

Genetic diversity and population restructuring of *Astronium fraxinifolium* Schott. (Anacardiaceae) in a heavily impacted Brazilian Savanna (Cerrado) anthropic area

Marcelo Augusto Mendes Alcantara

`marcelo.alcantara@unesp.br`

São Paulo State University (UNESP)

Maiara Ribeiro Cornacini

São Paulo State University (UNESP)

Ricardo Oliveira Manoel

São Paulo State University (UNESP)

Marcela Aparecida Moraes Silvestre

São Paulo State University (UNESP)

Bruno César Rossini

São Paulo State University (UNESP)

José Cambuim

São Paulo State University (UNESP)

Patrícia Ferreira Alves

São Paulo State University (UNESP)

Aparecida Juliana Martins Corrêa

São Paulo State University (UNESP)

Darlin Gonzalez Zaruma

National University of Loja (UNL), Universitaria Guillermo Falconi

Mario Luiz Teixeira Moraes

São Paulo State University (UNESP)

Celso Luis Marino

São Paulo State University (UNESP)

Research Article

Keywords: Genetic diversity, Gonçalves-Alves, natural population

Posted Date: December 18th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-5263336/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Brazil is a characteristic country for having an energy matrix essentially linked to its river courses and the forest fragmentation resulting from these impacts causes a decrease in the number of individuals in a population, favoring the loss of genetic variation. Therefore, preserving the diversity of native plant populations in these impacted ecosystems is extremely important, such as *Astronium fraxinifolium* Schott (Anacardiaceae), a dioecious and an endangered species typical of the Cerrado. In this context, from a natural population of *A. fraxinifolium*, located in a highly degraded area of Cerrado, the present work aimed to: i) estimate the genetic variation from the measurement of silvicultural characters; ii) determine the sex ratio; iii) investigate genetic diversity and structure using eight microsatellite loci. All growth characters were significant, with average height and diameter at breast height of 10.99 m and 21.30 cm, respectively, with females being more developed than males. The coefficient of genetic variability was above 25% in all traits. The sex ratio of the population is close to balance from 1:1 to 5% of significance. The population has a total of 101 alleles from 384 individuals analyzed with 8 microsatellite loci, resulting in an average of 12.5 allelic richness, 0.706 observed heterozygosity, 0.793 expected heterozygosity and 0.111 fixation index. The results of this work show the presence of genetic variation and diversity in the quantitative and molecular analyses, with use for mother trees to collect seeds for use in conservation programs, recovery of degraded areas and breeding.

Introduction

Brazil is a country known for its energy matrix closely tied to its river systems. Environmental licensing in Brazil between 1985 and 2015 mostly involved highly complex cases focused on hydroelectric power plants, leading to social and environmental impacts in their construction sites, often altering the microclimate of the region. These impacts result in a slow recovery of the area, taking decades for the adopted treatments to take effect (Alves *et al.*, 2012; Gasques *et al.*, 2014; Cruz *et al.*, 2016; Fainguelernt, 2016; Duarte *et al.*, 2017).

The forest fragmentation resulting from these impacts causes a decrease in the number of individuals in a population, leading to a loss of genetic variation. In these small impacted populations, genetic drift can occur in the short term, meaning that gene frequencies become different from those of the original population, resulting in the loss of alleles. In the long term, there may be an increase in inbreeding due to a higher probability of self-fertilization and mating between related individuals (Kageyama *et al.*, 1998; Caxambú *et al.*, 2015).

Therefore, preserving the diversity of native plant populations in these impacted ecosystems is extremely important. Conservation methods, especially those involving germplasm banks, help contribute to and ensure the preservation of species developed in these areas. Assessing the genetic representation of these banks can be done through the effective population size, which is a crucial parameter for evaluating the impact of drift on population genetic structure. Understanding the relationship between effective size and the actual size of a plant population is essential for conservation

planning. This data allows for identifying the number of remaining individuals in the preserved area, as well as estimating considerable genetic diversity among individuals, making it possible to certify the genetic variation that may be found in future batches of seedlings obtained from seed collection in trees present in the area (Moraes *et al.*, 1999; Ribeiro *et al.*, 2016; Souza, 2017).

Commonly known as “Gonçalo-Alves”, *Astronium fraxinifolium* Schott belongs to the Anacardiaceae family. It is a typical species of the Brazilian Savanna (Cerrado) and can also be found in ecotones between the Cerrado and the Caatinga, Atlantic Forest, and Pantanal biomes. It is a tree with a height of 25 to 30 meters, featuring a cylindrical and straight trunk with a diameter of 60 to 80 cm. The branches are branching, elevated, and sparse, with open foliage and a sturdy trunk that sheds all its leaves during winter (Lorenzi, 1998; Lorenzi, 2002; Machado *et al.*, 2006; Fernandes *et al.*, 2017; Zavala *et al.*, 2017).

The species has shown to be a promising agent for environmental recovery, as evidenced by studies in areas impacted by soil removal, which have yielded satisfactory results regarding plant diameter, height, and dry biomass assessments. These studies demonstrate the species' adaptation to low soil moisture conditions and improvement in observed soil physical conditions (Alves *et al.*, 2007; Campelo *et al.*, 2015).

