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Effects of forest fragmentation on natural populations of *Anadenanthera colubrina* (Vell.) Brenan: Insights for conservation and sustainable management

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SILVICULTURE

ABSTRACT

Background: The objective of this study is to characterize the diversity and genetic structure of *A. colubrina* in three Atlantic Forest fragments in the south of Espírito Santo state, using Inter Simple Sequence Repeats (ISSR) molecular markers. Genomic DNA from 85 trees was analyzed using 12 ISSR primers, generating 147 fragments, of which 109 were polymorphic (74%). Markers were characterized as moderately informative, with a mean polymorphic informational content of 0.34.

Results: Low genetic diversity was found for the three fragments and in the total sample, based on Nei's diversity parameters (H^* = 0.26) and Shannon's index (I^* = 0.38). In the dissimilarity analysis, four groups were observed in the dendrogram with an exclusive clustering trend by location. The analysis of molecular variance confirmed that most of the genetic variation is found within populations (73.50%), however, with high genetic differentiation between them (Φ_{ST} = 0.26). This result was supported by the Bayesian approach that indicated genetically structured populations.

Conclusion: The data obtained reveal that forest fragmentation affected the diversity and genetic structure of *A. colubrina* and allow expanding knowledge for the development of effective strategies for the conservation and management of the species.

Keywords: Genetic diversity; genetic structure; forest fragments; molecular markers; tree species.

HIGHLIGHTS

The ISSR markers were efficient in the genetic characterization of *A. colubrina*.
Forest fragmentation affected the diversity and genetic structure of populations.
The genetic sharing between areas 1 and 3 is possibly being produced by genetic drift.
Effective strategies can be applied to conserve and manage populations of *A. colubrina*.

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INTRODUCTION

The dynamics of plant biodiversity consists of the combination of interactions between abiotic and biotic factors and anthropic actions. However, native vegetation cover has historically been reduced mainly by disturbances caused by human exploitation. As a result, fragmentation and habitat loss in human-modified landscapes could lead to local extinction, due to subdivision increase the effects of inbreeding and the genetic erosion by drift (Rocha et al., 2020).

In order to manage, protect and conserve species exposed to anthropic and evolutionary factors, knowledge of genetic diversity and structure has become the basis for conservation and sustainable management programs (Gonçalves et al., 2019). Such knowledge has allowed the establishment of strategies such as *in situ* and *ex situ* conservation and even the selection of genetically dissimilar matrix trees for the composition of base populations (Silva Júnior et al., 2022).

In Brazil, the Atlantic Forest is a model of fragmented vegetation cover, with only 12.4% of its original extension remaining, characterized by a mosaic of forest fragments (SOS Mata Atlântica and Inpe, 2022). Among the tree species occurring in the Atlantic Forest, *Anadenanthera colubrina* (Vell.) Brenan., stands out, also occurring in the Caatinga and Cerrado biomes, that is, with a wide distribution between the Northeast, Central-West, Southeast and South regions, being known popularly known as "Angico Branco" (Morim, 2022).

A. colubrina is a tolerant species to soils with low fertility and has a high capacity for natural regeneration, which are desirable characteristics in projects for the recovery and restoration of degraded, fragmented and eroded areas (Araújo et al., 2018). Furthermore, in economic terms, the *A. colubrina* has potential for logging due to the high quality of its wood, being used in civil construction, in the manufacture of furniture, as well as in the supply of firewood and charcoal (Dias et al., 2015; Coradin, 2018). Currently, the species is also under strong anthropic pressure, mainly due to the extraction of tannin, used in traditional medicine (Coradin, 2018).

To formulate policies for the conservation and sustainable management of biological and genetic diversity, especially in fragmented landscapes, forest conservation and improvement programs seek knowledge about the magnitude of diversity and genetic structure. Thus, the use of molecular markers for this purpose becomes an efficient tool. Among them, the Inter Simple Sequence Repeats (ISSR) markers allow an economical and reproducible study (Turchetto-Zolet et al., 2017).

In this sense, the objective was to characterize the diversity and genetic structure of *Anadenanthera colubrina* in three fragments of the Atlantic Forest, using ISSR molecular markers. This information will make it possible to determine the levels of genetic diversity and its distribution among and within populations, allowing the establishment of conservation strategies and sustainable management of the species.

MATERIAL AND METHODS

Sampling strategy

Adult individuals of the species *A. colubrina* were evaluated in three fragments of Atlantic Forest in the south of Espírito Santo state (Figure 1). The first forest fragment (Area 1), called Rosal Forest, is characterized as a Permanent Preservation Area, located in the municipality of Guaçuí (20º 53' S 41º 42' W), with 93 hectares (ha), composed of Montane Seasonal Semideciduous Forest vegetation and rugged relief with an average altitude of 650 meters. Until the 1990s, Rosal Forest was the target of selective cutting, also impacted by livestock activities. Currently, it is part of the hydroelectric plant (Rosal HP), belonging to the Energy Company of Minas Gerais (CEMIG) (Curto et al., 2013).

