KELMAN, A. (1954): The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology **44**: 693–695.

KUSNANDAR, D., GALWEY, N. W., HERTZLER, G. L. and BUTCHER, T. B. (1998): Age trends in variances and heritabilities for diameter and height in maritime pine (*Pinus pinanster* Ait.) in Western Australia. Silvae Genet. **47**: 136–141.

LOPEZ, G. A., POTTS, B. M., VAILLANCOURT, R. E. and APIOLAZA, L. A. (2003): Maternal and carryover effects on early growth of *Eucalyptus globulus*. Can. J. For. Res. **33**: 2108–2115.

MONMA, S. and SAKATA, Y. (1993): Inheritance of resistance to bacterial wilt in tomato. *In:* Bacterial Wilt. ACIAR proceedings No. 45. Edited by HARTMAN, G. L. and HAYWARD, A. C. pp. 149–153.

OSORIO, L. F., WHITE, T. L. and HUBER, D. A. (2001): Age trends of heritabilities and genotype-by-environment interactions for growth traits and wood density from clonal trials of *Eucalyptus grandis* Hill ex Maiden. Silvae Genet. **50**: 30–37.

PAUL, A. D., FOSTER, G. S., CALDWELL, T. and MCRAE, J. (1997): Trends in genetic and environmental parameters for height, diameter, and volume in a multilocation clonal study with loblolly pine. For. Sci. **43**: 87–98.

ROUX, J., COUTINHO, T. A., BYABASHAIJA, D. M. and WINGFIELD, M. J. (2001): Diseases of plantation *Eucalyptus* in Uganda. South Afri. J. Sci. **97**: 16–18.

SAS INSTITUTE INC. (1997): SAS/STAT® user's guide. Version 6.0, 4th ed. SAS Institute Inc., Cary, N.C.

SATHYANARAYANA, H. V. and ANAND, N. (1993): Developing bacterial wilt resistant F_1 hybrids for processing in tomato (*Lycopersicon exculentum*). *In:* Bacterial Wilt. ACIAR proceedings No. 45. Edited by HARTMAN, G. L. and HAYWARD, A. C. pp. 158–162.

SORIA, F. and BORRALHO, N. M. G. (1997): The genetics of resistance to *Phoracantha semipunctata* attack in *Eucalyptus globulus* in Spain. Silvae Genet. **46**: 365–369.

SUDO, S., OLIVEIRA, G. H. N. and PEREIRA, A. C. (1983): Eucalipto (*Eucalyptus* spp.), bracaatinga (*Mimosa scrabella* Penth.) novas hospedeiras de *Pseudomonas solanacearum* E. F. Smith. Fitopatologia Brasileira **8**: 631.

TIKOO, S. K., ANAN, N. and RAMKRISHNA (1983): Presence of two independent genetic systems for resistance to bacterial wilt (*Pseudomonas solanacearum*) in tomato. In: Proceedings of the 15th International Genetic Congress, 12–23 December 1983, New Delhi, India.

WEI, X. and BORRALHO, N. M. G. (1998): Genetic control of growth traits of *Eucalyptus urophylla* S. T. Blake in southeast China. Silvae Genet. **47**: 158–165.

WINSTEAD, N. N. and KELMAN, A. (1952): Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. Phytophthology **42**: 628–634.

WU, Q. and LIANG, Z. (1988a): Selection of species and provenances of *Eucalyptus* for resistance to bacterial wilt (in Chinese). J. South China Agri. Univ. **9**: 41–45.

WU, Q. and LIANG, Z. (1988b): Identification and pathogenic tests of the causal organism of the bacterial wilt of *Eucalyptus* (in Chinese). J. South China Agri. Univ. **9**: 59–67.

YABUUCHI, E., KOSAKO, Y., YANO, I., HOTTA, H. and NISHIUCHI, Y. (1995): Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. Nov.: Proposal of *Ralstonia pickettii* (RALSTON, PALLERONI and DOUDOROFF, 1973) comb. Nov., *Ralstonia solanacearum* (SMITH, 1896) comb. Nov. and *Ralstonia eutropha* (DAVIS, 1969) comb. Nov. Microbiol. Immunol. **39**: 897–904.

YU, Q., YANG, D-Q., ZHANG, S. Y., BEAULIEU, J. and DUCHESNE, I. (2003): Genetic variation in decay resistance and its correlation to wood density and growth in white spruce. Can. J. For. Res. **33**: 2177–2183.

YUAN, Z., CHEN, F., CHEN, X. and FANG, S. (1999): Inheritance of bacterial wilt resistance and its correlation with yield traits in sweet potato (in Chinese). J. Fujian Agri. Univ. **28**: 413–416.

ZHU, J. (1992): Mixed model approaches for estimating genetic variances and covariance. J. Biomath. **7**: 1–11.

Patterns of Pollen Flow and Genetic Differentiation Among Pollen Pools in *Quercus salicina* in a Warm Temperate Old-growth Evergreen Broad-leaved Forest

By A. NAKANISHI[1*], N. TOMARU[1], H. YOSHIMARU[2], T. KAWAHARA[3], T. MANABE[4] and S. YAMAMOTO[1]

Forest Ecology and Physiology, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

(Received 5th November 2004)

Abstract

Paternity analysis and analysis of molecular variance were used to determine patterns of pollen flow and genetic differentiation among pollen pools in *Quercus salicina* in an 11.56-ha plot in a temperate old-growth evergreen broad-leaved forest. The genotypes at seven microsatellite loci were determined for 111 adult trees and 276 seeds collected from under eight seed parents. The proportion of pollen flow from outside the plot (further than 100 m) was 52.2%, indicating that long-distance pollen flow occurred frequently in this species, as observed in other *Quercus* species. The pollen pools from inside and outside the plot differed genetically, and genetic structure was detected in the population of adult trees within the plot. Therefore, long-distance pollen flow from outside the plot may introduce new or low-frequent alleles, and increase genetic diversity in this population. However, the actual average distance of pollen flow within the plot was significantly shorter than the average potential distance, and negative exponential curves explained well the frequencies of matings as functions of the distance

[1] Laboratory of Forest Ecology and Physiology, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan.

