CHANGES IN THE MATING SYSTEMS OF POPULATIONS OF *PINUS CARIBAEA* MORELET VAR. *CARIBAEA* UNDER DOMESTICATION

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Received May 9, 1997; accepted August 26, 1997

ABSTRACT

The mating system was analysed using isozyme markers and the mixed mating model in four populations of *Pinus caribaea* var. *caribaea* which differed in origin and management. In a wild but unmanaged population no significant deviation from random mating ($t = 1$) was found using either single or multi-locus estimators of the outcrossing rate. The same result was found for a seed orchard population. However in a population managed for seed production by thinning, the mean single-locus estimate of outcrossing rate ($t_s = 0.907$) was substantially lower than the corresponding multi-locus estimate ($t_m = 1.009$), indicating biparental inbreeding. Within a very heavily logged population the multi-locus estimate of outcrossing ($t_m = 0.894$) was significantly less than 1.0, showing that self fertilisation was occurring. In the same population the mean single-locus estimate was substantially lower ($t_s = 0.798$), providing evidence for additional biparental inbreeding. We conclude that management and exploitation of populations may have highly significant and often undesirable effects on the breeding system of forest trees under domestication, but that the establishment of well designed seed orchards can overcome these problems.

Key words: mating system, *Pinus caribaea* var. *caribaea*, outcrossing rate, inbreeding, isozyme loci

INTRODUCTION

Many commercially important forest tree species are in an active phase of exploitation and domestication. Natural populations are being managed for immediate production of timber by logging, and of seed by establishing seed stands through selective thinning. Domestication of natural genetic resources for long term production is also in progress and this involves establishing seed orchards and transferring germplasm between sites. Possible consequences of these forms of management and domestication are that unplanned changes in mating systems occur involving alterations in processes such as pollen production and dispersal. Any changes in mating systems may lead to significant changes in genetic structure and variability of the populations (HAMRICK 1989; SAVOLAINEN & KARKKAINEN 1992).

In forest trees, certain type of unplanned changes in mating system are highly undesirable. An obvious example of such undesirable genetic change is an elevated frequency of self-fertilisation leading to significant inbreeding depression expressed as decreased survival and growth which is particularly marked in coniferous forest species (FRANKLIN 1970; SORENSEN & MILES 1982). The ultimate impacts of these changes on genetic improvement programme are reduced quality and quantity of forest products which utilise the materials derived from the managed and domesticated genetic resources of the species. It is therefore important to monitor the changes in mating system for any tree improvement programme.

Codominant isozyme loci provide useful genetic markers to investigate such changes in mating systems (SHAW & ALLARD 1981; EL-KASSABY *et al.* 1981; ENNOS 1996). A first step in monitoring the changes is to estimate the single- and multi-locus outcrossing rates and the selfing frequencies of the unmanaged wild populations and the populations under domestication or exploitation. It is then possible to quantify the changes in mating systems that have occurred due to the management and exploitation of the wild populations.

P. caribaea is one of the most important softwood species in the tropics. It has been extensively exploited as a tropical plantation species for the last several decades. Domestication and genetic improvement programmes for the species have been undertaken in many countries. In this study, we are concerned with one of its three varieties, var. *caribaea*. This variety is playing an important role in plantation forestry in both

native and exotic environments, (*e.g.* Cuba and China) as well as some other south-eastern Asian countries. One consequence of this is that several natural populations have been managed for both seed and timber production. The other is that the genetic resources from natural populations have been utilised to establish a seed orchard in its native habitat. Exotic plantations are also now producing seeds.

The purpose of this research is to investigate the genetic impacts of management measures and domestication on the mating systems of contrasting populations of *P. caribaea* var. *caribaea*. Using isozyme markers, we first calculate the single- and multi-locus outcrossing rate estimates in both the unmanaged wild and the managed populations. Inferences can then be made about the extent and nature of changes in the mating systems associated with the management measures taken in the different populations.

