High Diversity and Low Genetic Structure of Remnants from Hancornia speciosa Gomes in Two Savanic Formations of the Cerrado Biome in the State Park of Serra de Caldas Novas - Goiás

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Abstract

Human actions over Cerrado biomes have been causing decline of several natural species. To assist in strategies that can mitigate the deterioration of biodiversity, it is necessary to understand how the genetic diversity of native species is maintained under natural conditions, particularly in protected areas. This work aimed to evaluate the diversity and genetic structure of two groups of Hancornia speciosa Gomes separated in two savanna formations of the Cerrado inside the State Park of Caldas Novas, Goiás. A sample was collected consisting of 45 individuals, 19 originated from the Cerrado Rupestre and 26 from the Cerrado Típico. Some genetic parameters were calculated using 21 microsatellite markers. Both groups showed high values of genetic diversity, observed heterozygosity and low values of inbreeding. The genetic differentiation between them was extremely low, ratifying the clusters inferred from genotypes of both groups observed in the UPGMA and PCoA cluster analysis, which did not correspond with the physical location of the samples. From the analysis made with STRUCTURE, a value of $\Delta K = 3$ was obtained, even though there were no genetically separated groups, but a high proportion of mixture of three gene pools among all individuals. In this way, it was demonstrated, for the first time, a weak genetic structure between two groups of mangabeira geographically close but located in two different savanna formations of the Cerrado within PESCAN. These results are relevant for the development of management strategies and conservation of the genetic diversity of natural mangabeira remnants.

Keywords: Hancornia speciosa; Conservation genetics; Natural species; Microsatellite markers

Introduction

Despite being considered one of the hotspot global biodiversity [1], the Brazilian Cerrado presents an embarrassing scene of a socio-environmental spoil promoted by substitution of natural areas for agricultural and livestock commodities besides mining [2-4]. The expansion of these activities brought with it the practice of aggressive human interference in the natural course of the existence and perpetuation of species, which is guaranteed, in particular, by genetic
variability [5, 6]. Therefore, this interference leads to the decline followed by the extinction of several natural species in the biome [7, 8].

When analyzing a sample of more than 800 thousand properties producing commodities, Rajão et al (2020) [9] found that 2% of these properties are responsible for 62% of all illegal deforestation in the Amazonia and the Brazilian Cerrado. The latter is considered the second largest biome in South America and is precisely that one with the lowest percentage of legally protected areas, presenting only 8.21% of its territory under protection in conservation units [10]. In a scenario where deforestation is constant in the country, according to [11], the Cerrado was the most affected biome in recent years, losing about 48 thousand hectares just from October 2018 to March 2019. Emphasizing this disaster, many deforestation alerts in this interstice were recorded in Conservation Units, Indigenous Lands, springs and other Permanent Preservation Areas [11, 12]. For this reason, part of the biome’s biodiversity is critically endangered and may be extinct even before being known [13-15].

Thus, apart from the fact that “passing the cattle” has proved to be a priority measure in the Brazilian State’s environmental policy (as inferred from the ministerial meeting held on April 22, this year), studies on fragments of vegetation and/or unexplored areas of the Cerrado are essential to guide actions for conserving both the genetic resources of the remaining species of the biome and all the biodiversity in latu sensu. Those studies can help in designing strategies for managing areas and programs for the recovery of degraded areas, the restoration of associated fauna, the orientation of sustainable extraction, the formation of agroecosystems and composition of germplasm banks [6, 16-20].

Species genetics studies make it possible to quantify and evaluate the spatial and temporal organization of the heterogeneous distribution of the allelic and genotypic variation in species, as well as the dynamics of this variation, influenced by natural or anthropic factors [21-24]. Thus, studies that contribute to the monitoring of the dynamics of genetic composition in natural species and threatened species have become necessary to understand the ability to maintain variability in the face of existing interferences.

The distribution of species, of the same native species, within a biome, can reveal a genetic and phenotypic plasticity manifested, for example, by the ability to adapt to different phytophysiognomies. Numerous studies of the diversity and genetic structure of natural species have been carried out in the Cerrado of Brazil [25-29]. However, studies of genetics that establish comparisons between the different phytophysiognomic formations in the Cerrado are scarce in the country.