[2] Ecological Genetic Laboratory, Department of Forest Genetics, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan.

[3] Forest Dynamics and Diversity Group, Hokkaido Research Center, Forestry and Forest Products Research Institute, Hitsujigaoka-7, Toyohira, Sapporo, Hokkaido 062-8516, Japan.

[4] Kitakyushu Museum of Natural History and Human History, Kitakyushu 805-0071, Japan.

*) Corresponding author: ATSUSHI NAKANISHI, E-mail: tomaru@agr.nagoya-u.ac.jp

DOI:10.1515/sg-2004-0048
edited by Thünen Institute of Forest Genetics

between parents, as found in other *Quercus* species. The genetic composition of pollen pools differed among the eight seed parents. The genetic differentiation of pollen pools between the seed parents showed significant positive correlation with the spatial distance between them, indicating that neighboring seed parents tend to accept similar pollen pools, probably because matings are frequently mediated by pollen transported over short distances

Key words: AMOVA, gene flow, microsatellite, paternity analysis, pollen, *Quercus*.

Introduction

Gene movement within and between populations determines genetic variation and structure over space and time, which in turn influences their evolutionary potential. In plant species with large, relatively immobile seeds, pollen dispersal is probably the most important component of gene movement. Pollen dispersal has been studied by following the physical movement of pollen using traps (GREENWOOD, 1986), dyes (LINHART *et al.,* 1987), and the movement of pollinators (LEVIN and KERSTER, 1969; MOSQUIN, 1971). However, the results of these studies may not accurately reflect actual fertilization events and gene movement within populations. Allozyme studies of gene movement have been conducted, but allozymes generally lack the resolution required to directly determine gene movement. Therefore, in most allozyme studies, estimates of F_{ST} (and variants of this parameter) based on allele frequencies have been used to indirectly infer the extent of gene flow among populations by estimating *Nm*, the number of migrants successfully entering a population per generation. Unfortunately, F_{ST} values can rarely be translated into accurate estimates of *Nm* because the mathematical model underlying this translation makes many biologically unrealistic assumptions (WHITLOCK and MCCAULEY, 1999).

Recently, gene movement in plant populations has been studied directly by parentage analysis using microsatellite markers with very high polymorphism and codominant expression (CHASE *et al.,* 1996; DOW and ASHLEY, 1996; DOW and ASHLEY, 1998; STREIFF *et al.,* 1999; ISAGI *et al.,* 2000). Parentage analysis is a methodology that determines the parents of offspring by comparing the genotypes of offspring and candidate parents. To determine the paternal parent, one usually compares the genotypes of the offspring, the known maternal parent, and the candidate paternal parents. Allelic diversity tends to be very high at microsatellite loci and, in most cases, either all adults or all but one adult in the population can be excluded as possible paternal parents (CHASE *et al.,* 1996; DOW and ASHLEY, 1998; STREIFF *et al.,* 1999). The movement of successful pollen can then be traced by examining the relative locations of the identified pollen and seed parents.

The natural vegetation in the warm-temperate zones of East Asia is evergreen broad-leaved forest. This type of forest is widespread in humid areas at mid-latitude in the Northern Hemisphere and is dominated by the families Fagaceae, Lauraceae and Hamamelidaceae (KIRA, 1991; TAGAWA, 1995). These forests once covered most lowland areas in southwest Japan, but because of human disturbance, old-growth stands are now found only around Shinto shrines, on islands, and on steep mountain slopes that are unsuitable for human development. Remnants of these forests are scattered and small, covering at most a few hectares, and rarely on level ground. Data on stand structure, population structure and population dynamics of these forests are scarce because of the rarity of suitable old-growth forests for study.

The research site in this study was in the Tatera Forest Reserve, on the South Island of Tsushima, which is located between the Japanese Archipelago and the Korean Peninsula. The reserve, protected as a National Natural Monument, has an area of approximately 100 ha and is situated on the north-facing slope of Mt. Tatera. There has been no human interference in the reserve for several centuries, and an old-growth evergreen broad-leaved forest is well developed in it (ITOW, 1991). At the research site, *Quercus salicina* (an evergreen oak) belonging to the subgenus *Cyclobalanopsis* constitutes one of the major species and forms the highest canopy, but is present at low density (MANABE *et al.,* 2000). This species is suitable for parentage analysis because of the low frequency of candidate parents. Therefore, in this study, we investigated the patterns of pollen flow and genetic differentiation among pollen pools in *Quercus salicina* by paternity analysis and analysis of molecular variance (AMOVA).

The genus *Quercus* (oak) comprises approximately 500 species of trees and shrubs distributed throughout much of the Northern Hemisphere (NIXON, 1993). Oaks are conspicuous members of the temperate deciduous forests of North America, Europe and Asia, in addition to being important evergreen elements of Mediterranean woodlands and subtropical forests. Most of them are important members of the forest ecosystem. Because of their economic and ecological importance, they have been included in many ecological and genetic studies. For this reason, oaks have become important model species in plant evolutionary genetics, but most genetic investigations have focused on a small number of European and North American species. There have been few genetic studies on Asian *Quercus* species, and most of them have examined members of the subgenus *Quercus* (deciduous oak), while southeast Asian members of the subgenus *Cyclobalanopsis* (evergreen oak) have been largely neglected.

