

PATTERNS OF GENETIC STRUCTURE AND VARIATION OF MERKUS PINE (*PINUS MERKUSII*) IN INDONESIA

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SIREGAR, I. Z. & HATTEMER, H. H. 2004. Patterns of genetic structure and variation of merkus pine (*Pinus merkusii*) in Indonesia. The genetic structure and variation of *Pinus merkusii* from two natural populations in Sumatra and one artificial population (seed orchard) in Java were evaluated using isoenzymes as genetic markers. Moderate levels of genetic variation were found in a conservation stand in Aceh (Sumatra) and a seedling seed orchard in Java included in the present study, while no variation was observed in a population from a national park in Kerinci (Sumatra). Genetic variation measures of the seed trees, progenies (embryos) and effective pollen in these populations were determined using eight isoenzyme loci (GOT-B, GOT-C, GOT-D, PGM-A, PGM-B, SKDH-A, NDH-A and FDH-A). In both of the variable populations 80% of the loci were polymorphic. Analysis of the seed trees showed that effective number of alleles per locus (A/L), allelic diversity (v), gametic diversity (v_{gam}) and total population differentiation ($\delta_r = H$) in the population from Aceh (Sumatra) were 2.0, 1.544, 40.953 and 0.361 respectively, while in the population of Java they were 2.4, 1.630, 62.516 and 0.395 respectively. At the progeny level (embryo level), the above measures for the population from Aceh were 2.0, 1.565, 46.023 and 0.362 respectively, while in the population from Java they were 2.4, 1.636, 63.448 and 0.389 respectively.

Key words: *P. merkusii* – isoenzymes – genetic marker – genetic conservation

SIREGAR, I. Z. & HATTEMER, H. H. 2004. Corak struktur dan variasi genetik pinus merkus (*Pinus merkusii*) di Indonesia. Struktur dan variasi genetik *Pinus merkusii* daripada dua populasi semula jadi di Sumatra dan satu populasi buatan (dusun biji benih) di Jawa dinilai menggunakan isoenzim sebagai penanda genetik. Variasi genetik sederhana didapati di dirian pemuliharaan di Aceh (Sumatra) dan di dusun biji benih di Jawa, sementara tiada variasi dicerap dalam populasi Taman Negara Kerinci-Seblat (Sumatra). Ukuran variasi genetik pokok biji benih, progeni (embrio) dan debunga efektif dalam populasi ini ditentukan dengan menggunakan lapan lokus isoenzim (GOT-B, GOT-C, GOT-D, PGM-A, PGM-B, SKDH-A, NDH-A dan FDH-A). Dalam kedua-dua populasi yang mempunyai kadar variasi yang tinggi ini, 80% lokus adalah polimorf. Analisis pokok biji benih menunjukkan bilangan alel yang efektif dalam setiap lokus (A/L), kepelbagaian alel (v), kepelbagaian gamet (v_{gam}) dan pembezaan jumlah populasi ($\delta_r = H$) dalam populasi Aceh (Sumatra) ialah masing-masing 2.0, 1.544, 40.953 dan 0.361. Sebaliknya dalam populasi Jawa, nilainya ialah masing-masing 2.4, 1.630, 62.516 dan 0.395. Pada aras progeni

(embryo), nilai ukuran ini untuk populasi Aceh ialah masing-masing 2.0, 1.565, 46.023 dan 0.362 sementara dalam populasi Jawa, nilainya ialah masing-masing 2.4, 1.636, 63.448 dan 0.389.

Introduction

Pinus merkusii is a tropical pine of Southeast Asia. It occurs naturally in Myanmar, Thailand, Laos, Cambodia, Vietnam, Indonesia and the Philippines (Critchfield & Little 1966). *Pinus merkusii* does not occur in the Malay Peninsula but can be found in an area extending from north-eastern India to southern Tibet, although little is known about its characteristics there (Lamprecht 1989). In Indonesia, *P. merkusii* occurs naturally in three separate locations on the island of Sumatra, namely, Aceh, Tapanuli and Kerinci. In the northern parts of Sumatra, namely in Aceh and Tapanuli, *P. merkusii* occurs mainly in 12 well-studied localities, occupying an area of about 130 000 ha of savanna-like terrain, where pines are scattered over repeatedly burned areas. In central Aceh alone, *P. merkusii* can be found in 10 main locations with a total area of about 70 000 ha (Anonymous 1988).

