III.6 Forest Management and Conservation Using Microsatellite Markers: The Example of *Fagus*

Y. Tsumura[1], M. Takahashi[2], T. Takahashi[3], N. Tani[1], Y. Asuka[4], and N. Tomaru[4]

1 Introduction

The genetic diversity and mating systems of tree species have been studied extensively during the last two decades by allozyme analysis (Hamrick 1989). The resulting information is important for forest management and conservation. In particular, information on genetic differentiation among populations of forestry species is necessary for the conservation of genetic resources. Highly polymorphic genetic markers such as microsatellite markers (Litt and Luty 1989; Weber and May 1989), are highly sensitive at detecting the dynamics of gene flow within and among populations (Dow and Ashley 1996). These markers give us information on selfing rate and biparental inbreeding (Kelly and Willis 2002; Obayashi et al. 2002), the differential paternal contribution of each individual tree to future populations, and the fine-scale genetic structure within forests (Ueno et al. 2000). As we can understand pollen flow within forests when we use these markers, we can determine the best management system to maintain genetic diversity among fragmented natural populations and man-made populations for the purposes of forestry and conservation.

Fagus crenata and *F. japonica* are monoecious, long-lived, woody angiosperm species with an outcrossing breeding system based on wind pollination, with gravity- and animal-dispersed seeds (Kitamura and Murata 1979). Both grow in Japan. *F. crenata* is considered to be important as an ecosystem component and for the conservation of bio-diversity; the World Heritage listed Mt. Shirakami is dominated by *F. crenata* forests (UNESCO 2002). However, since the 1950s, many areas of beech forest have been logged and converted to coniferous forests for timber production. Coniferous forests planted in high-altitude or heavy-snowfall regions sometimes are not adapted to the severe environmental conditions and do not grow well. Forest rehabilitation

[1] Department of Forest Genetics, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan
[2] Forest Tree Breeding Center, Juo, Ibaraki 319-1301, Japan
[3] Graduate School of Science and Technology, Niigata University, Niigata 950-2181, Japan
[4] Laboratory of Forest Ecology and Physiology, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

Biotechnology in Agriculture and Forestry, Vol. 55
Molecular Marker Systems (ed. by H. Lörz and G. Wenzel)

has been used to recover such forests by the planting of native species such as *F. crenata*. To maintain the genetic diversity of planted populations and to adapt them to the prevailing conditions, superior tree selection of *F. crenata*, clonal propagation by grafting, and construction of an experimental seed orchard have been started in the Forest Tree Breeding Center of Japan. Microsatellite markers are a very powerful and sensitive tool for evaluating the genetic diversity of collected superior trees in a seed orchard, and, unlike allozyme markers, can detect subtle changes in genetic diversity.

In this chapter, we describe an effective method for developing microsatellite markers, and discuss forest conservation and management based on information such as the genetic structure and gene flow of *Fagus* populations. We demonstrate that the pattern of gene flow is influenced by several factors – reproductive system, mating system, environment, and others – by using data obtained from fine-scale genetic structure studies between closely related species in different environmental conditions, and from a gene flow study in a seed orchard.

2 Development and Evaluation of Microsatellite Markers in *Fagus*

Several improvements in methodology have been reported recently for efficient development of simple-sequence repeat (SSR) markers, including the vectorette polymerase chain reaction (PCR) strategy (Lench et al. 1996), the random amplified hybridization microsatellites (RAHM) method (Cifarelli et al. 1995), and the library enrichment method for SSR regions (Ostrander et al. 1992; Karagyozov et al. 1993; Lyall et al. 1993; Kirkpatrick et al. 1995; Takahashi et al. 1996). The library enrichment method for the development of microsatellite markers is much more efficient and less labor-intensive than nonenrichment methods. Several procedures have been developed for enrichment of DNA libraries, among which the magnetic bead method is well established and has a very high efficiency of enrichment. We adopted two modified methods to develop microsatellite markers in *Fagus*, the RAHM method (Cifarelli et al. 1995) and the enrichment method using magnetic beads (Fischer and Bachmann 1998; Hamilton et al. 1999), and compared their efficiency.