Materials and Methods

Study site and field methods

A 4-ha permanent plot (200 x 200 m) was established in the Tatera Forest Reserve in 1990. Tree censuses were performed in 1990, 1992, 1997, and 2002 for all stems ≥ 5 cm in diameter at breast height (d.b.h.) of all woody plant species. The plot contained a total of 45 species and 4570 living stems ≥ 5 cm d.b.h., with a total basal area of 63.9 m^2/ha. The dominant species in terms of stem density was *Distylium racemosum* (Hamamelidaceae), at 410.0 stems/ha, but in terms of basal area *Castanopsis cuspidata* (Fagaceae) was dominant, at 24.9 m^2/ha. *Quercus salicina* was one of the major species in the plot; its basal area was the third largest at 4.7 m^2/ha, while its density was the ninth highest, at 13.5 stems/ha (MANABE *et al.,* 2000). In 2003, the 4-ha plot was expanded to 11.56 ha (340 x 340 m) for the census of *Quercus salicina*, and 111 *Quercus salicina* individuals were found with a d.b.h. ≥ 5 cm. Our previous field observations of *Q. salicina* in the plot indicated that all individuals with a d.b.h. ≥ 5 cm can flower, but individuals with a d.b.h. < 5 cm are not yet ready to flower. Thus, all the 111 individuals could be defined as adult trees with the potential to act as pollen parents. Leaf samples for all 111 adult trees and 278 seed samples located under the canopies of eight adult *Q. salicina* trees (*Fig. 1*) were collected, transported to our laboratory and stored at –30 °C until DNA extraction.

DNA extraction and microsatellite genotyping

Genomic DNA was extracted using a modification of the hexadecyltrimethyl ammonium bromide (CTAB) method (MURRAY and THOMPSON, 1980). Briefly, leaves or embryos of seeds were frozen in liquid nitrogen and ground to a fine powder using a mortar and pestle. Several spoonfuls of the powder (ca.

DOI:10.1515/sg-2004-0048
edited by Thünen Institute of Forest Genetics

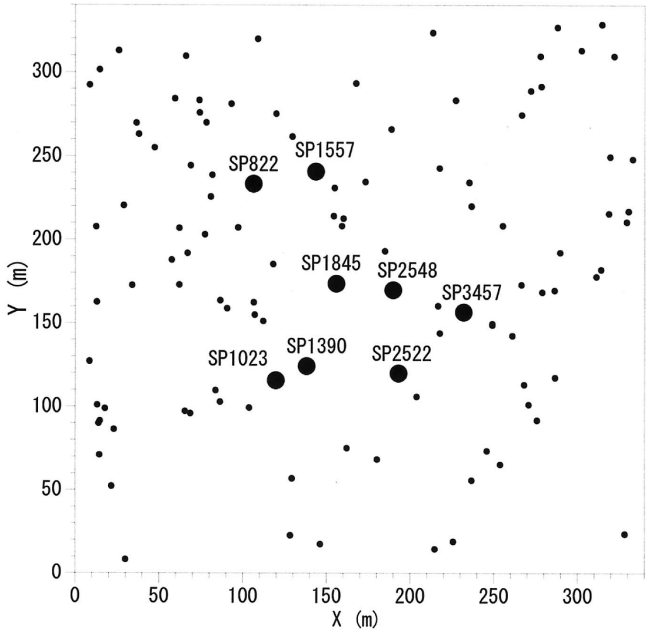

Figure 1. – Location of the eight seed parents (large circles with ID numbers) and the other 103 adults (small circles) of *Q. salicina* in the 11.56-ha plot.

100 mg) were mixed with 500 µl of CTAB extraction buffer [2% CTAB, 1.4 M NaCl, 0.1 M Tris-HCl (pH 8.0), 0.02 M EDTA, 4% β-mercaptoethanol] and incubated at room temperature for 30 min. A chloroform-isoamyl alcohol (24:1) extraction and an isopropanol precipitation were then performed. The precipitates were resuspended in TE buffer [10 mM Tris-HCl (pH 7.5) and 0.1 mM EDTA] followed by a single phenol-chloroform extraction. The DNA was then precipitated with 99.5% ethanol and 3 M sodium acetate, followed by a 70% ethanol wash and resuspension in TE buffer. Each DNA solution was standardized to 10 ng/µl for use in microsatellite amplification, by dilution with an appropriate amount of TE buffer, after quantifying the DNA concentration by 0.8% agarose gel electrophoresis in TAE buffer [40 mM Tris-acetate (pH 8.0) and 1 mM EDTA], staining with ethidium bromide and measuring the resulting fluorescence under UV illumination.

Seven polymorphic microsatellite loci were selected for genotyping *Q. salicina* adults and seeds: *QsalCT15* and *QsalCT33* were developed for *Q. salicina* by T. KAWAHARA (personal communication), *QpZAG119* for *Q. petraea* (STEINKELLNER *et al.,* 1997), *MSQ4* for *Q. macrocarpa* (DOW *et al.,* 1995), *QrZAG7* and *QrZAG101* for *Q. robur* (KAMPFER *et al.,* 1998), and *QM69-2M1* for *Q. mysiniforia* (ISAGI and SUHANDONO, 1997). The sequences of forward and reverse primers for the two loci *QsalCT15* and *QsalCT33* were CGGTAAGACGTTTGGTGTAG and TTGTACGGACGCCATTGAAA, and GCCTCTAGCCAGTTCAGTTG and CATTATTACTACCATTACAGTTTTGT, respectively.

For each sample, a 10-µl mixture containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of each primer, 0.25 units of Taq polymerase, and 10 ng of template DNA was used for polymerase chain reaction (PCR) amplification. PCR was performed using a thermal cycler (GeneAmp PCR System 9700, ABI) under the following conditions: initial denaturation at 94°C for 3 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 50–57°C for 30 s, and extension at 72°C for 30 s; followed by a final extension at 72°C for 2 min. Genotyping at the microsatellite loci was conducted by capillary electrophoresis using a 3100-Avant Genetic Analyzer and GeneScan™ analysis software (ABI).