In this context, it was deemed appropriate to study the natural regeneration of *A. fraxinifolium*, located in an area that was devastated for the construction of a hydroelectric power plant. Specifically, the following objectives were proposed: i) Estimate genetic variation based on silvicultural traits; ii) Determine the sex ratio within the population, as it is a dioecious species; iii) Investigate fixation index, diversity, and genetic structure using eight microsatellite loci; iv) Determine spatial genetic structure. These pieces of information are of great importance for a better understanding of adaptive mechanisms in populations under strong stresses in degraded areas.

Materials and methods

The study was conducted in a natural population of *A. fraxinifolium* located at the Teaching, Research, and Extension Farm - FEPE "Bovinos" of the Faculty of Engineering of Ilha Solteira - FEIS/UNESP in the municipality of Selvíria - MS, within a pre-delineated plot of approximately 170 hectares (Figure 1). This area is a remnant of a "loan area" used for the construction of the Ilha Solteira Hydroelectric Power Plant - SP (UHE Ilha Solteira) between 1967 and 1978 by the São Paulo Power Company - CESP and later incorporated by FEIS/UNESP (CESP, 2009; UNESP, 2017).

All sampled trees were identified and georeferenced with the aid of a GPS device. After this procedure, the height (ALT, cm) of the trees was measured using the Vertex IV hypsometer from Haglöf. The evaluation of diameter at breast height (DAP, cm) was conducted by converting the circumference at breast height (CAP, cm), conventionally at 1.30 m, by dividing the measured value by π (3.14). This procedure was carried out using a traditional measuring tape. Three measurements were taken for each of the traits at different points around each individual to standardize the data across three different periods. The sexing or sex ratio of the population under study was determined based on visual

observation of tree flowering. In total, 401 specimens were sampled, with 188 female trees and 213 male trees.

Leaf collection for DNA extraction was performed between the juvenile phase and the dehiscent period, totaling 384 trees. Genomic DNA extraction followed the protocol proposed by Doyle & Doyle (1990) with adaptations. Subsequently, quantification was done using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Products, DE, USA). To verify DNA integrity, electrophoresis was conducted on 3.5% agarose gels with TBE (1x) at a constant voltage of 120V. Leaf collection was authorized by the Institute for Biodiversity Conservation (ICMBio), linked to the Ministry of the Environment (MMA) under the number 73998–1.

Amplification was carried out through polymerase chain reaction (PCR) reactions. These were performed using primer sets developed by Cornacini *et al.* (2021). The 10 μ L amplification reaction included 1 μ L of genomic DNA (approx. 50 ng), 5 μ L of GoTaq Master Mix® (2x) (Promega/Cat. #M7133), 0.3 μ L of forward primer (2 pmol), 0.3 μ L of reverse primer (8 pmol), and 0.3 μ L of fluorescent primer with M13 tail (8 pmol; 6FAM, VIC, PET, or NED, Applied Biosystems), 0.5 μ L of Bovine Serum Albumin (BSA), 0.5 μ L of Magnesium Chloride, and 2.6 μ L of autoclaved Milli-Q ultrapure water.

The amplification reactions for all primers were carried out in an Eppendorf thermocycler programmed under the following conditions: initial denaturation step at 96°C for 5 minutes, followed by 35 amplification cycles (96°C [1 min], 2 min at the specific annealing temperature for each primer pair, 72°C for 2 min), followed by 12 cycles of 96°C for 1 min, 53°C for 2 min, 72°C for 2 min, and a final extension step at 72°C for 30 min. The amplifications were performed using a Mastercycler (Eppendorf, Hamburg, Germany). The PCR final product was subjected to capillary electrophoresis on the ABI3130xl Genetic Analyzer automatic sequencer (Applied Biosystems), along with the GeneScan 500 LIZ marker (Applied Biosystems). Genotype reading was done using GeneMapper software v.5.0 (Applied Biosystems).

Estimates of variance components and genetic parameters for the silvicultural traits ALT and DAP were obtained using the REML/BLUP (restricted maximum likelihood/best linear unbiased prediction) procedure, employing the genetic-statistical software SELEGEN-REML/BLUP (Resende, 2016). The sex ratio between male and female individuals within the population was analyzed using the chi-square (χ^2) statistical test (Opler & Bawa, 1978).

Genetic diversity was characterized by dividing the population into different clusters (Escudero *et al.*, 2003) based on the distance from the central point of the area, in circles with a radius of 150 meters (Figure 2), analyzing by locus and, on average, across all loci using the following indices: average number of alleles per locus (k), allelic richness (R), observed heterozygosity (H_o), and expected heterozygosity (H_e) according to expected proportions of Hardy-Weinberg equilibrium (HWE). The presence of inbreeding in all sampled generations was evaluated using the fixation index (F) within the population. The statistical significance of the values was tested using allele permutation resampling between individuals. These analyses were conducted using the FSTAT program (Goudet, 1995).

