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ESTIMATION OF THE MATING SYSTEM OF *Eucalyptus benthamii* Maiden at Cambage PROGENY[1]

ESTIMATIVA DO SISTEMA REPRODUTIVO DE PROGÊNIES DE *Eucalyptus benthamii* Maiden at Cambage

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RESUMO - *Eucalyptus benthamii* é uma importante espécie utilizada em plantios florestais monoclonais e híbridos em todo o mundo. Poucas populações naturais da espécie permanecem e, assim, foi colocada na lista de espécies ameaçadas de extinção. Foram amostradas 111 sementes de *E. benthamii* de nove progênies em duas procedências (Candói - duas progênies; Vale do Corisco - sete progênies). As amostras foram genotipadas usando 19 locos microssatélites. Um alto número de alelos (de 80 a 114) foi encontrado nas progênies estudadas. O índice médio de fixação foi baixo para todas as nove progênies e a taxa de cruzamento multiloco individual (t_m) variou de 0,990 até 1,0. Apesar do alto t_m, as progênies apresentaram altos níveis de acasalamento entre parentes ($t_m - t_s$), variando de 0,077 até 0,244. A coancestria de grupo (Θ= 0,181) atingiu valores maiores que os esperados para progênies de meios-irmãos (Θ= 0,125). Algumas progênies de polinização aberta são compostas de meio-irmãos e irmãos completos. Este estudo fornece informações importantes para programas de manejo e melhoramento de *E. benthamii* que podem subsidiar novas estratégias de conservação e produção de híbridos desta espécie.

Palavras-chave: Marcadores microssatélites; Genética de populações; Coancestria.

ABSTRACT - *Eucalyptus benthamii* is an important species used in monoclonal and hybrid forest plantations around the world. Few natural populations of the species remain, and as such it has been placed on the list of endangered species. We sampled 111 seeds of *Eucalyptus benthamii* from nine progenies across two provenances (Candói - two progenies; Vale do Corisco - seven progenies). Samples were genotyped using 19 microsatellite loci. A high number of alleles (from 80 to 114) were found in the studied progenies. The average fixation index was low for all nine progenies and the individual multilocus outcrossing rate (t_m) ranged from 0.990 to 1.0. Despite the high t_m, progenies showed high levels of mating among relatives ($t_m - t_s$), ranging from 0.077 to 0.244. The group coancestry (Θ= 0.181) reached values greater than those expected for half-sib progeny (Θ= 0.125). Some open-pollinated progenies are composed of both half-sibs and full-sibs. This study provides important information for management and breeding programs of *E. benthamii* that can help support new strategies for conservation and hybrid production of this species.

Keywords: Microsatellite markers; Population genetics; Coancestry.

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1 INTRODUCTION

In Brazil, the use of eucalypts by the forest industry has been increasing steadily, with plantations currently established on more than seven million hectares (ha) (IBÁ, 2021). The wood is mainly used for pulp and paper, firewood, and charcoal production, with the latter being used to fuel to steel mills (Júnior et al., 2013). Over the last 50 years, as a result of efforts focusing on genetic improvement and the use of advanced silvicultural techniques, the biomass production capacity of eucalypts has grown significantly (Andrade et al., 2018). The versatility of its wood, which is the main product, is another feature that explains the prevalence of Eucalyptus plantations compared to other species.

Temperate region Eucalyptus species, such as *Eucalyptus benthamii*, have received special attention from the industry, particularly for planting in colder regions with the occurrence of frosts. The species is resistant to cold (Higa and Pereira, 2003; Diniz et al., 2019), and is able to withstand temperatures of -10 °C and up to 25 annual frosts. As such, it has potential for use in reforestation in southern Brazil, providing an option for locations in which frequent and severe frosts occur (Paludzyszyn Filho et al., 2006). Thus, *E. benthamii* has been showing pronounced growth and development in the region.