The second fragment (Area 2) is called the Atlantic Forest Environmental Education Pole, being an area of the Instituto Federal de Educação, Ciência e Tecnologia do Espírito Santo, located in the municipality of Alegre, near the ES 482 highway (20°46' S and 41°27' W), with 109.6 ha. Area 2 has Semideciduous Seasonal Forest vegetation, impacted by selective logging, agricultural activities and surrounding pasture areas. Finally, the third fragment (Area 3) is a small private forest area, located in the municipality of Jerônimo Monteiro (20°48' S and 41°21' W), with only 2 ha. Among the impacts observed in area 3, the reduction of the fragment due to population growth, selective logging and pasture areas in the surroundings stand out.

The populations are spatially separated by 29.23 kilometers (km) (area 1 x area 2), 36.02 km (area 1 x area 3) and 9.68 km (area 2 x area 3). In addition, 36, 19 and 30 individuals were sampled for areas 1, 2 and 3, respectively, totaling 85 trees, spaced at a minimum distance of 50 m. Leaf tissue samples were collected for each individual for further process of genomic DNA extraction.

DNA extraction and amplification by ISSR markers

DNA was extracted following the CTAB (Cetyltrimethylammonium Bromide) method developed by Doyle and Doyle (1987), adjusting the concentrations to 1% polyvinylpyrrolidone (PVP) and 2% cetyltrimethylammonium bromide, suitable for plant species with high concentrations of polysaccharides. The DNA concentration and purity of each sample was estimated using a Nanodrop spectrophotometer (Thermo Scientifc 2000C), adopting as a quality criterion the relation A_{260}/A_{280} (Aguilar et al., 2016).

For the Polymerase Chain Reaction (PCR) assays, 12 primers (UBCs 807; 809; 810; 812; 815; 822; 827; 834; 836; 840; 842; 868) developed by the University of British Columbia were used, and a total reaction volume of 20 µL, containing: 1X buffer (10 mM Tris-HCL (pH 8.5) and 50 mM KCl), $MgCl_2$ (2.5 mM), dNTP (1 mM), primer (0.2 µM), 1 unit of Taq DNA polymerase and 50 ng of genomic DNA.