MATERIALS AND METHODS

Seed material and population information

Four populations which have undergone different forms of management were chosen. These comprised a wild population (JAG), a heavily logged stand (IDJ), a wild stand managed for seed production (MBJ), and a clonal seed orchard (SOR) (Table 1). One population, IDJ, was located on an island (Isle of pines, now Isle of Youth). The others were located on the Cuban mainland (Figure 1).

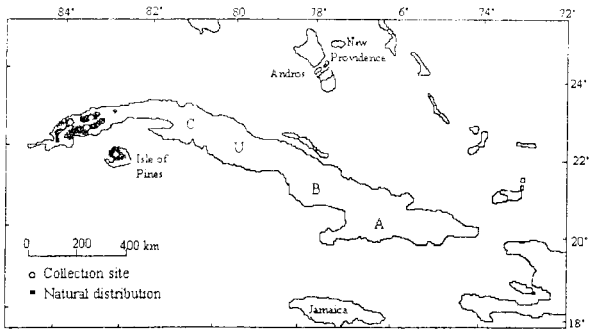

Figure 1. Natural distribution and seed collection sites of *Pinus caribaea* var. *caribaea* in Cuba

The JAG population is within a mixed pine forest. *P. caribaea* var. *caribaea* dominates on the better soils, typically the ridge tops and valley bottoms. The forest type on the Island of Youth (IDJ) is mixed pine and broad-leaved forest dominated by *P. caribaea* and *P. tropicalis*. Sites have been heavily degraded by clear felling and selective logging (BRODIE 1994). Samples of the IDJ population were collected from various sites in the remaining scattered stands on the island. Location with in the IDJ population was not considered in the analysis in this study. The MBJ population is managed for seed production. *P. caribaea* var. *caribaea* forms an almost pure stand in the upper canopy. Open pollinated seeds were sampled from 16–18 maternal trees in these three populations.

The clonal seed orchard (SOR) in Malas Aguas is located within the natural range of the species but is isolated from natural stands and plantations of *P. caribaea*. The orchard consists of 109 clones of plus trees selected from four provenances in Pinar del Rio Province and Topes de Collantes, with the majority of plus trees (68 out of 109) coming from the seed stands MBJ. The planting design of the seed orchard is random placement of single tree plots. The trees in each block were located with the restriction that ramets of the same clone are at least 30 m apart (BRODIE 1994). Open pollinated seed was sampled from 43 clones in the orchard which had performed above the progeny average in volume production. Most sampled clones were represented by 3 or 4 ramets, but for eleven clones seed from only one ramet was available. Seeds of different ramets of a clone were combined together to represent the clone in the electrophoresis.

Electrophoresis

Prior to electrophoresis, seeds were germinated at room temperature until the radicle was at least 5 mm long. Both haploid (megagametophyte) and diploid (embryo) tissues were used for enzyme extraction and starch horizontal gel (11%) was used to separate allozymes. At least 6 germinated seeds for each family were excised by separating the endosperms and embryos. The haploid endosperms and diploid embryos were then individually homogenised in 0.2 M Sodium Phosphate extraction buffer (with 1 $mg \cdot ml^{-1}$ dithiothreitol) and wicks were loaded onto the gel run for about 5 hours.

Electrophoresis and gel staining follows the method described by CHELIAK and PITEL (1984). All the enzyme systems were assayed simultaneously with the same batch of samples. The isozyme loci scored were *Aat–A* (Aspartate aminotransferase, E.C.2.6.1.1), *Idh* (Isocitrate dehydrogenase, E.C.1.1.1.42), *Mdh–B* (Malate dehydrogenase, E.C.1.1.1.37), *6–Pgd–B* (6-phosphogluconate dehydrogenase, E.C.1.1.1.44), and *Pgm* (Phosphoglucomutase, E.C.2.7.5.1). For the seed orchard population, *Aat–A* was not recorded.

