FOREST GENETICS 11(1):55–61, 2004

CORRELATED MATINGS IN DIOECIOUS TROPICAL TREE, *MYRACRODRUON URUNDEUVA* FR. ALL.

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Recieved July 28, 2003; accepted June 21, 2004

ABSTRACT

The correlated matings in two populations (Selvíria – SEL and Paulo de Faria – PFA) of dioecious *Myracrodruon urundeuva* were studied in the Southwest of Brazil, by allozyme analysis of progeny arrays using the sibling-pair model. Open-pollinated seeds were collected from 25 to 30 trees within populations. Departure from random matings were evident from the differences in pollen and ovule allele frequencies. The high and significant correlation of paternity (SEL $\hat{r}_p = 0.671 \pm 0.074$; PFA $\hat{r}_p = 0.371 \pm 0.062$) and a low number of effective pollinating trees (ranging from 2 to 3) were detected in the populations, suggesting high proportion of full-sibs progenies. According to these results, the estimate of coancestry within families ($\hat{\theta} = 0.209$ – SEL; $\hat{\theta} = 0.171$ – PFA.) exceeded the expectation of the half-sib progenies ($\hat{\theta} = 0.125$). Result outcomes are discussed from a conservation and breeding point of view.

Key words: *Myracrodruon urundeuva*, allozyme, correlated mating, coancestry, inbreeding

INTRODUCTION

Detailed knowledge and understanding of the mating system in natural and artificial plant populations are fundamental prerequisites for understanding their genetic architecture and evolutionary potential (EL-KASSABY & JAQUISH 1996). The mating system and the genetic structure are intrinsically related to species ecology and genetics (LOVELESS *et al.* 1998). In tree species, spatial distribution, relatedness and mating system can be associated. Especially in animal-pollinated species, close individuals tend to intercross more than widely spaced individuals, and if the latter are related by the dispersal of the seeds next to the mother tree, inbreeding is likely to increase in the population (COLES & FOWER 1976).

The mating system has traditionally been characterized by mixed-mating model. Most models of mixed-mating systems have assumed that progenies descended from common mothers are constant mixtures of selfs and half-sibs (RITLAND 1986). However, in dioecious species self-pollination do not occur and mating can occur by a mixture of random and correlated outcrossings. Thus, progenies descended from a common mother may consist of half and full-sibs mixtures. RITLAND (1989) developed a method including patterns of paternity and assumed that progenies may be mixtures of selfs, half-sibs and full-sibs (sibling-pair model or correlated matings model). The correlated mating model may be adequate to characterize the mating system in dioecious species, due that progenies may be mixtures of half-sibs and full-sibs.

Myracrodruon urundeuva Fr. All. (Anacadiaceae) is dioecious tropical tree species (SANTIN & LEITÃO FILHO 1991) of large geographic distribution, extending from 3°30' S (Brazil) to 25° S (Argentina), also found in Bolivia and Paraguay. It occurs in various phytoecological regions ranging from 17 m to 1,200 m altitude, including: Seasonal Semideciduous Forest; Seasonal Deciduous Forest; Savannah Woodland and Denser Savannah Woodland; Thorn Forest; Wetland (CARVALHO 1994). *M. urundeuva* timber is commonly employed in constructions, furnitures, and coal and firewood production. Nevertheless, *M. urundeuva* has became scarce in the Central-South region of Brazil, especially in the State of São Paulo, where its wild form is no longer found in great numbers, except in small private or governmental reserves.

M. urundeuva pollination occurs by animals and the fruits contain only one seed. Thus, there is possibility that open-pollinated seeds can be half-sibs. However, the lower density of trees in the populations may cause correlated matings (SURLES *et al.* 1990). In this way, the mating system was evaluated in two populations of *M. urundeuva* in the Southwest regions of Brazil, in order to estimate the levels of correlation of paternity, using allozyme markers.

MATERIALS AND METHODS

Sampling

Open-pollinated seeds were randomly collected from 25 to 30 trees, within two populations of *M. urundeuva* distanced approximately 250 km in the Southwest region of Brazil (Paulo de Faria, SP - PFA, 19°55' S, 49°31' W; Selvíria, MS - SEL, 20°19' S, 49°31' W). These areas are Savannah woodlands where *M. urundeuva* occurs in clusters and sometimes in isolated trees. The SEL population is in a large area (> 500 hectares) and has a high number of individuals (~300), but present a low density of individuals per hectare (~ 0.6) when compared to the PFA population. The PFA population occupies a site of approximately 50 hectares containing approximately 100 trees. Both populations underwent some sort of anthropic intervention, where the SEL population was intensively maneged and seed mother trees were origenated from natural regeneration. The PFA population, on the other hand, was less developed and may be considered a natural population.