Results and discussion

3.1. Quantitative Traits

The silvicultural traits showed an overall mean of 10.99 m (ALT) and 21.30 cm (DAP). Female trees exhibited greater growth, with 11.37 m and 22.71 cm for height and DAP, respectively (Table 1). The presence of genetic variation among individuals in the population was confirmed through the likelihood ratio test (LTR). In the literature, Cornacini *et al.* (2017) found in *A. fraxinifolium*, at 18 years of age and from Ilha Solteira - SP and Selvíria - MS planted in progeny tests, average heights of 9.08 m and 11.12 cm for DAP. Araújo *et al.* (2014) studying progenies planted in an open-pollinated test of *A. graveolens*, a species closely related, at 19 years of age found values of 9.34 m for height and 6.03 cm for DAP. Otsubo *et al.* (2015) in a mixed planting experiment of *A. fraxinifolium* with Aroeira (*Myracrodruon urundeuva*) and Capitão do Campo (*Terminalia argenea*) at 14 years, obtained average values of 8.60 m for height and 9.40 cm for DAP. Cambuim *et al.* (2021) reported an average DAP of 13 cm for *A. fraxinifolium* in a forest fragment near the study area.

These pieces of information highlight the genetic and productive potential of this population, whose individuals have undergone natural selection over generations within the degraded area. It is worth noting that, in experimental plantings like progeny tests, to promote gains and development in height and DAP, breeders and researchers carry out selective thinning within the experimental populations, aiming to eliminate individuals with inferior genotypes within each repetition and highlighting individuals with better results in these traits as potential candidates for species genetic improvement programs (Cambuim, 2017). The values of the experimental coefficient of variation and accuracy reveal robustness and reliability in the results obtained from the dataset used in this study.

Initially, it was expected that the present population founded in the study area would be composed of very few seed supplier individuals. However, the genotypic coefficients of variation (CV_{gi}) were of high magnitude, being 25.93% for height and 38.24% for DAP within the population. These values are important as they suggest a robust genetic basis that provides a low probability of inbreeding, which would be detrimental to the population over generations.

The heritability coefficients (h_g^2 and h_m^2) showed values above 0.90 for the evaluated traits, regardless of population or sex, suggesting strong genetic control of the assessed characteristics. Miranda *et al.* (2015) described heritability coefficients at the progeny means level above 0.90 for ALT and DAP traits, indicating that a large part of the total and mean phenotypic variation among progenies is of genetic origin and that, therefore, the genetic control of traits is high and there is a great possibility of altering the population mean through progeny selection.

Table 1: Variance components and genetic parameters of height (ALT, m) and diameter at breast height (DAP, cm) traits in a natural population of *A. fraxinifolium*, located in a degraded Cerrado area.

	Height (m)			DAP (cm)		
	Pop.	Female	Male	Pop.	Female	Male
σ_g^2	8.12	8.95	7.08	66.33	68.70	60.52
σ_e^2	0.81	0.89	0.73	4.13	4.28	4.03
σ_f^2	8.93	9.83	7.81	70.47	72.98	64.56
h_g^2	0.90 ± 0.09	0.90 ± 0.13	0.90 ± 0.12	0.94 ± 0.09	0.94 ± 0.13	0.93 ± 0.13
h_m^2	0.97	0.97	0.97	0.98	0.98	0.98
$r_{\hat{a}a}$	0.98	0.98	0.98	0.99	0.99	0.99
$CVg(\%)$	25.93	26.31	25.06	38.24	36.50	39.11
$CVe(\%)$	8.18	8.29	8.06	9.55	9.11	10.10
$CVr(\%)$	3.17	3.18	3.11	4.01	4.01	3.87
m	10.99	11.37	10.62	21.30	22.71	19.89
$LRT (\chi^2)$	1077.65*	535.93*	529.12*	1319.44*	655.39*	641.96*

*significant at 95% probability with 2 degrees of freedom; σ_g^2 : variance component associated with the genotypic factor; σ_e^2 : variance component associated with the experimental factor; σ_f^2 : variance component associated with the phenotypic factor; h_g^2 : broad-sense individual plot heritability, i.e., total genotypic effects; h_m^2 : heritability of genotype means, assuming no plot loss; $r_{\hat{a}a}$: accuracy of genotype selection, assuming no plot loss; $CVg(\%)$: genotypic coefficient of variation; $CVe(\%)$: residual coefficient of variation; $CVr(\%)$: relative coefficient of variation; m : overall mean; χ^2 : Chi-Square of the deviance; LRT : Likelihood Ratio Test.

3.2. Sex Ratio

The sex ratio of the natural population showed a ratio of 1.13:1 (Table 2), indicating that for every female specimen, there are 1.13 male specimens in this population. The chi-square test ($\chi^2 = 0.21$) allowed us to infer that this population is in a 1:1 equilibrium at 5% significance level, a value considered not significant.

Table 2: Sex ratio of *A. fraxinifolium* sampled in a natural population located in a degraded Cerrado area.

		N	$N_m:N_f$	χ^2
	188	213	401	1.13:1
				0.21 ^{ns}

N_f : Female census number, N_m : Male census number, N : Total census number, $N_m:N_f$: Sex ratio, χ^2 : Chi-square test, ^{ns}: Not significant.

The observed result aligns with the expected sex ratio of 1:1 in dioecious tropical forest species, theorized in a study by Opler & Bawa (1978). The authors associated both mortality and reproductive maturity as contributors to the observed sex ratios in multiple species, mainly because the ratios are consistent from one species to another in most reported cases.

In comparison, Cambuim (2017) found a sex ratio close to equilibrium (1.8:1), with 1.8 male individuals for every female tree in mixed progeny tests. Cornacini *et al.* (2020) reported a ratio close to 1:1 when evaluating the flowering of *A. fraxinifolium* over various reproductive periods. It is interesting to note that, although the population established itself in a degraded area with strong natural selection, the ratio of female and male individuals is consistent with what is expected in nature, meaning they are fit to reproduce and leave descendants for the next generation, ensuring long-term survival.

