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Genetic diversity of parental and offspring populations in a *Pinus merkusii* seedling seed orchard detected by microsatellite markers

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Introduction

P. merkusii is the only pine that occurs naturally south of the Equator, but has a much restricted distribution, being found solely in the Philippines and three areas of Northern Sumatra (Indonesia): Aceh, around Lake Toba in Tapanuli and around Mt. Kerinci (2° south of the Equator) (COOLING 1968). It is considered as conspecific to *Pinus latteri* that widely spread in South East Asia, occurring in Southwest China, Cambodia, Laos, Myanmar, Thailand and Vietnam (FARJON 2005). However, *P. merkusii* is one of the most important tree species in Indonesia for the production of fuel, pulp, paper, timber, and non-wood products such as resin and turpentine. It is also the preferred species for rehabilitating land on Sumatra and other Indonesian islands (HIDAYAT and HANSEN 2002). For these reasons it has been extensively planted in Indonesia, mainly by Dutch interests (MIROV 1967), from 1931 onwards (FERQUSON 1953).

A breeding strategy for *P. merkusii* in Indonesia was established in the mid-1970s, mainly to reduce stem crookedness – a major defect of this species (SOESENO 1988) in which three progeny test sites were established by planting the half-sib offspring of selected trees from thirteen artificial forests in Java following a randomized complete block design. Test plantations were then established in the six years between 1978 and 1983, and in each plantation a thousand families were eventually planted. The progeny trials were converted into open-pollinated seedling seed orchards (SSOs) after a few rounds of selective rouging (SOESENO 1988). Nowadays, most *P. merkusii* seeds are provided by these SSOs in Indonesia.

In natural populations of *P. merkusii* in Thailand and Vietnam, Szmidt *et al*. (1996) indicated that levels of intra-population genetic diversity are low, while levels of inter-population diversity are high. However, after surveying two natural Sumatran forests of *P. merkusii* (one in Aceh and one on Mt. Kerinci) and a seed orchard in Java using isozyme markers, SIREGAR and HATTERMER (2004) concluded that the Aceh and seed orchard populations were genetically similar, probably because Javan plantations of the species were established using materials almost exclusively originating from a sub-population of the natural forest in Aceh. If so, the genetic diversity in the SSOs (and thus the Javanese plantations) may be limited. Therefore, it is essential to clarify the genetic relationships among parent trees in the SSO, the plantations in Java and the natural

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populations to ensure that the available diversity is optimally used to the established SSO.

To promote high genetic diversity and maximize genetic gain, all of the genotypes in a seed orchard should ideally contribute equally to the seed crops (STOEHR *et al*. 1998). However, it is well known that the paternal contributions of different clones to seed production can vary widely in clonal seed orchards (CSOs) (MORIGUCHI *et al*. 2004; GOTO *et al*. 2002; SCHOEN and STEWART 1987; BURCZYK *et al*. 1997).

Furthermore, although conifers generally have high outcrossing rates, CHANGTRAGOON and FINKELDEY (1995) reported that selfing rates in natural forests of *P. merkusii* in Thailand are extraordinarily high. Thus, selfing rates in *P. merkusii* SSOs may be substantially higher than in most temperate *Pinus* seed orchards. Paternity analysis is useful for quantifying levels of selfing, and determining precise relationships among parental trees and their offspring in CSOs (CHAIX *et al*. 2003). However, larger number of genotypes is included in SSOs than in CSOs, therefore such paternity analysis in SSOs requires the use of hyper-polymorphic DNA markers. Alternatively, comparisons of the genetic diversity of parent and offspring populations may provide useful indications of levels of selfing and mating status in CSOs, even using markers with low levels of polymorphism such as allozymes (as shown, for instance, by MORAN *et al*. 1980). This approach can also be readily applied to SSOs.

