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Genetic Structure and Diversity of *Dalbergia nigra* from Brazilian Atlantic Forest Fragments

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Abstract: *Dalbergia nigra* is a long-living tree endemic to the Atlantic Rain Forest. Due to its high commercial value, this species has been widely exploited for timber production and is now endangered. It is widely known that understanding patterns of genetic structure is paramount for conserving threatened species. We analyzed the genetic diversity of 140 individuals from four different forest fragments in the southern region of Bahia, Brazil, to verify the possible effects of fragmentation on these populations and provide information for conservation initiatives. High polymorphism levels were detected from the genotyping of nine microsatellite loci (mean H_E = 0.733). All populations showed high genetic diversity; however, a reduction of genetic diversity was detected in each population ($H_O < H_E$). The average fixation index was high and significant (f = 0.167), which could be due to the occurrence of inbreeding, the Wahlund effect, reproductive system, or from null alleles. Genetic differentiation among populations was high (mean θ_P = 0.118), suggesting strong isolation, a pattern consistent with historically low gene flow. The Bayesian analysis revealed five different genetic groups, among which three groups correspond to three different forest fragments, and two groups showed the genetic subdivision of individuals from the other forest fragment. Based on our results, the suggested conservation strategy for *D. nigra* populations in the southern region of Bahia, Brazil, involves high environmental investments to protect all sampled forest fragments and individuals. Another strategy would be to collect seeds from all individuals from the sampled fragments and start a new population with human interference in its evolutive history inside a protection unit.

Keywords: biodiversity; conservation genetics; leguminosae; microsatellite markers; Brazilian rosewood; Bayesian analysis; forest fragmentation

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1. Introduction

Dalbergia nigra (Vell.) Allem. ex Benth. (Leguminosae: Papilionoidae) (Brazilian rosewood or "jacarandá-da-Bahia", in Portuguese) is a highly valued tree species endemic to the Atlantic Forest and is restricted to an area from southern Bahia to northern São Paulo [1,2]. Trees of this species can reach 15–25 m in height while the trunk ranges from 15 to 45 cm in diameter at breast height [3]. *D. nigra* wood is highly prized worldwide, both for luxury furniture and musical instruments such as guitars and pianos [1]. The intense extractive exploitation associated with deforestation in the Atlantic Forest resulted in the inclusion of the Brazilian rosewood in the IUCN Red List of Threatened Species as a vulnerable species [4] and also as a threatened species in the Official List of Endangered

Species of the Brazilian Flora [5]. Moreover, this species was also included in the Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora [6], which resulted in the prohibition of commercialization and international trade of its wood [2].

Although Brazilian rosewood occurs in a broad geographical region, the destruction of its natural habitat threatening this species necessitates immediate efforts for its future genetic conservation. The Atlantic Forest is one of the 34 biodiversity hotspots recognized worldwide [7]. Since the colonial period in Brazil, the Atlantic Forest has had a history of intensive land use for harvesting natural resources and human occupation, contributing to forest degradation. Currently, the remaining 7.5% of the original Atlantic Forest in Brazil persist as forest fragments [8], and the remnant plants in the primary forest are commonly found as clusters of low density in hard-to-reach places, all of which have contributed to the rarity of this species. Moreover, these forest fragments are embedded in an anthropized landscape matrix, which harms the species over time.

Habitat loss and fragmentation strongly increase species extinction rates [9–13]. Fragmented populations are particularly vulnerable to the effects of genetic drift and inbreeding, and the intensity of these effects depends on fragment size and degree of isolation [14]. The consequences of isolation in forest fragments also include a decrease in the heterozygote frequency. Additionally, this effect is accentuated in small fragments since the removal and fixation of some alleles may occur through generations due to oscillations in allele frequencies [15,16]. Conservation geneticists widely use the effective population size (Ne) to estimate populations' genetic representativeness. Populations with continuously small effective population sizes are more susceptible to the effects of inbreeding and genetic drift, and the higher inbreeding and genetic drift levels result in lower genetic representativeness of the population [14,17,18].

Despite the urgent need for actions to conserve and manage the remaining Brazilian rosewood populations, there are a lack of studies to estimate population genetic parameters. *D. nigra* is an ornamental tree widely used in landscaping for its delicate foliage and open canopy shape. As it is a rustic plant adapted to dry lands, this species is excellent for mixed planting on disturbed lands that are permanently protected [3]. A sustainable alternative to preserve Brazilian rosewood is the maintenance of the agroforestry system.

The first study to assess the genetic variability of *D. nigra* was carried out using RAPD markers to estimate the diversity of a germplasm bank [19] and native tree populations [20] from Estação Ecológica Pau-Brasil (ESPAB), Porto Seguro, state of Bahia. Later, allozyme markers were used for the genetic diversity study of Brazilian rosewood in forest fragments in Minas Gerais [2]. After the development of a set of ten polymorphic microsatellite loci for Brazilian rosewood [21], a broader genetic analysis could be carried out. For example, a higher population spatial genetic structure was reported in the adults rather than in saplings from the secondary forest fragment [22]. Recently, a study using adult trees from 20 different regions of the State of Espírito Santo revealed only three distinct genetic groups [23]. However, few studies have focused on the conservation genetic status of this species along its area of occurrence in the Atlantic Forest.

The remaining populations of Brazilian rosewood in different regions could differ genetically within each area of occurrence. A survey of 19 Brazilian rosewood populations using the variation in chloroplast DNA (cpDNA) sequences along the geographical range of the species revealed 15 different haplotypes which can be separated into three main geographical groups, each of which can be considered as a distinct management unit [24]. Therefore, in the present study, we investigated if nearby populations with different levels of current conservation status show significant genetic structuring. Hypothetically, any significant structuring that was found within populations of a particular phylogeographic group would support that population to be considered an independent evolutionary unit in regards its conservation. Our study assessed the genetic diversity of populations of Brazilian rosewood in remnants of the Atlantic Forest in southern Bahia using microsatellite markers, aiming at helping to provide recommendations for strategies for the conservation

of this species. According to the characteristics of the species, it was expected that the process of logging had promoted the reduction of genetic variability, increased the levels of inbreeding, and thus altered the genetic structure of natural populations, even in populations close to each other. The result of such a finding could require regional management strategies.

