PATTERNS OF GENETIC VARIATION AND CHARACTERIZATION OF THE MATING SYSTEM OF *PINUS MERKUSII* IN THAILAND

Suchitra Changtragoon [1] & Reiner Finkeldey [2]

[1)] DNA and Isozyme Laboratory, Silvicultural Research Division, Royal Forest Department, 61 Phaholyothin Road, Chatuchak, Bangkok 10900, Thailand
[2)] UNDP/FAO Project RAS/91/004, ERDB Building, P.O. Box 157, College, Laguna 4031, Philippines

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ABSTRACT

A genetic inventory in 11 natural populations of *Pinus merkusii* in Thailand revealed only little genetic diversity at 14 isozyme gene loci (average $\delta_T = H_e = 0.058$). Allelic differentiation among populations is also small ($\delta = 0.034$), but higher than the differentiation reported from many other conifers, if measured as a proportion of the total variation ($F_{ST} = 0.104$). Genotypic structures of seed samples are characterized by a deficiency of heterozygotes relative to Hardy-Weinberg expectations in most populations, while the genotypic structures of the seed trees, which represent the adult forest stand, do not differ significantly from Hardy-Weinberg proportions. Estimation of outcrossing rates revealed extraordinary high proportion of selfing ($0.017 < t_m < 0.65$ for 9 out of 10 analyzed populations), which account for the high inbreeding coefficients. The low genetic diversity is explained by bottlenecks and consequently genetic drift in the evolutionary history of the populations. Scarcity of foreign pollen available for fertilization of ovules due to low population density, poor synchronization of flowering periods, and over-mature of most stands resulting in limited flower production are mentioned as probable reasons for the high inbreeding.

Key words: *Pinus merkusii*, isozyme, genetic variation, mating system, inbreeding

INTRODUCTION

Pinus merkusii Jungh. and de Vriese is a tropical pine of Southeast Asia. It occurs naturally in Myanmar, Thailand, Laos, Cambodia and Vietnam as well as on the islands Sumatra (Indonesia), Luzon and Mindoro (Philippines) (CRITCHFIELD & LITTLE 1966). Extensive plantations exist on Sumatra and Java, but not on the Southeast-Asian mainland.

Provenance trials have revealed considerable genetic variation among "races" of *Pinus merkusii* (*e.g.*, CHANGTRAGOON 1984). The most conspicuous variation refers to the duration of the "grass stage", a growth depression of the shoot during early seedling development. Provenances from northern Thailand uniformly show a long lasting (3–5 years) grass stage, which makes the establishment of plantations very laborious. The grass stage of north-eastern provenances is much shorter, and the Sumatra races of *P. merkusii* do not exhibit the grass stage at all.

The natural distribution of *P. merkusii* in Thailand is shown in Figure 1. The pine occurs mainly in the northern mountain range west of Chiang Mai, north of Petchabun, in northeastern Thailand close to Laos and Cambodia, and in two small relic populations west of Suphan Buri and south-west of Petchaburi on the Gulf of Siam. The distribution is not continuous, but scattered in relics of forests in otherwise deforested areas (mostly in northeastern Thailand) or in forested dominated by hardwoods (mostly in northern Thailand). *P. merkusii* typically occurs in Pine-Dipterocarp Forests (northern Thailand) as well as in Seasonal Pine Rainforests and Pine Savannas, mostly in northeastern Thailand (WERNER 1993).

Currently there are no plantations of *P. merkusii* in Thailand, although the species is included in the pine plantation programme of the Royal Forest Department (RFD) of Thailand for timber and resin production (POUSAJJA *et al.* 1989). Due to the short grass stage of northeastern provenances and their good performance in provenance trials, their use is recommended for plantation establishment; *in situ* gene conservation measures are confined to natural populations in northeastern Thailand (Kong Chiam and Nong Khu).

An analysis of the genetic control of isozyme patterns of 12 enzyme systems controlled by 18 gene loci is described elsewhere (CHANGTRAGOON & FINKELDEY 1995). In this paper we describe and interpret patterns of genetic diversity and differentiation of 11 natural populations of *P. merkusii* in Thailand at 17 of these isozyme gene loci. One presumably monomorphic locus (*Ndh–A*) was excluded from the survey due to low staining intensity in some samples. This genetic inventory aims to provide basic information for future

