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CORRELATED MATING IN POPULATIONS OF A DIOECIOUS BRAZILIAN CONIFER, *ARAUCARIA ANGUSTIFOLIA* (BERT.) O. KTZE.

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ABSTRACT

The mating system in thirteen *Araucaria angustifolia* (Bert.) O. Ktze populations from three regions in Brazil was investigated by using seven enzyme gene loci. The mating system of this wind-pollinated dioecious sub-tropical conifer was analyzed for correlated matings. The pollen allele frequencies were heterogeneous, indicating deviations from random mating. Differences between multilocus and single-locus mating system parameters ($\hat{t}_m - \hat{t}_s$) ranged from 0.018 to 0.061 and were significant for all populations, suggesting a spatial genetic structure and biparental inbreeding. The estimated maternal fixation indices ($\hat{F}_m$) were statistically different from zero. Negative indices were observed in Campos do Jordão-exploited forest, whereas in Irati and Caçador populations they were positive. The estimates of paternity correlations ($\hat{r}_p$) were significant and ranged from 0.106 to 0.602, with an average of 0.293 among subpopulations. These results indicated that part of the sampled progeny were full-sibs and only a restricted number of pollen donors effectively contributed to the reproduction. The estimates of average coancestry within progenies was 0.162, ranging from 0.138 to 0.20. This was higher than expected for half-sibs. The average variance-effective size for progenies ranged between 2.50 and 3.62, with an average of 3.09 among populations. The variation among effective pollen clouds contributed markedly to the differentiation of the seed progeny collected from individual trees. These results are discussed in the context of genetic improvement strategies, gene conservation, and seed collection for restoring degraded forests.

Key words: mating system, paternity correlation, coancestry, effective pollen clouds, population size.

INTRODUCTION

The genetic structure of tree populations and their dynamics in consecutive generations are related to their mating systems (CHELIAK *et al.* 1985). In tree species, random mating usually prevails favoring recombination and high levels of genetic variation. On the other hand, when such species inbreed by either mating among relatives or selfing, often show strong inbreeding depression (FRANKLIN 1970). Knowledge of the mating system can be applied to seed collection programs if seed of good quality is required for further improvement and operational plantings.

RITLAND (1989) developed the "single pairs" model which describes reproductive events in which selfing and paternity correlations are considered. According to this model, two individuals in a given family may both have originated from selfing, one from self- and one from cross-fertilization, or both from cross-pollination. In the latter case, they may have a common pollen parent (full sibs) or only a common seed parent (half-sibs). The selfing correlation is induced by the variation in outcrossing rates between individuals and measures the excess of plants within progenies that results from selfing compared to the proportion expected under random mating. The genetic correlation between two individuals in a family depends on main aspects of the mating system such as: (1) biparental inbreeding, (2) selfing, (3) selfing correlation, r_s, and (4) paternity correlation, r_p. The paternity correlation results from non-random mating and measures the proportion of full-sibs within progenies (RITLAND, 1989; BROWN, 1990). In obligatory outcrossing systems such as in dioecious species, the biparental inbreeding and paternity correlation alone explain the genetic relationship between sibs in a progeny.

Araucaria angustifolia is a dioecious wind-pollinated species which plays an important economic, ecologic and social role in southern Brazil (CARVALHO, 2003). The wide natural distribution range (between 19° 15' S and 31° 30' S) possibly contributes to the degree of genetic variation found in this species (SHIMIZU *et al.* 2000, AULER *et al.* 2002, SOUSA *et al.* 2002). This reflects on evolutionary events during its migration history (SOUSA *et al.* 2004). However, the natural forests have been so strongly exploited that only isolated fragments remain today. Due to its unsustainable exploitation, the species was included in the list of endangered species. Urgent measures of Araucaria gene conservation are necessary to guide restoration programs (SOUSA *et al.* 2002). Studies on the reproduction system are important as aids for planning *ex situ* conservation and the restoration of degraded forests. As in other obligatory outbreeders, some factors other than chance influence reproduction of *A. angustifolia*. Seed dispersion in this species is autochorous. The heavy seeds fall close around the seed tree (CARVALHO 2003) and this leads to spatial genetic structures within natural populations and inbreeding among offspring, since the effective pollen is also suspected to be dispersed over a limited distance (SOUSA 2000, SOUSA & HATTEMER 2003). Another aspect to be considered is the possibility of increased biparental inbreeding as a consequence of reduced population size in the fragments. In this case, coancestry increases within populations. These factors strongly affect natural forest regeneration, tree improvement practices, *ex situ* conservation and seed collection for restoration of degraded forests.

The present study is focused on the determination of the mating system of *A. angustifolia*, specifically with regard to correlated mating. The objectives were to estimate parameters of paternity correlation, variation in effective pollen clouds, average coefficient of coancestry, and variance-effective population size.

MATERIAL AND METHODS

Araucaria populations were sampled to represent (1) Campos do Jordão, located in São Paulo State, at 22° 44' S, 43° 44' W at an altitude of 1,800 m above sea level; (2) Irati, located in the State of Paraná, at 25° 30' S, 50° 36' W and altitude of 880 m; and (3) Caçador, located at 26°50' S, 50° 55' W at 1,100 m elevation. The Caçador population is the oldest and the most exuberant forest in this study in which araucaria trees reach 77 cm d.b.h. on average (Table 1), and young trees are almost absent. This particular stand has been set aside for *in situ* conservation of araucaria and associated species. Differences in age structure between populations can be readily recognized from the average d.b.h. (Table 1) and from the number of non-reproductive trees in each population (SOUSA, 2000). The forest in Campos do Jordão showed the highest density (204 trees ha^{-1}) and the highest

Table 1. Details on sampling plots in *Araucaria angustifolia* populations and supopulations involved in the mating system study.