2. Materials and Methods

Leaf samples were collected from adult trees from four natural populations of Brazilian rosewood in the southern region of Bahia, Brazil. Sample sites were selected within a maximum distance of 80 km of each other, and leaves were collected from 35 individuals per population, totaling 140 trees (Figure 1). The populations are found in areas with different degrees of anthropic disturbance in the Atlantic Forest. The population of Ibicaraí (14°51′54″ S 39°35′16″ W) lies in a preserved forest in the Manacá—RPPN Manacá (RPPN means "Private Natural Heritage Reserve" from the Portuguese Reserva Particular do Patrimônio Natural). The population of Barro Preto (14°48′36″ S 39°28′15″ W) lies in a rainforest region with evidence of human occupation in the RPPN Pedra Lascada. The population of Arataca (15°15′46″ S 39°24′50″ W) includes a cabruca typical of southern Bahia and is located on a farm (Fazenda Jaci). The cabruca is an agroforestry system that keeps a thinned layer of native canopy species that provide shade for the cacao trees [25] and is a more permeable matrix in comparison to other crops [26]. The population of Uruçuca (14°35′34″ S 39°17′02″ W) lies within a pasture area. The sampled individuals had a diameter at breast height (DBH) equal or superior to 15 cm and were from 60 to 100 m apart.

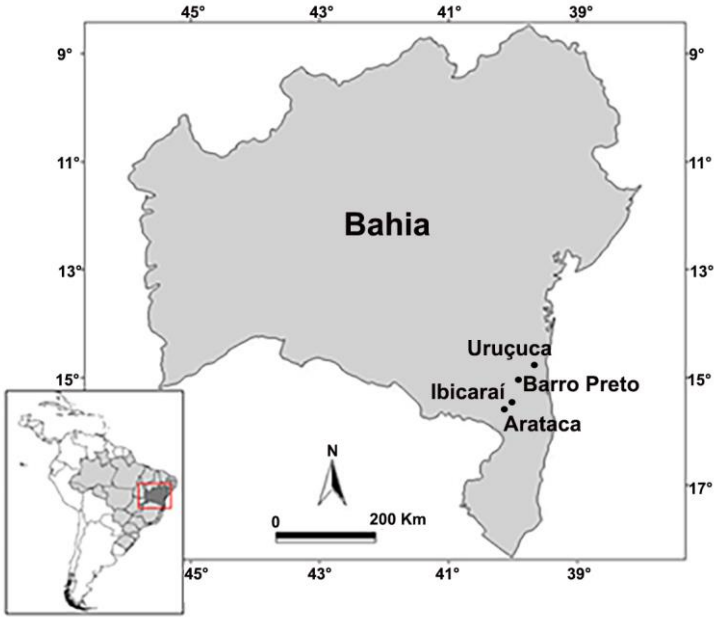

Figure 1. Geographic distribution of four Brazilian rosewood populations in southern Bahia, in Atlantic Forest remnants. The red box represents the enlarged area on the map.

Plant leaves were dried in silica gel and stored at −20 °C. Leaf DNA samples were extracted following the 2% CTAB protocol that Novaes et al. [27] adjusted for species with high amounts of polyphenols, polysaccharides, and secondary compounds. DNA quantification was performed using a Gene Quant Pro Spectrophotometer (Amersham Biosciences, Amersham, UK). Standard DNA samples (λ DNA) were used to compare and assess the extracted DNA.

Nine out of ten microsatellite primer pairs previously developed and optimized for Brazilian rosewood [21] were used to analyze 140 individuals representing the four populations sampled in this study. We used the methodology of primer tailing according

to the PCR conditions described by Ribeiro et al. [21]. Microsatellite locus amplification, electrophoresis, and allele-size determination by fluorescence detection in multiplexed assays were carried out following Lemes et al. [28].

The detection and estimation of allele size were carried out using GeneScan (Applied Biosystems v.3.1.2). The values were imported into Genotyper (Applied Biosystems v.2.5.2) for filtering peaks, interpretation, and final compilation of data, hence defining the genotype of each individual.

MICRO-CHECKER [29], parameterized with a 95% confidence interval and 10,000 randomizations, was used to determine the frequency of null alleles per locus both at the subpopulation and general levels, based on the Brookfield methodology [30]. FREENA [31], executing 10,000 replicates, was used to calculate global and per-locus Fst, using the ENA null allele correction methodology, as well as without correction.

Using the MICRO-CHECKER-corrected data, the following intrapopulation genetic diversity parameters were estimated using Genealex v.6.51b2 [32]: average number of alleles per locus ($\hat{A}$), private alleles, gene diversity or expected heterozygosis (H_E), observed heterozygosity (H_O), maxim genetic diversity (H_{max}), and the effective number of alleles per population. The latent genetic potential (LGP) was computed as the difference between the total and effective number of alleles summed over all loci by population [33]. Additionally, the structuring of genetic variability was investigated by the estimation of hierarchical F coefficients $f = F_{IS}$, $\Theta_P = F_{ST}$ and $F = \mathrm{F_{IT}}$ [34] using. F_{ST} values between population pairs were estimated, and the significance tests of multilocus pairwise were carried out using the log-likelihood statistic G implemented in Genealex v.6.51b2, with Bonferroni corrections [35]. Genetic differentiation according to the stepwise mutation model was assessed using an estimation of Slatkin's R_{ST} [36] analogous to F_{ST}. Given that some populations have suffered anthropic disturbances, the genetic differentiation was estimated using the analysis of diversity in subdivided populations of Nei [37] (G'_{ST}) with Hedrick's [38] correction. In order to estimate these parameters, a random model was assumed so that the sampled populations were considered local representatives of the species and thus assumed to have a common evolutionary history [39].

The historical gene flow among populations was quantified using indirect measures according to the model of Crow and Aoki [40] using the formula $Nm = \left(\frac{1}{4\alpha}\right)\left[\left(\frac{1}{F_{ST}}\right) - 1\right]$, where the estimated genetic divergence, F_{ST}, was replaced by θ_P, R_{ST}, and $\mathrm{G_{ST}}'$ parameters, and the correction for a finite number of populations (n) was calculated as $\alpha = \left[\frac{n}{(n-1)}\right]^2$.

