Acta Botanica Brasilica, 2023, 37: e20220176
doi: https://doi.org/10.1590/1677-941X-ABB-2022-0176

Review

Microsatellites are important for forest genetic resources conservation in Brazilian biomes

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Received: July 06, 2022
Accepted: January 14, 2023

ABSTRACT

Microsatellites are short sequence repeats that make up the genomes of eukaryotes and prokaryotes. They are of great importance as DNA markers for studies in several fields of genetics. In the present review, we searched for studies published in the five years period of 2017 to 2021 regarding the use of microsatellites in studies with forest tree species from the Brazilian biomes, in order to examine the importance of these markers for forest resources conservation. We searched scientific papers in journals indexed on the Scopus and Web of Science databases. There were found 38 peer reviewed articles that used microsatellites in the Brazilian biomes. The Atlantic Forest was the biome with more studies (35.9 %) and most of the studies were published in 2018 (34.2 %). In addition, most of the studied species belonged to the Fabaceae family (34.2 %). The conclusions and recommendations made in these studies ratify the great contribution of microsatellite markers in the conservation of native forest species in Brazilian biomes.

Keywords: Brazilian ecosystems, genetic diversity, genetic structure, forest conservation, SSR markers.

Introduction

Habitat fragmentation is one of the most issues of concern in conservation biology. Once large and continuous populations are split into smaller fragments, primarily by human disturbances such as land clearing and conversion, the genetic diversity is negatively affected (Franklin *et al.* 2002). Genetic diversity is a key component for the sustainability of species as it enables communities to adapt to changing environments. For this reason, efforts in forest conservation include genetic tools to analyze the genetic diversity among individuals and populations (Jump *et al.* 2009).

The genetic diversity and its distribution among groups can be quantified through the use of genetic markers, such as Simple Sequence Repeats – SSR. This class of markers contributes to increase efficiency in genetic studies as they are neutral, can be accessed regardless of the plant's development stage and the environment, and does not compromise the viability of the specimens under study since small amounts of tissue are necessary, allowing additional analyzes to be performed (Garcia *et al.* 2004).

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Since its development in the 1980s by Litt & Luty (1989), microsatellites or Simple Sequence Repeats (SSRs) have been used in genetic studies. These markers are abundant and broadly distributed in eukaryotic and prokaryotic genomes. Due to high rates of DNA replication error within microsatellites, the length of a microsatellite shows intra- and interspecific variation. For this reason, microsatellites are used for designing PCR-based markers for population genetic characterizations, genome mapping, tagging trait-associated genes during marker-assisted selection, among other applications (Wang *et al.* 2018). The use of SSRs is qualified by the information obtained, mainly because of their co-dominant inheritance, allowing access to complete genetic information (Garrido-Cardenas *et al.* 2018).

The aim of this study was to analyze the state of the art of using microsatellite markers in scientific articles regarding genetic studies of natural forest populations in Brazilian biomes published from 2017 to 2021.

Material and methods

This article is a bibliographic review on the studies regarding the use of microsatellite markers in genetic analyzes of Brazilian forest ecosystems. It brings together manuscripts published in the last five years (2017-2021). The research was restricted to scientific articles in journals indexed on the Scopus and Web of Science databases. The access to these databases was performed through the Coordination for the Improvement of Higher Education Personnel (CAPES, Brazil) platform. The following keywords were used: "SSR", "microsatellites", "forest" and "Brazil", in association with the Boolean operator AND. Only publications with some application in forest tree species conservation were selected.

The obtained data were analyzed in order to get the dimension of the relevance of this class of DNA markers in studies for forest resources conservation.

Results

There were 38 peer reviewed scientific articles that used microsatellite markers in forest tree species from different Brazilian biomes from 2017 to 2021. The Atlantic Forest was the biome with more studies (n = 14; 35.9 %), followed by Cerrado (n = 11; 28.2 %) and Amazon (n = 10; 25.6), while Pantanal (n = 2; 5.1 %) and Caatinga (n = 2; 5.1 %) were the less studied biomes. No study was recorded in the Pampa biome (Table 1).

