

## Article

# Mating System Analysis and Genetic Diversity of *Parkia multijuga* Benth. One Native Tree Species of the Amazon

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**Abstract:** The Amazonian native tree species *Parkia multijuga* has potential silvicultural characteristics that can be utilized to productive plantations. Understanding its mating system is necessary to delineate the methods for the breeding of the species, the collection of seeds for conservation, and the use of seedlings for production plantations. The aim of this study is to evaluate the mating system and population genetic diversity of *P. multijuga*, using molecular markers. The DNA of 221 plants was extracted and genotyped with nine microsatellite loci using capillary electrophoresis in an automated DNA sequencer. The estimates for single and multilocus crossing rates were 0.998 and 1.0, respectively. The paternity correlation was low ( $\hat{r}_{p(m)} = 0.307$ ). The fixation index ( $f$ ) showed values below zero, indicating an excess of heterozygotes. The cluster number  $K = 2$  shows a better grouping among families for genetic structure. *P. multijuga* families consist mainly of half-sibs, and the reproductive strategy of the species is allogamy.

**Keywords:** coancestry; conservation; crossing rate; forest breeding; forest genetics; microsatellite marker



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## 1. Introduction

*Parkia multijuga* Benth., family Fabaceae, also known as “faveira”, “faveira-benguê”, “pinho-cuiabano”, or “fava-arara-tucupi”, is one of many species native to the Amazon of the initial secondary ecological group, being able to reach 40 m in height and 100 cm in diameter in adulthood [1]. The species is important for ecological succession in degraded areas, targeting the recomposition of vegetation by providing good seed germination, high plant regeneration, good ground cover, and fast growth [2], considering that for the years 2021 and 2022, based on satellite monitoring, the consolidated rates of deforestation for the nine states of the Brazilian Legal Amazon were 13,038 km<sup>2</sup> and 11,594 km<sup>2</sup>, respectively [3].

The inflorescence of *P. multijuga* is described as a panicular type, with flowers arranged in sections and bracts at its base. The chapter has an average of 421 flowers ( $\pm 59$ ;  $N = 50$ ), distributed as stem flowers in greater numbers and hermaphroditic flowers at the apex.

The diplostemonous androecium is composed of 10 stamens and filaments measuring, on average, 1.72 cm long ( $\pm 0.19$ ;  $N = 50$ ). The anthers are longitudinally dehiscent and dorsifixed. At the apex of each anther runs a glandular appendage [4,5]. Examination of the floral structure of the species does not allow us to make conclusions about its mating system; in-depth study and analysis of the reproductive system is necessary. The presence of pollinators can also influence outcrossing rates. In Asia, the pollination of the genus *Parkia* is performed by nectarivorous bats. The nonvolant mammals and birds also visit flowers but their contribution to reproductive success is still unknown [6].

It should be noted that in *P. multijuga*, it has been observed that its flowering is highly influenced by its geographical distribution [5], which could also be affected by climate types *Af* and *Am* within the Köppen–Geiger climatic classification [7,8]. Thus, flowering occurs in August and September in the states of Acre (AC) and Rondônia (RO), respectively, from September to April in Amazonas (AM), and from September to May in Pará (PA) [1].

It must also be pointed out that *P. multijuga* is not present in the literature regarding the estimates of the parameters of the crossing systems, crossing rate ( $t$ ), selfing rate ( $s$ ), and inbreeding coefficients. Such estimates make allowances for the understanding of other genetic parameters of quantitative characters. The study of the reproductive system and genetic diversity of a natural population is important for the design strategies of seed collection, for genetic improvement, for the establishment of production plantations with variability, and for the conservation of its germplasm [9,10].

Recent research focused on the *Parkia* genus, in Brazil, has revealed significant advancements in our understanding of its ecology and importance within tropical ecosystems [10,11]. Novel findings regarding the seed dispersal ecology of *Parkia platycephala* Benth. [12] and seed germination of *Parkia gigantocarpa* Ducke [13] also emphasize genus role in forest regeneration and biodiversity maintenance. Recent studies have delved into *P. platycephala* pollination ecology, highlighting the intricate web of interactions between the plant and its specific pollinators [12]. Furthermore, the species populations present genetic differences with each other; a significant variation between progenies for all characters was evaluated, and the variation was greater within the populations [11]. Ultimately, these advances underscore the need for targeted management and conservation approaches to ensure the continued survival and vital contribution of *Parkia* species to forest ecosystems [10,12].

Studies of reproductive systems can be made mainly from the analysis of genetic markers. Moreover, utilizing microsatellites is optimal because they are codominant [14], multiallelic [15], and were applied in several plant species, such as *Acrocomia aculeata* [16], *Euterpe precatoria* [17], *Theobroma grandiflorum* [18], and *Carapa guianensis* [19], for example.