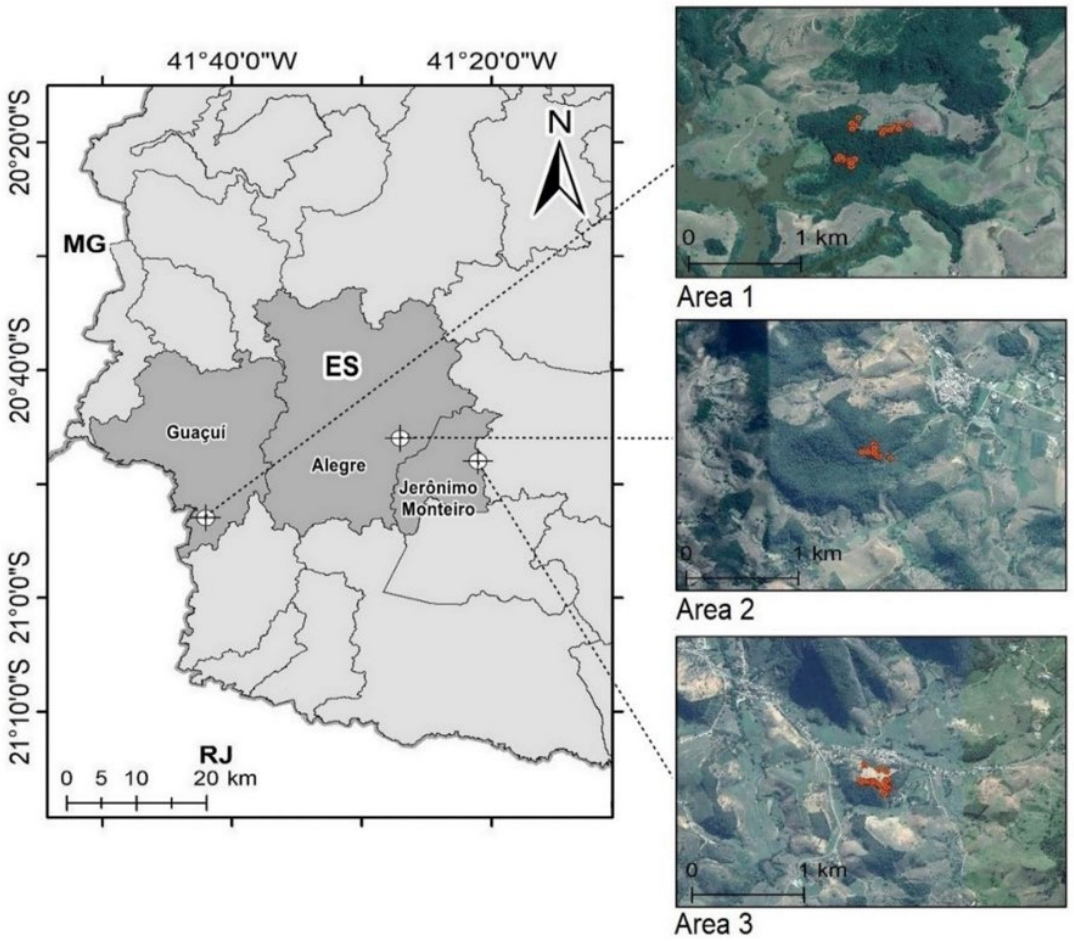

Figure 1: Location map of the study areas, indicating the location of cities within the of Espírito Santo state, the three fragments and the location of each individual of the species *Anadenanthera colubrina*.

Amplifications were performed in a thermocycler (Veriti 96 Thermal cycler), with denaturation steps at 94 °C for 5 min, followed by 35 cycles of 94 °C for 45 s, annealing at 52 °C for 45 s and 72 °C for 90 s, with a final extension of 72 °C for 7 minutes. The amplification products were separated by electrophoresis in a 2% agarose gel and subsequently stained by immersion in ethidium bromide solution (0.50 µg/mL - 20 min) and photographed under ultraviolet light using photodocumentation (ChemiDoc MP Imaging System – BioRad). The size of the fragments was established with the aid of the Ladder 100 base pair molecular weight marker.

Statistical analysis

The visualized gel amplification was converted into a binary matrix, assuming the presence (1) and absence (0) of bands. Then, from the matrix, descriptive analyzes were performed, involving: number of total bands (NTB), number of polymorphic bands (NPB), private markers (PM), percentage of polymorphic bands (PPB), size variation of the fragments generated in base pairs (SVF) and polymorphic information content (PIC).

Genetic diversity was evaluated for each population and for the combined data using the parameters: Number of observed alleles (A_O), number of effective alleles (A_E), Nei genetic diversity (*H**) and Shannon diversity index (*I**). For these analyses, the program Popgene version 3.2 was used (Yeh and Boyle, 1997). The genetic dissimilarity between individuals was measured using the arithmetic complement of the Jaccard coefficient. From the numerical matrix of dissimilarity, the dendrogram was obtained, considering the total sampling, by the method of unweighted pair group with arithmetic mean (UPGMA), with the cut-off point following that proposed by Mojena (1977), with coefficient k = 1.25. A new numerical matrix is generated during the formation of the dendrogram, composed by the distances of the intersections that connect to the individuals. Therefore, the analysis of the cophenetic correlation coefficient (CCC) was performed to verify the consistency between the dissimilarity matrix and the clusters formed. The matrix of genetic dissimilarity between the populations was also obtained, and its correlation (r) with the geographic distances between them was evaluated, performed using the Mantel test, with 1000 permutations. For these analyses, the statistical

software Genes (Cruz, 2016) and R (R Development Core Team, 2016) were used.

To evaluate the genetic structure, the Genes software (Cruz, 2016) was used to perform the Analysis of Molecular Variance (AMOVA) with two hierarchical levels: between and within populations (Excoffier et al., 1992), considering pairwise and total sampling. The genetic differentiation parameter (Φ_{ST}), characterized as a parameter analogous to the F statistic (Wright, 1951), was obtained from AMOVA. To indirectly infer the levels of gene flow the number of migrants (Nm) was estimated as Nm=1/4 ($1/F_{ST}$ -1) (Whitlock and Mccauley, 1999). The effective size (Ne) of the populations and joint data was estimated using the Colony version 2.0 software (Jones and Wang, 2010), via the sibling assignment method (Wang, 2009).

In addition, the analysis of genetic structure was performed using the Bayesian approach using Structure 2.3 software (Falush et al. 2007), performing 20 runs for each value of K, establishing the number of groups (K) ranging from K = 1 to K = 6, with 7,500 iterations and an initial discard (burn-in) of 2,500 iterations, totaling 10,000 iterations via Markov chain Monte Carlo (MCMC) methods. The number of genetic groups was estimated based on the Neperian logarithm of probability [LnP(K)], considering the mean and standard deviation for each value of K, and by the ad hoc method ΔK proposed by Evanno et al. (2005).

RESULTS

Descriptive analysis and efficiency of molecular markers

Genotyping resulted in 147 total bands, of which 109 were polymorphic, corresponding to approximately 74% polymorphism. Among the primers used in this study, UBC 818 presented the highest number of total bands, while UBC's 810 and 812 had the lowest values for NTB. Despite these results, the primers UBC's 810 and 812 were the ones that resulted in the highest percentage of polymorphism, with the lowest percentage observed for the primer UBC 834 (Table 1).