Data analysis

Isozyme phenotypes were recorded for both the haploid

Table 1. Summary of seed collection data of the populations of *Pinus caribaea* var. *caribaea*

Site name	Site code	Latitude (N)	Longitude (W)	Altitude (m)	No of trees
La Jagua	JAG 0601-0618	22° 43'	83° 38'	200-280	18
Isla de la Juventud	IDJ 2001-2016	21° 25'	83° 00'	50-100	16
		21° 46'	83° 02'		
		21° 43'	83° 55'		
Marbajita	MBJ 2101-2117	22° 49'	83° 28'	50-70	17
Malas Aguas	SOR 1001-1117	22° 41'	83° 53'	50	43

endosperm and the diploid embryo of each seed. Maternal genotype was inferred at each locus from segregation of the haploid megagametophytes which are genetically identical to the maternal gamete. The probability of correct identification of maternal genotype from n megagametophytes is $1-(0.5)^{n-1}$ (TIGERSTEDT 1973; CHELIAK & PITEL 1984). In this study, at least 6 megagametophytes were scored per family giving a probability of 0.97 of correct identification of maternal heterozygotes.

Method used for mating system estimation follows the way described by RITLAND and JAIN (1981) and RITLAND (1986). The main model used for multi-locus outcrossing rate estimation is given here. With the mixed mating model, for n unlinked loci and outcrossing rate t_m, the progeny genotype frequencies **f** at equilibrium can be related to the Hardy-Weinberg genotypic frequencies $\mathbf{f}_0$ as:

$$f = t_m[I - (1 - t_m)\, S)^{-1} f_0$$

where **S** is the Kronecker product of matrices, at each of the n loci, of self fertilised progenies from a given maternal parent, and **I** is the identical matrix of suitable dimension. Multi-locus outcrossing rate and pollen pool gene frequencies were estimated using maximum likelihood procedures (see RITLAND & JAIN 1981 and RITLAND 1986 for details). Data on progeny-genotype arrays from the individual families were used to estimate pollen allele frequencies (p) and single- and multi-locus outcrossing rates (t_s, t_m) for each population by the computer program by RITLAND (1990). At *Pgm–B* the 4th allele with the lowest frequency was combined with the allele having the nearest mobility because the computer program can only process a maximum of 3 alleles per locus. Variances of estimates were obtained by conducting 100 to 500 bootstraps in which resamplings were performed between families. Spatial heterogeneity of pollen pool allele frequencies over the maternal trees was tested by χ^2 tests. The degree of freedom (df) of the χ^2 is 1 and 9 for a diallelic and a triallelic locus respectively plus an approximation of $1/n$ (n is the number of loci used in multilocus estimation) (RITLAND 1991). Differences between allele frequencies of ovule and pollen pools were examined by the t-test. Values of t were calculated as:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S_{\bar{x}_1 - \bar{x}_2}} = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{2\frac{s_P^2}{n}}}$$

where n is the number of bootstraps used for the estimation of standard error, S^2_P is the pooled variance for the differences and calculated as (when df for both estimates are equal):

$$S_P^2 = \frac{df_1\sigma_1^2 + df_2\sigma_2^2}{f_1 + df_2} = \frac{1}{2}(\sigma_1^2 + \sigma_2^2)$$

where σ^2_1 and σ^2_2 are the variances of the allele frequencies in ovule and pollen pool respectively. The heterogeneity of single-locus outcrossing rates was conducted by a χ^2 test in which the χ^2 value is given by:

$$\chi^2_{k-1} = \sum_{i=1}^{k} I_i(t_i - t^c)^2$$

with k–1 degree of freedom; t^c is minimum variance mean of estimates of t_s, the information measure (I_i) is the inverse of the variance (V_i) for the ith estimate of t_s (KAHLER *et al.* 1984).