Twelve botanic families were represented in the studies. Most of the studied species belonged to the Fabaceae family (n = 13; 34.2 %), followed by the Lecythidaceae family (n = 8; 21.1 %). On the other hand, Caryocaraceae, Meliaceae, Apocynaceae, Rhizophoraceae and Vochysiaceae were represented by only one species (2.6 %). The other studied families were Myrtaceae (n = 3; 5.3 %), Anacardiaceae (n = 3; 5.3 %), Salicaceae (n = 2; 2.6 %), Rubiaceae (n = 2; 26 %) and Malvaceae (n = 2; 2.6 %). In addition, most of the studies were published in 2018 (n = 13; 34.2 %), followed by 2021 (n = 8; 21.1 %), 2017 (n = 7; 18.4 %), 2019 (n = 7; 18.4 %), while in 2020 least number of studies (n=3; 7.9 %) were published.

Atlantic Forest biome

Most of the published studies with microsatellite markers were performed in the Atlantic Forest. There was a record of 14 studies (36.8 %) in this biome. In a study that involved two species of the *Cariniana* genus, microsatellite markers were used to access the gene flow pattern in fragmented populations of *Cariniana estrellensis* and *C. legalis*. For both species, there were high levels of seed (38.5–61.5 %) and pollen (80.1–100 %) immigration. No self–fertilization was detected, but there was evidence of mating among related trees (8.9 - 12.5 %). The effective size in most of the populations varied from 10 to 33 and these values are lower than suggested for short-term conservation (Ne < 70) (Souza *et al.* 2018).

In another study that involved two species, microsatellite markers were used to study the mating system and gene flow for *Anadenanthera colubrina* and *A. peregrina*. The analyses revealed that *A. colubrina* is a mixed mating species (multilocus outcrossing rate = 0.619) while *A. peregrina* is a predominantly outcrossing species (multilocus outcrossing rate = 0.905). For both species, high indices of biparental inbreeding were observed (0.159 and 0.216 respectively), resulting in low effective pollination neighborhood sizes (Feres *et al.* 2021).

Sujii *et al.* (2017) used nuclear and plastid microsatellite markers to assess genetic parameters of juvenile and adult individuals in two *Centrolobium tomentosum* restoration areas, one corresponding to a disturbed fragment and the other, a large and well-preserved protection area. The authors reported that the restoration program was successful as they observed high genetic diversity and low inbreeding in the restoration areas, whose values were similar to the natural remnants, suggesting gene flow between those areas.

In characterizing two *Casearia sylvestris* populations, microsatellite markers revealed high allelic variation in both populations (number of alleles = 101 and 117; allelic richness = 12.5 and 14.4), despite what the authors considered high inbreeding (F_{IS} = 0.640 and 0.363). Due to low gene flow, the authors found significant genetic divergence between populations (F_{ST} = 0.103) (Araujo *et al.* 2017).

In genotyping *Myroxylon peruiferum* populations from reforested and remnant natural areas, Schwarcz *et al.* (2018) evaluated the potential of forest restoration for the production of high genetic diversity tree populations in previously deforested areas. Due to the intense gene flow, no significant differences were found between areas in terms of inbreeding (F_{IS} = 0.20) or genetic diversity (H_E = 0.31 - 0.43; allelic richness = 2.41 - 2.94).

Table 1. List of studies published in the Brazilian biomes using microsatellite markers from 2017 to 2021.