A potential problem of the latter approach is that genetic diversity in the seed crops could become the same (or even higher) levels as those of the parent trees if background pollen from external sources made substantial contributions to the fertilization events in the orchards. For instance, MUONA and HARJU (1989) detected similar levels of expected heterozygosity in parental populations and seed crops in *Pinus sylvestris* CSOs, but they also found that the total number of alleles was significantly higher in the seed crops than in the parental populations due to background pollination. Significant contributions from background pollination have also been detected in a *Pseudotsuga menziesii* CSO (STOEHR *et al.* 1998) and a *Eucalyptus grandis* SSO (CHAIX *et al.* 2003) by cpDNA and nuclear microsatellite analyses, respectively. The SSO we examined included 600 families in randomized blocks, planted in six consecutive years, surrounded by a 200 m wide pollen dilution zone. SIREGAR and HATTEMER (2004) observed very similar levels of expected heterozygosity between parent trees and embryos in the same orchard, and suggested that there was little gene flow into it from external sources. However, their conclusion may be problematic, because the extent of outside pollen flow may not be directly reflected in the heterozygosity of the offspring population. Thus, further information on the genetic differentiation among parent trees and the genetic diversity of both parent trees and offspring is essential for establishing appropriate management regimes for *P. merkusii* seed orchards in order to produce genetically sound seed crops.

The aims of the study presented here were to use microsatellite markers to confirm the genetic background of parental trees selected from different artificial plantations, to determine the levels of genetic diversity maintained in the SSO and the offspring produced in it, and to assess the status of the mating in it.

Materials and methods

Description of the examined seed orchard

The examined SSO was established between 1978 and 1983 at a site covering 96 ha with flat topography, 600 meters above sea level, at Jember, East Java, in Indonesia (113° 52'E, 7° 67'S), surrounded by commercial plantations of *Swietenia mahogany*, *Eucalyptus* spp. and *P. merkusii*. The parent trees in the SSO are about 20-30 meters in height and 30-80 cm in diameter.

The SSO was originally established as a progeny trial of phenotypically selected trees from artificial plantations in thirteen districts scattered throughout Java Island (Table 1, Fig. 1). Half-sib offspring of 200 selected trees (200 families) were planted following a randomized complete block design, with ten replications of five tree row-plots with 4 × 4 m spacing in the first planting year. In each of the following planting years, 40 of the 200 families were used again, with 160 newly selected families. Thus, 1,000 (200+160×5) families were planted in total. As illustrated in Fig. 2, the area occupied by most blocks is 80 m (north to south) × 200 m (east to west) with some exceptions due to topographical constraints. Within-tree plot selections and among-family selections have been conducted (on a phenotypic basis) twice and once, respectively, to date. Consequently, the present breeding population size of the SSO encompasses 600 families and about 6,000 parent trees, with a spacing of 4 to 16 m.

Table 1. Districts where plus trees were selected, and numbers of existing families in the present seed orchard.

District	Planting year (Population)						Total
	1978	1979	1980	1981	1982	1983	
South Bandung		12			1	1	14
North Bandung		8			18	12	38
Sumedang		4			16	1	21
Majalengka			9	8	5	2	24
West Pekalongan		9	40	38	10	10	107
East Banyumas		5		5		3	13
East Pekalongan	60	9	10	6		11	96
Magelang						7	7
Surakarta		9		1		19	29
Lawu DS		4	28	11	7	7	57
Kediri				2	30	11	43
Malang		16				3	19
Jember	70	38	11	30	14	13	176
Total	130	114	98	101	101	100	644

