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GENETIC INSIGHTS FOR CONSERVATION AND RESTORATION OF TROPICAL TREES IN THE ATLANTIC RAINFOREST

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DEDICATION

Firstly, I want to thank ALMIGHTY GOD that have been a source of strength, inspiration, and unwavering support throughout this journey.

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Finally, I dedicate this thesis to the pursuit of knowledge and the quest for understanding. May this work contribute in some small way to the betterment of our world and inspire others to continue exploring, questioning, and learning.

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ABSTRACT

Deforestation and fragmentation are serious threats to tropical forests because they can adversely affect ecosystem services, biodiversity loss, and degradation of genetic resources. Originally very widespread, and very diverse with the Brazilian Atlantic Forest vegetation formations have drastically disappeared due to intense human activities, leaving fragmented remains that try to keep their ecological functions. This has consequently reduced the genetic diversity of the remaining populations, hence putting in jeopardy their long-term survival and adaptability. Conventional practices for restoration and reforestation usually ignore the genetic aspect, hence they are likely to have limited success and persistence. We have studied, then, the genetic diversity and structure of three native forest species, ecologically and economically important, by means of state-of-the-art genetic techniques: *Manilkara multifida*, *Lecythis pisonis*, and *Plathymenia reticulata*. We aimed to detect key genetic metrics that are crucial for choosing the appropriate species and populations in conservation and restoration purposes by using microsatellite markers. Partially unexpected for these species, such complex genetic patterns were found. This also points out the importance of those genetic factors for the preservation of ecological integrity for the Atlantic Forest. We show that long-term stability of restored ecosystems is greatly enhanced by choosing species with adaptive capacity and promoting landscape connectivity, which also strongly increase genetic resilience. Our work provides a framework, where we compare native trees grown in different landscapes to check whether they are suitable for restoration strategy optimization, not only of the degraded area itself, but also supporting the broader objectives of the Atlantic Forest for conserving its valuable biodiversity. We do this by embedding genetic considerations into rescue planning. Finally, our study highlights how genetic insights of native trees grown in protected areas, forest and cabruca are crucial in maintaining resilience and longevity in the unique ecosystems of the Atlantic Forest. It is indeed a strategy towards the reversal of negative fragmentation outcomes and long-term conservation of this global biodiversity hotspot. Our findings may also benefit future forest restoration programs like the Pact for the Restoration of the Atlantic Forest, launched in 2009, which aimed at recovering a total area of 15 million hectares by 2050.

Keywords: Neotropical trees, genetic diversity, reproductive system, conservation, agroforest.

GENERAL INTRODUCTION

Human-induced deforestation and fragmentation greatly threaten tropical forests, where roughly two-thirds of terrestrial biodiversity occur on Earth. Such widespread degradation has resulted in the decline of ecosystem services and reduced biodiversity (Faria et al., 2023). More than half of the tropical forests are currently being lost, hence becoming a big concern for conservation (Aronson et al., 2020). A remarkable reduction in biomass and species variety has been noticed in the highly biodiverse Brazilian Atlantic Forest, and merely 11.7% of the original cover remains of this forest cover (Ribeiro et al., 2009, de Lima et al., 2024). Despite these challenges, Brazil is trying to mitigate the effects of deforestation by implementing a number of conservation and restoration projects (Ditt et al., 2010).

Genetic data may indicate the source population that may harbor the necessary information for adaptive variations and gene flow to maintain the evolutionary potential of the species (Mijangos et al., 2015; Almeida-Rocha et al., 2020). Genetic information differentiates a lot in the success of restoration projects because genetic diversity is the important for fitness and persistence of populations, and it is adversely affected by anthropogenic disruptions (Reynolds et al., 2012). These resources also provide historical and modern data on genetic dynamics, connectivity, and species distribution that are particularly valuable for assessing ecosystem health and understanding impacts of habitat loss (Diekmann et al., 2010; Thomas et al., 2014). When genetic divergence among populations is assessed, scientists can learn about evolutionary history and adapt strategies to cope with forest fragmentation and connectivity (Sork & Smouse, 2006; Kremer et al., 2012).

Restoration and agroforestry systems help link up the fragments of remaining forests by increasing connectivity, a measure that may reduce the genetic impacts of forest fragmentation.

When landscape connectedness is reduced, genetic diversity is often lost and inbreeding depression worsens (Baguette et al., 2013; Herrera et al., 2017). In fact, the effective conservation of genetic diversity requires appropriate selection of species and knowledge about their genetic properties (Maloney et al., 2011; Weeks et al., 2011).

Although the urgency of conserving the Atlantic Forest is widely recognized, it is essential to provide a clear scientific foundation to society, ensuring that these conservation actions are implemented effectively. In this regard, our study aimed to select the forest with the highest levels of tree diversity, based on genetic attributes, to be restored. For instance, it can be put forward those different members of the species come together at different sites to promote genetic variability in the sites in which they are to be planted and then to the overall restored population.

The traditional methods of restoration and reforestation are efforts often overlook the critical importance of genetics issues; this can hugely jeopardize their potential for success as well as viability. In the case of our study, we addressed this knowledge gap by applying modern genetic techniques to research genetic diversity and structure in three important tree species native to the region that play an important role in ecology and the economy: *Manilkara multifida* Penn., *Lecythis pisonis* Cambess, and *Plathymenia reticulata* Benth.

We have studied the genetic patterns of *M. multifida* in two protected areas in the first chapter. We observed reduction in genetic diversity among juveniles of *M. multifida* populations and small distance gene flow due to anthropogenic disturbances indicates that even protected populations are vulnerable to genetic erosion. A conservation-focused action would be needed to address those impacts on habitat degradation, thereby assuring the survival of genetic diversity.

In chapter two, we stated the necessity of researching the reproductive patterns of *L. pisonis*. This study presents additional information on how the reproductive behavior of *L. pisonis* really depends on pollinators and cross-pollination dependence. The results gathered on inbreeding show that there is no existence of inbreeding among the analyzed families; therefore, a distance of six kilometers among the pollen dispersal will allow adequate outcrossing events. This stresses, therefore, the importance of maintaining habitat connectedness and the conservation of active pollinators to encourage gene flow and protect genetic diversity. These findings underline the need for conservation strategies that promote and enhance habitat connectivity and pollinator activity, respectively, to safeguard *L. pisonis* genetic integrity and the long-term survival of this species in agroforestry systems.

In third chapter, we compared the genetic indices of *P. reticulata* populations grown in cabruca and natural forests. Cabruca are the cacao agroforest that are grown under the shade of native trees species. The present work has therefore also evidenced that agroforestry systems, mainly with a cabruca, have opened scope for conservation opportunities. Genetic diversity of cabruca systems is as high as in natural forests, though these are altered landscape types. This therefore, underlines the fact that, if appropriately managed, agroforestry can really act as a pioneer in the conservation of genetic resources in degraded and fragmented settings. The second thing is that the genetic difference observed among populations grown in forests and cabruca habitats underlines the impact of environmental factors and management strategies on genetic composition. These results underscore the need for adaptive planning of conservation that recognizes the unique ecological and genetic contexts of different landscapes.

