

GENETIC STUDIES IN A TROPICAL PINE - *PINUS KESIYA* III. THE MATING SYSTEM IN FOUR POPULATIONS FROM NORTHERN THAILAND

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BOYLE, T.J.B., LIENGSIRI, C. & PIEWLUANG, C. 1991. Genetic studies in a tropical pine - *Pinus kesiya* III. The mating system in four populations from northern Thailand. Single and multilocus estimates of outcrossing rates in four populations of *Pinus kesiya* from northern Thailand were obtained using isozyme data. Multilocus estimates ranged from 0.68 to 0.97, which are comparable with estimates for other coniferous species. Comparisons of expected and observed inbreeding coefficients indicated excesses of heterozygotes over the frequencies expected from random mating in two populations. Evidence for spatial heterogeneity in allele frequencies was strongest in the same two populations, suggesting the possibility of matings occurring among trees in genetically distinct groups. In one population (Samoeng) there was an excess of homozygotes, compared with the equilibrium frequency expected from the estimated outcrossing rate.

Key words: *Pinus kesiya* - isozymes - mating system - fixation index - population variation

Introduction

A species' mating system summarises the pattern of gametes that unite to form the next generation (Stern & Roche 1974). The most important parameter of the mating system is the outcrossing rate. Although species are generally termed either as inbreeders or outcrossers, mating systems can vary widely in time and space and may be affected by a large number of biotic and abiotic factors. Many aspects of the population genetics of a species, for example, the population structure, are affected by the mating system. Restricted pollen movement results in high levels of inbreeding and, combined with limited seed dispersal, leads to the development of genetically distinct neighbourhoods. Hamrick *et al.* (1979), in a review of mating system studies, demonstrated that species characterised by high levels of outcrossing typically maintain high genetic diversity, with relatively small differences among populations and high within-population variation. The distribution of genetic variation within populations is thus critical in determining efficient sampling procedures for both genetic improvement and genetic conservation.

In addition, the estimation of many genetic parameters using open pollinated progeny is often based on an assumption of random mating, which may not be valid (Squillace 1974). Significant deviations from random mating affect the coefficients of relationship among relatives and may inflate estimates of

heritability and genetic gain, leading to unrealistic expectations from genetic improvement programmes. The performance in field trials of progeny sampled from populations may also be affected by the mating system that produced them. The ranking of black spruce (*Picea mariana*) provenances, based on 15-year height and survival, has been found to be related to estimates outcrossing rates (Petawawa National Forestry Institute Canada, unpublished data). Without such information, provenance selection recommendations could be biased. Therefore, knowledge of the mating system is needed for a better assessment of genetic entries in field trials.

Although the estimation of mating system parameters in natural populations is possible using morphological traits (e.g. Morgenstern 1972, Park & Fowler 1984), the procedure is greatly facilitated by the use of allozymes, because of their co-dominant expression and the large number of loci that can be assayed simultaneously (Lewontin 1974). Many statistical methods for estimation of mating system parameters are available, making use of both single locus and multi-locus data. Multilocus estimators are more reliable because they require fewer assumptions to be made concerning the data.

Tree improvement programmes for *Pinus kesiya* have been initiated recently in Southeast Asia and Africa (Guldager *et al.* 1980). Substantial numbers of provenance trials have also been established (Burley & Armitage 1980). However, estimates of mating system parameters for the species are required before decisions concerning genetic management can be made with confidence (Guldager *et al.* 1980). In the present study, the mating systems in four natural populations of *P. kesiya* from northern Thailand were investigated using allozyme data from 14 loci.

Material and methods

Seed was obtained from 38 individuals of *P. kesiya*, representing four populations, from the Royal Forest Department Pine Improvement Centre, Huay Kaew Arboretum, Chiang Mai, Thailand. Details of the four populations are given by Boyle *et al.* (1991).

Maternal and progeny allele frequencies at 14 loci from nine enzyme systems were obtained for at least 15 embryos per parent as described by Boyle *et al.* (1990), with the pollen contribution to each embryo being deduced by comparisons with the haploid maternal gametophyte. These data were used to estimate both single locus and multilocus outcrossing rates (t) using the method of Ritland and El-Kassaby (1985). The estimation of multilocus outcrossing rates assumes no associations among loci (Shaw *et al.* 1981). Two loci, 6PG-1 and 6PG-2, have been shown to be tightly linked in *P. kesiya* (Boyle *et al.* 1990) so, for the multilocus estimates, 6PG-1 was omitted from the data set. The mean of the single locus estimates, inversely weighted by their variances, was also calculated. Comparisons of single and multilocus estimates can provide information on the occurrence of inbreeding other than selfing in the populations (Shaw & Allard 1981). In support of such inferences, heterogeneity in the frequency of detectable outcrosses among homozygous parents was

investigated by means of a heterogeneity G-test (Sokal & Rohlf 1969).