Allozyme Analysis

Leaf tissues of germinating embryos of 20 seedlings per progeny array were evaluated by allozyme electrophoresis at the Population Genetics and Silviculture Laboratory of the Phytotechny, Food Technology and Social-Economy Department of the FEIS/UNESP, Ilha Solteira, São Paulo State, Brazil. The electrophoresis was horizontal (Table 1), conducted on 12 % starch gel using gel electrode tris-citrate buffer (ALFENAS 1998). The runs (Table 1) were performed in a refrigerator, at 5°C, keeping constant currents in the electrodes. The allozymes were extracted from leaf tissues using approximately 20 mg leaf blade tissue, 10 mg washed urea, 7 mg polyvinyl pyrrolidone (PVP 40), 7 mg polyvinyl pyrrolidone (PVP 60), and 200 microliters of number 1 extraction solution of ALFENAS (1998). Five allozyme systems were assayed: aspartate aminotransferase (AAT, EC 2.6.1.1), leucine aminopeptidase (LAP, EC 3.4.11.1), phosphoglucoisomerase (PGI, EC 5.3.1.9), 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.44) and phosphoglucomutase (PGM, 2.7.5.1). The allozymes staining procedures were the same as those described by ALFENAS (1998). The loci were sequentially designed with the highest anode migration allele named 1, the next one 2 and so forth. Nevertheless, the genetic base of the loci was not tested through controlled mating, but the selected enzymes expressed phenotypes consistent in subunits and genetic interpretation of other studies in plants, as documented by WENDEL & WEEDEN (1989) and ALFENAS (1998). In addiction, these loci have been assayed in large number of plants and generally shown to follow Mendelian ratios.

Data Analysis

Correlated mating parameters were estimated using the MLTR computer program developed by RITLAND (1994) to calculate the mixed-mating model (RITLAND & JAIN 1981) and correlated-mating model (RITLAND 1989). Since the species is dioecious, multilocus ($\hat{t}_m$) and unilocus ($\hat{t}_s$), outcrossing rates were constrained to one, and the selfing rate correlation ($\hat{r}_s$) was constrained to zero. The estimated parameters were: the ovule (o) and outcrossing pollen allele frequencies (p); the average inbreeding coefficient of maternal parents ($\hat{F}_m$); and the correlation of outcrossed paternity within progeny arrays ($\hat{r}_p$). The standard errors of the estimates parameters were obtained from 1,000 bootstraps, where the sampling unit was the families.

Differences between allele frequencies in the

Table 1. Buffer and running condictions for starch-gel electrophoresis of *M. urundeuva* populations.

Electrode buffer	Gel buffer	Running condictions	Enzyme systems
Tris-citrate system	12 % starch	20 Vcm^{-1}	AAT
0.135 M Tris	0.0034 M Tris	for 5:3 h	LAP
0.043 M citric acid	0.011 M citric acid	maximum	PGI
pH 7.0	water pH 7.5	38 mA	6PGDH
			PGM

maternal tree populations (ovule) and their corresponding outcrossing pollen pool allele frequencies were checked by computing chi-square distribution statistics (Table 2) as $\chi^2 = 2n\hat{F}_{ST}(\alpha - 1)$, with ($a$–1) degree of freedom, where n is the number of seeds examined, a is the number of alleles for locus, and $\hat{F}_{ST}$ is the genetic divergence between groups (ovule and pollen) (Murawski *et al.* 1994). $\hat{F}_{ST}$ parameter was estimate from each locus according to Nei (1977).

The coancestry within families ($\hat{\theta}$) was estimated from the coefficient of correlation among offsprings within families ($\hat{r}_{xy}$), where according to Ritland (1989),

$$\hat{r}_{xy} = 0.25(1+\hat{F})[4\hat{s}+(\hat{t}^2+\hat{s}\hat{t}\hat{r}_s)(1+\hat{r}_p)]$$

and, as $\hat{\theta}=\hat{r}_{xy}/2$ (Lynch & Walsh, 1998) and assuming $\hat{t}_m = 1{,}0$ and $\hat{r}_s = 0$, then $\hat{\theta} = 0.125(1+\hat{F})(1+\hat{r}_p)$ where, $\hat{F}$ is the inbreeding coefficient in the parental generation.