LITERATURE REVIEW:

1. Anthropogenic activities and Forest loss:

Human disturbances cause major forest loss around the globe during the last century (Curtis et al., 2018). This has major implications from changing ecological dynamic of local small environment to 95% of rapid rise of earth temperature (Viñals et al., 2023). According to recent estimates by 2050, over half of the human population will live in tropics and other half depends on tropics for fresh water, agricultural products such as food, timber and medicines (Archer et al., 2020). Destruction of tropical forest not only impacting plants, animals and insects but also human population (Roberts et al., 2017). Drivers of global change are disrupting ecosystem services, causing decline in population at fast-pace worldwide (García et al. 2016). Disturbances due to human activities has often promoted change in natural habitat of species (Gomez et al. 2010). There is a relationship between biodiversity loss and human well-being, which is related to ecology and economy, as increase of one could affects the other (Brockerhoff et al. 2017).

The world has 4.06 billion hectares of forest area, and it is estimated during five-year period from 2015-2020, the rate of net-forest lost is 10 million ha per year (Global Forest Resources Assessment 2020). Loss of tropical forest remain high, it was estimated 4.1-million-hectare loss occurred in 2022 (Pro et al., 2023). During recent decades decline in forest leads to habitat fragmentation and directly effecting species population size, genetic variation and ultimately its long-term persistence (Tan et al., 2018). It is important to understand the richness of biological diversity on Earth, the threats that human activities pose to this richness, and the negative consequences that further loss of diversity may have to humankind and to the Earth biomes.

Land degradation threatens food security, biodiversity loss and climate change crises by emission of greenhouse gases. Forest restoration has gain lots of attention in recent years as a strategy for addressing environmental and social problems caused by human degradation (Brancalion et al., 2022). Understanding the loss of biodiversity by anthropogenic activities is important to plan conservation strategies accordingly. After decades of research delineate universal conservation strategy are rarely effective and more effective if they are peculiar to local context (Barreto et al., 2023a, Meyfroidt et al., 2022, Wells et al., 2020).

2. Biodiversity Conservation Initiatives:

Biodiversity indicators to track the rate of loss of biodiversity has been underway for over two decades, started in 1992 first with the adoption of the Convention on Biological Diversity (CBD) and now updated Strategic Plan for Biodiversity 2011–2020 (UNEP 2010). There is a relationship between biodiversity loss and human well-being, which is related to ecology and economy, as an increase of one could affects the other (Brockerhoff et al. 2017). According to CBD, biological diversity is commonly distinguished at three levels: ecosystems, species, and genes (Graudal et al. 2014). The three levels of biodiversity all have a potential of a huge impact and can lead to serious economic and ecological losses if biodiversity decline. The animals, plants and microbial life are well adapted according to changing environment (Verma 2020).

United Nation Decade on Ecosystem Restoration (2021-2030) (Unep, 2020), forest restoration initiatives scale-up by many governments, NGOs etc. to address conservation of biodiversity, climate change and associated socio-economic concerns. This leads to Bonn Challenge, that encourages 60 countries to restore >200 million hectares of forest by 2030 (Aronson et al., 2020, Holl et al., 2020). Forest restoration programs can provide different benefits in addition to creating jobs, it can provide forest products and services (Di Sacco et al., 2021, Batista et al., 2021). The payments programs for ecosystem services, timber and non-timber products can make sustainable and income sources for farmers and stakeholders (Brancalion et al., 2022). So, restoration projects can help in sustainability, adaptability, natural disaster and can act as crucial opportunity to emerge as forest-based bioeconomy (Santos et al., 2023, Bastos Lima and Palme, 2021).

3. Brazil’s role in restoration project specifically loss in Atlantic Forest in Southern Bahia:

In Brazil, approximately 50 million hectares of native forests have been damaged by inappropriate land use (Brancalion et al., 2022). This area accounts for over 25% of the overall Bonn Challenge target and is a major component of what will be required by 2050 to supply the expanding demand for forest products worldwide (FAO, 2022). As a result, Brazil can play a significant role in advancing the demands for forest restoration and meeting the world's demand for forest products (Brancalion et al., 2022). Brazil has a very strong chance of providing the globe with multifunctional restoration models that include socioeconomic benefits like income production, job creation, and forest products, together with ecological benefits like water security, biodiversity protection, and climate mitigation. Around 14.5% of the world's known tree species are found in Brazil (Beech et al., 2017), with at least 100 species known for highgrade timber as well as a number of forest products, including fibers, oils, medicines, and resins (Rolim et al., 2019). Because of this, the native species now have more economic diversity, and Brazil is leading the way in extending restoration efforts by utilizing a bioeconomy based on restoration products (Santos et al., 2023).

Atlantic Forest has ~11.7% of its original forest cover remaining (Ribeiro et al., 2009) and it has long history of deforestation dating back to 16th Century. It is among the top five global biodiversity hotspots, and benefitting almost 70% population by conserving biodiversity, water and mitigating climate change (Rosa et al., 2021; Strassburg et al., 2019). A large portion of Atlantic Forest turned into matrix of degraded areas, agroforest, pasture and urban areas, threatened almost 60% of the entire flora and fauna in Brazil (Rezende et al., 2018, Joly et al., 2014a). Due to ongoing deforestation and its anticipated effects on both plant life (de Lima et al., 2015) and vertebrates (Grelle et al., 2005), the Brazilian Atlantic Forest has become a focal point for numerous conservation initiatives. These initiatives include the establishment of national and state protected areas (ranging from categories I to VI according to the IUCN), as well as private protected areas known as RPPNs, the creation of ecological corridors, agroforestry and the implementation of action plans for endangered species (Joly et al., 2014b). The Atlantic Forest in southern Bahia is among the most well-preserved regions in the state and is part of the "Central Corridor of the Atlantic Forest" (Romário et al., 2020). The landscape of southern Bahia underwent visual changes between 1985 and 2019, marked by a mix of human activities and natural areas. This blend has created a complex dynamic between conservation, preservation, and environmental impact. The landscape of southern Bahia experienced significant changes that led to the expansion of pasture areas, which became predominant between 1990 and 2005. Today, pastures and natural forests cover similar proportions of the region. The fragmentation of the

Atlantic Forest into small patches, along with edge effects, has diminished its ability to sustain ecosystems (Escarlett de Arruda et al., 2022).