Deforestation and flooding has had a significant impact on natural *E. benthamii* populations, and today only three small populations of the species remain. These populations are located south-west of Sydney, Australia, with a total of about 10,400 trees (Skinner, 2003). Because of this strong genetic bottleneck, studies on the species are necessary. Understanding how inbreeding can affect these populations and documenting the reproductive system to inform seed collection, conservation, and genetic improvement is of utmost importance.

Genetic bottlenecks can lead to increased inbreeding and kinship within populations (Tambarussi et al., 2017). Related individuals may crossbreed, resulting in inbreeding depression and causing subsequent generations to have diminished adaptive capacity. In general, species of the Eucalyptus genus present mixed mating systems, which can make it easier for related alleles to be passed on to the next generation. For improvement, understanding the mating system has a direct impact on the estimation of genetic parameters (Tambarussi et al., 2018). When genetic variance is estimated for open-pollinated progenies (half-sibs), additive genetic variance is σ_A^2=4σ_p^2, where σ_p^2 is variance between progenies. According to Cockerham and Weir (1984): $\sigma_p^2=2\theta\sigma_A^2+0\sigma_D^2+0D_1+0D_2+0H^2= \sigma_A^2 = \sigma_p^2/2\theta$; where, σ_D^2 is dominance genetic variance; θ is the coancestry coefficient; D_1 is the sum of the covariance between additive and dominance effects for all loci; and D_2 is the squared average of the dominance effects for all loci. Thus, in perfectly allogamous species with F=0 and θ=1/8, the $\sigma_A^2 = \sigma_p^2/(2.1/8) = 4\sigma_p^2$ or $\sigma_A^2 = 4\sigma_p^2$. However, with a mixed mating system corrections must be applied to estimate additive genetic variance (Tambarussi et al., 2018), which reduces genetic gains.

Thus, the present study aimed to analyze the *E. benthamii* mating system and understand how inbreeding is distributed among and within populations.

2 MATERIAL AND METHODS

2.1 Study progenies and sampling

The studied material was sampled in a provenance and progeny test established in 2015 in Mafra, Santa Catarina (26º10’N and 50º04’W). Two progenies from Candói (C) and seven from Vale do Corisco (VC), Paraná, Brazil, were sampled. Leaves from 111 individuals, of which 24 were from Candói and 87 from Vale do Corisco, were collected to obtain DNA.

2.2 Microsatellite analysis

DNA plant tissue was extracted using the CTAB protocol described by Ferreira and Grattapaglia (1998) with 19 microsatellite loci (Table 1) (Brondani et al., 1998; Brondani et al., 2002; Brondani et al., 2006; Faria et al., 2010; Grattapaglia et al., 2015). After extraction, total DNA was genotyped and quantified. The loci transferred from other Eucalyptus species performed well.

2.3 Genetic diversity and effective population size

Genetic diversity was characterized by the total number of alleles per locus (k), allelic richness (R), observed heterozygosity (H_o), and expected heterozygosity (H_e), assuming Hardy-Weinberg equilibrium. The intrapopulation fixation index (F_{IS}) and the statistical significance of its value was estimated by the Monte Carlo permutation of alleles between individuals. All analyses were performed using the program FSTAT 2.9.3.2 (Goudet, 1995). Group coancestry (Θ) was estimated from the pairwise coancestry coefficient (θ_{xy}) between pairs of adult individuals, within and among provenances, using the Lynch and Ritland (1999) method and the SPAGEDI 1.3 software (Hardy and Vekemans, 2002). The effective population size was estimated based on Sebbenn et al. (2011).

2.4 Mating system

Mating system at the population and progeny level was analyzed using the MLTR software (Ritland, 2002). The following indices were estimated: multilocus outcrossing rate (t_m); single locus outcrossing rate (t_s); mating among relatives (t_m-t_s); selfing correlation at the population level (r_s); and multilocus paternity correlation ($r_{(p(m)}$). The 95% confidence interval for these indices was obtained from 1,000 bootstrap resampling, with progeny as the resampling unit. The effective number of pollen donors was estimated as $N_{ep}=1/r_{(p(m))}$ (Ritland, 1989), and mean coancestry coefficient within progeny as θ $\bar{\theta} = 0.125(1+F_p)\left[4s+(t_m+st_mr_s)(1+r_{p(m)})\right]$, half-sibs ($P_{hs}=t_m^2(1-r_{p(m)})$) and full-sibs ($P_{fs}=t_m^2 r_{p(m)}$) and within family variance effective size was estimated as described in Sebbenn (2006).

