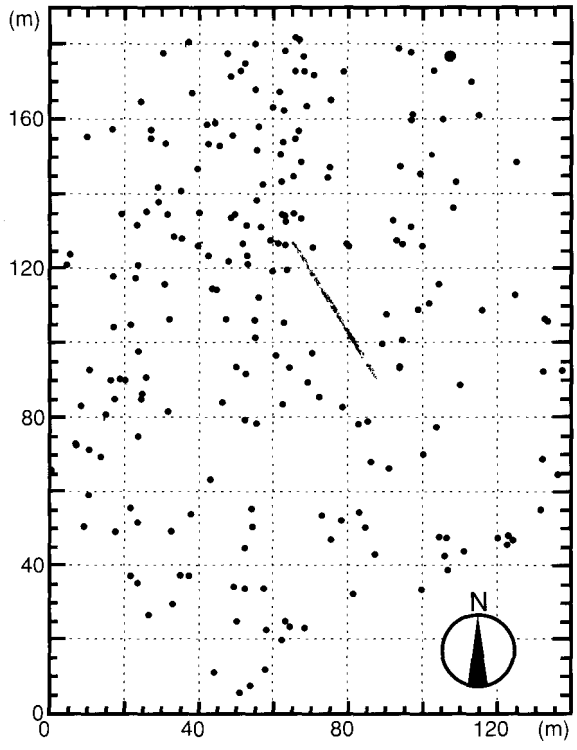


Fig. V-1　Scattergram of investigated *Fagus crenata* current-year seedlings and adult trees in a *F. crenata* forest at Mt. Kurikoma, in northern Honshu, Japan.
Solid circles and small dots denote investigated adult trees ($n$ = 220) and current-year seedlings ($n$ = 1,408), respectively.

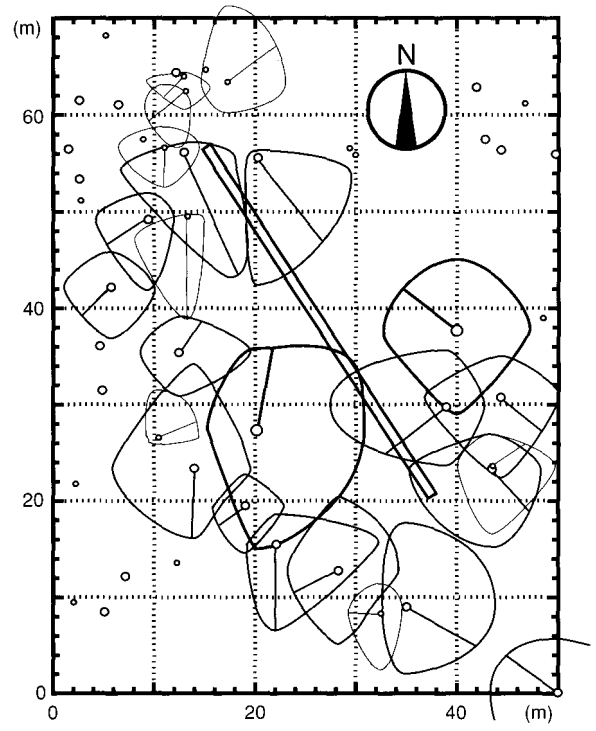


Fig. V-2　Location of a seedling plot and adult *Fagus crenata* trees and the crown cover of the adult trees that surrounded the plot.
Rectangle denotes the seedling plot (50m long x 1m wide). Open circles denote adult trees and the sizes of the circles represent the relative DBH.

### 2) Isozyme analysis

Fifteen mg of hypocotyl from each seedling were used for isozyme analysis, as described previously (Tsumura *et al.* 1990; Tomaru *et al.* 1997). The following six loci, encoding five enzyme systems, were analyzed: *Mdh-3* (EC 1.1.1.37), *Dia-1* and *Dia-2* (EC 1.8.1.4), *Amy-3* (EC3.2.1.1), *Fm* (EC 4.2.1.2) and *Pgi-1* (EC 5.3.1.9).

### 3) Data analysis

Cumulative germination and survival rates were

calculated for five blocks of the OP, and the changes were compared using the pairwise Kolomogorov-Smirnov test. All germinating seedlings in the OP were divided into four sub-cohorts by sampling date (5 June, 13 June, 20 June and 5 July). The sub-cohort observed on 30 July was excluded from the analysis because of its small sample size. Differences in the final survival rate on 21 October were compared among the five blocks and among the four sub-cohorts using an analysis of variance (ANOVA). Additionally, temporal changes in the survival rate of current-year seedlings were analyzed in relation to rainfall data, collected from June to October in 1996 at the nearest meteorological station, Komanoyu (3.5 km east of the study forest, 570 m in altitude). The Kolomogorov-Smirnov and ANOVA tests were performed using the SAS procedures nparlway and glm (SAS 1988, 1996).

As the sampling and observation subplots were side by side, I assumed that they retained similar amounts of genetic variation. The following polymorphism indices, described in Chapter Ⅲ, were calculated to determine the amount of genetic variation in the two seedling sub-populations and the adult population: the proportion of polymorphic loci (*Pl*; regarded as the loci where the frequency of the most common allele < 0.95); the average number of alleles per locus (*Na*); the effective number of alleles per locus (*Ne*); the observed and expected heterozygosities ($H_o$ and $H_e$); and the inbreeding coefficient ($F_{IS}$). Differences in values of polymorphism indices of five blocks both between two seedling sub-populations and among three sub-cohorts (the 5 July and 30 July sub-cohorts in the observation subplot were excluded because of their small sample sizes) were tested using the ANOVA. The genetic variation within the seedling population was compared with two adult tree populations: 1) All 220 mapped *F. crenata* adult trees (referred to as the whole adult population or ADW), and 2) 14 adult trees from which the distances to the seedling subplot were less than 10 m (referred to as the local adult population or ADL). To assess the degree of genetic differences between seedling and adult tree populations, Nei's genetic identity (*I*) was calculated (Nei 1987).

### 3. Results

#### 1) Demography of seedlings

A total of 1,179 seedlings germinated in the OP between June 5 and October 21 (Table V-1). Most

Table V-1 Demography of *Fagus crenata* current-year seedings in the observation subplot at Mt. Kurikoma, northern Honshu, Japan.

| | 5 Jun. | 13 Jun. | 20 Jun. | 5 Jul. | 30 Jul. | 30 Aug. | 21 Oct. |
|---|---|---|---|---|---|---|---|
| No. of emerging seedlings | 579 | 505 | 59 | 34 | 2 | | |
| No. of dead seedlings | | 11 | 26 | 334 | 508 | 20 | 9 |
| Cumulative no. of emerging seedlings | 579 | 1,084 | 1,143 | 1,177 | 1,179 | 1,179 | 1,179 |
| Cumulative no. of dead seedlings | | 11 | 37 | 371 | 879 | 899 | 908 |
| No. of living seedlings | 579 | 1,073 | 1,106 | 806 | 300 | 280 | 271 |
| Mortality factors | | | | | | | |
| Insects | 6 | 15 | 8 | 1 | | 2 | |
| Damping-off | | 1 | 4 | 326 | 477 | | 4 |
| Unknown | | 4 | 7 | 0 | 30 | 20 | 3 |