The effective population size (N_e) was estimated for each population using the formula $N_e = \frac{n}{(1+f)}$ [41], N_e is the population genetic representativeness, n is the population size, and f is the fixation index [42]. The genetic representativeness of the samples was tested by the ratio $\frac{N_e}{n}$. This parameter is the estimated number of individuals representing an effective size of 500 plants in an ideal panmictic population. The number of populations to be conserved for the maintenance of genetic diversity in 10 to 15 generations was obtained following the model proposed by Vencovsky and Crossa [41].

STRUCTURE v2.3.4 [43] was used to investigate the population structure of the four populations without a priori hierarchy. The number of clusters was defined from K = 1 to K = 10, and 20 runs of each K were performed with a 100,000 burn-in period and 400,000 MCMC (Markov Chain Monte Carlo) and a model that allowed admixture and correlated allele frequencies. The optimal number of clusters was estimated using ΔK values, following Evanno et al. [44], using the Structure Harvest software [45].

A pairwise population matrix of Nei Unbiased Genetic Distance and a pairwise genetic distance matrix at the individual level were calculated using Genealex v.6.51b2. The distance matrix between pairs of genotypes used to construct the dendrogram was calculated based on the proportion of common alleles [46]. Dendrograms were constructed using the MEGA4 program [47]. For the population analysis, the UPGMA clustering method was applied and the neighbor-joining method was used at the individual level.

3. Results

A total of 154 alleles was detected in nine microsatellite loci across the four populations studied (Table S1). The mean number of alleles per locus was 5.944, ranging from 3.5 (locus Dnig7) to 8.5 (Dnig9) alleles (Table 1). The mean total expected heterozygosity (H_E = 0.733) was higher than the mean total observed heterozygosity (H_O = 0.554). H_E values ranged from 0.618 (locus Dnig7) to 0.817 (locus Dnig9), whereas H_O values ranged from 0.342 (locus Dnig3) to 0.692 (locus Dnig5). These estimates revealed a diversity reduction in the sampled populations, despite the high number of alleles detected (Table 1). Nevertheless, the observed heterozygosity detected 68% of the maximum possible genetic diversity (mean $H_{E\ MAX}$ = 0.820). The mean fixation index over all loci was high (f = 0.167), ranging from 0.068 (locus Dnig9) to 0.192 (locus Dnig7), showing considerable inbreeding.

Table 1. Estimates of genetic parameters obtained from the genotyping of nine microsatellite loci in 140 Brazilian rosewood individuals.

	Dye	pb Range	Ta	n_A	n_{pa}	$\hat{A}$	H_{MAX}	H_E	H_O	f
Dnig1	6FAM-G1	241–265	55	25	9	6.25	0.84	0.743	0.576	0.181
Dnig2	6FAM-G2	152–244	55	29	7	7.25	0.86	0.777	0.605	0.130
Dnig3	6FAM-G3	174–208	55	18	6	4.5	0.78	0.659	0.342	0.405
Dnig4	HEX-G2	197–241	55	24	5	6	0.84	0.765	0.634	0.121
Dnig5	HEX-G1	236–288	55	29	7	7.25	0.86	0.785	0.692	0.097
Dnig6	HEX-G3	263–269	55	22	3	5.5	0.82	0.718	0.479	0.181
Dnig7	NED-G3	198–220	57	14	2	3.5	0.71	0.618	0.558	0.056
Dnig9	NED-G1	216–270	55	34	6	8.5	0.88	0.817	0.590	0.163
Dnig10	NED-G2	251–277	54	19	1	4.75	0.79	0.712	0.510	0.170
Average	-	-	-	-	5.1	5.944	0.82	0.733	0.554	0.167

Ta is the annealing temperature (°C), n_A is the total number of alleles per locus, n_{pa} is the number of private alleles, $\hat{A}$ is the mean number of alleles per locus, H_{MAX} is the *maxim* genetic diversity, H_E is the mean genetic diversity, H_O is the mean heterozygosity, f is the mean fixation index.

All populations showed equally high genetic diversity with mean H_E = 0.738, ranging from 0.730 in Ibicaraí to 0.748 in Uruçuca. The genetic diversity was similar both for the most conserved populations close to RPPNs (Barro Preto and Ibicaraí) and for populations undergoing significant anthropic disturbances (Uruçuca and Arataca) (Table 2). Although the mean observed heterozygosity calculated among populations was high (H_O = 0.622), the H_O values for each population were lower than H_E values. The differences found in estimates of H_E and H_O corroborate the excess of homozygotes found in those populations (f = 0.167) (Table 2). The highest inbreeding coefficient (f) values were found in the less preserved areas (0.192 in Uruçuca and 0.228 in Arataca populations). The N_e was similar in the four populations analyzed, whereas the genetic representativeness was superior to 70%. The estimate of effective size to maintain inbreeding stability for 10–15 generations revealed that at least 600 Brazilian rosewood trees are necessary for each population. Many private alleles were identified, and the Uruçuca (n_{ap} = 1.444) and Arataca (n_{ap} = 1.333) populations showed the highest values.

The Brazilian rosewood populations were highly structured as estimated by the statistic θ_p value equal to 0.118 (Table 3). Both the F_{ST} values calculated without considering null alleles and the F_{ST} values with ENA correction showed that there was genetic differentiation between the populations. Genetic differentiation for all pairwise combinations between populations was significant, and the populations of Uruçuca and Barro Preto showed the greatest differentiation from the others (Table 4). Additionally, these two populations were genetically more distant in the UPGMA clustering analysis (Figure 2). The lowest genetic distance was observed between Ibicaraí and Arataca populations, compared to the Uruçuca population. Notably, the population pairs found to be most genetically divergent were those that were most geographically distant (Figures 1 and 2). These estimates can be con-

sidered moderate and suggest a limited historical gene flow among these four populations ($Nm_{\theta_p} = 1.025$).