The inbreeding coefficients of offsprings ($\hat{F}_o$) were estimated based on Wright's fixation indices $\hat{F}_o = 1-(\hat{H}_o/\hat{H}_e)$, where $\hat{H}_o$ is the observed heterozygote frequencies and $\hat{H}_e$ is the expected heterozygote frequencies under Hardy-Weinberg equilibrium (Nei, 1977).

The variance effective size of a simple family was estimated according to Cockerham (1969),

$$\hat{N}_e = \frac{0.5}{\hat{\theta}\left(\frac{n-1}{n}\right)+\frac{1+\hat{F}_o}{2n}}$$

where n is the number of individuals of each family (20).

RESULTS AND DISCUSSION

Allele Frequencies

Allele frequencies in the pollen and ovule pools are presented in Table 2. The chi-square statistical analysis showed that pollen and ovule allele frequencies contributing to the progeny sampled were significantly different at the 0.01 level for *Aat-1, Aat-2, Lap* and *Pgm* in the SEL population and at the *Aat-1, Aat-2, Pgi* and *6pgdh* ($\alpha = 0.05$) in the PFA population. These discrepancies in allele frequencies between the ovule and outcrossing pollen pool may probably be caused by biparental inbreeding, correlated outcrossing and pollen migration derived from outside the population. Another cause of the discrepancies may be natural selection that occurred between the time of actuals pollination and the progeny sampling.

Inbreeding Coefficient

The average inbreeding coefficient ($\hat{F}_o$) of offsprings was high in both populations (minimum 0.122), but was not differento from zero in SEL population (Table 3). Since inbreeding is caused by coancestry (θ) among mates, the offspring inbreeding coefficient ($\hat{F}_o$) corresponds to coancestry of the precedent generation (Lindgren *et al.* 1997). The high $\hat{F}_o$ index observed in the offsprings of PFA (0.180) population suggest that the mean coancestry of the parental generation was between half-sibs (0.125) and full-sibs (0.25), indicating the possibility of spatial genetic structure in population.

Signs of selection against homozygotes were detected between the adult phase and that of the progenies (Table 3). The fixation index was about zero in maternal trees ($\hat{F}_m$) and positive, high and significantly different from zero (1.96 SE) in the progenies of the PFA population. The absence of inbreeding in maternal trees and the positive and high inbreeding in the progenies indicated selection against homozygous progeny. The biparental mating is the only cause of inbreeding in dioecious species and probably may lead to inbreeding depression in *M. urundeuva.*

Indications of selection against homozygous have also been reported in *Pinus ponderosa* (Linhart *et al.* 1981), *Abies balsamea* (Neale & Adams 1985), *Eucalyptus rhodantha* (Sampson *et al.* 1989), *Ceiba pentandra* (Murawski & Hamrick 1992), *Shorea trapezifolia* (Murawski *et al.* 1994), *Metrosideros excelsa* (Schimidt-Adams *et al.* 2000) and *Cariniana legalis* (Sebbenn *et al.* 2000).

Correlated Mating

The correlation of outcrossed paternity (Table 3) was high and significantly different from zero in both populations (SEL $\hat{r}_p = 0.671 \pm 0.074$; PFA $\hat{r}_p = 0.371 \pm 0.062$). The correlation of outcrossing paternity within progeny arrays ($\hat{r}_p$) is the proportion of pairs of outcrossing progenies that are full-sibs (Ritland, 1989). Thus, theses results show that part of the progenies were full-sibs. If t measures the proportion of progeny produced for outcrossing and $\hat{r}_p$ the proportion of progeny pairs that are full-sibs, and since t in dioecious species, consequently $\hat{r}_p$ also measures the correlated mating rate, and ($1-\hat{r}_p$) measures the random mating rate. Hence, the proportion of full-sibs is about 67% in SEL and 37% in PFA populations.

Table 2. Allele frequencies for six polymorphic loci in maternal (Ovule pool) and outcrossing pollen pools of M. urundeuva populations.

