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GEOGRAPHIC GENETIC PATTERN OF *PINUS MERKUSII* IN THAILAND BASED ON A PROVENANCE TRIAL – IMPLICATION FOR CONSERVATION OF GENETIC RESOURCES

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ABSTRACT

A provenance trial comprising 14 provenances of *Pinus merkusii* was analysed using multivariate analysis of 12 quantitative traits. The aim of the study was to examine the geographic pattern of genetic variation of the species based on the single, existing provenance trial to support a genetic resource conservation plan of *Pinus merkusii* in Thailand. Correlations at the provenance level between the 12 traits were calculated, and canonical analysis of variance as well as cluster analysis was applied to discriminate between the provenances.

Large differentiation between populations was revealed, and four clusters of provenances were identified. To a large extent, the genetic between-provenance pattern reflects the geographic distribution. A tendency to a Northwest-Southeast transect was identified for several traits. Economically important traits like branch coarseness, foxtailing and forking are highly correlated at the provenance level. The genetic pattern with large differentiation between provenances is discussed. The results have been used in a genetic conservation plan for the species.

Key words: *Pinus merkusii*, provenance trial, multivariate statistics, quantitative traits, geographic pattern of intraspecific genetic variation, conservation.

INTRODUCTION

Pinus merkusii (Jungh. Et de Vriese) is one of the two tropical pines indigenous to Thailand. It grows exclusively in the seasonally dry climatic zone of Thailand, characterized by the remarkable alternation of the hot dry (November – April) and rainy (May – October) season (SANTISUK 1997; COOLING 1968). The area of distribution of *P. merkusii* has its western limit in eastern Burma, includes Thailand, Laos and Cambodia and southern Vietnam. Then a disjunction in the distribution area is seen, before *P. merkusii* is found again at Luzon and Mindoro (islands of the Philippines). The species also occurs in the northern end of the Malaysian peninsula, isolated from populations in northern Sumatra (WERNER 1993). The main altitudinal range of *P. merkusii* extends from 400 to 1200 m., in some cases even lower – e.g. the southernmost location at Pa Chumchon Khao Son (12° 45'N) is only 70-100 m.a.s.l. (WERNER 1993) and Tha Yang, Phetchaburi only 30 m.a.s.l while in the Philippines it is reported up to 2100 m.a.s.l. (see Table 1).

Genetic conservation activities for *P. merkusii* in Thailand have been done and are still in progress (SAARDAVUT *et al.* 1989). The conservation plan is presently being revised (THEILADE *et al.* 2000) based on the concept of genecological zonation, where a genecological zone is defined as an area with sufficiently uniform ecological conditions to assume similar phenotypic or genetic characters within a species (GRAUDAL *et al.* 1997). Among other things, the genecological zonation builds on existing information of genetic variability in the species. The analysis presented in this paper shall be considered as an input to the genecologial zonation of *Pinus merkusii* based on analysis of an existing field trial. The aim of the present analysis is thus to describe the genetic variation pattern of *P. merkusii*, especially in Thailand, in a way that facilitates conservation of major gene pools. In particular, the analysis is meant as an instrument to *discriminate* between different populations in order to facilitate and support the genecological zonation.

Provenance trials are normally associated with breeding activities, but the link to genecology and thereby genetic conservation is evident, because the trials deal to a large extent with traits of adaptive importance. LANGLET (1971) states that provenance studies and genecology seem to be synonymous and TIGERSTEDT (1974) also emphasises the close connection between provenance research and genecology.