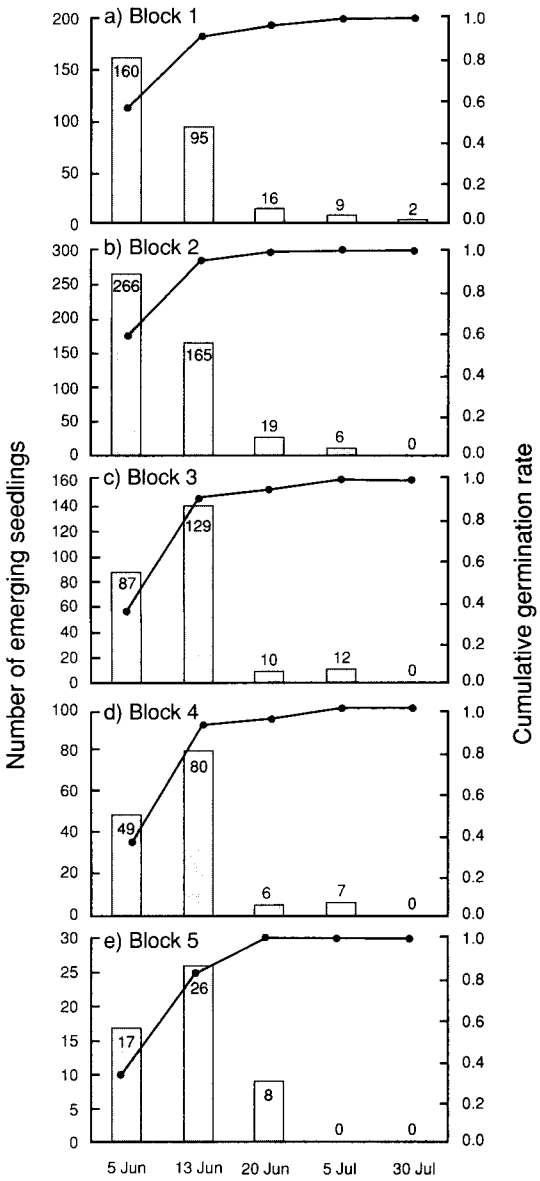


Fig. V-3　The number of emerging *Fagus crenata* current-year seedlings and their cumulative germination rates at five seedling blocks at Mt. Kurikoma, northern Honshu, Japan.

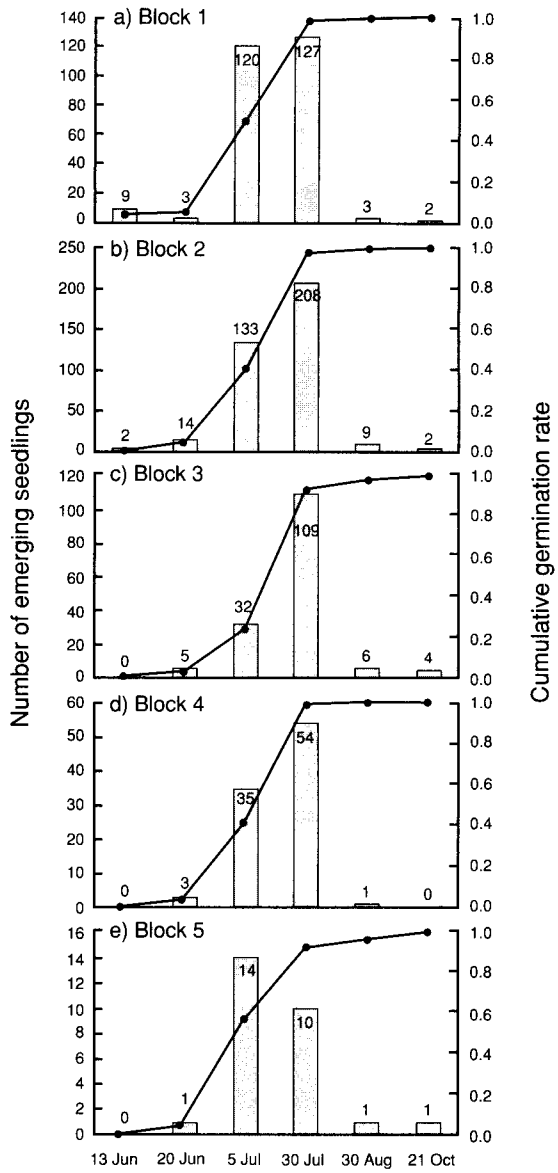


Fig. V-4　The number of dead *Fagus crenata* current-year seedlings and their cumulative mortality rates at five seedling blocks, Mt. Kurikoma, northern Honshu, Japan.

seedlings germinated before 13 June (91.9%), although emergence continued until July 30. Their numbers and cumulative germination rates are shown in Fig. V-3. The number of germinating seedling was highest on June 5 in blocks 1 and 2, and on June 13 in blocks 3, 4, and 5. In addition, the distribution patterns of the cumulative germination rate were compared and, out of ten pairwise comparisons, six pairs (between blocks 1 or 2 and blocks 3, 4, or 5) were significant, suggesting that the rate changes differed among them. Of the 1,179 germinating seedlings, 271 seedlings (23.0%) survived until October 21 (Table V-1). The deaths of the remaining 908 seedlings were concentrated between June 20 and July 30, and the numbers and cumulative mortality rate are shown in Fig. V-4. The drastic decline in survival between July

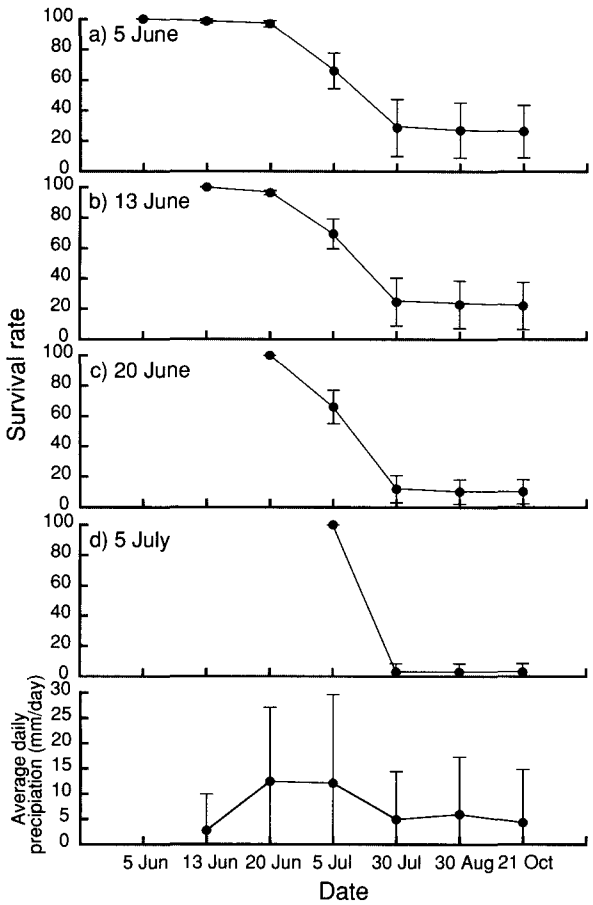


Fig. V-5 Changes in survival rate for four *Fagus crenata* current-year seedling sub-cohorts and the average daily rainfall during the observation period, at Mt. Kurikoma, northern Honshu, Japan.
Error bars denote one standard deviation. The rainfall data from June to October 1996 are from the nearest meteorological station, Komanoyu, 3.5 km east of the surveyed forest at an altitude of 570 m.

5 and July 30 was consistent throughout the five blocks, as shown by pairwise Kolomogorov-Smirnov tests, in which three out of ten comparisons were significant, suggesting that real changes in the cumulative mortality rates between block 3 and blocks 1, 2, and 5. However, the lower mortality rate in block 3 on July 5 contributed to the significant differences. The average survival rates and standard deviations of the four sub-cohorts in all blocks are presented in Fig. V-5 (excluding the small July 30 sample). Before June 20, herbivores were primarily responsible for seedling death, whereas between July 20 and 30, damping-off was the main cause of death. The proportion of the causes of seedling disappearance was temporally heterogeneous ($P<0.01$; Chi-square test). The decline of seedlings due to damping-off was associated with abundant rainfall (Fig. V-5). The final survival rate, which was significantly lower in seedlings that emerged later ($P<0.05$, ANOVA), ranged from 2.94% (the 5 July sub-cohort) to 26.25%. (the 5 June sub-cohort). Thus, seedlings that emerged earlier were predominant in the surviving population.