The Serra de Caldas Novas State Park (PESCAN) is an integral protection area located in the center-south of the State of Goiás, with 125 km² presenting an ellipsoidal base elevation and a flattened apex in the form of a plateau, 100 m from that base. It is a conservation unit (UC) composed predominantly of the Typical Cerrado, but which also exhibits other characteristic formations of the biome, such as Cerradão, Campo Cerrado, Campo Rupestre, Vereda and Mata de Galeria [30]. The Park is surrounded by areas devastated by the most diverse human demands. All those subdivisions, summer clubs, rural properties, grain production, cattle ranching, real estate speculation and the intense tourist activity were made possible by deforestation and burning of previously existing vegetation. In addition, many of these enterprises were established showing a blatant disregard even of the geographic limits legally signed for the Park [30, 31]. As an aggravating factor, at least from the ecosystem point of view, the APA was incorporated into the tourist context too early. Indeed, depending on the location of its accommodation, the tourist could access the park directly and unrestrictedly from their accommodations, which makes it mandatory to execute the Park’s management plan, elaborated in 1997. Among other measures, the plan established the zoning of the UC, resulting in five different zones, with their respective possibilities of access and use [30]. Within these zones there are countless native species such as Hancornia speciosa Gomes.
Belonging to the Apocynaceae family, *H. speciosa* is a native fruit that appears as the only species of its genus, presenting six varieties [32] that occur from the North to the Southeast, associated with different types of vegetation, such as: restingas, coastal and Cerrado regions, as can be seen in countless floristic surveys carried out in Brazil [2, 33-36]. This wide distribution is compatible with the high adaptability shown by the species, which is able to survive in places, natural or anthropic that, for many other beings, are considered inhospitable, such as hillsides and road borders [35].

As a resource of agricultural interest, mangaba has a greater expression in the state of Sergipe, the only one in Brazil that has a productive chain of the fruit [37]. In other regions, extractivism still predominates in truly improved species, that bring phenotypes defined by the evolutionary pathways [38]. Several of these mangabeira are found in the Brazilian Cerrado and some of them have already been investigated regarding their genetic diversity [26, 27, 39-41]. Nevertheless, countless other species still need this type of studies, such as those existing in PESCAN.

In order to carry out a genetics study that allows the comparison of groups of individuals of the same species, allocated in different phytophysionomies, it is necessary to seek the composition of the genetic variation of these groups by analyzing the dynamics of the genotypic and allele frequencies, as well as the action of forces capable of altering such frequencies over the generations (species size, geographic isolation, mating stocking, migration, mutation, natural selection, genetic drift) [42, 43]. The study of these frequencies, in turn, depends on the detection of alleles and their alternative forms (or polymorphisms) among individuals in the species. To this end, this area of genetics has used the resources of Molecular Biology, such as molecular markers based on the amplification of SSR microsatellites [44-47]. Codominance, high degree of polymorphism, abundant genomic coverage and non-susceptibility to environmental oscillations are advantages that justify the preference for the use of this marker in studies of biological diversity [48]. However, it is still possible to consider that there are few studies on natural types of mangabeiras that used SSR's markers [39, 49, 50]. This gap may be related to the intrinsic difficulty in accessing natural species, to the fact that scientific communication about *H. speciosa* specific markers occurred only sometime after its development [51] and the possible prevalence of the use of biotechnology in studies aimed at species related to the future market.

That said and considering the importance of regular monitoring to understand the recovery processes after disturbances subsidized by information obtained from molecular tools and in situ observations of native species, the objective of the present work was to produce, for the first time, information concerning the genetic diversity and the distribution of remnants of *H. speciosa* in two savanna formations of the State Park of Caldas Novas - Goiás, through 21 microsatellite markers. Our hypothesis for this study was that, although geographically close, the two groups of mangaba trees would be genetically distinct (structuring) and so explaining the phenotypic differences and keeping coherence with the different underlying savanna formations.