3.3. Genetic Diversity and Inbreeding

Firstly, genetic diversity parameters were estimated at the population level and then at strata level with a distance of 150m (Figure 2). The population was characterized by having 101 alleles across 8 microsatellite loci, allelic richness of 12.5, observed heterozygosity of 0.706, expected heterozygosity of 0.793, and a fixation index of 0.111 (Table 3).

Table 3: Genetic diversity and fixation index (F) in microsatellite loci of a natural population of *A. fraxinifolium* separated into 8 groups at a fixed distance of 150 m from a degraded Cerrado area.

G	N	T_(a)	k	R	H_o	H_e	F
1	3	28	4	3,1	0,833	0,750	-0,130*
2	40	86	11	3,1	0,710	0,834	0,151*
3	132	104	13	3,1	0,703	0,819	0,146*
4	111	101	13	3,1	0,738	0,818	0,100*
5	66	89	11	3,1	0,800	0,821	0,021*
6	22	72	9	3,0	0,701	0,809	0,131*
7	3	29	4	2,9	0,625	0,802	0,225*
8	7	41	5	2,9	0,798	0,778	-0,036*
M	48	68,75	8,75	3,04	0,739	0,804	0,076*
Pop	384	101	12.6	12.5	0.706	0.793	0.111*

G: Groups; **N**: Census of the reproductive adult population per group; **T_(a)**: Total number of alleles found per group; **k**: Average alleles per locus; **R**: Allelic richness; **H_o**: Observed heterozygosity; **H_e**: Expected heterozygosity; **M**: Overall mean of strata; **Pop**: Population diversity estimate without strata division; *: Bonferroni correlation, significant at $P < 0.05$.

In the literature, studies conducted on natural populations of various species report similar results. For instance, Silva *et al.* (2018) found 136 alleles with an overall average of 5.9 per locus in *Croton linearifolius*, Aguiar *et al.* (2013) reported 111 alleles, with 92 (83%) being polymorphic, and an average of 10.1 alleles per locus in *Eugenia uniflora* in a degraded area in an early successional stage, Lima *et al.* (2015) inferred from 71 to 101 alleles in *Senna reticulata*, in studies with a natural population of *Copernicia prunifera*, Vieira *et al.* (2015) detected a total of 110 loci with variation between 3 to 18 alleles per locus and an average of 9.17 alleles per locus, Chagas *et al.* (2015) detected 68 loci ranging from 8 to 14 loci per primer, with an average of 11.3 in *Elaeis guineensis*. These results indicate that the present population possesses substantial genetic diversity, even when subjected to strong natural selection over time.

The stratification was carried out with the hypothesis that as individuals establish farther from the population center, there might be fewer pollinators and seed dispersers, leading to a higher presence of related individuals with more loci in homozygosity. However, this hypothesis is incorrect. It can be observed that although the average number of alleles per locus is different in the strata, allelic richness is similar. This occurs because, even though there are many alleles, they are common in the population, thus not affecting the allelic richness value.

The observed heterozygosity (H_o) was significantly lower than the expected heterozygosity (H_e) at all loci, resulting in positive fixation index values significantly different from zero for all loci, indicating

inbreeding. Using the Bonferroni sequential correction (95%, $\alpha = 0.05$), the fixation index was significantly different from zero in six of the eight strata loci (Table 3). Negative values indicate high heterozygosity while their inverses represent the occurrence of inbreeding, genetic drift, founder effect combined with the small number of individuals, and fixation of deleterious alleles, leading to a reduction in species adaptability in cases where gene dominance interaction prevails (Sebbenn *et al.*, 2000; Frankham *et al.*, 2006). In this study, only the strata located at the extremes (1 and 8) showed negative fixation index, suggesting that even with a low number of individuals, they are half-sibling individuals.

However, the results presented in this study differ significantly when compared to those found in the literature for impacted or human-altered species, as described by Silva *et al.* (2016) who observed average values for observed heterozygosity (H_o) ranging from 0.34 to 0.40 in *Psidium guineense*, Martins-Corder *et al.* (2009) obtained values of 0.25 in *Euterpe edulis*, Tiago *et al.* (2018) found a value of 0.26 in *Hymenaea courbaril*, and Bernardi (2015) found a value of 0.043 in *Podocarpus lambertii*.

Furthermore, these crossings may have occurred due to a strong intrapopulation spatial genetic structure (SGS) in the parents, which would have resulted in increased inbreeding in this population (Sebbenn *et al.*, 2011). This is supported by the Nei's statistic (G_{st}), where for the population, genetic diversity is distributed only 1.7% among subpopulations and 98.3% within the sampled subpopulations, another factor contributing to the stratification not being significant in allelic richness.

The fixation index (F) graph suggests a differentiated correlation pattern across the studied area. The extreme values indicate low correlation, while the center of the distribution shows higher correlation, suggesting a scenario of inbreeding. However, this inbreeding does not prevent considerable genetic diversity within the central area of the population. This variation in the fixation index indicates gene flow occurring from the center of the population towards the edges. Allele dispersion appears to begin in the central core, where inbreeding is more pronounced, expanding towards the peripheral areas, which, in turn, display greater diversity due to mixing with adjacent populations.