4. Agroforestry-Cabruca, Forest Fragments:

Ecosystem restoration is widely recognized as one of the most impactful methods for addressing climate change (Bastin et al., 2019). Additionally, there is significant potential to reintroduce trees into farmlands and urban areas, emphasizing the importance of agroforestry in climate change mitigation (Gupta et al., 2023). Agroforestry is relevant at three levels: individual plots, multifunctional landscapes, and the intersection of agricultural and forestry policies (Van Noordwijk, 2019). Forest and landscape restoration (FLR) is a comprehensive strategy that seeks to harmonize various forms of tree cover to deliver multiple benefits (Mansourian el at., 2018). FLR efforts encompass a range of actions, including tree planting, assisted natural regeneration, agroforestry, and enhanced land management (Gupta et al., 2023). Agroforestry and FLR involve practices that integrate trees into agricultural settings, which may include crops or livestock (Chazdon el at., 2019). These efforts can lead to a range of land-use outcomes, from extensive natural forests to productive agroforestry systems and patches of wooded areas within agricultural fields (IUCN et al., 2014; Hanson et al., 2015).

In climate-smart environment, agroforestry plays a vital role in mitigation and adaptation. Agroforestry, with its numerous environmental and economic advantages, has the potential to support the agricultural and forestry industries by providing innovative solutions to current challenges such as financial instability, environmental issues, and a negative public perception (Jatav et al., 2024). The Brazilian Native Vegetation Protection Law, often referred to as the "New Forest Code," (Filho et al., 2014) mandates that Atlantic Forest farmers maintain at least

20% of their land with native vegetation. Farmers who fall short of this requirement must comply by either restoring native vegetation on their own land or by funding conservation or restoration projects within the same biome. If properly implemented, this could result in up to 5.17 million hectares being restored, which is the target area considered in all scenarios (Strassburg et al., 2019). Bahia, have smallholder farming of historical cocoa plantation (Bahia de Aguiar el at., 2019). This cocoa plantation is under the shade of native Atlantic Forest trees known as Cabruca. The majority of Brazil's cacao production (53%) is centered in the southern region of Bahia state, where small-scale farmers with properties of less than 10 hectares (Heming et al., 2022). Cabruca is rich in biodiversity and plays an important role in restoration projects.

5. Genetics as tool for conservation:

Over the past forty years, conservation geneticists have created numerous concepts, methodologies, and tools to guide molecular biodiversity conservation. The relationship between genetics and restoration covers various areas, with genetics offering essential insights for decision-making and monitoring in restoration projects (Mijangos et al., 2014). The application of genetic information to guide decision-making in restoration initiatives is especially crucial for developing tropical countries. The outcome of restoration activities is influenced by the connectivity of the restored areas to the surrounding landscapes (Lindenmayer et al., 2010, Proft et al., 2018) and its degree to which landscape facilitates movement of species across its existing resource. The isolation between habitat patches as a result of deforestation reduces levels of genetic diversity and effective population size. In this scenario, forest restoration and agroforest systems could help increasing the connectivity among remanent forest fragments reducing the genetic consequences of forest fragmentation. The reduction in landscape connectivity between habitat patches can also increase inbreeding depression (Baguette et al., 2012) (Herrera et al., 2017). Also, to choose suitable species to identify that population suffered low genetic diversity or suffered from inbreeding due to habitat fragmentation (Maloney et al., 2011, Weeks et al., 2011). Therefore, genetic parameters are important ecosystem indicators for landscape connectivity as by forest restoration or agroforest systems. The landscape connectivity can influence gene flow, population size and genetic diversity (Proft et al., 2018). Gene flow can occur through species but for the most tropical trees the effect of isolation and contribution of gene flow is not understood (Dick et al., 2008). By working with these elements, it will ensure adaptive potential and long-term fitness of species and to determine best approach for restoration projects.

The loss of genetic diversity impacts species by affecting their reproduction and survival rates, increasing their susceptibility to climate change, and elevating the risk of extinction (Hoban et al., 2021, Des Roches et al., 2021). Now is a crucial moment for conserving genetic diversity, especially through the United Nations Convention on Biological Diversity (CBD) post-2020 Kunming-Montreal Global Biodiversity Framework (GBF), which was adopted in December 2022 during COP15 (15th Conference of the Parties) (Hoban et al., 2023b).

The Convention on Biological Diversity proposed practical strategies to enhance genetic diversity also different indicators to measure genetic diversity. There are several strategies for improving genetic connectivity: increasing the size of populations that are geographically proximal enough to allow gene flow, removing barriers such as roads or fences, and others (Frankham et al., 2017). Habitat corridors link fragmented habitats so that species can move more easily across a landscape. Wildlife underpasses and overpasses are structures to enable wildlife to cross over, or under human-made barriers that act as corridors (Frankham et al., 2017). All these methods work to lessen the impact of population fragmentation on species genetic health (Frankham, 2022). The post-2020 biodiversity conservation goals and action targets emphasize that monitoring species using DNA-based methods serves as an additional indicator for assessing genetic diversity (Sean et al., 2020). To gather this information, techniques in molecular biology, such as molecular markers, are essential. Collecting genetic data through these techniques enhances the management of genetic diversity, underscoring the crucial role of genetic evaluations in conservation strategies (Hoban et al., 2023a).

6. Microsatellite markers

Genetic diversity and its distribution among populations can be effectively measured using genetic markers like Simple Sequence Repeats (SSRs) as known as microsatellite (Serrote et al., 2023). These markers enhance the efficiency of genetic studies due to their neutrality, accessibility across different plant developmental stages and environments, and minimal impact on specimen viability, as only small tissue samples are required, facilitating further analysis (Garcia et al., 2004)

Developed in the 1980s by (Litt and Luty, 1989), microsatellites, or SSRs, are widely employed in genetic research. They are prevalent in both eukaryotic and prokaryotic genomes, and due to frequent DNA replication errors within microsatellites, their lengths vary both within and between species. This variability makes microsatellites ideal for creating PCR-based markers for tasks like population genetic studies, genome mapping, and marker-assisted selection. By using a specific set of primers (20-30 base pairs) that match unique sequences flanking the microsatellite, the resulting amplified segments vary in size, each representing a distinct allele from the same locus. Additionally, microsatellites are abundant, uniformly spread throughout the genome, and are among the most polymorphic molecular markers currently available (Jarne and Lagoda, 1996, Ferreira and Grattapaglia, 1998). The effectiveness of SSRs is largely due to their co-dominant inheritance, which provides comprehensive genetic information (Garrido-Cardenas et al., 2018).

SSR markers are among the most optimal for population genetic studies due to their high allele count and heterozygosity, which surpass those of other markers like isoenzymes (Rafalski et al., 1996). SSR markers have been introduced in ecological research to assess relatedness between individuals (Melo, 2000, Yang et al., 1994) and have been utilized to investigate genetic structure and diversity across individuals, populations, and species (Collevatti et al., 2001, Dayanandan et al., 1999, White et al., 1999).