The expected coefficient of inbreeding at equilibrium was calculated as the minimum variance average over individual loci (Ritland & El-Kassaby 1985). This expected value was then compared with the observed inbreeding coefficient of Wright (1965), calculated as:

$$F = 1 - (h_o / [1 - \sum_{i=1}^n p_i^2])$$

where h_o is the observed heterozygosity, p_i is the allele, and n is the total number of alleles.

Results

Monomorphic loci provide no information for the estimation of outcrossing rates. Single locus estimates are presented for nine loci that proved to be polymorphic in one or more populations (Table 1). No estimates are shown in Table 1 for loci at which no outcrossed embryos were detected in a given population. Inter-locus heterogeneity in outcrossing rates is typical in many studies of conifers (Brown *et al.* 1975, El-Kassaby *et al.* 1981, Mitton *et al.* 1981, Shaw & Allard 1981). In this study heterogeneity was detected for every population except in Wat Chan. Weighted means of single locus estimates ranged from 0.63 for Nong Krating to 1.00 for Wat Chan. Multilocus estimates of outcrossing rates ranged from 0.68 for the Nong Krating population to 0.97 for Wat Chan. The estimates for Nong Krating and Huay Tong were significantly less than 1.00 (*i.e.* [$t + 2S.E.$] < 1.00). Multilocus estimates were similar to the means of single locus estimates for all populations except Samoeng, for which the multilocus estimates was significantly higher.

Table 1. Single and multilocus estimates of outcrossing rates for four sampled populations (standard errors in parentheses)

Locus	Samoeng	Huay Tong	Nong Krating	Wat Chan
AAT	-	1.00 (0.57)	1.11 (0.07)	0.90 (0.13)
G6P	-	-	0.97 (0.70)	0.91 (0.07)
LAP	1.00 (0.27)	0.70 (0.25)	0.51 (0.14)	0.81 (0.12)
6PG-1	0.32 (0.25)	0.72 (0.20)	0.65 (0.17)	0.89 (0.14)
6PG-2	0.94 (0.19)	1.02 (0.17)	0.74 (0.16)	0.95 (0.14)
6PG-3	0.77 (0.29)	0.99 (0.13)	1.09 (0.15)	0.94 (0.10)
PG1-2	-	-	-	1.07 (0.75)
PGM	0.94 (0.13)	0.82 (0.16)	0.39 (0.10)	1.03 (0.00)
SDH-1	0.60 (0.26)	0.29 (0.17)	0.25 (0.05)	0.75 (0.10)
Mean	0.66	0.80	0.63	1.00
Multilocus	0.84 (0.08)	0.81 (0.09)	0.68 (0.05)	0.97 (0.07)

The degree of heterogeneity in pollen pool allele frequencies varied across loci and for different populations (Table 2). For Samoeng there was no detectable heterogeneity, but there was significant heterogeneity at LAP for the

remaining three populations. In two out of three populations there was also heterogeneity detected at 6PG-1, 6PG-2 and SDH-1. For Nong Krating, only two loci exhibited heterogeneity, whereas for Huay Tong and Wat Chan four loci were heterogeneous, although the significance level only reached 5% for two of these loci at Huay Tong.

Table 2. Significance of heterogeneity G-tests for the frequency of detectable outcrosses across homozygous parents

Locus	Samoeng	Huay Tong	Nong Krating	Wat Chan
AAT	-	NS	NS	NS
G6P	-	-	NS	NS
LAP	NS	**	**	**
MR-2	-	*	NS	NS
6PG-1	NS	*	NS	**
6PG-2	NS	**	NS	NS
6PG-3	NS	NS	NS	NS
6PG1-2	-	-	-	NS
PGM	NS	NS	NS	NS
SDH-1	NS	NS	**	**

Note: Significance levels: NS - not significant at the 5% level; * - significant at the 5% level; ** - significant at the 1% level

The calculated values of observed fixation indices (F) indicate the frequency of homozygotes observed, compared with expectations under random mating conditions. The negative values obtained for Huay Tong and Wat Chan (Table 3), therefore, indicate an excess of heterozygotes. At Nong Krating and Samoeng, more homozygotes were observed than expected under random mating and this difference was particularly marked for Samoeng.

Table 3. Observed and expected inbreeding coefficients (from both multilocus and the mean of single locus estimates)

	Samoeng	Huay Tong	Nong Krating	Wat Chan
F_{obs}	0.140	-0.077	0.037	-0.080
F_{exp} (multi)	-0.122	-0.478	0.015	-0.330

The relative frequency of homozygotes observed, compared with the expectations based on the estimated outcrossing rate, is indicated by a comparison of observed and expected fixation indices ($F_{obs} - F_{exp}$). For all four populations more homozygotes were observed than expected (Table 3).