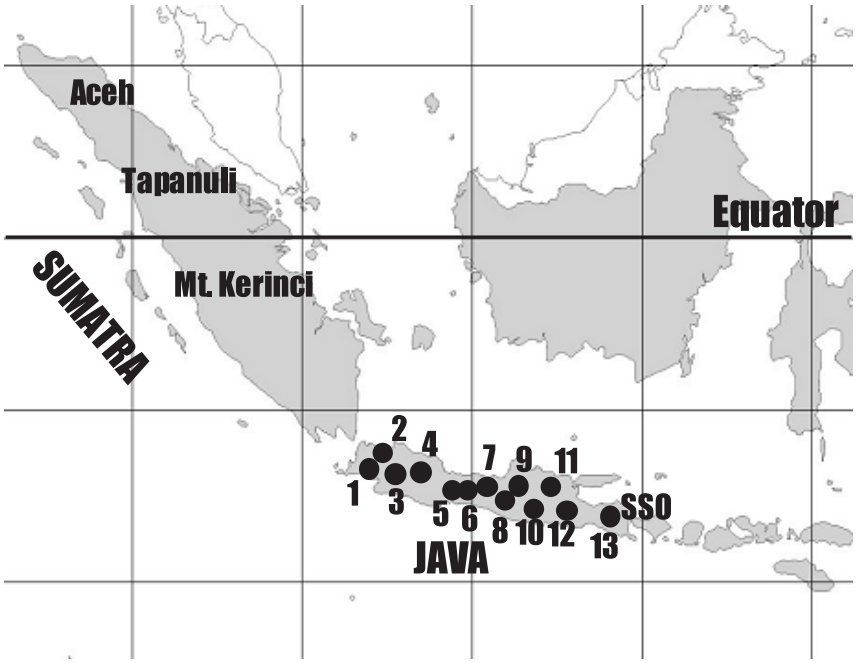

Fig. 1. Map of distribution of *Pinus merkusii* in Indonesia

P. merkusii occurs naturally only in three regions in Indonesia, all of which are on Sumatra Island: Aceh, Tapanuli and around Mt. Kerinci. All *P. merkusii* forests on Java Island are artificial plantations. Parent trees in the SSO originated from 13 Javan plantations at the following locations: (1) South Bandung, (2) North Bandung, (3) Sumedang, (4) Majalengka, (5) West Pekalongan, (6) East Banyumas, (7) East Pekalongan, (8) Magelang, (9) Surakarta, (10) Lawu DS, (11) Kediri, (12) Malang, (13) Jember. The SSO is located in Jember.

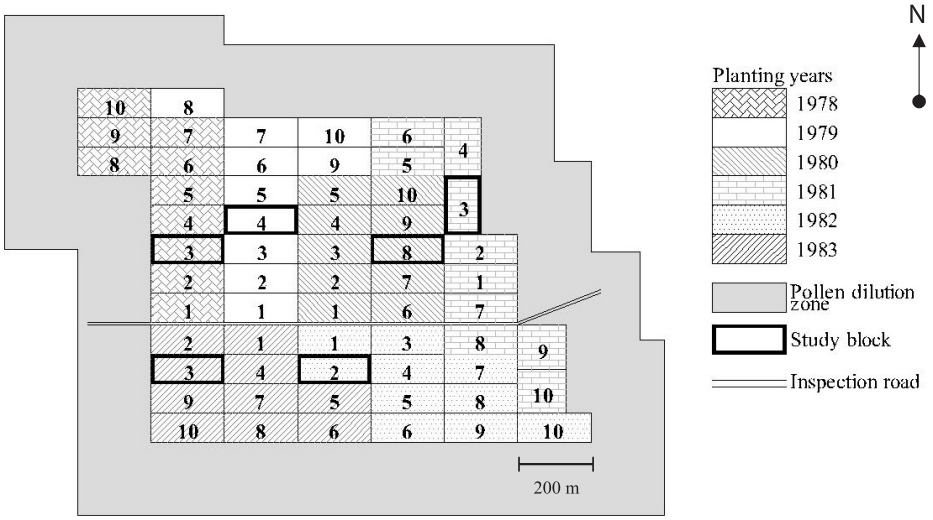

Fig. 2. Map of the *Pinus merkusii* seedling seed orchard, indicating the six blocks studied.

The six planting years are indicated by the different patterns in the cells. Numbers inside the blocks indicate replications in each planting year. Blocks included in this study are enclosed by thick lines.

Sample collection

To estimate the genetic diversity of the parental tree and progeny populations, we selected one representative block for each planting year, as shown in Fig. 2. Needles were collected from all parent trees in the studied blocks in August 2003. They were dried in small plastic bags with silica gel and kept at room temperature until DNA extraction. Cones were collected from each study block in June 2004. In order to avoid sampling from a limited part in each block, we set 10 sampling lines in the SSO, across two diagonal lines (from the North West corner to the South East corner and from the North East corner to the South West corner), four North to South lines (with 20 to 30 m intervals), and three West to East lines (again with 20 to 30 m intervals) and designated five sample trees along each line. A hundred cones were randomly collected from the canopies of each sample tree. The set of cones collected from each block were mixed and dried in sunlight until the seeds could be extracted. The seeds were extracted from the cones, mixed again, and about 2500 seeds randomly chosen to represent each block were germinated on wet filter paper at room temperature for approximately one month. Then, 48 seedlings per study block were randomly chosen, dried in a plastic bag with silica gel and stored at room temperature until DNA extraction.

Samples from each block were denoted by numerals indicating the planting year and p (for parent trees; parental populations hereafter) or o (for seedlings, the offspring populations hereafter), e.g. 79p and 83o, respectively.