Pinus merkusii has been planted quite extensively in Java; the total area of plantations is approximately 900 000 ha, of which 570 000 ha serve as production forests and 330 000 ha as protection forests (Anonymous 1998). Nowadays, it is the second most extensively planted species after teak (*Tectona grandis*), making up more than 30% of the total plantations in Java. The species has also proven to perform satisfactorily in the reforestation and afforestation of areas marginal to tree growth and it can be used as a pioneer species in plantations of shade tolerant species (Harahap 1995).

Due to its present and potential future ecological and economic importance, attempts to conserve *P. merkusii*, especially in its natural habitats, have been initiated. In Aceh and Tapanuli (Sumatra), forest degradation and deforestation occur due to over-logging for the supply of wood to paper mills. Therefore, in 1994 conservation efforts were taken up in the Aceh region and 13 natural stands were chosen as *in situ* conservation stands. The stands range from 20 to 425 ha, amounting to a total of 900 ha. These stands are located in a logging concession of Alas Helau Ltd. at elevations varying from 500 to 1350 m above sea level (Hardiyanto 1994). Appropriate management of the stands, including silvicultural treatment, was proposed when establishing the conservation stands. However, the management of these stands was not regularly and properly conducted (pers. observation). The Kerinci population is also threatened due to its very low population size.

Harahap (1995) has surveyed and identified the important localities where natural stands of *P. merkusii* in Indonesia required urgent conservation efforts; the numbers of localities were 17, 7 and 4 in Aceh, Tapanuli and Kerinci respectively. The areas proposed to be conserved range from 10 to 20 ha, with at least 50 trees per stand. However, up till now, conservation measures have not been carried out, nor has a rational basis of conservation been available. Surveying the patterns of genetic variation of tree species is necessary to provide basic information for future conservation of genetic resources. This has become a fundamental principle of conservation just as the conviction that genetic variation

or diversity is an important consideration when managing forest stands, ecosystems and landscapes. A common method is the investigation into the spatial distribution of genetic variation, which can be carried out by analysing variation at gene marker loci.

Analysis of variation at several markers such as restriction fragment length polymorphisms (RFLP) or isozymes is useful in providing rough approximations to the total amount of genetic variation within forest tree species. On the other hand, analysis of genetic variation can also be done by observing phenotypes of quantitative traits in specifically designed trials. Comparisons of patterns of variation of these two groups of traits are rarely conducted. However, Hamrick *et al.* (1993) demonstrated that the proportion of total genetic variance found among populations for four quantitative traits was quite similar to that observed for 19 polymorphic allozyme loci by Duba (1985).

The aim of this study was to determine the patterns of genetic structure and variation of *P. merkusii* found in natural stands and in a seed orchard in Indonesia. The results were aimed at providing basic information for future activities in tree breeding and genetic conservation.

Materials and methods

Seed samples

Seeds were harvested in two natural populations and one artificial population, i.e. seedling seed orchard of *P. merkusii*, covering the most important populations in Indonesia. Details of the investigated populations are given in Table 1 and their approximate locations are indicated in Figure 1.

Both of the natural populations are protected, one is found in a conservation stand in Aceh and the other, within a national park in Kerinci. The artificial stand is regarded as the most productive seedling seed orchard in Java. It was established in Java in 1980 as a progeny test of originally 200 selected plus trees. After evaluation of the test, progenies of the 120 best trees were left in the orchard.

The seeds for the present investigation were collected from 21 seed trees in the conservation stand in Aceh, 25 seed trees in the national park in Kerinci and 30 seed trees in the seedling seed orchard in Java. The variation was studied in megagametophytes (the female gametophytes) and the embryos developing in them. At least eight seeds per seed tree were investigated to determine its genotype.