Statistical data analyses

The genetic diversity of *Q. salicina* adults in the 11.56 ha plot was analyzed using standard population genetic parameters. The number of alleles (A), observed heterozygosity (H_O), unbiased expected heterozygosity (H_E) and fixation index (F_{IS}) were calculated for each locus and over all the loci. Deviations from Hardy-Weinberg equilibrium at each locus and over all loci were tested by the Markov-chain method using the Genepop program ver.3.3 (RAYMOND and ROUSSET, 1995).

The genetic structure of the adult trees in the plot was examined by calculating a correlation between the genetic and spatial distances between them. The genetic relatedness between two adult trees was used as a measure of the genetic distance. In this study, the relatedness proposed by QUELLER and GOODNIGHT (1989) was applied, the average of which is assumed to be zero as a whole within a population, and ranges from –1 to 1. The relatedness of all pairs of the 111 adult trees was calculated using the RELATEDNESS program (QUELLER and GOODNIGHT, 1989). MANTEL's r statistic was calculated to quantify the correlation between the genetic and spatial distances (MANTEL, 1967), and the significance was tested based on 5000 randomizations using the R PACAGE program (CASGRAIN and LEGENDRE, 1999).

Paternity analysis was performed by simple exclusion (CHAKRABORTY *et al.,* 1974) based on multilocus genotypes for all 111 adult trees and 278 seeds, assuming that each of the eight adult trees whose crowns covered the seed sampling site was the seed parent of the seeds collected there. This analysis was conducted using the CERVUS program (MARSHALL *et al.,* 1998).

The distances of pollen flow within the plot were analyzed by the following three methods. First, the frequency of matings with each pollen parent over all matings was separately calculated for each seed parent. The frequency distribution was plotted as a function of the distance between the parents. Data were bulked and fitted to an exponential function. Second, following the method by STREIFF *et al.* (1999), subdividing all matings into distance classes, the frequency of matings over all matings was calculated at each distance class for each seed parent. The frequency distribution was then plotted as a function of the distance between the parents. Data were averaged over all seed parents and fitted to an exponential function. In this study, because the density of adults was low, each distance class had a 40-m interval. Third, to test whether the average distance of pollen flow within the plot deviated significantly from random expectations it was compared with the distribution of the average values generated by the permutation procedure. The permutation was repeated 1000 times, as follows. If the ith seed parent had n_i seeds whose pollen parents could be assigned by paternity analysis (so the total number of seeds, N, was the sum of n_i over all eight seed parents), a sample for the seed parent was generated with n_i randomly extracted adult trees from the 110 potential pollen parents, with replacement. The distances between the seed parent and the n_i extracted adult trees were then estimated, and an average value over all eight seed parents (an average value for N distances) was calculated.

Similarly, to test whether the average relatedness between mating pairs of parents within the plot differed significantly from random expectations, it was compared with the distribution of the average values generated by the same permutation procedure as used to test the average distance of pollen flow within the plot.

DOI:10.1515/sg-2004-0048
edited by Thünen Institute of Forest Genetics

To quantify genetic heterogeneity among the pollen pools of the seed parents, AMOVA of pollen haplotypes (EXCOFFIER *et al.,* 1992; SMOUSE *et al.,* 2001) was performed as follows. Pollen haplotypes were obtained by subtracting the contribution of the seed parent from the genotypes of the seeds, and pollen profiles were constructed using the pollen haplotypes. In ambiguous cases, in which seeds and their seed parents were heterozygotes, with the same alleles at the same loci, the pollen profiles were constructed using the likelihood of pollen parent contribution (SMOUSE *et al.,* 2001). Variation in the pollen profiles was then examined using AMOVA based on the pairwise squared Euclidean distances between the pollen profiles. AMOVA allowed us to calculate both variance components within and among the pollen pools of the seed parents and "Φ_{FT}" values (analogs of Wright's F_{ST} values). This analysis was conducted for the total pollen pools and for the separate pollen pools originating inside and outside of the plot. A Φ_{FT} value was also calculated between the pollen pools originating inside and outside of the plot by bulking all seeds of the eight seed parents. The significance of the Φ_{FT} values was tested by 1000 randomizations (EXCOFFIER *et al.,* 1992). Pairwise Φ_{FT} values as genetic distances between the pollen pools of the seed parents were also obtained from the AMOVA, and tested by 1000 randomizations after Bonferroni correction. Spearman's rank correlation coefficients were determined between the pairwise Φ_{FT} and the spatial distance or relatedness between the seed parents. The correlation analysis relevant to the spatial distance was also conducted for the total pollen pools and for the separate pollen pools originating inside and outside of the plot.

Results

Characteristics of seven polymorphic microsatellite loci

The microsatellites amplified by the seven primer pairs were highly variable, with 7-25 alleles per locus and mean H_E of 0.754 (*Table 1*). F_{IS} values for each locus and all the loci were –0.117 to 0.087 and –0.004, respectively, and the deviations from Hardy-Weinberg equilibrium were not significant. The total exclusion probability for the second parent (MARSHALL *et al.,* 1998), i.e., the exclusion probability if one parent was known, was 0.9993 over the seven loci. The probability of correctly excluding all unrelated adult trees (111 trees) within the plot was 0.925 calculated by 0.9993^{111}.

Genetic structure of adult trees

A significant negative correlation was detected between the spatial distance and relatedness between the adult trees in the plot (*Fig. 2*). The absolute value of MANTEL's *r*-value was, however, low ($r = -0.1028$, $P < 0.0005$, Mantel test), indicating that weak genetic structure existed among the adult trees.