Study number	Biome	Target species	Family	Authors and publication year
1	Atlantic Forest	*Cariniana estrellensis* and *Cariniana legalis*	Lecythidaceae	Souza *et al.* (2018)
2	Atlantic Forest	*Anadenanthera colubrina* and *Anadenanthera peregrina*	Fabaceae	Feres *et al.* (2021)
3	Atlantic Forest	*Centrolobium tomentosum*	Fabaceae	Sujii *et al.* (2017)
4	Atlantic Forest	*Casearia sylvestris*	Salicaceae	Araujo *et al.* (2017)
5	Atlantic Forest	*Myroxylon peruiferum*	Fabaceae	Schwarcz *et al.* (2018)
6	Atlantic Forest	*Myroxylon peruiferum*	Fabaceae	Silvestre *et al.* (2018)
7	Atlantic Forest	*Rhizophora mangle*	Rhizophoraceae	Francisco *et al.* (2018)
8	Atlantic Forest	*Eschweilera ovata*	Lecythidaceae	Santos *et al.* (2019)
9	Atlantic Forest	*Cedrela fissilis*	Meliaceae	Gandara *et al.* (2019)
10	Atlantic Forest	*Eugenia involucrata*	Myrtaceae	Stefanel *et al.* (2021)
11	Atlantic Forest	*Luehea divericata*	Malvaceae	Silva *et al.* (2021)
12	Atlantic Forest	*Campomanesia xanthocarpa*	Myrtaceae	Petry *et al.* (2021)
13	Atlantic Forest	*Anadenanthera peregrina*	Fabaceae	Cortelete *et al.* (2021)
14	Atlantic Forest	*Schinus terebinthifolia*	Anacardiaceae	Velasques *et al.* (2021)
15	Cerrado	*Dipteryx alata*	Fabaceae	Berti *et al.* (2017)
16	Cerrado	*Dipteryx alata*	Fabaceae	Guimarães *et al.* (2019)
17	Cerrado	*Casearia grandiflora*	Salicaceae	Costa *et al.* (2017)
18	Cerrado	*GenipaAmericana*	Rubiaceae	Manoel *et al.* (2017)
19	Cerrado	*Eugenia dysenterica*	Myrtaceae	Boaventura-Novaes *et al.* (2018)
20	Cerrado	*Hymenaea stigonocarpa*	Fabaceae	Moraes *et al.* (2018)
21	Cerrado	*Qualea grandiflora*	Vochysiaceae	Potascheff *et al.* (2019)
22	Cerrado	*Dimorphandra wilsonii*	Fabaceae	Muniz *et al.* (2020)
23	Cerrado	*Hancornia speciosa*	Apocynaceae	Chaves *et al.* (2020)
24	Cerrado	*Astronium fraxinifolium*	Anacardiaceae	Manoel *et al.* (2021)
25	Amazon	*Bertholletia excelsa*	Lecythidaceae	Cabral *et al.* (2017)
26	Amazon	*Bertholletia excelsa*	Lecythidaceae	Giustina *et al.* (2017)
27	Amazon	*Bertholletia excelsa*	Lecythidaceae	Giustina *et al.* (2018)
28	Amazon	*Bertholletia excelsa*	Lecythidaceae	Martins *et al.* (2018)
29	Amazon	*Bertholletia excelsa*	Lecythidaceae	Vieira *et al.* (2019)
30	Amazon	*Bertholletia excelsa*	Lecythidaceae	Baldoni *et al.* (2020)
31	Amazon	*Genipa Americana*	Rubiaceae	Ruzza *et al.* (2018)
32	Amazon	*Theobroma speciosum*	Malvaceae	Dardengo *et al.* (2018)
33	Amazon	*Hymenaea courbaril*	Fabaceae	Rocha *et al.* (2019)
34	Amazon	*Caryocar villosum*	Caryocaraceae	Francisconi *et al.* (2021)
35	Caatinga	*Prosopis palida* and *Prosopis juliflo*	Fabaceae	Freitas *et al.* (2019)
36	Caatinga	*Spondias tuberosa*	Anacardiaceae	Santos *et al.* (2021)
37	Pantanal	*Prosopis rubriflora* and *Prosopis ruscifolia*	Fabaceae	Alves *et al.* (2018a)
38	Pantanal	*Prosopis rubriflora*	Fabaceae	Alves *et al.* (2018b)

Eight microsatellite loci used to study the reproductive system and genetic diversity in *Myroxylon peruiferum* revealed a mixed reproductive system in this species with evidence of biparental inbreeding at the rate of 0.118. Genetic diversity was low (allelic richness = 1.40 - 4.82; H_E = 0.29 - 0.52) and the effective sizes for seedlings were much lower (Ne = 27.54 - 34.86) to those recommended for short-term conservation (Ne ≥ 100) (Silvestre *et al.* 2018).

In studying the genetic diversity and the reproductive system of adult individuals and seeds from a *Rhizophora mangle* population, four microsatellite loci yielded a fixation index of -0.222 and 0.030 for adults and seeds respectively, and a multilocus outcrossing rate (tm) of 0.921. The coancestry coefficient was 0.180, similar to the expected for half-sib progenies (0.125). Based on these results, the authors estimated that 62 adult trees are needed for seed collection for short-term conservation (Francisco *et al.* 2018).

The genetic variability and gene pool sharing analysis of *Eschweilera ovata* revealed that there was moderate genetic diversity, particularly in conservation units with full protection, and that there was gene pool sharing between the subpopulations, which might reflect the historical gene flow that occurred before forest fragmentation (Santos *et al.* 2019).