### 2) Genetic variability

Isozyme analysis was used to estimate genetic variation in 271 seedlings in the OP, 1,137 seedlings in the SP, and 220 nut-producing adult trees, totaling 1,628 individuals. As the sampling and observation subplots were side by side, it was assumed that they retained similar amounts of genetic variation. Twenty-seven alleles were detected at the six surveyed loci encoding five enzyme systems (Tables V-2, V-3, and V-4). The polymorphism indices of the SP were: $Pl$ = 83 %, $Na$ = 3.8, $Ne$ = 1.73, $H_e$ = 0.370, and $H_o$ = 0.387, those for the OP were 83 %, 3.5, 1.65, 0.331, and 0.347, respectively (Table V-5), and those for the four sub-cohorts in the SP are presented in Table V-6. With the exception of $Pl$, all the indices in the OP were smaller than those in the SP. ANOVA showed that the $Ne$ values in the five blocks of the OP were significantly smaller than those of the SP ($P < 0.01$), while differences in the other indices were not significant between the two subplots.

For the ADL, the polymorphism indices, except the $Na$ value, did not differ significantly from those for the OP and SP. The $Na$ value for the ADL was

smaller than for the two subplots, and when it was compared, using the pairwise *t*-test, it was significantly lower than the values for the SP ($P < 0.05$), while it did not differ from those for the OP ($P < 0.10$). For the ADW, the polymorphism indices, except the *Pl* value, were consistently lower than those for the two subplots. The locus-based pairwise *t*-test indicated that the *ne*, $h_e$, and $h_0$ values for the ADW were significantly lower than those of the sampling subplot ($P < 0.05$).

The $F_{IS}$ values were compared between the ADW and two subplots of seedlings (Fig. V-6). Although most of the individual $F_{IS}$ values were not significantly different from zero, the $F_{IS}$ for *Dia-1* in

Table V-2 Allele frequencies at six loci in the current-year seedlings and adult trees of *Fagus crenata* at Mt. Kurikoma, northern Honshu, Japan.

| Locus | Allele | Seedling Subplot | | Adult trees | |
|---|---|---|---|---|---|
| | | SP | OP | ADL | ADW |
| *N* | | 1,137 | 271 | 14 | 220 |
| *Mdh-3* | *b* | 0.730 | 0.770 | 0.893 | 0.861 |
| | *c* | 0.270 | 0.230 | 0.107 | 0.137 |
| | *d* | | | | 0.002 |
| *Dia-1* | *a* | 0.185 | 0.192 | 0.214 | 0.075 |
| | *b* | 0.810 | 0.808 | 0.786 | 0.920 |
| | *c* | 0.001 | | | |
| | *d* | 0.001 | | | 0.005 |
| | *e* | 0.001 | | | |
| *Dia-2* | *a* | 0.101 | 0.006 | | 0.016 |
| | *b* | 0.821 | 0.943 | 0.893 | 0.907 |
| | *c* | 0.074 | 0.035 | 0.107 | 0.061 |
| | *d* | 0.004 | 0.016 | | 0.014 |
| | *e* | | | | 0.002 |
| *Amy-3* | *a* | 0.192 | 0.199 | | 0.041 |
| | *b* | 0.259 | 0.235 | 0.286 | 0.352 |
| | *c* | 0.012 | 0.009 | | 0.009 |
| | *d* | 0.023 | 0.026 | 0.036 | 0.014 |
| | *e* | 0.503 | 0.515 | 0.642 | 0.566 |
| | *f* | 0.008 | 0.005 | | 0.011 |
| | *g* | 0.003 | 0.011 | 0.036 | 0.007 |
| *Fm* | *a* | 0.427 | 0.407 | 0.357 | 0.266 |
| | *b* | 0.573 | 0.593 | 0.643 | 0.734 |
| *Pgi-1* | *b* | 0.007 | 0.009 | | 0.011 |
| | *c* | 0.962 | 0.954 | 0.893 | 0.941 |
| | *d* | 0.031 | 0.035 | 0.107 | 0.048 |
| | *e* | | 0.002 | | |

SP and OP are the sampling and observation subplots for seedlings, respectively. ADL included only the 14 adult trees within 10 m of the seedling plot, and ADW included all mapped adult trees.

Table V-3 Allele frequencies at the six loci in the five blocks within the two seedling subplots of *Fagus crenata* current-year seedling at Mt. Kurikoma, northern Honshu, Japan.

| Locus | Allele | Blocks in the SP subplot | | | | | Blocks in the OP subplot | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| *N* | | 74 | 125 | 243 | 569 | 126 | 25 | 47 | 93 | 88 | 18 |
| *Mdh-3* | *b* | 0.912 | 0.831 | 0.746 | 0.687 | 0.691 | 0.920 | 0.859 | 0.739 | 0.738 | 0.625 |
| | *c* | 0.088 | 0.169 | 0.254 | 0.313 | 0.309 | 0.080 | 0.141 | 0.261 | 0.262 | 0.375 |
| | *d* | | | | | | | | | | |
| *Dia-1* | *a* | 0.115 | 0.136 | 0.216 | 0.207 | 0.119 | 0.140 | 0.106 | 0.263 | 0.193 | 0.111 |
| | *b* | 0.885 | 0.860 | 0.782 | 0.787 | 0.881 | 0.860 | 0.894 | 0.737 | 0.807 | 0.889 |
| | *c* | | 0.004 | | 0.001 | | | | | | |
| | *d* | | | 0.002 | 0.002 | | | | | | |
| | *e* | | | | 0.003 | | | | | | |
| *Dia-2* | *a* | 0.023 | 0.071 | 0.116 | 0.122 | 0.042 | | | 0.017 | | |
| | *b* | 0.901 | 0.868 | 0.823 | 0.792 | 0.866 | 0.900 | 0.913 | 0.932 | 0.980 | 1.000 |
| | *c* | 0.068 | 0.061 | 0.056 | 0.085 | 0.075 | 0.100 | 0.087 | 0.017 | 0.010 | |
| | *d* | 0.008 | | 0.005 | 0.001 | 0.017 | | | 0.034 | 0.010 | |
| | *e* | | | | | | | | | | |
| *Amy-3* | *a* | 0.139 | 0.188 | 0.258 | 0.193 | 0.091 | 0.140 | 0.106 | 0.253 | 0.227 | 0.111 |
| | *b* | 0.410 | 0.220 | 0.213 | 0.254 | 0.329 | 0.360 | 0.309 | 0.188 | 0.210 | 0.222 |
| | *c* | 0.014 | 0.012 | 0.019 | 0.009 | 0.012 | | 0.011 | 0.005 | 0.017 | |
| | *d* | | 0.016 | 0.010 | 0.032 | 0.024 | | 0.011 | 0.016 | 0.045 | 0.056 |
| | *e* | 0.430 | 0.540 | 0.492 | 0.503 | 0.532 | 0.480 | 0.520 | 0.528 | 0.495 | 0.583 |
| | *f* | 0.007 | 0.008 | 0.004 | 0.009 | 0.012 | 0.020 | 0.011 | 0.005 | | |
| | *g* | | 0.016 | 0.004 | | | | 0.032 | 0.005 | 0.006 | 0.028 |
| *Fm* | *a* | 0.414 | 0.411 | 0.447 | 0.437 | 0.367 | 0.480 | 0.468 | 0.376 | 0.414 | 0.278 |
| | *b* | 0.586 | 0.589 | 0.553 | 0.563 | 0.633 | 0.520 | 0.532 | 0.624 | 0.586 | 0.722 |
| *Pgi-1* | *b* | 0.041 | 0.004 | 0.006 | 0.005 | 0.004 | 0.060 | | 0.005 | 0.006 | |
| | *c* | 0.898 | 0.940 | 0.959 | 0.977 | 0.956 | 0.860 | 0.926 | 0.968 | 0.977 | 0.971 |
| | *d* | 0.061 | 0.056 | 0.035 | 0.018 | 0.040 | 0.060 | 0.074 | 0.027 | 0.017 | 0.029 |
| | *e* | | | | | | 0.020 | | | | |

SP and OP are the sampling and observation subplots for seedlings, respectively. *N* denotes sample numbers of four populations.

the ADW was positively significant ($P < 0.01$), and those for the *Dia-1* and *Amy-3* in the SP were negatively significant ($P < 0.001$). The average $F_{IS}$ value with its standard error for the ADW was 0.031 ± 0.039, and the corresponding values for the SP and OP were -0.047 ± 0.027 and 0.048 ± 0.038, respectively, although none of the averages were significantly different from zero.