Dicussion

Estimates of outcrossing rates for *P. kesiya* are similar to or slightly lower than those obtained for other conifers. Boyle and Yeh (1988) listed published

estimates that ranged from 0.58 for tamarack (*Larix laricina*) to over 1.00 for several species. Typically, species such as tamarack occurring in small, isolated populations, tend to have lower outcrossing rates than species that tend to form large monocultures. It is not surprising, therefore, that the estimates for these small, isolated populations in northern Thailand tend to be low, as *P. kesiya* typically occurs in small stands, often at low population density (Turnbull *et al.* 1980). For eight tropical species of tree and shrubs, Hamrick and Murawski (1990) reported outcrossing estimates from 0.35 to 1.08. Moran *et al.* (1989a, 1989b) report high outcrossing rates for three species of tropical *Acacia*.

The mixed mating model used in the estimation of outcrossing rates makes certain genetic assumptions concerning the population structure and genetics of the species (Shaw *et al.* 1981). The effect of failure in one or several of these assumptions would be to bias the estimates obtained. For example, Wahlund's effect (Wahlund 1928), family clustering, or other forms of positive assortative mating would induce a downward bias in estimates, whereas gametic or postzygotic selection or negative assortative mating would bias them upwards (Boyle & Morgenstern 1986). All these various effects have been documented in temperate conifers (Sarvas 1962, Mitton *et al.* 1981, Park & Fowler 1984). As mentioned previously, multilocus estimates are less severely affected by failures in assumptions. Samoeng is the only population for which there appears to be a downward bias in outcrossing estimates, based on comparison of the mean of single locus against multilocus estimates.

Heterogeneity in pollen pool allele frequencies, as found in the Huay Tong and Wat Chan populations, is usually interpreted as evidence for Wahlund's effect which, as noted previously, would normally be expected to bias estimates of outcrossing downwards. However, Wahlund's effect can originate from several different causes, such as restricted gene flow, temporally distinct generations, or spatial variation in selection intensity. Restricted gene flow would certainly result in positive assortative mating and a downward bias but, based on information about the population structure of *P. kesiya* (Boyle *et al.* 1991), gene flow does not appear to be restrictive. A spatially highly heterogeneous environment, resulting in differential selection intensities during the life cycle of the trees, could also cause Wahlund's effect and when combined with high levels of pollen exchange, could result in negative assortative mating and upward bias in outcrossing estimates. The fact that these two populations are also the ones having an excess of heterozygotes compared with expectations under random mating would further support the existence of negative assortative mating (strictly, negative assortative mating applies only to non-random mating based on phenotypes, but the term is applied here to genotypic non-random mating).

Measures of genetic diversity indicated that, despite problems with sample size, the Samoeng population appears to be the least variable of the four, and the most dissimilar from the other three (Boyle *et al.* 1991). It was also the only population for which single locus outcrossing estimates were biased downwards. Observed frequencies of homozygotes were greater than expecta-

tions based on random mating and there was also no indication of heterogeneity in pollen pool allele frequencies. All of these factors suggest a genetically depauperate population, either as a result of repeated inbreeding or from some form of bottleneck in its recent genetic history. It is indeed the population with by far the highest observed fixation index, but it also yielded a high multilocus outcrossing rate indicating that inbreeding is unlikely to be the only explanation.

The Samoeng population was represented in the provenance test at Huay Tong, reported by Thaiutsa (1981). In terms of height at age five years, the trees at Samoeng were above average, but this was in comparison with many exotic, slow growing provenances. When compared with provenances from the same locality, the Samoeng population sample was the poorest. Four neighbouring provenances were the four tallest in the experiment, averaging between 12 and 49 cm taller than those at Samoeng. Three of the four also had a larger diameter. It appears, therefore, that the Samoeng population is inferior to those at other local provenances, and that this is likely a result of a high degree of inbreeding. The Nong Krating population was also represented in the provenance test and, although being 14 cm shorter than the Samoeng provenance at age five, it ranked second overall in terms of diameter.

Conclusion

P. kesiya is a predominantly outcrossing species, although outcrossing rates are somewhat lower than in many other conifers, probably as a result of low population density. However, in most populations, effective pollen transfer is adequate to maintain fixation indices near zero. There is some evidence that spatial heterogeneity in selection intensity in some populations may result in local genetic differentiation during the life cycle.

The Samoeng population is unique in having a higher proportion of homozygotes than expected. Although this conclusion is based on a small size, the evidence is consistent and is supported by relatively poor field performance. Estimated outcrossing rates suggest that inbreeding alone is not responsible for this situation, and it is possible that the population has passed through the recent genetic bottleneck. Therefore, although among-population differentiation may be low (Boyle *et al.* 1991), careful assessment of populations is necessary for both tree improvement and conservation efforts to identify those populations that may have abnormal levels of inbreeding. Of course, one generation of controlled outbreeding will break down any inbreeding depression that may have accumulated.

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