3 RESULTS

The loci used herein presented genotypic linkage equilibrium (data not shown). A high number of alleles were found for all (19) loci, ranging from 3 to 14 (average of 9.58; Table 1). For progenies from Condoi (C) and Vale do Corisco (VC), the H_o ranged from 0.049 to 0.907 and H*e* from 0.162 to 0.787, with an average of 0.618 and 0.624, respectively (Table 1)

The intrapopulation fixation index was low for all progenies, but only negative for VC3 (Figure 1). The average across all progenies was 0.04 (ranging from - 0.17 to 0.07).

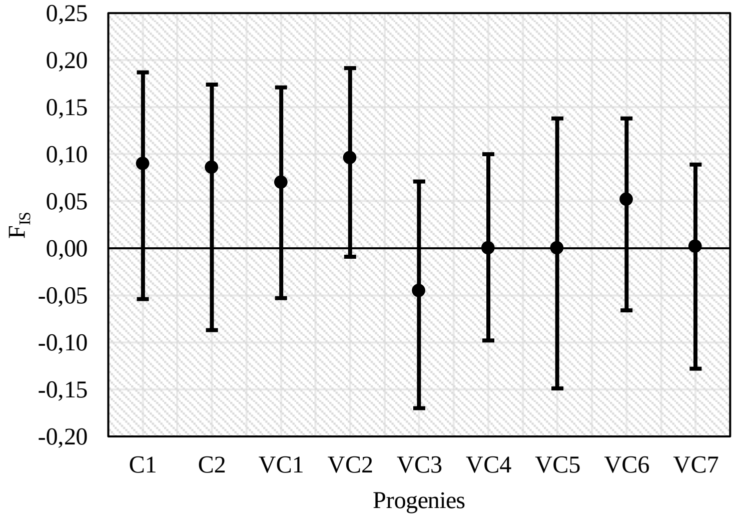

Figure 1. Intrapopulation fixation index (F_{IS}) estimated using 19 microsatellite loci for two *Eucalyptus benthamii* Maiden et Cambage progenies from Candói (C) and seven progenies from Vale do Corisco (VC) Paraná, Brazil.

Figura 1. Parâmetros de índice de fixação (F_{IS}) estimado com 19 *loci* microssatélites em duas progênies de Candói (C) e sete progênies de Vale do Corisco (VC) de *Eucalyptus benthamii* Maiden et Cambage, Paraná, Brasil.

Table 1. Microsatellite primers used for *Eucalyptus benthamii* Maiden et Cambage progenies from Candói (C) and Vale do Corisco (VC), Paraná.

Table 1. *Primers* microssatélites usados em progênies de *Eucalyptus benthamii* Maiden et Cambage, from Candói, Paraná and Vale do Corisco, Paraná.