CHANGTRAGOON & FINKELDEY (1995) made an allozyme study of *P. merkusii* including 11 natural populations and 14 allozyme loci revealing only little genetic diversity in the allozymes markers compared to other *Pinus* species, and a relatively large proportion of this variation was allocated between populations. CHANGTRAGOON & FINKELDEY (1995) suggest that the low genetic diversity is caused by bottlenecks and subsequent genetic drift in the populations. Another allozyme study on *P. merkusii* is given by SZMIDT *et al.* (1996) in which *P. kesiya* was included. The investigation supports the findings of CHANGTRAGOON & FINKELDEY (1995); *P. merkusii* possesses exceptionally low genetic diversity compared to *P. kesiya*. Much of the diversity originates from differences between populations, causing a high G_{ST} value. In general, allozyme markers cannot stand alone when it comes to guiding gene conservation effort, because quantitative traits and allozyme markers are likely to show different levels and patterns of genetic differentiation (ERIKSSON 1995; KARKU *et al.* 1996; YANG *et al.* 1996; KJÆR & GRAUDAL in press). Natural selection is often a strong evolutionary force causing directional selection (and thereby genetic differentiation between populations) in fitness related characters, whereas genetic markers in general are neutral, and as such insensitive to natural selection.

Conservation of adaptive genetic variation and processes is the major objective of most gene conservation programmes for trees (ERIKSSON *et al.* 1995), and traits analysed in field trials (e.g. survival and growth) are therefore especially suitable for guiding conservation effort (ERIKSSON 1995). Despite this, quantitative genetic studies are surprisingly little applied to problems in conservation biology (LYNCH 1996). Establishment and analysis of provenance trials takes time, but the numerous provenance trials established around the world, often involving heavy costs, offer important information, and should therefore be used in conservation planning whenever possible. This was the objective of the present study.

A multivariate approach was applied as it was considered to be well suited for this purpose.

MATERIALS AND METHODS

The provenance trial of *Pinus merkusii*, Exp. 2.104.1 is located at the Huey Bong Experimental Station, Chiang Mai in northern Thailand 790 m above sea level (Figure 1). The trial was established in June 1971 and was laid out as a randomised complete block design with 6 x 6 trees per plot and four replications.

The trial includes fourteen provenances. Eight of these are from the natural forest of Thailand, four originate from the Philippines, one is from Papua New Guinea (Sumatra origin) and one is from Zambia (Vietnam origin). The two latter have thus been growing outside the natural distribution area of the species, and formation of land races may have taken place (see Table 1, Figure 1).

Table 1. Location of provenances included in the Huey Bong trial.

Seed source no.	Locality of seed collection	Latitude	Longitude	Elevation m (a. s. l.)
1005	Tha Yang, Phetchaburi, Thailand	12°45N	99°15E	30
1008	Phu Kradung, Loei, Thailand	16°51N	101°47E	1300
1012	Khun Yuam, Mae Hong Son,	18°50N	97°47E	600
1014	Thailand	18°21N	99°20E	800
1015	Mae Tha, Lamphun, Thailand	19°52N	99°15E	5–600
1018	Fang, Chiang Mai, Thailand	18°04N	98°10E	1100
1019	Hot, Chiang mai, Thailand	14°43N	103°50E	180
1020	Sangkha, Surin, Thailand	14°50N	104°32E	150
1022	Huey Ta, Si Sa Ket, Thailand	15°45N	120°02E	13–1600
1023	Santa Cruz, Zambales, Philippines	15°47N	120°01E	7–900
1024	Santa Cruz, Zambales, Philippines	13°03N	120°51E	12–1600
1025	Panas, Mindoro Occ., Philippines	13°04N	120°50E	18–2100
1026	Kipkipan, Mindoro Occ., Philippines	7°S	146°E	700
1027	Bulolo, Papua New Guinea	12°S	27°E	?
	Dola Hill, Zambia			