RESULTS

Gene frequencies in pollen and ovule pools

The only significant differences between the pollen and ovule allele frequencies were for one allele of *Pgm–B* in the logged population (IDJ) and the managed seed stand (MBJ) (Table 2). There was no evidence of unequal spatial distribution of pollen in the seed orchard (SOR) as shown by the χ^2 tests. However, significant departure from homogeneous pollen distri

Table 2. Estimates of allele frequencies in pollen and ovule pools and their differences (with t-test) and χ^2-tests for spatial heterogeneity of pollen distribution (*n* is the number of seeds scored in the electrophoresis; *: 5% significance level

Population	Locus	Allele	*n*	Ovule		Pollen		Difference	χ^2
				frequency	s. e.	frequency	s. e.		
JAG	*Aat-A*	1	98	0.278	0.086	0.343	0.055	-0.065	6.84*
		2		0.722	0.086	0.657	0.055	0.065	
	Idh	1	110	0.111	0.049	0.191	0.030	-0.080	0.52
		2		0.889	0.049	0.809	0.030	0.080	
	Mdh-B	1	110	0.028	0.021	0.029	0.014	-0.001	-
		2		0.972	0.021	0.971	0.014	0.001	
	Pgd-B	1	110	0.444	0.073	0.461	0.054	-0.017	1.33
		2		0.222	0.060	0.272	0.050	-0.050	
		3		0.333	0.065	0.267	0.051	0.066	
	Pgm-B	1	110	0.139	0.051	0.100	0.024	0.039	19.56*
		2		0.583	0.083	0.515	0.043	0.068	
		3		0.278	0.081	0.385	0.045	-0.107	
IDJ	*Aat-A*	1	149	0.500	0.085	0.323	0.053	-0.177	9.36*
		2		0.500	0.085	0.677	0.053	-0.177	
	Idh	1	149	0.094	0.049	0.128	0.033	-0.034	0.79
		2		0.906	0.049	0.872	0.033	0.034	
	Mdh-B	1	149	0.063	0.038	0.039	0.022	0.024	-
		2		0.938	0.038	0.961	0.022	-0.023	
	Pgd-B	1	149	0.313	0.053	0.315	0.044	-0.002	13.44
		2		0.469	0.080	0.429	0.055	0.040	
		3		0.219	0.081	0.256	0.058	-0.037	
	Pgm-B	1	149	0.031	0.025	0.208	0.047	-0.177*	26.21*
		2		0.500	0.077	0.447	0.069	0.053	
		3		0.469	0.071	0.345	0.103	0.124	
MBJ	*Aat-A*	1	84	0.441	0.087	0.1453	0.079	-0.012	7.18*
		2		0.559	0.087	0.547	0.079	0.012	
	Idh	1	110	0.206	0.058	0.197	0.041	0.009	3.55
		2		0.794	0.058	0.803	0.041	-0.009	
	Mdh-B	1	110	0.206	0.077	0.116	0.034	0.090	10.13
		2		0.794	0.077	0.884	0.034	-0.090	
	Pgd-B	1	110	0.471	0.084	0.481	0.053	-0.010	8.70
		2		0.206	0.052	0.166	0.054	0.040	
		3		0.324	0.074	0.353	0.042	-0.029	
	Pgm-B	1	110	0.206	0.087	0.032	0.014	0.174*	4.55
		2		0.265	0.073	0.431	0.066	-0.166	
		3		0.529	0.089	0.538	0.070	-0.009	
SOR	*Idh*	1	259	0.229	0.026	0.163	0.037	-0.066	3.02
		2		0.771	0.026	0.837	0.037	-0.066	
	Mdh-B	1	241	0.131	0.035	0.070	0.026	-0.061	1.41
		2		0.869	0.035	0.930	0.026	0.061	
	Pgd-B	1	247	0.488	0.045	0.500	0.036	0.012	16.14
		2		0.295	0.031	0.279	0.049	-0.016	
		3		0.217	0.032	0.221	0.049	0.004	
	Pgm-B	1	275	0.119	0.030	0.140	0.034	0.021	10.41
		2		0.446	0.030	0.465	0.049	0.019	
		3		0.435	0.040	0.395	0.050	-0.040	

bution among maternal trees was found at *Aat–A* in all the other three populations and at *Pgm–B* in the wild population (JAG) and the logged population.