Table 2. Estimates of genetic parameters for four Brazilian rosewood populations of southern Bahia based on the analysis of nine microsatellite loci.

	n	n_A	n_{pa}	n_e	LGP	H_E (SD)	H_O (SD)	f	N_e	N_e/n	N_e = 500
Ibicaraí	35	52	1.222	36.075	15.925	0.730 (0.028)	0.638 (0.034)	0.125	28.44	0.813	615.33
Barro Preto	35	51	1.111	34.714	16.286	0.732 (0.019)	0.638 (0.016)	0.124	30.22	0.863	579.09
Arataca	35	58	1.333	41.653	16.347	0.744 (0.047)	0.605 (0.078)	0.228	26.11	0.746	670.24
Uruçuca	35	53	1.444	38.705	14.295	0.748 (0.025)	0.606 (0.037)	0.192	24.44	0.698	716.04
Average	-	-	-	-	-	0.738	0.622	0.167	-	-	-

n is the number of accessions analyzed for each population, n_A is the number of alleles per locus, n_{pa} is the mean of number of private alleles, n_e is the number of effective alleles per locus, LGP is the Latent Genetic Potential, H_E is the mean genetic diversity, H_O is the mean heterozygosity, f is the mean fixation index, N_e is the effective population size, (SD) is the standard deviation. Significance levels were based on 10,000 permutations ($p < 0.0014$).

Table 3. Estimates of genetic parameters for four populations of Brazilian rosewood trees. Confidence interval (CI) at 95% probability.

F (SD)	Θ_p (SD)	F (SD)
0.159 (0.025)	0.118 (0.013)	0.258 (0.026)

F coefficients: the $f = F_{IS}$, $\Theta_p = F_{ST}$ and $F = F_{IT}$ (Weir and Cockerham, 1984).

Table 4. Pairwise Θ_p among populations of Brazilian rosewood from four forest fragment.

	Barro Preto	Arataca	Uruçuca
Ibicaraí	0.094	0.089	0.106
Barro Preto		0.068	0.069
Arataca			0.076

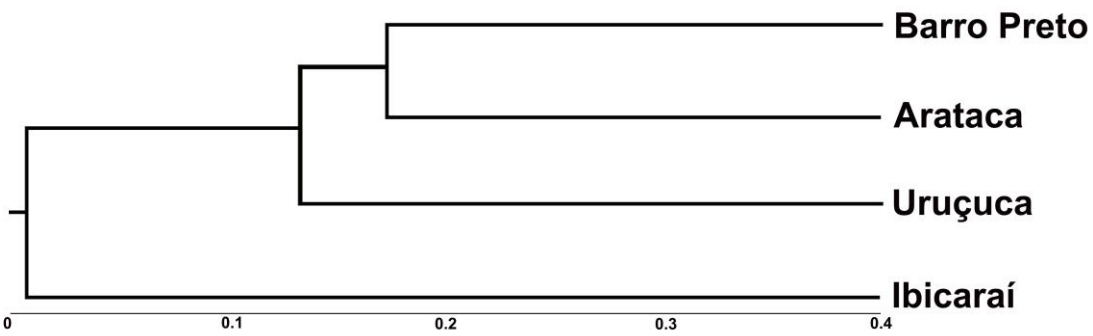

Figure 2. Graphic representation of the clustering analysis using UPGMA based on Nei's unbiased minimum genetic distance (1978) for four Brazilian rosewood populations based on microsatellite markers.

The clustering-based Bayesian model was applied to detect the genetic structure of 140 individuals of Brazilian rosewood. According to the ΔK values [44], the highest log likelihood scores were found when the number of groups was set at five (Figure 3 and Figure S1), ΔK = 5, indicating the assemblage of most individuals within their respective collection population. Clustering analysis confirmed the isolation pattern detected with θ statistic. In agreement with the structure analysis, the neighbor-joining tree showed that the individuals tended to form groups similar to their respective collection population, evidencing a significant population structure (Figure 4).

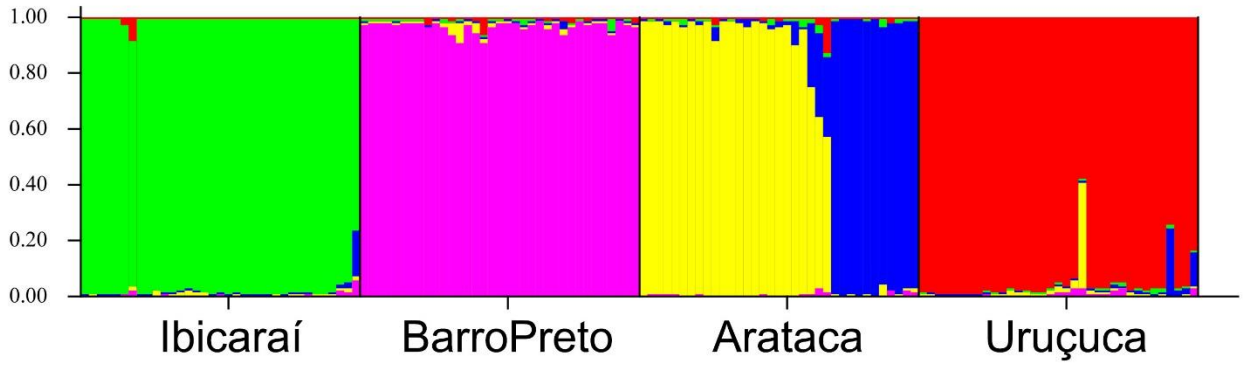

Figure 3. Clustering of individuals by structure at K = 5. Individuals are represented by vertical bars. The same colors in different individuals indicate that they belong to the same cluster. Different colors in the same individual indicate the percentage of the genome shared with each cluster, according to the admixture proportions.