Table 1 Geographic location of the *Pinus merkusii* populations sampled for a survey of the variation encountered at isozyme gene loci

Locality	Altitude (m asl)	Longitude (E)	Latitude (N)	Forest type
Aceh (A)	900	96° 51'	4° 30'	Natural pure forest
Kerinci (K)	900	101° 26'	2° 02'	Natural mixed forest
Java (J)	600	113° 52'	7° 67'	Seedling seed orchard



Figure 1 Geographic distribution of *Pinus merkusii* natural populations in Sumatra and plantations in Java. Sampled populations are marked with A = Aceh, K = Kerinci and J = Java (Critchfield & Little 1966, Anonymous 1998).

Electrophoresis

Seeds were immersed overnight in water, dissected and the embryos were then carefully separated from the megagametophytes. The embryo and the megagametophyte were ground in one and two drops of homogenising buffer (0.97 g Tris-HCl, 30 mg DTT and 2.5 g PVP in 100 ml H₂O, pH 7.3) respectively. Paper wicks saturated with the homogenate were inserted into starch gels. Extracts from the megagametophyte and the embryo of the same seed were positioned adjacent to each other.

Horizontal starch gel electrophoresis of seeds (10.5% starch concentration plus 2.5–3.5% sucrose) was performed as described by Feret and Bergmann (1976), Conkle *et al.* (1982) and Liengsiri *et al.* (1990). The buffer system of Ashton with pH 8.7 for glutamate oxaloacetate transaminase (GOT) and phosphoglucomutase (PGM) as well as Tris-Citro with pH 7.4 for shikimate dehydrogenase (SKDH), nicotinamide adenine dinucleotide dehydrogenase (NADH) and formiate dehydrogenase (FDH) were used as electrode and gel buffers.

Genetic interpretation of zymograms

A total of eight polymorphic enzyme gene loci were recovered by analysing the segregation of megagametophytes. The methods were largely those used by

Changtragoon and Finkeldey (1995). Interestingly, the enzyme system GOT turned out to be controlled by four gene loci. This result is in contrast with other pine species hitherto investigated and reported (Mejnartowicz & Bergmann 1985, Kim *et al.* 1994). The results of the genetic control of enzyme systems is published in Siregar (2000).

Measures of variability

Data on seed trees, embryos and pollen contributions were utilised in the statistical analysis. Genetic variabilities of seed trees and embryos/progenies were calculated using the following measures: number of alleles per locus (A/L); percentage of polymorphic loci (PPL); genetic diversity (v); gametic diversity (v_g) and observed or expected heterozygosity (H_o or $H_e = \delta_p$). On the other hand, allelic frequencies and diversities (v) were calculated for seed trees, embryos and pollen contributions (pollen cloud) according to Gregorius (1978). The degree of differentiation between and among populations was analysed with the genetic distance measures d_o (Gregorius 1974) and D (Nei 1972) as well as the genetic differentiation measures D_i and δ (Gregorius & Roberds 1986) and the fixation index F_{ST} (Wright 1965). The computer programs of GSED version 1.1 and BIOSYS-2 were used to calculate the above values. No software was required to determine the number of alleles per locus and percentage of polymorphic loci for seed trees and embryos.

Results

Genetic variability of seed trees and progenies (embryos) within populations

Measures of genetic variation of the three investigated populations were based on seed trees and their progenies (embryos) (Table 2). The population from the Kerinci National Park showed no variability in the eight gene loci investigated. Comparisons of the measures of genetic variability were, therefore, made only between the populations from Aceh and Java. In general, the population from Java had higher variability than that from Aceh. Only one measure, that is the percentage of polymorphic loci, showed no difference between the two populations.

Allelic structures among seed trees, embryos and pollen contributions

Allele frequencies in different populations at the loci studied are presented in Table 3. Of 10 loci examined, eight were polymorphic (0.95 criterion) in at least one population. The population of Kerinci, surprisingly, showed no polymorphism at any loci in any of the materials, i.e. seed trees, embryos and pollen contributions. The alleles of monomorphic loci found in the Kerinci population were GOT-B₂, GOT-C₂, GOT-D₁, PGM-A₂, PGM-B₂, SKDH-A₂, NDH-A₂ and FDH-A₁.