SEL

Locus	Allele	Ovule pool	Pollen pool	$\hat{F}_m$	n	df	χ^2
Aat-1	1	0.880	0.752				
	2	0.120	0.248	0.028	430	1	24.12**
Aat-2	1	0.880	0.937				
	2	0.010	0.061				
	3	0.020	0.003	0.009	422	2	14.95**
Lap	1	0.540	0.499				
	2	0.280	0.441				
	3	0.180	0.060	0.018	430	2	30.83**
Pgi	1	0.412	0.381				
	2	0.569	0.607				
	3	0.020	0.012	0.001	378	2	1.88
6Pgdh	1	0.980	0.979				
	2	0.020	0.021	0.000	373	1	0.01
Pgm	1	0.820	0.738				
	2	0.180	0.262	0.010	392	1	7.73**

PFA

Locus	Allele	Ovule pool	Pollen pool	$\hat{F}_m$	n	df	χ^2
Aat-1	1	0.717	0.892				
	2	0.283	0.108	0.051	582	1	59.56**
Aat-2	1	0.617	0.904				
	2	0.383	0.096				
	3	–	–	0.127	576	1	146.84**
Lap	1	0.450	0.499				
	2	0.467	0.450				
	3	0.083	0.051	0.002	578	2	3.83
Pgi	1	0.317	0.203				
	2	0.667	0.786				
	3	0.017	0.011	0.017	591	2	39.74**
6Pgdh	1	0.900	0.938				
	2	0.100	0.062	0.005	578	1	5.63*
Pgm	1	0.800	0.797				
	2	0.200	0.203	0.000	579	1	0.05

Correlated outcrossing was lower in PFA population than the SEL population. The lowest proportion of correlated outcrossing in the PFA population may be caused by the high density of individual in this population, whereas the lowest density of individuals in the SEL population may favor the highest correlated outcrossing.

There are only few studies of tropical tree species reporting correlation of paternity estimates. Significant paternity correlation has been detected in *Eucalyptus rameliana* ($\hat{r}_p$= 0.260±0.050; SAMPSON 1998), *Eucalyptus marginata* ($\hat{r}_p$ variation of 0.530 ± 0.100 to 0.920 ± 0.100; MILLAR *et al.* 2000), *Enterolobium cyclocarpum* ($\hat{r}_p$ variation of 0.174 ± 0.105 to 0.462 ± 0.151;

Table 3. Estimates of inbreeding, correlated mating and coancestry parameters in *M. urundeuva* populations.

Parameters	SEL (s.e.)	PFA (s.e.)
Number of families	25	30
Inbreeding coefficient of maternal trees – $\hat{F}_m$	0.013 (0.010)	0.000 (0.000)
Inbreeding coefficient of progenies – $\hat{F}_o$	0.122 (0.141)	0.180 (0.095)
Correlated paternity – $\hat{r}_p$	0.671 (0.074)	0.371 (0.062)
Effective number of pollinators – $1/\hat{r}_p$	2	3
Coancestry within families – $\hat{\theta}$	0.209	0.171
Variance effective size – $\hat{N}_e$	2.21	2.60

(SE) – standard error.

Rocha & Aguilar 2001), *Cariniana legalis* ($\hat{r}_p$ variation of 0.219 ± 0.033 to 0.324 ± 0.045; Sebbenn *et al.* 2000), *Esenbeckia leiocarpa* ($\hat{r}_p$ variation of 0.749 ± 0.080 to 0.986 ± 0.037; Seoane *et al.* 2001), *Tabebuia cassinoides* ($\hat{r}_p$ variation of 0.295 ± 0.070 to 0.547 ± 0.095; Sebbenn *et al.* 2001) and *Eucalyptus camaldulensis* ($\hat{r}_p$ variation of 0.079 ± 0.020 to 0.365 ± 0.117; Butcher & Willians 2002). Even though current research represents little of the diversity in animal-pollinated tree species, it shows that correlated mating are common, sometimes high and that the assumption that half-sibs in open-pollinated progeny in from tropical tree is probably never true.