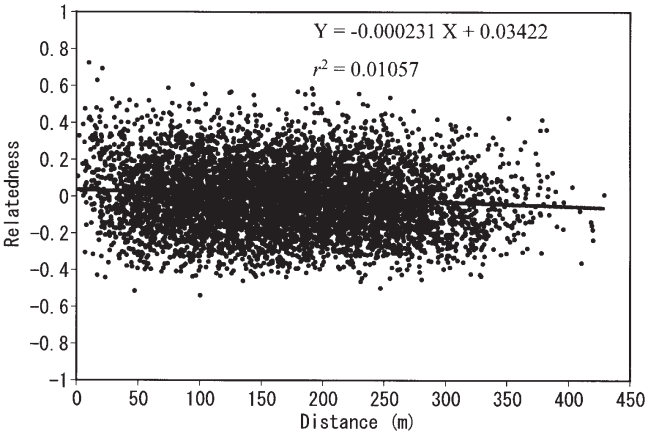

Figure 2. – Correlation between spatial distance and relatedness between the adult trees in the plot. The correlation was significantly negative (Mantel test with 5000 permutation, $r = -0.1028$, $P < 0.0005$). The solid line is the slope of a least-squares fit ($r^2 = 0.01057$).

Paternity analysis

Genotypes at the seven loci were determined for all of the 278 seeds sampled. The haplotypes of 276 seeds matched those of the respective putative seed parents, and the haplotypes of only two seeds (sampled under the canopy of SP1023 and SP2548) did not match their putative seed parents. The 276 seeds were defined as the offspring of each putative seed parent, and the remaining two seeds were probably brought under the canopy from the canopy of other adult trees. Paternity analysis was conducted for the 276 seeds; 132 had at least one candidate pollen parent in the plot and were therefore considered to have been pollinated by adult trees within the plot, including one case of self-pollination (*Table 2*). Thus, 144 (52.2% of the total) of the gene movement events analyzed were apparently mediated by pollen from outside the plot. In the 11.56-ha plot, all the seed parents were located at least 100 m from any edge of the plot. Therefore, 52.2% of the pollen flow occurred over a distance greater than 100 m.

According to the results of the simple exclusion analysis, 11 of the 132 seeds that apparently originated from pollination by trees within the plot had multiple candidate pollen parents and were excluded from the analysis of pollen flow within the plot. Each of the other 121 seeds had only one candidate pollen par-

Table 1. – Characteristics of seven polymorphic microsatellite loci revealed by genotyping 111 adult trees in the plot.

Locus	*A*	H_O	H_E	F_{IS}
QsalCT15	16	0.865	0.880	0.017
QsalCT33	9	0.577	0.599	0.037
QpZAG119	25	0.766	0.839	0.087
QM69-2M1	7	0.441	0.418	-0.055
QrZAG101	11	0.910	0.815	-0.117
QrZAG7	12	0.928	0.884	-0.050
MSQ4	14	0.802	0.844	0.050
Mean	13	0.756	0.754	-0.004

A, Number of alleles detected; H_O, observed heterozygosity; H_E, expected heterozygosity; and F_{IS}, Fixation index.

Table 2. – Profiles of mating events in the eight seed parents revealed by paternity analysis.

Seed parent	Self[a]	Mating within the plot[b]	Pollen migration[c]
SP822 (20)	0.000 (0)	0.450 (9)	0.550 (11)
SP1023 (58)	0.000 (0)	0.586 (34)	0.414 (24)
SP1390 (42)	0.000 (0)	0.524 (22)	0.476 (20)
SP1557 (31)	0.000 (0)	0.323 (10)	0.677 (21)
SP1845 (39)	0.000 (0)	0.564 (22)	0.436 (17)
SP2522 (13)	0.000 (0)	0.462 (6)	0.538 (7)
SP2548 (43)	0.000 (0)	0.349 (15)	0.651 (28)
SP3457 (30)	0.033 (1)	0.433 (13)	0.533 (16)
Total (276)	0.004 (1)	0.475 (131)	0.522 (144)

[a] Proportion of self-pollinated seeds.
[b] Proportion of mating events that occurred within the plot.
[c] Proportion of mating events for which no compatible pollen parent was found within the plot. The numbers of seeds are in parentheses.

DOI:10.1515/sg-2004-0048
edited by Thünen Institute of Forest Genetics

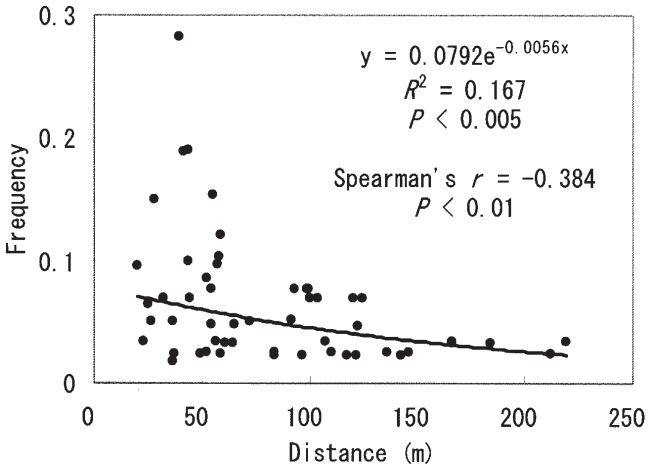

Figure 3. – Distribution of the frequencies of matings with each pollen parent over all matings for each seed parent as a function of the distance between the parents. The frequencies of matings were calculated as the number of matings with each pollen parent divided by the total number of matings for the mated seed parent. Data were bulked and fitted to an exponential function.

