GENETIC STUDIES IN A TROPICAL PINE - *PINUS KESIYA* II. GENETIC VARIATION AMONG FOUR POPULATIONS IN NORTHERN THAILAND

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Received March 1990

BOYLE, T.J.B., LIENGSIRI, C. & PIEWLUANG, C. 1991. Genetic studies in a tropical pine - *Pinus kesiya* II. Genetic variation among four populations in Northern Thailand. The genetic diversity and population structure of four populations of *Pinus kesiya* from Northern Thailand were investigated by means of isoenzyme analysis. Expected heterozygosities were similar to those obtained for other tropical tree species. Estimates of population differentiation by means of G-statistics indicated comparable levels of differentiation with other conifers and somewhat lower levels than other tropical tree species. Genetic distances indicated that one population in particular (Samoeng) was the main contributor to population differentiation.

Key words: *Pinus kesiya* - isozymes - genetic variation - genetic differentiation - F-statistics - genetic distance

Introduction

The organisation of genetic variation within a species is of critical importance for effecient management of the resource. For example, in devising an optimum sampling strategy for genetic conservation, it is highly benefical to obtain estimates of the amount and distribution of genetic variation among populations (Marshall & Brown 1975). Sampling of genetically similar populations can then be limited and highly variable population can be sampled more intensively. Similarly, genetic improvement involves sampling of the natural resource and in order to maximize the rate of improvement, the same information concerning the distribution of genetic variation is required (Guries & Ledig 1977). Clearly, such investigations should be completed before management of the resource begins. Since this is seldom possible, it is important to develop flexible programmes that can incorporate subsequent genetic information.

The arrangement of genetic variation among populations has been termed 'population structure', and the use of biochemical techniques such as isoenzyme analysis has greatly facilitated its investigation. This is due to co-dominant expression of isoenzymes and the large number of loci that can be simultaneously assayed (Lewontin 1974). The relationship between isoenzymes and commercially important traits is usually weak, and morphological traits typically exhibit greater levels of variation among population (Boyle & Yeh

1988) than do isozyme loci. However, some studies have demonstrated that the same intrinsic pattern can be detected in both morphological and biochemical traits (Wheeler & Guries 1982).

Pinus kesiya has been widely planted in tropical Africa, especially in Madagascar and Zambia (Armitage 1980). Substantial areas of plantations have also been established in the Philippines, and planting programmes have been initiated in Thailand (Armitage 1980). As noted by Pousujja (1986) the wide but discontinuous natural range of *P. kesiya* would be expected to result in large provenance differences. In addition, the altitudinal variation in its distribution would be expected to result in genetic differentiation (Burley & Amitage 1980). Indeed substantial variation in growth and morphology from trees planted in a wide variety of countries is well documented (Burley & Armitage 1980). For example, *5-y* results from a provenance testatHuay Tong in Chiang Mai, Thailand demonstrated substantial difference in height growth among the 18 provenances (Granhof 1978). Several of the local sources ranked among the best. Some tests indicate a lack of provenance differences; for example, Das and Stephan (1986) reported that 11-*y* results from a trial of 12 provenances from the Philippines, Thailand, Vietnam, Zambia and Assam in India, revealed no significant differences among provenances for either height or diameter growth.

In our study, the population structure and genetic diversity of four populations of *P. kesiya* from Northern Thailand were investigated by means of isoenzyme analysis.