Population	Subpopulation	Area (m^2)[1]	N[2]	d[3]	Average d.b.h. (cm)	Seed trees[4]
Campos de Jordão	Natural forest	10,000	204	204	25.09	35
	Natural forest exploited	10,000	166	166	31.30	35
Irati	Natural Forest	10,000	109	109	39.30	10
	Natural Forest – Stand 1	5,000	39	78	42.10	10
	Natural Forest – Stand 2	3,000	31	104	25.40	10
	Natural forest exploited	3,000	17	57	32.70	10
	Plantation	3,000	88	294	29.96	10
Caçador	Natural Forest	10,000	72	72	77.00	10
	Natural Forest – Stand 1	3,000	25	84	68.00	10
	Natural Forest – Stand 2	3,000	31	104	73.50	10
	Natural Forest – Stand 3	3,000	23	77	71.60	10
	Natural Forest – Stand 4	3,000	31	104	67.10	10
	Natural forest exploited	3,000	17	57	65.50	10

1) plot area
2) number of trees in the area
3) number of trees per hectare
4) number of sample trees

frequency (55 %) of young trees; the Irati population had 64 % young trees. The average d.b.h. was the smallest in Campos do Jordão, intermediate in Irati and the largest in Caçador, whereas the number of non-reproductive trees followed the opposite trend. The sex ratio over all populations did not differ significantly from 1:1. Only one subpopulation showed significant deviation from the 1:1 sex ratio, with an excess of females (SOUSA 2000).

The number of trees from which seed was sampled ranged from 10 to 35 per subpopulation (Table 1). Seeds from 35 trees were collected in two Campos do Jordão populations (natural and exploited forest). In other populations (Irati and Caçador) seeds from 10 trees in a large forest and 10 trees in surrounding smaller fragments (subpopulations) were collected. The forest fragment area ranged from 12 to 60 hectares. The smaller stands within locations were at least 3 km apart from each other. The sampling included three selectively exploited araucaria stands in each location and a 37 year-old plantation in Irati which had been subjected to two thinnings. Sexually mature trees were identified as either male or female and had their stem diameter (d.b.h.) measured and the distances from each other recorded. The sampling at each location consisted of one-hectare plots and 0.3 ha plots in each sub-population.

Genotyping of seed trees and seeds

Twenty megagamethophytes from each of the 70 seed trees in Campos do Jordão (35 trees per stand) and eight megagamethophytes from each of 110 trees in Irati and Caçador were used to infer about genotypes of the standing trees. Joint enzyme electrophoresis of megagamethophytes and embryo tissues provided identification of the respective pollen contributions. Seven enzyme gene loci were scored: *Got-B, Got-C, Pgm-A, Mdh-B, Skdh-A, Skdh-B* and *6Pgdh-B*. *Skdh-A* locus showed very little variation and was fixed in some subpopulations. There was no evidence of linkage among these loci. A total of 2,280 zymograms were prepared for this study. The methodology for enzyme electrophoresis of embryo and megametophyte tissues was reported by SOUSA *et al.* (2002) together with details of the genetic interpretation of the zymograms.

Data analysis

Due to the large sample size (20 seeds from each of 35 seed trees), the natural and exploited Campos do Jordão subpopulations were analyzed separately. Only three seeds from the natural and 28 from the exploited stands in Campos do Jordão did not produce clear bands and were omitted from the data set. Due to small sample sizes representing the subpopulations (8 seeds each from 10 trees), the analyses were performed in two stages: samples were analyzed separately for each subpopulation and location. Subsequently, samples from natural subpopulations within the main forests were pooled. This resulted in a sample size of 70 trees in Campos do Jordão, 50 in Irati and 60 in Caçador. Prior to pooling, allele frequencies were tested for homogeneity by using the F_{ST} statistics (SWOFFORD & SELANDER 1989).

The maternal genotypes as inferred from megagametophytes were used in mating system analysis. As araucaria is a dioecious species, the analysis was based on the estimation of correlated mating (RITLAND 1989) by using the MLTR software (RITLAND 1994). Estimates of the fixation index of seed trees ($\hat{F}_m$), the single-locus ($\hat{t}_s$) and multilocus outcrossing rates ($\hat{t}_m$), and the differences between the single-locus and multilocus outcrossing rates ($\hat{t}_s - \hat{t}_m$) were drawn for Campos do Jordão (natural and exploited) and Irati and Caçador natural populations. The true outcrossing rate was considered to be unity ($t = 1$) and the correlation of selfing to be zero ($r_s = 0$). Only pollen and ovule allele frequencies and paternity correlations ($\hat{r}_p$) were estimated. The standard deviation was obtained in 500 bootstrap runs and employed to estimate the standard error (SE) at 95 % probability. According to sample sizes, the analysis was performed with two numerical methods, as suggested by RITLAND (1994, 2002). The natural and exploited forests of Campos do Jordão were analyzed by using the "Newton-Raphson" method, since the sample size was large enough. For the other populations and subpopulations with small sample sizes (10 progenies per subpopulation or 50 per population with 8 seeds each) the method of “expectation-maximization” was adopted. The heterogeneity of pollen and ovule allele frequencies was interpreted as a characteristic of the species mating system which indicates deviation from random mating. The hypothesis of homogeneity of pollen and ovule allele frequencies was tested by using the $\hat{F}_{ST}$-statistics (WRIGHT 1965). The chi-square test was applied to determine whether $\hat{F}_{ST}$ values were different from zero. The adopted test statistics was $\chi^2 = 2n\hat{F}_{ST}(k-1)$ with $(k-1)(s-1)$ degrees of freedom, where n is the number of sampled individuals (seeds) in both groups (pollen and ovules), k is the number of alleles at a given gene locus, and $s = 2$ the number of groups (*i.e.* pollen and ovules).