The high proportion of full-sibs in the arrays of open-pollinated *M. urundeuva* suggests that each maternal tree received pollen from a limited number of paternal parents. According to Ritland (1989), the probable number of pollinators can be calculated by the ratio $1/\hat{r}_p$ (Table 3), which indicates that the individual outcrossing occurred in at least three pollinating genotypes. The possible explanations for the observed correlated outcrossing in *M. urundeuva* are flowering asynchronism, low number of potentially pollinating trees, low density of individuals in the populations and pollinator insects visiting neighboring trees. All these explanations are acceptable for *M. urundeuva*. However, it is difficult to exactly determine the real causes of correlated mating. This would require a detailed study of the reproductive event. The most likely hypothesis may be related to flowering asynchronism and low density of individuals in populations combined to the behavior of pollinators visiting neighboring trees. *M. urundeuva* presents an extensive and variable bloom, spreading from June through August in the state of São Paulo (Carvalho 1994). Since most of the time the flowering period is characterized by an initial phase in which few trees have bloomed, followed by a period of flowering peak and a final stage of flowering shortage, it can be inferred that the initial and final asynchronism may favor correlated mating. The high proportion of correlated mating implies in the increased probability of full-sibs individuals becoming established in the populations.

Coancestry Within Families

Correlated matings increases the coancestry among offsprings within families. Coancestry (θ) between two diploid individuals is defined as the probability that two alleles sampled from the same locus in each of the individuals are identical by descend (Lindgren *et al.* 1997). The estimate of the average coancestry among offsprings within families was 0.209 from SEL and 0.171 from PFA (Table 2). The θ parameters show values of 0.125 in half-sib progenies and 0.25 in full-sibs progenies. In mixed half-sibs and full-sibs progenies in populations without inbreeding and relatedness, θ assumes intermediary values between 0.125 and 0.25. This value also increases when parental genotypes show inbreeding and relatedness. In the populations θ was higher than expected for half-sib progenies (0.125), confirming the hypothesis of correlated mating in the populations. The increase of average coefficient of coancestry within families reduces the genetic diversity, variance effective populations size ($N_{e(v)}$) and status number (N_s) in the populations. In an ideal population (infinite size, random matings, without inbreeding, relatedness, selection, mutation and migration), $N_{e(v)}$ in a simple half-sib family is $4 (N_{e(v)} = 0.5/\theta_{half\text{-}sibs})$. Thus, $N_{e(v)}$ was 35 % to 45 % lower than expected in ideal population.

Genetic Conservation and Breeding

The results observed in *M. urundeuva* mating system have important implications for its conservation and genetic breeding. For *ex situ* genetic conservation, the correlated matings requires the maintenance of larger sample sizes than those predicted for Hardy-Weinberg's equilibrium populations. For example, assuming that the goal of *ex situ* conservation is to retain a reference effective populations size ($N_{e(reference)}$) of 50 and that infinite open-pollinated seed ($n > 1{,}000$) will be collected per tree, then 23 mother tree are sufficient to obtain the effective size required ($m = N_{e(reference)}/N_{e(v)}$).

In breeding, the use of classic models that assume open-pollinating progenies being half-sibs is not appropriate for *M. urundeuva* populations, and may lead to overestimates in the additive genetic variance (σ_A^2), heritability (h^2) and genetic gains in the selection (Gs). The correlated matings observed require the adoption of appropriate genetic-statistical models to analysis of quantitative trais. RITLAND (1989) presented specific genetic models for populations with mixed mating system, including correlated matings. Calculations of the r_{xy} parameter from coancestry ($r_{xy} = 2\theta$) in *M. urundeuva* populations (SEL = 0.209; PFA = 0.171) obtained values of 0.418 in SEL and 0.342 in PFA populations. Since the additive genetic variance (σ_A^2) in half-sibs is estimated by $\sigma_f^2/0.25(\sigma_f^2/r_{xy})$, where σ_f^2 is family genetic variance, clearly the false assumption that of half-sibs families will lead to 40% and 27% overestimate of σ_A^2 in SEL and PFA populations, respectively. Finally, these results illustrate the importance of knowing the mating system in programs of genetic breeding and genetic conservation, even in dioecious species.

ACKNOWLEDGMENTS

The authors would like to thank Selma Maria Bozzite de Moraes and Elza Martins Ferraz for the allozyme analyses and Alexandre Marques da Silva, Gelson Dias Fernandes, Ailton dos Reis, Manoel Fernandes da Rocha Bonfim and José Cambuim for the assistance with the field work. This project was supported by the FAPESP. We gratefully acknowledge the two anonymous reviewers.

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