The most variable loci found in the other two populations were GOT-D, PGM-B, SKDH-A, FDH-A and NDH-A. GOT-C locus was moderately polymorphic while the remaining two loci, GOT-B and PGM-A, had minor polymorphism. Of

Table 2 Genetic variation measures of seed trees and progenies (embryos) in the investigated populations of *Pinus merkusii* based on a survey of 10 enzyme gene loci

Population	Sample		Genetic multiplicity		Allelic diversity	Gametic diversity	Heterozygosity	
	Material	Size (N)	A/L	PPL	ν	ν_{gm}	H_a	$H_t = \delta_r$
Aceh	Seed trees	21	2.0	80.0	1.544	40.953	0.369	0.361
	Embryos	253	2.0	80.0	1.565	46.023	0.348	0.362
Java	Seed trees	30	2.4	80.0	1.630	62.516	0.433	0.395
	Embryos	797	2.4	80.0	1.636	63.448	0.355	0.389
Kerinci	Seed trees	25	1.0	00.0	1.000	1.000	0.000	0.000
	Embryos	200	1.0	00.0	1.000	1.000	0.000	0.000

A/L = Number of alleles per locus

PPL = Percentage of polymorphic loci (among 10 loci studied)

H_a = Actual heterozygosity

H_t = Expected heterozygosity

δ_r = Total population differentiation

Table 3 Allelic structures of seed trees, their embryos and pollen contributions at the polymorphic gene loci in the investigated populations of *Pinus merkusii*

Gene locus	Allele	Population								
		Aceh			Java			Kerinci		
		Seed tree	Embryo	Pollen	Seed tree	Embryo	Pollen	Seed tree	Embryo	Pollen
GOT-B	1	0.000	0.000	0.000	0.000	0.001	0.003	0.000	0.000	0.000
	2	0.950	0.927	0.931	0.850	0.899	0.896	1.000	1.000	1.000
	3	0.050	0.073	0.069	0.150	0.100	0.102	0.000	0.000	0.000
GOT-C	1	0.100	0.057	0.060	0.050	0.067	0.063	0.000	0.000	0.000
	2	0.875	0.826	0.714	0.800	0.759	0.763	1.000	1.000	1.000
	3	0.000	0.022	0.039	0.067	0.107	0.155	0.000	0.000	0.000
	4	0.025	0.059	0.188	0.083	0.067	0.019	0.000	0.000	0.000
GOT-D	1	0.525	0.486	0.518	0.517	0.425	0.420	1.000	1.000	1.000
	2	0.475	0.514	0.482	0.483	0.575	0.580	0.000	0.000	0.000
PGM-A	1	0.071	0.036	0.024	0.017	0.029	0.011	0.000	0.000	0.000
	2	0.929	0.960	0.968	0.983	0.953	0.949	1.000	1.000	1.000
	3	0.000	0.004	0.008	0.000	0.018	0.041	0.000	0.000	0.000
PGM-B	1	0.286	0.340	0.397	0.400	0.422	0.430	0.000	0.000	0.000
	2	0.714	0.660	0.603	0.600	0.578	0.570	1.000	1.000	1.000
SKDHA	1	0.524	0.518	0.530	0.417	0.372	0.390	0.000	0.000	0.000
	2	0.476	0.482	0.470	0.583	0.628	0.610	1.000	1.000	1.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NDH-A	1	0.381	0.379	0.393	0.333	0.327	0.315	0.000	0.000	0.000
	2	0.619	0.619	0.603	0.617	0.635	0.668	1.000	1.000	1.000
	3	0.000	0.002	0.004	0.050	0.038	0.017	0.000	0.000	0.000
FDH-A	1	0.571	0.486	0.538	0.583	0.526	0.450	1.000	1.000	1.000
	2	0.429	0.514	0.462	0.417	0.474	0.550	0.000	0.000	0.000

the five most polymorphic loci, NDH-A and PGM-B loci possessed alleles (NDH-A₂ and PGM-B₂) that were predominant in all populations examined. These alleles were also found in the fixed population of Kerinci. No single allele was predominant at the four other loci (GOT-D, PGM-B, SKDH-A, FDH-A) in the Aceh and Java populations. However, there was a tendency for alleles present in the Kerinci population to also be predominant in the other two populations.