DNA extraction and microsatellite analysis

Five microsatellite loci for *P. merkusii* (Pm01, Pm05, Pm07, Pm09a and Pm12) developed by NURTJAHJANINGSIH *et al*. (2005) were amplified and analyzed in this study, as follows. DNA was extracted from the samples using a DNeasy Plant Mini Kit (QIAGEN). The PCR amplifications were performed using a PCR thermal cycler (Takara) and Gene amp PCR system 9700 (Applied Biosystems), in reaction mixtures (10 μL) containing 5 ng of template DNA, 1 × PCR buffer, consisting of 2.5 mM $MgCl_2$, 0.2 mM dNTP, 0.5 μM of each designed primer pair, and 0.25 U of Ampli *Taq* Gold (Applied Biosystems). The PCR cycling conditions were as follows; 10 min at 95°C followed by 30 cycles of 30 sec. at 94°C, 30 sec. at a primer-specific annealing temperature, 30 sec. at 72°C and a final elongation step of 12 min at 72°C. Amplified fragments were detected and sequenced using an ABI 3100 genetic analyzer (Applied Biosystems).

Statistical analysis

The data acquired were analyzed as follows. First, we checked that the primers had successfully amplified microsatellites from all of the analyzed samples. Parent trees were then grouped according to their source districts, as shown in Table 1, and compared with respect to the genetic parameters shown in Table 2 to evaluate the genetic differentiation among districts. Finally, the parental and offspring populations were compared with respect to the same genetic parameters.

All the genetic parameters used in this paper were calculated using FSTAT software version 2.9.3.2 (GOUDET 2001). For each population, genetic diversity was evaluated in terms of the

Table 2. Genetic diversity of microsatellite loci over all parent (bold) (N=644) and offspring samples (N=288) used in this study.

Locus	A		$A_{(576)}$		H_O		H_E		F_{IS}	
pm01	**4**	3	**3.70**	3.00	**0.503**	0.691	**0.471**	0.517	**-0.069**	-0.337
pm05	**4**	3	**3.70**	3.00	**0.304**	0.441	**0.317**	0.448	**0.040**	0.015
pm07	**5**	6	**4.97**	6.00	**0.563**	0.728	**0.624**	0.672	**0.097***	-0.084
pm09a	**8**	9	**7.75**	9.00	**0.574**	0.716	**0.654**	0.670	**0.123***	-0.068
pm12	**3**	3	**2.70**	3.00	**0.412**	0.427	**0.475**	0.488	**0.133***	0.125
Mean	**4.8**	4.8	**4.56**	4.80	**0.471**	0.601	**0.508**	0.559	**0.073***	-0.075

* significant according to 200 randomizations at the P<0.05 level
A, number of detected alleles; $A_{(576)}$, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, fixation index.

number of detected alleles (A), allelic richness (R_S; EL MOUSADIK and PETIT 1996), observed heterozygosity (H_O), Nei's unbiased expected heterozygosity (H_E; Nei 1987), fixation index (F_{IS}), the number of locus pairs showing significant genotype disequilibrium, which is synonymous with linkage disequilibrium (*L-D*), and the effective population size (*N*e). Allelic richness is a parameter indicating the allelic diversity at a locus for a fixed sample size (EL MOUSADIK and PETIT 1996). Here, allelic richness was evaluated with a fixed sample size for each population. The significance of deviations from Hardy-Weinberg equilibrium (HWE), as evidenced by deviations of F_{IS} from zero, was tested by randomization. The value of *L-D* among loci may indicate mating status, so it was tested for all locus pairs in each population by randomization and the P values obtained (< 0.05) were adjusted by sequential Bonferroni correction (RICE 1989) to avoid false positives. The value of *N*e needed to maintain the current level of H_E was determined using the formula of CROW and KIMURA (1970), i.e. $Ne = H_E/[4\mu(1-H_E)]$, where H_E is the expected heterozygosity and μ is the neutral mutation rate. Here we used the mutation rate of 5×10^{-4} for microsatellites, according to previous reports (EDWARDS *et al.* 1992, ELLEGREN 1992, SHLOTTER and TAUTZ 1992).

The genetic differentiation among populations was evaluated by calculating F_{ST} values (WEIR and COCKERHAM 1984), the significance of which was tested by comparing them to 95% confidence intervals acquired by 1000 bootstrap permutations. The significance of differentiation at each locus was tested by the log-likelihood (G)-based exact test (GOUDET *et al*. 1996). Pairwise F_{ST} values were also calculated, the significance of which was tested by randomizing multilocus genotypes between the two populations with standard Bonferroni corrections.