The molecular markers provide a means to decipher the various and complex facets of the mating system dynamics and genetic diversity of *P. multijuga* in depth. To validate this hypothesis, the study was conducted with the objective of performing a comprehensive evaluation of the mating system and genetic diversity of the species. The study allows us to understand the dynamics of matings occurring between closely related individuals, and to discern the presence and implications of correlated matings. By exploring these dimensions, we show a deeper understanding of genetic intricacies inherent in the open-pollinated progenies sourced from an ex situ conservation area.

## 2. Materials and Methods

### 2.1. Study Area and Sampling

This research used plant material (leaves and seeds) of 13 families of *P. multijuga*. Each family was composed of a mother plant and 16 progeny plants, totaling 221 plants. The seeds and leaves of each mother plant were collected in March 2014 (families 1 to 13) in the Apuí region ( $07^{\circ}11'25''$  S and  $59^{\circ}52'51.4''$  W). The seeds were collected from parent trees (mother plant) with a minimum distance of 300 m between them to avoid the collection of genetically related material.

The seedling production was carried out at the Forest Nursery of the Federal University of Amazonas during a period of twelve months. Polyethylene containers were used, each with a volume of 2 L (16 cm wide and 26 cm high). They were filled with substrate in a 2:1 ratio of black soil and yellow latosol fertilized with limestone, simple superphosphate, potassium chloride, ammonium sulfate, and micronutrients (FTE BR 12) in dosages of 0.891, 3.170, 0.235, 0.151, and 0.185 g dm<sup>-3</sup>, respectively [11].

The seedlings of *P. multijuga* were installed at a spacing of 3 m × 3 m, totaling an area of 0.3 ha. At the experimental Farm of the Federal University of Amazonas (FAEXP-UFAM) located 38 km from the BR-174 highway in the city of Manaus, AM (02°37'17.1" S and 02°39'41.4" S; −60°03'29.1" W and −60°07'57.5" W), with a climate type of Am—tropical monsoon [20]. Of this material, leaves from 16 plants were collected from each family installed, totaling 208 plants from 13 families.

The leaves of each mother plant (year 2014) and its progenies (year 2015) were individually stored in a previously labeled Ziplock plastic bag, containing silica gel until the samples could be stored at −20 °C in the Laboratory of Genetics and Plant Breeding of the Department of Agronomy at the Federal University of Amazonas.

These collections were carried out within the rules of Resolution number 21 of 31 August 2006. The Genetic Heritage Management Council, Brazil. The study into the relationships between genetic diversity and gene flow in geographic areas of the Amazonas state with the species *P. multijuga* is classified as scientific research. The species is registered in *SisGen* (National System for the Management of Genetic Heritage and Associated Traditional Knowledge, Decree n° 8772, of 11 May 2016, which regulates Law n° 13,123, of 20 May 2015) with number A0AE37F.

## 2.2. Amplification of Microsatellite Markers

For the extraction of DNA, hexadecyltrimethylammonium bromide CTAB protocol was used as described by [21] and then quantified [17]. A total of 9 polymorphic and informative microsatellite primers developed for *Parkia panurensis* (Parpan 3, Parpan 4, Parpan 5, Parpan 9, Parpan11, Parpan13, Parpan14, Parpan15, and Parpan 21) (Table 1) were validated in heterologous amplification [22] and were used in the study. These microsatellite loci were amplified by polymerase chain reaction (PCR) using a Veriti thermal cycler (Applied Biosystems, Foster City, CA, USA) with a total volume of 10 µL per reaction (containing 10 ng of genomic DNA, 1× buffer, 210 µM of each dNTP, 1.5 mM MgCl<sub>2</sub>, 0.16 µM forward primer and M13 (FAM or NED) broth [23], 0.32 µM reverse primer, 1.05 U Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), and 3.49 µL ultrapure water. These PCR amplifications consisted of two steps, of which the first was primer-specific and the second M13 binding [24]. The amplification products were checked by electrophoresis on 1.5% agarose gels stained with GelRed (Biotium, Fremont, CA, USA) in 1× TBE buffer (pH 8.0). The amplified PCR products were subjected to an automated DNA analyzer by capillary electrophoresis, model ABI 3130XL (Applied Biosystems, Foster City, CA, USA). A standard size GeneScan™-500 ROX® (Applied Biosystems, Foster City, CA, USA) was used to determine the allele size. The amplified fragments were observed and analyzed with GeneMapper v4.0 (Applied Biosystems, Foster City, CA, USA) software.