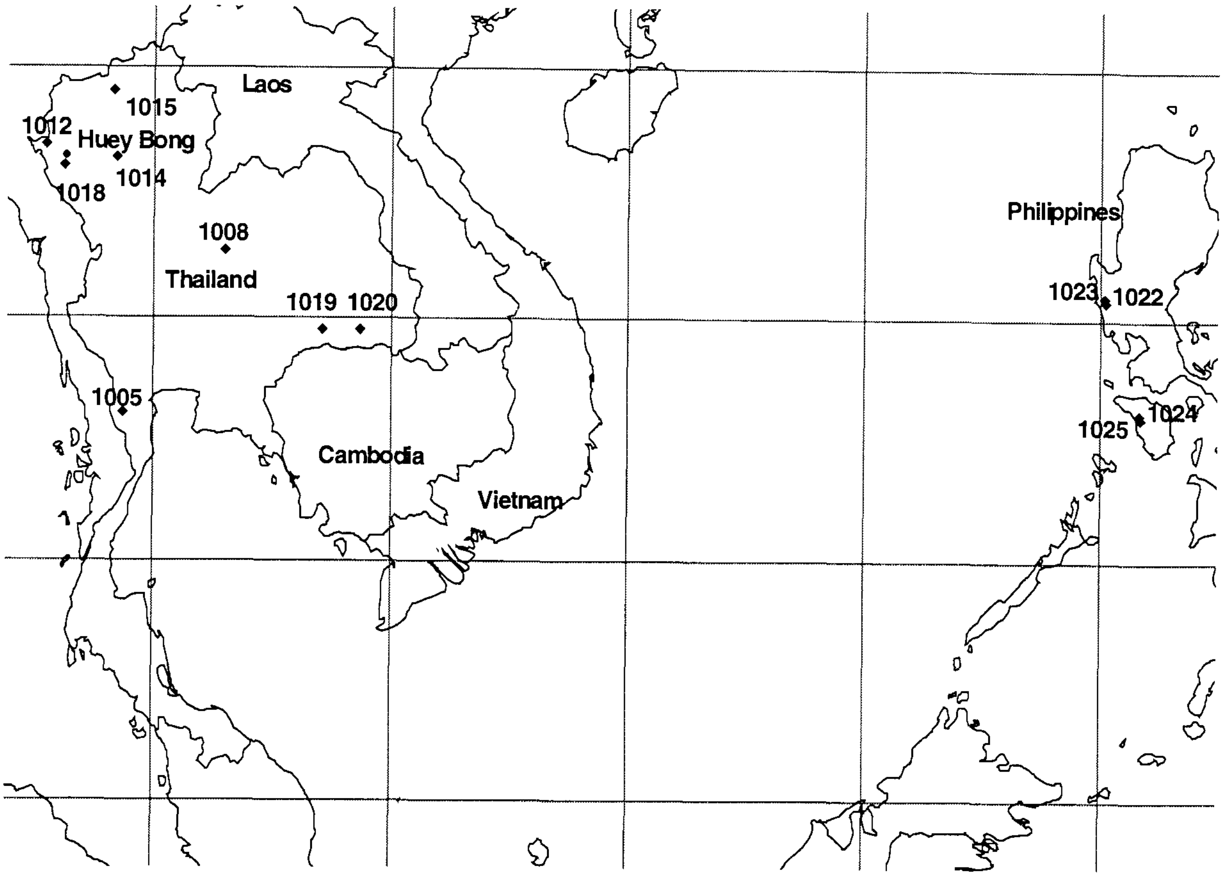

Figure 1. Map showing the location of site of the Huey Bong experiment and the origin of the eight Thai and four Philippine provenances included in the field trial of *Pinus merkusii*.

assessed in 1999, but data from previous evaluations of the trial were also included in the analysis.

The traits analysed in the present study are: (1) stem crookedness 1999 (CRO) based on scores from a five level scale, where 1 means a straight stem and 5 means a very crooked stem, (2) branch coarseness 1999 (BC) assessed by the use of a five level scale, where 1 means fine branches compared to the stem and 5 means coarse branches compared to the stem, (3) diameter at 1.3 m 1999 (DBH), (4) height 1996 (H96), (5) frequency of foxtailing in any part of the tree – assessed 1999 (FOX), (6) frequency of forks (FORK) as the percentage of trees in 1999 with forking in the lower 4 m section of the stem, (7) bark thickness (BAT) in the bark fissures (the thinnest part of the bark) at breast height, (8) indirect wood density measure (WPD) measured in 1999 by using a Pilodyne wood density tester (the instrument injects a steel needle into the wood by a constant force, and penetration of the needle is read – low values reflect high density. For further details see COWN (1978), HANSEN (2000). Two measurements at the Southern side of each tree at a height of 1.3 m were made after removing the bark, (9) height in 1976 (H76), (10) survival assessed in 1976 (SUR76), (11) height in 1978 (H78), and (12) survival in 1972 (SUR72).