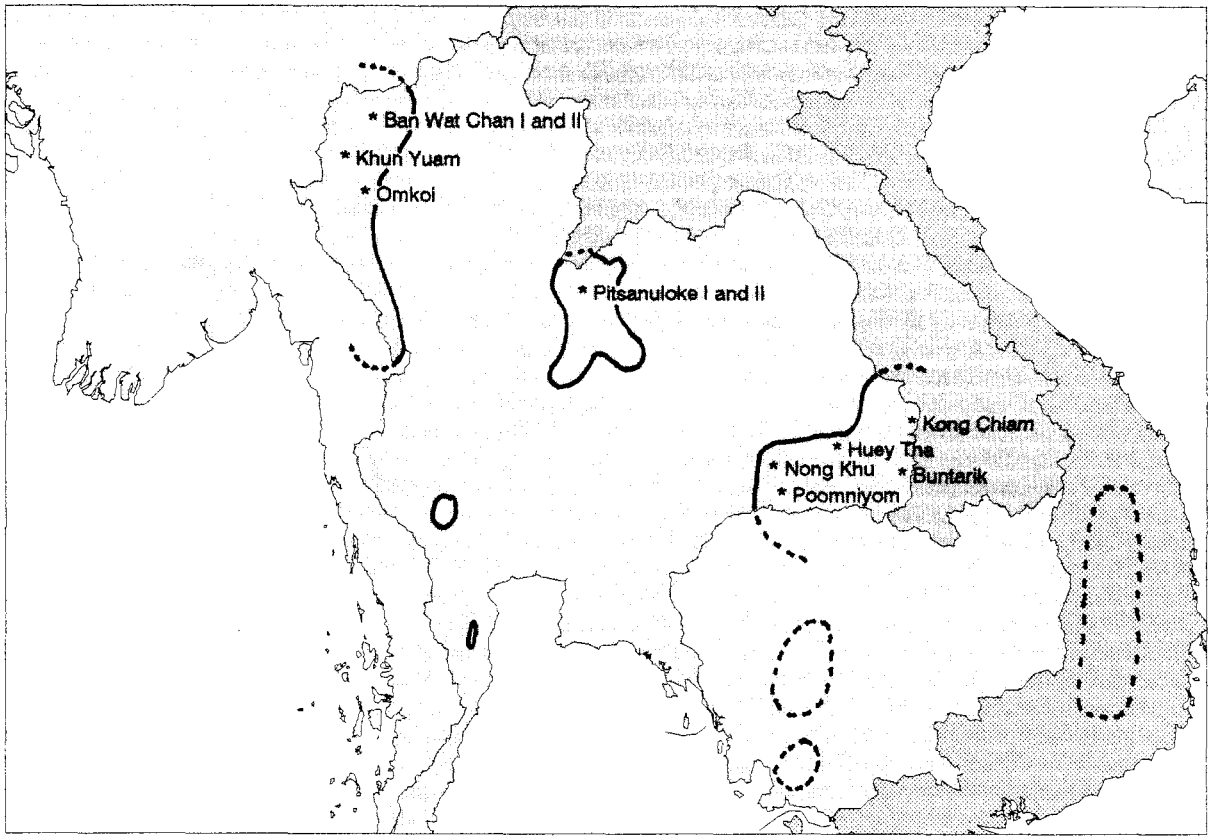

Figure 1 Natural distribution of *Pinus merkusii* in Thailand and neighboring countries (modified from CRITCHFIELD & LITTLE 1966, COOLING 1968, WERNER 1993)

activities in tree breeding and conservation of genetic resources.

MATERIAL AND METHODS

Seeds were harvested in 11 natural populations of *P. merkusii* covering the most important distribution areas of the species in Thailand. No viable seeds were produced by the southernmost population near Petchaburi at the Gulf of Thailand, which consequently could not be included in the survey. Details of the investigated populations are given in Table 1, their approximate location is indicated in Figure 1.

Seed-bearing trees were selected in all populations at random. The number of seed trees had to be limited due to the considerable efforts to climb the trees for seed harvesting (Table 1). In case of population 8 (Pommniyom) only 8 seed-bearing trees could be identified. The number of investigated seeds per tree was increased for this population in order to compensate for the very small number of investigated seed trees.

Details of laboratory procedures, isozyme phenotypes, and genotyping are given elsewhere (CHANGTRAGOON & FINKLEDEY 1995). The investigated enzyme systems, their abbreviations, E.C.-Nos., and controlling gene loci are listed in Table 2.

From all seeds both the endosperm and the embryo were investigated. However, due to weak staining intensity of most embryo tissue extracts for the GOT system, the interpretation of the GOT loci was confined to endosperm variation. The genotypes of the seed trees were determined by an investigation of at least 6 of their endosperms, as in that case the probability of misclassification of a heterozygous tree as a homozygote is below 5% (exactly $0.5^{(6-1)} = 0.03125$ assuming regular segregation; *cf.*, RITLAND & EL-KASSABY 1985).

Allelic and genotypic structures were computed for all 11 populations for the seed trees, which are a random sample of the adult stands, and for the seeds, which represent the offspring generation.

Based on allelic structures of the seeds, the following parameters of genetic diversity within populations were measured for each population: proportion of polymorphic gene loci (both 95 % criterion, *i.e.*, a gene locus is considered to be a polymorphic only, if the frequency of the most frequent allele is below 95 %, and "no" criterion, *i.e.*, all gene loci with more than one allele are considered as polymorphic), average number of alleles per locus, genetic diversity $\upsilon=(\Sigma p_i^2)^{-1}$ (GREGORIUS 1987), which equals the "gene diversity" or

expected heterozygosity" H_e of NEI (1973), and hypothetical gametic diversity $\upsilon_{gam}=\Pi\upsilon_i$.

Allelic differentiation among populations was measured both for the adult stands and the seed populations using F_{ST} statistics (NEI 1973; FINKELDEY 1994) and the concept of GREGORIUS and ROBERDS (1986; δ and D_j). All populations were equally weighted both for the computation of δ and F_{ST}. A hierarchical partitioning of the total genetic diversity in a proportion due to differences among regions (north, Pitsanuloke, northeast), among populations within regions, and within populations was performed (NEI 1973)as well as a cluster analysis using UPGMA (Unweighted Pair-Group Method with Arithmetic Averaging; SNEATH & SOKAL 1973) based on Nei's genetic distance (NEI 1972).