According to Frankel and Soulé (1981), two important components of diversity should be taken into account when analyzing genetic variability using microsatellite markers: allelic richness, which is based on the total number of alleles in a population or sample (A), and the distribution of allele frequencies within the population, which is measured by the gene diversity coefficient (H_E). Additional crucial metrics include of: (i) Wright's fixation index (f), which assesses the greater or lesser value of heterozygotes relative to expectations under the Hardy-Weinberg equilibrium; (ii) the percentage of polymorphic loci (P), which is used in conjunction with the number of alleles per locus to assess and compare genetic variation levels across populations; and (iii) observed heterozygosity (H_O), which is essential for measuring genetic diversity because it reflects the presence of different alleles in heterozygotes, representing variation in both self-pollinating and cross-pollinating populations. Evolution is driven by contrasts between a Hardy-Weinberg "ideal" population and real-world populations, as stated by Futuyma (1992). The findings showed that SSR loci are effective instruments for studying population structure and that they offer a way to precisely estimate gene flow and paternity, two crucial conservation biology characteristics (Changtragoon et al., 2017).

7. Impact on different tropical trees.

One of the most striking features of the Atlantic Forest in southern Bahia is its impressive richness of native tree species. In a single hectare of forest, in some locations, it is possible to find more than 450 different woody species with DBH (diameter at 130 cm from the ground) ≥ 5.0 cm, one of the highest rates of richness of this type in forests in the world. In addition, the species that occur in each location can vary greatly according to the environmental variations in soil, relief and climate existing in the region. It is also important to emphasize that many of these species are endemic, being found only in the Atlantic Forest strip that covers southern Bahia and northern Espírito Santo. Studies show that more than 20% of the plant species found in surveys are endemic to this region (Sambuichi et al., 2009). A major concern is the lack of knowledge about newly discovered species. Many remain botanically undescribed, and their ecological characteristics are largely unknown. This poses a significant challenge for biodiversity conservation, as understanding environmental preferences, reproductive strategies, and population dynamics is crucial for effective conservation efforts.

The harvesting of large, old trees has led to the dominance of exotic timber production in short-rotation industrial plantations (Pedro Medrado et al., 2023), particularly in the highly degraded and fragmented landscapes of southern Brazil's Atlantic Forest and Cerrado regions (de Moraes Gonçalves et al., 2013). In 2020, timber from native forests generated $518 million, while commercial plantations yielded $3.48 billion (IBGE, 2020). Enhancing the production of native timber and non-timber resources in restoration efforts could significantly boost large-scale restoration (Lamb et al., 2005, Hua et al., 2022).

Assessing the genetic diversity within and between these natural populations, along with a deeper insight into their mating systems, can guide the development of management strategies, conservation efforts, and genetic enhancement of the species. This approach ensures the species' genetic diversity is maintained without being compromised. Gaining deeper insights into genetic diversity both within and among natural populations can significantly aid in the conservation of native trees.

In the Atlantic Forest, understanding the genetic parameters of native tree species is essential for their conservation. We use three important native tropical trees in Atlantic Forest, *Manilkara multifida* Penn., *Lecythis pisonis* Cambess , *Plathymenia reticulata* Benth. The three species are restricted to Dense Rainforest of Southern Bahia. The species has small scale dispersion as the main pollinators for these species are bees (Maués, 2007, Monteiro et al. 2020, Goulart et al., 2005). Another similarity between these trees is their role in bioeconomy. These trees are important for their timber production. Their wood is used for construction purposes, because of their resistance. They are grown in forest as well as in cabruca system (Almeida, 2023), which makes them excellent target species to study their genetic parameters for restoration projects and to study their long-term fitness and sustainability.

Manilkara multifida Penn.

Historically, the genus *Manilkara*, described by Adanson in 1763, derives from some species of the genera *Mimusops* and *Achras* described by Linnaeus in 1753, with *Manilkara kauki* being the first species of the genus attributed by Dubard in 1915 (Kukachka, 1981). Along with other genera such as *Pouteria*, *Chrysophyllum*, *Ecclinusa*, and *Pradosia*, the genus *Manilkara* is included in the Sapotaceae family proposed by Jussieu in 1789. The Sapotaceae family belongs to the order Ericales and forms a sister group with the families Ebenaceae, Maesaceae, Theophrastaceae, Primulaceae, and Myrsinaceae (Stevens, 2008). Sapotaceae have arboreal or shrubby habits and exhibit important vegetative characteristics, such as white latex (rarely yellow), which is very frequent in the trunk, branches, and fruits, as well as alternate phyllotaxy with a spiral distribution of leaves (Pennington, 1990). They are commercially valued for the latex and lipid compounds used in the production of chewing gum, balata, oils, and butters (Monteiro et al., 2007). However, despite the results of a study contracted under the IBAMA/Fundação Biodiversitas agreement, the Brazilian government does not recognize the international status established for this species, even though Fundação Biodiversitas used the same evaluation criteria as the IUCN.

Lecythis pisonis Cambess.

Lecythidaceae is a family of trees with a pantropical distribution, found in the tropics of Central and South America, Southeast Asia, and Africa, including Madagascar. In South America, there are 10 genera and 202 known species in the subfamily Lecythidoideae, along with a single species, *Asteranthos brasiliensis*, from the Central African subfamily Scytopetaloideae. The largest genus in the subfamily Lecythidoideae is *Eschweilera* with 85 species, while the third-largest genus by number of species is *Lecythis* with 26 species (Mori, 2004). *L. pisonis* occurs in both the Amazon and Atlantic forests, and in the latter, it is distributed from Pernambuco to São Paulo, where it is common, especially in southern Bahia and northern Espírito Santo (Mori and Prance, 1990; Brasília, 2002). The sapucaia (*Lecythis pisonis*) occurs more frequently than other trees in agroforestry systems of the cacao-cabruca type (Sambuichi et al., 2009), possibly due to its beauty and value among local farmers. *L. pisonis* can be classified as a flagship species according to criteria proposed by Bowen-Jones and Entwistle (2002), such as being locally important for the conservation of other species and typical in the landscape; having characteristics that make it easily recognizable; being charismatic, which can influence public opinion; having cultural significance; and occurring in traditional landscapes (cabruca), among others. Furthermore, it has ecological importance and is recommended for forest restoration, as its seeds, appreciated by fauna for their flavor, make it an attractive tree (Lorenzi, 2002; Sambuichi et al., 2009).

Plathymenia reticulata Benth.