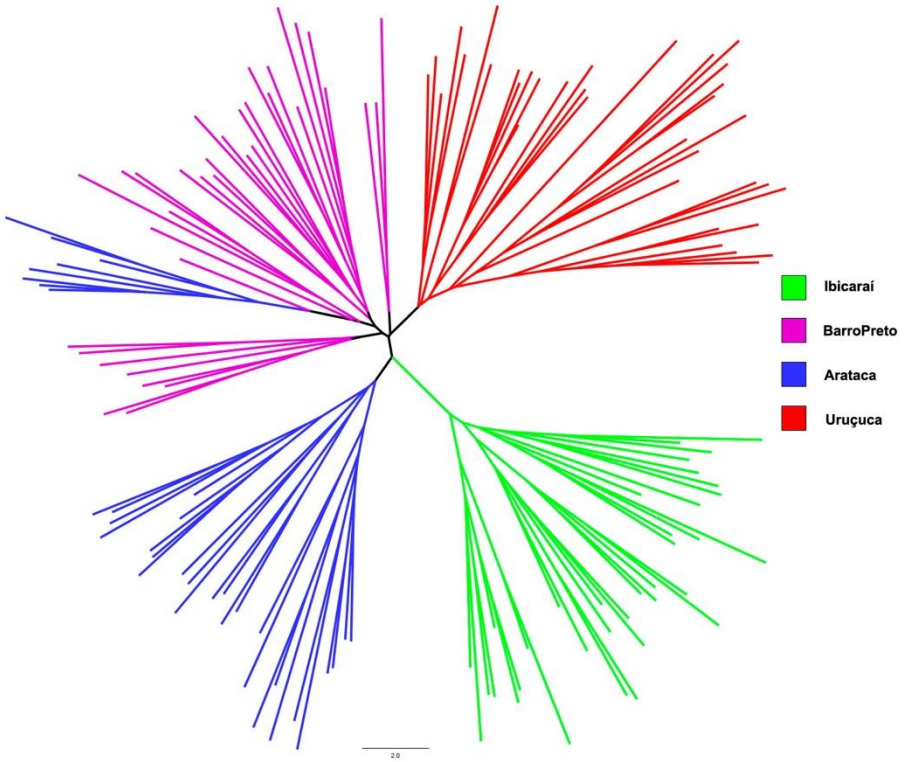

Figure 4. Unrooted neighbor-joining (NJ) phenogram based on the distances between genotype pairs, based on PCR amplification of DNA samples of 140 Brazilian rosewood individuals with nine SSR primers.

4. Discussion

The allelic diversity parameters observed for Brazilian rosewood were similar to values commonly reported for other wild plants analyzed using microsatellite markers. Nybom [48] reviewed several intraspecific genetic diversity studies in plants based on microsatellites, and in this review, he reported an average of 83.2 alleles with $\hat{A}$ = 9.9 alleles per loci, genetic diversity H_E = 0.61 (DV $\pm$ 0.21) and heterozygosity H_O = 0.58 (DV $\pm$ 0.22). Brazilian rosewood samples from different regions of the state of Espírito Santo also revealed values similar to these diversity estimates [23]. The results found in our study are important because, even though the habitat remains under fragmented conditions, the forest fragments still retain some critical reserves of Brazilian rosewood trees with

high genetic variability, as also related to other small fragment forests by Leite et al. [22]. Nevertheless, it is essential to consider that the sampling comprised individuals with DBH above 15 cm. Therefore, these Brazilian rosewood individuals with such growth patterns were probably generated in different flowering periods over 30 years ago, representing the genetic diversity of a recent fragmentation. Thus, the effects of genetic drift have not been sufficient to cause a significant loss and/or fixation of alleles. Results of studies evaluating chronic habitat fragmentation revealed the strong genetic structure and inbreeding in the remaining species pollinated or dispersed by the wind. They showed that the effects of fragmentation are detected after a few generations because of the longevity of most tree species [49]. The fragmentation in the region of the present study is recent. Fragmentation started in the 1950s with the construction of a north–south Brazilian highway (BR 101). Therefore, the elapsed time has probably been too short for genetic drift and selection to cause pronounced differences between populations of a long-lived tree.

The fixation index differed significantly from zero (f = 0.159, $p > 0.05$). This result might derive from biparental inbreeding or the Wahlund effect or yet from null alleles. Long-distance pollen dispersal and tolerance to selfing are two strategies to overcome demographic restrictions to allow efficient cross-pollination [50]. In contrast, *D. nigra* is mainly pollinated by bees [51,52], which tends to restrict pollen flow compared to the foraging habits of specialized pollinators. The seed dispersal system for Brazilian rosewood is anemochoric and/or barochoric [53], which is less effective in terms of distance. Thus, seeds likely germinate near the parent tree, leading to the formation of families. Adult Brazilian rosewood trees bloom and bear fruit within 2–3 years, and their fruit ripens before the new leafing [54,55]. The characteristic supra-annual flowering of this species should also be considered as it is shared among long-lived trees and may intensify the fragmentation effects, resulting in a smaller effective population size in small fragments [56]. The reproductive system of Brazilian rosewood is probably outcrossing. Thus, the observed coefficient of inbreeding could have derived from mating between half-sib (0.125) and/or full-sib families (0.25), but the occurrence of the Wahlund effect cannot be entirely discarded due to the possible existence of temporal breeding subunits within the sampled regions. Alves et al. [57] showed that 93% of the individuals from open-pollinated progenies appeared to be full sibs.

Studies on Brazilian rosewood from undisturbed primary forest vegetation revealed results different from those obtained in this study. Using different markers, Resende et al. [58] detected a fixation index for a rosewood population that was not significant (f = 0.047). Buzatti et al. [59] reported a fixation index significantly different from zero (f = 0.087, $p < 0.05$), probably associated with the reproductive system since the Bayesian analysis showed a single gene cluster. Resende et al. [58] found no evidence of inbreeding in their study.

The estimates of genetic diversity (H_E, H_O, and f) between the human-disturbed populations of rosewood (Uruçuca and Arataca) and the better-preserved populations located near the RPPNs (Barro Preto and Ibicaraí) were positive and significant. Our data showed that the causes for the different estimates between Brazilian rosewood populations analyzed was the direct loss of genetic diversity from fragmentation. This suggests that these populations are evolving independently but that they can still preserve the species' genetic diversity. Our results were consistent with those obtained by Ribeiro et al. [24], confirming the predictions of theoretical studies that populations from most preserved habitats hold greater diversity, and that populations in more disturbed areas tend to have lower levels of diversity [11]. Resende et al. [58] carried out a phylogeographic study of populations of Brazilian rosewood and showed that conserved populations had more haplotypes than small populations that have suffered disturbances. The present study showed a relatively high genetic variation in the populations studied. This suggests that, even when small fragments are analyzed, the degree of their conservation is vital for the maintenance of variability. It also indicates the possibility of conserving these populations in situ, especially in the case of an endangered species such as the Brazilian

rosewood. Currently, human occupation and habitat fragmentation represent a significant cause of biodiversity erosion, and thus understanding their effects is the key to successful conservation programs.