Due to small sample sizes and low levels of genetic variation, tests of homogeneity of effective pollen clouds fertilizing the seed trees were difficult to perform. In the two larger subpopulations of Campos do Jordão, conventional contingency tests were run for homogeneity of pollen alleles, embryo alleles and embryo genotypes, although some expectations were less than 5. Therefore, significance statements were based on the test statistics G. As an approximate procedure, the numbers of homozygous and heterozygous offspring of homozygous seed trees were arranged in $2 \times f$ contingency tables, where f is the number of seed trees, and analyzed by using the chi-square test.

The differentiation between individual effective pollen clouds in the two Campos do Jordão subpopulations was estimated by using D_j statistics as derived by GREGORIUS & ROBERDS (1986) with the use of GSED software (GILLET 1998). This statistics strongly appeals to our intuition, since it measures the proportion of genes that a family does not share with all others under study. The average of D_j estimates over progenies is denoted by δ.

The coancestry within families ($\hat{\theta}_{xy}$) was estimated from the coefficient of correlation among offsprings within families ($\hat{r}_{xy}$), derived by RITLAND (1989)

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

and, $\theta = \hat{r}_{xy}/2$ as (RITLAND 2002) and, inserting $\hat{t}_m = 1.0$ and $\hat{r}_s = 1.0$, then,

$$\hat{\theta}_{xy} = 0.125(1+\hat{F})(1+\hat{r}_p),$$

where F is the parental inbreeding coefficient and $\hat{F}$ is the inbreeding coefficient in the parental generation.

The variance-effective size ($N_{e(v)}$) of a single family was estimated from the sampling variance ($\hat{\sigma}_p^2$) of an allele frequency p in an ideal population,

$$\sigma_p^2 = \frac{p(1-p)}{2N}.$$

In an ideal population, it is assumed that there is no inbreeding nor relatedness among individuals. Therefore, the census number is equal to its effective population size ($N = N_e$), and σ_p^2 can be defined as a function of N_e,

$$\sigma_p^2 = \frac{p(1-p)}{2N_e}.$$

However, this expression is not valid in populations with structured progenies. COCKERHAM (1969) defined the variance σ_p^2 of the mean gene frequency p in terms of the coefficient of coancestry (θ_{xy}) in a structured population (progenies) as

$$\sigma_p^2 = \theta_{xy}p(1-p).$$

By equating the latter two expressions, it is possible to estimate the variance-effective size of a single family by the average coefficient of coancestry within families:

$$\theta_{xy}p(1-p) = \frac{p(1-p)}{2N_e}$$

and

$$\hat{N}_e = \frac{0.5}{\hat{\theta}_{xy}}.$$

The last equation is similar to the status effective number defined by LINDGREN *et al.* (1997).

RESULTS

Allele frequencies in pollen and ovules

Seven enzyme systems were variable in thirteen populations (cf. SOUSA 2000 for data). Six gene loci had two active alleles (*Got-B, Got-C, Pgm-A, Mdh-B, Skdh-A,* and *Skdh-B*) and one locus showed three alleles (*6Pgdh-B*). Comparisons among allele frequencies in pollen and ovule pools are presented in Table 2 in terms of WRIGHT's (1965) measures of population differentiation ($\hat{F}_{ST}$). The chi-square statistics showed that pollen and ovule allele frequencies were significantly heterogeneous in 29 out of 105 comparisons (27 %). WRIGHT's (1965) $\hat{F}_{ST}$-statistics ranged from 0.000 to 0.175 (*6Pgdh-b* in Campos do Jordão, exploited population). The observed heterogeneity of allele frequencies of pollen and ovule pools indicated deviations from the assumption of random mating, presumably as a consequence of their biparental inbreeding, correlated mating and/or differentiation between allele frequencies in adult males and females.

The allele frequencies were significantly heterogeneous in Caçador subpopulation but not in

Table 2. Estimates of genetic divergence F_{ST} between outcrossing pollen gene pools and ovule gene pool in populations of *Araucaria angustifolia* and their subpopulations. Numbers indicate sample sizes.