Table 3. Single-locus (t_s) and multi-locus (t_m) estimates of outcrossing rate in four populations of *Pinus caribaea* var. *caribaea*. t^b is arithmetic mean; t^c is minimum variance mean. (*: 5% significance level; –: maximum likelihood estimation did not converge due to the high monomorphism at the locus

Estimate	Locus	JAG		IDJ		MBJ		SOR	
		t_s	s. e.	t_s	s. e.	t_s	s. e.	t_s	s. e.
Single-locus	*Aat–A*	0.860	0.181	0.798	0.124	0.678*	0.133		
	Idh	0.942	0.277	0.849	0.173	0.827	0.169	0.977	0.079
	Pgd–B	0.888	0.111	0.654*	0.116	0.919	0.097	0.964	0.106
	Pgm–B	1.049	0.097	0.762*	0.095	1.004	0.223	0.900	0.088
	Mdh–B	–		–		0.959	0.138	1.000	0.254
				0.071		0.141		0.000	
	χ^2 *of* t_s	0.079							
	t^b	0.935	0.167	0.766*	0.127	0.877	0.152	0.962	
	t^c	0.954	0.066	0.798*	0.052	0.907	0.065	0.941	
Multi-locus	t_m	0.984	0.046	0.894*	0.038	1.009	0.086	0.985	0.050
	t_m–t_c	0.029	0.058	0.096*	0.020	0.102*	0.049	0.044	0.028

Population estimates of outcrossing rates

Single- and multi-locus estimates of outcrossing rate (Table 3) were examined by comparing the bounds of confidence intervals to test for departure from complete outcrossing ($t = 1.0$). In the unmanaged wild population and the seed orchard, all single- and multi-locus estimates showed non-significant departure from complete outcrossing. In the managed seed stand the outcrossing rate estimate for *Aat–A* was significantly less than one, though the multi-locus estimate did not deviate from unity. In the logged population, single-locus estimates at *6Pgd–B* and *Pgm–B* and the multi-locus estimate were significantly lower than complete outcrossing. All the estimates of outcrossing rates were lower in the logged population than in the other three populations.

Heterogeneity tests (Table 3) of single-locus estimates of outcrossing rates showed no significant differences (P>0.05) among loci in any of the 4 populations. In the unmanaged wild population, single-locus estimates ranged from 0.86 at *Aat–A* to 1.049 at *Pgm–B* with a minimum variance mean of 0.954. The multi-locus estimate was 0.984 showing a low level (1.6%) of selfing. In the managed seed stand, the minimum variance mean single-locus outcrossing rate was 0.907 with a range from 0.678 at *Aat–A* to 1.004 at *Pgm–B* The multi-locus estimate was 1.009 indicating virtually no selfing. In the logged population, the single-locus estimates of outcrossing rate ranged from 0.654 at *6Pgd–B* to 0.849 at Idh with a minimum variance mean of 0.798. The multi-locus estimate was 0.894 indicating 10.6% selfing. In the seed orchard, the single-locus estimates ranged from 0.90 at *Pgm–B* to 1.007 at *Mdh–B* with a minimum variance mean of 0.941. The multi-locus outcrossing rate was 0.985, suggesting a low level of selfing (1.5%) in the seed orchard.

The significance of the difference between multi-locus and minimum variance mean single-locus estimates of outcrossing rate (t_m–t_c) was examined by comparing the confidence intervals for all populations (Table 3). Significant (P > 0.05) differences were found in the logged island population and the managed seed stand indicating significant levels of biparental inbreeding (mating with relatives). There was no evidence for biparental inbreeding in the unmanaged population or the seed orchard.