## 2.3. Data Analysis

For analyzing mating systems, the software MLTR 3.4 was used, employing mixed mating and correlated mating models [25]. Analyses were based on probabilities of maximum expectations “ME” [25]. The following indices were estimated: multilocus outcrossing rate ( $\hat{t}_m$ ), single-locus outcrossing rate ( $\hat{t}_s$ ), biparental inbreeding or mating between relatives ( $\hat{t}_m - \hat{t}_s$ ), multilocus paternity correlation ( $\hat{f}_{p(m)}$ ), selfing correlation ( $\hat{f}_s$ ), and maternal fixation index ( $\hat{F}_m$ ) utilizing the moment method for the  $\hat{t}_m$  of each family [26]. The 95% confidence interval (CI) of each index was calculated from 1000 bootstrap replications, where the sampling units were represented by plants within progenies for individual analysis and by progenies for population analyses. The mean

effective number of pollen donors was estimated by  $\hat{N}_{e(p)} = 1/r_{p(m)}$  [27], and the average proportions of pairwise self-sibs ( $\hat{P}_{ss}$ ), half-sibs ( $\hat{P}_{hs}$ ), full-sibs ( $\hat{P}_{fs}$ ), and self-half-sibs ( $\hat{P}_{shs}$ ) within families were estimated as  $\hat{P}_{ss} = \hat{s}^2$ ;  $\hat{P}_{hs} = \hat{f}_m^2(1 - \hat{r}_{p(m)})$ ;  $\hat{P}_{fs} = \hat{f}_m^2\hat{r}_{p(m)}$  and  $\hat{P}_{shs} = 2\hat{s}\hat{f}_m$  [28]. The coancestry coefficient within family ( $\Theta$ ) was estimated by  $\Theta = 0.125(1 + \hat{F}_m) \left[ 4\hat{s} + (\hat{f}_m^2 + \hat{f}_m\hat{s}\hat{r}_s) (1 + \hat{r}_{p(m)}) \right]$ , where  $s$  is the selfing rate ( $\hat{s} = 1 - \hat{f}_m$ ) and variance effective size within family, according to [29]. This was based on the assumption of an idealized reference population (infinite size, random mating, without selection, mutation or migration):  $\hat{N}_e = 0.5 / \{ \Theta[(n - 1/n)] + [(1 + \hat{F}_o)/2n] \}$ , where  $n$  is the number of analyzed offspring within families and  $\hat{F}_o$  is the within-families fixation index,  $F_o = F = 1 - (H_o/H_e)$ , where  $H_o$  and  $H_e$  are observed and expected heterozygosities, respectively [30,31]. The number of mother trees ( $m$ ) for seed collection aiming to retain the reference effective population size of 150 [32] was calculated by  $m = \hat{N}_{e(reference)} / \hat{N}_e = 150 / \hat{N}_e$  and was based on three assumptions: (i) nonrelatedness of the mother trees; (ii) no intermating of the sampled mother trees; (iii) no overlapping of the pollen pools and no pollen from the same fathers received by the mother trees.

**Table 1.** Locus, repeat motif, microsatellite sequences of *P. multijuga* primers used for mating system analysis.

Locus	Repeat Motif	Primer Sequences (5'-3')	AT °C	(bp)	AN GenBank
Parpan 3	(GT) <sub>15</sub>	F: CACGTTAATCAATCAAAATGGTG R: TTTGCCTTTTCGGACTTG	56.5	155–209	GU735073
Parpan 4	(GT) <sub>13</sub> (GA) <sub>15</sub>	F: TTGATGGGAGTGGGAAAAAG R: CAGGAGGTGGTCTCTCAGG	54.0	148–210	GU735074
Parpan 5	(CA) <sub>17</sub>	F: CTCAATAAGATAACCCTTTACATTGC R: TTGAATCGAGGAATGAGATTATTG	60.0	166–200	GU735075
Parpan 9	(AC) <sub>12</sub>	F: GGGGCTTGTGTCTCTCACTG R: ACTTTGAAGGCACGAGATGG	58.0	204–262	GU735076
Parpan 11	(TG) <sub>5</sub> CA(TG) <sub>16</sub>	F: ACGTAGGGAATAGGGCCATC R: CTACGTACGAGCCGACACTC	58.5	94–214	GU735077
Parpan 13	(GT) <sub>17</sub> TT(GT) <sub>8</sub>	F: CCTCCCTCGCTTCAACAATC R: CACATGCAAATGAAAATGGTG	58.5	86–194	GU735078
Parpan 14	(GT) <sub>20</sub>	F: ACATCAAAAATGGTCGCTCAAC R: CAAATGTTCTTGTATGGAGCAAG	60.0	76–116	GU735079
Parpan 15	(AC) <sub>24</sub>	F: TGGCCTCACTGCATACTGAC R: TGGGATGAACAAAACCTGTGC	55.0	104–152	GU735080
Parpan 21	(GT) <sub>15</sub>	F: TGCTTTGTGCGACTTGAATC R: CATTGTTACAGCATATAGGCATACAG	58.0	153–185	GU735081

AT = annealing temperature; AN GenBank—GenBank access number. Source: Luettmann et al. [22].

The genetic diversity compared among the families was obtained using the following genetic parameters: average number of alleles/locus ( $A$ ), average number of effective alleles ( $N_E$ ), number of private alleles ( $N_P$ ), observed heterozygosity ( $H_O$ ) and expected heterozygosity ( $H_E$ ), and inbreeding coefficient ( $f$ ). These parameters were calculated using the GenAIEx v. 6.5 [33,34].