Results

Genetic diversity of microsatellites

Genetic diversities at the five microsatellite loci across all samples of parent trees (N=644) and their offspring (N=288) are summarized in Table 2. The numbers of alleles (A) for each marker found in parent trees and offspring ranged from three to eight and from three to nine, respectively.

Observed and expected heterozygosity levels for parent trees were 0.471 and 0.508, respectively; corresponding figures for the offspring were 0.601 and 0.559. The mean coefficients of inbreeding (F_{IS}) for parent trees and offspring were 0.073 and -0.075, respectively. Significant deviations from Hardy-Weinberg equilibrium (HWE) were observed at loci pm07, pm09a, pm12 and in the across-loci mean for the parent trees.

Genetic differentiation among source districts of the parent trees

Parent trees were grouped according to their source districts (Table 1), and values of genetic diversity parameters were calculated for each group (Table 3) to evaluate the genetic differentiation among districts. While the number of alleles (*A*) deviated from 13 to 22, reflecting the sample size for each district, $A_{(14)}$, H_O and H_E were similar among all districts. Inbreeding coefficients ranged from -0.228 to 0.230 and significantly deviated from zero ($P < 5\%$) in the trees that originated from East Pekalongan and Jember. Only one pair of loci from trees originating from Kediri showed significant linkage disequilibrium (*L-D*). The mean value of F_{ST} was 0.008 (95% confidence interval, 0.004 – 0.015) for the districts overall, indicating a low level of genetic differentiation.

Table 3. Genetic diversity parameters of parent trees grouped according to their source districts.

Area	N	*A*	$A_{(14)}$	H_O	H_E	F_{IS}	*L-D*
South Bandung	14	15	2.77	0.514	0.503	-0.022	0
North Bandung	38	17	2.70	0.563	0.502	-0.122	0
Sumedang	21	15	2.68	0.520	0.501	-0.038	0
Majalengka	24	16	2.67	0.404	0.445	0.094	0
West Pekalongan	107	19	2.65	0.414	0.460	0.100	0
East Banyumas	13	14	2.67	0.375	0.486	0.230	0
East Pekalongan	96	21	2.92	0.462	0.521	0.112*	0
Magelang	7	13	2.60	0.629	0.512	-0.228	0
Surakarta	29	17	2.99	0.559	0.557	-0.002	0
Lawu DS	57	19	2.75	0.454	0.479	0.052	0
Kediri	43	15	2.68	0.567	0.534	-0.063	1
Malang	19	17	2.79	0.400	0.474	0.156	0
Jember	176	22	2.85	0.467	0.528	0.116*	0
Overall	644	24	2.75	0.487	0.500	0.030	0.08

* significant F_{IS} with 1300 randomizations, significant *L-D* with 2600 permutations at the P<0.05 level

N, sample size; *A*, number of detected alleles; $A_{(14)}$, Allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, fixation index; *L-D*, number of locus pairs in significant linkage disequilibrium.

Table 4. Genetic diversity parameters of parental (bold) and offspring populations over five microsatellite loci.

Population	N		*A*		$A_{(96)}$		H_O		H_E		F_{IS}		*L-D*		*Ne*	
78	**130**	48	**23**	21	**4.18**	4.20	**0.468**	0.654	**0.545**	0.561	**0.142***	-0.165	**0**	0	**599.4**	640.0
79	**114**	48	**20**	19	**3.58**	3.80	**0.452**	0.658	**0.480**	0.579	**0.059**	-0.137	**3**	0	**461.9**	687.6
80	**98**	48	**20**	21	**3.58**	4.20	**0.361**	0.604	**0.405**	0.594	**0.108**	-0.017	**0**	0	**340.1**	730.9
81	**101**	48	**17**	21	**3.22**	4.20	**0.416**	0.534	**0.470**	0.513	**0.114**	-0.041	**1**	0	**443.4**	525.9
82	**101**	48	**16**	15	**2.99**	3.00	**0.512**	0.633	**0.503**	0.519	**-0.018**	-0.220	**4**	0	**506.0**	539.9
83	**100**	48	**17**	20	**3.30**	4.00	**0.618**	0.521	**0.532**	0.506	**-0.162**	-0.029	**4**	0	**568.4**	513.0
Overall	**644**	288	**24**	24	**3.48**	3.90	**0.471**	0.601	**0.489**	0.545	**0.041**	-0.102	**2.00**	0.00	**486.5**	606.2

* significant F_{IS} with 1200 randomizations, significant *L-D* with 2400 permutations at the P<0.05 level
N, number of samples; *A*, number of detected alleles; $A_{(96)}$, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, fixation index; *L-D*, number of locus pairs in significant linkage disequilibrium; *Ne*, effective population size.