The RAHM procedure, based on PCR, is very convenient because it is not necessary to prepare high-quality genomic DNA to make a genomic DNA library (Cifarelli et al. 1995), so we merely selected the RAPD fragments containing SSR regions. The screening efficiency is very high compared with that of the colony hybridization method, because one random primer amplifies many DNA fragments, which can be screened at the same time. We detected 60 positive fragments by using 38 out of 360 random primers; thus, 10.6% of primers yielded positive fragments (Tanaka et al. 1999). Some plant species

have high contents of secondary metabolites in their cells, so if we use the colony hybridization method, we have to exclude the components during the DNA extraction before we can prepare the genomic library. We developed nine polymorphic microsatellite markers in *F. crenata* by using the RAHM method, eight of which are available also in *F. japonica*. The polymorphic level was extremely high in both species; the average heterozygosity was 0.615 and 0.660, respectively.

Our method for enriching a genomic DNA library containing SSR regions is based on the methods of Fischer and Bachmann (1998) and Hamilton et al. (1999). We used the following procedure to develop SSR markers in *F. crenata*. Genomic DNA of *F. crenata* was extracted by using a modified CTAB method (Murray and Thompson 1980) and purified by ultracentrifugation. Ten micrograms of DNA was digested with *Nde*II and electrophoretically separated on a 1.2% agarose gel. DNA fragments ranging from 300 to 1000 bp were recovered. Approximately 600 ng of the fragments was ligated to 5 pmol of *Sau*3AI cassette (TaKaRa). The nick between the genomic DNA and the cassette sequence was filled by using DNA polymerase I. One hundred ng of DNA fragments with *Sau*3AI cassettes were denatured at 95 °C for 15 min and hybridized at 70 °C overnight to 2 pmol of biotinylated oligonucleotides, $(CT)_{15}$, in 100 ml of buffer containing 6×SSC and 0.05% SDS at 55 °C. These hybrids were captured with 20 mg of pre-washed streptavidin-coated magnetic beads (Dynal), and microsatellite-containing fragments were enriched and recovered in eluate as described by Hamilton et al. (1999). Double-stranded conformation was performed by PCR with the Primer C1 (Takara). The PCR products were digested with *Sau*3AI to remove the cassette, ligated into pUC118 (Takara) plasmid vectors, and cloned into competent cells of *E. coli*. Plasmid DNA was extracted from positive clones and sequenced on a 3100 Genetic Analyzer by using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems). PCR primer pairs for microsatellites were designed by using OLIGO software (National Biosciences). After PCR optimization, successful forward primers were fluorescently labeled, and amplifications were carried out in 10-μl reactions containing 1×PCR buffer (10 mM Tris·HCl (pH 8.3), 50 mM KCl, 100 mM of each dNTP), 1.5 mM $MgCl_2$, 0.25 U *Taq* polymerase, 0.2 mM of each primer, and 5–10 ng of template DNA. The PCR conditions were 3 min at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at primer-specific annealing temperature, 30 s at 72 °C; and final extension at 72 °C for 7 min. The PCR products were run on a 3100 Genetic Analyzer with GeneScan software (Applied Biosystems), and genotypes were determined. We obtained a highly enriched DNA library containing SSR regions. The proportion of SSR-containing clones was about 61%: sequence data were obtained from 806 clones, of which 496 contained SSR regions. PCR primers to amplify SSR regions were designed by using sequence data that had pure repeats after redundancy was excluded. Finally, we designed PCR primers for at least 96 loci in *F. crenata*; 16 of the markers showed clear patterns and high polymorphisms (Asuaka et al. 2004a).

Both methods are effective for developing SSR markers. The RAHM method is much simpler than the enrichment method using magnetic beads, but the latter is much more efficient; in particular, many markers are needed for construction of the linkage map. The RAHM procedure is a step-by-step method that can be followed by an operator who is not a specialist in molecular biology techniques. The genomic library is not necessary in this method because of the PCR based-method, thus, high quality DNA is also not needed. Conversely, the enrichment method using magnetic beads requires a relatively high level of skill in molecular biology techniques. For the purpose of genetic monitoring of a forest population, fewer than ten loci of SSR markers are probably adequate if the markers show high polymorphism because we can determine the pollen and seed dispersal using these markers. Therefore, both methods can be used for the purpose of genetic monitoring, but, for the construction of a genetic linkage map, the latter method is much more effective.