Plathymenia reticulata Benth. (Leguminosae-Mimosoideae) is a canopy tree species, characterized by its diploid chromosome number of 2n = 26 (Moore, 1977). It is commonly referred to as “vinhático,” a name inspired by the reddish hue of its trunk, reminiscent of red wine (Silve Jr., 2005). Native to South America, this species is predominantly found in Brazil, where it is distributed across states like Bahia, Ceará, Maranhão, Espírito Santo, São Paulo, Minas Gerais, and others, including regions in the Amazon. Beyond Brazil, *P. reticulata* is also present in Bolivia, Suriname, and northern Paraguay (Warwick & Lewis 2003).

The tree's high-quality wood has led to its vulnerability, as it is often subjected to selective logging. This, combined with habitat loss, has put the species at risk of extinction. Despite these challenges, *P. reticulata* demonstrates a strong potential for regeneration in degraded areas and has been utilized in cocoa agroforestry systems in southern Bahia, Brazil. These attributes could support its conservation, although there remains a gap in understanding the evolutionary potential of its populations.

CHAPTER 1

Gene Flow and Genetic Structure Reveal Reduced Diversity between Generations of a Tropical Tree, *Manilkara multifida* Penn., in Atlantic Forest Fragments

genes

MDPI

Article

Gene Flow and Genetic Structure Reveal Reduced Diversity between Generations of a Tropical Tree, *Manilkara multifida* Penn., in Atlantic Forest Fragments

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1. Introduction

Tropical forests retain the greatest biodiversity on Earth, yet they have been drastically deforested and degraded due to anthropogenic activities [1,2]. Currently, one-half of tropical and sub-tropical forests have been altered [3], and about 10% of forest area in all continents consists of fragments smaller than 10,000 ha [4]. The increased demand for timber, energy, food, and other agricultural products for human consumption is leading to intense deforestation, inducing global warming, and consequently contributing to biodiversity loss [3]. The remaining biota stranded in forest patches, especially those strictly associated with high-quality forest environments, is forecast to become locally extinct or suffer drastic population decline, with subsequent loss of diversity [5].

Understanding the effects of anthropogenic disturbances on ecological and evolutionary processes is vital to perceive the long-term viability of current populations, especially threatened and endemic ones. From this perspective, the importance is evident of integrating knowledge of the influence of habitat loss in a genetic approach to broaden the understanding of ecological and evolutionary processes. The population genetics approach allows inferences about connectivity between forest fragments by analyzing genetically distinct populations [6]. Estimations of genetic differences between populations can help to

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Abstract: The Atlantic Forest remnants in southern Bahia, Brazil, contain large tree species that have suffered disturbances in recent decades. Anthropogenic activities have led to a decrease in the population of many tree species and a loss of alleles that can maintain the evolutionary fitness of their populations. This study assessed patterns of genetic diversity, spatial genetic structure, and genetic structure among *Manilkara multifida* Penn. populations, comparing the genetic parameters of adult and juvenile trees. In particular, we collected leaves from adults and juveniles of *M. multifida* in two protected areas, the Veracel Station (EVC) and the Una Biological Reserve (UBR), located in threatened Atlantic Forest fragments. We observed a substantial decay in genetic variability between generations in both areas i.e., adults' H_O values were higher (EVC = 0.720, UBR = 0.736) than juveniles' (EVC = 0.463 and UBR = 0.560). Both juveniles and adults showed genetic structure between the two areas ($\theta = 0.017$ for adults and $\theta = 0.109$ for juveniles). Additionally, forest fragments indicated an unexpectedly short gene flow. Our results, therefore, highlight the pervasive effects of historical deforestation and other human disturbances on the genetic diversity of *M. multifida* populations within a key conservation region of the Atlantic Forest biodiversity hotspot. **Keywords:** biodiversity; molecular ecology; conservation; fragmentation; microsatellites

understand the evolutionary history and adaptive potential of species faced with forest loss and fragmentation [7]. Hence, a direct relationship exists between population dynamics and genetic diversity [8,9].

The decrease in species dispersion rate can affect the mating system and spatial genetic structure of trees. A decrease in species dispersion rate occurs because disconnection and increasing distance between populations increase the probability of mating between closely related individuals, leading to a reduction in genetic diversity due to inbreeding [10]. Additionally, a spatial genetic structure consisting of a nonrandom distribution of genotypes of certain species in a given space can be affected by natural selection, demographic history, and gene flow of species [11].

Since gene flow indicates the movement of genetic material between populations, its interruption increases the genetic divergence between populations, likely leading to their isolated among habitats [9]. Therefore, conservation strategies might be more effective by understanding the connectivity between forest fragments, including investigation of gene flow. Habitat loss can disrupt this gene flow, affecting breeding and dispersal success in plant assemblages in human-modified landscapes [12]. The negative impact on habitat connectivity due to anthropogenic activities can cause isolation of populations, with consequences on intercrossing between individuals and effective gene flow [13]. According to genetic variation dynamics, over space and time [14] a few genotypes of a population can become dominant over others due to natural selection, genetic drift, and inbreeding.

Additionally, inbreeding can generate drastic consequences for populations, such as increased homozygosity and decreased allele frequency, leading to allele fixation and inbreeding depression [15]. Genetic drift is one of the strongest evolutionary factors, since populations are composed of a finite number of individuals, and random allele frequency fluctuations are inevitable [16]. Small population size also increases the likelihood of allelic fixation due to inbreeding. As a result, it can reduce long-term viability.

It is challenging to understand the effect of the environment and human activities on species' past and current genetic diversity [10,17]. Species' evolutionary history is important to understand their capacity to resist stresses. In particular, the ability of species to cope with environmental disturbances is directly linked to their genetic diversity. Species with low genetic diversity likely have less chance to adapt to severely disturbed environments [10,18,19]. To ascertain the fitness of any individual genotype of a population and its evolutionary processes [20], it is necessary to quantify its survival and reproduction parameters. If changes in allele frequency are detected in a certain population due to anthropogenic activities, it is fundamental to understand whether they are caused by mechanisms such as migration, natural selection, and genetic drift [21]. The last two mechanisms can be related to human activities, such as hunting and fragmentation caused by logging or clearance for crops. In this scenario, estimation of genetic diversity parameters is a way to understand the human impact. As such, it is one of the maim challenges, particularly in small populations in tropical forests. To overcome this challenge, some authors [22] have published frameworks to detect genetic change between generations of a population by calculating allele frequency, to obtain detailed knowledge of gene flow within and between populations [23]. As mentioned before, analyses of genotypic frequencies also provide important information to understand the diversity and evolutionary processes between generations of certain species.