Genetic diversity of parental and offspring populations

Allele frequencies of parental and offspring populations are shown in Appendix 1. The total number of alleles per locus ranged from two to eight in parental populations and from two to nine in offspring populations. At all loci, frequencies of several alleles were higher in offspring than in parental populations. At loci pm07 and pm09a, alleles appearing in offspring genotypes were absent from the parental populations (Appendix 1). Values of genetic parameters of the parental and offspring populations in the SSO are summarized in Table 4. Although the values differed among planting years, the differences between parental and offspring populations were more pronounced. Twenty-four alleles were recorded in both parental and offspring populations. Allelic richness was higher in the offspring populations (mean=3.90) than in the parental populations (mean=3.48) in every block. H_O and H_E were higher in the offspring (mean=0.601 and 0.545, respectively) than in the parental populations (mean=0.471 and 0.489, respectively) in most of the blocks. The F_{IS} values did not significantly deviate from zero, except for the 78p population. Significant linkage disequilibrium was only observed in the parental populations (mean=2.00). The effective population size calculated under the mutation rate 5×10^{-4} was higher in the offspring populations (mean=606.22) than in the parental populations (mean=486.53). The F_{ST} values among parental populations and offspring populations were 0.038 (95% confidence interval, 0.02 - 0.062) and 0.029 (95% confidence interval, 0.016 - 0.041), respectively.

Discussion

Utility of the microsatellite markers

Usually genetically independent selected trees are used to establish seed orchards, and thus the observed heterozygosity (H_O) values of their offspring populations are higher than expected (H_E) under HWE (EL-KASSABY and RITLAND 1996). However, significant positive deviations from HWE were detected at three of the five examined loci in the parent trees. Sometimes high estimates

of H_E can be attributed to the presence of null alleles when microsatellite loci are applied for genetic analysis (CHAKRABORTY *et al*. 1997). If there is any null allele, F_{IS} values can significantly deviate from zero, even though offspring are derived from panmictic processes in SSOs. However, in this case, no such deviation was found in the offspring. Thus, there is no evidence of null alleles at these loci and we considered the results obtained using them to be reliable.

Genetic differentiation among source districts of the parent trees

The SSO was established using families selected from thirteen districts of Java Island (SOESENO 1988, Fig. 1), but differing combinations of districts were represented in each planting year, as shown in Table 1. Although the artificial forests on Java Island were derived from sources with limited natural distributions in Aceh, Sumatra Island (SIREGAR and HATTERMER 2005), the genetic similarity of the populations in the districts used in this SSO was uncertain. If there was significant genetic differentiation among trees selected from different districts, the genetic diversity of the materials planted in the SSO each year would be different, and the resulting genetic structure in the SSO is likely to be reflected in variations in the diversity of their offspring. In contrast, the results show that the genetic diversity parameters were quite similar among districts (Table 3), and the very low F_{ST} value obtained (0.008) indicates that there was a high degree of genetic uniformity among districts. Thus, our data support the suggestion by SIREGAR and HATTERMER (2005) that the trees in the SSO originate from materials with limited genetic diversity.

Genetic diversity of parental and offspring populations

Locus pairs in linkage disequilibrium (*L-D*) were found in four of the six parental populations, but none in any of the offspring populations. Since significant *L-D* occurs when populations with different gametic frequencies are admixed (HARTL 1999), the *L-D* observed in the parental population was presumably due to the admixture of different families in it, which is consistent with the mixture of plus trees from various districts. Values of H_E were higher in all of the offspring populations than the parental populations, and F_{IS} was not significantly different from zero in any offspring population (Table 4), presumably due to extensive pollination both within and among blocks, since the studied blocks were in the centre of the SSO, surrounded by other blocks. Genetic differentiation (F_{ST}) among populations was higher in the parental populations than in the offspring populations. These findings suggest that seed production in the SSO may be nearly panmictic and that genetic exchange freely occurs among the parent trees. In addition, values of effective population size (*N*e) were higher for the offspring populations than the parental populations, suggesting that pollen from other blocks or outside the SSO has contributed to matings within the blocks we examined. This inference is supported by the appearance of new alleles and higher allele frequencies in the offspring populations (Appendix 1). High values of H_E in seed crops have been attributed in large part to pollen contamination from external sources in both *P. sylvestris* (MUONA and HARJU 1989) and *Eucalyptus grandis* (CHAIX *et al*. 2003) seed orchards. PLOMION *et al*. (2001) estimated that pollen was dispersed according to a parabolic

Appendix 1. Allele frequencies of parental (bold) and offspring populations over five microsatellite loci.