3 Spatial Analysis of Genetic Structure Within Forests by Microsatellite Markers

3.1 Differences in Fine-Scale Genetic Structure Between *F. crenata* and *F. japonica*

We compared the spatial genetic structures of *F. crenata* and *F. japonica* by using four microsatellite markers (Takahashi et al. 2004, in press). The study site was a 2-ha plot within a mixed population on Mt. Takahara, central Honshu, Japan. Two statistics, genetic relatedness and number of alleles in common, were used to detect spatial genetic structure. A significant negative correlation between genetic relatedness and spatial distance was detected among all individuals in each species. However, this correlation was weak; the genetic structures likely resulted from extensive pollen flow caused by wind pollination. Similarly, Merzeau et al. (1994) and Streiff et al. (1998) also detected weak genetic structure in one of three *Fagus sylvatica* stands and in *Quercus petraea* and *Q. robur*, which are wind-pollinated species whose seeds are gravity-dispersed, like those of *Fagus*. Spatial genetic clustering in *F. japonica* was stronger than in *F. crenata* over short distance classes. The presence of self-incompatibility may also influence genetic structure (Loveless and Hamrick 1984; Doligez et al. 1998). Self-incompatibility can induce decreases in spatial genetic structure within populations (Doligez et al. 1998). Furthermore, if self-incompatibility combines with family structure, pollen dispersal becomes large and genetic subdivision is less likely (Loveless and Hamrick 1984). Although only two studies investigated self-incompatibility in *F. crenata* (Kouno and Mukouda 1985) and *F. japonica* (Igarashi 1996), they suggested that the ratios of mature and immature seeds in *F. japonica*

were higher than in *F. crenata* in controlled pollination experiments. The reproductive system might also influence the difference in spatial genetic structure between the two species. The regeneration of *F. crenata* depends mainly on the growth of seedlings or saplings under canopy gaps (e.g., Nakashizuka 1987). On the other hand, *F. japonica* forms stools by vigorous sprouting, and the stools help to maintain trees (Ohkubo 1992). Thus, an *F. japonica* stool can reach a substantial age – about 1000 years, against about 200–300 years for *F. crenata*. Differences in genetic structure between those species would be caused by different periods of generation overlap.

3.2 Influence of Environmental Differences and Forest History on Spatial Genetic Structure

The spatial genetic structure of *F. crenata* in a 4-ha plot (200×200 m^2) of an old-growth beech forest was analyzed by using microsatellite markers (Asuka et al. 2004b). Two types of coefficient were used to assess the genetic structure: Moran's *I* spatial autocorrelation coefficients and genetic relatedness. The correlation between spatial distance separating individuals and genetic relatedness was tested by a Mantel test. Correlograms of both Moran's *I* and Mantel's *r* values showed significant positive values for short distance classes, indicating weak genetic structure, the same as in the previous studies (Kitamura et al. 1997; Takahashi et al. 2000). The genetic structuring within the population is probably created by limited seed dispersal, but likely weakened by extensive pollen flow and overlapping seed shadows. Genetic structure was detected in an eastern subplot of 1 ha (50×200 m^2) with immature soils and almost no dwarf bamboo (*Sasa* spp.), but none was found in a western subplot of the same size with mature soils and *Sasa* cover. The apparent genetic structure detected in the 4-ha plot was, therefore, due to the structure in the western portion of the plot. The heterogeneity of genetic structure presumably reflects variation in regeneration, which is strongly influenced by heterogeneity of environmental conditions.

Takahashi et al. (2000) examined the effect of logging on within-population genetic structure by comparing two forests (selectively logged and unlogged) by allozyme analysis. They found that logging slightly, but significantly decreased the genetic variability and reinforced the spatial genetic structure by reducing the mixing of half-sib progeny derived from a limited number of reproductive trees. They also found that linkage disequilibrium was higher in the logged forest than in the unlogged forest, and suggested that this value might be a good indicator of forest decline. However, linkage disequilibrium is influenced by population history and natural selection as well. Therefore, by considering the history and natural selection of each forest, we will be able to use the value of linkage disequilibrium for suitable management.