Molecular studies are widely used to provide insights on genetic structure, diversity, and relationships among species by investigating species at the population level. The Atlantic Forest in southern Bahia, Brazil, is a hotspot biome, including endemic and endangered species [24]. Due to anthropogenic disturbance, the original portion of the forest has been reduced to less than 10% [25,26]. Despite forming crucial areas of endemism, the Atlantic Forest fragments of southern Bahia have suffered long periods of anthropogenic disturbances of the biota. For example, [27] discussed the impact of human activities on species diversity of *Dalbergia nigra* (Vell.) Allemao ex Benth. Popularly known as Brazilian rosewood, it is an endemic Atlantic Forest species. Since 1998, it has been listed as “Vulnerable” due to overexploitation of trees for its high-quality timber. Some species affected by anthropogenic disturbances are likely to go extinct if they cannot adapt [5]. Despite forming an important area of endemism, the biota of Atlantic Forest remnants in southern Bahia has suffered the incidence of long periods of anthropogenic disturbances, mainly related to deforestation. Consequently, the populations of tree species such as brauna (*Melanoxylon brauna*), Bahia rosewood (*D. nigra*), and brazilwood (*Paubrasilia echinata*) have a declined [11,27,28].

According to previous studies [24,26], botanical surveys suggest that this region has the highest species richness per unit area and highly endemic plants. Endemic plants are generally adapted to a specific geographic area [29,30], which makes them prone to inbreeding due to isolation, so they generally have low genetic diversity [29,31]. Endemic plants have characteristics that make them vulnerable to anthropogenic disturbances, such as limited geographic distribution and small population size, ultimately leading to their local extinction [32,33].

Therefore, these endemic plant species have high global priority for preservation [30,32,33]. To achieve this, it is important to know whether the species has reduced genetic diversity or restricted gene flow due to small and isolated population [34,35]. Protected areas play a vital role in effective species conservation strategies [36], especially in the regions where endemic plant species are present, requiring extra biodiversity protection [37]. In this study, we assessed the genetic diversity of an endemic tropical tree, *M. multifida*, in two protected Atlantic Forest fragments in southern Bahia. Our focal tree species belongs to the genus Manilkara (Sapotaceae family), which is exploited for its hard and heavy wood, edible fruits, and latex. The fruits are consumed by a wide range of forest vertebrates, providing key food resources, especially for primates [38]. *M. multifida* is a large tree that can reach approximately 30 m in height, and only occurs in Bahia within remaining Atlantic Forest areas, classified as one of the most threatened biomes in the world. Due to the severe forest loss and fragmentation, the species is currently classified as “Endangered” by the International Union for Conservation of Nature (IUCN) [39], with remaining populations stranded in fragmented forest landscapes.

In this study, we (i) compared the genetic patterns among juvenile and adult trees within each protected area; (ii) estimated the genetic structure among *M. multifida* populations in the two protected areas; (iii) estimated the gene flow of this tree species within and between protected areas, to evaluate the dispersal distance. In particular, we predicted (i) lower genetic diversity of juveniles than adults, given they represent the generation under the most intense impact of forest loss and anthropogenic disturbances; (ii) a high level of genetic structure between populations due to the intense history of human disturbances in the region combined with the long distance between the two sampled protected areas, causing us to believe that both populations are derived from expansion from an original gene pool with consecutive migration events; (iii) short distance of gene flow of the populations due to isolation of the protected areas. Based on our findings, we finally discuss the effects of human disturbances on the loss of genetic diversity and genetic structure of *M. multifida* populations, comparing genetic parameters of adult and juvenile trees, to account for the period before and after forest loss.

2. Materials and Methods

2.1. Study Areas and Tree Sampling

The Atlantic Forest is the second-largest tropical forest in South America. Its original area was approximately 1,500,000 km^2, ranging along the Brazilian Atlantic coast, with additional patches in Argentina and Paraguay [40]. Currently, only 11.4% to 16% of its original area remains, distributed in fragmented landscapes [40]. We conducted the samplings in two protected areas located in the southern region of Bahia, Brazil—the Veracel Station (EVC) and the Una Biological Reserve (UBR), 145 km apart. The EVC was created in 1998 and retains a total area of 6069 ha, and UBR was established in 1980, encompassing 11,400 ha (Figure 1). Both protected areas are evergreen tropical forests and present similar floristic and vegetation structures, composed mainly of large and mediumsized trees, with a great abundance of lianas and epiphytes [41]. EVC has forest mostly of advanced age, considered a well-preserved Tabuleiro forest, with a uniform canopy typical of tropical lowland rainforests. The average temperature is 22.6 °C and mean annual rainfall is 919 mm [42]. UBR is embedded in human-modified landscapes, consisting of dense Ombrophylous forest. The region's average annual temperature is 24 °C, and its mean yearly rainfall is 1600 mm [43].