Locus	Observed Range (bp)	$Ta\ (^oC)$	$k(\pm SE)$	$H_o(\pm SE)$	$H_e(\pm SE)$	Author
EMBRA3	111-149	56	13	0.801(0.128)	0.719(0.060)	Brondani et al. (1998)
EMBRA4	99-199	56	8	0.399(0.120)	0.637(0.141)	Brondani et al. (1998)
EMBRA10	117-141	56	10	0.789(0.024)	0.684(0.055)	Brondani et al. (1998)
EMBRA11	101-199	56	9	0.498(0.205)	0.491(0.120)	Brondani et al. (1998)
EMBRA12	120-152	56	8	0.790(0.145)	0.684(0.055)	Brondani et al. (1998)
EMBRA28	185-235	56	14	0.525(0.072)	0.654(0.068)	Brondani et al. (2002)
EMBRA38	108-142	56	13	0.834(0.027)	0.773(0.030)	Brondani et al. (2002)
EMBRA63	173-205	58	8	0.736(0.139)	0.714(0.053)	Brondani et al. (2002)
EMBRA128	106-146	58	14	0.519(0.063)	0.633(0.162)	Brondani et al. (2006)
EMBRA175	158-174	54	3	0.155(0.080)	0.163(0.084)	Brondani et al. (2006)
EMBRA204	122-146	56	9	0.789(0.175)	0.740(0.043)	Brondani et al. (2006)
EMBRA210	192-212	57	5	0.553(0.133)	0.622(0.077)	Brondani et al. (2006)
EMBRA219	237-590	57	8	0.680(0.097)	0.636(0.094)	Brondani et al. (2006)
EMBRA333	216-242	56	9	0.776(0.116)	0.726(0.043)	Brondani et al. (2006)
EMBRA350	204-240	56	13	0.907(0.094)	0.787(0.046)	Brondani et al. (2006)
EMBRA681	192-214	58	3	0.049(0.062)	0.229(0.091)	Brondani et al. (2002)
EMBRA979	396-452	56	8	0.835(0.112)	0.752(0.076)	Faria et al. (2010)
EMBRA1071	268-272	60	4	0.144(0.061)	0.554(0.491)	Grattapaglia et al. (2015)
EMBRA1924	323-339	56	7	0.657(0.134)	0.674(0.041)	Faria et al. (2010)
Mean			9.58 (4.15)	0.618 (0.256)	0.624 (0.168)	

Bp = Fragment size; $Ta\ (^oC)$ = annealing temperature; SE= standard error; H_o and H_e = observed and expected heterozygosities, respectively.

Bp = Tamanho do fragmento; $Ta\ (^oC)$ = temperatura de recozimento; SE= erro padrão; H_o and H_e = heterozigosidades observadas e esperadas, respectivamente.

The outcrossing rate (t_m) for the seven progenies from VC was 1.0 and the rate of mating among relatives ($t_m - t_s$) was significantly higher than zero (0.122). The average coancestry coefficient among progenies ($\bar{\theta}$) was 0.129 (Table 2).

A total of 155 alleles were identified for the entire sample, with 91 and 80 alleles observed in progenies from C1 and C2, respectively, and for VC progenies the total ranged from 87 to 114 alleles. The allelic richness (R) was similar among progenies with the lowest value found for progeny C2 (3.71) and the highest (4.66) for VC7 (Table 3). The effective population size (N_e) for each progeny ranged from 2.85 to 3.20. The individual outcrossing rate (t_m) ranged from 0.990 to 1.0, and mating among relatives (tm-ts) ranged from 0.077 to 2.44. The selfing correlation (r_s) was significantly higher than zero for progeny C2, VC2, VC4, and VC7 (Table 3). The multilocus paternity correlation ($r_{(p(m))}$) was significantly higher than zero for C2, VC6, and VC7, suggesting that the effective number of pollen donors (N_{ep}) that fertilized the mother trees was highly variable (4.3 to 1,000) (Table 3). The proportion of half-sibs (($P_{hs} = t_m^2(1 - r_{p(m)})$)) was high (97%) and a small number (3%) of full sibs ($P_{fs} = t_m^2 r_{p(m)}$) were identified (data not shown). For all progeny, the coancestry coefficient (Θ) within family, ranged from 0.069 to 0.181.

Table 2 Mating system parameters for *Eucalyptus benthamii* Maiden et Cambage progenies from the provenance Vale do Corisco (VC), Paraná, Brazil.

Tabela 2. Parâmetros do sistema reprodutivo para progênies de *Eucalyptus benthamii* Maiden et Cambage da procedência Vale do Corisco (VC), Paraná, Brasil.