3.3 Gene Flow Within Seed Orchard Revealed by Microsatellite Analysis

To understand gene flow within a seed orchard of *F. crenata*, we investigated seedlings derived from open-pollinated seeds of six clones by using four microsatellite loci (Tanaka et al. 1999). The seed orchard consisted of 38 clones, which were established by grafting in 1979. We searched for pollen donor candidates for each seedling. When we found a single match between seedling haplotype and pollen donor haplotype, we could determine the pollen donor.

Finally, we could determine the pollen donor for 172 out of the 217 seedlings. The assigned paternity rate for each parent clone ranged from 71.4 to 100.0 %, with an average of 79.3 % (Table 1; "1 male parent" column). Thirty-one out of the remaining 45 seedlings had more than one candidate paternal clone in the seed orchard ("2 possible male parents" and "3 or more possible male parents" columns). We could not find a candidate within the six parent clones in the seed orchard for 13 seedlings. The pollen responsible would have traveled a long distance, because no reproductive mature trees grow within 500 m of the seed orchard. The degree of long-distance pollen transport is generally high in wind-pollinated species (contamination rate; 69–71 % in *Picea abies*, Pakkanen et al. 2000; 48 % in *Pinus taeda*, Friedman and Adams 1985; about 70 % in *Quercus robur*, Buiteveld et al. 2001). Thus, this seed orchard seems to be isolated from beech forests, because the pollen contamination was only 6 %. We also found only one selfed seedling, and thus the outcrossing rate is very high (99.5 %), which reconfirms that *Fagus* is an allogamous species.

The contribution of the parent clones to seedling paternity differed greatly: Sanbongi-103 fathered 43.4 % of all seedlings (Table 2), followed by Fukaura-101 (17.3 %), Ajigasawa-102 (7.5 %), and the rest (<5 %). Sanbongi-

Table 1. Paternity analysis of seed orchard of *Fagus crenata*

Parent clone	No. seedlings investigated	No. seedlings with paternity assigned			No. selfed seedlings	Male parent not in nursery
		One male parent	Two possible male parents	Three or more possible male parents		
Ajigasawa 102	40	29	3	0	1	7
Fukaura 101	14	14	0	0	0	0
Fukaura 102	42	30	8	3	0	1
Hirosaki 103	40	35	3	0	0	2
Iwaizumi 103	49	37	8	2	0	2
Tayama 104	32	27	4	0	0	1
Total	217	172	26	5	1	13
Proportion (%)		79.3	12.0	2.3	0.5	6.0

Table 2. Paternity contribution of parent clones in the seed orchard

Female clone	Male clone							
	Sanbongi 103	Fukaura 101	Ajigasawa 102	Tayama 102	Tayama 104	Hirosaki 102	Tohno 101	Other 15 clones
Ajigasawa 102	15	5	1	0	0	1	0	8
Fukaura 101	7	0	2	0	0	0	0	5
Fukaura 102	6	16	5	0	1	2	0	0
Hirosaki 103	15	7	1	0	3	1	0	8
Iwaizumi 103	19	0	1	0	1	0	5	11
Tayama 104	13	2	3	5	0	1	0	3
Total	75	30	13	5	5	5	5	35
Proportion (%)	43.35	17.34	7.51	2.89	2.89	2.89	2.89	20.23

103 has the highest pollen fecundity in the orchard. Pollen fecundity and flowering phenology might be highly related to success of mating.

Microsatellite analysis showed that *F. crenata* is an almost completely outcrossing species that *F. crenata* trees with high pollen fecundity may contribute disproportionately to future generations, and that *F. crenata* pollen travels long distances. In our seed orchard, three clones fathered 68.2% of all seedlings. However, to maximize genetic diversity, it is important to select several clones with synchronous flowering and comparable pollen fecundity. With regard to long-distance gene flow, allozyme study showed that genetic differentiation between populations is very low (G_{ST}=0.038; Tomaru et al. 1997), because gene migrants from neighboring populations frequently come into a population in pollen. Pollen fecundity, flowering phenology, and pollen contamination are critical issues for genetic diversity and production of high-quality seedlings in seed orchards.