Rare alleles ($p < 1\%$) were found at GOT-B and NDH-A loci and they were GOT-B₁ and NDH-A₃ with frequencies of 0.3% and 0.4% respectively. These alleles were found more frequently in pollen contributions than in embryos. It seemed also that alleles with low frequencies were found more often in pollen contributions than in the seed trees and embryos.

Allelic diversities of seed trees, embryos and pollen contributions

The allelic diversity for each locus and averaged over all gene loci, is given in Table 4. The gene pool data showed slightly different patterns of diversity between populations. In the Aceh population, the allelic diversities of the effective pollen clouds were higher than in the seed trees and embryos, while in the Java population the allelic diversities were similar in all samples.

The single-locus diversities were similar and low at the gene loci GOT-B, PGM-A and PGM-B. At GOT-C, allelic diversity of the pollen cloud was considerably higher ($v = 1.817$) than that of the seed trees ($v = 1.250$) and embryos ($v = 1.288$).

Genetic distances between populations

The two measures of genetic distance applied most widely were compared and the values are presented in Table 5. Results show that the genetic distances of d_0 (Gregorius 1974) are higher than that of D (Nei 1972). However, a very strong correlation between the two distances is observed as shown in Figure 2.

The difference between the two measures was low when comparing distances between populations from Java and Aceh. However, the differences were considerably higher when calculating the distances between Kerinci and Java populations as well as Kerinci and Aceh populations.

Table 4 Allelic diversity of seed trees, their progenies (embryos) and their effective pollen clouds in the investigated populations

Gene locus	Allelic diversity $v = (\sum_i p_i^2)^{-1}$								
	Aceh			Java			Kerinci		
	Seed tree	Embryo	Pollen	Seed tree	Embryo	Pollen	Seed tree	Embryo	Pollen
GOT-B	1.105	1.157	1.147	1.342	1.222	1.230	1.000	1.000	1.000
GOT-C	1.288	1.250	1.817	1.529	1.676	1.638	1.000	1.000	1.000
GOT-D	1.995	1.998	1.997	1.998	1.956	1.950	1.000	1.000	1.000
PGM-A	1.152	1.084	1.066	1.035	1.100	1.108	1.000	1.000	1.000
PGM-B	1.690	1.814	1.919	1.923	1.952	1.962	1.000	1.000	1.000
SKDH-A	1.995	1.997	1.993	1.946	1.877	1.908	1.000	1.000	1.000
NDH-A	1.893	1.898	1.930	2.024	1.955	1.832	1.000	1.000	1.000
FDH-A	1.960	1.998	1.989	1.946	1.995	1.980	1.000	1.000	1.000
Gene pool	1.544	1.582	1.631	1.630	1.635	1.625	1.000	1.000	1.000

Table 5 Matrix of average gene pool distances (d_g) according to Gregorius (1974, below diagonal) and genetic distances (D) according to Nei (1972, above diagonal)

Population		Aceh			Java			Kerinci		
		Seed tree	Embryo	Pollen	Seed tree	Embryo	Pollen	Seed tree	Embryo	Pollen
Aceh	Seed tree	***	0.000	0.000	0.000	0.008	0.012	0.122	0.122	0.122
	Embryo	0.096	***	0.001	0.001	0.009	0.010	0.154	0.154	0.154
	Pollen	0.042	0.031	***	0.000	0.009	0.011	0.154	0.154	0.154
	Seed tree	0.071	0.065	0.047	***	0.000	0.003	0.119	0.119	0.119
Java	Embryo	0.092	0.066	0.060	0.047	***	0.001	0.147	0.147	0.147
	Pollen	0.105	0.073	0.077	0.065	0.025	***	0.165	0.165	0.165
	Seed tree	0.293	0.315	0.321	0.308	0.325	0.337	***	0.000	0.000
Kerinci	Embryo	0.293	0.315	0.321	0.308	0.325	0.337	0.000	***	0.000
	Pollen	0.293	0.315	0.321	0.308	0.325	0.337	0.000	0.000	***