Deviations form Hardy-Weinberg expectations in the seeds and seed trees were tested for statistical significance using the Log-Likelihood-Test (G-test) testing an excess or deficiency of observed heterozygotes relative to Hardy-Weinberg proportions for statistical significance. The G-test requires a minimum of at least 5 for all classes of expected frequencies (SOKAL & ROHLF 1983). Accordingly, it was not performed if less than 5 heterozygotes were expected, *i.e.*, for monomorphic or only slightly polymorphic gene loci. Inbreeding coefficients ($F = 1 - H_o / H_e$)were calculated for those gene loci, for which the Log-Likelihood-Test for the significance of deviations from Hardy-Weinberg expectations could be performed. The same set of polymorphic gene loci, which differed among the investigated populations, was used to estimate average single-locus (t_s) and multilocus (t_m) outcrossing rates following the procedure of RITLAND and JAIN (1981). Standard errors of the outcrossing estimates were calculated by bootstrapping (100 bootstraps; resampling between families). Absence of linkage is one of assumptions of their estimation method, which is based on a mixed mating model. This assumption could not be tested, as no information has been obtained regarding linkage relationships among the investigated isozyme gene loci.

Table 1 Investigated populations of *Pinus merkusii* from Thailand

No	Population	Abbrev.	Region	Year of seed harvest	Approx. altitude	Vegetation type	# of seed trees harvested	# of seeds per tree investigated
1	Ban Wat Chan 1	BW 1	North	1992	1,000	PD	23	6
2	Ban Wat Chan 2	BW 2	North	1993	1,000	PD	18	6
3	Khun Yuam	KY	North	1992	600	PD	21	6
4	Omkoi	OM	North	1992	1,000	PD	23	6
5	Pitsanuloke 1	PI 1	Pitsanuloke	1993	800	PS	30	6
6	Pitsanuloke 2	PI 2	Pitsanuloke	1993	800	PS	26	6
7	Nong Khu	NK	Northeast	1992	170	SPR	25	6
8	Poomniyom	PO	Northeast	1992	170	SPR	8	15
9	Huey Tha	HT	Northeast	1992	150	SPR	23	6
10	Kong Chiam	KC	Northeast	1992	130	SPR	18	6
11	Buntarik	BU	Northeast	1992	140	SPR	30	6

a) From WERNER (1993): PD – Pine-Dipterocarp Forest; PS – Pine Savannah; SPR – Seasonal Pine Rainforest

Table 2 Investigated enzyme systems, their abbreviations, E. C. numbers, and controlling gene loci

Enzyme system	Abbreviation	E.C. Number	Gen Loci
Leucine aminopeptidase	*LAP*	3.4.11.1	*Lap-A, Lap-B*
Glutamate-oxaloacetate transaminase	*GOT*	2.6.1.1	*Got-A, Got-B, Got-C*
Glutamate dehydrogenase	*GDH*	1.4.1.3	*Gdh-A*
Formate dehydrogenase	*FDH*	1.2.1.2	*Fdh-A*
Malate dehydrogenase	*MDH*	1.1.1.37	*Mdh-A, Mdh-B, Mdh-C*
Shikimate dehydrogenase	*SKDH*	1.1.1.25	*Skdh-A*
6-phosphogluconate dehydrogenase	*6-PGDH*	1.1.1.44	*6-pgdh-A, 6-pgdh-B*
Phosphoglucomutase	*PGM*	2.7.5.1	*Pgm-A*
Glucose-6-phosphate-dehydrogenase	*G-6-PDH*	1.1.1.49	*G-6-pdh-B*
Isocitrate dehydrogenase	*IDH*	1.1.1.42	*Idh-A*
Diaphorase	*DIA*	1.6.4.3	*Dia-A*

The computations of frequency distributions and all diversity and differentiation measures were performed by the computer programs BIOSYS (SWOFFORD & SELANDER 1981) and GSED (GILLET 1994). The MLT computer program of RITLAND (1990) was used to estimate the outcrossing rates.

RESULTS

Genetic Frequency Distributions and Variation within Populations

The allelic frequency distributions of the seed trees and the seed samples are given in Appendix I and II respectively for all polymorphic gene loci. *Got–B* (recorded only for the seed trees) and *Dia–A* were monomorphic in all populations; these gene loci were included in the computation of gene pool variation and differentiation parameters. Measures of genetic variation within the seed populations based on 14 gene loci (*i.e.*, without GOT loci) are summarized in Table 3.

Out of the 13 variable gene loci investigated only two (*Fdh–A* and *Gdh–A*) proved to be polymorphic in all populations (Appendix II). These loci are dominated by two frequent alleles in some populations (major polymorphisms), *cf.* LEWONTIN 1985), while only one allele is frequent in other populations (minor polymorphisms). The remaining 11 loci are dominated by the same frequent allele in all populations, which is accompanied by one to three rare alleles in some populations (minor polymorphisms). The only exceptions from this observation are the *Skdh–A* and *6-pgdh–A* locus in the population Huey Tha (HT), for which frequencies of roughly 20% of the rare types were recorded.

The maximum number of alleles is 4 for any gene locus; it was observed only at the *Skdh–A* locus, where one of the four alleles was a "null-allele", which was identified only in a single seed tree. Patterns of genetic diversity within populations are very similar to those reported in Table 3, if they are based on genetic structures of the seed trees instead of the seeds (data not presented). The gene pool diversity measures are slightly higher, if the three GOT loci are included, as one of them (*Got–C*) is polymorphic with two frequent alleles in all populations (major polymorphism), while *Got–A* and *Got–B* are monomorphic or minor polymorphisms.

The population Huey Tha stands out with its high percentage of polymorphic gene loci (especially if the 95% criterion is applied), and the highest gene pool diversity and hypothetical gametic diversity estimates. Very low levels of genetic variation within populations were recorded for the populations Khun Yuam and Poomniyom.