Gene locus	Campos de Jordão		Irati						Caçador						
	Nat.	Expl.	Nat.	Stand 1	Stand 2	Expl.	Plant	ICNat.	Nat.	Stand 1	Stand 2	Stand 3	Stand 4	Expl.	CCNat.
	35	35	10	10	10	10	10	30	10	10	10	10	10	10	50
Got-B	0.024**	0.017**	0.010	0.011	0.011	0.010	0.009	0.004	0.010	0.011	0.006	0.000	0.011	0.011	0.000
Got-C	0.002	0.000	0.0.36*	0.001	0.011	0.009	0.003	0.002	0.012	0.001	0.050*	0.003	0.010	0.001	0.003
Pgm-A	0.001	0.001	0.011	0.001	0.011	0.001	0.010	0.001	0.001	0.011	0.015	0.011	0.011	0.011	0.004
Pgm-B	0.008**	0.002	0.004	0.010	0.011	0.010	0.010	0.000	0.011	0.010	0.010	0.010	0.011	0.011	0.002
Skdh-A	0.005	0.006**	0.011	0.011	0.011	0.010	0.015	0.004	0.003	0.011	0.037*	0.01*	0.011	0.010	0.005*
Skdh-B	0.000	0.013**	0.064**	0.010	0.011	0.010	0.001	0.027**	0.019	0.011	0.010	0.001	0.036*	0.034*	0.027**
6-pgdh-B	0.009**	0.175**	0.083**	0.049**	0.076*	0.023	0.112**	0.074**	0.020	0.118**	0.053**	0.062**	0.058**	0.053*	0.065**

** $P < 0.01$; * $P < 0.05$;

Nat. = Natural; Expl. = exploited; ICNat. = Irati – natural pooled; CCNat. = Cacador – natural pooled.

Irati. The $\hat{F}_{ST}$-statistics was 0.019 ± 0.022 in Irati population and 0.024 ± 0.014 in Caçador. The range of these values was similar to those observed by MITTON *et al.* (1997) in *Pinus washoensis* ($\hat{F}_{ST}$ = 0.021 to ± 0.022). Although the allele frequencies varied statistically among subpopulations in Caçador, the differences were small. Therefore, subpopulation data sets were pooled in order to determine the mating system parameter.

Inbreeding estimation

The fixation indices $\hat{F}_m$ of seed trees (Table 3) ranged from –0.116 ± 0.031 (Campos do Jordão: Exploited) to 0.101 ± 0.020 (Irati: Natural pooled). These estimates were significantly different from zero for Campos do Jordão exploited, Irati and Caçador populations, as judged by the standard error for the average $\hat{F}_m$. In the exploit-ed population in Campos do Jordão, the fixation index indicated an excess of heterozygotes (–0.116); in other populations (natural populations in Campos do Jordão Caçador, and Irati), excesses of homozygotes were detected.

Estimation of mating system parameters

In a dioecious species, every multilocus mating parameters varies from zero to one. In this study, multilocus estimates of outcrossing rates ($\hat{t}_m$) ranged from 0.972 ± 0.007 to 1.012 ± 0.007 (Table 4). However, these values were statistically not different from 1.0 for all populations. An exception was the natural population in Caçador, where the outcrossing rate was high and different from 1.0, within 95% confidence interval. The small difference observed (0.028) between $\hat{t}_m$ and 1.0 can be attributed to sampling errors due to either low polymorphism at some gene loci, restricted sampling within progenies (8 seeds) or biparental inbreeding. With biparental inbreeding, the multilocus outcrossing estimate can be significantly lower than one, even for complete outcrossing species, because the bias due to biparental inbreeding is only gradually removed as the number of loci increases. In spite of these deviations, *A. angustifolia* can be considered a perfectly outcrossing species.

The values of single-locus outcrossing estimates ($\hat{t}_s$) ranged from 0.911 ± 0.005 (Caçador) to 0.995 ± 0.011 (Campos do Jordão – Exploited) (Table 3). By using the criterium of the 95% confidence interval, it was observed that $\hat{t}_s$ were signifi

Table 3. Estimates of the mating system parameters in *Araucaria angustifolia* populations (means ± standard errors at the 95 % probability level).

Population	n	$\hat{F}_m$	$\hat{t}_m$	$\hat{t}_s$	$\hat{t}_m - \hat{t}_s$
Campos do Jordão – natural	35	0.029 ± 0.037	0.997 ± 0.007	0.950 ± 0.008	0.047 ± 0.006
Campos do Jordão – exploited	35	–0.116 ± 0.031	1.012 ± 0.007	0.994 ± 0.011	0.018 ± 0.007
Irati – natural pooled	30	0.101 ± 0.020	0.998 ± 0.001	0.959 ± 0.002	0.039 ± 0.002
Caçador – natural pooled	30	0.043 ± 0.005	0.972 ± 0.007	0.911 ± 0.005	0.061 ± 0.005

n – number of families; $\hat{F}_m$ – fixation index in maternal trees; $\hat{t}_m$ – multilocus outcrossing rate; $\hat{t}_m$ – single-locus outcrossing rate.

Table 4. Estimates of paternity correlation ($\hat{r}_p$), of coancestry coefficients (θ_{xy}) and variance-effective size ($\hat{N}_{e(v)}$ of single families in *Araucaria angustifolia* (means ± 95 % confidence intervals).