DISCUSSION

Multi-locus estimates of outcrossing rate are robust to many violations of the mixed-mating model assumptions (MORGANTE *et al.* 1991) and provide a true picture of the amount of self-fertilisation within a population. Our results on multi-locus estimates of outcrossing rates (t_m) indicate that *P. caribaea* var. *caribaea* shows low levels of self-fertilisation in all the populations. This is as expected for a tropical conifer species (LOVELESS 1992). Although no previous study on mating system of var. *caribaea* has been reported, multi-locus estimates of outcrossing rates obtained from an earlier investigation on the other two varieties of *P. caribaea* indicated from 15 % to 7 % selfing in var. *bahamensis* and from 11 % to 8 % in var. *hondurensis*, based on analysis of 11 loci (MATHESON *et al.* 1989). These selfing estimates fall between the values obtained from our study and are slightly higher than the results for the wild population, the seed stand and the seed orchard but slightly lower than the estimates for the logged island population

The multi-locus analysis indicates a clear difference between self-fertilisation rates in the logged population IDJ and elsewhere. Over-exploitation of the population IDJ is associated with a significant increase in self-fertilisation rates. This may be due to the low density in the population which has been subjected to intensive logging (BRODIE 1994). Pollen supply may be limited in such a low density population leading to an increase in self pollination. Such effects of density on outcrossing rate have also been found in other tree species (KNOWLES *et al.* 1987; HARDNER *et al.* 1996).

The second striking result of this study concerns the difference between single and multi-locus outcrossing estimates. For both the managed seed stand and the heavily logged population the mean single-locus estimates of t are significantly lower than the multi-locus estimates of t. Such a result implies that substantial biparental inbreeding is occurring in these situations. While not as serious in its effects as self-fertilisation, biparental inbreeding will lead to loss of fitness as a consequence of inbreeding depression. A possible reason for these results is that there is substructuring or family clustering in the two populations which have been subjected to logging and genetic thinning (SHAW & ALLARD 1982; ENNOS & CLEGG 1982; ELLSTRAND & FOSTER 1983).

In the logged population, the better individuals have been selectively cut for timber production and a small number of badly growing and possibly related individuals have been left to provide the seed for the next generation. In such a situation it is likely that groups establishing after natural regeneration will be derived from a very limited number of parents and will be related to one another. Preferential mating between adjacent individuals within such groups will then give rise to biparental inbreeding.

In the stand managed for seed production it is less clear how family structure may be generated in the population to give rise to biparental inbreeding. Management removes badly performing individuals, phenotypically superior individuals are left for seed production, and overall tree density is reduced. The remaining individuals within an area could be more closely related to one another than would a random sample of trees chosen from that area if variation in performance has a genetic basis. Preferential mating among these related individuals within different areas of the population could generate biparental inbreeding. We should note that the rate of self fertilisation (as measured by the multi-locus outcrossing estimate) in the seed stand has not been significantly reduced by the genetic thinning, which is in contrast to the logged population. This probably indicates that only biparental inbreeding is important in the seed stand whereas both true selfing and consanguineous mating are occurring in the logged population.

The increased level of biparental inbreeding due to management in the seed stand can be prevented by establishing a well planned seed orchard. The high rate of outcrossing and the very low level of true selfing detected in the seed orchard may be attributable to the optimal spacing and deployment of ramets in the seed orchard which promotes pollen movement and mixing and prevents matings between relatives. Our results for the seed orchard are in accordance with findings from other studies which have also revealed higher outcrossing rates (SHAW & ALLARD 1982; RUDIN *et al.* 1986; MUONA & HARJU 1989) and the lack of difference between single-locus and multi-locus estimates of selfing in seed orchards (SHAW & ALLARD 1982). It confirms that orchard design has been successful in preventing biparental inbreeding (RITLAND & EL-KASSABY 1985).

We conclude that management measures can have significant impacts on the mating systems of forest tree populations. The management measures may have either positive or negative effects on the outcrossing rate and the level of consanguineous mating. Intensive selective logging causing dramatic reduction in density may lead to elevated levels of both true selfing and biparental inbreeding. Converting a natural stand for seed production by thinning may increase the degree of biparental inbreeding. Good seed orchard design on the other hand can realise the goal of preventing both of these undesirable effects and optimising the delivery of genetic resources in the seed crop.

ACKNOWLEDGEMENTS

This research was supported by the Overseas Development Administration through a grant for co-operative research between the Chinese Academy of Forestry and Edinburgh University.

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