Genetic Differentiation among Populations

A summary of measures of genetic differentiation among the seed samples is presented in Table 4. Most gene loci are dominated by the same frequent allele in all populations; consequently, the differentiation among populations is low for these loci. Higher differentiation is observed for those gene loci, for which a major polymorphisms (two frequent alleles) was observed in at least one population, while other populations are characterized by the dominance of the same frequent type. These loci are *Gdh–A, Skdh–A, 6-pgdh–A*, and *Fdh–A*.

Table 3 Measures of genetic variation within populations; percentage of polymorphic gene loci (PPL; "no" and 95% criterions), mean number of alleles per locus (A/L), gene pool allelic diversity (υ), gene pool differentiation within populations δ_T (= "expected heterozygosity" H_e), average observed heterozygosity, and hypothetical gametic diversity (υ_{gam})

Population	N	PPL (no)	PPL (95%)	A/L	υ	δ_T (=H_e)	H_o	υ_{gam}
Ban Wat Chan 1	126	50.0	21.4	1.6	1.040	0.038	0.019	1.781
Ban Wat Chan 2	108	57.1	21.4	1.6	1.050	0.048	0.037	2.019
Khun Yuan	126	35.7	7.1	1.4	1.029	0.029	0.006	1.552
Omkoi	139	50.0	21.4	1.7	1.082	0.076	0.042	3.532
Pitsanuloke 1	180	50.0	21.4	1.8	1.097	0.088	0.063	4.602
Pitsanuloke 2	156	42.9	14.3	1.4	1.051	0.048	0.032	2.270
Nong Khu	150	42.9	21.4	1.6	1.075	0.070	0.051	3.257
Poomniyon	120	28.8	7.1	1.3	1.041	0.039	0.030	2.093
Huey Tha	138	57.1	35.7	1.6	1.112	0.109	0.061	6.213
Kong Chiam	108	50.0	21.4	1.5	1.058	0.055	0.044	2.361
Buntarik	180	42.9	14.3	1.6	1.044	0.042	0.025	1.932
Average	139.1	46.1	18.8	1.6	1.062	0.058	0.037	2.873

Table 4 Genetic differentiation among populations; D_j and δ (GREGORIUS & ROBERDS 1986) and F_{ST} (NEI 1973) for all polymorphic gene loci and the gene pool

Gene locus	D_j											δ	F_{ST}
	BW 1	BW 2	KY	OM	PI 1	PI 2	NK	PO	HT	KC	BU		
Lap–A	0.000	0.015	0.015	0.015	0.019	0.015	0.019	0.015	0.005	0.047	0.015	0.016	0.024
Lap–B	0.048	0.076	0.048	0.032	0.059	0.026	0.018	0.038	0.094	0.022	0.038	0.045	0.040
Gdh–A	0.014	0.049	0.031	0.199	0.102	0.033	0.016	0.115	0.013	0.108	0.024	0.064	0.056
Pgm–A	0.083	0.002	0.013	0.013	0.013	0.013	0.013	0.009	0.013	0.017	0.013	0.019	0.053
Mdh–A	0.018	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.005	0.002	0.002	0.004	0.013
Mdh–B	0.000	0.025	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.015	0.005	0.007	0.018
Mdh–C	0.004	0.026	0.000	0.001	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.006	0.016
Skdh–A	0.038	0.038	0.038	0.038	0.030	0.038	0.067	0.038	0.211	0.038	0.017	0.054	0.131
6-pgdh–A	0.046	0.046	0.046	0.010	0.043	0.043	0.046	0.046	0.176	0.074	0.076	0.059	0.101
6-pgdh–B	0.004	0.034	0.027	0.025	0.016	0.016	0.014	0.016	0.014	0.016	0.016	0.018	0.019
G-6-pdh–B	0.009	0.009	0.034	0.009	0.034	0.009	0.009	0.009	0.009	0.009	0.004	0.013	0.023
Fdh–A	0.193	0.221	0.240	0.018	0.242	0.090	0.163	0.343	0.067	0.040	0.195	0.165	0.173
Idh–A	0.002	0.055	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.010	0.048
Gene pool	0.033	0.043	0.036	0.027	0.041	0.021	0.027	0.046	0.045	0.029	0.030	0.034	0.104

The gene pool D_j are uniformly small in all populations, ranging from 0.021 to 0.046. δ is the (unweighted) mean of the D_j; it is an absolute measure of the differentiation among (sub-) populations. F_{ST} measures the relative amount of the total genetic diversity, which is due to differentiation among populations. F_{ST} is higher than D_j due to the low genetic diversity observed at most gene loci.

A hierarchical partitioning of the total genetic diversity H_T in a component among regions (north, Pitsanuloke, northeast; *cf.* Table 1; G_{RT}),among populations within regions (G_{PR}), and within populations shows that the genetic differentiation among regions is small (G_{RT} = 0.017) if compared to the differentiation among populations within regions (G_{PR} = 0.085).

The cluster analysis based on Nei's genetic distance and using UPGMA does not reflect the geographic distributions of the populations (Figure 2). The small genetic differentiation among populations is reflected by the short branches of the dendrogram. Only two clusters are fairly well separated: The four populations from the north cluster with the two of the northeastern populations (Kong Chiam and Buntarik), while the remaining populations from northeastern Thailand are connected to the Pitsanuloke populations.