Parameter	**Estimates (±SE)**
Number of individuals per progeny (total)	11-16 (87)
Multilocus outcrossing rate: t_m	1.00 (0.00)
Single locus outcrossing rate: t_s	0.878 (0.022)
Mating among relatives: $t_m - t_s$	0.122 (0.022)
Selfing correlation: r_s	0.044 (0.015)
Multilocus paternity correlation: $r_{p(m)}$	0.031 (0.020)
Effective number of pollen donors: N_{ep}	119
Average coancestry: $\bar{\theta}$	0.129

Table 3. Diversity and mating system parameters for open-pollinated progeny arrays of *Eucalyptus benthamii* Maiden et Cambage from two provenances: Candói (C) and Vale do Corisco (VC), Paraná, Brazil.

Tabela 3. Parâmetros de diversidade e sistema reprodutivo para progênies de polinização aberta de duas procedências de *Eucalyptus benthamii* Maiden et Cambage in Candói and Vale do Corisco, Paraná, Brasil.

Progeny/Source	n	k	$R(\pm SE)$	$t_m(\pm SE)$	$t_s(\pm SE)$	$t_m - t_s(\pm SE)$	$r_{p(m)}(\pm SE)$	$r_s(\pm SE)$	N_{ep}	N_e
Candói										
C1	12	91	4.56 (1.40)	1.000 (0.00)	0.923 (0.015)	0.077 (0.015)	-0.205 (0.398)	-0.009 (0.025)	50.0	3.15
C2	12	80	3.71 (1.24)	0.991 (0.01)	0.835 (0.022)	0.156 (0.030)	0.661 (0.473)	-0.661 (0.472)	11.1	2.95
Vale do Corisco										
VC1	12	99	4.29 (1.60)	1.000 (0.00)	0.898 (0.024)	0.101 (0.024)	0.006 (0.009)	0.079 (0.008)	1,000	3.20
VC2	12	92	4.19 (1.20)	1.000 (0.00)	0.880 (0.035)	0.120 (0.035)	0.017 (0.026)	0.083 (0.013)	62.5	3.16
VC3	13	87	4.20 (1.40)	1.000 (0.00)	0.840 (0.033)	0.160 (0.033)	0.019 (0.026)	0.080 (0.008)	58.8	3.16
VC4	11	98	4.43 (1.42)	0.990 (0.00)	0.887 (0.037)	0.113 (0.037)	0.029 (0.038)	0.089 (0.011)	38.5	3.14
VC5	12	92	4.33 (1.59)	1.000 (0.00)	0.893 (0.026)	0.106 (0.026)	0.002 (0.000)	0.073 (0.008)	1,000	3.37
VC6	11	97	4.53 (1.45)	1.000 (0.00)	0.756 (0.041)	0.244 (0.041)	0.253 (0.134)	0.159 (0.061)	4.3	2.85
VC7	16	114	4.66 (1.48)	1.000 (0.00)	0.907 (0.019)	0.093 (0.019)	0.040 (0.037)	0.091 (0.012)	38.5	3.14

n = number of individuals per progeny; k = total number of alleles; R = allelic richness; t_m= multilocus outcrossing rate; t_s= single locus outcrossing rate; $t_m - t_s$= mating among relatives; $r_{p(m)}$ = paternity correlation; r_S= selfing correlation; N_{ep}= number of pollen donors; N_e= effective population size; SE = standard error.

n = número de indivíduos por progênie; k = número total de alelos; R = riqueza alélica; t_m= taxa de ultrapassagem multilocus; t_s= taxa de cruzamento de locus único; $t_m - t_s$= acasalamento entre parentes; $r_{p(m)}$ = correlação de paternidade; r_S= correlação de auto-estar; N_{ep}= número de doadores de pólen; N_e= tamanho efetivo da população; SE = erro padrão.