4 Genetic Management of *Fagus* Forests for Conservation and Sustainable Use

Forests frequently experience fragmentation or isolation by human disturbance and thus suffer genetic bottlenecks. Severe bottlenecks, such as drastic reduction in population size, result in genetic erosion and loss of adaptation to environmental change. In a small and isolated population, inbreeding depression as a result of mating between relatives reduces the variability and viability of forest. These forces cause forests to decline and can change the forest environment. To maintain relatively high genetic diversity within forests, long-term genetic monitoring is needed. Microsatellite markers are very suitable for this purpose due to their high polymorphism. This marker can be traced to the pollen and seed movements. This kind of information is neces-

sary to maintain adequate genetic diversity within forests especially for the conservation and management of forest tree species.

Fagus forests are widely distributed in Europe, eastern Asia, and eastern North America (Peters 1997), but their area has been decreased and fragmented by exploitation for land and timber. In particular, suburban spread has promoted fragmentation. For the conservation of such forests, maintenance of genetic diversity is very important. Data on the heterozygosity, allelic richness, genetic differentiation between local populations, outcrossing rate, and genetic structure of those forests are important for conservation. Gene flow through pollen is not strongly restricted in *Fagus*, because this species is wind-pollinated. An allozyme study of 23 populations of *Fagus* forest showed that the genetic differentiation between populations was low (Tomaru et al. 1997). However, gene flow through seeds is strongly restricted: genetic differentiation revealed by both mtDNA and cpDNA polymorphisms between populations was extremely high (Tomaru et al. 1998; Fujii et al. 2002; Okaura and Harada 2002). Guidelines for gene conservation within populations can be based on information on maternal inherited DNA markers such as mtDNA and cpDNA. However, an understanding of gene flow within a forest through pollen and seed is necessary for conservation and sustainable use. Fine-scale genetic structure is influenced by the regeneration system, forest history, and microenvironment heterogeneity, such as forest floor conditions. Generally, long-lived, wind-pollinated, dominant species such as *Fagus*, *Quercus*, and conifers in temperate regions show weak spatial genetic structure (Merzeau et al. 1994; Streiff et al. 1998; Takahashi et al. 2000; Epperson and Chung 2001). If the structure becomes strong, this indicates that a forest is declining.

Tree density is an important factor in maintaining a high outcrossing rate and genetic diversity (Rajora et al. 2000; Obayashi et al. 2002). Inbreeding increases in an isolated or low-density forest, and the forest declines owing to inbreeding depression. Most forest tree species are predominantly allogamous, and some are self-incompatible.

The outcrossing rate and a fixation index can be used to assess the integrity of a forest. Takahashi et al. (2000) suggested that reduced genetic variability and linkage disequilibrium would have a significant influence over several generations. Reductions in genetic 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 to the conservation of important genetic resources. For rehabilitation programs, indicators such as spatial genetic structure, outcrossing rate, fixation index, and linkage disequilibrium can be used to assess the integrity of planted populations.

5 Conclusions

We could develop a sufficient number of microsatellite markers in *F. crenata* using the enrichment method of the microsatellite region. These markers will provide important information for the conservation and management of *F. crenata* forests.

Fine-scale genetic structure was influenced by life history such as the regeneration system, microenvironment, and forest history. *Fagus crenata* had a weaker fine-scale genetic structure than that of *F. japonica*, probably due to the different level of self-incompatibility and longevity. The genetic structure of *F. crenata* has also been changed by their microenvironment such as soil type and forest floor, which are closely related to forest history. The parameter of linkage disequilibrium could be one of better indicators to understand the forest history, which might show the maturity and stability of forest population. Combining ecological and environment data together with genetic data, we can understand the integrity of forest population and may take a suitable strategy for conservation and management. For the rehabilitation program of *Fagus* forest, seed sources with high genetic diversity and genetic similarity to introduced population is critically important to maintain original genetic component of the population. For that purpose, the suitable seed orchard is necessary to supply such seedlings for the plantation.