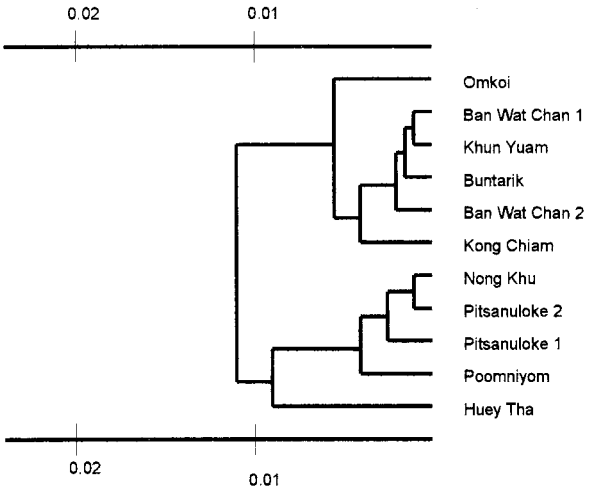

Figure 2 Cluster analysis (UPGMA based on Nei's genetic distance for 14 gene loci) for the 11 investigated populations

Genotypic Structures and Analysis of the Mating System

The comparison of the observed heterozygosities to those expected under Hardy-Weinberg proportions (H_o and $\delta_T = H_e$ in Table 3) reveals a substantial deficiency of heterozygotes (averaged over all gene loci) for all seed populations. In order to test a surplus or deficiency of heterozygotes as compared to Hardy-Weinberg proportions for statistical significance, a log-likelihood test (G-test) was performed comparing the observed distribution of homozygotes and heterozygotes to the expected distribution. Since the G-test requires a minimum of 5 for all frequency classes, it was restricted to those loci, which were sufficiently polymorphic to expect at least 5 heterozygotes. The set of loci, which met this criterion, differed among populations. For the same loci inbreeding coefficients (F-values) were computed (Table 5).

The frequency of observed heterozygotes is always smaller than the expected frequency under Hardy-Weinberg proportions (*i.e.*, inbreeding coefficients are positive) with only two statistically non-significant

Table 5 Observed (H_o) and "expected" (H_e) heterozygosities at single gene loci, results of a statistical test for significance of the differences between observed and expected heterozygosities ($K_{\alpha=0.05;\,1DF} = 3.841$; $K_{\alpha=0.01;\,1DF} = 6.635$; $K_{\alpha=0.001;\,1DF} = 10.828$; n.s.: not significant), and inbreeding coefficients (F) for the seed population

Population	Gene locus	H_o	H_e	G	F
Ban Wat Chan 1	*Gdh–A*	15	22.40	3.314 ns	0.332
	Fdh–A	10	11.45	0.210 ns	0.125
Ban Wat Chan 2	*Lap–B*	8	9.07	0.152 ns	0.118
	Gdh–A	9	12.06	0.971 ns	0.254
Khun Yuam	*Gdh–A*	6	30.86	36.056 ***	0.806
	6-pgdh–B	1	8.68	11.527 ***	0.885
	6-pgdh–C	1	8.86	11.527 ***	0.885
Omkoi	*Lap–B*	10	18.92	5.731 *	0.471
	Gdh–A	18	57.81	54.560 ***	0.689
	6-pgdh–A	8	7.74	0.274 ns	–0.034
	6-pgdh–B	7	8.72	0.392 ns	0.198
	Fdh–A	31	45.31	7.173 ns	0.361
Pitsanuloke 1	*Lap–B*	19	32.33	7.630 **	0.412
	Gdh–A	45	60.89	6.574 *	0.261
	Skdh–A	12	15.51	0.936 ns	0.226
	G-6-pdh–B	3	6.67	2.792 ns	0.556
	Fdh–A	64	88.27	13.364 ***	0.275
Pitsanuloke 2	*Lap–B*	7	10.61	1.488 ns	0.340
	Gdh–A	19	22.83	0.802 ns	0.168
	Fdh–A	37	63.40	20.279 ***	0.416
Nong Khu	*Lap–B*	10	9.96	0.011 ns	–0.032
	Gdh–A	9	27.90	20.215 ***	0.680
	Fdh–A	50	68.05	9.173 **	0.265
Poomniyom	*Fdh–A*	46	59.73	6.347 *	0.235
Huey Tha	*Lap–B*	11	25.95	12.933 ***	0.576
	Gdh–A	11	25.95	12.933 ***	0.576
	6-pgdh–A	19	40.84	19.667 ***	0.535
	Skdh–A	27	39.56	6.533 *	0.316
	Fdh–A	34	55.96	15.447 ***	0.392
Kong Chiam	*Fdh–A*	30	32.59	0.295 ns	0.075
	6-pgdh–A	19	20.55	0.144 ns	0.080
Buntarik	*Gdh–A*	19	42.33	19.968 ***	0.551
	6-pgdh–A	26	36.20	3.895 *	0.282
	Skdh–A	7	6.88	0.002 ns	–0.017
	Fdh–A	8	15.29	0.477 *	0.477

exceptions. Inbreeding is plausible interpretation for high (positive) F-values only if the values are homogenous among the loci for each population. This holds true for most populations; exceptions, *i.e.*, F-values clearly deviating from each other, are confined to low polymorphic gene loci. Hence, results clearly point towards strong inbreeding in several of the investigated populations (*e.g.* Huey Tha, Khun Yuam, and Pitsanuloke 1), while others (*e.g.*, Kong Chiam) seem to be less affected by inbreeding.