4 DICUSSION

Given the number of alleles found in the sample, genetic diversity was high for these studied *E. benthamii* provenances and progenies. For 10 of the 19 analyzed microsatellite markers, the values of observed heterozygosity (H_o) were higher than those of expected heterozygosity (H_e). This resulted in negative values of intrapopulation fixation index (F_{IS}) and indicates an excess of heterozygotes in relation to Hardy-Weinberg equilibrium. The average heterozygosity estimates for C and VC progenies are similar to those obtained in studies on the diversity of *E. benthamii* populations in Australia (Skinner, 2003; Butcher et al. 2005; Han et al. 2020). Nevertheless, the tendency is for the genetic diversity of populations to decrease across breeding cycles, given that selection aims to increase the frequency of favorable alleles and reduce unfavorable alleles. This results in a reduction in the number of alleles as well as heterozygosity in populations under selection (Miranda et al., 2019).

Low levels of inbreeding were detected for the studied progenies, and the results obtained for the fixation index were similar to those found in isolated, natural, and planted *E. benthamii* populations in Australia (Skinner, 2003; Han et al., 2020). However, higher inbreeding levels were obtained for progenies from natural populations of the same species (Butcher et al., 2005) and for open-pollinated families of *Eucalyptus grandis* Hills ex Maiden in São Paulo, Brazil (Miranda et al., 2019).

A high number of alleles were found for progenies from C and VC provenances, mainly considering the sample size, and these results are comparable to those obtained for other *Eucalyptus* species in Brazil, such as improved populations of *Eucalyptus dunnii* (Miranda et al., 2019) and *Eucalyptus urophylla* S. T. Blake (Silva et al., 2019). Although the allelic richness did not differ between progenies, the number of alleles detected for VC7 (k= 114, n= 16) is noteworthy, as it is higher than that found for the other progenies. This may suggest the loss of rare or private alleles within progenies of both provenances.

For the seven VC progenies, the outcrossing rate (t_m) was high (= 1.00), despite the fact that 12.2% of mating occurred between related trees and 3.1% were correlated. This may indicate a generation of inbred offspring and low levels of non-random mating. For the total sample, t_m was also high (>0.99) between progenies, and the highest rates of mating among relatives ($t_m - t_s$) occurred in progenies C2, VC3, and VC6. The highest multilocus paternity correlation ($r_{p(m)}$) were observed for C2 and VC6, representing 66.1 and 25.3% of correlated mating, respectively. Progeny C2 and VC2 were among the four progenies that showed significant selfing correlation, which indicates low individual variation in the outcrossing rate. Unlike the present study, Butcher et al. (2005) obtained outcrossing rates of t_m< 0.7 and did not detect evidence of selfing for *E. benthamii.* However, variations in the mating system pattern of a species can be explained by environmental and genetic factors (Francisco et al., 2018), with different individuals, populations, and even reproductive events showing variations in rates of selfing (Tambarussi et al., 2016; Tambarussi et al., 2017).

As a result of the rates of selfing and correlated mating observed for some progenies, the coancestry coefficient within families were higher (Θ= 0.181), and the variance effective size lower (N_e= 2.85) , than those expected for half-sib progenies (Θ= 0.125; N_e= 4). These results indicate the occurrence of inbreeding and the existence of some full-sib individuals within progenies. This is particularly the case for C2 and VC6, in which the number of pollen donors (N_{ep}) was quite restricted, ranging from 11.1 to 4.3, respectively.

The mating system has been described for several species of the *Eucalyptus* genus, including *E. benthamii* (Butcher et al., 2005), *E. dunni* (Miranda et al., 2019), *Eucalyptus camaldulensis* Dehnh. (Gonzaga et al., 2016), *Eucalyptus globulus* Labill. (Mimura et al., 2009), *E. urophylla* (Pupin et al., 2019), *Eucalyptus regnans* F. Muell. (Griffin et al., 2019), and *Eucalyptus pellita* F. Muell. (House and Bell, 1996), as well species of *Corymbia* (Tambarussi et al., 2018). The vast majority are considered to have mixed mating systems.

5 CONCLUSIONS

Despite the significant reduction in the population size in the region of its origin, the studied *E. benthamii* progenies show high levels of genetic diversity. Although a high rate of outcrossing was observed, some open-pollinated progenies show elevated rates of correlated mating, in addition to evidence of selfing, low levels of inbreeding, and high levels of coancestry. Our results suggest that progeny are composed of both half-sibs and full-sibs.