Population	Subpopulation	$\hat{r}_p$	$1/\hat{r}_p$	θ_{xy}	$\hat{N}_{e(v)}$
Campos de Jordão	Natural	0.209 ± 0.028	4–5	0.151	3.31
	Exploited	0.396 ± 0.033	2–3	0.174	2.86
Irati	Natural	0.432 ± 0.085	2–3	0.179	2.79
	Stand 1	0.447 ± 0.060	2–3	0.181	2.76
	Stand 2	0.211 ± 0.058	4–5	0.151	3.30
	Exploited	0.511 ± 0.148	1–2	0.189	2.65
	Plantation	0.602 ± 0.175	1–2	0.200	2.50
	Pooled	0.395 ± 0.028	2–3	0.174	2.87
Caçador	Natural	0.237 ± 0.055	4–5	0.155	3.20
	Stand 1	0.173 ± 0.068	5–6	0.147	3.41
	Stand 2	0.110 ± 0.037	9–10	0.139	3.60
	Stand 3	0.222 ± 0.037	4–5	0.153	3.27
	Stand 4	0.106 ± 0.037	9–10	0.138	3.62
	Exploited	0.160 ± 0.063	6–7	0.145	3.45
	Pooled	0.226 ± 0.014	4–5	0.154	3.24
General average		0.293	3–4	0.162	3.09

Table 5. Chi square tests (χ^2) for homogeneity of allelic frequencies of effective pollen between seed trees.

Locus	Campos do Jordão Nat.		Campos do Jordão Exp.		Irati		Caçador	
	χ^2	d.f.	χ^2	d.f.	χ^2	d.f.	χ^2	d.f.
Got-B	26.15	18	33.52*	19	30.49	34	67.01**	48
Got-C	50.96*	23	40.45*	30	49.56*	32	55.53**	46
Pgm-A	21.45	25	41.45**	21	86.74**	33	42.65	43
Mdh-B	8.57	5	4.38	13	54.50**	34	61.42**	49
Skdh-B	36.55	24	2.92	3	0.00	34	40.85	43
Skdh-C	0.0	34	50.03**	25	148.47**	34	82.62**	49
6pgdh-B	65.60**	44	94.30**	48	0.00	24	39.50	49

** $P < 0.01$; * $P < 0.05$.

Table 6. Average genetic differentiation (δ) of effective pollen clouds among 35 seed trees based on data from different tissues in each subpopulation at Campos de Jordão.

Gene locus	Pollen alleles	Ovule alleles	Embryo alleles	Unordered embryo genotypes
		Natural subpopulation		
Got-B	0.101*	0.291***	0.173***	0.295--
Got-C	0.027 n.s.	0.057***	0.036***	0.070 –
Pgm-A	0.068 n.s.	0.204***	0.119***	0.196***
Mdh-B	0.080 n.s.	0.221***	0.120***	0.185***
Skdh-B	0.123***	0.227***	0.160***	0.231***
6pgdh-B	0.229***	0.239***	0.148***	0.285–
Average	0.105	0.207	0.126	0.215
		Exploited subpopulation		
Got-B	0.101***	0.243***	0.154***	0.260***
Got-C	0.045*	0.103***	0.061***	0.115***
Pgm-A	0.088**	0.219***	0.112***	0.192***
Mdh-B	0.088 n.s.	0.323***	0.179***	0.246***
Skdh-B	0.081**	0.171***	0.093***	0.174***
6pgdh-B	0.251**	0.152***	0.150***	0.297 –
Average	0.109	0.202	0.125	0.214

Asterisks denote the results of pertinent G-tests with 34 or 68 (gene locus *6-Pgdh-b*) degrees of freedom.

cantly different from 1.0 in three out of four populations (Campos do Jordão – Natural, Irati and Caçador).

The differences between single-locus and multilocus outcrossing rates $(\hat{t}_s - \hat{t}_m)$ allowed to infer about biparental inbreeding. These differences ranged from 0.018 ± 0.007 (Campos do Jordão, Exploited) to 0.061 ± 0.005 (Caçador, cf. Table 4) and were statistically significant for all populations with 95% probability. These suggest the occurrence of outcrossing among relatives and some degree of spatial genetic structure within populations of araucaria.

Paternity correlation

The estimated paternity correlations (Table 4) ranged from 0.106 ± 0.037 (Irati: Stand 5) to 0.602 ± 0.175 (Irati: Plantation) with an average of 0.293. These were statistically significant in all populations and subpopulations with 95 % probability. The estimates of 29 % on average suggested deviations from random mating and indicated notable proportions of full-sibs. The numbers of effective pollen donors estimated by the reciprocal of this correlation varied among subpopulations but were rather small and ranged from 1 or 2 (Irati: Plantation) to 9 or 10 (Irati: Stand 2), with an average of 3 to 4 male mating partners.

Coancestry and variance-effective population size

The coancestry coefficients $(\hat{\theta}_{xy})$ exceeded 0.125 which is the expected value in ideal half-sib families in all populations. Estimates ranged from 0.138 (Caçador: Stand 4) to 0.200 (Irati: Plantation), with an average of 0.162 (Table 4). The variance-effective size $(\hat{N}_e)$ was lower than expected in half-sib families of infinite size (4). Estimates varied between 2.50 (Irati: Plantation) and 3.62 (Caçador: Stand 4).

Differentiation of effective pollen clouds and seeds

Based on chi-square test results, it is suggested that the effective pollen clouds varied among seed trees in the Campos do Jordão subpopulations (Table 5). Fifteen out of 28 tests showed statistically significant differences between the observed and the expected values.