Such a pattern is consistent with a colonization process that likely began in the center of the regenerating area, with native *Astronium fraxinifolium* trees expanding towards the peripheral regions. The higher genetic diversity at the edges can be explained by gene flow from external populations, promoting greater genetic variability in these peripheral areas. Thus, the graph reflects not only the structure of inbreeding within the population but also the processes of dispersion and admixture that may be occurring as colonization expands into an area previously degraded by soil removal. This scenario is particularly relevant for conservation and management strategies, suggesting that the center of the population, despite showing signs of inbreeding, may play a fundamental role in maintaining genetic diversity through allele dispersion towards the edges, where genetic variability is higher.

Studies on plant populations in regenerating areas have demonstrated that genetic structure can be strongly influenced by colonization and spatial dispersion processes, as observed in tree species in areas of environmental restoration (Young *et al.*, 1996). Furthermore, populations regenerating in degraded environments often exhibit greater variability at the edges due to gene flow from neighboring

populations, promoting higher genetic diversity in the periphery (Lowe et al., 2005). This pattern aligns with dispersion processes involving the mixing of inbred central populations with external populations, facilitating adaptation to the recovering environment. Thus, the genetic variation observed at the edges, with a lower inbreeding index, reflects an important mechanism for maintaining genetic diversity and resilience in plant populations colonizing restored areas (Gamba-Moreno, 2020).

In their studies, Cornacini et al. (2020 e 2021) demonstrated that *Astronium fraxinifolium* flowering is highly variable across reproductive events, with environmental and genetic factors playing important roles in this variability, including climatic factors and the species' intrinsic relationship with its pollinators. According to the authors, phenotypic plasticity in tropical tree species is a common response to variable environmental conditions, which may explain the differences observed between consecutive years. Similarly, Manoel et al. (2021) highlighted the importance of genetic diversity in natural populations as a crucial factor for species resilience in the face of climate change and anthropogenic impacts, such as large-scale construction. The authors also emphasize the importance of preserving these regenerating populations as a source of diversity and seed collection within a radius of at least 170 meters, as pollen flow in this species shows average dispersion patterns of 300 to 500 meters from mother trees, while seeds, dispersed by wind, can be found up to 4 km away.

Conclusion

There are significant differences between male and female subpopulations within the population for growth traits, height, and diameter at breast height (DAP). Therefore, it can be inferred that both traits are influenced by the genetics of each individual. This suggests that the population has high potential for selection in a breeding program.

The natural population of *A. fraxinifolium* in the degraded area, a former loan area of the UHE Ilha Solteira, possesses sufficient genetic diversity, and consequently, shows evolutionary potential for many generations, with a balanced expected sex ratio of 1:1.

The population exhibits low fixation index and substantial genetic diversity, which supports the species' long-term survival in this loan area. Despite strong selection pressure, there was low genetic differentiation among the population, resulting in similar diversity parameters across strata.

The results of this study reinforce the potential of the *A. fraxinifolium* population in the degraded area for natural regeneration and are indicated for selection of parent trees for seed collection, highlighting the species' adaptive value in the local study area.

Declarations

ACKNOWLEDGMENTS

We would like to thank Coordination for the Improvement of Higher Education Personnel (CAPES) for the Masters Degree granted to the student during the period.

CONTRIBUTIONS

Marcelo Augusto Mendes Alcantara contributed to fieldwork, laboratory analyses, statistical analysis and manuscript writing. Maiara Ribeiro Cornacini contributed to fieldwork and laboratory analyses, Ricardo de Oliveira Manoel and Marcela Aparecida de Moraes Silvestre contributed to statistical analysis and manuscript writing, Aparecida Juliana Martins Corrêa and Patrícia Ferreira Alves contributed to laboratory analyses, Darlin Gonzalez Zaruma and José Cambuim contributed to fieldwork, Bruno Cesar Rossini, Mario Luiz Teixeira de Moraes and Celso Luis Marino contributed to project conception, supervision, direction, and manuscript review.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

References

1. Aguiar RV, Cansian RL, Kubiak GB, Laura Benetti Slaviero LB, Tomazoni TA, Budke JC, Mossi AJ (2013) Genetic variability of *Eugenia uniflora* L. in forest remnants at different successional stages. *Revista Ceres* 60(2):226-233.
2. Allem AC (1991) Study of the reproductive biology of two forest species (aroeira and gonçalo-alves) from the Cerrado region. *Embrapa/CENARGEN*, Brasília, pp 1-5.
3. Alves MC, Nascimento V, Souza ZM (2012) Recovery in a borrow area used for the construction of a hydroelectric plant. *Revista Brasileira de Engenharia Agrícola e Ambiental* 16(8):887-893.
4. Araujo D, Sebbenn AM, Zanatto ACS, Zanata M, Morais E, Moraes MLT, Freitas MLM (2014) Genetic variation for silvicultural traits in open-pollinated progenies of *Astronium graveolens* Jacq. (Anacardiaceae). *Cerne* 20(1):61-68.
5. Bernardi AP (2015) Genetic and demographic structure of *Podocarpus lambertii* Klotzch ex Endl. in a field landscape. Master's dissertation. Postgraduate Program in Plant Genetic Resources, Federal University of Santa Catarina, Florianópolis, 128p.
6. Bertonha LJ, Moraes MLT, Sebbenn AM, Freitas MLM (2016) Progeny selection of *Myracrodruon urundeuva* based on phenological and growth traits for the restoration of legal reserve areas. *Scientia Forestalis* 44(109):108-116.
7. Bittencourt JM, Sebbenn AM (2007) Patterns of pollen and seed dispersal in a small fragmented population of a wind-pollinated *Araucaria angustifolia* in southern Brazil. *Heredity* 99:580-591.
8. Campelo DH, Lacerda CF, Sousa JA, Correia D, Bezerra AME, Araújo JDM, Neves ALR (2015) Gas exchange and efficiency of photosystem II in adult plants of six forest species as a function of soil water supply. *Revista Árvore* 39(5):973-983.