The genetic variation in the four sub-cohorts was compared using the polymorphism indices (Table V-6). They showed that the more lately germinating cohorts (June 20 and July 5) possessed less genetic diversity. The ANOVA comparisons among the four sub-cohorts indicated that the differences in the *Na* and *Ne* values were statistically significant ($P < 0.001$ for the *Na* and $P < 0.05$ for *Ne*).

Differences in genetic variation between the two subplots for seedlings and the adult population were examined using Nei's genetic identity, *I* (Fig.V-7). The adult trees included in the population depended on the distance from the seedling plot. The *I* values were high between the OP and ADL (0.995 ± 0.007)

and between the SP and ADL (0.990 ± 0.007). The $I$ values decreased with increasing distance between the adult trees and the seedling plot, and the difference was significant when the distance was within 20 m. A comparison of the $I$ values for OP and ADW (0.989 ± 0.005, $P < 0.05$) and for SP and ADW (0.986 ± 0.005, $P < 0.01$) suggests that the genetic variation of the ADW differed from that in the two subplots. Additionally, the $I$ values show that genetic variation of seedlings was most homogeneous with adult trees within 10 m of the seedling plot.

Table V-4 Allele frequencies at the six loci in the seven sub-cohorts within the two seedling subplots of *Fagus crenata* at Mt. Kurikoma, northern Honshu, Japan.

| Locus | Allele | Sub-cohorts in the SP subplot | | | | Sub-cohorts in the OP subplot | | |
|---|---|---|---|---|---|---|---|---|
| | | 5 Jun | 13 Jun | 20 Jun | 5 Jul | 5 Jun | 13 Jun | 20 Jun |
| *N* | | 622 | 415 | 75 | 25 | 152 | 111 | 6 |
| *Mdh-3* | *b* | 0.714 | 0.727 | 0.882 | 0.840 | 0.741 | 0.806 | 0.833 |
| | *c* | 0.286 | 0.273 | 0.118 | 0.160 | 0.259 | 0.194 | 0.167 |
| | *d* | | | | | | | |
| *Dia-1* | *a* | 0.178 | 0.208 | 0.140 | 0.120 | 0.178 | 0.207 | 0.205 |
| | *b* | 0.817 | 0.791 | 0.846 | 0.880 | 0.822 | 0.793 | 0.750 |
| | *c* | 0.001 | | 0.007 | | | | |
| | *d* | 0.002 | 0.001 | | | | | |
| | *e* | 0.002 | | 0.007 | | | | |
| *Dia-2* | *a* | 0.144 | 0.022 | 0.079 | 0.091 | 0.012 | | |
| | *b* | 0.789 | 0.882 | 0.842 | 0.818 | 0.934 | 0.957 | 0.875 |
| | *c* | 0.067 | 0.085 | 0.079 | 0.091 | 0.036 | 0.036 | |
| | *d* | | 0.011 | | | 0.018 | 0.007 | 0.125 |
| | *e* | | | | | | | |
| *Amy-3* | *a* | 0.180 | 0.208 | 0.203 | 0.188 | 0.197 | 0.212 | 0.083 |
| | *b* | 0.252 | 0.257 | 0.311 | 0.333 | 0.211 | 0.257 | 0.417 |
| | *c* | 0.013 | 0.012 | 0.007 | | 0.007 | 0.014 | |
| | *d* | 0.020 | 0.029 | 0.014 | | 0.023 | 0.027 | 0.083 |
| | *e* | 0.527 | 0.478 | 0.458 | 0.479 | 0.542 | 0.476 | 0.417 |
| | *f* | 0.007 | 0.010 | 0.007 | | 0.010 | | |
| | *g* | 0.001 | 0.006 | | | 0.010 | 0.014 | |
| *Fm* | *a* | 0.426 | 0.440 | 0.362 | 0.353 | 0.394 | 0.432 | 0.417 |
| | *b* | 0.574 | 0.560 | 0.638 | 0.647 | 0.606 | 0.568 | 0.583 |
| *Pgi-1* | *b* | 0.003 | 0.016 | | | 0.013 | 0.005 | |
| | *c* | 0.971 | 0.948 | 0.953 | 0.960 | 0.964 | 0.940 | 0.917 |
| | *d* | 0.026 | 0.036 | 0.047 | 0.040 | 0.023 | 0.050 | 0.083 |
| | *e* | | | | | | 0.005 | |

SP and OP are the sampling and observation subplots for seedlings, respectively. *N* denotes sample numbers of seven sub-cohorts within two seedling subplots.

Table V-5 Polymorphism indices and inbreeding coefficients in the seedlings and two *F. crenata* adult populations at Mt. Kurikoma, northern Honshu, Japan.

| Parameter | Seedling subplot | | Adult population | |
|---|---|---|---|---|
| | SP | OP | ADL | ADW |
| *N* | 1,137 | 271 | 14 | 220 |
| *Pl* | 83 | 83 | 100 | 100 |
| *Na* | 3.8 | 3.5 | 2.3 | 3.8 |
| | (0.8) | (0.8) | (0.3) | (0.7) |
| *Ne* | 1.73 | 1.65 | 1.51 | 1.45 |
| | (0.24) | (0.26) | (0.14) | (0.17) |
| $H_e$ | 0.370 | 0.331 | 0.324 | 0.270 |
| | (0.078) | (0.087) | (0.061) | (0.070) |
| $H_o$ | 0.387 | 0.347 | 0.310 | 0.262 |
| | (0.085) | (0.095) | (0.093) | (0.070) |
| $F_{IS}$ | -0.047 | -0.048 | 0.043 | 0.031 |

SP and OP are the sampling and observation subplots for seedlings, respectively. ADL included only the 14 adult trees within 10 m of the seedling plot, and ADW included all mapped adult trees. N denotes the sample size. *Pl*, *Na*, *Ne*, $H_e$, $H_o$, and $F_{IS}$ denote the proportion of polymorphic loci (0.95 criterion), number of alleles per locus, effective number of alleles per locus, expected heterozygosity, observed heterozygosity, and inbreeding coefficient, respectively. Standard errors are in parentheses.

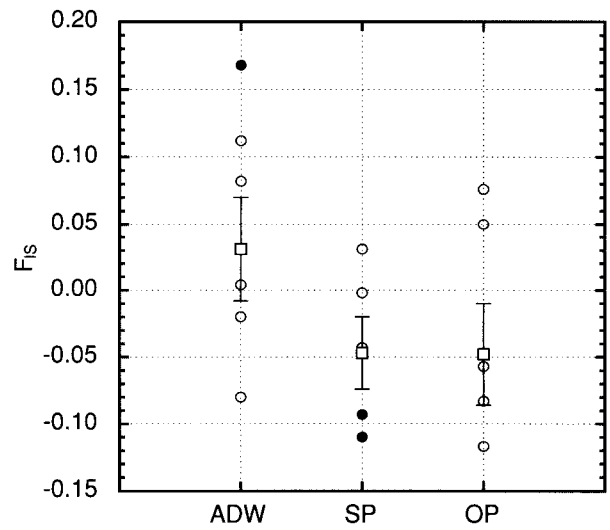


Fig. V-6 $F_{IS}$ values within the whole adult population (ADW), sampling subplot (SP), and observation subplot (OP).

Circle denotes individual $F_{IS}$ values at polymorphic loci. Solid circles indicate significant values at the 1 % level. Squares denote average $F_{IS}$ values over the loci, with error bars of one standard error.