For analysis of the genetic structure, Wright's  $F$  statistics were used [35], including  $F_{IT}$  (total inbreeding levels in individuals of all populations),  $F_{IS}$  (inbreeding index within subpopulations owing to the reproductive system), and  $F_{ST}$  (genetic differentiation owing to subdivision), and calculations were performed using the algorithms of Weir and Cockerham [36] and were calculated and evaluated with significance ( $p \leq 0.05$ ) from 20,000 bootstrapped samples, implemented in GenAIEx v. 6.5 [33,34].

For the genetic structure analysis of families, Bayesian analysis was performed to determine the number of clusters within the set of samples evaluated through the use of the software structure [37], configured in the admixture model for its usual application with natural populations. The number of clusters ( $K$ ) was set from 1 to 15, and for each  $K$ ,

ten iterations were performed, with a burn-in of 200,000 followed by 1,000,000 iterations of Markov chain Monte Carlo (MCMC). The number of clusters was estimated using the Evanno  $\Delta K$  method, indicating that the most likely  $K$  is where the change is greatest in the second-order rate of change in  $\Pr(X|K)$  among successive  $K$  values [38]. With the value of  $K$  selected, a consensus was reached on the number of iterations performed in this cluster through the CLUMPP v. 1.1.2—Cluster Matching and Permutation Program [39]. Using the program Distruct v. 1.1 [40], the graphical visualization of the population structure was performed.

### 3. Results

#### 3.1. Crossing System

The results of the analysis of the mating system of the faveira-benguê tree in relation to three parameters—the maternal fixation index ( $\hat{F}_m$ ), the multilocus outcrossing rate ( $\hat{t}_m$ ), and the single-locus outcrossing rate ( $\hat{t}_s$ )—are presented in the Table 2.

**Table 2.** Mating system at the population level.

Parameters	Mean (95% CI) <sup>1</sup>
Maternal fixation index: $\hat{F}_m$	0 (0–0)
Multilocus outcrossing rate: $\hat{t}_m$	1.0 (1.0–1.0)
Single-locus outcrossing rate: $\hat{t}_s$	0.998 (0.997–1.000)
Mating among relatives: $\hat{t}_m - \hat{t}_s$	0.002 (0.003–0.000)
Selfing correlation: $\hat{r}_s$	0.11 (0.11–0.11)
Paternity correlation: $\hat{r}_{p(m)}$	0.307 (0.193–0.338)
Number of pollen donors: $\hat{N}_{e(p)}$	3.3 (3.0–5.2)
Frequency of self-sibs: $\hat{P}_{ss}$	0 (0–0)
Frequency of self-half-sibs: $\hat{P}_{shs}$	0 (0–0)
Frequency of half-sibs: $\hat{P}_{hs}$	0.693 (0.807–0.662)
Frequency of full-sibs: $\hat{P}_{fs}$	0.307 (0.193–0.338)
Coancestry within family: $\Theta$	0.163 (0.149–0.167)
Variance effective size: $\hat{N}_e$	2.730 (2.676–2.945)
Number of seed trees: $\hat{m}$	55 (51–56)

<sup>1</sup> 95% CI—confidence interval 95%.

The fact that the maternal fixation index was not significantly different from zero suggests that there is no maternal preference in selecting mating partners. The high values observed for both the multilocus and single-locus outcrossing rates (1 and 0.998, respectively) provide strong evidence that faveira-benguê predominantly employs an outcrossing mating system. The results of multilocus outcrossing rate ( $\hat{t}_m$ ) at the family level presented in all were equal to 0.998 (Table 3).

The observed high multilocus outcrossing rate ( $\hat{t}_m$ ) in most families indicates that the majority of faveira-benguê families primarily engage in outcrossing, contributing to genetic diversity within populations. The multilocus outcrossing rate observed in the families, represented by a value of 0.998, suggests a higher occurrence of mating between and within those families.

The study's findings on the mating system of faveira-benguê at the family level provide insights into the genetic dynamics and breeding strategies within distinct familial groups. The mixture of high outcrossing rates adds to understanding of the species' reproductive biology. Such an insight can guide conservation efforts by emphasizing the importance of considering genetic diversity at the family level along with developing strategies that account for the variability in mating behaviors, ultimately contributing to the long-term viability of faveira-benguê populations.

The difference between the multilocus and the single-locus outcrossing rate was low (0.002). The selfing ( $S = 1 - \hat{t}_m$ ) was zero (0.000). The estimated selfing correlation ( $\hat{r}_s$ ) among progenies was low (0.11). The paternity correlation ( $\hat{r}_{p(m)}$ ) was moderate (0.307), and effective pollen donors ( $\hat{N}_{e(p)}$ ) fertilized the seed trees in the investigated reproductive

event were 3.3 (Table 1), indicating that mating was not random and that the families were mainly composed of half-sibs (69.3%). Nonrandom matings were also evidenced by variations among families for mating among relatives ( $\hat{t}_m - \hat{t}_s$ : between 0.007–0.247), paternity correlation rates ( $\hat{r}_{p(m)}$ : 0.091–0.656), and effective number of pollen-donor trees ( $\hat{N}_{e(p)}$ : 1.52–10.99) (Table 2).