The population of Uruçuca showed the highest estimates of private alleles, thereby supporting the hypothesis that fragmentation and isolation provoke genetic differentiation. This population was the most divergent of all populations when Nei's genetic distances were calculated (Figure 2). A small and isolated population will sharply suffer the effects of genetic drift, genetic bottleneck, and inbreeding in all loci [14]. Thus, fragmented and isolated populations become very vulnerable and may also become endangered. *Ne* is an important estimator in genetic structure studies and contributes to the design of conservation strategies. The *Ne* depends on the number of individuals who effectively contribute to the next generation; then, the *Ne* indicates the genetic representativeness of the population [14]. Thus, genetic drift may occur in the short term when the Ne is reduced. In the long term, inbreeding resulting from the mating system may be higher due to the higher crossing probability among related individuals in populations with reduced N_e. The ratio between effective (*Ne*) and actual (*n*) population size is a parameter that conservation planners use to assume that the population is still viable to preserve. The ratio Ne/n is generally between 0.25 and 1.0 [60]. In this study, the minimum viable population was higher than 0.74, indicating the possibility of conserving these populations in situ.

Govindaraju [61] distinguished three levels of gene flow: high ($Nm > 1$), intermediate ($0.25 < Nm < 0.99$), and low ($Nm < 0.25$). The gene flow detected in this study ($Nm_{\theta_p} = 2.092$) and the number of private alleles (46) was relatively high. According to Wright [62], a single migrant per generation is sufficient to avoid the effects of genetic drift and inbreeding among small populations. However, gene flow disruption plays an essential role in speciation. The low gene flow after the fragmentation between populations analyzed may explain the high estimates of F_{ST}. As few cpDNA haplotypes were observed in southeastern Bahia [24], we hypothesized that there would have been a metapopulation in the past. But, as fragmentation is recent, no such high levels of genetic structuring were found. However, our data revealed that different populations within 80 km diverge when analyzed by SSR. In this study, besides the frequency statistic (F_{ST} and N_m), we used probabilistic statistics (Bayesian), which revealed that these populations are evolving independently but can still preserve the species' genetic diversity. The results obtained by Bayesian statistics are consistent with the estimates of parameters of genetic structure based on frequency statistics (F_{ST} and N_m). The divergence might be caused by inbreeding, genetic drift, and reduced gene flow. Results obtained from the analysis of genetic structure without a priori hierarchy confirmed that conserving the genotypes of the populations studied would be prudent. We reaffirm the need for the creation of corridors for the maintenance of gene flow and hence homogenization of allele frequencies of these populations.

The results obtained here are correlated to the stochastic effects of fragmentation and complement previous studies for this species [2,20,24,58,59]. In previous studies, it has been verified that the maintenance of germplasm banks is a strategy for preserving genetic diversity suitable for Brazilian rosewood, according to data on diversity from the germplasm bank of the Estação Ecológica Pau-brasil, Porto Seguro, BA [19,20]. Other studies showed that the maintenance of continuous forest fragments is vital to preserving the genetic diversity of Brazilian rosewood [59]. Also, continuous forests may be connected with smaller and disturbed fragments offsetting the effects of fragmentation since, as demonstrated, these populations have high levels of genetic diversity, and even low flow still occurs [58]. Our results regarding Brazilian rosewood's genetic diversity and structure also indicate that conservation strategies should be aimed at all the sampled populations since, as demonstrated, these populations have high levels of genetic diversity and some gene flow still occurs. One of the difficulties in maintaining the connectivity between forest fragments is the considerable geographical distance between fragments (80 km at least), which prevents gene flow. A way of connecting forest fragments would be to create a porous matrix for pollinators.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14112165/s1, Figure S1: Log-likelihood graph showing the optimal number of clusters of 140 *Dalbergia nigra* individuals from different sites in Atlantic Forestry, Bahia, Brazil. Figure S2: Clustering of individuals by structure at K = 4, numbering the field and lab identity. Table S1: Microsatellite loci amplified from individuals of four different *Dalbergia nigra* populations. Table S2: Estimates of inbreeding coefficient in four populations of *Dalbergia nigra* for 10 SSR loci.

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References

1. Carvalho, A.M.V. A synopsis the genus *Dalbergia* (Fabaceae: Dalbergieae) in Brazil. *Britonia* **1997**, *49*, 87–109. [CrossRef]
2. Ribeiro, R.A.; Ramos, A.C.S.; Lemos Filho, J.P.; Lovato, M.B. Genetic variation in remnant populations of *Dalbergia nigra* (Papilionoideae) on endangered tree from the Brazilian Atlantic Forest. *Ann. Bot.* **2005**, *95*, 1171–1177. [CrossRef] [PubMed]
3. Rêgo, G.M.; Possamai, E. Jacarandá-da-Bahia (*Dalbergia nigra* Vellozo) Leguminoseae—Papilionoideae: Produção de mudas. Colombo: Embrapa Florestas 1–3. (Comunicado Técnico, 106). 2003. Available online: http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/309652 (accessed on 18 February 2018).
4. Varty, N. *Dalbergia nigra*. In IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. 1998. Available online: www.iucnredlist.org (accessed on 17 January 2013).
5. IBAMA—Instituto Brasileiro de Meio Ambiente. *Instrução Normativa, 19 de Setembro de 2008, O IBAMA Torna Pública a Lista Oficial de Espécies da Flora Brasileira Ameaçadas de Extinção*; IBAMA: Brasília, DF, Brazil, 2008.
6. CITES. *CITES: Appendices I, II and III of the Convention on International Trade in Endangered Species of Wild Fauna and Flora*; US Fish and Wildlife Service: Washington, DC, USA, 1992.
7. Lagos, A.R.; Muller, B.L.A. Hotspot brasileiro: Mata Atlântica. *Saúde Ambiente Rev.* **2007**, *2*, 35–45.
8. Guimarães, M.; Alexandrino, J.; Haddad, C.F.B. Conservação da biodiversidade. *Ciência Hoje* **2007**, *233*, 60–61.
9. Ehrlich, P.R. The loss of diversity: Causes and consequences. In *Biodiversity*; Wilson, E.O., Ed.; National Academy Press: Washington, DC, USA, 1998; pp. 29–35.
10. Kareiva, P.; Wennergren, U. Connecting landscape patterns to ecosystem and population process. *Nature* **1995**, *373*, 299–302. [CrossRef]
11. Young, A.; Boyle, T.; Brow, T. The population genetic consequences of habitat fragmentation for plants. *Trends Ecol. Evol.* **1996**, *11*, 413–418. [CrossRef]
12. Freire, J.M.; Piña-rodrigues, F.C.M.; Lima, E.R.; Sodré, S.R.C.; Corrêa, R.C. Estrutura genética de populações de *Schizolobium parahyba* (Vell.) Blake (guapuruvu) por meio de marcadores RAPD. *Sci. For.* **2007**, *74*, 27–35.
13. Raposo, A.; Martins, K.; Ciampi, A.Y.; Wadt lho Veasey, E.A. Diversidade genética de populações de andiroba no baixo Acre. *Pesqui. Agropercuária Bras.* **2007**, *42*, 1291–1298. [CrossRef]
14. Ellastrand, N.C.; Elam, D.R. Population genetics consequences of small population size: Implications for plant conservation. *Annu. Rev. Ecol. Syst.* **1993**, *24*, 217–242. [CrossRef]
15. Tonsor, S.J.; Kalisz, J.; Fisher, J.; Holtsford, T.P. A lifehistory based study of population genetic structure: Seed bank to adults in plantago lanceolata. *Evolution* **1993**, *47*, 833–843.