Statistical analyses

Plot average values were calculated and used as basis for further analysis. Multivariate analysis of variance (MANOVA) based on the multivariate normal distribution was applied testing the null hypothesis of no difference between provenances based on four different, approximated F-tests: Lawley-Hotelling's T, Wilks' Λ, Pillai's V, and Roy's largest root (cf. PIMENTEL 1979; SHARMA 1996). Also, MANOVA was used to estimate the variance and co-variance structure for the assessed traits.

Next, canonical variates analysis (CVA) was applied to see if a reduction in the dimensionality of data was possible, thereby making it possible to illustrate the results and to cluster the provenances into groups. CVA can be seen as a low-dimensional approximation to data (SKOVGAARD & BROCKHOFF 1998). With that it has a goal similar to e.g. principal component analysis (PCA), but in contrast to the principal components, the canonical variates are absorbing variance between provenances rather than just variance *per se* and CVA is therefore also invariant to change of origin and scale (CHATFIELD & COLLINS 1980).

PROC GLM in the SAS software package was used for the statistical analyses (SAS INSTITUTE INC. 1990). The option **printh** and **printe** in the **manova** statement in **PROC GLM** requests calculation of Sums of Squares and Cross Products matrices for the main effect (here provenance differences) and error components, respectively. Dividing by the matching degrees of freedom gives the Mean Squares and Cross Products matrices, from which estimated covariance matrices were obtained (see e.g. BECKER 1992). Then it was straightforward to find the correlation between two traits at the provenance level, here given in the notation of VAN BUIJTENEN (1992) for 'genetic correlation':

$$r_{A_1,A_2} = \frac{cov_{A_1,A_2}}{\sqrt{V_{A_1}\ V_{A_2}}}$$

Values above 1 were truncated to 1.00. In general, 'genetic correlations' between quantitative traits refer to covariance between pairs of traits caused by genes with additive effects. Genetic correlations thereby refer to correlation between "breeding values" within populations (FALCONER 1989). The correlations calculated here are different from traditional genetic correlations in the sense that they refer to covariance at the provenance level – for example $r_{(\mathrm{DBH;FOX})}$ quantifies to what extent provenances with high average diameter in general also have above average foxtailing. The estimated correlations in the present analysis thus reflect combined effects of (i) any covariance between selective forces that have caused *between population* differentiation, and/or (ii) any genetic covariance at the *within population* level (e.g. caused by plagiotrophic functioning genes or gamete phases dis-equibrillia). These two types of effects are of different origin, and have different evolutionary implications, but cannot be separated in the present analysis, as no progeny structure within provenances is included in the trial.

Cluster analysis

Hierarchical clustering was performed on the provenance means of significant canonical variates. In the current study, three methods have been used: the centroid method, WARD's method and the single-linkage (nearest-neighbour) method.

The hierarchical cluster analysis was performed using the **PROC CLUSTER** procedure in the SAS programme. Some statistics used to evaluate the cluster solutions were requested (e.g. Root-mean-square standard deviation (RMSSTD) of the new cluster and RSQUARE). These statistics were plotted to look for a large jump in value, indicating the "right" number of clusters – see SHARMA (1996 p.197 –202) for details.

RESULTS

The MANOVA gave a highly significant effect of provenances. The result of the MANOVA is presented in Table 2.

The CVA resulted in four significant canonical variates (Table 3). The first two canonical variates account for 81 % of the variance in the data. Provenance means of these two variates are plotted against each other in Figure 2. The standard error of a canonical variate mean is $1/\sqrt{n}$, where n is the number of replications (here 4). This comes from the fact that canonical variates are normalised so that the variance equals 1 (CHATFIELD & COLLINS 1980) and from the calculation of standard error of means from n replications. An approximate 95 % confidence region for a single pro-venance value plotted by 1st and 2nd canonical variates is therefore a circle with a radius of $2/\sqrt{n} = 2/\sqrt{4} = 1$ centred on the means (Figure 2).