This study provides key information regarding the mating system of *E. benthamii* that can be used to support conservation, management, and breeding and help to develop new strategies for conservation and hybrid production for this species.

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REFERENCES

ANDRADE, F.W.C.; TOMAZELLO FILHO, M.; MOUTINHO, V.H.P. Influence of Wood Physical Properties on Charcoal from *Eucalyptus* spp. **Floresta e Ambiente**, v. 25, n. 3, e20150176, 2018.

BRONDANI, R.P.V. et al. Development, characterization and mapping of microsatellite markers in *Eucalyptus grandis* and *E. urophylla*. **Theoretical and Applied Genetics**, v. 97, n. 5, p. 816-827, 1998.

BRONDANI, R.; BRONDANI, C.; GRATTAPAGLIA, D. Towards a genus-wide reference linkage map for *Eucalyptus* based exclusively on highly informative microsatellite markers. **Molecular Genetics and Genomics**, v. 267, n. 3, p. 338-347, 2002.

BRONDANI, R.P. et al. A microsatellite-based consensus linkage map for species of *Eucalyptus* and a novel set of 230 microsatellite markers for the genus. **BMC Plant Biology**, v. 6, n. 1, p. 1-16, 2006.

BUTCHER P.A.; SKINNER A.K.; GARDINER C. A. Increased inbreeding and inter-species gene flow in remnant populations of the rare *Eucalyptus benthamii*. **Conservation Genetics**, v. 6, n.2, p. 213-226, 2005.

COCKERHAM, C.C.; WEIR, B.S. Covariances of relatives stemming from a population undergoing mixed self and random mating. **Biometrics**, v. 40, n. 1, 157-164, 1984.

DINIZ, C.P. et al. Near-infrared-based models for lignin syringyl/guaiacyl ratio of *Eucalyptus benthamii* and *E. pellita* using a streamlined thioacidolysis procedure as the reference method. **Wood Science and Technology**, v. 53, n. 3, p. 521-533, 2019.

FARIA, D.A. et al. A selected set of EST-derived microsatellites, polymorphic and transferable across 6 species of *Eucalyptus*. **Journal of Heredity**, v. 101, n. 4, p. 512-520, 2010.

FERREIRA, M.E.; GRATTAPAGLIA, D. **Introdução ao Uso de Marcadores Moleculares em Análise Genética**. Brasília: EMBRAPA, 1998. 220 p.

FRANCISCO, P.M. et al. Genetic diversity and mating system of *Rhizophora Mangle* L. (Rhizophoraceae) in Northern Brazil revealed by microsatellite analysis. **Cerne**, v. 24, n. 4, p. 295-302, 2018.

GONZAGA, J.M.S. et al. Pollen contamination and nonrandom mating in a Dehnh seedling seed orchard. **Silvae Genetica**, v. 65, n.1, p. 1-11, 2016.

GOUDET, J. FSTAT (version 1.2): a computer program to calculate F-statistics. **Journal of Heredity**, v. 86, n. 6, p. 485-486, 1995.

GRATTAPAGLIA, D. et al. A novel genome-wide microsatellite resource for species of *Eucalyptus* with linkage-to-physical correspondence on the reference genome sequence. **Molecular Ecology Resources**, v. 15, n. 2, p. 437-448, 2015.

GRIFFIN, A.R. et al. Life cycle expression of inbreeding depression in *Eucalyptus regnans* and inter-generational stability of its mixed mating system. **Annals of Botany**, v. 124, n. 1, p. 179-187, 2019.

HARDY, O.J.; VEKEMANS, X. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. **Molecular Ecology Notes**, v. 2, n. 4, p. 618-620, 2002.

HAN, L. et al. Origin of planted *Eucalyptus benthamii* trees in Camden NSW: checking the effectiveness of circa situm conservation measures using molecular markers. **Biodiversity and Conservation**, v. 29, n. 4, p. 1301-1322, 2020.