Locus *Skdh-C* was omitted from the analysis due to lack of variation in the sample from the natural subpopulation. Although significance statements on pollen cloud differentiation in natural subpopulations were not consistent (Table 6) with those based on Chi-square test (Table 5), the results on the exploited population were similar. The estimated degrees of genetic differentiation varied widely among gene loci.

The allelic variance among ovules reflects only the variation among seed trees. Therefore, it is expected to be highly significant in all instances. It is also natural that allelic structures of the female contributions to the seeds vary more strongly than those of male alleles, because they represent the effective gametes of only one individual all the time. In comparison, the variation in individual contribution to the pollen clouds had less influence on variation among embryos at most gene loci. Also, at most gene loci, the degree of variation increased from pollen alleles to embryo alleles and over to the unordered genotypes of these embryos. The locus *6Pgdh-b* was remarkable in this respect. The allele b_1 is related to sex expression (SOUSA *et al.* 2004). This allele is extremely rare in female trees, as inferred from their megagametophytes. However, it is not strictly confined to male trees (SOUSA *et al.* 2002). Among the pollen contributions to the seeds collected from two stands, the frequency of allele b_1 was 0.361. This is less than 0.5, as expected in the case of stringent sex determination. This indicates that only 36 % of the offspring carry this allele in heterozygous condition. Most unlike the pooled Irati and Caçador populations (Table 5), the variable frequency of allele b_1 among the effective pollen clouds has led to their strong average differentiation (δ) within both stands. The respective estimates were almost equal to those of the female contributions (0.229 in the natural stand) or even exceeded those (0.251 in the exploited stand). Although at all other gene loci δ was greater in embryos in comparison to male alleles, this pattern was reversed at gene locus *6Pgdh-b*, where differences were leveled out to only 0.150 and 0.148, respectively, by the female contributions which largely lacked b_1.

The variation among unordered genotypes was high. This observation has a bearing on seed collection. Although the natural and the exploited subpopulations differed markedly in paternity correlations, the pollen cloud differentiation averaged over seed trees and gene loci were very much the same (0.109 and 0.105, respectively).

DISCUSSION

Pollen and ovule pool differentiation

The allele frequencies in the effective ovules and pollen varied significantly among subpopulations and populations in 28 percent of the tests (Table 2). The reason can be related to allelic differences between reproducing females and males, influx of external pollen, selection between the stages of pollination and census, or small numbers of sampled seeds (MURAWSKI & HAMRICK 1992; LEE *et al.* 2000). The difference in allele frequencies between sexes may be plausible, since *Araucaria* is dioecious.

Inbreeding

By comparing the single-locus to multilocus ($\hat{t}_m - \hat{t}_s$) outcrossing rates, it is possible to quantify the rate of biparental inbreeding in populations (RITLAND & JAIN 1981, RITLAND & EL-KASSABY 1985). The average single-locus outcrossing rate was lower than the multilocus outcrossing rate for all populations, suggesting biparental inbreeding as a presumable consequence of clustering of related trees in combination with limited pollen flow within populations. *A. angustifolia* normally disperses its heavy wingless seeds by autochory around the seed trees (CARVALHO, 2003), forming dense sapling clusters. This situation is expected to favor a family structure within populations, since it increases the probability of establishment of relatives in close neighborhood. The probability of neighbor mating is increased by the presumably short transport distances of effective pollen within populations.

Knowledge on the genetic structure of *A. angustifolia* populations can be a tool for rational exploitation. The logging of neighbor trees eventually has a positive effect by blurring existing family structures, since it reduces the chance of mating among relatives. This possibility may have occurred in the exploited population of Campos do Jordão. However, for successful management of a population, it is desirable to quantify the degrees of relatedness among trees. The use of highly variable genetic markers such as microssatellites in combination with specific programs to estimate the coefficients of relatedness could contribute to the sustainable management of this species.

In dioecious species, a mating preference among relatives is the only thinkable source of inbreeding. Evidence of inbreeding was detected by fixation indices of seed trees ranging between 0.029 to 0.101 estimated in three populations. However, for the natural population in Campos do Jordão, this index did not prove significant (Table 3). Inbreeding has been detected in other studies involving natural and fragmented populations of *A. angustifolia*. Shimizu *et al.* (2000) detected a fixation index of 0.063. Fixation indices between –0.120 and +0.402 were observed by Auler *et al.* (2002) in populations with different levels of disturbance. In more conserved populations, the indices tended to be lower or even negative whereas, in exploited forest, positive and higher values were observed. According to Auler *et al.* (2002), structures of all populations with excess of inbreeding shows close fit to the structure expected under inbreeding, indicating the mating system as the cause of excess in homozygotes. Considering that in outcrossing species the inbreeding in the progeny generation is the same as the coefficient of coancestry between outcrossing parents ($F = \theta_{xy}$), estimates of the fixation coefficient ranging between 0.043 and 0.101 in the Caçador and Irati populations could be attributed to mating between second-degree cousins ($\theta_{xy} = 0.031$) or ideal half-sibs ($\theta_{xy} = 0.125$).