**Table 3.** Mating system at the mother tree level.  $\hat{t}_m$  multilocus outcrossing rate;  $\hat{t}_m - \hat{t}_s$ : mating among relatives;  $\hat{r}_{p(m)}$ : paternity correlation;  $\hat{N}_{e(p)}$ : effective number of pollen donors;  $\hat{\Theta}$ : coancestry coefficient;  $\hat{N}_e$ : effective size; SD: standard deviation, calculated from 1000 bootstrap samples.

Mothers	$\hat{t}_m$ (SD)	$\hat{t}_m - \hat{t}_s$ (SD)	$\hat{r}_{p(m)}$ (SD)	$\hat{N}_{e(p)}$	$\hat{\Theta}$	$\hat{N}_e$
Family 1	0.998 (0.005)	0.247 (0.033)	0.656 (0.106)	1.52	0.207	2.271
Family 2	0.998 (0.005)	0.014 (0.001)	0.224 (0.079)	4.46	0.153	3.025
Family 3	0.998 (0.005)	0.020 (0.002)	0.153 (0.065)	6.54	0.145	3.170
Family 4	0.998 (0.005)	0.029 (0.004)	0.383 (0.136)	2.61	0.173	2.725
Family 5	0.998 (0.005)	0.021 (0.002)	0.127 (0.077)	7.87	0.141	3.302
Family 6	0.998 (0.005)	0.034 (0.006)	0.267 (0.099)	3.75	0.159	2.963
Family 7	0.998 (0.005)	0.053 (0.011)	0.251 (0.114)	3.98	0.157	2.941
Family 8	0.998 (0.005)	0.059 (0.013)	0.281 (0.090)	3.56	0.161	2.850
Family 9	0.998 (0.005)	0.034 (0.007)	0.091 (0.044)	10.99	0.137	3.355
Family 10	0.998 (0.005)	0.062 (0.013)	0.233 (0.097)	4.29	0.155	3.022
Family 11	0.998 (0.005)	0.096 (0.029)	0.198 (0.062)	5.05	0.150	3.060
Family 12	0.998 (0.005)	0.007 (0.000)	0.192 (0.069)	5.21	0.149	3.070
Family 13	0.998 (0.005)	0.045 (0.006)	0.291 (0.092)	3.44	0.162	2.879

*n*: sample size;  $\hat{t}_m$ : multilocus outcrossing rate;  $\hat{t}_m - \hat{t}_s$ : mating among relatives;  $\hat{r}_{p(m)}$ : paternity correlation;  $\hat{N}_{e(p)}$ : effective number of pollen donors;  $\hat{\Theta}$ : coancestry coefficient;  $\hat{N}_e$ : effective size; SD: standard deviation, calculated from 1000 bootstrap samples.

The mean coancestry coefficient within progenies ( $\Theta = 0.163$ ) was higher than expected in half-sib progenies (0.125). To obtain estimates of additive genetic variance and heritability, a relatedness coefficient of 0.326 ( $2\Theta$ ), rather than 0.25, must be used. The estimate of the variance effective size ( $\hat{N}_e$ ) was lower ( $\hat{N}_e = 2.73$ ). In *P. multijuga*, the seeds must be collected from at least 52 trees to retain the effective reference population size of 150 in progeny array samples.

### 3.2. Diversity and Genetic Structure

Of the nine microsatellite loci used, all were polymorphic. Thus, the average number ranged from 3 to 5.33 alleles per locus, on average, for families 1 and 11, respectively. The expected heterozygosity ( $H_E$ ) ranged from 0.543 (family 1) to 0.659 (family 3). High values were observed for observed heterozygosity ( $H_O$ ), with  $H_O$  values exceeding  $H_E$  in all 13 families under study. The fixation index ( $f$ ) showed values below zero, indicating an excess of heterozygotes (Table 4). The analyses also revealed that private alleles were observed in families 3, 8, 9, 10, 11, and 12.

The results of Wright's [35]  $F$ -statistics analyses in the 13 sampled families indicated that the total inbreeding ( $F_{IT} = -0.073$ ) and the estimate of inbreeding due to reproductive or intrapopulation system ( $F_{IS} = -0.190$ ) were lower when compared to inbreeding due to subdivision ( $F_{ST} = 0.098$ ). The  $F_{ST}$  fixation indices showed significance (Table 5). However,  $F_{IT}$  and  $F_{IS}$  were not significant.

When estimating the number of genetically homogeneous families ( $K$ ) through Bayesian analysis performed by the Structure software version 2.3.3, the best result observed was  $K = 2$  (Figure 1).