As illustrated in Figure 2, the first canonical variate separates the insular provenances from the Philippines and Papua New Guinea, and the continental provenances from Thailand and Zambia. The second canonical variate separates the northeastern Thai provenances from the northwestern Thai provenances, the insular provenances and in particular the Zambian (Vietnam origin) provenance is somewhere in the middle. How-

Table 2. Approximated mutlivariate F-tests of differences between provenances.

Test statistic	Value	*F*	*df*	*P*
Lawley-Hotelling's *T*	36.58	5.90	156, 260	0.0001
Wilks' Λ	0.00006	3.31	156, 456	0.0001
Pillai's *V*	1	2.05	156, 302	0.0001
Roy's largest root	4.94	63.52	13, 38	0.0001
	21.73			

Table 3. Results from canonical analysis of variance. Eigenvalues (λ), proportion of the total variance accounted by the nth variate (*P*), the cumulated proportion of the total variance accounted for by 1st to nth variates (Σ) and a F-test of the canonical dimension. Variates above the dotted line is significant on 5 % level.

Canonical variate	λ	*P*	Σ	*F*	Df	*P*
1st	21.729	0.594	0.594	3.31	156, 260.8	0.0001
2nd	7.991	0.218	0.812	2.30	132, 246.0	0.0001
3rd	2.699	0.074	0.886	1.67	110, 230.4	0.0007
4th	1.265	0.035	0.921	1.36	90, 213.7	0.0371
5th	1.008	0.028	0.948	1.22	72, 196.1	0.1420
6th	0.703	0.019	0.968	1.06	56, 177.6	0.3739
7th	0.508	0.014	0.981	0.92	42, 158.2	0.6139
8th	0.286	0.008	0.989	0.76	30, 138	0.8047
9th	0.206	0.006	0.995	0.67	20, 117.0	0.8442
10th	0.165	0.005	0.999	0.54	12, 95.5	0.8816
11th	0.020	0.001	1.000	0.14	6, 74	0.9912
12th	0.002	0.000	1.000	0.04	2, 38	0.9647

ever, it is also seen that clearly significant differences exist within the western Thai provenances, where the southern Petchanburi provenance (1005) is separated from the northernmost Mae Hong Son (1012) and Fang (1018) provenances along the first canonical variate.

Cluster solutions

All three clustering methods gave similar results (centroid, WARD's and single linkage).

After studying plots of Root-mean-square standard deviation and R-square of the clusters it was decided to use four as the proper number of clusters. The four clusters from the solution obtained by the centroid method are sketched in figure 2. The clustering seems in good concordance with the pattern found from the two first canonical variates (cf. Figure 2). Cluster 2 (western Thai provenances) includes fairly large variation, but is still identified as one by all three applied cluster algorithms, probably because the variation between the provenances within cluster 2 is of a somehow continuous nature.

Correlation due to provenance differences

Correlations due to provenance differences are presented in Table 4.

High correlations at provenance level are found between heights in different ages. It is interesting that high correlation in general exists between the economically important traits. Branch coarseness (BC), foxtails (FOX) and forking (FORK) are highly correlated at the provenance level. The association between BC, FOX, FORK and growth is more complicated because they correlate positively to diameter (DBH) but negatively to height (H96) (Table 4). This reflects a low correlation between H96 and DBH at the provenance level. The correlation between height and diameter seems to vary between different parts of the distributional area, the H96/DBH ratio being substantially higher for the Philippino and NE Thai provenances (lowland) when compared to other Thai provenances (Figure 3). This might be due to different rhythms of growth between provenances with or without foxtails. There could also be a minor effect of different spacing, following differences in mortality.