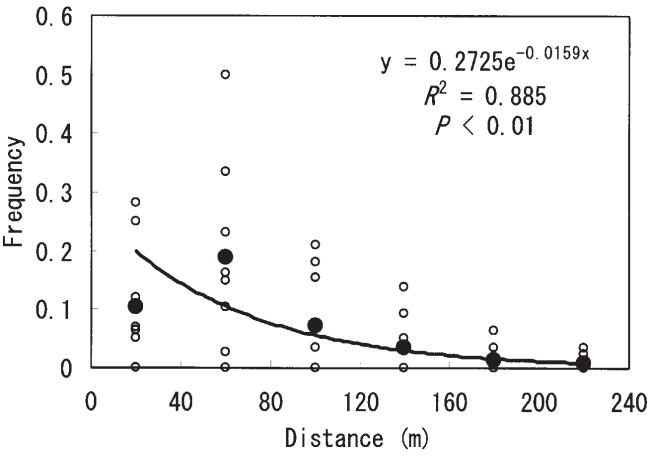

Figure 4. – Distributions of the frequencies of matings over all the matings at each distance class for each seed parent (open circles) and of the averages over all seed parents (filled circles) as functions of the distance between the parents. The frequencies of matings were calculated as the number of matings at each distance class divided by the total number of matings for the mated seed parent. The averages as a function of the distance were fitted to an exponential function.

ent, but one appeared to be self-pollinated and was also excluded from the analysis, since the aim was to evaluate cross-pollination. The average distance for pollen flow within the plot, based on the remaining 120 pollination events, was found to be 66.7 m.

Pattern of pollen flow within the plot

A negative exponential function of the distance between parents explained significantly the frequencies of matings with each pollen parent over all matings for each seed parent (R^2 = 0.167, $P < 0.005$), and there was also a significant, negative correlation between the distance and these frequencies (Spearman's $r = -0.384$, $P < 0.01$) (*Fig. 3*). Furthermore, a negative exponential function of the distance explained significantly the averages of the frequencies of matings over all matings at each distance class for each seed parent ($R^2 = 0.885$, $P < 0.01$) (*Fig. 4*). The actual average distance (66.7 m) of pollen flow within the plot was significantly shorter than that (133.7 m) generated by the permutation procedure ($P < 0.001$).

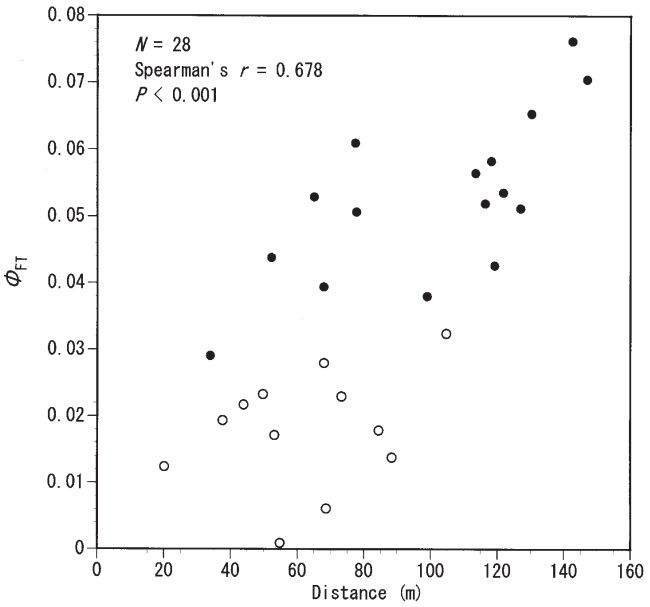

Figure 5. – Correlation between the pairwise Φ_{FT} values in the total pollen pools and the spatial distance between two seed parents. Open and filled circles indicate non-significant and significant Φ_{FT}, respectively ($P < 0.05$ after Bonferroni correction).

The actual average relatedness (0.103) between mating pairs of parents within the plot was significantly higher than that (0.053) generated by the permutation procedure ($P < 0.005$).

Genetic heterogeneity among the pollen pools

The total pollen pools and the separate pollen pools from inside and outside the plot genetically differed significantly among the seed parents. (Φ_{FT} = 0.034, 0.070 and 0.035, $P < 0.001$, 0.005 and 0.005, respectively). There were also significant genetic differences between the pollen pools from inside and outside the plot that were bulked over the seed parents ($\Phi_{FT} = 0.017$, $P < 0.001$). Furthermore, there were significant positive correlations between the pairwise Φ_{FT} values (for the total pollen pools and the pollen pools from inside the plot) and the spatial distance between the seed parents (Spearman's $r = 0.678$ and 0.547, $P < 0.001$ and 0.01, respectively) (*Fig. 5*). However, no such correlation was detected for the pollen pools from outside the plot. There was no significant correlation between the pairwise Φ_{FT} values of the pollen pools and the relatedness between the seed parents.

Discussion

Patterns of pollen flow from inside and outside the plot.

Genotyping errors and mutations cause the exclusion of actual pollen parents within the plot. However, in this study, genotyping was conducted twice or more times and thus genotyping error rate should be low. At all the loci used in this study, observed genotype frequencies did not deviate from Hardy-Weinberg equilibrium, and the haplotypes of all seeds but two matched those of the respective putative seed parents. Therefore, the possibility of existence of null alleles may be low at the loci in this population. The mutation rate of microsatellite sequences has been estimated at 10^{-4} to 10^{-5} per locus and generation (EDWARDS *et al.,* 1992; ELLEGREN, 1992; DOW and ASHLEY, 1996), indicating that the probability of mutating between parents and offspring is negligible. Therefore, the risks of excluding actual pollen parents within the plot should not be serious in this study.

DOI:10.1515/sg-2004-0048
edited by Thünen Institute of Forest Genetics

The average distance of pollen flow within the plot was 66.7 m. Since the proportion of pollen flow from outside the plot (and therefore further than 100 m) was 52.2%, the average distance of actual pollen flow was greater than this. Therefore, long-distance pollen transport by wind probably occurs among the *Quercus salicina* individuals in this temperate old-growth evergreen broad-leaved forest, as it does in other *Quercus* species (Dow and Ashley, 1996; Dow and Ashley, 1998; Streiff *et al.,* 1999).