**Material and Methods**

*In situ collection, plant material and DNA extraction*

Expeditions to collect and monitor natural species of *H. speciosa* were carried out in the State Park of Caldas Novas, Goiás, Brazil, between the months of September and October 2019. Direct observations of environmental conditions and groups of *H. speciosa* were performed, and some leaf branches from each specimen found were collected. The *in situ* observations of the identified accessions were considered in the detection of the Cerrado phytophysionomy type in which the mangabeiras were established. The leaf branches collected during the expeditions were enveloped with aluminum foil and packed in a thermal box with ice for transport to the Molecular
Genetics Laboratory of the Federal Goiano-Urutaí Institute, where they were kept in a freezer until its subsequent genomic DNA extraction.

Two accesses were located with the aid of the biologist and director of the Park, one being part of the savanna formation of Cerrado Rupestre and the other of the Cerrado Típico. Respectively, collections of 19 and 26 adult plants properly identified and designated according to the Cerrado phytophysiognomic matrix to which each one of them integrates.

The extraction of genomic DNA was performed from small portions of the leaf tissue of each plant, separately. With the aid of crucibles and liquid nitrogen, these portions were macerated until they became powder allowing the separation between the tissue and its ribs. A sample of the macerate was deposited to the mark of 1/4 volume in 2.0mL tubes, following the DNA extraction protocol of CTAB 2%. A sample of each extracted genomic DNA was quantified on a 1% agarose gel, using a standard lambda DNA marker.

**Obtaining microsatellite markers**

A set of 21 out of 34 pairs of microsatellite primers developed specifically for the species *H. speciosa* were selected for the characterization of the genetic diversity of the PESCAN mangrove species [51]. The selection of these 21 primers followed the order of presentation of the genetic characterization published by Rodrigues *et al.* [51], since there is a high level of polymorphic information from all the evaluated loci. Each PCR was produced to a final volume of 20µL, containing 5ng of DNA, 1x of PCR buffer, 1.5mM MgCl₂, 0.2mM dNTP, 0.2µL of each primer pair (forward and reverse), 1U of Taq DNA Platinum polymerase and RNAase free water qsp 20µL. PCR was conducted in a thermocycler for 35 cycles for the following conditions and steps after initial denaturation at 94°C for 5 min: (i) denaturation (1 min at 94 °C), (ii) annealing (1min at annealing temperature for each primer pair recommended by the authors [51]), (iii) extension (1min at 72°C), and, finally, (iv) final extension of 72°C for an additional 7 min, then the amplification product it was separated by vertical electrophoresis in a 6% polyacrylamide gel, stained with silver nitrate [52] and its size was estimated by comparison using a standard 50-base pair (bp) DNA ladder (Invitrogen™, USD).

**Data analysis**

The descriptive analysis of genetic diversity from mangabeira accessions was carried out by estimating the allele frequencies of the polymorphic loci, number of alleles per locus (*A*), number of private alleles (*A*ₚ - alleles found in a single species), gene diversity or expected heterozygosity (*Hₑ*), observed heterozygosity (*Hₒ*), fixation index (*Fᵦ*), probability of identity (*Pᵯ*) and exclusion (*Pₑ*) unilocus and multilocus using the *GenAlEx* v6.5 software [53].

The genetic divergence between the pairs of individuals, as well as between the accessions, was calculated based on the genetic distance (DG) of Roger [54] modified by Wright [55] using the software *Bood* [56]. The generation of the DG matrix among the pairs of individuals made it possible to form a dendrogram using the Neighbor joining grouping method from the DARwin program v5.0158 [57]. To assist in the cluster analysis, the principal coordinate analysis (PCoA) was also performed based on the modified Roger genetic distance, using the program *GenAlEx* v6.5 [53]. This software was also used to perform an analysis of molecular variance (AMOVA) to verify the partitioning of genetic variation between and within accessions, as well as to estimate genetic differentiation (*Fₛₜ*) between accessions, according to Wright's *F* statistic [55].