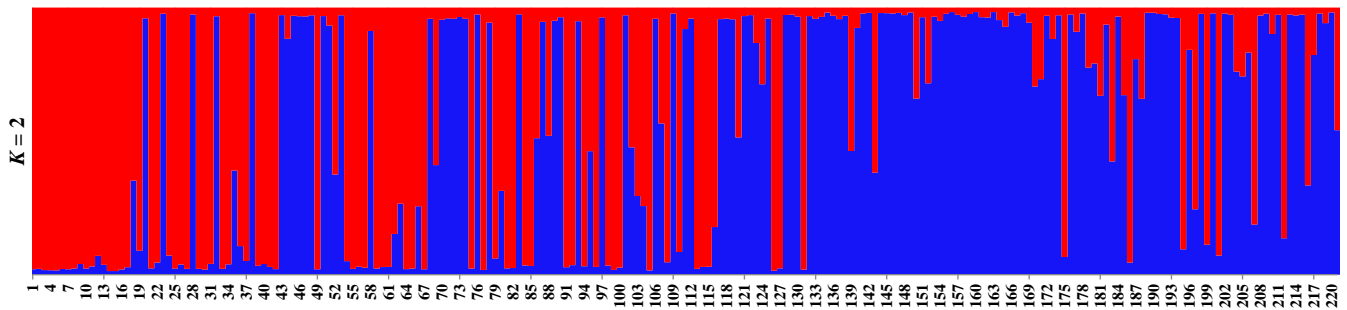
**Table 4.** Average estimates of genetic diversity parameters and fixation index ( $f$ ) estimated at the family level for individuals of *P. multijuga*.

Family	$N$ (SE)	$A$ (SE)	$N_p$ (SE)	$H_O$ (SE)	$H_E$ (SE)	$f$ (SE)
Fam1	15.111 (0.716)	3.000 (0.373)	0.000 (0.000)	0.655 (0.085)	0.543 (0.038)	−0.201 (0.126)
Fam2	17.000 (0.000)	4.667 (0.441)	0.000 (0.000)	0.837 (0.057)	0.647 (0.034)	−0.291 (0.056)
Fam3	17.000 (0.000)	4.556 (0.294)	0.111 (0.111)	0.837 (0.065)	0.659 (0.038)	−0.265 (0.062)
Fam4	16.222 (0.434)	4.000 (0.408)	0.000 (0.000)	0.820 (0.080)	0.613 (0.047)	−0.323 (0.052)
Fam5	16.778 (0.147)	5.111 (0.633)	0.000 (0.000)	0.906 (0.038)	0.661 (0.031)	−0.379 (0.054)
Fam6	16.778 (0.222)	4.333 (0.333)	0.000 (0.000)	0.876 (0.047)	0.649 (0.031)	−0.350 (0.047)
Fam7	16.556 (0.242)	4.889 (0.611)	0.000 (0.000)	0.802 (0.080)	0.633 (0.043)	−0.249 (0.075)
Fam8	16.556 (0.338)	4.111 (0.389)	0.111 (0.111)	0.725 (0.084)	0.601 (0.043)	−0.187 (0.087)
Fam9	16.778 (0.147)	5.222 (0.596)	0.222 (0.147)	0.850 (0.089)	0.624 (0.051)	−0.317 (0.081)
Fam10	16.556 (0.176)	4.556 (0.242)	0.222 (0.147)	0.814 (0.056)	0.613 (0.034)	−0.331 (0.082)
Fam11	16.333 (0.441)	5.333 (0.500)	0.111 (0.111)	0.766 (0.081)	0.599 (0.057)	−0.268 (0.061)
Fam12	16.111 (0.423)	5.000 (0.333)	0.111 (0.111)	0.836 (0.081)	0.647 (0.049)	−0.270 (0.062)
Fam13	15.556 (0.930)	4.444 (0.530)	0.000 (0.000)	0.788 (0.072)	0.600 (0.051)	−0.307 (0.040)

$N$  is the number of adult individuals used for the estimated parameters;  $A$  is the average number of effective alleles;  $N_p$  is the number of private alleles;  $H_E$  is expected heterozygosity;  $H_O$  is observed heterozygosity; and  $f$  is the fixation index, calculated as  $f = (H_E - H_O)/H_E$ , representing inbreeding within the population.

**Table 5.** Results of estimates from Wright's F statistics obtained for 13 families of *P. multijuga* using nine microsatellite loci.

	$F_{IS}$	$F_{IT}$	$F_{ST}$
Below all loci	−0.190	−0.073	0.098
$p$ value	1.000	1.000	0.001

**Figure 1.** Structure of individuals from 13 families of *P. multijuga* when grouped toward the north, south, and center of Amazonas.