Negative exponential functions of distance could explain significantly the mating events observed in the plot, and the actual average distance of pollen flow within the plot was significantly shorter than the potential average distance. In wind-pollinated *Quercus* species, many adult trees near seed parents tend to contribute frequently as pollen parents at the local scale and, consequently, the pollen flow follows an exponential dispersion curve (Streiff *et al.,* 1999). The results in this study confirm the findings by Streiff *et al.* (1999), and support the hypothesis that mating in *Quercus* species may be strongly affected by wind. Streiff *et al.* (1999) indicated that effective pollen dispersal in oaks could involve a combination of two processes: local dispersion and long-distance transport. We found evidence for both of these processes in *Quercus salicina,* i.e. evidence that large amounts of pollen came from outside the plot and that pollination over short distances frequently occurred, as indicated by the negative exponential curves of pollen flow versus distance within the plot.

The actual average relatedness between mating pairs of parents within the plot was significantly higher than that generated by the permutation procedure. The high average relatedness may be caused by the genetic structure of the adult trees and the above-mentioned relationship between spatial distance and pollen flow (i.e. that nearby adult trees tend to be genetically related to seed parents due to the genetic structure, and may often be pollinated by the seed parents because of the short distances between them). Consequently, neighboring trees that are genetically related to the seed parents may have frequently mated with them.

Genetic differentiation of pollen pools

The pollen pools from inside and outside the plot differed genetically. This finding differs from the results of a study by Streiff *et al.* (1999), in which the difference between allele frequencies from inside and outside the plot they examined was very weak. In the site studied here, genetic structure may be somewhat stronger, and long-distance pollen flow from outside the plot probably transfers different alleles into it, thus increasing the genetic diversity within the plot.

The genetic composition of not only the total pollen pools and but also the pollen pools from inside the plot differed significantly among the eight seed parents, and the genetic differentiations were correlated with the distance between seed parents. The genetic differentiation of these pollen pools is due to variations in the contributions of pollen parents to each seed parent. Different seed parents located near to each other may be frequently pollinated by the same pollen parents near them due to the limited pollen dispersal, so the pollen pools accepted by neighboring seed parents may be genetically similar. Furthermore, although the pollen pools from outside the plot also genetically differed significantly among the seed parents, the genetic differentiation was not correlated with the distance between the seed parents. Therefore, variations in flowering phenology among trees may also contribute to the differentiation amongst the pollen pools. Studies of flowering phenology conducted in populations of different oak species such as *Q. robur* and *Q. petraea* (Bacilieri *et al.,* 1995) and *Q. alba* (Sharp and Chisman, 1961) have revealed high levels of variation among individuals. To clarify the effect, field observations of the flowering phenology would be necessary. Hardy *et al.* (2004) discussed different mechanisms causing differentiation among pollen pools and indicated limited pollen dispersal as a main factor and phenological heterogeneity among plants as a second factor limiting diversity of pollen parents.

This study of the *Q. salicina* population allowed us to reconstruct actual pollination events within the plot and link them with long-distance pollen flow from outside the plot, which may increase genetic diversity in the study population. On the other hand, the likelihood of trees mating within the plot was strongly correlated with the distance between them, which is a characteristic feature of wind-pollination. The distance-dependence of pollen flow may be causally reflected in the pattern of genetic differentiation of the pollen pools between the seed parents, since the genetic similarity of their pools decreases as the distance between them increases.

As well as the long-distance pollen flow, year-to-year variations in pollen flow (due to year-to-year fluctuations in flowering phenology or wind direction during the flowering season) may be important factors in the maintenance of genetic diversity (especially since it is known that the flowering of *Quercus* species fluctuates from year to year). In this study, all analyses focused on pollination events that occurred in only one year. Analyses of multi-year pollination coupled with field observations of flowering phenology would further enhance our understanding of pollen flow and its effects on genetic diversity amongst *Q. salicina* populations.

Acknowledgements

Field and laboratory assistance was provided by M. Miura, T. Fujita, and other members of the Laboratory of Forest Ecology and Physiology of Nagoya University and by C. Yamanaka, whose help is greatly acknowledged. We thank the Tsushima District Forest Office for permitting this study, and T. Torimaru and S. Ueno for their statistical assistance. This study was supported by a Grant-in-Aid for Scientific Research (Nos. 11460069 and 14206017) from the Japanese Society for the Promotion of Science.

References

Bacilieri, R., A. Ducousso and A. Kremer (1995): Genetic, morphological, ecological and phenological differentiation between *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. in a mixed stand of northwest of France. Silvae Genetica **44**: 1–10.

Casgrain, P. and P. Legendre (1999): The R package for multivariate and spatial analysis version 4.0 (development release 4) user's manual. Department of Biological Sciences, University of Montreal, Montreal.

Chakraborty, R., M. Shaw and W. J. Schull (1974): Exclusion of paternity: the current state of the art. Am J Hum Genet **26**: 477–488.

Chase, M. R., C. Moller, R. Kesseli and K. S. Bawa (1996): Distant gene flow in tropical trees. Nature **383**: 398–399.

Dow, B. D. and M. V. Ashley (1996): Microsatellite analysis of seed dispersal and parentage of saplings in bur oak, *Quercus macrocarpa.* Mol Ecol **5**: 615–627.

Dow, B. D. and M. V. Ashley (1998): High levels of gene flow in bur oak revealed by paternity analysis using microsatellites. Journal of Heredity **89**: 62–70.

Dow, B. D., M. V. Ashley and H. F. Howe (1995): Characterization of highly variable (GA/CT)n microsatellites in the bur oak, *Quercus macrocarpa.* Theor Appl Genet **91**: 137–141.

Edwards, A., H. A. Hammond, L. Jin, C. T. Caskey and R. Chakraborty (1992): Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics **12**: 241–253.