16. Rossum, F.V.; Triest, L. Fine-scale genetics structure of the common *Primula elatior* (Primulaceae) at an early stage of population fragmentation. *Am. J. Bot.* **2006**, *93*, 1281–1288. [CrossRef] [PubMed]
17. Wang, J.I. A new method for estimating effective population sizes from a single sample of multilocus genotypes. *Mol. Ecol.* **2009**, *18*, 2148–2164. [CrossRef]
18. Schwartzl, M.K.; Tallmon, D.A.; Luikart, G. Review of DNA-based census and effective population size estimators. *Anim. Conserv.* **1998**, *1*, 293–299.
19. Almeida, M.P. Avaliação da Diversidade Genética de Acessos Ex Situ de Jacarandá-da-Bahia (*Dalbergia nigra* Vell. Allemão ex Benth.) por meio de Marcadores RAPD, Como Subsídio para sua Conservação. Master's Thesis, Universidade Estadual de Santa Cruz, Ilhéus, BA, Brazil, 2001.
20. Juchum, F.S.; Leal, J.B.; Santos, L.M.; Almeida, M.P.; Ahnert, D.; Corrêa, R.X. Evaluation of the genetic diversity in a natural rosewood population (*Dalbergia nigra* Vell. Allemão ex Benth.) using RAPD markers. *Genet. Mol. Res.* **2007**, *6*, 543–553. [PubMed]
21. Ribeiro, R.A.; Rezende, M.F.S.; Resende, L.C.; Lemos-Filho, J.P.; Kalapothakis, E.; Lovato, M.B. Development of polymorphic microsatellite markers for *Dalbergia nigra* (Papilionoideae), an endangered tree from the Brazilian Atlantic Forest. *Mol. Ecol. Resour.* **2008**, *9*, 203–206. [CrossRef] [PubMed]
22. Leite, F.A.B.; Brandão, R.L.; Buzatti, R.S.O.; Lemos-Filho, J.P.; Lovato, M.B. Fine-scale genetic structure of the threatened rosewood *Dalbergia nigra* from the Atlantic Forest: Comparing saplings versus adults and small fragmente versus continuous forest. *Tree Genet. Genomes* **2014**, *10*, 307–316. [CrossRef]
23. Silva-Júnior, A.L.; Cabral, R.L.R.; Sartori, L.; Miranda, F.D.; Caldeira, M.V.W.; Moreira, S.O.; Oliveira-Godinho, T.; Oliveira, F.S. Molecular markers applied to the genetic characterization of *Dalbergia nigra*: Implications for conservation and management. *Trees* **2022**, *36*, 1539–1557. [CrossRef]
24. Ribeiro, R.A.; Lemos-Filho, J.P.; Ramos, A.C.S.; Lovato, M.B. Phylogeography of the endangered rosewood *Dalbergia nigra* (Fabaceae): Insights into the evolutionary history and conservation of the Brazilian Atlantic Forest. *Heredity* **2011**, *106*, 46–57. [CrossRef]
25. Sambuichi, R.H.R. Fitossociologia e diversidade de espécies arbóreas em cabruca (mata Atlântica raleada sobre plantação de cacau) na região sul da Bahia, Brasil. *Acta Bot. Bras.* **2002**, *16*, 89–101. [CrossRef]
26. Sambuichi, R.H.R. Estrutura e dinâmica do componente arbóreo em área de cabruca na região cacaueira do sul da Bahia, Brasil. *Acta Bot. Bras.* **2006**, *20*, 943–954. [CrossRef]
27. Novaes, R.M.L.; Rodrigues, J.G.; Lovatom, B. An efficient protocol for tissue sampling and DNA isolation from the stem bark of leguminosae trees. *Genet. Mol. Res.* **2009**, *8*, 86–96. [CrossRef] [PubMed]
28. Lemes, M.R.; Brondani, R.P.V.; Grattapaglia, D. Multiplexed systems of microsatellite markers for genetic analysis in mahogany, *Swietenia macrophylla* King (Meliaceae), a threatened Netropical timber species. *J. Hered.* **2002**, *93*, 287–290. [CrossRef] [PubMed]
29. Van Oosterhout, C.; Hutchinson, W.F.; Wills, D.P.M.; Shipley, P. micro-checker: Software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* **2004**, *4*, 535–538. [CrossRef]
30. Brookfield, J.F.Y. A simple new method for estimating null allele frequency from heterozygote deficiency. *Mol. Ecol.* **1996**, *5*, 453–455. [CrossRef] [PubMed]
31. Chapuis, M.P.; Estoup, A. Microsatellite Null Alleles and Estimation of Population Differentiation. *Mol. Biol. Evol.* **2007**, *24*, 621–631. [CrossRef]
32. Peakall, R.; Smouse, P.E. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—An update. *Bioinformatics* **2012**, *28*, 2537. [CrossRef]
33. Bergmann, F.; Gregorius, H.R.; Larsen, J.B. Levels of genetic variation in European silver fir (*Abies alba*)—Are they related to the species' decline? *Genetica* **1990**, *82*, 1–10. [CrossRef]
34. Weir, B.S.; Cockerham, C.C. Estimating F-statistics for the analysis of population structure. *Evolution* **1984**, *38*, 1358–1370. [PubMed]
35. Rice, W.R. Analyzing tables of statistical tests. *Evolution* **1989**, *43*, 223–225. [CrossRef]
36. Slatkin, M. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **1995**, *139*, 457–462. [CrossRef]
37. Nei, M. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **1978**, *89*, 583–590. [CrossRef] [PubMed]
38. Hedrick, P.W. A standardized genetic differentiation measure. *Evolution* **2005**, *59*, 1633–1638.
39. Weir, B.S. *Genetic Data Analysis*; Sinauer Associates: Sunderland, MA, USA, 1996; Volume II.
40. Crow, J.F.; Aoki, K. Group selection for a polygenic behavioral trait: Estimating the degree of population subdivision. *Evolution* **1984**, *81*, 6073–6077. [CrossRef] [PubMed]
41. Vencovsky, R.; Crossa, J. Measurements of representativeness used in genetic resources conservation and plant breeding. *Crop Sci.* **2003**, *43*, 1912–1921. [CrossRef]
42. Wright, S. The genetical structure of populations. *Ann. Eugen.* **1951**, *15*, 323–354. [CrossRef]
43. Pritchard, J.K.; Stephens, M.; Donnelly, P.J. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959. Available online: http://pritch.bsd.uchicago.edu/structure.html (accessed on 18 February 2018). [CrossRef]
44. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef]