Correlated mating

The paternity correlation measures the proportion of full-sibs in outcrossed progenies (Ritland 1989). The results showed significant correlated mating in all populations and subpopulations. The paternity correlation in *A. angustifolia* originated from a restricted number of male trees contributing effective pollen. Mixing of this pollen shed by different trees in an anemophilous species and multi-paternity of progenies of a single seed tree depend also on the transfer distance that is presumably short in *A. angustifolia*. Also, in dioecious species such as this, only half of the individuals shed pollen. The female trees only impede pollen dispersal, as was pointed out in a report on the reproduction of the European yew, *Taxus baccata* L., another dioecious gymnosperm, by Rajewski *et al.* (2000). Other reasons for low numbers of male mating partners have been mentioned above. These reasons and asynchrony of flowering phenology, limitation of pollen dispersal and possible variation of male fecundity can more strongly reduce the effective pollinator number of a given seed tree.

Sharing of paternity within a family could arise from multiple contributions of pollen from a single male parent, or from mating to a small number of near-neighbors in general (Sun and Ritland 1998). In the latter case, if there were n male neighbors to a seed tree and each had equal chances of becoming an effective male parent, and if consecutive matings were independent (eventually independent pollinator visits), then the probability of outcrossing twice to the same mate would be $1/n$ and the effective number of males is $1/r_p$ (Ritland 1989). The number of effective pollinators (Table 4) was unexpectedly low.

In dense populations where abundant pollen is produced, one might expect lower paternity correlation and a higher proportion of ideal half-sibs within progeny than in less dense populations, except if pollen dispersal is inefficient and if there is much mismatch of flower phenology. However, the results of the present study did not support this hypothesis. The estimated paternity correlations tended to be higher in populations with higher density. For example, in the Irati plantation with almost 294 trees per hectare (Table 1), the paternity correlation was high (0.602). For both natural and exploited populations in Campos do Jordão, with equally high density, 204 and 166 trees per hectare, respectively, the paternity correlation was also high (natural: $\hat{r}_p = 0.209$; exploited: $\hat{r}_p = 0.396$). In the natural population as well as stand 2 in Irati, the density was 109 and 104 trees per hectare, respectively, and the paternity correlations were 0.211 or higher. On the other hand, in populations with density of only 57 (Exploited population, Caçador) and 84 (Stand 1, Caçador) per hectare, the paternity correlation tended to be lower, ranging from 0.160 to 0.173. However, the Spearman correlation coefficient between density (*d*, see Table 1) and the coefficient of paternity correlation was positive ($r_{Spearman} = 0.417$) (Table 5), although not significant, according to a Student test ($t = 1.52$, $P > 0.05$). This condition suggests that increasing density in natural populations must lead to an increase in correlated matings and stronger deviation from random mating in *A. angustifolia*. It is possible that the density of individuals per unit area contributes to correlated mating together with other elements of the biology of the species such as asynchronous flowering and differential male fertility. Therefore, in less dense populations, the distribution and the number of paternal trees contributing to given progenies can be higher than in more dense populations. Also, increased atmospheric turbulence might support the dispersal of the relatively heavy pollen which has no air sacs and sediments with a high speed (Sousa & Hattemer 2003). El-Kassaby &

JAQUISH (1996) noted this trend when comparing populations of *Larix occidentalis* Nutt. with different densities. These authors detected low (0.001 and 0.024) and non-significant ($P > 0.05$) paternity correlation in low-density populations. However the paternity correlation was higher (0.062 and 0.104) and significant ($P < 0.05$) in high density populations. They suggested that high density supposedly limits the pollen movement within populations. The same has possibly occurred in *A. angustifolia.*

Wind is apparently a more efficient pollen dispersal agent to promote multi-paternal mating when compared to animal dispersion agents. With the exception of Irati plantation that seems to be atypical in this respect because of high tree density, all other paternity correlations ranged from 0.106 to 0.511. Estimated paternity correlations have been lower for other wind-pollinated species such as *Larix occidentalis* Nutt. (EL-KASSABY & JAQUISH 1996), *Pinus washoensis* Manson & Stockewell (MITTON *et al.* 1997), *Pinus martinezii* T.F. Patterson (LEDIG *et al.* 2000), *Pinus pinceana* Gordom (LEDIG *et al.* 2001), *Picea mariana* (Mill.) BSB (PERRY & BOUSQUET 2001), *Abies amabilis* (Dougl. ex Forbes) and *Tsuga heterophylla* (Raf.) Sarg. (EL-KASSABY *et al.* 2003) and ranging from 0.001 to 0.389. On the other hand, estimated paternity correlations for animal-pollinated tropical trees have been higher. In *Eucalyptus marginata* (MILLAR *et al.* 2000), $\hat{r}_p$ estimates ranged from 0.530 to 0.920, in *Cariniana legalis* (Mart.) Ktze (SEBBENN *et al.*, 2000) from 0.212 to 0.324, in *Dryobalanops aromatica* Gaertn. (LEE 2000), from 0.107 to 0.428, in *Eucalyptus camaldulensis* Dehnh (BUTCHER & WILLIAMS 2002), from 0.079 to 0.365, in *Enterolobium cyclocarpum* Jacq. (ROCHA & AGUILAR, 2001) from 0.174 to 0.462, in *Caryocar brasiliense* Camb. (Dougl. ex Forbes) (COLLEVATTI *et al.*, 2001) from 0.042 to 0.205, and in *Pachira quinata* (Jacq.) Alverson (FUCHS *et al.*, 2003) from 0.470 to 0.777. Under the given conditions, wind is obviously a more effective pollen vector than animals.