Population		**78p**	78o	**79p**	79o	**80p**	80o	**81p**	81o	**82p**	82o	**83p**	83o
N		**130**	48	**114**	48	**98**	48	**101**	48	**101**	48	**100**	48
Locus	Allele												
pm01	1	**0.123**	0.063	**0.057**	0.042	**0.010**	0.083	**0.084**	0.031	**0.084**	0.146	**0.100**	0.073
	2	**0.677**	0.563	**0.776**	0.594	**0.918**	0.469	**0.703**	0.646	**0.550**	0.635	**0.475**	0.813
	3	**0.200**	0.375	**0.167**	0.365	**0.066**	0.448	**0.213**	0.323	**0.366**	0.219	**0.420**	0.115
	4	**0.000**	0.000	**0.000**	0.000	**0.005**	0.000	**0.000**	0.000	**0.000**	0.000	**0.005**	0.000
pm05	1	**0.188**	0.198	**0.110**	0.323	**0.036**	0.302	**0.129**	0.094	**0.158**	0.240	**0.090**	0.354
	2	**0.735**	0.802	**0.864**	0.667	**0.867**	0.646	**0.812**	0.792	**0.782**	0.667	**0.850**	0.615
	3	**0.069**	0.000	**0.026**	0.010	**0.097**	0.052	**0.059**	0.115	**0.059**	0.094	**0.060**	0.031
	4	**0.008**	0.000	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000
pm07	1	**0.038**	0.042	**0.026**	0.094	**0.010**	0.021	**0.015**	0.021	**0.005**	0.031	**0.090**	0.000
	2	**0.012**	0.010	**0.009**	0.000	**0.000**	0.000	**0.000**	0.042	**0.005**	0.000	**0.000**	0.000
	3	**0.500**	0.375	**0.434**	0.344	**0.505**	0.365	**0.629**	0.490	**0.535**	0.583	**0.400**	0.354
	4	**0.342**	0.313	**0.338**	0.260	**0.372**	0.438	**0.248**	0.271	**0.243**	0.354	**0.420**	0.479
	5	**0.108**	0.219	**0.193**	0.271	**0.112**	0.146	**0.109**	0.167	**0.213**	0.031	**0.090**	0.167
	6	**0.000**	0.042	**0.000**	0.031	**0.000**	0.031	**0.000**	0.010	**0.000**	0.000	**0.000**	0.000
pm09a	1	**0.012**	0.063	**0.000**	0.073	**0.000**	0.042	**0.000**	0.000	**0.000**	0.000	**0.000**	0.031
	2	**0.000**	0.000	**0.000**	0.031	**0.000**	0.021	**0.000**	0.010	**0.000**	0.000	**0.000**	0.031
	3	**0.004**	0.021	**0.009**	0.021	**0.005**	0.021	**0.000**	0.010	**0.000**	0.000	**0.000**	0.010
	4	**0.023**	0.010	**0.004**	0.000	**0.021**	0.000	**0.010**	0.000	**0.000**	0.000	**0.000**	0.010
	5	**0.031**	0.021	**0.004**	0.000	**0.042**	0.031	**0.005**	0.021	**0.000**	0.000	**0.000**	0.010
	6	**0.269**	0.146	**0.204**	0.156	**0.286**	0.135	**0.163**	0.042	**0.255**	0.115	**0.085**	0.083
	7	**0.338**	0.417	**0.527**	0.490	**0.526**	0.406	**0.638**	0.427	**0.570**	0.542	**0.410**	0.510
	8	**0.300**	0.323	**0.239**	0.229	**0.115**	0.292	**0.184**	0.427	**0.175**	0.344	**0.395**	0.281
	9	**0.023**	0.000	**0.013**	0.000	**0.005**	0.052	**0.000**	0.063	**0.000**	0.000	**0.110**	0.031
pm12	1	**0.354**	0.510	**0.482**	0.469	**0.281**	0.521	**0.470**	0.302	**0.272**	0.344	**0.430**	0.302
	2	**0.008**	0.021	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000	**0.000**	0.000
	3	**0.638**	0.469	**0.518**	0.531	**0.719**	0.479	**0.530**	0.698	**0.728**	0.656	**0.570**	0.698