45. Earl, D.A.; Vonholdt, B.M. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* **2012**, *4*, 359–361. [CrossRef]
46. Bowcock, A.M.; Ruiz-Linares, A.; Tomfohrde, J.; Minch, E.; Kidd, J.R.; Cavalli-Sforza, L.L. High resolution of human evolution with polymorphic microsatellites. *Nature* **1994**, *368*, 455–457. [CrossRef]
47. Tamura, K.; Dudley, J.; Nei, M.; Kumar, S. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **2007**, *24*, 1596–1599. Available online: http://www.megasoftware.net/mega4/mega.html (accessed on 18 February 2018). [CrossRef]
48. Nybom, H. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Mol. Ecol.* **2004**, *13*, 1143–1155. [CrossRef] [PubMed]
49. Dubreuil, M.; Riba, M.; González-Martínez, S.C.; Vendramin, G.G.; Sebastiani, F.; Mayol, M. Genetic effects of chronic habitat fragmentation revisited: Strong genetic structure in a temperate tree, *Taxus baccata* (Taxaceae), with great dispersal capability. *Am. J. Bot.* **2010**, *97*, 303–310. [CrossRef] [PubMed]
50. Latouche-Hallé, C.; Ramboer, A.; Bandou, E.; Caron, H.; Kremer, A. Long-distance pollen flow and tolerance to selfing in a neotropical tree species. *Mol. Ecol.* **2004**, *13*, 1055–1064. [CrossRef] [PubMed]
51. Bortoluzzi, R.L.C.; Carvalho-okano, R.M.; Garcias, F.C.P. Tozzi AMGA Leguminosae, Papilionoideae no Parque Estadual do Rio Doce, Minas Gerais, Brasil. II: Árvores e arbustos escandentes. *Acta Bot. Bras.* **2004**, *18*, 49–71. [CrossRef]
52. Mathilde, I.; Oliveira, B.; Sigrist, M.R. Fenologia reprodutiva, polinização e reprodução de *Dipteryx alata* Vogel (Leguminosae-Papilionoideae) em Mato Grosso do Sul, Brasil. *Rev. Bras. Bot.* **2008**, *31*, 95–207.
53. Donadio, N.M.M.; Demattê, M.E.S.P. Morfologia de frutos, sementes e plântulas de canafístula (*Peltophorum dubium* (Spreng.) Taub.) e jacarandá-da-bahia (*Dalbergia nigra* (Vell.) Fr.All. ex Benth.)—FABACEAE. *Rev. Bras. Sementes* **2000**, *22*, 64–73. [CrossRef]
54. Leão, A.C.; Vinha, S.G. Ocorrência de Jacarandás no Sul da Bahia. *Cacau Atual.* **1975**, *12*, 22–27.
55. Carvalho, A.M.V. Os Jacarandás da Bahia. *Agrotrópica* **1989**, *2*, 1–10.
56. Hall, P.; Walker, S.; Bawa, K. Effect of forest fragmentation on genetic diversity and mating system in a tropical tree, *Pithecellobium elegans*. *Conserv. Biol.* **1996**, *10*, 757–768. [CrossRef]
57. Alves, R.M.; Artero, A.S.; Sebbenn, A.M.; Figueira, A. Mating system in a natural population of *Theobroma grandiflorum* (Willd. Ex Spreng.) Schum., by microsatellite markers. *Genet. Mol. Biol.* **2003**, *26*, 373–379. [CrossRef]
58. Resende, L.C.; Ribeiro, R.A.; Lovato, M.B. Diversity and genetic connectivity among populations of a threatened tree (*Dalbergia nigra*) in a recently fragmented landscape of the Brazilian Atlantic Forest. *Genetica* **2011**, *139*, 1159–1168. [CrossRef] [PubMed]
59. Buzatti, R.S.O.; Ribeiro, R.A.; Lemos-Filho, J.P.; Lovato, M.B. Fine-scale spatial genetic structure of *Dalbergia nigra* (Fabaceae), a threatened and endemic tree of the Brazilian Atlantic Forest. *Genet. Mol. Biol.* **2012**, *35*, 838–846. [CrossRef] [PubMed]
60. Nunney, L.; Campbell, K.A. Assessing minimum viable population size: Demography meets population genetics. *Trends Ecol. Evol.* **1993**, *8*, 234–239. [CrossRef] [PubMed]
61. Govindaraju, D.R. Estimates of gene flow in forest trees. *Biol. J. Linn. Soc.* **1989**, *37*, 345–357. [CrossRef]
62. Wright, S. Evolution in mendelian populations. *Genetics* **1931**, *16*, 111–123. [CrossRef]