## 4. Discussion

### 4.1. Crossing System

The mating system of the faveira-benguê tree provides valuable insights into the genetic dynamics and reproductive strategies of this species. These findings reveal significant details about mating preferences and gene flow within faveira-benguê populations. The result zero in  $\hat{F}_m$  indicates that the mother trees do not show any level of inbreeding. It underscores a significant aspect of the study, that faveira-benguê does not exhibit selective mating strategies based on maternal lineage, and that reproductive outcomes are more likely influenced by other factors, such as partner availability and environmental conditions. Inbreeding, or the mating between closely related individuals, can lead to the expression of recessive deleterious alleles, resulting in reduced fitness and potentially compromising the survival and reproductive success of the offspring. The absence of inbreeding in mother trees suggests that the population is avoiding such detrimental effects by promoting outcrossing, which can help maintain genetic diversity and overall fitness. The similar behaviors in other species, such as *Handroanthus heptaphyllus* [41] and *Euterpe precatoria* [17],

reinforces the consistency of the findings across different plant species. This suggests that the preference for outcrossing and the avoidance of inbreeding might be a common adaptive strategy in various plant populations. The comparison to species like *A. aculeata* [16] and *E. precatoria* [17], which exhibit similar crossbreeding behaviors, further supports the idea that allogamy (cross-pollination) might be a prevalent reproductive strategy in these plant communities.

The multilocus and single-locus outcrossing rates ( $\hat{t}_m$  and  $\hat{t}_s$ ) reinforce the notion that the progenies in the study were the result of crosses, indicating an allogamous mating system. Allogamy enhances genetic diversity and provides an advantage by reducing the risk of inbreeding depression. The consistent presence of allogamy across various species and populations suggests that this strategy offers selective advantages in terms of adapting to changing environments and maintaining fitness.

The observation of low mating presence among relatives of the plants sampled from *P. multijuga* is particularly interesting. This implies that these plants are effectively avoiding mating with close relatives, which is essential for avoid inbreeding depression. This behavior might be influenced by mechanisms that promote mate recognition and selective mate choice, ultimately leading to a balanced genetic composition within the population. In the multilocus and the single-locus outcrossing ( $\hat{t}_m - \hat{t}_s$ ), low mating presence was observed among relatives of the plants sampled from *P. multijuga*.

The species *P. multijuga* also indicates that there is low variation in  $\hat{t}_m$  between the mother trees, due to a low estimated selfing correlation ( $\hat{r}_s$ ). In this sense, the  $\hat{t}_m$  among the mother trees was all equal to 0.998 (Table 2).

The high presence of half-siblings indicates that there are correlated crosses, which could be caused by the behavior of pollinators who systematically visit nearby trees [42]. It also shows nonrandom mating variations obtained between families mating among relatives. In the families, independently, the nonrandom crossings were also evidenced by the variations between families mating among relatives, paternity correlation rates, and effective number of pollen-donor trees. Moreover, the values observed in  $\hat{N}_{e(p)}$  were also reported in *Euterpe edulis* between 4.5 and 9 donors [43].

The estimate of the effective size of the variance, from the coancestry coefficient  $\Theta$ , was lower than expected in the open or panmictic crossing system [42], considering that in infinite samples the effective size of the variance varies from 1 to 4, where the value 1 indicates progenies generated by self-fertilization, value 2 are complete siblings, and 4 are half-siblings [42]. The effective size of the variance  $\hat{N}_e$  is important to be able to determine the minimum number of seed donor trees in the collection process. Thus, the results show the importance of estimating the size of the samples designated for use in breeding programs, genetic conservation, seed collection, and addressing environmental recovery, as well as for the monitoring of genetic diversity in manipulated populations [24,42,44].

Considering that individuals of *P. multijuga* interbreed randomly, obtaining a representative sampling of the gene pool within individuals from natural populations in their center of diversity in the Amazon is necessary for their conservation. Given the pattern of genetic variability found in outcrossing species, this might result in a large number of individuals constituting an ex situ collection. Collections established in the field require ongoing resources for maintenance. Experience with in-field conservation of long-cycle species native to the Amazon region suggests that in situ conservation of *P. multijuga* should be promoted in areas where this species is distributed. With in situ conservation, efforts and resources can be focused on small-scale working collections, including only the highest-potential genotypes selected in situ, which, in the very first selection cycle, could become the initial seed production fields for plantations [45].

This research focused on the mating system of the faveira-benguê tree, leveraging the advantages of molecular markers to demonstrate the genetic complexity of this species. Through analysis of microsatellites and specific genomic sequence markers, these investigations highlight the extensive genetic diversity within faveira-benguê progenies. By un-raveling intricate mating patterns, molecular markers have unveiled a previously un-



known network of genetic interactions among individuals of the species. The integration of these data enable a more precise understanding of gene flow between progenies, underscoring the importance of genetic connectivity in maintaining the diversity and resilience of the faveira-benguê. As conservation gains prominence, these advancements provide vital insights for adaptive management strategies that consider genetic richness, ensuring the long-term survival of this keystone species in forest ecosystems.

The mating system indices estimated for *P. multijuga* in this study indicate that the species is allogamous but also self-compatible. The studied progenies were primarily represented by half-sibs. However, matings were not random, due to the occurrence of some correlated mating, resulting in few full-sibs within progenies. This study emphasizes important nuances of the mating system in *P. multijuga*. The allogamous nature of the species suggests that the majority of crosses occur between genetically distinct individuals, contributing to genetic diversity and potential adaptation to environmental changes. Furthermore, self-compatibility suggests that the species possesses the ability to self-pollinate, which can be advantageous in situations of pollinator scarcity or when the distance between breeding individuals is significant. The mating system in *P. multijuga* is a complex balance between a preference for allogamous mating to maintain genetic diversity and adaptation and the ability for self-pollination to ensure reproduction under unfavorable conditions. These findings have important implications for the ecology and evolution of the species, while also informing conservation strategies aimed at preserving the genetic variability of *P. multijuga* populations.