Material and methods

Open-pollinated seeds from 38 individuals, representing four populations (Table 1, Figure 1), were provided by the seed bank of the Royal Forest Department Pine Improvement Centre, Huay Kaew Arboretum, Chiang Mai, Thailand. The small sizes were limited by seed availability in the seed bank. Germinating embryos and haploid megagametophytic tissue were used to determine genotypes at ten enzyme systems, representing 18 loci, as described by Boyle *et al.* (1990) and using the method of Liengsiri *el al.* (1990). The loci analyzed were aspartate aminotransferase (AAT), glucose-6-phosphate dehydrogenase (G6P), isocitrate dehydrogenase (IDH), leucine aminopeptidase-2 (LAP-2), menadione reductase-1 and -2 (MR-1 and MR-2), 6-phosphoglutamate dehydrogenase-1,-2, and -3 (6PG-1, 6PG-2, and 6PG-3),phosphoglucose isomerase-1 and-2 (PGI-1 and PGI-2), phosphoglucomutase (PGM), shikimic acid dehydrogenase-1 and -2 (SDH-1 and SDH -2), and malate dehydrogenase-1, -2, -3 and -4 (MDH-1, MDH-2, MDH-3 and MDH-4).

For the analyses of genetic diversity, both maternal and progeny allele frequencies were used. However, at all four MDH loci, the embryos did not stain sufficiently to allow scoring and data for the progeny are based on only 14 loci.

Table 1. Location data of the four populations from which the parent trees were sampled

Name	Latitude ("N)	Longitude ("E)	Elevation *(m)*	Number of trees
Samoeng	19"00'	98"45'	1,100	4
Huay Tong	18"35'	98"10'	1,200	8
Nong Krating	17"56'	98"17'	1,080	10
WatChan	19°05'	98"18'	900	16