Table V-6 Polymorphism indices and inbreeding coefficients of four *Fagus crenata* current-year seedling sub-cohorts within the sampling subplots at Mt. Kurikoma, northern Honshu, Japan.

| Parameter | Sub-cohort | | | |
|---|---|---|---|---|
| | 5 June | 13 June | 20 June | 5 Jul |
| *N* | 622 | 415 | 75 | 25 |
| *Pl* | 83 | 100 | 83 | 83 |
| *Na* | 3.7 | 3.5 | 3.2 | 2.3 |
| | (0.8) | (0.8) | (0.7) | (0.2) |
| *Ne* | 1.72 | 1.74 | 1.64 | 1.61 |
| | (0.23) | (0.27) | (0.27) | (0.23) |
| $H_e$ | 0.372 | 0.366 | 0.329 | 0.333 |
| | (0.079) | (0.082) | (0.082) | (0.080) |
| $H_o$ | 0.400 | 0.369 | 0.353 | 0.328 |
| | (0.084) | (0.095) | (0.097) | (0.085) |
| $F_{IS}$ | -0.069 | -0.018 | -0.074 | 0.016 |

*N* denotes the sample size. *Pl*, *Na*, *Ne*, $H_e$, $H_o$, and $F_{IS}$ denote the proportion of polymorphic loci (0.95 criterion), number of alleles per locus, effective number of alleles per locus, expected heterozygosity, observed heterozygosity, and inbreeding coefficient, respectively. Standard errors are in parentheses.

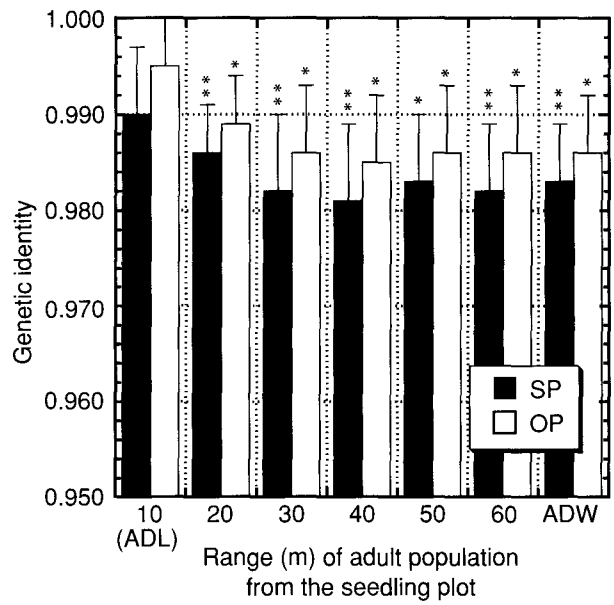


Fig. V-7 Nei's genetic identities between the two subplots for seedlings (the observation subplot (OP) and sampling subplot (SP)) and the adult tree populations in a *Fagus crenata* forest, northern Honshu, Japan.

The ADW is the whole adult population in the adult tree plot. Error bars show one standard error. $*P < 0.05$, $**P < 0.01$.

## 4. Discussion

### 1) Genetic variation within the *Fagus crenata* seedling population

The genetic composition of the seedling population was compared with that of the adult population for *F. crenata* using Nei's genetic identity (Fig.V-7). This decreased with increasing distance between the adult trees and the seedling plot. The genetic variation in the seedling population differed significantly from the adult population (ADW; $P < 0.01$), in that the seedling population was homogeneous with respect to neighbouring adult trees within a 10-m range. The *Ne*, $H_e$, and $H_o$ values for the sampling and observation subplots also suggest that the seedling population was more genetically similar, which may be attributed to the limited seed dispersal in this species, with the neighbouring adult trees. Nuts of *F. crenata* are dispersed by gravity, and sound nuts are not usually dispersed beyond 20 m from the edge of the mother tree crown (Maeda 1988; Yanagiya *et al.* 1969). Thus, it appears that the genetic variation of a small number of adult trees contributes disproportionately to that found in the seedling population.

The genetic variability within the surviving seedlings in the OP was as large as that of the seedlings that germinated in the SP during the summer, while the *Ne* value for the OP was slightly, but significantly, smaller than that for the SP. The $H_e$ and $H_o$ values for the OP were smaller than those for the SP, but not significantly so. It appears that little genetic variation was lost by the massive death of seedlings (Fig.V-5).

The *Na* values of the SP (3.8) and OP (3.5) were larger than that of the ADL (2.3), and as large as that of the ADW (3.8). Furthermore, the seedling population possessed three rare alleles (*Dia-1*$^d$, *Dia-1*$^e$, and *Pgi-1*$^e$) that were not observed in adults within 60 m of the seedling plot. This suggests that pollen dispersal is very large (> 60 m), which is concordant with the mean distance of pollen flow in *F. sylvatica* (31.7 m) (Troggio *et al.* 1996) and in *Quercus macrocarpa* (76.9 ± 45.0 m) (Dow and Ashley 1996).

Despite extensive pollen flow, the genetic variation of seedlings was most similar to that of neighbouring adult trees. Due to limited seed dispersal, nearby seedlings were a mixture of half and full siblings (Hamrick *et al.* 1993). Even if pollen donors represent a large number of adult trees scattered over a wide range, a large proportion of the genetic variation is represented by a small number of just neighbouring reproducing trees, showing the large influence of limited seed dispersal, even in the face of high pollen flow.

### 2) Massive mortality of current-year seedlings

Most *Fagus crenata* seedlings emerged between 5 - 13 Jun, and the patterns differed between blocks 1 and 2, and blocks 3, 4, and 5, suggesting that micro-environmental factors, important for seedling emergence, are heterogeneous among the blocks. Although the disappearance pattern of seedlings in block 3 differed from that in blocks 1, 2, and 5, overall, the differences in these patterns were more obscure than the emergence patterns, suggesting that macro-environmental factors play a greater role in the disappearance when compared to the emergence process. The drastic disappearance of *F. crenata* current-year seedlings was concentrated between June 20 and July 30 in all blocks and in four sub-cohorts (Figs V-4 and V-5), and is consistent with studies by Maeda (1988) and Sahashi *et al.* (1994) that

found that seedling death, due to damping-off, occurred from early June to mid-July. Here, more than half of seedling disappearances was due to herbivory before June 20, and damping-off was predominant during the subsequent rainy season ($P < 0.01$) (Table V-1; Fig. V-3). Sahashi *et al.* (1995) revealed that *Colletotrichum dematium*, which exists in the litter layer of the *F. crenata* forest floor, was responsible for damping-off of *F. crenata* seedlings through contact during germination in the litter layer. They conducted inoculation experiments with *C. dematium* under high humidity, and observed a high incidences of damping-off in the seedling samples (Sahashi *et al.* 1995). The massive death of the *F. crenata* current-year seedling population in my study is probably related to humidity and fungus.

The earlier-emerging seedling sub-cohorts showed higher final survival rates compared to the later-emerging seedling sub-cohorts ($P < 0.05$; Fig. V-5). The survival rate of all four sub-cohorts decreased similarly during the rainy season, between June 20 and July 30, with little decrease thereafter (Fig. V-2). However, the final survival rate on October 21 was differed among the sub-cohorts. A similar tendency has been observed in hinoki cypress (*Chamaecyparis obtusa*; Yamamoto and Tsutsumi 1979), four *Carpinus* species (Shibata and Nakashizuka 1995), ironwood (*C. caroliniana*), sweetgum (*Liquidambar styraciflua*), red maple (*Acer rubrum*) and American elm (*Ulmus americana*) (Streng *et al.* 1989). Streng *et al.* (1989) enumerated three possible explanations for the phenomenon: 1) temporal changes occur in the environment so that early conditions are more favorable for seedling survival; 2) spatial differences occur at the time of emergence, so that early seedlings emerge in more favorable microhabitats; and 3) there is a tendency for intrinsically vigorous seedlings to emerge earlier in the season. However, the first two explanations can be ruled out, because disappearance pattern did not differ among blocks in this study (Fig. V-4). Yamamoto and Tsutsumi (1979) found that mortality was highest in the growth stage, when seedlings have cotyledons, and subsequently decreases along with the growth stage, while the mortality rate of seedlings with scaly leaves was zero. The findings suggested that dampness on the forest floor just after the seedlings emerge affects their survival, and is tightly related to their growth stage until the onset of the rainy season.