9. Canuto DSO, Zaruma DUG, Moraes MA, Silva AM, Moraes MLT, Freitas MLM (2015) Genetic characterization of a progeny test of *Dipteryx alata* Vog. from a forest remnant in the Paulo de Faria Ecological Station, SP, Brazil. *Hoehnea* 42(4):641-648.
10. Cambuim J (2017) Forest fragments and progeny tests: options for seed collection in native Cerrado tree species in the southern Mato Grosso do Sul region. PhD Thesis, Postgraduate Program in Agronomy, Faculty of Engineering, São Paulo State University, Ilha Solteira, 103p.
11. Cambuim J, Zaruma DG, Silva AM, Canuto DSO, Moraes MA, Moraes MLT (2021) Dynamics of tree species in a Cerrado fragment in the southern Mato Grosso region. 1st ed. Appris, Curitiba, 71p.
12. Caxambu MG, Geraldino HCL, Dettke GA, Silva AR, Santos EN (2015) Native palms (Arecaceae) in the municipality of Campo Mourão, Paraná, Brazil. *Rodriguésia* 66(1):259-270.
13. Chagas KPT, Sousa RF, Fajardo CG, Vieira FA (2015) Selection of ISSR markers and genetic diversity in a population of *Elaeis guineensis*. *Revista Brasileira de Ciências Agrárias* 10(1):147-152.
14. Cockerham CC (1969) Variance of gene frequencies. *Evolution* 23:72-84.
15. Companhia Energética de São Paulo – CESP (2009) Available at: http://www.cesp.com.br/portalCesp/portal.nsf/V03.02/Empresa_Usinallha?OpenDocument. Accessed: 28 March 2017.
16. Cornacini MR, Alcantara MAM, Silva JR, Corrêa AJM, Cambuim J, Manoel RO, Alves PF, Rossini BC, Aguiar AV, Moraes MLT, Marino CL (2020) Flowering in a provenance and progeny test of *Astronium fraxinifolium* Schott (Anacardiaceae) in three reproductive events. In: Felsemburgh CAA (ed) *The Production of Knowledge in Forest Engineering*. Atena, Ponta Grossa, pp 82-90.
17. Cornacini MR, Silva JR, Luz KC, Cambuim J, Santos W, Moraes MLT, Aguiar AV (2017) Selective thinning in a provenance and progeny test of *Astronium fraxinifolium* Schott based on genetic variability. *Scientia Forestalis* 45(115):581-591.
18. Costa DF, Vieira FA, Fajardo CG, Chagas KPT (2015) Genetic diversity and ISSR primer selection in a natural population of mangaba (*Hancornia speciosa* Gomes) (Apocynaceae). *Revista Brasileira de Fruticultura* 37(4):970-976.
19. Cruz AR, Teixeira CS, Pereira JA, Amorim R, Pinheiro JR (2016) Impact of the implementation of hydroelectric plants and PCHs in the southern Amazon. *Revista Eletrônica Geoaraguaia* 6(1):19-29.
20. Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 12:13-15.
21. Duarte CG, Dibo APA, Sánchez LE (2017) What does academic research say about impact assessment and environmental licensing in Brazil? *Ambiente & Sociedade* 20(1):245-278.
22. Escudero A, Iriondo JM, Torres ME (2003) Spatial analysis of genetic diversity as a tool for plant conservation. *Biological Conservation* 113:351-365.
23. Fainguelernt MB (2016) The historical trajectory of the environmental licensing process for the Belo Monte hydroelectric plant. *Ambiente & Sociedade* 19(2):247-265.
24. Fernandes MM, Oliveira TM, Fernandes MRM (2017) Natural regeneration of a Caatinga forest fragment in the semi-arid region of Piauí. *Scientia Plena* 13(2):1-7.