Coancestry coefficient

The coefficient of coancestry expected in open-pollinated progenies of an ideal population (infinite size, random mating, discrete generations, absence of selection, mutation and migration) is 0.125. This means that the probability to sample two alleles that are identical by decedent at a locus in two individuals of the same family is 12.5 %. The estimated coefficients of coancestry ranged from 0.138 to 0.200. These were 9.4 % to 37.5 % higher than expected in progenies drawn from an ideal population. This result implies that although *A. angustifolia* is a typical outcrossed species, at each generation new individuals are introduced in the populations with higher degrees of relationship than expected by chance. Consequently, there is an increasing chance to create a spatial genetic structure and to generate biparental inbreeding. Although there is no study on inbreeding depression in *A. angustifolia*, this is believed to occur and to produce the same effects as those detected in other conifers.

The correlation of relatives (r_{xy}) within half-sib family estimates one quarter of the additive genetic variance (NAMKOONG 1966; SQUILLACE 1974). In random-mating populations, the coefficient of relatedness (r_{xy}) between individuals within families would be twice the respective coefficient of coancestry ($r_{xy} = 2\theta_{xy}$). In the present study, the estimated correlations of relatives within open-pollinated families ranged from 0.276 to 0.400 averaging 0.324. Based on the average estimate of the coefficient of coancestry, it would be more suitable to consider the correlation coefficient of 0.324 instead of 0.25 in heritability studies and improvement programs in which open-pollinated families are used.

Variance-effective population size

The variance-effective size measures the genetic representation of progeny arrays in a given population relative to that of an ideal population (KIMURA & CROW 1963; CABALLERO 1994). In an ideal population, the variance-effective size of a single progeny with infinite size attains its maximum value, $\hat{N}_e = 0.5/0.125 = 4$, where 0.125 is the expected coefficient of coancestry in half-sib progenies. The estimates of variance-effective size in this study ranged from 2.50 to 3.62 and were consistent with the coefficient of coancestry, *i.e.*, lower than expected for half-sib progenies. The variance-effective size is affected by the progeny sample size (n), by inbreeding, and by the average coancestry within progenies. In the present study, the estimates were made assuming that the sample size tends to infinity ($n \rightarrow \infty$) in such a way that they are affected only by the average coancestry between individuals within progenies.

The variance-effective size is an important parameter to determine sample sizes required for *ex situ* gene conservation and seed collection to restore degraded populations. This parameter is also important in tree breeding for monitoring the size of selected populations and to predict the number of generations that a population can be subjected to selection. Based on the relationships between effective size ($N_{e(required)}$) required for the conservation of a given population and the variance-effective size ($N_{e(v)}$) of a single progeny, it is possible to estimate the number of seed trees ($\hat{m} = N_{e(required)} / N_{e(v)}$) required for seed collection. For example, if the objective were to maintain an effective population size of 150 and a large number of seeds were collected per tree, the number of seed trees has to be between 42 to 60 in the present populations of *A. angustifolia*.

Differentiation of effective pollen clouds and seeds

Genetic heterogeneity of pollen allelic frequencies among seed trees (Tables 5 and 6) has been detected by SOUSA & HATTEMER (2003) in the Campos do Jordão population where only a moderate percentage of male trees (20 %) contributed with effective pollen. The same was true for other populations, although it could not be verified at the time of seed collection. Genetic variation in effective pollen clouds was implied based on paternity correlations, since any reduction in the number of male mating partners leads to a chance effect on the allelic structure of pollen clouds. This can be associated with very low density of reproducing male trees, lack of flowering synchrony, variation in male tree fertility or predominant neighbor mating in combination with a spatial genetic structure within natural populations. An additional reason can be the exceptionally low floatation potential of *Araucaria* pollen (SOUSA & HATTEMER, 2003). Whatever the reason for this condition, the variation in effective pollen clouds represents a deviation from random outcrossing and thus violates the assumptions made for estimating mating system parameters. Therefore, estimates of $\hat{t}_m$ and $\hat{t}_s$ have to be taken with a grain of salt.

The general relationship $\hat{\delta}_{ge} < \hat{\delta}_{ga} < \hat{\delta}_{go}$ which refer to differentiation at genic, gametic, and genotypic levels, respectively, as reported by GREGORIUS & ROBERDS (1986), does not hold in all instances. There are obvious deviations from the statement that more complex organization of the genetic material leads to additional opportunities for differentiation. However, $\hat{\delta}_{ge} < \hat{\delta}_{go}$ does hold, since the estimated genotypic differentiation is up to twofold of that of the alleles.

Nonetheless, the variation in effective pollen clouds has an impact on designing procedures of seed collection. Given that seeds collected from various trees differ genically and even more so genotypically, they should be collected from a large number of trees. This supports the strong contribution of the variation among female alleles, reflecting the variation among seed trees. Furthermore, seeds should be collected after the flowering of many males in the population in order to achieve better sampling of the male gene pool which eventually differs from that of the seed trees (Table 2). In particular, this is true in view of the eventual indication of male sex by the presence of allele b_1 at the locus *6Pgdh-B*. Differentiation statistics D_j of embryo genotypes at this locus averaged 0.285 and 0.297 in the two subpopulations and ranged from 0.109 to 0.460 and from 0.098 to 0.509, respectively. One may speculate that in view of highly variable sex ratios within progenies, the risk of interfering on population sex ratio is lowest if equal proportions of seed are collected from many trees.

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