F-values were computed for the populations of seed trees as well (Table 6). Due to the smaller sample sizes, the set of gene loci, for which a log-likelihood test for significance of differences in the frequency distributions of observed and expected heterozygotes / homozygotes could be performed, had to be reduced further. Out of the 20 tests, which could be performed, only one showed significant deviation from Hardy-Weinberg heterozygosity at the 5 % level, and a (non-significant) excess of heterozygotes was recorded in 8 cases as compared to 12 cases with an excess of homozygotes. Hence, strong inbreeding seems to be confined to the seed generation, but is not evident for the seed trees.

In order to investigate the suspicion of strong inbreeding in at least some populations further, (average) single-locus (t_s) and multilocus (t_m) population outcrossing rates were estimated based on a mixed mating model (Table 7).

The estimation of outcrossing rates shows a high amount of selfing in most populations. For 8 out the 11 investigated populations, multilocus outcrossing rates

Table 6 Observed (H_o) and "expected" (H_e) heterozygosities at single gene loci, results of a statistical test for significance of the differences between observed and expected heterozygosities ($K_{\alpha = 0.05;\ 1DF} = 3.841$; n.s.: not significant), and inbreeding coefficients (F) for the seed trees

Population	Gene locus	H_o	H_e	G	F
Ban Wat Chan 1	*Got–C*	7	9.41	1.304 ns	0.257
Ban Wat Chan 2	*Got–C*	5	5.38	0.052 ns	0.070
Omkoi	*Gdh–A*	7	9.33	0.907 ns	0.249
	Fdh–A	9	10.10	0.272 ns	0.110
Pitsanuloke 1	*Got–C*	11	12.09	0.418 ns	0.090
	Gdh–A	12	10.74	0.452 ns	–0.118
	Fdh–A	16	14.93	0.153 ns	–0.071
Pitsanuloke 2	*Gdh–A*	7	5.98	1.201 ns	–0.171
	Fdh–A	9	10.50	0.496 ns	0.143
Nong Khu	*Fdh–A*	14	11.67	0.976 ns	–0.200
Huey Tha	*Got–C*	7	9.85	1.932 ns	0.290
	Lap–B	4	5.21	0.989 ns	0.233
	Gdh–A	6	5.21	0.903 ns	–0.150
	Skdh–A	7	7.81	0.202 ns	0.104
	6-pgdh–A	7	7.98	0.289 ns	0.122
	Fdh–A	11	9.32	0.802 ns	–0.179
Kong Chiam	*Fdh–A*	10	7.22	1.740 ns	–0.385
Buntarik	*Got–C*	8	12.80	4.059 *	0.375
	6-pgdh–A	6	5.40	0.668 ns	–0.111
	Gdh–A	8	8.33	0.040 ns	0.040

Table 7 Estimates of single-locus (t_s) and multilocus (t_m) outcrossing rates and their standard errors

Population	t_s	t_m
Ban Wat Chan 1	0.424 ± 0.307	0.444 ± 0.322
Ban Wat Chan 2	0.571 ± 0.204	0.593 ± 0.202
Khun Yuan	0.014 ± 0.011	0.017 ± 0.013
Omkoi	0.385 ± 0.102	0.422 ± 0.120
Pitsanuloke 1	0.600 ± 0.134	0.644 ± 0.154
Pitsanuloke 2	0.372 ± 0.099	0.395 ± 0.095
Nong Khu	0.433 ± 0.114	0.455 ± 0.115
Poomniyon	0.767 ± 0.145	–
Huey Tha	0.395 ± 0.068	0.468 ± 0.077
Kong Chiam	0.863 ± 0.068	0.843 ± 0.087
Buntarik	0.396 ± 0.089	0.400 ± 0.088

vary between 39.5% and 66.4%, *i.e.*, in most populations roughly half of all seed originate from selfing. The seeds of the population Khun Yuam seem to originate from selfing. The seeds of the population Khun Yuam seem to originate nearly completely from selfing (t_m = 0.017), while population Kong Chiam is predominantly outcrossing (t_m = 0.843). No multilocus outcrossing rate could be computed for population Poomniyom, as the estimation procedure had to be based on only one gene locus (*Fdh–A*) for this population. With only one exception (Kong Chiam), multilocus outcrossing rates are slightly higher than average single locus estimates. This outcome is usually interpreted as a hint of inbreeding other than selfing (SHAW & ALLARD 1982). Since all investigated populations are natural forests, the existence of a family structure resulting in mating among relatives is a reasonable explanation for the slight differences between the outcrossing estimates.

DISCUSSION

Genetic Diversity

Most pines show a high levels of genetic variation within populations, "expected heterozygosities" at isozyme gene loci usually being higher than 0.15 (*e.g.*,

Table in LEDIG 1986). Thus *P. merkusii* is a pine of comparatively low genetic diversity. Unfortunately, to the best of our knowledge there is currently no information available on patterns of genetic variation in other parts of the natural distribution area and especially for the island provenances in Indonesia and the Philippines. Likewise, the evolutionary history of *P. merkusii* is largely unknown. We do not know when the ancestors of *P. merkusii* started to migrate from the north to the tropical zone in Asia, nor we are sure about migration routes.

WERNER (1993) concludes from his studies on distribution and ecology of pines in Thailand, that the distribution range of *P. merkusii* in Thailand is shrinking not only because of human influence, but also because of climatic changes after the last glacial period favoring hardwood species. Occasional fires play a crucial role, since they promote the regeneration of *P. merkusii* by removing the grass cover from the top soil and restraining competition of hardwoods. The past and current distribution of *P. merkusii* forests in Thailand has been shaped by the occurrence and frequency of forest fires. Hence, it is easily conceivable that even extensive forests dominated by *P. merkusii*, which existed for example in Ban Wat Chan and Omkoi, were derived from only few parent trees, which found favorable conditions for reproduction after extensive forest fires.