Regarding the total number of private markers (PM), two, one and two markers were observed for Area 1, Area 2 and Area 3, respectively. The primers that allowed the identification of private bands were UBC's 812, 815, 834, 842 and 868. The polymorphic information content (PIC) ranged from 0.35 (UBC's 807, 815 and 836) to 0.32 (UBC's 809 and 827), with an average value of 0.34 (Table 1).

Genetic diversity and dissimilarity

The number of observed alleles (A_O) for the individual populations was greater and equal for Areas 1 and 2, but the highest value for the number of effective alleles (A_E) was only for Area 2. As for the joint data, the values of A_O and A_E were higher than those observed for the populations evaluated individually. The other parameters that indicate the level of genetic diversity (*H** and *I**) were higher for Area 2, however, close to the values observed for the other populations (Table 2).

For genetic dissimilarity, initially quantified for pairs of individuals within populations, the minimum value (IDG_{min} = 0.08) was between individuals 49 and 50, and the maximum value (IDG_{max} = 0.45) was between individuals 38 and 44, both the peers located in Area 2. Considering the combined data, the IDG_{min} remained between the pair 49 x 50, as expected, however, the resulting IDG_{max}

Table 1: Descriptive analysis of ISSR primers used in the genetic characterization of individuals of the *Anadenanthera colubrina*.

Primers	Sequence*	NTB	NPB	PPB (%)	PM_{Area1}	PM_{Area2}	PM_{Area3}	SVF	PIC
UBC 807	(AG)8T	15	11	73.33	0	0	0	1160 – 320	0.35
UBC 809	(AG)8G	12	9	75.00	0	0	0	1300 – 480	0.32
UBC 810	(GA)8T	10	9	90.00	0	0	0	1200 – 320	0.34
UBC 812	(GA)8A	10	9	90.00	0	1	0	1100 – 420	0.34
UBC 815	(CT)8G	11	8	72.72	1	0	0	1500 – 520	0.35
UBC 822	(TC)8A	11	8	72.72	0	0	0	1200 – 620	0.34
UBC 827	(AC)8G	11	7	63.63	0	0	0	1200 – 490	0.32
UBC 834	(AG)8YT	13	8	61.53	0	0	1	1220 – 340	0.34
UBC 836	(AG)8YA	14	10	71.42	0	0	0	1180 – 460	0.35
UBC 840	(GA)8YT	12	9	75.00	0	0	0	1200 – 340	0.33
UBC 842	(GA)8YG	10	7	70.00	0	0	1	1100 – 230	0.34
UBC 868	(GAA)6	18	14	77.77	1	0	0	1080 – 380	0.34
Total	-	147	109	74.14	2	1	2	-	0.34

NTB: Number of total bands; NPB: Number of polymorphic bands; PPB: Percentage of polymorphic bands; PM: Private markers; SVF: Size variation of the fragments generated in base pairs by 100 bp molecular weight marker; PIC: Polymorphic information content. *A = Adenine; T = Thymine; C = Cytosine; G = Guanine and Y = (C or T).

in the value of 1.71 was between individuals 28 and 48, located in Area 1 and Area 2, respectively (Table 2). As for the genetic dissimilarity between populations, the minimum value (IDGmin = 0.10) was between populations 1 and 3, and the maximum value (IDGmax = 0.17) was between populations 1 and 2. The correlation analysis by Mantel test between population dissimilarity values and geographic distances showed significance at 1% probability, resulting in a moderate correlation between matrices (r = 0.51, p = 0.01).

The cluster analysis performed by the UPGMA method, based on the values of genetic dissimilarity between individuals pair by pair and a cutoff value of 0.33, resulted in the formation of 4 groups. The largest group, called G1, was composed of 34 individuals, all located in Area 1. The G2 group was composed of 31 individuals, containing only two individuals from Area 1, while the others were sampled from Area 3. The G3 group was composed of 3 individuals located in Area 3. Finally, the G4 group was composed of 17 individuals, one located in Area 3 and the others located in Area 2. The cophenetic correlation coefficient (CCC) was 81%, indicating consistency between the matrix of numerical values of genetic dissimilarity and the groups shown in the dendrogram (Figure 2).

Genetic structure

Determination of genetic structure based on the AMOVA resulted in unequal division of genetic variation, which 26.5% is between populations and 73.5% is within populations, with an overall estimate of Φ_{ST} equal to 0.26. The analysis of the number of migrants (Nm) resulted in values ranging from 0.46 (Area 1 x Area 2) to 1.31 (Area 1 x Area 3). For the joint data, the value of Nm was 0.71 (Table 3). The effective population size (Ne) was 25, 14, 20 and 26 individuals for Areas 1, 2, 3 and total sampling, respectively.

The Bayesian approach performed using the Structure software, considering the total sampling, resulted in the best K = 2. Therefore, is possible to observe from the different colors the distribution of genetic groups in relation to individuals within and between populations, equivalent to the proportion of ancestry or evolutionary and demographic events that may have occurred in the respective populations of the species (Figure 3).