## Chapter Ⅵ Development of software calculating indices for characterizing within-population genetic structure

### 1. Introduction

The investigation of within-population genetic structure has been an important aspect of population genetics since Sokal and Oden (1978a,b) first introduced the use of spatial autocorrelation analysis. The technique allows the determination of the major genetic processes that have produced the genetic structure within a population. This is achieved by examining together with relevant parameters of population genetics (Berg and Hamrick 1995; Sokal and Jacquez 1991; Sokal *et al.* 1997 and literature cited therein). The calculation of spatial indices for several distance classes, covering the whole of a study site, is laborious. Software designed to perform such calculations is, therefore, valuable. The software described here, the Program for Spatial Autocorrelation for Windows 95/98, Delphi version 1.1.1 (PSAwinD version 1.1.1), can calculate three spatial indices: Moran's $I$ (Sokal and Oden 1978a),

SND (Standard Normal Deviates; Sokal and Oden 1978a) and NAC (Number of Alleles in Common; Surles *et al.* 1990; Berg and Hamrick 1995). These indices are frequently used in spatial genetic studies of plant populations.

This version of the software was developed from PSAwin version 4.3.2 (unpublished). Prior to version 4.3.2, PSAwin had been developed using Visual Basic version 5.0 (Microsoft Inc.). This resulted in the program taking a long time to calculate indices from large data sets, i.e. those containing more than 500 samples. To resolve such problems, the software was converted from Visual Basic into Delphi version 5.0 (Borland Inc.) and renamed PSAwinD ver. 1.0.0. PSAwinD ver. 1.1.1 includes most of the functions in PSAwin ver. 4.3.2 and produces the results rapidly, even with a large data set.

## 2. System requirements and installation

PSAwinD ver. 1.1.1 must be run on a PC, with Windows 95 or higher. Software installation is very simple: the application file, PSAwinD111.exe, can be copied into any directory on the computer's hard disk. No additional files are needed to run the software. Tests confirm that the software will run successfully on the following computers: 1) Dell Dimension XPS H233 with Windows ME (English version), CPU: Pentium II, RAM: 64 MB, 2) NEC NX VS30D model (DOS 5 machine) with Windows 98 (Japanese version), CPU: GenuineIntel Family 6 Model 8(733 MHz), RAM: 158 MB, and 3) Epson Direct EDi Cube with Windows 98 (Japanese version), CPU: GenuineIntel Family 6 Model 8 (501 MHz), RAM: 128 MB.

## 3. Data files

PSAwinD requires at least two data files for calculating the spatial indices: a DAT file and a LOC file. The DAT file should include genetic data relating to each individual, and the LOC file, the location data for each individual. The data format for each of the two data files is described in the users' manual. These files must have the same filename with the relevant extensions, ".dat" and ".loc" (for example, "pine.dat" and "pine.loc"). An optional data file, LYR, can be created. This file allows the user to define layers (data sub-sets) within the data set. Using the layers, it is possible to calculate the spatial indices of specific data sub-sets.

The simplest way to create the data files is to use a spreadsheet application, such as Excel (Microsoft Corp.) or Lotus (Lotus Development Corp.). These programs allow the data to be saved as a comma delimited text file. After saving the data in this format, the file can be opened using any text editor, and modified as required. The file can then be saved with the correct file name, and the appropriate extension added subsequently.

## 4. Calculation Steps

### 1) Conversion of the DAT file into a GEN file

Once the data files have been created, the user should double-click on the rocket icon of PSAwinD, to start the software. The main window contains a button labeled "File conversion of DAT file into GEN file (C)". Select the relevant DAT file, then click on this button. The software begins processing the DAT file to create a GEN file. Conversion failure suggests that the DAT file includes one or more errors. If this is the case, check the DAT file, correct any errors and click on the button again. PSAwinD uses the

resulting GEN file for its calculations. The GEN file can be viewed in any text editor.

#### 2) Selection of distance classes and calculation of *W* values

Three types of distance class are supported by this software: 1) sequential: 0-10, 10-20, 20-30 etc; 2) cumulative: 0-10, 0-20, 0-30 etc; 3) Gabriel network (See Sokal and Oden 1978a). From the "Distance class" menu, select the type of distance class required. Selecting the "DC option (O)" on the menu bar gives the choice of five options. Once the correct option has been selected, click on the button "Determine distance classes (DC; W)". The software produces a matrix of distance classes between all the individuals, and calculates *W* values. Several dialog boxes will appear during the process, for each, select the appropriate answer and click the "OK" button. Rock's move, Bishop's move, and Queen's move are not supported in this version (See Sokal and Oden 1978a).

#### 3) Calculation of the spatial indices

Next step is to calculate the spatial indices. In this version of the software, three spatial indices are available: Moran's *I* (Sokal and Oden 1978a), SND (Standard Normal Deviates; Sokal and Oden 1978a), and NAC (Number of Alleles in Common; Surles *et al.* 1990; Berg and Hamrick 1995).

Three methods for calculating SND are provided: genotypic; same allele; and different alleles. For examining the spatial pattern of dominant markers, such as flower color or presence/absence of dominant DNA markers, one of the latter two methods should be used. These represent the join count statistics between identical and different types, respectively. In genetic studies examining the spatial pattern of genotypes using isozyme markers, the first method of calculating SND should be used. This procedure calculates SND for every combination of genotypes at each locus, and, therefore, tends to be more time consuming than the other two methods. To ensure calculations are performed as quickly as possible, the "Identical genotypes ONLY" button should be checked, as required. When this button is checked, the software calculates SND only between identical genotypes, thus reducing the processing time.

It is possible to include either all the loci in the data file or only the polymorphic loci (95% criteria) when calculating the NAC. The user can specify which is required.

### 5. Output

The results of the calculations are output as comma delimited text files with the extensions: "RT1" and "RT2" for Moran's *I*; "RPS" and "SND" for the SND; and "NAC" for the NAC data. To view the results, it is recommended that the files are opened in a spreadsheet application such as Excel or Lotus, using the function for opening comma delimited text files in a worksheet. The results can then be readily used to produce tables and figures.

### 6. Other features

#### 1) Auto-calculation

When the "Auto-calculations" button is checked, the software automatically calculates the Moran's *I* and the SND, immediately after calculating the distance class matrix and *W* values.

#### 2) Auto-drive

The variance of each distance class is influenced by

the *W* value, with twice the sum of the join number included in each class. Therefore, to equalize the statistical power of the indices in each distance class, the *W* values for every class must be standardized. This is usually achieved by repeatedly calculating the *W* values, and changing the lower and upper limits of each distance class, until suitable ranges are determined. The auto-drive function, within this software, determines distance classes with equal *W* values automatically.

#### 3) Summary files

The software can create summary files from the RT2 and SND files. The summary files present the number and the proportion of significant alleles (or genotypes) at three levels of significance. This facilitates the identification of general trends within the results.

#### 4) Defining layers

Spatial indices for sub-sets within the data file can be calculated by defining layers (data sub-sets). It is possible to calculate the spatial indices for various combinations of layers, by including or excluding certain data sub-sets from the calculation. To use this function, an additional data file, LYR, is required. If three layers are created, for example, on the basis of the size of each individual: Large (L), Medium (M), and Small (S), three calculation procedures are possible. These are 1) between identical layers; 2) between different layers; 3) between specific combinations of layers. The first procedure includes only joins between identical layers in the calculations; joins between different layers are excluded. The second procedure includes only joins between different layers in the calculations. The last procedure includes joins only between specific combinations of layers. In this case, it is possible to calculate the SND and the Moran's *I* for various combinations of layers. In the example above, selecting "L－M" , would include joins between the L layer and the M layer. Inputting "L－not L" , would include joins between the L layer and the M layer and joins between the L layer and the S layer in the calculation. After calculating the *W* values, an SDS file is created. Using this, it is possible to calculate the Moran's *I* and SND for the defined layers. This version of PSAwinD, does not support the calculation of NAC with defined layers.

## Chapter Ⅶ General discussion

### 1. Genetic variation and structure within *Fagus crenata* populations

The geographic genetic variation in populations of *Fagus crenata* in northern Honshu and Hokkaido was studied in Chapter Ⅱ. The genetic variation was homogeneous among populations ($G_{ST}$ = 0.015), although five rare alleles were absent from four populations in Hokkaido, probably due to founder effects through colonization from Honshu to Hokkaido after the last glacial period. A species' range wide study on geographic genetic variation by Tomaru *et al.* (1997) found higher degree of differentiation among populations ($G_{ST}$ = 0.038), indicating that most of the nuclear genetic variation is due to the within-population component.