In conclusion, the current distribution and ecology of *P. merkusii* suggests a dynamic migration history in Thailand. All populations might have experienced severe bottlenecks in their evolutionary past, which reduced the genetic variation within populations (cf. LEDIG 1986). Hence, genetic drift is the most probable cause of the comparatively low genetic diversity of *P. merkusii* in Thailand.

Differentiation among Populations

Since the genetic diversity within populations is low and with only few exceptions (*Fdh–A* and *Gdh–A*) all populations are dominated by the same frequent allele, genetic differentiation among populations is small. The patterns of differentiation among populations are dominated by only two loci (*Fdh–A* and *Gdh–A*), which show a major polymorphism in some populations and a minor polymorphism in the others. The small number of variable gene loci might explain, why the strong differentiation between provenances from the North and the Northeast of Thailand, which has been observed for quantitative traits, is not reflected at the enzyme gene loci.

Another more genera explanation for the lack of congruence between the patterns of differentiation at isozyme and quantitative trait loci is the presumably different selective regime to which both types of genetic traits are exposed (FINKELDEY 1993).

Inbreeding and Selfing Rates

The dynamics of genotypic structures of many forest tree species is characterized by an excess of homozygotes relative to Hardy-Weinberg proportions (*i.e.* positive inbreeding coefficients), which is gradually diminished or even reversed during the development of the stand. However, the inbreeding coefficients of the seed populations reported here are extraordinarily high and the estimates of outcrossing rates very low for most populations. Conifers are wind-pollinated and usually outbreeding. To the best of our knowledge *P. merkusii* is the first pine for which high selfing proportions in most natural populations have been reported.

Three factors may lead to a scarcity of foreign pollen available for fertilization of ovules in *P. merkusii:*

Low population density. The population density in natural populations of *P merkusii* is usually very low. Typically, pines occur in small clusters or even as a single tree in mixed forests. The density of trees is very low in population Khun Yuam, for which very high level of selfing was estimated. However, the density of population Huey Tha is high; this population is a small relic of nearly pure pine forest in an area dominated by agriculture. Inbreeding coefficients were uniform and high (between 0.316 and 0.576) for 5 polymorphic loci, indicating substantial inbreeding, and the outcrossing rate t_m was estimated to be 0.486. Hence, results indicate a considerable amount of selfing even in this population of high density.

Poor synchronization of flowering periods. Detailed phenological studies on flowering of *P. merkusii* in Thailand are still lacking. However, the great variation among trees within populations with regard to the time of seed ripening (COOLING 1968; pers. obs.) suggests also a considerable variation in flowering times. The receptive period of female flowers of many trees might be in a period of low or no availability of foreign pollen.

Over-maturity populations. Due to increased human pressure on most forests in Thailand, including those dominated by *P. merkusii*, during the last decades natural regeneration is completely or almost completely lacking in most populations and the populations are over-mature. Trees of most populations and namely those from the Northeast produce only few male and female flowers.

Low seed production, frequent abortions of female flowers, high proportions of empty seeds, and low seed germination have been reported from *P. merkusii* (SAARDAVUT *et al.* 1989). Evidence suggesting high

inbreeding and selection against inbred individuals uring the development of stands is presented in this paper.

Most pine species grow in stands of high density in a seasonal climate, factors which favor outcrossing for a wind-pollinated tree species. Only few species were able to invade tropical regions, *P. merkusii* being the only pine occurring naturally in the southern hemisphere. Although *P. merkusii* has been one of the few successfully invading pines from the north, we assume that at least the provenances from the south-east Asian mainland still are in a process of evolutionary adaptation of their genetic system to the aseasonal climate of the tropics and the growing in stands of low population density.

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Appendix I Allelic distributions of the seed trees