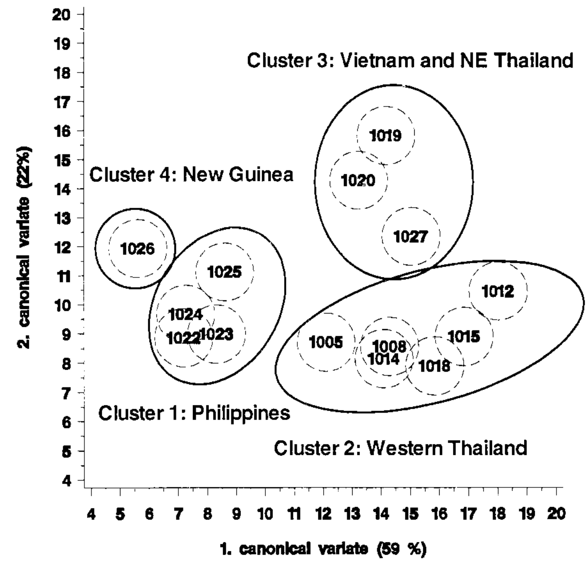

Figure 2. Average canonical values for all 14 provenances resulting from canonical variates analysis.

Within the Thai provenances, it is the two seed sources from the eastern lowland (1019 & 1020) that are identified to be economical superior in traditional

Table 4. Correlations due to provenance effects ('genetic correlations'). ***A positive correlation to WPD corresponds to a negative correlation to wood density.***

	CRO	BC	DBH	H96	FOX	FORK	BAT	WPD	H76	SUR76	H78	SUR72
CRO	1											
BC	–0.66	1										
DBH	–0.80	0.75	1									
H96	–0.29	–0.20	0.56	1								
FOX	–0.45	0.97	0.63	–0.18	1							
FORK	–0.56	1.00	0.59	–0.36	0.73	1						
BAT	–0.56	1.00	0.76	–0.12	1.00	1.00	1					
WPD	–0.68	0.30	0.69	0.58	0.21	0.00	0.31	1				
H76	0.20	–0.83	0.04	0.82	–0.63	–0.71	–0.63	0.06	1			
SUR76	–0.28	–0.30	0.32	0.84	–0.42	–0.53	–0.35	0.53	0.52	1		
H78	0.11	–0.68	0.16	0.88	–0.54	–0.67	–0.52	0.20	0.99	0.57	1	
SUR72	–0.86	0.68	0.74	0.37	0.50	0.48	0.54	0.83	–0.55	0.73	–0.36	1

CRO = stem crookedness, BC = branch coarseness, DBH = diameter, H96 = height 1996, FOX = frequency of fox tailing, FORK = frequency of forks, BAT = bark thickness, WPD = indirect wood density measure, H7 = height 1976, SUR76 = survival in 1976, H78 = height 1978, SUR72 = survival in 1972.

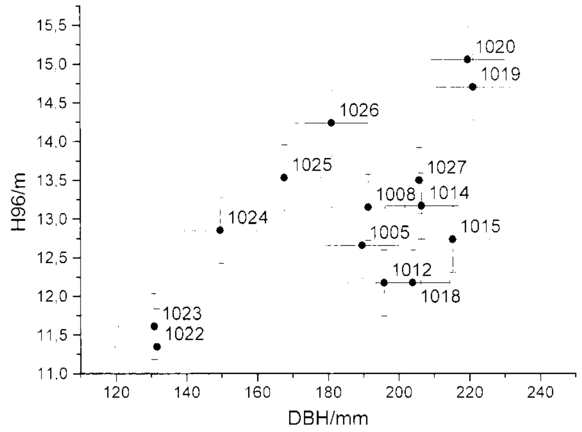

Figure 3. Plot illustrating the correlation between height 1996 (H96) and diameter (DBH) at the provenance level. Vertical and horizontal error bars indicate the standard deviation of the provenance means for the two characters.

provenance investigations (HÜBERTZ & SIRIKUL 1983; HANSEN 1999; cf. also discussed below). These two provenances are certainly not local provenances when considering the location of the trial site (cf. Figure 1). However, from an adaptive point of view, it is interesting to see that the "superior traits" at the provenance level are associated with thin bark (negative correlations to BAT), because thick bark has been suggested as a result of adaptation to frequent forest fire (POSUJJA *et al.* 1986).