HIGA, R.C.V.; PEREIRA, J.C.D. Usos potenciais do *Eucalyptus benthamii* Maiden et Cambage. Embrapa Florestas: Comunicado Técnico (INFOTECA-E), 2003.

HOUSE, A.P.N.; BELL, J. C. Genetic diversity, mating system and systematic relationships in two red mahoganies, *Eucalyptus pellita* and *E. scias*. **Australian Journal of Botany**, v. 44, n. 2, p. 157-174, 1996.

INDÚSTRIA BRASILEIRA DE ÁRVORES. São Paulo, SP: Relatório IBÁ 2021, 2021. Disponível em: https://www.iba.org/publicacoes. Acesso 10 jun. 2022.

JONES, M. E. et al. Pollen flow in *Eucalyptus grandis* determined by paternity analysis using microsatellite markers. **Tree Genetics & Genomes**, v. 4, n. 1, p. 37-47, 2008.

JÚNIOR, D. L.; COLODETTE, J. L. Importância e versatilidade da madeira de eucalipto para a indústria de base florestal. **Pesquisa Florestal Brasileira**, v. 33, n. 76, p. 429-438, 2013.

LYNCH, M.; RITLAND, K. Estimation of pairwise relatedness with molecular markers. **Genetics**, v. 152, n. 4, p. 1753-1766, 1999.

MIMURA, M. et al. Comparison of contemporary mating patterns in continuous and fragmented *Eucalyptus globulus* native forests. **Molecular Ecology**, v. 18, n. 20, p. 4180-4192, 2009.

MIRANDA, A. C. et al. Investigating the origin and genetic diversity of improved *Eucalyptus grandis* populations in Brazil. **Forest Ecology and Management**, v. 448, p. 130-138, 2019.

PALUDZYSZYN FILHO, E.; SANTOS, P.E.T.; FERREIRA, C.A. Eucaliptos indicados para plantio no Estado do Paraná. Embrapa Florestas: Documentos (INFOTECA-E), 2006.

PUPIN, S. et al. Effects of pollen contamination and non-random mating on inbreeding and outbreeding depression in a seedling seed orchard of *Eucalyptus urophylla*. **Forest Ecology and Management**, v. 437, p. 272-281, 2019.

RITLAND, K. Correlated matings in the partial selfer *Mimulus guttatus*. **Evolution**, v. 43, n. 4, p. 848-859, 1989.

_____. Extensions of models for the estimation of mating systems using *n* independent loci. **Heredity**, v. 88, n. 4, p. 221-228, 2002.

SEBBENN, A.M. Sistemas de reprodução em espécies tropicais e suas implicações para a seleção de árvores matrizes para reflorestamentos ambientais. In: HIGA, A. R.; SILVA, L. D. (Ed.) **Pomar de sementes de espécies florestais nativas**. Curitiba: FUPEF, 2006. p. 93-138.

_____. et al. Low levels of realized seed and pollen gene flow and strong spatial genetic structure in a small, isolated and fragmented population of the tropical tree *Copaifera langsdorffii* Desf. **Heredity**, v. 106, n. 1, p. 134-145, 2011.

SKINNER, A. The effects of tree isolation on the genetic diversity and seed production of Camden White Gum (*Eucalyptus benthamii* Maiden et Cambage). **Centre for Plant Biodiversity Research**, v. 10, 2003.

TAMBARUSSI, E.V. et al. Several small: how inbreeding affects conservation of *Cariniana legalis* Mart. Kuntze (Lecythidaceae) the Brazilian Atlantic Forest’s largest tree. International Forestry Review, v.18, n.4, p.502-510, 2016.

_____. et al. *Dipteryx alata* Vogel (Fabaceae) a neotropical tree with high level of selfing: implication for conservation and breeding programs. **Annals of Forest Research**, v. 60, n. 2, p. 243-261, 2017.

_____. et al. Are tree breeders properly predicting genetic gain? A case study involving *Corymbia* species. **Euphytica**, v. 214, n. 8, p. 1-11, 2018.