With the aid of the STRUCTURE v2.2.4 program [58], the structuring of the studied genotypes was inferred, through grouping based on the Bayesian model. The analysis was performed considering the admixture model, with frequency correlated to *K* ranging from 1 to 6, with 15 repetitions for each *K* value. A burn-in of 50,000 was defined for each race, followed by 500,000 iterations of MCMC (Markov Chain Monte Carlo). To determine the number of genetic groups (*AK* most likely) regarding the genetic organization of accessions, the procedure used followed the approach proposed by Evanno *et al.* (2005) [59] through the Structure Harvester application [60].
Results and Discussion

Characterization of the sample collection location of mangaba groups

The analysis of the geographic disposition of the accessions, based on the georeferenced positioning of the specimens, reveals that these groups are separated but at a minimum distance between individuals of both sets of the order of 100m (Figure 1). The sampled specimens are part of a region of savanna formations within PESCAN.

![Diagram of State Park of Serra de Caldas Novas](http://www.ijcs.ro)

*Fig. 1. State Park of Serra de Caldas Novas. Black: group of mangaba trees located in Cerrado Rupestre; white: group of mangaba trees located in Cerrado Típico*

However, in view of the edaphic and phytophysiognomic conditions identified *in situ*, it was observed that the nineteen mangabeiras of group 1 compose a fragment of Cerrado Rupestre with transition to Typical Cerrado. In this region the soil has a very light color, without depth and with outcrop of quartzite. These attributes are compatible, according to SiBCS [61], with the lithic Neossol, which is distinguished from other Neossols by the fragmentary lithic or lithic contact within 50cm from the surface. In this context, it was observed that the mangaba trees of the Cerrado Rupestre are relatively more elongated (reaching about two meters in height) and denser although in the middle of a thin vegetation. The soil under the twenty-six specimens from group 2 is deeper, with a smooth slope, a yellowish to reddish-yellow color and without cuttings (quartzite), signaling the presence of a Latosol [61]. In this scenario, the local vegetation is more diverse and larger, since individuals can form deeper roots. This provides greater shading in the area, leading to the trees to be distributed in a more spaced way and to be shorter in comparison.
to individuals from the Cerrado Rupestre, since the species has preference for exposure to sunlight [62].

All individuals located in both groups were adult and, although studies predominating suggesting the August - December interstice (with peak in October) as the most expressive flowering and fruiting period of *H. speciosa* in natural conditions in the Cerrado region [63], at the time of the collections carried out in September and October, there was no evidence of these phenophases. Pilon *et al* (2015) [64] also observed similar phenology, even for mangabeiras under cultivation, in four years of monitoring of native Cerrado. The study found that, in the months of September, sprouting was more frequent after the fall of leaves followed by flowering of the species. In October, flowering was more frequent, followed by fruiting and sprouting, which were also recurrent.

In addition to the fact that the entire Park is completely surrounded by an area of great anthropization and despite the fact that both groups submitted to our study are located within the Intangible Zone - ZI (the most restricted in the park because it is dedicated to the integral protection of the ecosystem and where it is prohibited the public circulation), these groups are divided by a Special Use Zone - ZUE (formed by some circulation routes for tourism and visitation points) and close to the Intensive Use Zone - ZUI and the Primitive Zone - ZP (where installation of any infrastructure is prohibited, even though walking is allowed as well as certain activities such as educational and scientific research) [30]. Thus, even though the literature reports mangabeira as highly adaptable to impacted areas [35], the perpetuation of the species in altered *habitats* may encounter difficulties in terms of the ecological interactions necessary for the renewal of individuals in the group, since the adaptability of *H. speciosa* to disturbed and/or constantly disturbed environments does not necessarily extend to Hoary Fox, Crab-eating Fox and Maned Wolf, as well as to moths, bees and butterflies essential to intermediate dispersion [65, 66] and the pollination of mangabeiras, whose flowers are hermaphrodite but self-incompatible [62, 67].