DOI:10.1515/sg-2004-0048
edited by Thünen Institute of Forest Genetics

ELLEGREN, H. (1992): Polymerase-chain reaction (PCR) analysis of microsatelliets-a new approach to studies of genetic relationships in birds. Auk **109**: 886–895.
EXCOFFIER, L., P. E. SMOUSE and J. M. QUATTRO (1992): Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics **131**: 479–491.
GREENWOOD, M. S. (1986): Gene exchange in loblolly pine: the relation between pollination mechanism, female receptivity and pollen availability. Am J Bot **73**: 1443–1451.
HARDY, O. J., S. C. GONZALES-MARTINEZ, B. COLAS, H. FRVILLE, A. MIGNOT and I. OLIVIERI (2004): Fine-scale genetic structure and gene dispersal in *Centaurea corymbosa* (Asteraceae). II. Correlated paternity within and among shibships. Genetics **168**: 1601–1614.
ISAGI, Y., T. KANAZASHI, W. SUZUKI, H. TANAKA and T. ABE (2000): Microsatellite analysis of the regeneration process of *Magnolia obovata* Thunb. Heredity **84**: 143–151.
ISAGI, Y. and S. SUHANDONO (1997): PCR primers amplifying microsatellite loci of *Quercus mysinifolia* Blume and their conservation between oak species. Mol Ecol **6**: 897–899.
ITOW, S. (1991): Species turnover and diversity patterns along an evergreen broad-leaved forest cenocline. J Veg Sci **2**: 477–484.
KAMPFER, S., C. LEXER, J. GLOSSL and H. STEINKELLNER (1998): Characterization of $(GA)_n$ microsatellite loci from *Quercus robur*. Hereditas **129**: 183–186.
KIRA, T. (1991): Forest ecosystem of east and southeast Asia in a global perspective. Ecol Res **6**: 185-200.
LEVIN, D. A. and H. W. KERSTER (1969): The dependence of bee-mediated pollen and gene dispersal upon plant density. Evolution **23**: 560–571.
LINHART, Y. B., W. H. BUSBY, J. H. BEACH and P. FEINSINGER (1987): Forager behavior, pollen dispersal and inbreeding in two species of hummingbird-pollinated plants. Evolution **41**: 679–682.
MANABE, T., N. NISHIMURA, M. MIURA and S. YAMAMOTO (2000): Population structure and spatial patterns for trees in a temperate old-growth evergreen broad-leaved forest in Japan. Plant Ecol **151**: 181–197.
MANTEL, N. (1967): The detection of disease clustering and a generalized regression approach. Cancer Res **27**: 209–220.
MARSHALL, T. C., J. SLATE, L. E. B. KRUUK and J. M. PEMBERTON (1998): Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol **7**: 639–655.
MOSQUIN, T. (1971): Competition for pollinators as a stimulus for the evolution of flowering time. Oikos **22**: 398–402.
MURRAY, M. G. and W. F. THOMPSON (1980): Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res **8**: 4321–4325.
NIXON, K. C. (1993): Infrageneric classification of *Quercus* (Fagaceae) and typification of sectional names. Annales des sciences forestieres (Suppl) **50**: 25–34.
QUELLER, D. C. and K. F. GOODNIGHT (1989): Estimating relatedness using genetic markers. Evolution **43**: 258–275.
RAYMOND, M. and F. ROUSSET (1995): GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity **86**: 248–249.
SHARP, W. M. and H. H. CHISMAN (1961): Flowering and fruiting in the white oaks. I. Staminate flowering through pollen dispersal. Ecology **42**: 365–372.
SMOUSE, P. E., R. J. DYER, R. D. WESTFALL and V. L. SORK (2001): Two-generation analysis of pollen flow across a landscape. I. Male gamete heterogeneity among females. Evolution **55**: 260–271.
STEINKELLNER, H., S. FLUCH, E. TURETSCHEK, C. LEXER, R. STREIFF, A. KREMER, K. BURG and J. GLOSSL (1997): Identification and characterization of (GA/CT)n – microsatellite loci from *Quercus petraea*. Plant Mol Bio **33**: 1093–1096.
STREIFF, R., A. DUCOUSSO, C. LEXER, H. STEINKELLNER, J. GLOESSL and A. KREMER (1999): Pollen dispersal inferred from paternity analysis in a mixed oak stand of *Quercus roubur* L. and *Q. petraea* (Matt.) Liebl. Mol Ecol **8**: 831–841.
TAGAWA, H. (1995): Distribution of lucidophyll oak-laurel forest formation in Asia and other areas. Tropics **5**: 1–40.
WHITLOCK, M. C. and D. E. MCCAULEY (1999): Indirect mesures of gene flow and migration: $F_{ST} \neq 1 / (4Nm + 1)$. Heredity **82**: 117–125.

Variation in Growth Performance of *Acacia nilotica* Willd. ex Del. Provenances of Wide Geographical Origin : Six Year Results

By H. S. GINWAL* and A. K. MANDAL

Division of Genetics and Tree Propagation, Forest Research Institute, P.O. I.P.E. Kaulagarh Road, Dehradun (Uttaranchal) – 248195 INDIA

(Received 8th November 2004)

Abstract

Results of a provenance trial of *Acacia nilotica* Willd. ex Del. laid out in 1993 at Tropical Forest Research Institute Campus, Jabalpur (23°N lat., 79°E long. and 400 m altitude) Madhya Pradesh, a semi-arid region of India are reported and discussed. Nineteen provenances from India, Pakistan, Sudan, Senegal and Yemen were evaluated in the field at age three and six years. Local source of *A. nilotica* (S.F.R.I., Jabalpur) was used as check material for comparison. Significant differences between the provenances ($P < 0.05$) were observed for height, diameter at breast height (DBH), number of branches and field survival. The provenances from Gujrat (Punjab), Pakistan, ranked first for growth traits namely height, DBH and survival. The next superior provenance was from Beihan, Yemen, which scored second highest values of height and DBH and had good survival at age six years. Results indicate that

*) Author for correspondence
(e-mail: ginwalhs@icfre.org; ginwalhs@rediffmail.com)

DOI:10.1515/sg-2004-0049
edited by Thünen Institute of Forest Genetics