DISCUSSION

Descriptive analysis and efficiency of molecular markers

The use of ISSR markers was efficient to evaluate the percentage of polymorphism in populations of the *A. colubrina*. Similar and lower percentages of polymorphisms than those found in this study were observed in studies with other forest species such as *Astronium concinnum*, where the genetic characterization of two populations of the species via ISSR markers revealed a polymorphism rate of approximately 73% (Vieira et al., 2022). For the species *Dalbergia nigra*, the polymorphism rate considering two populations was 68% (Silva Júnior et al., 2020). These results indicate the efficiency and reproducibility of ISSR markers in genetic analyzes in forest species, including the species under study. Regarding the PIC, according to Tatikonda et al. (2009), for molecular markers characterized as dominant, such as ISSR markers, the values can vary from 0 to 0.25 for poorly informative markers, above 0.25 to 0.45 for moderately informative and above 0.45 to 0.5 for highly informative. Therefore, it is noteworthy that for this study the markers evaluated individually (PIC = 0.32 – 0.35) and together (PIC = 0.34) are classified as moderately informative, being sufficient to determine the genetic dissimilarity between individuals and the diversity and genetic structure of the evaluated populations.

Genetic diversity and dissimilarity

The highest values for the number of observed and effective alleles (A_O and A_E) and the indices of genetic diversity of Nei (H^*) and Shannon (I^*) resulting for Area 2 reveal the distribution of alleles and greater genetic diversity in comparison with the other evaluated populations. This observation may be related to greater degree of conservation of this population when compared to the others, located in an area that is currently protected, called Pole of Environmental Education of the Atlantic Forest, monitored by the Instituto Federal de Educação, Ciência e Tecnologia do Espírito Santo.

Table 2: Parameters of genetic diversity and dissimilarity determined for the populations and for the total sampling of individuals of *Anadenanthera colubrina*.

Population	N° of individuals	A_O	A_E	H^*	I^*	IDG_{min}	IDG_{max}	$DG_{min-max}$
Area 1	1 -36	1.63	1.37	0.21	0.32	33 - 34	11 - 28	0.10 – 0.38
Area 2	37 - 55	1.63	1.39	0.22	0.33	49 – 50	38 - 44	0.08 – 0.45
Area 3	56 - 85	1.61	1.36	0.20	0.30	77 - 78	58 - 67	0.13 – 0.38
Joint Data	1 - 85	1.74	1.48	0.26	0.38	49 - 50	28 - 48	0.08 – 0.51

$DG_{min-max}$: Minimum and maximum genetic dissimilarity; IDG_{min}: Pair of individuals with minimal genetic dissimilarity; IDG_{max}: Pair of individuals with maximum genetic dissimilarity; A_O: Number of observed alleles; A_E: Number of effective alleles; H^*: Nei's genetic diversity index; I^*: Shannon index.