Furthermore, the potential negative impact on these interspecific relationships caused by the use of the ZUE that cross groups, as can be inferred from the degree of road compaction observed at the site, needs to be assessed. This could also guide the redesign of PESCAN's conservation zones, as well as exposing the need for technological advances, such as the replacement of human traffic related to monitoring and inspection by the adoption of modern alternatives such as drone type vehicles [68, 69].

**Genetic characterization**

From twenty-one microsatellite markers, we sought to genotype 45 individuals of the species *Hancornia speciosa* Gomes belonging to PESCAN, nineteen from the Rupestre group and twenty-six from the Typical group. And, although three of the markers used (HS19, HS20 and HS21) did not compete for genotyping due to amplification problems, all the others were polymorphic. Thus, a total of 107 alleles were detected among the sampled individuals, with the number of alleles per locus (NA) varying between three (HS12; HS18) and eight (HS01) with an average detection of 5.94 alleles per locus, a value similar to that found by Amorim *et al* (2015) [39] when analyzing remaining groups of mangaba in Northeast Brazil. The observed heterozygosity (*H*o) ranged from 0.489 (HS18) to 0.889 (HS01), while the expected heterozygosity (*H*e) varied between 0.652 (HS12) and 0.880 (HS01). Thus, the average fixation index (*F*) was around 0.060, indicating a slight excess of homozygotes in this group. The eighteen markers used exhibited a high power of discrimination of genotypes as seen from the probabilities of identity and exclusion, calculated for the studied loci [69] (Table 1).

Considering the variability attributes in each group, we obtained the means of the number of polymorphic alleles per locus (*A*), the quantities of private alleles (*Apr*) for each set, the means of the expected heterozygosities (*H*E), observed (*H*D), as well as the averages of the fixation index for each of them, as described in Table 2.
Table 1. Descriptors of the genetic variability of eighteen polymorphic SSR markers in the total sample of 45 adult accessions

<table>
<thead>
<tr>
<th>Loci</th>
<th>(N_A)</th>
<th>(H_E)</th>
<th>(H_O)</th>
<th>(F_{IS})</th>
<th>PI</th>
<th>PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS01</td>
<td>8</td>
<td>880</td>
<td>889</td>
<td>-22</td>
<td>30</td>
<td>890</td>
</tr>
<tr>
<td>HS02</td>
<td>6</td>
<td>833</td>
<td>667</td>
<td>190</td>
<td>60</td>
<td>820</td>
</tr>
<tr>
<td>HS03</td>
<td>7</td>
<td>860</td>
<td>667</td>
<td>216</td>
<td>40</td>
<td>860</td>
</tr>
<tr>
<td>HS04</td>
<td>8</td>
<td>879</td>
<td>886</td>
<td>-21</td>
<td>30</td>
<td>890</td>
</tr>
<tr>
<td>HS05</td>
<td>8</td>
<td>861</td>
<td>841</td>
<td>12</td>
<td>40</td>
<td>870</td>
</tr>
<tr>
<td>HS06</td>
<td>7</td>
<td>853</td>
<td>778</td>
<td>78</td>
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<td>HS08</td>
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<td>867</td>
<td>-17</td>
<td>40</td>
<td>860</td>
</tr>
<tr>
<td>HS09</td>
<td>7</td>
<td>858</td>
<td>864</td>
<td>-18</td>
<td>40</td>
<td>860</td>
</tr>
<tr>
<td>HS10</td>
<td>7</td>
<td>864</td>
<td>822</td>
<td>38</td>
<td>40</td>
<td>870</td>
</tr>
<tr>
<td>HS11</td>
<td>4</td>
<td>749</td>
<td>622</td>
<td>160</td>
<td>120</td>
<td>670</td>
</tr>
<tr>
<td>HS12</td>
<td>3</td>
<td>652</td>
<td>523</td>
<td>189</td>
<td>200</td>
<td>500</td>
</tr>
<tr>
<td>HS13</td>
<td>4</td>
<td>745</td>
<td>822</td>
<td>-116</td>
<td>120</td>
<td>660</td>
</tr>
<tr>
<td>HS14</td>
<td>5</td>
<td>769</td>
<td>682</td>
<td>103</td>
<td>100</td>
<td>710</td>
</tr>
<tr>
<td>HS15</td>
<td>4</td>
<td>748</td>
<td>778</td>
<td>-51</td>
<td>120</td>
<td>670</td>
</tr>
<tr>
<td>HS16</td>
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<td>817</td>
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<td>38</td>
<td>70</td>
<td>790</td>
</tr>
<tr>
<td>HS17</td>
<td>6</td>
<td>828</td>
<td>867</td>
<td>-58</td>
<td>60</td>
<td>810</td>
</tr>
<tr>
<td>HS18</td>
<td>3</td>
<td>671</td>
<td>489</td>
<td>263</td>
<td>190</td>
<td>520</td>
</tr>
</tbody>
</table>