25. Frankel OH, Soulé MS (1981) *Conservation and Evolution*. Cambridge University Press, Cambridge, 327p.
26. Frankham R, Ballou JD, Briscoe DA (2006) *Introduction to Conservation Genetics*, 6th edn. Cambridge University Press, New York, 617p.
27. Gasques ACF, Okawa CMP, Angelis Neto G, Miotto JL, Castro TR (2014) Environmental impacts of construction materials: a brief theoretical review. *Revista Tecnológica Maringá* 23:13-24.
28. Goudet J (1995) Fstat (version 2.9.3.2): a computer program to calculate F-statistics. *Journal of Heredity* 86(6):485-486.
29. Kageyama PY, Gandara FB, Souza LMI (1998) Genetic consequences of fragmentation on tree populations. *Série Técnica IPEF* 12(32):65-70.
30. Lima RA, Lopes MTG, Bentes JLS, Valente MSF, Pereira JO, Muniz GIB (2015) Genetic diversity and structure of *Senna reticulata*. *Floresta* 45(3):507-514.
31. Loiselle BA, Sork VL, Nason J, Graham C (1995) Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany* 82:1420-1425.
32. Lorenzi H (1998) *Brazilian Trees: Manual for Identification and Cultivation of Native Trees of Brazil*. Nova Odessa, 368p.
33. Lorenzi H (2002) *Brazilian Trees: Manual for Identification and Cultivation of Native Trees of Brazil*, 3rd edn. Nova Odessa, 352p.
34. Manoel RO, Rossini BC, Cornacini MR, Moraes MLT, Cambuim J, Alcantara MAM, Silva AM, Sebbenn AM, Marino CL (2021) Landscape barriers to pollen and seed flow in the dioecious tropical tree *Astronium fraxinifolium* in Brazilian savannah. *PLoS ONE* 16(8).
35. Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7:639-655.
36. Martins-Corder MP, Fialho LEB, Zambiasi DC, Konzan ER (2009) Genetic diversity analysis of populations of palm tree (*Euterpe edulis*) through isoenzyme markers. *Revista Ceres* 56(2):204-213.
37. Miranda AC, Moraes MLT, Silva PHM, Sebbenn AM (2015) Genetic gains in selection by the multi-effect index method in open-pollinated progenies of *Eucalyptus grandis* Hill ex Maiden. *Scientia Forestalis* 43(105):1-7.
38. Moraes PLR, Monteiro R, Venkovsky R (1999) Genetic conservation of populations of *Cryptocarya moschata* Nees (Lauraceae) in the Atlantic Forest of São Paulo state. *Brazilian Journal of Botany* 22(2)(Suppl):237-248.
39. Opler PA, Bawa KS (1978) Sex ratios in tropical forest trees. *Evolution* 32:812-821.
40. Otsubo HCB, Moraes MLT, Moraes MA, José Neto M, Freitas MLF, Costa RB, Resende MDV, Sebbenn AM (2015) Genetic variation for silvicultural traits in three tree species from the southern Mato Grosso do Sul region. *Cerne* 21(4):535-544.
41. Pupin S, Ribeiro Júnior WA, Alzate-Marín M, Moraes MA, Silva JR, Moraes MLT (2017) Genetic variation for biochemical compounds in seeds of *Schinus terebinthifolia* from an anthropized

- population. *Nativa* 5(5):349-354.
42. Resende MDV (2002) *Biometric and Statistical Genetics in the Breeding of Perennial Plants*. EMBRAPA Information Technology, Brasília, 975p.
 43. Resende MDV (2007a) *Mathematics and Statistics in the Analysis of Experiments and Genetic Improvement*. EMBRAPA Florestas, Colombo, 561p.
 44. Resende MDV (2007b) *SELEGEN-REML/BLUP: Statistical System and Computerized Genetic Selection via Mixed Linear Models*. EMBRAPA Florestas, Colombo.
 45. Ribeiro NP, Sanches CC, Oliveira MAC, Costa RB (2016) Biodiversity and conservation of genetic resources of tree species. *Multitemas* 21(50):31-49.
 46. Sant'Ana VZ (2017) Sexual proportion in *Myracrodruon urundeuva* populations for seed orchard formation. Master's dissertation. Postgraduate Program in Agronomy, Faculty of Engineering, São Paulo State University, Ilha Solteira, 64p.
 47. Sebbenn AM, Carvalho ACM, Freitas MLM, Moraes SMB, Gaino APSC, Silva JM, Jolivet C, Moraes MLT (2011) 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* 106:134-145.
 48. Sebbenn AM, Kageyama PY, Siqueira ACFM, Zanatto ACS (2000) Mating system in populations of *Cariniana legalis* Mart. O. Ktze.: implications for conservation and genetic improvement. *Scientia Forestalis* 58:25-40.
 49. Silva EF, Araújo RL, Martins CSR, Martins LSS, Veasey EA (2016) Genetic diversity and structure of natural populations of araçá (*Psidium guineense* Sw.). *Revista Caatinga* 29(1):37-44.
 50. Silva FL, Baffa DCF, Rezende JC, Oliveira ACB, Pereira AA, Cruz CD (2015) Genetic variability among *Coffea robusta* genotypes in Minas Gerais. *Coffee Science* 10(1):20-27.
 51. Silva TS, Freitas JS, Santos ESL, Cardoso TS, Cerqueira-Silva CBM (2018) Characterization and selection of molecular markers in *Croton linearifolius* Mull. Arg. as a basis for genetic studies. *Multi-Science Journal* 1(10):4-8.
 52. Souza LC (2017) Genetic diversity and structure in a natural population of *Plathymenia reticulata* Benth. in southern Espírito Santo. Master's dissertation. Postgraduate Program in Genetics and Breeding, Federal University of Espírito Santo, Alegre, 82p.
 53. Tiago PV, Rossi AAB, Carpejani AA, Tiago AV, Rocha VD, Fernandes JM, Silva IV (2018) Genetic diversity and population structure of jatobá: a species with economic potential for the Amazon region. *Ciência Florestal* 28(2):515-524.
 54. Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP (2017) Historical Overview. Available at: <http://www.feis.unesp.br/#!/instituicao/historico>. Accessed: 28 March 2017.
 55. Vieira FA, Sousa RF, Silva RAR, Fajardo CG, Molina WF (2015) Genetic diversity of *Copernicia prunifera* using ISSR molecular markers. *Revista Brasileira de Ciências Agrárias* 10(4):525-531.