Through the use of nine microsatellite loci, Gandara *et al.* (2019) investigated the genetic structure and diversity of undisturbed and disturbed *Cedrela fissilis* fragments. Genetic diversity was higher within than among fragments, with observed and expected heterozygosities ranging from 0.48 to 0.63 and from 0.55 to 0.70, respectively. The fragments showed moderate genetic structure (F_{ST} = 0.10). Therefore, authors suggested protecting all fragments instead of single isolated fragments.

A set of microsatellites used to analyze the variability and genetic structure in *Eugenia involucrata* fragments revealed high levels of genetic variability (3.67 alleles per locus; H_O = 0.815; H_E = 0.625), most of which (93 %) was distributed within the fragments (Stefanel *et al.* 2021).

Silva *et al.* (2021) studied the genetic variability of three *Luehea divaricata* natural fragments and observed high genetic variability, most of which (77 %) distributed within fragments, high gene flow (Nm= 3.853) and low genetic differentiation (F_{ST} = 0.072).

The evaluation of the patterns of genetic diversity, fine-scale spatial genetic structure and historical gene flow in *Campomanesia xanthocarpa* fragments revealed that the fragments presented moderate to high levels of genetic diversity and there was observed the isolation by adaptation pattern, which implied the need for maintenance of the current remnants to assure the conservation of the private alleles (Petry *et al.* 2021).

The diversity and genetic structure of *Anadenanthera peregrina* were used as strategies for *ex situ* conservation. From a planted population, 42 alleles were detected and negative values for F_{IS} were observed, indicating escape of inbreeding in the population. According to the authors, the findings revealed the importance of *ex situ* conservation of the evaluated genotypes, allowing future use of the population as a seed orchard (Cortelete *et al.* 2021).

Microsatellite markers were used in studies of genetic structure among *Schinus terebinthifolia* populations from different ecological groups. Genetic structure revealed differences among populations (37.72 %) and significant fixation rates based on F_{ST} ($P < 0.001$). The patterns of distribution for the species did not follow the isolation by distance or similarity by environmental conditions. The most divergent genotype group was found at the ombrophilous forest, which indicates that conservation efforts should be undertaken to prevent losses of biodiversity in that area (Velasques *et al.* 2021).

Cerrado biome

Eleven studies (28.9 %) used microsatellites to analyze the genetic diversity in forest tree species from Cerrado, one of which was performed also in the Pantanal biome. Two of these studies involved *Dipteryx alata*. The analysis of the genetic diversity of three natural populations of *Dipteryx alata* revealed that these populations presented moderate genetic diversity (H_O = 0,618; H_E = 0,715) which is fundamental for their survival along the generations (Berti *et al.* 2017). In another study, the genetic diversity of a *Dipteryx alata* progeny from a germplasm collection, revealed that the number of alleles was 50 and, due to the high effective population size (Ne = 96), the germplasm collection had sufficient representativeness for use as a base population for breeding programmes (Guimarães *et al.* 2019).

In studying the genetic structure of *Casearia grandiflora* in conserved and disturbed populations, there was observed moderate divergence between populations (F_{ST} = 0.14) and higher proportion of genetic diversity (85 %) was distributed within populations, which were not structured. In addition, less urbanized populations had greater genetic diversity, confirming the effectiveness of protected areas in genetic diversity conservation (Costa *et al.* 2017).

Microsatellites used to investigate the impact of spatial isolation on pollen and seed flow in a *Genipa americana* population detected a minimum immigration of pollen (6 %) and seeds at 4 % and mating among relatives (20-40 %), indicating genetic connectivity with other populations (Manoel *et al.* 2017).

The assessment of the pattern of phenotypic and molecular genetic divergence among natural subpopulations of *Eugenia dysenterica* suggested that the species has a spatial genetic structure which must be taken into account for managing its genetic resources for both conservation and breeding purposes (Boaventura-Novaes *et al.* 2018).

Microsatellite loci were used to investigate the pollen and seed dispersal and mating patterns in *Hymenaea stigonocarpa*. The species presented a mixed mating system, with variations in the outcrossing rate (0.53 - 1.0). Pollen and seed dispersal occurred over long distances (>8 km) and the dispersal patterns were isolated by distance. Selfing resulted in a higher inbreeding depression than mating among relatives (Moraes *et al.* 2018).

The analysis of reproductive success, pollen dispersal and mating system of *Qualea grandiflora* trees revealed that the mean pollen dispersal distance (524.7 m) and the effective number of pollen donors per mother-tree (Nep = 12.7) were higher than for roadside trees (60.9 m, Nep = 4.6). The results indicated that the spatial isolation of roadside trees decreased pollinator movements (Potascheff *et al.* 2019).