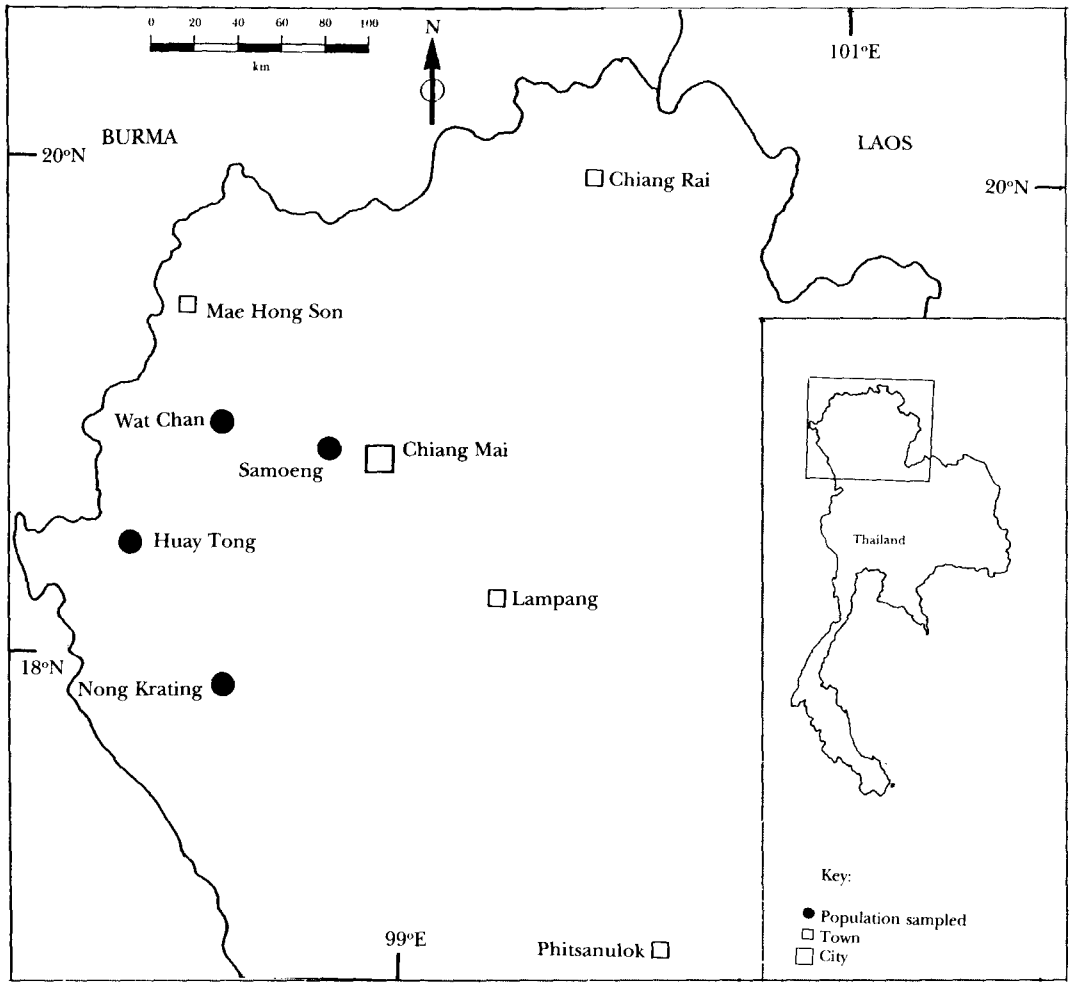

Figure 1. Map of Thailand showing the location of four sampled populations

The two loci 6PG-1 and 6PG-2 are strongly linked (Boyle *et al.* 1990) and, for analyses of population differentiation requiring an assumption of independent inheritance, only one of these loci (6PG-2) was used.

Several methods of quantifying genetic diversity are frequently used. These include the proportion of polymorphic loci (loci for which the frequency of the most common allele is < 95%), percentage of heterozygous loci per individual, and average number of alleles per locus. All of these statistics are self-descriptive. In addition, observed and expected heterozygosities (Nei 1975) and the effective number of alleles per locus (Crow & Kimura 1970) were calculated. The expected heterozygosity is defined as:

$$h_e = 1 - \sum_{i=1}^{n} P_i^2$$

where P_i is the frequency of the i^{th} allele. The average heterozygosity per population (h_e) is then the arithmetic mean of expected heterozygosities over all loci. The effective number of alleles per locus is calculated in a similar fashion as:

$$n_e = I/\sum_{1=1}^{n} P_i^2$$

but population averages are calculated as the geometric means (Lundkvist 1979).

Genetic differentiation has also frequently been characterised by several different methods. Two of the most common approaches have been the G-statistics of Nei (1975), an adaptation of Wright's (1965) F-statistics, and various measures of genetic distance, such as that proposed by Nei (1972). Results using both G-statistics and Nei's genetic distance (D) are presented here. Sampling variances of D were derived by Nei and Roychoundhury (1974). Both methods require the assumption of a random sample of genes from the populations. Therefore, only maternal allele frequencies can be used, because genotypes from the parents are clearly correlated.

Results

Three of the 14 loci which could be scored for the embryos were monomorphic over all populations (Table 2). For the maternal allele frequencies, the same three loci were monomorphic over all populations and, in addition, one of the four MDH loci (MDH-2) was monomorphic. In almost every case, there was close agreement between maternal and progeny allele frequencies. The only exceptions to this occurred for the Samoeng population, where the small maternal population sample (four trees) resulted in some deviations of maternal allele frequencies from the progeny estimates. At most loci, a very common allele was supplemented by one or two rare alleles. However, at some loci, especially 6PG-1, 6PG-2, 6PG-3 and MR-2 the frequency of the most common allele was often < 0.75. The only locus for which more than three alleles were recorded in a single population was for MDH-4 in Nong Krating, where five

alleles were detected among the parents.