Mean ± SE  5.94 (±0.40)  0.809 (±0.017)  0.755 (±0.029)  0.060 (±0.025)  -  -

Total 107 - - - 3.3x10^{-22} 1.00

\(N_A\), number of alleles; \(H_E\), expected heterozygosity or gene diversity; \(H_O\), observed heterozygosity; \(F_{IS}\), fixation index; PI, probability of identity; PE, probability of exclusion; SE, Standard Error

Table 2. Descriptors of genetic variability by access of *Hancornia speciosa* collected in the State Park of Serra de Caldas Novas.

<table>
<thead>
<tr>
<th>Accession</th>
<th>A</th>
<th>(N_A)</th>
<th>(A_p) (SE)</th>
<th>(A_r)</th>
<th>(H_E) (SE)</th>
<th>(H_O) (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerrado Rupestre</td>
<td>19</td>
<td>99</td>
<td>5.5 (±0.40)</td>
<td>4</td>
<td>0.795 (±0.021)</td>
<td>0.752 (±0.028)</td>
</tr>
<tr>
<td>Cerrado Típico</td>
<td>26</td>
<td>103</td>
<td>5.7 (±0.40)</td>
<td>8</td>
<td>0.810 (±0.016)</td>
<td>0.757 (±0.036)</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>107</td>
<td>-</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Mean (SE) - - - 0.802 (±0.013) 0.755 (±0.022)

A, individuals in the sample; \(N_A\), number of alleles; \(A_p\), average of the number of polymorphic alleles per locus; \(A_r\), private alleles; \(H_E\), expected heterozygosity or gene diversity; \(H_O\), observed heterozygosity; \(F_{IS}\), fixation index; SE, Standard Error

The means of polymorphic alleles of the Rupestre and Típico sets, respectively, were 5.5 and 5.7, both of which were higher than the means of five of the six species observed by Amorim *et al* (2015) [39] in remnants of mangabeira from Northeast Brazil. Nogueira *et al* (2015) [49] highlighted that it is possible to infer the ability of species to adapt to the environment from the rates of polymorphism per locus in their samples, since the polymorphism implies genetic variation and genetic variation, in turn, is strongly related to the species success of selection pressures.

As for heterozygosities, the two groups showed higher means of expected heterozygosity \((H_E)\) compared to that observed, suggesting an excess of homozygotes, as expected for a species in Hardy-Weinberg equilibrium, which is confirmed by the positive means of fixation indices.

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The values found for these descriptors point to a greater genetic diversity in these PESCAN remnants, in comparison with the natural species studied by Amorim et al. (2015) [39].

The adult mangabeira plants of each species showed a high genetic divergence, as suggested by the average estimate of the genetic distance of Roger modified by Wright [55], with a value of 0.646, ranging from 0.527 to 0.799 between the mangaba trees of the Rupestre group and 0.655, varying from 0.500 to 0.825 between the mangaba trees of the Typical group. The study of dissimilarity between these same groups, on the other hand, using the Roger coefficient [54] modified by Wright [55] resulted in a low genetic distance (0.172), indicating a slight divergence between them. The molecular analysis of variance (AMOVA) allowed to observe the genetic variation among mangabeira individuals, as well as between groups, which correspond, respectively, to approximately 7% and 1%, while 92% is organized within individuals, confirming the extremely low structure observed between the accessions, and as suggested by the low genetic differentiation revealed by the F ST equal to 0.012 (p-value = 0.011 to 9999 permutations) confronted with Wright [55].