The genetic diversity evaluation of ten *Dimorphandra wilsonii* populations resulted in 4 to 13 alleles per locus, and heterozygosity values per locus ranged from 0.113 to 0.940 for H_O and from 0.219 to 0.796 for H_E (Muniz *et al.* 2020).

A study aiming to compare quantitative and molecular variation within and among botanical varieties and subpopulations of *Hancornia speciosa* revealed a low degree of divergence among the botanical varieties and significant structuring among the subpopulations within varieties. According to the authors, divergent selection shaped the genetic structure among the botanical varieties for some traits, while genetic drift and uniform selection influenced the variation among the subpopulations (Chaves *et al.* 2020).

Pollen and seed flow for *Astronium fraxinifolium*, investigated through parentage analysis and microsatellite loci, revealed that a large proportion of pollen (76.5 %) and seeds (57 %) immigrated from trees outside the sampled populations and the dispersion followed a pattern of isolation by distance (Manoel *et al.* 2021).

Amazon Forest biome

In this biome were recorded ten studies (26.3 %) using microsatellite markers in forest tree species. *Bertholletia excelsa* was the most studied species with six studies. Cabral *et al.* (2017) assessed the genetic diversity of a *B. excelsa* population and observed high genetic diversity ($H_O = 0.512$; $H_E = 0.491$) and no inbreeding. The analysis of half-sib progenies from different *B. excelsa* trees, by Giustina *et al.* (2017), revealed greater genetic diversity between families than among progenies from the same family. In studying the mating system in a *B. excelsa* population, Giustina *et al.* (2018) observed that outcrossing rates varied between trees (0.49–1.0) and fruits (0.53–1.0), but seeds were predominantly produced by outcrossing (0.92). Martins *et al.* (2018) observed moderate genetic diversity and high seed-dispersal distances in *B. excelsa* populations. Studying two *B. excelsa* populations, Vieira *et al.* (2019) found 70 alleles, H_O was 0.43 and H_E was 0.82. The analysis of the genetic diversity of *B. excelsa* revealed greater genetic diversity between populations than within populations, allelic variation ranged from four to nine alleles, and heterozygosity ranged from 0.32 to 0.80 (Baldoni *et al.* 2020).

The analysis of the genetic diversity and population structure of three *Genipa americana* populations revealed 17 alleles, the expected heterozygosity ranged from 0.35 to 0.67 and remained higher than the observed heterozygosity. The populations presented high inbreeding ($F_{IS} = 0.40$), probably because of fragmentation (Ruzza *et al.* 2018).

In studying the effects of fragmentation on the genetic structure of *Theobroma speciosum* populations, Dardengo *et al.* (2018) found that most of the genetic diversity was distributed within groups (83 %), which means that no significant effect of fragmentation was observed. However, given the small number of reproductive individuals in the population, the authors warned that the process of continuous fragmentation might increase inbreeding and favor genetic drift, leading populations to inbreeding depression and diversity loss.

In another study, SSR markers used to analyzed the genetic diversity of five *Hymenaea courbaril* populations detected 10.29 alleles per locus, H_E and H_O averaged 0.85 and 0.29 per locus, respectively (Rocha *et al.* 2019).

The genetic diversity and structure analysis of *Caryocar villosum* revealed low inbreeding ($F_{IS} = 0.127$; $F_{IT} = 0.173$) and low differentiation between regions ($F_{ST} = 0.06$). Most of the variation (89 %) was found to occur within regions (Francisconi *et al.* 2021).

Caatinga biome

In Caatinga, only two studies (5.3 %) were found in our search. Freitas *et al.* (2019) found low levels of genetic diversity (two alleles per locus and $H_E = 0.181$) and inbreeding ($F_{IS} = -0.007$) for *Prosopis palida* and *Prosopis juliflora*, suggesting the presence of genetic bottleneck and probable events of founders.

The diversity and genetic structure analysis of *Spondias tuberosa* accessions resulted in similarity coefficients from 0.30 to 0.84, indicating the existence of divergence among the accessions which can be used to increase the germplasm bank genetic diversity of this species (Santos *et al.* 2021).

Pantanal biome

Only two studies (5.3 %) with microsatellites were recorded in Pantanal, one of which was performed also in Cerrado. Microsatellites employed by Alves *et al.* (2018a) in collections of *Prosopis rubriflora* and *Prosopis ruscifolia* from Cerrado and Pantanal resulted in similar levels of genetic diversity for both species ($H_E = 0.59$ and $H_E = 0.60$ respectively) and there was evidence of genetic bottleneck in 64 % of *P. rubriflora* sampled area and in 36 % of *P. rusciflia* sampled areas.