#### 4.2. Diversity and Genetic Structure

This study demonstrated that the progenies exhibit high levels of genetic diversity across the 13 families of *P. multijuga*. There is no existing study evaluating an ex situ collection of this species. The observed high diversity also supports the species' reproductive system, where the fixation index ( $f$ ) showed negative values for all thirteen families, indicating an excess of heterozygosity with higher observed heterozygosity ( $H_O$ ) values compared to expected heterozygosity ( $H_E$ ) [46]. Therefore, this information is significant as it confirms that *P. multijuga* reproduces through outcrossing, and its progenies do not exhibit self-fertilization. The consistently higher  $H_O$  over  $H_E$  in *P. multijuga* indicates a high diversity pattern for outcrossing species [35,47].

The majority of mating events occur between genetically unrelated individuals, promoting genetic diversity within the population. This high rate of outcrossing is typically associated with strategies to avoid inbreeding depression and adapt to changing environmental conditions through the generation of diverse genotypes. The high multilocus outcrossing is a way to prevent the accumulation of deleterious alleles and inbreeding depression. This adaptive reproductive strategy can enhance the ability of populations to respond to changing environmental conditions and maintain overall fitness [48].

The private alleles identified in the progenies of each sampled family suggest the importance of genetic conservation for this species. These alleles come from different potential pollinators, as delineated in the study of the reproductive system. It is important to carry out special management for these families considering this allelic difference [49].

The genetic differentiation due to subdivision ( $F_{ST}$ ), as indicated by Wright's F-statistics, suggested the presence of a moderate genetic structure [47] among the 13 sampled families of *P. multijuga*. The majority of the sampled genetic variability was within families, as shown through observed inbreeding within subpopulations due to the reproductive system ( $F_{IS}$ ). This result supports the idea that this may be a characteristic of the species, influenced by the capacity for genetic material dispersal, the degree of isolation of each family, the reproductive system, and the allelic diversity it exhibits [50–52].

The genetic structure of the 13 families of *P. multijuga* would be related to gene flow through pollen dispersal, as observed in the distribution of progenies, where closer proximity suggests higher relatedness. Since these progenies come from a collection and were established in the final location, it is possible that the parents are still influencing the

formation of these groupings. This is because they could be undergoing an evolutionary process independently of each other, considering that groups of individuals occupying different parts of a species' distribution may evolve relatively independently under the influence of genetic drift and local selection [48].

#### 4.3. Implications for Conservation

These findings have important implications for the population's genetic structure, as well as for conservation and management strategies. A predominantly outcrossing mating system contributes to maintaining genetic diversity, which is crucial for the adaptability and resilience of populations in the face of environmental challenges [41]. Additionally, understanding the mating patterns of faveira-benguê assists in designing effective conservation strategies that can enhance genetic variability and maintain healthy population sizes.

The absence of maternal preference and the high outcrossing rates emphasize the adaptive reproductive strategies of this species, which could be a key factor in its ability to thrive in diverse habitats. These insights can guide conservation efforts by highlighting the importance of maintaining genetic diversity and fostering strategies that promote outcrossing, ultimately ensuring the long-term survival and conservation of faveira-benguê populations.

In the species management, this initial study related to the reproductive system, diversity, and genetic structure of *Parkia* in a collection of different families would suggest that species conservation should be primarily carried out considering the identified groupings within the collection. Conservation efforts should also be undertaken at the in situ/on-farm level and at the ex situ level by conducting research that enables the initiation of the domestication and improvement process for this species. The use of the species in the recovery of disturbed areas within the process of ecological succession is recommended to be carried out with materials collected from the greatest possible number of parent trees to avoid the risk of inbreeding depression due to the possibility of mating between close relatives [53].

## 5. Conclusions

The mating system indices estimated for *P. multijuga* in this study indicate that the species is allogamous but also self-compatible. The studied progenies were primarily represented by half-sibs. However, matings were not random due to the occurrence of some correlated mating, resulting in few full-sibs within progenies. The allogamous nature of the species suggests that the majority of crosses occur between genetically distinct individuals, contributing to genetic diversity. Furthermore, self-compatibility indicates that the species possesses the ability to self-pollinate, which can be advantageous in situations of pollinator scarcity or when the distance between breeding individuals is significant. The mating system in *P. multijuga* is a complex balance between a preference for allogamous mating to maintain genetic diversity and adaptation and the ability for self-pollination to ensure reproduction under unfavorable conditions. These findings have important implications for the ecology and evolution of the species, while also informing conservation strategies aimed at preserving the genetic variability of *P. multijuga* populations.

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