CONCLUSION AND DISCUSSION

Discussion of methods

The cluster results illustrated the combination of using cluster analysis and low-dimensional approximation to data like canonical variates. The use of cluster analysis on canonical variates gives the opportunity of including more than two dimensions of the data in grouping the solutions, whereas visual grouping is limited to use only two. The cluster solutions obtained obviously can be a starting point when choosing populations to conserve, once an appropriate number of clusters is decided on. However, the present study shows important differences between the provenances within the clusters (especially cluster 2, cf. Figure 2). Therefore, it must be recommended to take a closer look at the provenances within the cluster, before deciding on conservation units.

Biochemical and DNA markers can contribute interesting information as a supplement to observations from the present kind of field trial, but are not considered a real alternative to field trials or ecological surveys. Genetic markers in general deal with neutral, rather than adaptive genetic variation (MILLAR & WESTFALL 1992; ERIKSSON 1995), and important genetic differentiation following divergent natural selection in a few generations may therefore not be detected by the markers. This is supported by the fact that several studies of forest trees have shown larger differentiation between adaptive traits than between biochemical markers (MUONA 1990; KARHU *et al.* 1996; YANG *et al.* 1997). The markers can nevertheless supply important information on likely historic migration patterns and amount of pollen flow, and they are efficient tools for examination of breeding systems of target species.

Naturally, it is fair to ask whether the analysed material, consisting of only 14 provenances, is adequate to cover the whole natural distribution area of the *P. merkusii*. Indeed it is doubtful, but taking the low number into consideration, Knud Bryndum and Jens Granhof who established the trial (THAI-DANISH 1971) have actually succeeded in sampling in the extremes of the natural distribution area; e.g. sampling across the entire altitudinal range (Table 1).

Genetic pattern

The findings of the present study show that the genetic between-provenance pattern to a large extent reflects the geographic distribution, with provenances 1008 and 1012 being slight exceptions. For the rest of the provenances, the means of the canonical variates are positioned in a pattern rather similar to their geographic origin – compare Figure 1 and 2.

In example, the plot of canonical scores (Figure2) showed a clear separation between insular provenances in one group and continental provenances in a second group. Also, distinctive separation was observed between northeastern Thai provenances and northwestern Thai provenances.

The position in the plot of provenance 1027, Zambia (Vietnam origin), close to the north- eastern Thai provenances, further supports the reliability of the analysis. The Pecthanbun provenance (1008) from the northeastern part of Thailand is found to be closely related to the northwestern provenances, although separated geographically from these (cf. Fig. 1).

Earlier results

The *P. merkusii* provenance trial in Huey Bong was analysed earlier in a univariate way. CHUNTANAPARP *et al.* (1974) found significant differences in height, the provenance of Sumatra origin showed superior height growth. The latter was due to absence of the so-called *grass stage*, characterised by the seedling developing a thick carrot-like stem, and producing long, stout secondary needles tightly packed on the short main stem (SIRIKUL 1990). During the *grass stage*, the duration of which differs between individuals, there is very little height growth and significant height growth does usually not start before the third to fifth year (COOLING 1968). The distinct differences between provenance groups in *grass stage* habit, compared to the within group variation, were also found by SIRIKUL (1990).

HÜBERTZ & SIRIKUL (1983) observed significant difference in heights, stem form and defects and diameter. The two provenances from northeastern Thailand (nos. 1019 and 1020) were now the tallest, followed by the one of Sumatra origin (no.1026). The two northeastern Thai provenances were also superior regarding stem form, stem defects and diameter.

Furthermore, significant variation of branch characteristics was observed, the continental provenances having long internodes and few whorls with thick branches. In contrast, the insular provenances had many shorter internodes and thinner branches.