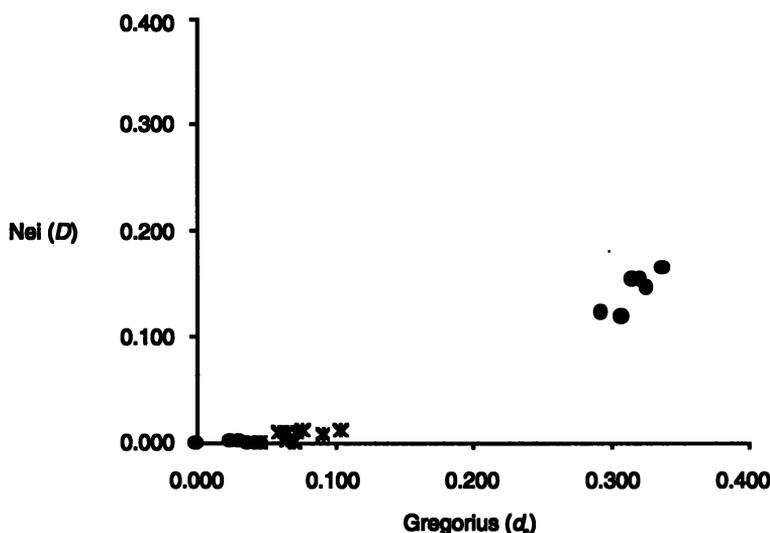


Figure 2 Relationship between average gene pool distances (d_g) according to Gregorius (1974) and genetic distances (D) according to Nei (1972).

- * = genetic distances between populations Java and Aceh,
- o = genetic distances between populations Kerinci and Java as well as populations Kerinci and Aceh

Genetic differentiation among populations

The measures of allelic differentiation (D) for each locus and the gene pool are presented in Table 6 and Figure 3. The level of average differentiation (δ) found in this study could be classified as moderate ($\delta = 16\%$) if compared with the results of other comparable studies, which showed low differentiation of only $\delta = 5\text{--}6\%$ (Changtragoon & Finkeldey 1995, Finkeldey 1995).

In general, δ has higher values than F_{ST} . However, the rankings of the gene loci in the gene pool differentiation measured by δ and F_{ST} are still comparable. Significant heterogeneity of allelic frequencies was observed consistently at all loci.

Table 6 Allelic differentiation (D_j) and their average (δ) among population samples of *Pinus merkusii* and fixation index F_{IT} . In a log likelihood ratio test of homogeneity (G_c test) the allelic frequency distributions were all significant at the 0.1% level of significance.

Gene locus	D_j									δ	F_{IT}
	Aceh			Java			Kerinci				
	Seed tree	Embryo	Pollen	Seed tree	Embryo	Pollen	Seed tree	Embryo	Pollen		
GOT-B	0.012	0.019	0.010	0.101	0.045	0.049	0.068	0.068	0.068	0.048	0.024
GOT-C	0.065	0.097	0.059	0.082	0.192	0.147	0.143	0.143	0.143	0.106	0.049
GOT-D	0.146	0.194	0.148	0.155	0.258	0.266	0.389	0.389	0.389	0.259	0.216
PGM-A	0.054	0.019	0.004	0.014	0.021	0.021	0.032	0.032	0.032	0.025	0.009
PGM-B	0.037	0.106	0.156	0.165	0.190	0.199	0.285	0.285	0.285	0.190	0.192
SKDH-A	0.243	0.256	0.251	0.122	0.072	0.093	0.347	0.347	0.347	0.231	0.148
NDH-A	0.163	0.157	0.175	0.152	0.131	0.094	0.279	0.279	0.279	0.190	0.100
FDH-A	0.128	0.213	0.165	0.114	0.179	0.264	0.354	0.354	0.354	0.236	0.181
Gene pool	0.106	0.125	0.121	0.113	0.128	0.142	0.237	0.237	0.237	0.161	0.135