We believe that microsatellite markers are the best markers to monitor the gene flow within a forest, fine-scale genetic structure, and to investigate genetic diversity and similarity between the seed source and the rehabilitation forests for conservation and management of *Fagus* populations.

References

Asuka Y, Tani N, Tsumura Y, Tomaru N (2004a) Development and characterization of microsatellite markers for *Fagus crenata* Blume. Mol Ecol Note 4:101–103

Asuka Y, Tomaru N, Nishimura N, Tsumura Y, Yamamoto S (2004b) Spatial genetic structure of *Fagus crenata* (Fagaceae) in an old-growth beech forest revealed by microsatellite markers. Mol Ecol 13:1241–1250

Buiteveld J, Bakker EG, Bovenschen J, de Vries SMG (2001) Paternity analysis in a seed orchard of *Quercus robur* L. and estimation of the amount of background pollination using microsatellite markers. For Genet 8:331–337

Cifarelli RA, Gallitelli M, Cellini F (1995) Random amplified hybridization microsatellites (RAHM): isolation of a new class of microsatellite-containing DNA clones. Nucleic Acid Res 23:3802–3803

Doligez A, Baril C, Joly HI (1998) Fine-scale spatial genetic structure with nonuniform distribution of individual. Genetics 148:905–919

Dow BD, Ashley MV (1996) Microsatellite analysis of seed dispersal and parentage of sapling in bur oak, *Quercus macrocarpa*. Mol Ecol 5:615–627

Epperson BK, Chung MG (2001) Spatial genetic structure of allozyme polymorphisms within populations of *Pinus strobus* (Pinaceae). Am J Bot 88:1006–1010

Fischer D, Bachmann K (1998) Microsatellite enrichment in organisms with large genomes (*Allium cepa* L.). Biotechniques 24:796–802

Friedman ST, Adams WT (1985) Estimation of gene flow into two seed orchards of loblolly pine (*Pinus taeda* L.). Theor Appl Genet 69:609–615

Fujii N, Tomaru N, Okuyama K, Koike T, Mikami T, Ueda K (2002) Chloroplast DNA phylogeography of *Fagus crenata* (Fagaceae) in Japan. Plant Systemat Evol 232:21–33

Hamilton MB, Pincus EL, di Fiore A, Fleischer RC (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques 27:500–507

Hamrick JL (1989) Isozymes and the analysis of genetic structure in plant populations. In: Soltis DE, Soltis PS (eds) Isozymes in plant biology. Dioscorides Press, Portland, Oregon, USA, pp 87–105

Igarashi T (1996) The relationship between variation of fructification and efficiency of pollination in *Fagus crenata* and *F. japonica*. Msc Diss, University of Tokyo

Karagyozov L, Kalcheva ID, Chapman VM (1993) Construction of random small-insert genomic libraries highly enriched for simple sequence repeats. Nucleic Acids Res 21:3911–3912

Kelly JK, Willis JH (2002) A manipulative experiment to estimate biparental inbreeding in monkeyflowers. Int J Plant Sci 163:575–579

Kirkpatrick BW, Bradshaw M, Barendse W, Dentine MR (1995) Development of bovine microsatellite markers from a microsatellite-enriched library. Mamm Genome 6:526–528

Kitamura S, Murata G (1979) Colored illustrations of woody plants of Japan, vol II. Hoikusha Publ, Osaka, Japan

Kitamura K, Shimada K, Nakashima K, Kawano S (1997) Demographic genetics of the Japanese beech, *Fagus crenata*, at Ogawa forest preserve,Ibaraki, central Honshu, Japan. I. Spatial genetic substructuring in local population. Plant Species Biol 12:107–136

Kouno K, Mukouda M (1985) Flowering and seed-setting traits of three broadleaf trees, *Fagus crenata*, *Cornus controvera* and *Aesculus turbinata*. Bull Tohoku For Tree Breed Ctr 25:74–76

Lench NJ, Norris A, Bailey A, Booth A, Markham AF (1996) Vectorette PCR isolation of microsatellite repeat sequence using anchored dinucleotide repeat primers. Nucleic Acids Res 24:2190–2191

Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397–401

Loveless MD, Hamrick JL (1984) Ecological determinants of generic structure in plant populations. Annu Rev Ecol Sys 15:65–95

Lyall JEW, Brown GM, Furlong RA, Ferguson-Smith MA, Affara NA (1993) A method for creating chromosome-specific plasmid libraries enriched in clones containing [CA]n microsatellite repeat sequences directly from flow-sorted chromosome. Nucleic Acids Res 21:4641–4642

Merzeau D, Comps B, Thiebaut B, Cuguen J, Letouzey J (1994) Genetic structure of natural stands of *Fagus sylvatica* L. (beech). Heredity 72:269–277

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DANN. Nucleic Acids Res 8:4321–4325

Nakashizuka T (1987) Regeneration dynamic of beech forests in Japan. Vegetatio 69:169–175

Obayashi K, Tsumura Y, Ihara-Ujino T, Niiyama K, Tanouchi H, Suyama Y, Washitani I, Lee C-T, Lee S-L, Muhammad N (2002) Genetic diversity and outcrossing rate between undisturbed and selectively logged forests of *Shorea curtisii* (Dipterocarpaceae) using microsatellite DNA analysis. Int J Plant Sci 163:151–158

Ohkubo T (1992) Structure and dynamics of Japanese beech (*Fagus japonica* Maxim.) stools and sprouts in the regeneration of the natural forests. Vegetatio 101:65–80

Okaura T, Harada K (2002) Phylogeographical structure revealed by chloroplast DNA variation in Japanese beech (*Fagus crenata* Blume). Heredity 88:322–329

Ostrander EA, Jong PM, Rine J, Duyk G (1992) Construction of small-insert genomic DNA libraries highly enriched for microsatellite repeat sequences. Proc Natl Acad Sci USA 89:3419–3423

Pakkanen A, Nikkanen T, Pulkkinen P (2000) Annual variation in pollen contamination and outcrossing in a *Picea abies* seed orchard. Scand J For Res 15:399–404

Peters R (1997) Beech forests. pp. 169, Kluwer, Dordrecht

Rajora OP, Rahman MH, Buchert GP, Dancik BP (2000) Microsatellite DNA analysis of genetic effects of harvesting in old-growth eastern white pine (*Pinus strobus*) in Ontario, Canada. Mol Ecol 9:339–348

Streiff R, Labbe T, Bacilieri R, Steinkellner H, Glossl J, Kremer A (1998) Within-population genetic structure in *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. assessed with isozymes and microsatellites. Mol Ecol 7:317–328

Takahashi H, Nirawasa N, Furukawa T (1996) An efficient method to clone chicken microsatellite repeat sequences. Jpn Poultry Sci 33:292–299

Takahashi M, Mukouda M, Koono K (2000) Differences in genetic structure between two Japanese beech (*Fagus crenata* Blume) stands. Heredity 84:103–115

Takahashi T, Konuma A, Ohkubo T, Taira H, Tsumura Y (2004) Comparison of spatial genetic structures in *Fagus crenata* and *F. japonica* by the use of microsatellite markers. Silvae Genet (in press)

Tanaka K, Tsumura Y, Nakamura T (1999) Development and polymorphism of microsatellite markers for *Fagus crenata* and closely related species, *F. japonica*. Theor Appl Genet 99:11–15

Tomaru N, Mitsutsuji T, Takahashi M, Tsumura Y, Uchida K, Ohba K (1997) Genetic diversity in *Fagus crenata* (Japanese beech): influence of the distributional shift during the late-Quaternary. Heredity 78:241–251

Tomaru N, Takahashi M, Tsumura Y, Takahashi M, Ohba K (1998) Intraspecific variation and phylogeographic patterns of *Fagus crenata* (Fagaceae) mitochondrial DNA. Am J Bot 85:629–636

Ueno S, Tomaru N, Yoshimaru H, Manabe T, Yamamoto S (2000) Genetic structure of *Camellia japonica* L. in an old-growth evergreen forest, Tsushima, Japan. Mol Ecol 9:647–656

UNESCO (2002) Properties inscribed on the word heritage list world heritage centre. UNESCO, Paris

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396