The within-population distribution pattern of genetic variation was studied in two *F. crenata* populations that had contrasting forest histories, reviewed in Chapter Ⅲ. One population (KU) was in an old-growth beech forest at Mt. Kurikoma, while the other (AK) was in a secondary beech forest at Mt. Akitakomagatake, which was logged during the

1920s. The genetic structure of the two populations, examined by spatial autocorrelation, showed a positive autocorrelation at a distance of less than 20 m, probably due to limited seed dispersal. However, there were differences in their genetic structures and linkage disequilibrium between the two populations, suggesting that forest history is influential. I examined the northernmost marginal *F. crenata* population (UT) in a beech forest to determine how the population was established and how it was affected by generation time. The genetic structure of the population, examined by using spatial indices at three levels (genotypic, allelic, and multilocus basis), did not show a clear positive autocorrelation, even at shorter distance classes, in comparison to an old-growth beech population (KU), suggesting that the generation time was not sufficient to generate inherent genetic structure in the species. These results are concordant with predictions from previous simulation studies (Sokal and Wartenberg 1983; Epperson 1990). Additionally, AK had a stronger genetic structure than the UT population, indicating that generation time and the way by which the populations have been established have influenced the genetic structure. Thus, forest history is an essential component when one considers within-population genetic structure.

The genetic variation within an adult tree population and two current-year seedling populations was intensively examined in a *F. crenata* population in an old-growth beech forest at Mt. Kurikoma (Chapter V). Nei's genetic identity indicates that little genetic variation exists between the seedling population and neighbouring reproducing adult trees, suggesting that most of seeds disperse just beneath the crown of their mother trees. The difference in the number of alleles per locus between the adult trees and seedlings indicates that adult trees located 60 m away from seedlings participated in mating events.

For *F. crenata*, the distribution of genetic variation among populations was homogeneous, while variation was spatially structured within population. Although long-distance pollen flow is present within population, limited seed dispersal is associated with the spatial genetic structuring.

### 2. Population genetic and demographic analyses

Population genetic data are a cumulative record of the population dynamics within population, thus genetic variation within populations is affected by population dynamics. Genetic variation is influenced by genetic processes such as gene flow, random genetic drift, mutation, and selection, while demographic data track genetic variation through evolutionary selection processes. Thus, when population genetic data are integrated with demographic data, multi-dimensional information on population dynamics can be obtained. Population genetic and demographic analyses provide various ways to investigate the data, and multi-directional analyses are essential to their interpretation. A comparison of allelic and multilocus-based spatial analyses is important, because it provides information about the covariance-like component among loci for spatial genetic structure. Likewise, linkage disequilibrium analysis is important because it detects founding events. Thus, comprehensive analyses that integrate population genetic and demographic data are needed to fully understand population dynamics.

### 3. Future studies: conservation of *Fagus crenata* populations

The results presented in this study were from three *F. crenata* populations. This study showed that population structure and dynamics are influenced by forest history and discrete genetic processes. The quantity of data collected for this study may be insufficient to describe the population structure and dynamics in detail. Thus, further studies on multiple populations are needed for this species. Future conservation efforts for *F. crenata* should consider the amount of disturbance a population can withstand, how it can overcome a decrease in effective population size, and how many generations are needed to recover its inherent genetic status. To answer these questions, it is important to characterize secondary beech forests that have had their genetic variation modified by human intervention.

One generation of a forest tree species is usually much longer than human lifetime. Thus, population geneticists and conservationists need to make an intense systematic effort to characterize the population genetic and demographic changes in populations. Long-term monitoring of multiple populations would produce the comprehensive picture that is necessary for constructing appropriate management and conservation strategies for this species.

## Summary

### 1. Geographic genetic variation in *Fagus crenata* populations in northern Honshu and Hokkaido

The genetic variation of *Fagus crenata* Blume was investigated in 14 populations in the Hokkaido and Honshu regions of northern Japan. Within populations, averages for the proportion of polymorphic loci (95% criterion), the number of alleles per locus, the effective number of alleles per locus, the expected average heterozygosity, and the observed average heterozygosity were 58.2%, 2.63, 1.34, 0.197 and 0.160, respectively. The relative magnitude of gene differentiation and the average genetic distance among populations were 0.015 and 0.002, respectively. The inbreeding coefficient averaged 0.036. Overall, I detected 24 rare alleles (less than 5 % of the allele frequency). The genetic composition of the Honshu and Hokkaido populations was essentially the same, except for five rare alleles absent from the Hokkaido populations. These results indicate that the Hokkaido populations may have experienced a partial bottleneck in the process of seed dissemination from Honshu.

### 2. Differences in genetic structure and variability between two *Fagus crenata* populations with contrasting forest histories

To examine the effects of forest cutting on within-population genetic structure, the genetic structure and variability of two Japanese beech (*Fagus crenata* Blume) populations with contrasting disturbance histories were investigated. Six hundred and sixty beech trees, covering two hectares in total, were mapped and genetically analyzed using nine isozyme loci encoding eight enzyme systems. The proportion of polymorphic loci, the average number of alleles per locus, the effective number of alleles per locus, the expected heterozygosity, and the observed heterozygosity were 78%, 3.3, 1.31, 0.200, and 0.189, respectively, in a secondary population (designated AK) cut during the 1920s. The corresponding figures were 78%, 3.3, 1.33, 0.203, and 0.193, respectively, in an old-growth population designated KU. The inbreeding coefficient and grand mean of the number of alleles in common (NAC) were 0.055 and 1.684 in AK, and 0.042

and 1.649 in KU, respectively. The genetic variability was slightly, but significantly, lower in AK. The genetic structure of the two populations was strikingly different. The proportions of positively significant Moran's $I$ and SND values found in the shortest distance class were 0.86 and 0.38 for AK, and 0.14 and 0.29 for KU, respectively. Furthermore, significant linkage disequilibrium was observed in AK, but not in KU. A self-thinning process was simulated to examine differences in genetic structure that might influence future generations, and this suggested that reduced genetic variability and linkage disequilibrium would have a significant influence in the AK population for several generations.

### 3. Genetic structure of a northerly marginal population of *Fagus crenata*

Genetic variability and structure were examined in the northernmost marginal population of Japanese beech (*Fagus crenata* Blume), located in the Kuromatsunai lowland (Utasai), Hokkaido Island, Japan, with special regard to the influence of the founding process on its genetic structure. From palynological records, it appears that the population was established between 350 and 680 years ago. I investigated 119 trees, using 11 isozyme loci, which encoded eight enzyme systems. The proportion of polymorphic loci, average number of alleles per locus, effective number of alleles per locus, expected heterozygosity and observed heterozygosity in the population were 64%, 2.5, 1.30, 0.182 and 0.178, respectively. The average $F_{IS}$ was 0.046. Significant linkage disequilibrium was found for two pairs of loci ($P < 0.05$), probably related to founder effects during and after establishment of the population. Genetic structure in the population was examined by Moran's $I$, standard normal deviate (SND), and the number of alleles in common (NAC). The genetic clustering in the population was weaker and less clear than previously studied populations. The common genetic structure commonly seen in populations of this species has probably not yet emerged here because of the small number of generations since it was founded.