Discussion

Knowledge of the distribution of genetic variation within and between populations is of substantial benefit in tree breeding and in the conservation of plant genetic resources. The results on genetic variation reported in this chapter may contribute to the currently scarce information on the pattern of genetic variation in *P. merkusii*. It was found that *P. merkusii* in Indonesia harbours larger amounts of genetic variation in the mainland provenances than those found by Changtragoon and Finkeldey (1995). A high level of genetic variation within populations is a common phenomenon in most pines, as reported by Ledig (1986). The author found that of 20 conifer species, the mean for the percentages of polymorphic loci, the number of alleles per locus and the expected heterozygosity were 67.7, 2.29 and 2.07 respectively. The results of the present study indicated that, with the exception of the Kerinci population, the level of genetic variation in a natural population and a seed orchard was moderately high. A previous study of genetic variation of four artificial stands (plantations) of *P. merkusii* in Java was conducted by Na'iem and Indrioko (1996). They reported that the mean for the percentages of polymorphic loci, the number of alleles per locus and the expected heterozygosity were 85.7, 0.259 and 2.250 respectively. These values are similar to the values obtained from the seed orchard population in the present study ($PPL = 80.0$, $H_e = 0.395$ and $A/L = 2.40$). Muona and Harju (1989) attributed the high variation in pine species mainly to their wind-mediated pollination, considerable pollen migration and large effective population size. The genetic differentiation of the Kerinci population was larger than that of the other two populations (see Figure 3) because Kerinci population was fixed to the globally most frequent allele at all gene loci.

The absence of genetic variation found in Kerinci raises questions about its possible causes. It may be reasonable to adopt the same explanation as that referred to in regard to the observed low genetic variation in *P. merkusii* found in Thailand and Vietnam. Changtragoon and Finkeldey (1995) as well as Szmidi