In a study of the reproductive system of *Prosopis rubriflora*, Alves *et al.* (2018b) found that the species is preferably allogamous and the obtained progeny was composed predominantly by half-sibs (79 %). Coancestry coefficients ranged from 0.158 to 0.162 and there were high levels of crossings due to several mechanisms that prevent selfing.

Discussion

The Fabaceae family was the most studied in the Brazilian biomes. This finding is supported by the fact that Fabaceae is among the richest families in most Brazilian ecosystems. According to Lima *et al.* (2015), there are 222 native genera and 2807 species. In the Caatinga, for example, this family constitutes about a third of the richness of the biome with 86 genera and 320 species (Córdula *et al.* 2014).

Regarding the evolution of the number of studies published within the analyzed period, there was a trend of increase until 2018. However, the studies decreased in 2019 and 2020, coincidently, during the COVID-19 pandemic outbreak. This period was characterized by lower allocation of research resources and increased socio-political tension in Brazil. The syndemic theory by Merrill Singer could explain the negative effect of COVID-19 on researchers productivity (Singer 1996). In 2021, probably, due to the vaccination process and the return of many activities, the publications returned to growing.

Considering the importance of microsatellites and the growing need for genetic studies in Brazilian biomes, there were expected more studies for a five-year period. However, forest species are low studied in Brazil because they are neglected. On the other hand, the need for prior knowledge of the species genome limits the use of this class of molecular markers in genetic analysis. Thus, the abundance of alternative molecular markers, which are more accessible, may have contributed to the less use of microsatellites in genetic analyzes in Brazilian biomes.

Despite these limitations, research that uses microsatellites is important due to the quality of the information accessed, mainly in terms of genomic coverage, codominance characteristics and heritability.

Most of the studies were performed in the Atlantic Forest biome, while Pampa and Pantanal are the biomes with less studies. In general, the genetic diversity decreased in all biomes, primarily due to anthropic activities, according to the authors. In addition, the use of microsatellites was helpful to propose proper alternatives for conservation. However, there was a lack of standardization of the genetic diversity statistics. For example, Berti *et al.* (2017) considered $H_O = 0.618$ and $H_E = 0.715$ as moderate genetic diversity, while Cabral *et al.* (2017) considered $H_O = 0.512$ and $H_E = 0.491$ high genetic diversity. The probable reasons for this discrepancy may be the life history traits of each species and the heterogeneity between ecosystems. For example, Pantanal has richer ecosystems than Caatinga and Pampa.

Most of the genetic diversity in all biomes is distributed within groups. This pattern is consistent with the predominance of allogamy in the plant kingdom (Bawa *et al.* 1985) and the gene exchange allows recombination, increasing the genetic diversity within groups.

In spite of fragmentation, the studies ratified the role of gene flow in connecting isolated populations, thus preventing the loss of genetic diversity in the Brazilian biomes. In fact, allowing gene flow among populations of a species is one successful alternative to reduce the negative effects associated with small populations such as inbreeding and genetic drift. It is supported by studies carried out to compare disturbed and undisturbed areas, in which disturbance did not affect genetic diversity when high levels of gene flow were observed (Costa *et al.* 2017; Sujii *et al.* 2017; Gandara *et al.* 2019). According to Hellberg *et al.* (2002), gene flow is essential in connecting reproductively isolated populations, thereby reducing genetic differentiation among them. The studies ratified the predominance of crossing in the tropical forest tree species (Sobierajski *et al.* 2006). Although most of the flowering plants in nature are hermaphrodite, many species developed mechanisms, such as self-incompatibility, to prevent selfing in order to allow gene exchange and avoid gene erosion (Bawa *et al.* 1985; Sobierajski *et al.* 2006).

Final remarks

Our research highlighted the prominent contribution of microsatellites in genetic studies in the Brazilian forest biomes as well as for genetic conservation. In general, the studies reinforce that human activities are reducing genetic diversity in the Brazilian biomes. In addition, the studies ratified the role of gene flow in connecting isolated populations, thus reducing the probability of species extinction. Thus, in order to slow down the loss of genetic diversity, it is recommended to maintain a large number of individuals and allow connectivity among isolated fragments.

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