The changes in ranking of heights, certainly influenced by the presence of *grass stage* (GRANHOF 1983), underlines the need to be cautious with premature conclusions from field trials.

The conclusions of the study mentioned above are still valid today (HANSEN 1999). Thus, the two northeastern provenances seem to be the most promising and valuable seen from a tree breeding point of view.

When studying the values of the individual provenances for the quantitative traits, seen in HANSEN (1999), these indicate differentiation along a Northwest-Southeast transect for some of the traits. For example foxtailing occurs with high frequency in Northwest Thailand, with intermediate frequency in Northeast Thailand and not at all in Petchaburi, Thailand (1005) and in the insular provenances. The same pattern goes for forking, although 1005 has an exceptionally high forking percentage. In the case of bark thickness, the Northwest-Southeast transect is very clear without any deviations at all. Similarly, branch coarseness indicates this geographic tendency.

Interestingly, COPPEN *et al.* (1998) found the same Northwest to Southeast pattern of both turpentine composition and resin acid composition in a study treating the provenance variation in resin composition for 11 Thai provenances and 5 Filipino provenances of *P. merkusii.*

Regarding height-traits (H76, H78, H96), STF and DBH, the above mentioned geographic pattern is rather discontinuous. This is mainly caused by the two provenances from northeastern Thailand.

Origin of genetic pattern

An important question in relation to conservation is the likely origin of the genetic pattern: is this differentiation caused by local adaptation to prevailing ecological conditions or is the pattern a result of migration and drift. The present study cannot answer this question. Clearly, some of the traits analysed may have a close connection to the fitness of the individual tree and thereby be subject to directional selection. During the 28 years at the Huey Bong site, the local provenances (number 1018, 1012, 1015 and 1014) did *not* show superior growth (figure 3). Still, it is difficult to be decisive on whether the pattern reveals adaptation to local conditions. Bark thickness, for example, can be seen as an adaptation to frequent fires (POSUJJA *et al.* 1986). Forest fires may (at least in a historical perspective) have been less frequent in the insular populations, and one can therefore speculate whether the thicker bark represent a potential adaptational advantage of the highland provenances to forest fires, that has not been revealed in the Huey Bong trial? This underscores the importance of conserving the between population variation for future use, rather than only focusing on provenances that are assumed to be superior at present. Regarding foxtailing, this trait is a product of a free-growth phenomenon, this also being the case for the *grass stage* (HÜBERTZ & SIRIKUL 1983). Usually, the latter is regarded as an adaptation to seasonal drought or fires (KOSKELA *et al.* 1995). POSUJJA *et al.* (1986) also state that the rapid height growth in the early years of some of the insular provenances from near the equator is more likely to succeed in near-equatorial conditions where competition from hardwoods is strong.

Interesting is the large differentiation between provenances revealed in the quantitative traits. Relative high differentiation between Thai populations of *P. merkusii* was also found in the studies by CHANGTRAGOON & FINKELDEY (1995) and SZMIDT *et al.* (1996), using allozyme markers. As mentioned in the introduction, discrepancy between genetic patterns revealed in quantitative traits and genetic patterns found in allozyme markers is often seen. However, in the case of *P. merkusii*, both allozyme markers and quantitative traits point towards important differentiation between populations. It is therefore most likely that both natural selection (especially between highland and lowland conditions) and genetic drift (as a result of small population size and limited gene flow) have been important evolutionary processes involved in formation of the observed genetic patterns. From a gene conservation point of view, this observation favours a sampling strategy that includes populations from all major, geographically isolated, natural occurrences in Thailand. This is because genetic drift may have played an important evolutionary role, even for fitness related quantitative traits.

The results from this study and the allozyme study by CHANGTRAGOON & FINKELDAY (1995), combined with observations from field surveys in the remaining occurrences of *P. merkusii* in Thailand, and available information on variation within Thailand in vegetation types and biotic factors (physiographic, soil and climatic conditions), have been applied for developing a gene conservation plan for *P. merkusii* in Thailand (cf. THEILADE *et al.* 2000).

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