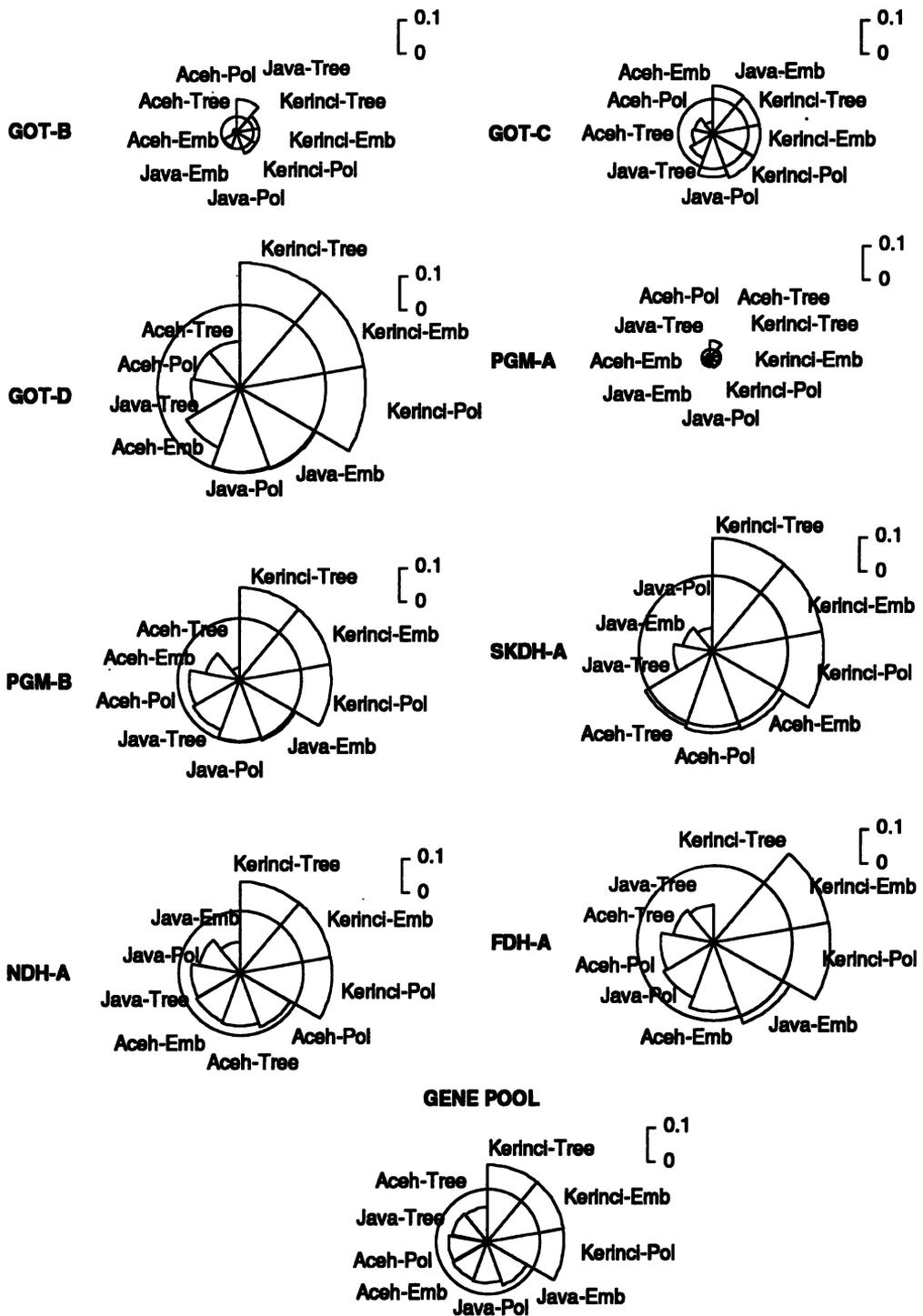


Figure 3 Allelic differentiation (D) among populations as shown by snail diagrams. The length of the radii denotes D , and the radius of the circle is equal to δ . Tree refers to seed trees, Emb refers to embryos and Pol refers to effective pollen.

et al. (1996) attributed the low level of genetic variation in *P. merkusii* in mainland Asia to bottlenecks and reduced gene flow among populations. With regard to the Kerinci population, an additional explanation might be the geological history of Mount Kerinci. Kerinci is a stratovolcano (steep, conical volcanoes built by the eruption of viscous lava flows, tephra and pyroclastic flows) which has erupted at least 20 times since 1838. The most recent eruption was in 1969–1970. An unconfirmed eruption was reported in 1971 (Simkin & Siebert 1994). It is hypothesised that there have been drastic reductions in the number of trees in Kerinci during this geological age, causing repeated bottlenecks that resulted in low genetic variation. The presumable geological reason for the genetic fixation of *P. merkusii* in Kerinci is likely to be similar to that which caused the low genetic diversity in *P. resinosa* in Quebec, Canada and *Amentotaxus formosana* in Taiwan. The low genetic diversity of these two species resulted from passing through a genetic bottleneck during glacial episodes of the Holocene (Simon *et al.* 1986, Wang *et al.* 1996). The observed low genetic diversity in Kerinci could then also be the result of small population size and bottlenecks. The majority of trees in the Kerinci area are reported to be at elevations 1500 and 2000 m above sea level. The exact size of the populations is not known, but it has long been assumed that the numerous small stands and groups of this pine trees may total less than 3000 ha (Cooling 1967). To date, the size of the total area is believed to be much smaller. In Kerinci, disturbance of *P. merkusii* is caused by illegal logging, shifting cultivation, land conversion for agriculture/horticulture, and forest fires. This probably results in reduced tree population size, more effective isolation and less gene flow among populations. Thus, some genes may be lost and, subsequently, some populations will become extinct; this will result in overall reduction in genetic diversity at the species level (Lee *et al.* 2000).

In any event, the protection and conservation of the Kerinci population may only be slightly effective to conserve the genetic resources of the species. However, a final decision should take into account also the expression of adaptive phenotypic traits, in addition to the genetic traits referred to in this study.

Low genetic distance between the seed orchard population in Java and the natural population in Aceh is considered to be due to history of the introduction of *P. merkusii* to Java. The *P. merkusii* plantations established in the early 1930s in Java originated in a sub-population of the Aceh natural forest. However, the exact origin of this sub-population is still not confirmed; presumably planting material was derived from the Blang Kejeren population (Hardiyanto 1996). Unfortunately, there exists no current information on patterns of genetic variation within the Aceh population. Similarity in gene pool diversities among seed trees, progenies (embryos) and the effective pollen which were found in the seed orchard population suggest little gene flow into the orchard from outside. On the other hand, higher gene pool diversity found in the effective pollen of the Aceh natural population suggests extensive gene pollen inflow from outside. This natural population has a total area of about 7000 ha (Anonymous 1988). The comparable genetic variation in the seed orchard and the Aceh population has implications for conservation. This suggests that starting new populations—in this case seed orchards—from a relatively small number of mother trees (200 or less) can

conserve most of the genetic diversity in the original population, in this case the much larger Aceh population.

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