56. Zaruma DUG, Canuto DSO, Pupin S, Cambuim J, Silva AM, Mori ES, Sebbenn AM, Moraes MLT (2015) Genetic variability in provenances and progenies of *Dipteryx alata* Vogel for conservation and seed production. *Scientia Forestalis* 43(107):609-615.
57. Zavala CBR, Fernandes SSL, Pereira ZV, Silva SM (2017) Phytogeographic analysis of shrub-tree flora in an ecotone on the Bodoquena plateau, MS, Brazil. *Ciência Florestal* 27(3):907-921.

Figures



Figure 1

Individuals of *A. fraxinifolium* sampled, sex identified as males/females and Clustering based on the distance from the central point of the area into circles with a 150-meter radius within the experimental area (20°22'26.4"S 51°24'06.9"W).

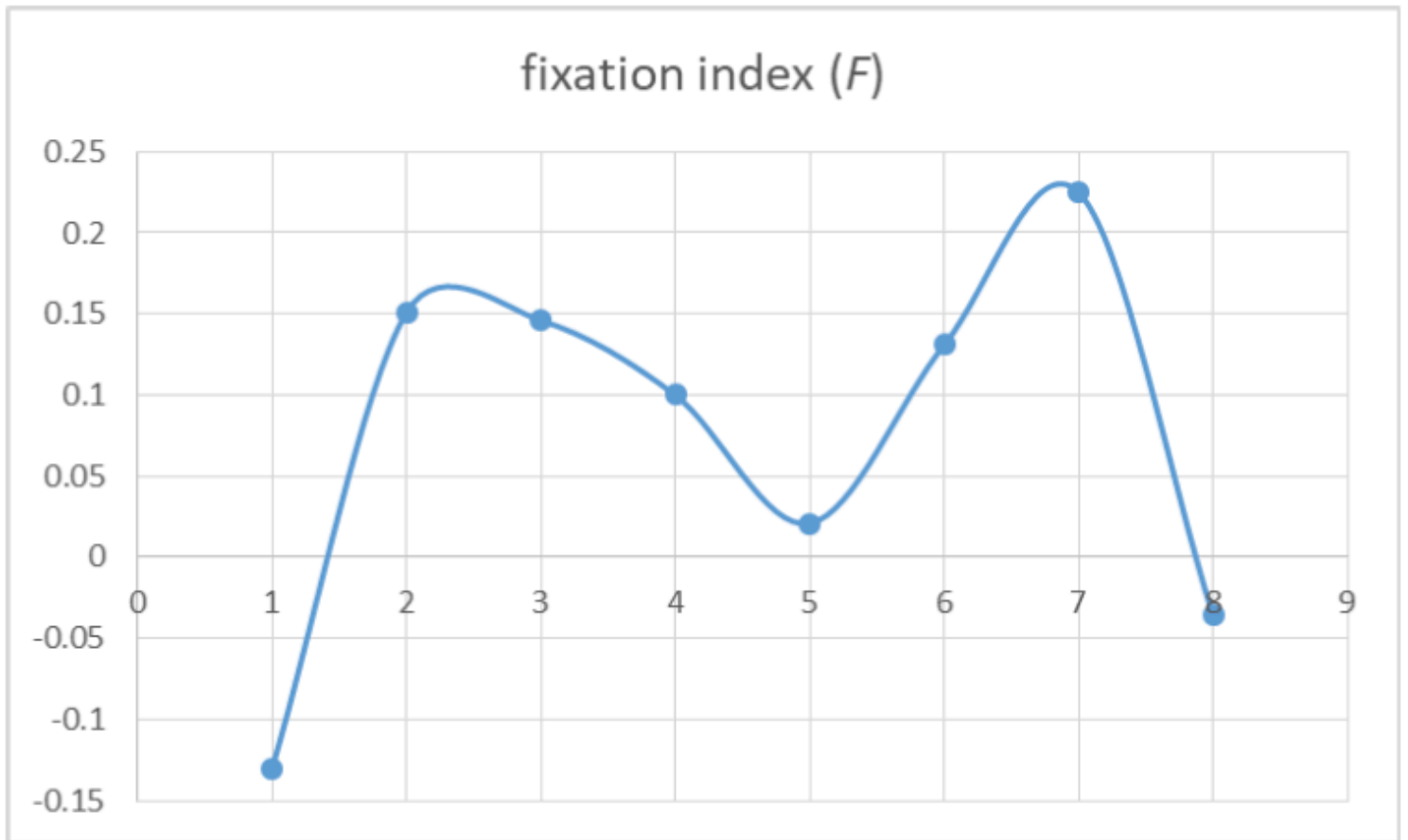


Figure 2

Fixation Index (F) in microsatellite loci of a natural population of *A. fraxinifolium* separated into 8 groups at a fixed distance of 150 m from a degraded Savanna (Cerrado) area.