After the cluster analysis using the neighbor joining methodology and in order to facilitate the identification of dissimilar groups, they were arranged cladistically as shown in figure 2. Based on this figure, the formation of three mixed groups is observed carrying out genetic influence of both studied species, indicating a significant cross-pollination between them, as demonstrated by the historical gene flow rate of Nm = 21.2.

![Fig. 2. Dendrogram built from cluster analysis by neighbor joining of 45 adult mangaba trees from two species of PESCAN based on the genetic distance of Roger modified by Wright to 18 microsatellite markers. The red lines define the plants collected in the Cerrado Rupestre and the green ones in the Cerrado Tipico.](image)

The groups identified by the dendrogram, when submitted to AMOVA, also resulted in a weak genetic structure (FST = 0.045; p-value = 0.000 for 9999 permutations). Consistently, the principal coordinates analysis (PCoA) of the 45 mangaba trees organized in the two groups confirms this lack of structure while illustrating the idea of mixed spatial organization among individuals in the species samples. These findings suggest that individuals belong to a single unstructured population or in an initial structuring process (Fig. 3).
HIGH DIVERSITY AND LOW GENETIC STRUCTURE OF REMNANTS FROM *Hancornia speciosa* Gomes

Not unlike the cluster analysis based on the genetic distances treated previously, the analysis of structuring based on the Bayesian method using the Structure software, did not define distinct genetic groups, nor similar to the clustering pattern observed in the environment, where a group is in the Cerrado Rupestre and the other in the Typical Cerrado. With the aid of the ΔK estimate proposed by Evanno *et al* (2005) [59] a high peak was observed for ΔK = 3 and another peak, of smaller amplitude, for ΔK = 5, suggesting a substructure of the studied species (Fig. 4).

This result reinforces the weak genetic structure indicated by the value of \( F_{ST} \), suggesting an expressive connectivity between groups. The hypothesis of this connectivity can be supported...
by the high multiple ancestry indicated among the genetic groups inferred in the analysis of Structure (Fig. 5). Thus, considering a limit value of the ancestry coefficient of each genotype $q \geq 0.70$, represented by the colors in each column as the probability of attribution in the genetic groups (vertical axis in figure 5), it was found that few individuals would be attributed to a single genetic group, whereas the mangaba plants of both groups share, at different levels, the genetic background of the three suggested gene pools, which, associated with the high heterozygosity observed between them, is compatible with the dependence on the exchange of gametes between different genotypes for their reproduction, as occurs in allogamous species due to self-incompatibility.

**Fig. 5.** Genetic structure of the 45 mangaba plants collected at PESCAN. The columns represent the individuals analyzed; the colors represent the probability of attribution in the genetic groups; the number 1 refers to the group of the Cerrado Rupestre and the number 2 refers to the group of the Cerrado Típico

**Conclusions**

The results obtained in this study demonstrate that both groups analyzed have high genetic diversity, however, with greater variability observed within each group. Research at the molecular level revealed that, although they are phenotypically distinct and geographically separated in a fragment of Cerrado Rupestre and Cerrado Típico, respectively, the two groups have a high connectivity, since their genetic materials recombine in such a way that any genetic structure is lost, demonstrating that the group functions as a single ecological species, making the edaphic conditions under each savanna formation in which they are contained as probable authors of this paradox.

The species presents highly polymorphic loci, revealing, therefore, a good adaptability to the environment. However, remnants exposed to fragmentation may initiate a process of loss of alleles, which makes the implementation of studies aimed at monitoring the dynamics of this species mandatory.

The genotypic structure of the species is shown to be characteristic of allogamous species by presenting high heterozygosity and low inbreeding coefficient after the individuals genotyping.

The interface of this remnant of mangaba trees with the different areas of the PESCAN’s interior potentially impacts its natural ecological interaction with their pollinators and dispersers and this must be investigated in order to orient the management of the area.

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