Table 2. Allele frequencies in the four sampled populations

		Population							
		Samoeng		Huay Tong		Nong Krating		Wat Chan	
Locus	Allele	Mat.	Prog.	Mat.	Prog.	Mat.	Prog.	Mat.	Prog.
AAT	1	1.00	1.00	1.00	0.99	0.90	0.92	0.94	0.92
	2	-	-	-	0.01	0.10	0.08	0.06	0.08
G6P	1	0.88	0.97	1.00	1.00	0.85	0.92	0.97	0.97
	2	0.12	0.03	-	-	0.15	0.08	0.03	0.03
1DH	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
LAP-2	1	1.00	0.88	0.83	0.76	0.80	0.78	0.84	0.79
	2	-	0.12	0.17	0.24	0.20	0.22	0.16	0.21
MR-1	0	-	-	0.06	0.02	-	-	-	-
	1	1.00	1.00	0.94	0.98	1.00	1.00	1.00	1.00
MR-2	0	0.50	0.28	0.25	0.13	0.10	0.07	0.25	0.22
	1	0.50	0.63	0.67	0.76	0.85	0.84	0.75	0.76
	2	-	0.09	0.08	0.11	0.05	0.09	-	0.02
6PG-1	1	0.75	0.90	0.71	0.72	0.70	0.71	0.78	0.76
	2	0.25	0.10	0.29	0.28	0.30	0.29	0.22	0.24
6PG-2	1	0.63	0.68	0.75	0.75	0.70	0.66	0.69	0.69
	2	0.37	0.32	0.25	0.24	0.30	0.34	0.31	0.31
	3	-	-	-	0.01	-	-	-	-
6PG-3	1	0.75	0.77	0.63	0.66	0.60	0.63	0.72	0.67
	2	0.25	0.23	0.37	0.34	0.40	0.37	0.28	0.33
PGI-1	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PG1-2	1	1.00	1.00	1.00	1.00	0.95	0.99	0.97	0.98
	2	-	-	-	-	0.05	0.01	0.03	0.02
PGM	1	0.25	0.19	0.13	0.13	0.15	0.16	0.03	0.05
	2	0.75	0.81	0.87	0.86	0.85	0.83	0.94	0.94
	3	-	-	-	0.01	-	-	0.03	0.01
	4	-	-	-	-	-	0.01	-	-
SDH-1	1	0.75	0.72	0.81	0.79	0.75	0.69	0.91	0.84
	2	0.25	0.27	0.19	0.21	0.25	0.28	0.09	0.16
	3	-	0.01	-	-	-	0.03	-	-
SDH-2	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
MDH-1	1	0.88		1.00		1.00		1.00	
	2	0.12		-		-		-	
MDH-2	1	1.00		1.00		1.00		1.00	
MDH-3	1	0.88		1.00		1.00		1.00	
	2	0.12		-		-		-	
MDH-4	1	0.75		0.94		0.80		0.91	
	2	0.25		-		0.05		0.09	
	3			0.06		0.05		-	
	4	-		-		0.05		-	
	5	-		-		0.05		-	

Note: Mat. - Maternal; Prog. - Progeny

Measure of genetic diversity can be used to compare the four populations (Table 3). For most of the measures, the Nong Krating population is the most variable, both for the maternal trees and the progeny, and the Samoeng is least variable, especially among the progeny. Again, however, the small sample size from Samoeng is likely to have biased the results. The observed maternal heterozygosities compared well with expected results at all populations but, for the progeny, fewer heterozygotes were observed than were

expected, this deficiency being especially severe for Nong Krating.

Genetic differentiation, as measured by G-statistics and Nei's genetic distance, are shown in Tables 4 and 5. Hs is a measure of mean diversity within populations, and the Gst value is an estimate of the proportion of the total variation accounted for by differences among populations. There is substantial variation among loci, as is typical for such studies, but the overall estimates indicate that 3.9% of the total variation is due to differences among populations. LAP, MR-2 and PGM have high levels of among-population differentiation, while 6PG-2 and PGI-2 are below average. All of the polymorphic MDH loci have high Gst values.