Population N / Locus	Allele	BW 1 21	BW 2 18	KY 21	OM 23	PI 1 30	PI 2 26	NK 25	PO 8	HT 23	KC 18	BU 30
Got–A	A_1	0.000	0.000	0.000	0.000	0.000	0.000	0.105	0.000	0.022	0.167	0.000
	A_2	1.000	1.000	1.000	0.978	1.000	1.000	0.895	1.000	0.978	0.833	1.000
	A_3	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Got–C	C_1	0.132	0.222	0.208	0.109	0.391	0.095	0.278	0.250	0.294	0.750	0.435
	C_2	0.868	0.639	0.708	0.891	0.565	0.857	0.722	0.750	0.559	0.250	0.500
	C_3	0.000	0.139	0.084	0.000	0.043	0.048	0.000	0.000	0.147	0.000	0.065
Lap–A	A_0	0.028	0.000	0.000	0.000	0.050	0.000	0.083	0.000	0.043	0.083	0.000
	A_1	0.972	1.000	1.000	1.000	0.950	1.000	0.917	1.000	0.957	0.917	1.000
Lap–B	B_1	1.000	0.833	1.000	0.957	0.917	0.923	0.938	1.000	0.870	0.944	0.983
	B_2	0.000	0.167	0.000	0.022	0.067	0.000	0.042	0.000	0.000	0.056	0.000
	B_3	0.000	0.000	0.000	0.022	0.017	0.077	0.021	0.000	0.130	0.000	0.017
Gdh–A	A_1	0.075	0.033	0.095	0.283	0.233	0.146	0.080	0.000	0.130	0.000	0.167
	A_3	0.925	0.967	0.905	0.717	0.767	0.854	0.920	1.000	0.870	1.000	0.833
Pgm–A	A_1	0.929	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.972	1.000
	A_2	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.000
Mdh–A	A_1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.119	0.000	0.000
	A_2	0.974	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.881	1.000	1.000
	A_3	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh–B	B_0	0.026	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028	1.000
	B_1	0.974	0.958	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.972	0.000
Mdh–C	C_1	1.000	0.958	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	C_2	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Skdh–A	A_0	0.000	0.0000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
	A_1	0.000	.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.017
	A_2	1.000	1.000	1.000	1.000	0.933	1.000	0.917	1.000	0.711	1.000	0.950
	A_3	0.000	0.000	0.000	0.000	0.017	0.000	0.083	0.000	0.289	0.000	0.033
6-pgdh–A	A_1	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.275	0.111	0.100
	A_2	1.000	1.000	1.000	0.975	1.000	0.981	1.000	1.000	0.725	0.889	0.900
	A_3	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000
6-pgdh–B	B_1	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000
	B_2	0.975	0.917	0.976	0.925	1.000	1.000	0.960	1.000	1.000	1.000	1.000
	B_3	0.025	0.083	0.024	0.075	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G-6-pdh–B	B_1	0.000	0.000	0.000	0.000	0.059	0.000	0.000	0.000	0.000	0.000	0.000
	B_2	1.000	1.000	0.976	0.975	0.941	1.000	1.000	1.000	1.000	1.000	1.000
	B_3	0.000	0.000	0.024	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fdh–A	A_2	0.925	0.958	1.000	0.674	0.467	0.700	0.583	0.437	0.717	0.722	0.933
	A_3	0.075	0.042	0.000	0.326	0.533	0.300	0.417	0.563	0.283	0.278	0.067
Idh–A	A_1	1.000	0.917	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	A_2	0.000	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Appendix II Allelic frequency distributions of the seed samples

Population N / Locus	Allele	BW 1 126	BW 2 108	KY 126	OM 139	PI 1 180	PI 2 156	NK 150	PO 120	HT 138	KC 108	BU 180
Lap–A	A_0	0.014	0.000	0.000	0.000	0.031	0.000	0.031	0.000	0.018	0.057	0.000
	A_1	0.986	1.000	1.000	1.000	0.969	1.000	0.969	1.000	0.982	0.943	1.000
Lap–B	B_1	1.000	0.907	1.000	0.928	0.903	0.965	0.966	0.992	0.895	0.977	0.992
	B_2	0.000	0.093	0.000	0.040	0.067	0.000	0.031	0.008	0.000	0.023	0.000
	B_3	0.000	0.000	0.000	0.032	0.031	0.035	0.003	0.000	0.105	0.000	0.008
Gdh–A	A_1	0.104	0.072	0.143	0.295	0.186	0.087	0.100	0.013	0.105	0.019	0.136
	A_2	0.000	0.000	0.000	0.000	0.022	0.000	0.003	0.000	0.000	0.000	0.000
	A_3	0.896	0.928	0.857	0.705	0.792	0.913	0.897	0.988	0.895	0.981	0.864
Pgm–A	A_1	0.913	0.986	1.000	1.000	1.000	1.000	1.000	0.996	1.000	0.972	1.000
	A_2	0.087	0.014	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.028	0.000
Mdh–A	A_1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000
	A_2	0.982	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.995	1.000	1.000
	A_3	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh–B	B_0	0.004	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	1.000
	B_1	0.996	0.972	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.981	0.000
Mdh–C	C_1	1.000	0.972	0.996	0.995	1.000	0.993	1.000	1.000	1.000	1.000	1.000
	C_2	0.000	0.028	0.004	0.005	0.000	0.007	0.000	0.000	0.000	0.000	0.000
Skdh–A	A_0	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000
	A_1	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.003
	A_2	1.000	1.000	1.000	1.000	0.956	1.000	0.908	1.000	0.776	1.000	0.981
	A_3	0.000	0.000	0.000	0.000	0.014	0.000	0.092	0.000	0.224	0.000	0.017
6-pgdh–A	A_1	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.199	0.106	0.106
	A_2	1.000	1.000	1.000	0.967	0.992	0.997	1.000	1.000	0.801	0.894	0.894
	A_3	0.000	0.000	0.000	0.000	0.008	0.003	0.000	0.000	0.000	0.000	0.000
6-pgdh–B	B_1	0.000	0.000	0.000	0.008	0.000	0.000	0.017	0.000	0.017	0.000	0.000
	B_2	0.988	0.958	0.964	0.963	1.000	1.000	0.983	1.000	0.983	1.000	1.000
	B_3	0.012	0.042	0.036	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G-6-pdh–B	B_1	0.000	0.000	0.000	0.004	0.034	0.000	0.000	0.000	0.000	0.000	0.000
	B_2	1.000	1.000	0.964	0.983	0.966	1.000	1.000	1.000	1.000	1.000	0.992
	B_3	0.000	0.000	0.036	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.008
Fdh–A	A_1	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	A_2	0.950	0.979	0.996	0.795	0.559	0.697	0.630	0.467	0.717	0.815	0.956
	A_3	0.046	0.021	0.004	0.205	0.441	0.303	0.370	0.533	0.283	0.185	0.044
Idh–A	A_1	1.000	0.944	1.000	1.000	1.000	0.977	1.000	1.000	1.000	1.000	1.000
	A_2	0.000	0.056	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000