### 4. Genetic and demographic changes in genetic variation in a *Fagus crenata* seedling population

I studied the genetic and demographic aspects of a *Fagus crenata* current-year seedling population. The demography of 1179 current-year seedlings that emerged within a 25-m$^2$ survey plot was observed in a *F. crenata* forest located at Mt. Kurikoma, northern Honshu, Japan. Most seedling emerged in early June, and seedling mortality was highest in the rainy period, between July 5 and July 30. The early-emerged seedling sub-cohort had a significantly higher survival rate than later ones. The survival of current-year seedlings was tightly related to their growth stage until the onset of rainy season. Using six polymorphic loci encoding five enzyme systems, 271 seedlings that survived until the first autumn in the OP, 1,137 seedlings that germinated in the SP, and 220 nut-producing adult trees within 60 m of the seedling plot (a total of 1,628 individuals) were analyzed. Seedling populations were more homogeneous with neighbouring adult trees located within 10 m of the seedling plot compared to the whole adult-tree population. Limited seed dispersal predominantly influenced the genetic variation of the seedlings, although long distance pollen flow was

incorporated into mating events. The OP population possessed slightly, but significantly, lower levels of genetic variation than the SP population, possibly due probably to the massive mortality during the rainy season.

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# ブナ（*Fagus crenata*）の集団内における遺伝変異と遺伝構造

高橋　誠

## 要　旨

森林樹木では，ほとんどの遺伝変異は集団内に保持されている。集団内の遺伝変異は，遺伝子流動や突然変異，遺伝的浮動，自然淘汰といった遺伝的なプロセスの影響を受けて次世代へ引き継がれる。このようなプロセスに影響を受ける集団内の遺伝的な構造と動態について理解を深めることは，その種の適切な管理と保全のために必要と考えられる。

ブナは，日本の冷温帯広葉樹林を構成する主要な樹種の一つである。本研究では，アイソザイムを遺伝マーカーとして，集団遺伝学的解析と空間的自己相関解析により，ブナの集団内における遺伝的な構造と動態を理解することを目的とした。

まずはじめに，北日本のブナの集団内と集団間における遺伝変異を明らかにするため，北海道黒松内低地に位置する歌才自生北限地の集団を含む14集団について14遺伝子座のアイソザイム分析を行った。解析の結果，多型的な遺伝子座の割合（*Pl*），1遺伝子座あたりの平均対立遺伝子数（*Na*），1遺伝子座あたりの有効な対立遺伝子数（*Ne*），平均ヘテロ接合体率の期待値（$H_e$）と観察値（$H_o$）は，それぞれ58.2%，2.63，1.34，0.197，0.160であった。また，集団間に遺伝的な分化はほとんど認められず（遺伝子分化係数：$G_{ST}$ = 0.015），遺伝変異のほとんどは集団内に保持されていることが明らかになった。ただし，頻度が0.05以下の対立遺伝子には地理的な分布の偏りがみられ，特に非常に頻度の低い５つの対立遺伝子（0.01以下）は，本州の集団のみで検出され，北海道の4集団では検出されなかった。これは最終氷期以降のブナの分布拡大の際に本州から北海道へ分布を拡大する際，弱いびん首効果を受けた可能性を示唆していると考えられた。

ブナの遺伝変異のほとんどは集団内に保持されていることが明らかとなったが，この変異が集団内にどのように分布しているのか，すなわち集団内の遺伝構造がどのようになっているのかを，履歴が異なる２集団において調査した。一方は栗駒山麓に位置し，老齢林と考えられる集団（KU）であり，他方は秋田駒ヶ岳山麓に位置し，1920年代に母樹保残施業により伐採された後に更新した二次林（AK）であった。これら2集団において合計2.0haの個体位置図を作成し，延べ660個体について9遺伝子座のアイソザイム分析を行った。

*Pl*, *Na*, *Ne*, $H_e$, $H_o$の値は，KUではそれぞれ78%，3.3，1.31，0.203，0.193，同様にAKでは78%，3.3，1.31，0.200，0.189で，2集団はほぼ同程度の遺伝変異を保持していた。しかし，NAC（the number of alleles in common）の平均はKUでは1.649，AKでは1.684で，AKの遺伝変異はわずかではあるが有意にKUよりも低かった。両方の集団に遺伝構造が認められ，これは限られた範囲への種子散布様式が密接に関連していると考えられた。一方，2集団における遺伝構造の程度は明らかに異なった。短い個体間距離の距離階級における有意な正のモランの*I*と同一遺伝子型間でのSND（基準正規偏差）の割合は，AKではそれぞれ0.86と0.38であったが，KUでは0.14と0.29であった。また，有意な連鎖不平衡がAKでは認められたが，KUでは検出されなかった。AK集団の個体密度が減少する際，その遺伝構造と連鎖不平衡の程度が減少するかどうかを自己間引きのシミュレ

ーションで調べた。その結果，連鎖不平衡が数世代の間影響し続けることが示唆された。

AKとKUの集団間での遺伝変異と遺伝構造の比較から，集団内の遺伝構造には，集団の履歴が影響することが明らかになった。最終氷期以降のブナの分布変遷については，花粉分析により比較的よく研究されており，現在の北限集団である歌才集団は，集団の成立から350～680年しか経過していないとされている。これはこの集団が成立後数世代しか経過していないことを意味する。この歌才集団に1.3haの調査プロットを設定し，119個体について11遺伝子座のアイソザイム分析を行い，集団の成立過程の遺伝構造に与える影響について解析を行った。

歌才集団における*Pl*，*Na*，*Ne*，$H_e$，$H_o$の値はそれぞれ64%，2.5，1.30，0.182，0.178であった。また2組の遺伝子座間で連鎖不平衡が認められた。この連鎖不平衡は集団の成立に伴う創始者効果に関係していると考えられた。この集団内での遺伝構造をモランの*I*とSND，NACを用いて解析した。遺伝子の集中分布の程度は，AKやKUに比べて弱く，不明瞭であった。歌才集団は成立後間もないため，ブナ集団内において本来形成されるべき遺伝構造がまだ形成されていないと考えられた。

集団内の遺伝変異を考える上で，個体群動態的な視点からも解析することが重要である。なぜならば，生活史の各段階における遺伝変異は個体数の変化に伴って変動していることが予想されるからである。そこで，栗駒山麓のブナ老齢林に設定した調査地に50m²の実生調査プロットを調査し，そこに発生した実生の動態と遺伝変異を調査した。実生調査プロットは，実生の動態を観察するためのOPサブプロットとアイソザイム分析のサンプルを採取するためのSPサブプロットに分割した。

OPサブプロットでは合計1,179個体の実生が発生した。発芽は7月上旬に集中し，枯死は7月5日～7月30日の梅雨期に集中していた。観察終了の10月21日まで271個体の実生が生残したが，発芽時期の早い個体群が発芽時期の遅い個体群よりも有意に高い生存率を示した。当年生実生の生残は，梅雨期の到来までにどの程度生育しているかと密接に関連していると考えられた。実生集団が保有する遺伝変異の調査は，OPサブプロットで生残した271個体，SPサブプロットに発生した1,137個体および実生プロットから60m以内の220個体の繁殖個体について6遺伝子座のアイソザイム分析により行った。

その結果，当年生実生個体群の遺伝変異は，10m以内の距離に位置するごく近隣の母樹と最も類似していた。調査地外からの花粉の流入が認められたが，限られた種子散布様式が，実生集団の遺伝変異に顕著に影響していると考えられた。

一連の研究の結果，ブナにおいてほとんどの遺伝変異は集団内に保持されていることが明らかになった。また，集団内における遺伝変異の分布はランダムではなく，重力による種子散布様式の影響により遺伝子の集中分布が形成されたと考えられた。履歴の異なる集団を解析することにより，このような遺伝構造には集団の履歴が深く関わってることが明らかになり，集団内の遺伝構造を考える上で，種子散布といった種の種特性の他に，森林の履歴も重要であることが分かった。当年生実生集団における動態と遺伝変異についても解析を行った。その結果，生存率は発芽時期に依存的であることが明らかになった。また，実生集団における遺伝変異は10m以内の繁殖個体と最も類似していることから，実生集団において種子散布様式が影響していることが明らかになった。

今回の一連の研究から，集団内の遺伝変異を空間統計学的手法や個体群統計学的手法を用いて解析する方法は，集団内の遺伝変異に影響を与えている遺伝的なプロセスを推定するのに有効であると考えられた。今後，

さまざまな集団における遺伝構造や個体群動態についてのデータを蓄積して行くことにより，集団内で遺伝変異に作用している遺伝的なプロセスについて，より深く理解することができるものと考える。