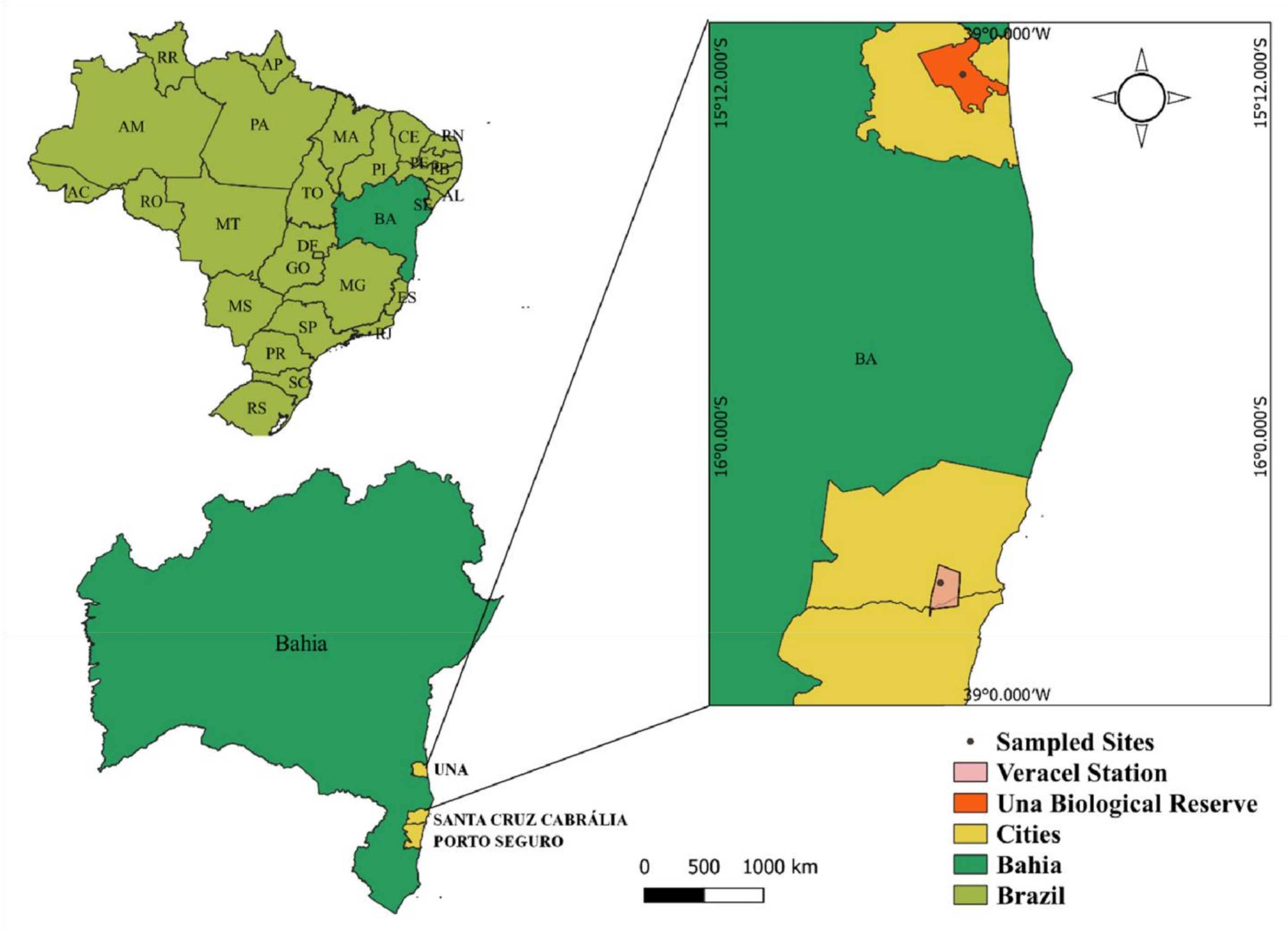

Figure 1. Study area, highlighting the Veracel Station (EVC) and Una Biological Reserve (UBR). Adapted from SOS Atlantic Forest Foundation/INPE. Geographic coordinate system Datum: WGS:84 Data Source: IBGE (2020) and Veracel Celulose (2021).

We performed an active search for juveniles and adults of *M. multifida* in each protected area, keeping a minimum distance of 10 m from any sampled tree, since the genus is considered to be dispersed over short distances [44]. We considered trees to be adults with circumference at breast height (CBH) ≥ 70 cm, and juveniles with CBH ≤ 10 cm. We collected healthy young leaves from each individual and obtained the geographic coordinates using a GPS device (Garmin®, Schaffhausen, Switzerland). All told, we sampled leaves of 50 adults and 50 juveniles in EVC, and 45 adults and 34 juveniles in UBR. Our sampling corresponded to approximately 1/3 of the total area of EVC and 1/8 of the UBR area. The ratio between adults and juveniles in these natural forests is 1:1.

2.2. Genetic Analysis

We used the CTAB protocol to extract DNA from leaf tissues [45], and genotyped it using eight specified microsatellite loci [46]. We performed PCR in a LifePro thermal cycler from Bioer (Hangzhou, China) with a 13 mL mix containing 7.5 ng of genomic DNA, ultrapure water, buffer 1× (Fermentas Life Sciences, Burlington, ON, Canada) (3.25 ng of BSA, 3.25 mM of dNTPs, and 20 mM of MgCl2), forward and reverse primers with M13 tail (3.9 µM) marked with NED TM, 6-FAM TM, PET® or VIC®, and 1U of Taq polymerase. The PCR followed the conditions: 94 ◦C for 3:30 min; 30 cycles of 94 ◦C for 1 min; specific annealing temperature of each primer for 45 s [46]; 72 ◦C for 1 min; a final extension of 72 ◦C for 7 min. We then verified the PCR product with an ABI 3130XL genetic analyzer (Applied Biosystems, Foster City, CA, USA) with GS500LIZ marker. We defined the size of the alleles by GeneMarker® (Applied Biosystems, Foster City, CA, USA).

For each generation from each protected area we first estimated the allelic richness, observed heterozygosity (H_O), and genetic diversity (H_E) under the Hardy–Weinberg equilibrium. The level of inbreeding within a population was assessed by the inbreeding coefficient (f). The mean values of the allelic richness, H_E, H_O, and f were estimated considering a 95% confidence interval (95% CI) for all loci. Additionally, we calculated the genetic structure for adults and juveniles in both conservation units considering the F-statistics. The fixation index f and differentiation index θ were determined by performing 1000 permutations for all loci with Bonferroni sequential correction for multiple comparisons ($\alpha = 0.05$). The results of all parameters were estimated by the FSTAT v.2.9.3.2 software [47].

We performed paternity analysis using the CERVUS 3.0.7 software [48] by combining genotypes from both protected areas, i.e., 95 adults and 83 juveniles, and all genotypes were georeferenced. We also calculated the distance between parents and their alleged progeny. For this, we considered that all sampled adults would be potential parents. For each juvenile, we used the likelihood method to account for the possible parents with 95% confidence. The likelihood ratio was expressed by the LOD score, where a positive score indicates that the candidate tree is likely to be a true parent. Only those adults and juveniles were considered with at least 6 out of 8 loci genotyped in the analysis. Paternity test results were estimated according to 95% strict and 80% relaxed confidence levels. We also performed analysis of spatial genetic structure for each protected area, for both juvenile and adult populations, using the SPAGeDi 1.3 software [49], to estimate the coefficient of relatedness or genetic distance between populations using genotype data from both protected areas, calculated according to Loiselle et al. [50].

3. Results

We observed that when comparing the number of alleles (Na) between generations, adults in both protected areas showed higher values (EVC = 12.9 and UBR = 10.9) than juveniles (EVC = 9.9 and UBR = 9.9). We also found that adults from EVC and UBR had similar values for allelic richness (9.6 and 8.8, respectively), H_E (0.815 and 0.818, respectively), and H_O (0.720 and 0.736, respectively). Although the inbreeding coefficients (f) were also similar (EVC = 0.103 and UBR = 0.097), they were positive and significant for both populations (*p*-value ≤ 0.01). In general, our genetic diversity estimations showed no statistical significance, considering only adults from both protected areas.

We also observed that juveniles of both protected areas presented similar values for allelic richness (EVC = 8.1 and UBR = 7.1), H_E (EVC = 0.731 and UBR = 0.729), and H_O (EVC = 0.463 and UBR = 0.560). However, the inbreeding coefficient was higher for juveniles (f = EVC = 0.366 and UBR = 0.231) than adults (EVC = 0.103 and UBR = 0.097). Moreover, we detected a significant difference (*p*-value ≤ 0.01) of the f-value for juveniles between EVC = 0.366 and UBR = 0.231 (Table 1). When comparing the genetic structure between generations, we found pronounced differentiation between the protected areas. Considering adults, we detected a lesser genetic structure between EVC and UBR (θ = 0.017; *p*-value ≤ 0.05), while juveniles showed accentuated differentiation between both areas (θ = 0.109; *p*-value ≤ 0.001).