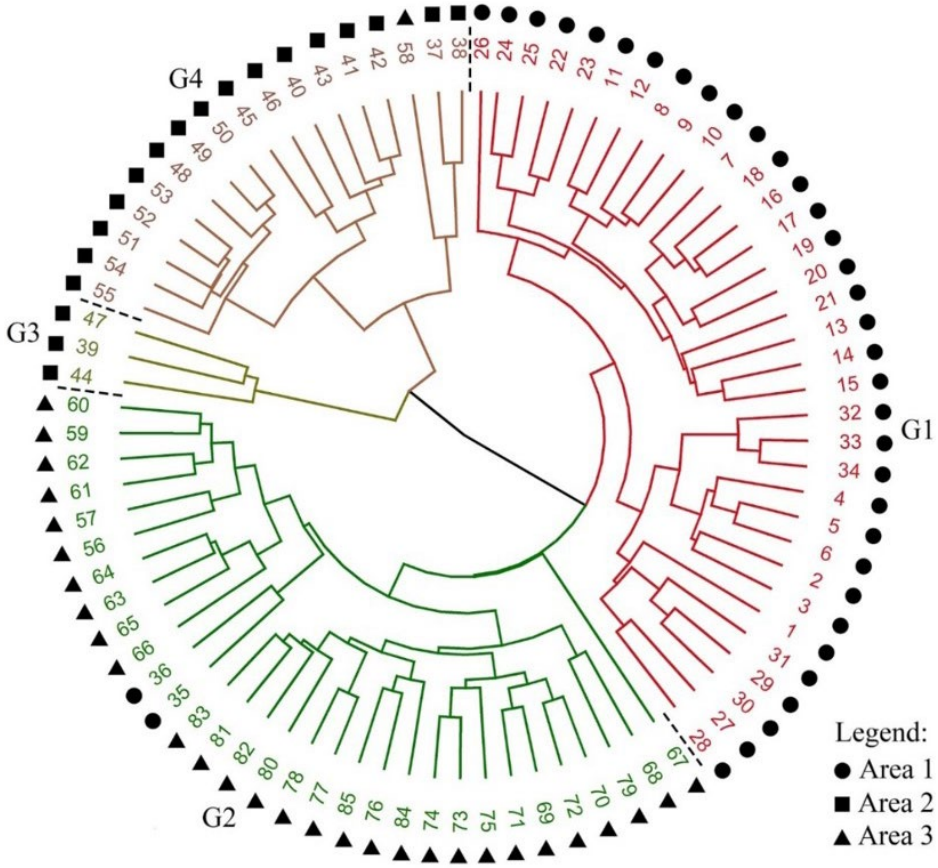

Figure 2: Cluster analysis between individuals of the species *Anadenanthera colubrina* evaluated by ISSR markers, obtained by the UPGMA method with a cut-off point of 0.33.

Table 3: Analysis of molecular variance (AMOVA), genetic differentiation (Φ_{ST}) and number of migrants (Nm) estimated for populations of the species *Anadenanthera colubrina*.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Variation (%)
Between populations	2	327.96	5.42	26.50
Within populations	82	1233.91	15.04	73.50
Total	84	1561.87	20.47	-
Sampling			**Φ_{ST}**	**Nm**
Area 1 x Area 2			0.35	0.46
Area 1 x Area 3			0.16	1.31
Area 2 x Area 3			0.31	0.55
Joint Data			0.26	0.71

However, for the three populations evaluated individually or together, the parameters indicate the presence of homozygous *loci*, since the presence of different alleles per locus in *A. colubrina* accessed by ISSR markers would result in an A_O value equal to two, where according to Ortolani et al. (2010) the species is characterized as diploid (2n = 26). In addition, A_E values indicate poor effective distribution of alleles among individuals, resulting in distant values when compared to A_O. Another observation should be made of the increase in A_O and A_E values for the joint data, indicating the presence of restricted loci among the populations, corroborating the presence of private markers evidenced in Table 1.

Regarding the genetic diversity parameters (*H** and *I**) estimated for individual and joint populations, the values indicate low genetic diversity. This classification is based on Lewontin (1972) who describes that *I** values can vary from 0 to 1, where 0 represents absence of genetic diversity and 1

represents high diversity. Furthermore, the characterization of genetic diversity is based on similar studies, considering the type of molecular marker used, in addition to the species, genus, family, or populations of forest species that have related characteristics such as biological, ecological and demographic factors. Such characteristics can be observed in a study with the species *Enterolobium contortisiliquum* (Fabaceae), where low genetic diversity was confirmed (*H** = 0.28 and *I** = 0.38; Moreira et al., 2015). As for the study with the species *Dalbergia nigra* (Fabaceae), the genetic diversity for the populations evaluated together was characterized as high (*H** = 0.35 and *I** = 0.53; Silva Júnior et al., 2022).

High levels of genetic diversity have already been found for populations of *A. colubrina* (Barrandeguy et al., 2014; Gonçalves et al., 2019), however, it is noteworthy that the populations were located in Natural Reserves, while this study evaluates populations in forest fragments marked by anthropic disturbances. In addition, the nature of the markers used in these studies, classified as codominant and called microsatellites, is highlighted. Such markers have the ability to detect different alleles per locus and, consequently, detect greater genetic variation, being considered as one of the most polymorphic classes of molecular markers present in genomes (Turchetto-Zolet et al., 2017).

Therefore, other comparisons can be extended to populations of forest species located in sampling areas evaluated in this study or that are close and that have been genetically characterized via ISSR markers, such as for *Dalbergia nigra* where low genetic diversity was identified in Area 2 (Silva Júnior et al., 2022), and *Paratecoma peroba* where the study also identified low genetic diversity in two Conservation Units close to Areas 2 and 3 (França et al., 2022).

Comparing the biological information of *A. colubrina* with *D. nigra*, it is highlighted that both are species of the Fabaceae family, with characteristics from late secondary to climax, semi-caducifolia, hermaphrodites and pollinated by bees (Rêgo and Possamai, 2003; Coradin, 2018). Compared to *P. peroba*, despite belonging to a different family, called Bignoniaceae, it is again a late secondary species, pollinated mainly by bees (Martins, 2011; Coradin, 2018). Furthermore, both species have anemochorous fruit dispersion (Carvalho, 2003; Braz et al., 2009; Lins and Nascimento, 2010).

Based on the previous comparisons, it is possible to identify that the forest fragmentation generated by anthropic activities is associated with the decrease in the levels of genetic diversity of forest populations, corroborating the moderate correlation between genetic dissimilarity and geographic distance of populations, obtained by the Mantel test. Among the different factors generated by fragmentation and which reduce the adaptive and maintenance potential of forest populations, we can highlight the subdivision of populations, increase in the inbreeding rate and the loss of alleles due to genetic drift (Almeida-Rocha et al., 2020). However, for *A. colubrina*, the low genetic diversity may also be influenced by ecological factors such as its main pollination vector, which is the bee *Trigona spinipes*, which has a small radius of action, nearly 840 m (Araújo et al., 2004). Furthermore, it features a 1:1 ratio for "number of pollen grains per polyad/number of ovules per flower", suggesting that a polyad can fertilize all the ovules in a flower, producing a fruit whose seeds produce complete siblings (Borges et al. 2017).