Table 3. Genetic diversity data for the four populations

Statitistic	Population									
	Samoeng		Huay Tong		Nong Krating		Wat Chan		Means	
	Mat.	Prog.	Mat.	Prog.	Mat.	Prog.	Mat.	Prog	Mat.	Prog.
Percent polymorphic loci	55.6	50.0	50.0	50.0	61.1	64.3	50.0	57.1	54.2	55.4
Percent heterozygous loci per tree	17.6	14.8	17.3	16.0	19.6	18.0	14.6	17.7	16.8	16.6
Average number of alleles per locus	1.56	1.71	1.56	1.86	1.83	1.93	1.67	0.86	1.65	1.84
Effective number of alleles per locus	1.24	1.22	1.18	1.23	1.23	1.27	1.16	1.22	1.21	1.23
Observed heterozygosity	0.166	0.158	0.167	0.165	0.183	0.168	0.149	0.173	0.166	0.166
Excepted heterozygosity*	0.222 (0.046)	0.177	0.166 (0.044)	0.187	0.200 (0.042)	0.214	0.144 (0.039)	0.183	0.169	0.190

* unbiased estimates for maternal data; standard errors in parentheses; Mat. - maternal, Prog. - progeny

Table 4. G-statistics for individual loci and overall results

Locus	Ht	Hs	Gst
AAT	0.078	0.074	0.047
G6P	0.120	0.115	0.043
LAP	0.227	0.215	0.052
MR-1	0.031	0.386	0.005
MR-2	0.445	0.407	0.086
6PG-1	0.388	0.386	0.005
6PG-2	0.427	0.423	0.009
6PG-3	0.440	0.432	0.018
PGI-2	0.040	0.039	0.023
PGM	0.253	0.242	0.043
SDH-1	0.279	0.271	0.027
MDH-1	0.061	0.055	0.097
MDH-3	0.061	0.055	0.097
MDH-4	0.296	0.253	0.061
Overall	0.173	0.166	0.039

Table 5. Nei's genetic distances (xlOO); Standard errors (x 100) are in parentheses

Population	Samoeng	Huay Tong	Nong Krating	Wat Chan
Samoeng	-	1.60 (0.002)	2.04 (0.011)	1.48 (0.003)
Huay Tong		-	0.51 (0.001)	0.31 (0.000)
Nong Krating			-	0.50 (0.000)
Wat Chan				-

Unlike the G-statistics, genetic distances (which are dimensionless numbers) allow comparisons among individual populations. Clearly, the Samoeng population is the main contributor to populations being two or three times the distances among the other populations. The most similar pair of populations are Huay Tong and Wat Chan.

Discussion

The average percentage of polymorphic loci (54.2% for maternal frequencies and 55.4% among the progeny) and the number of alleles per locus are slightly lower than figures reported by Loveless and Hamrick (1987) for both conifers (67.7%) and tropical tree species (65.5%). In another study of tropical tree species from Central America, however, Hamrick and Loveless (1989) found a mean percentage of polymorphic loci for 16 taxa of 60.9%, much closer to the value reported in this study. In a study of 11 populations of *Acacia mangium* Willd., Moran *et al.* (1989) found much lower percentages of polymorphic loci, averaging 12.7% when all variation was considered, and only 6.7% when the criterion of 95% frequency of the most common allele was used. Similar results apply to the average number of alleles per locus, with Loveless and Hamrick (1987) reporting 2.29 for conifers, and 2.02 for tropical species. Among the maternal plants in this study, the average was 1.65 and Moran *et al.* (1989) found only 1.14 for *A. mangium.* The magnitude of both of these statistics is strongly influenced by the selection of loci.

The values of observed heterozygosity and estimates of expected heterozygosity obtained for these four populations are similar to those found in other studies of coniferous species. In lodgepole pine *(Pinus contorta* ssp. *latifolid)* ,Yeh and Layton (1979) reported a figure of 0.160, compared with an estimate of 0.146 for Jack pine *(P. banksiana)* (Danzrnann & Buchert 1983). Hamrick *et al.* (1979) listed values for conifers ranging from 0.0 for red pine *(P. resinosa)* to 0.43 for Norway spruce *(Picea abies).* The values are also very similar to the mean value reported for tropical trees and shrub taxa by Hamrick and Loveless (1989) (0.211), though somewhat lower than the figure of Loveless and Hamrick(1987) (0.240). In other tropical tree species, much lower expected heterozygosities have been reported, for example for *A. mangium* (0.017) (Moran *et al.* 1989). The average for nine species *Acacia* was only 0.132. As discussed by Hamrick and Loveless (1989), the mode of pollen and seed

dispersal is expected to have a major influence on the degree of genetic diversity as reflected in observed and expected heterozygosities, as well as on Gst values (see below).