curve from receptor trees in a *P. pinaster* polycross seed orchard, with an optimum dispersal distance of 500 m, so pollen contamination was highest in the center of the orchard. Conversely, YAZDANI and LINDGREN (1991) found that contamination was higher (albeit only slightly) in the corners than in the centre of a *P. sylvestris* CSO. In addition, FRIEDMAN and ADAMS (1985) observed that at least 48% of the seeds produced in *Pinus taeda* seed orchards were fertilized by trees more than 100 m away. Thus, there is considerable support in the literature for the hypothesis that substantial proportions of pollination could be attributed to pollen originating from other blocks or outside the SSO. However, we were unable to quantify the extent of gene flow from within and outside the focal blocks, since the markers we used provided insufficient resolution for paternity analyses.

In conclusion, clear evidence of random mating and extensive gene flow in the SSO was found in the differences in genetic parameters between the parental and the offspring populations. Thus, although the SSO consists of genetically related trees, high genetic diversity is still maintained in the offspring generation. We recommend that desirable phenotypic traits with genetically unrelated trees should be selected in the advances generation of the SSO. In addition, to help ensure that appropriate management regimes are applied, the distance of pollen dispersal in the SSO should be clarified.

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Summary

A seedling seed orchard (SSO) of *Pinus merkusii* was established by planting representatives of selected families from thirteen districts of Java Island, Indonesia over six years (1978-1983). The genetic diversity of the parental populations planted each year, and their offspring populations, was analyzed and compared using five microsatellite markers. The genetic differentiation among parent trees representing different districts was very small (F_{ST}=0.008), indicating (in accordance with previous proposals) that the genetic origins of the material in the SSO are limited. Consequently, values of F_{IS} and *L-D* were significantly high in the parental populations. Differences in the values of genetic diversity parameters were statistically significant between the parental populations and their offspring populations. H_E and *N*e were higher in the offspring (mean=0.545 and 606.2, respectively) than in the parental populations (mean=0.489 and 486.5, respectively) in most of the blocks. The F_{ST} value of among population was smaller for the offspring populations that for the parental populations, 0.029 and 0.038, respectively. The results suggest that mating was random, or nearly random, and that gene flow was extensive in the orchard. New alleles were detected in offspring populations that were not found in the corresponding parental populations, indicating that gene flow from other blocks within the SSO, or from outside the SSO, has occurred.

Key words: *Pinus merkusii*, Seedling seed orchard, Genetic diversity, Parent trees, Offspring, Microsatellite marker

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マイクロサテライトマーカーにより検出されたメルクシマツ実生採種園における母樹および次世代集団の遺伝的多様性

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要　　旨

インドネシア，ジャワ島の 13 地域の人工林から選抜された家系を用いて 1978 年から 1983 年の間に造成された実生採種園において，母樹集団とそれから生産された次世代集団の遺伝的多様性を，5 個のマイクロサテライトマーカーにより明らかにした。選抜地域間の遺伝的分化は，F_{ST}=0.008 と小さかった。このことから，採種園の遺伝的由来は限られていることが分かった。その結果として，母樹集団では F_{IS} が有意に大きく *L-D* が高かった。遺伝的多様性のパラメーターの値は，母樹集団と次世代集団では有意に異なっており，ヘテロ接合度の期待値（H_E）と集団の有効サイズ（*N*e）は，次世代集団で平均 0.545，606.2 と，母樹集団のそれ（0.489 と 486.5）よりも高かった。F_{ST} の値は次世代集団間で 0.029，母樹集団間で 0.038 と次世代で集団間の分化程度が低かった。これらの結果から，採種園内で任意交配あるいはそれに近い交配が起こっており，遺伝子流動が盛んであることが推察された。次世代集団で母樹集団にはない新たな対立遺伝子が見られることから，他のブロックや採種園の外部からの園外花粉の流入が生じていることが示唆された。

キーワード： *Pinus merkusii*・実生採種園・遺伝的多様性・母樹・次世代・マイクロサテライト マーカー