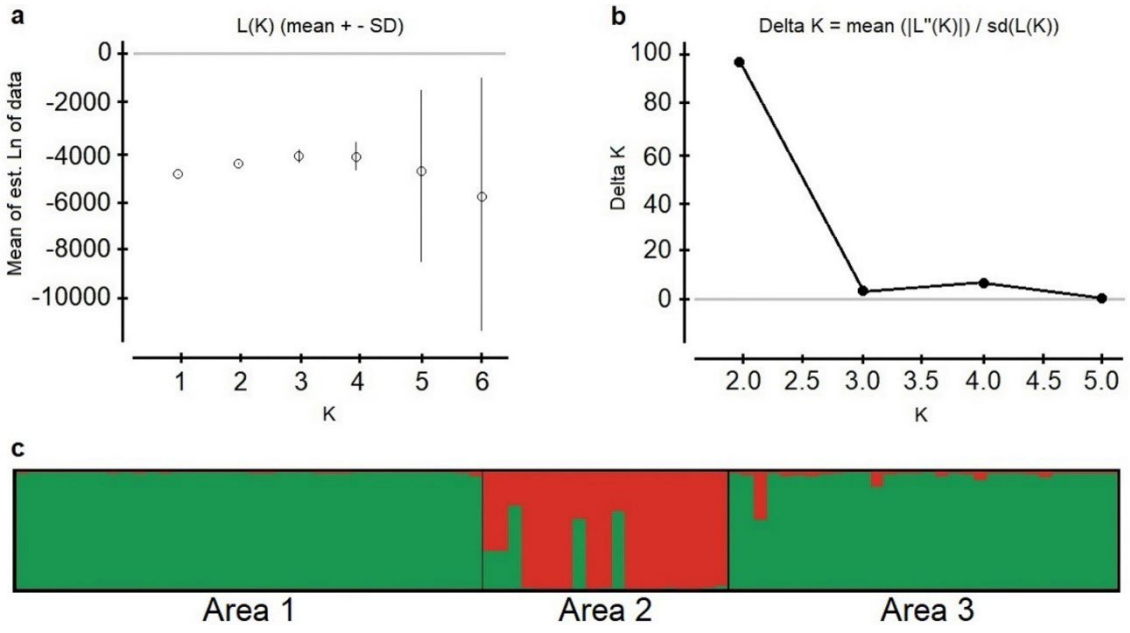

Figure 3: Genetic structure analysis performed by the Bayesian approach, considering three populations of *Anadenanthera colubrina*. a) Neperian logarithm of probability [LnP(K)], considering the mean and standard deviation for each value of K; b) Most likely number of K clusters, based on the ΔK method proposed by Evanno et al. (2005); c) Distribution of genetic groups in relation to individuals within and between populations.

Regarding the genetic dissimilarity between the individuals, it is possible to verify for the IDG_{min} a correlation with the geographic distance, that is, the genetically closest individuals for the three populations are also located geographically close (~50 meters), while the most genetically distant individuals (IDG_{max}) are also geographically distant (>100 meters). Thus, pollination by bees that fly over short distances may again be related to this observation (Araújo et al., 2004; Kill and Silva, 2016), in addition to the species being characterized as autochorous (Cruz et al., 2021), that is, the seeds are dispersed by the plants themselves, presenting an aggregate spatial distribution pattern, forming populations with high rates of related individuals (Silva and Barbosa, 2000).

Furthermore, it is possible to identify from the dendrogram the separation of the genetic variation between the populations, forming groups constituted almost exclusively by individuals located within the populations. This observation allows the inference that although populations share common alleles, currently the gene flow between them seems to be interrupted, leading to the formation of genetically structured populations.

Genetic structure

The Analysis of Molecular Variance (AMOVA) resulted in the highest percentage of genetic variation occurring within populations, confirming the genetic sharing between them. However, the value found for Φ_{ST} = 0.26 corresponds to high genetic differentiation between populations. According to Wright (1978) values of Φ_{ST} between 0 and 0.05 indicate low genetic differentiation; greater than 0.05 to 0.15 moderate differentiation; greater than 0.15 to 0.25 high differentiation and values above 0.25 indicate very high differentiation.