The Gst values obtained are also comparable with those for other conifers. For example, Boyle and Yeh (1988) list published values ranging from 0.01 for black spruce *(Picea mariana)* to 0.79 for Sitka spruce *(P. sithensis).* However there are also substantial differences in estimates for the same species from different studies, as the magnitude of the estimates depends on both sample of loci and populations. Clearly, there will be larger genetic differences among a sample of widely separated populations than there will be among populations from the same region.

It has often been sugggested that greater differentiation among populations should be expected in the tropics compared with the temperate zone. Bawa (1976) reviewed possible reasons for this, including lower population densities, more widely scattered populations reducing gene flow and increasing genetic drift, and greater spatial variation in natural selection pressure. However, published results have contradicted this, indicating that long distance pollen movement is possible for tropical species (Hamrick & Murawski 1990). Partly due to this potential long distance pollen dispersal, estimates of Gst [or the equivalent Fst of Wright (1965)] for most tropical tree species, although slightly higher than for most conifers, are not dissimilar (Hamrick & Loveless 1989, Hamrick & Murawski 1990). However, A. *mangium* is an exception for which the extremely disjunct distribution, combined with small population size, has been interpreted as being responsible for Gst value of 0.311 (Moran *et al.* 1989). The estimate of Gst obtained in this study is lower than all of the estimates of Fst presented for seven tropical tree species by Hamrick and Murawski (1990), but is similar to most of the estimates given by Hamrick and Loveless (1986).

As discussed by Loveless and Hamrick (1987), the mode of pollination and seed dispersal will affect genetic differentiation, and they noted that tropical species believed to be wind pollinated generally exhibited greater population differentiation than insect or bat pollinated species. They interpreted this observation to be evidence for the relative inefficiency of wind pollination in tropical environments. *P. kesiya* would, therefore, be expected to produce higher Gst values than either temperate conifers or animal pollinated tropical species. The fact that it has comparable or lower values, despite the populations being separated by far greater distances than those studied by Loveless and Hamrick (1987), indicates that possibly wind pollination in moderate elevations in the tropics is more effective than in sea-level habitats. Other species with similar distribution patterns to *P. kesiya,* but with less efficient pollen and seed dispersal mechanisms, would be expected to exhibit a greater degree of differentiation over a similar area.

A strong relationship between genetic and geographic distances has been interpreted to indicate that isolation by distance is the major cause of population differentiation (Yeh & O'Malley 1980). In this case, as can be seen from Figure 1, the distinctive Samoeng population is not geographically

separate from the others. The genetic distances calculated for *P. kesiya* are similar to those for *A. mangium* (Moran *et al.* 1989), although the *Acacia* populations were distributed over a far larger area. This suggests that the inclusion of *P. kesiya* populations from other parts of the range would yield larger estimates of genetic differentiation than those reported for most temperate conifers.

Conclusions

The four sampled populations from northern Thailand are genetically similar for non-adaptive allozyme loci, although one population (Samoeng) differs from the other three. The small population, however, makes this conclusion tentative. Nevertheless, the degree of population differentiation is comparable with other conifers species.

In comparison with other tropical species, population differentiation in *P. kesiya* is rather low, at least among populations in northern Thailand. This indicates efficient gene transfer among populations occupying similar ecological conditions within a geographic region.

The small sample sizes in most of the populations mean that interpretation of the data must be cautious. *P. kesiya* appears to be typical of most conifers in its genetic organisation but further studies are required to confirm this.

Acknowledgements

The assistance of S. Kijkar in securing the seed samples is gratefully acknowledged. Laboratory assitance was provided by S. Kheawkla. J. Lavereau assisted with data entry and checking.

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