Regarding the analysis of the number of migrants (Nm) that infers the occurrence of gene flow, Wright (1951) describes that values lower than 1 confirm the non-sharing of alleles between populations, being observed between Areas 1 x 2, 2 x 3 and for the joint data. However, we highlight the resulting Nm value between Areas 1 and 3 (Table 3), which indicates the presence of similar alleles, despite being more geographically distant (Figure 1). This event is possibly being produced by genetic drift, characterized as a stochastic factor (Basey et al., 2015), randomly maintaining more similar alleles when compared to those observed in Area 2. In addition, the analysis evaluates the historical gene flow, that is, when populations were connected in the past. Through this statement, it is understood that despite the genetic sharing between populations 1 x 3, confirmed by the value of Nm greater than 1, the current scenario of forest fragmentation may genetically distance them.

To estimate the effective population size (Ne), the sibling assignment estimator (Wang, 2009) was used, which, in comparison with the others based on information on linkage disequilibrium, excess heterozygotes and molecular coancestry, has stands out for allowing greater precision in the face of data obtained by genotyping with dominant markers (Wang, 2016). According to Vencovsky (1987), the effective population size is related to the genetic representation contained in a sample, in relation to the next generation, that is, the effective size is related to the genetic size of the population and not to the sample size (N). Usually, Ne will be smaller than N (Wright, 1938), as observed in this study. This means that a smaller number of individuals effectively participated in the generation of intercrosses that generated the current populations, indicating the occurrence of inbreeding, possibly associated with the low values obtained for the genetic diversity indices and the observation of genetic structuring of the populations.

The genetic structure analysis performed by the Bayesian approach supports the previous analyzes and confirms the genetic distance of the populations. Although two genetic groups are formed for the total sampling of individuals (Figure 3a and 3b), it is possible to observe the structuring of populations by the presence of a single majority genetic group, represented by the exclusivity of a certain color in each of them (Figure 3c). In addition, the greatest genetic distance from Area 2 is again observed, even though it is geographically located among the other populations.

Insights for the conservation and management of *Anadenanthera colubrina*

Although *A. colubrina* is classified as "least concern" (IUCN, 2022), the data generated show that forest fragmentation has affected the diversity and genetic structure of its populations, characterized in this study as low and structured, respectively. Therefore, the results obtained represent another advance in the knowledge of forest resources, allowing the establishment of conservation strategies and management of the species.

Species naturally have reproductive and ecological mechanisms that allow them to maintain the diversity and genetic structure of their populations. However, recurrent anthropic impacts in their places of occurrence can make them vulnerable and genetically isolated. Considering the studied areas and the history of selective cuts and agricultural activities, the information obtained highlights the importance of actions that can help in the conservation of the genetic resources of *A. colubrina*.

From the data obtained, further actions can be carried out with the introduction of germplasm obtained from other sources of the species, as a way of overcoming the low levels of genetic diversity found in local populations. In addition, genetically dissimilar individuals, confirmed in this study, may be used for seed collection and later production of seedlings to be implanted in these fragments as a way of conserving them *in situ*, expanding the genetic variation within populations and allowing greater sharing of alleles between them. It is expected, in this case, that genetic variability acts by increasing the survival and maintenance rates of individuals, with a consequent reduction in the probability of inbreeding depression events, which in turn decreases the risk of the species populations in the face of biotic, abiotic and/or evolutionary factors (Mukhopadhyay and Bhattacharjee, 2016).

Another effective strategy for the conservation of the species and populations evaluated is via *ex situ*, which, again, genetically dissimilar individuals can be used in seed collection programs or vegetative propagules, since according to Coradin (2018), the species can also be propagated by cuttings (Coradin, 2018). Furthermore, aiming at the conservation, management and subsequent genetic improvement of the species, the individuals sampled can be used in the formation of base populations from matrices with certified genetic variability. The studies can still be extended to test half-sister progenies, evaluated by the estimation of genetic parameters, allowing the selection of the best genotypes.

As the species allows its propagation via seminal and vegetative (Coradin, 2018), conducting seed orchards in the field, or greenhouse could conserve and provide superior genotypes. Seed orchards allow the development of materials with high genetic value in the full-sib category, helping to obtain clones (Funda and El-Kassaby, 2012). In addition, considering the propagation by the cutting method and aiming at the protection of genetic diversity, clonal minigardens can be formed, which are already being evaluated for the genus *Anadenanthera* (Dias et al., 2015). The implementation and management of mini-gardens require reduced labor and area and, on the other hand, can promote increased productivity, better nutritional and phytosanitary control, and can be used in the dissemination of knowledge among various research institutions (Carvalho and Silva, 2012).

CONCLUSIONS

The ISSR markers were efficient in the genetic characterization of *A. colubrina*. The data reveal that forest fragmentation affected *A. colubrina* populations, characterized by low levels of genetic diversity and a high degree of structuring. However, they make it possible to expand knowledge for the development of effective strategies for the conservation and management of the species.

AUTHORSHIP CONTRIBUTION

Project Idea: JALS; MFM

Database: SKDA; JALS; JALS; MFM

Processing: ACS

Analysis: JALS

Writing: SKDA; JALS; SMC; SLC; JALS; MFM; CMVW; STCB

Review: SMC; SLC; JALS; MFM; ACS; STCB

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