Paternity analysis revealed that 21 (49%) juveniles whose parents were detected showed positive LOD scores. We identified seven juveniles (16%) whose parents were detected with 95% confidence level. The remaining trees (84%) had low probability of paternity. The juveniles with the same parent label indicated that selfing has occurred in the sampled areas. We also observed that the maximum distance between individuals was 13.0 and 5.28 km in UBR and EVC, respectively. Additionally, the maximum distance between juveniles and adults of different populations was 127.64 km.

We did not find any spatial genetic structure in any of the trees from both protected areas (see Figure S1 in Supplementary Material).

Table 1. Genetic indices estimated for adult and juveniles of *M. multifida* trees recorded in the Una Biological Reserve (UBR) and Veracel Station (EVC). Allele number (Na), allelic richness (Ar), genetic diversity (H_E), observed heterozygosity (H_O), inbreeding coefficient (f), and differentiation index (θ).

	Site	Samples	Na	Ar	H_E	H_O	f	Θ
Adults	EVC	50	12.9	9.6	0.815	0.720	0.103 **	0.017 *
	UBR	45	10.9	8.8	0.818	0.736	0.097 **	
Juveniles	EVC	50	9.9	8.1	0.731	0.463	0.366 **	
	UBR	33	8.1	7.1	0.729	0.560	0.231 **	
								0.109 ***

* p-value ≤ 0.05, ** p-value ≤ 0.01, and *** p-value ≤ 0.001.

4. Discussion

We detected novel results on the patterns of genetic diversity of an endemic and threatened tropical tree species, *M. multifida*, in two protected areas of the Brazilian Atlantic Forest hotspot. Additionally, we contrasted the genetic diversity among adults and juveniles, revealing a strong decay in genetic variability between generations in both protected areas. This has occurred due to habitat loss and fragmentation of the forest remnants in the region, which has led to reduction in their populations, with negative effects on heterozygosity. We also found that in studied fragments of Atlantic Forest, juveniles showed greater genetic structure than adults, likely reflecting the responses of young life stages to forest fragmentation and isolation of the populations. Finally, paternity analysis revealed that most identified parents of juveniles are in the same protected area, indicating that gene flow occurs mainly over a small distance. Our findings, therefore, highlight the pervasive effects of historical deforestation and fragmentation on the genetic diversity of *M. multifida* populations, a native Atlantic Forest species present in the areas for a long period and hypothetically contemplating two periods (before and after fragmentation).

Analyses of genetic diversity revealed low observed heterozygosity among juveniles of *M. multifida* inhabiting each surveyed area (Table 1). As for allelic richness, only small differences between generations were observed, which indicates the adaptative potential of the species [51]. Indeed, reduction of genetic parameters evaluated in juvenile in relation to adult trees within each protected area demonstrated that the progenies have inherited genes from a reduced set of remaining trees. In particular, low genetic diversity has also been observed in another Neotropical tree, *Podocarpus sellowii* (Klotz.), due to the small size of the remnant population [52]. Before the intense deforestation and fragmentation, which mainly occurred in the last century in Bahia [53], it is likely that alleles were frequently exchanged across the state's southern region, because the Atlantic Forest was originally continuous. This potentially explains high genetic diversity among the adult population of *M. multifida.* Indeed, adult trees often show responses of past landscape conditions on their genetic diversity [54–56].

A myriad of anthropogenic disturbances including deforestation, fragmentation, and overexploitation have likely affected the remaining populations of *M. multifida* in the studied region, since this species has been massively logged due to its high timber value [46]. The synergistic effect of these anthropogenic activities potentially led to reduction of the abundance of adult trees in forest fragments of southern Bahia, ultimately affecting genetic diversity in recent populations (i.e., juveniles). According to a recent study [57], the negative impacts of habitat loss on genetic diversity are observed in the various taxonomic group. In particular, low genetic diversity in juveniles observed in our study cannot be attributed to allelic richness, which is good news for the conservation of *M. multifida* future generations [58]. Apart from habitat loss and fragmentation, the reduction of genetic diversity depends on factors including the absence or inefficacy of dispersers or pollinators and the reduced effective population size [30,59].

We found inbreeding of both adults and juveniles inhabiting both protected areas. However, juveniles belonged to the smallest gene pool that remained in the populations after human disturbances. Additionally, despite the fact that no spatial genetic structure was found in the sampled populations, high inbreeding values might be related to historical aggregated crossing between individuals due to small-scale dispersion, a phenomenon also observed in the Amazon Forest for *Manilkara huberi* (Ducke) [44]. We observed inbreeding even in adults, demonstrating a process of natural inbreeding in the past, perhaps due to selective logging [44,60]. Other studies have confirmed similar processes, such as in juveniles of *Prunus africana* (Hook.f.) Kalkman in Kenya’s Kakamega Forest [60], and in adults of *Swietenia macrophylla* King in the Brazilian Amazon [61]. Inbreeding, also, can occur due to natural and biological factors related to pollination. However, there are still no reports on the pollen or seed dispersal syndrome of *M. multifida*. Studies in this respect exist in the Amazon Forest, revealing the interaction of *M. huberi* with bees of the genera *Melipona*, *Trigona*, *Plebeia*, *Tetrapedia*, and *Augochloropsis* [62]. Thus, we believe that the limitation of dispersal and/or pollination is a key factor related to our results. These bee genera are also found in the Atlantic Forest in southern Bahia [63], suggesting the possible interaction of *M. multifida* with these pollinators. As for the potential dispersers [38], reported that other species of the genus *Manilkara* interact with the golden-headed lion tamarin (*Leontopithecus chrysomelas*) in our region, given that species from this genus provide resources such as fruit and nectar [38].

Regarding population genetic structure, our results revealed that the differentiation of adult populations between the two protected areas is

CHAPTER 2:

Insights into the Unveiling the Reproductive Ecology in *Lecythis pisonis* Cambess within Cocoa "cabruca" Agroforests in Brazil

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ABSTRACT:

Agroforestry systems are essential in sustainable land use in the face of the growing global food demand and climate change. The southern region of Bahia, Brazil is one of the places in the world where the tree species is particularly in abundance, primarily in cocoa agroforestry systems, contributing to biodiversity conservation. Knowing their reproductive patterns is important for trees to survive and be sustainable. This study dealt with *Lecythis pisonis* (Sapucaia) trees by applying microsatellite markers for mixed mating mode and paternity analysis for pollen dispersal. In particular, it was found that the *Lecythis pisonis* offspring is produced through outcrossing as the case may be while random crossings and no nearby trees fertilization are the remaining factors which play a crucial role in myriad genetic diversity inversions. This phenomenon was indicated by paternity in nine offspring, with full siblings being from the same parents. The average distance of pollen flow was 6 km which is as the pollinator, the bee *Xylocopa frontalis*, has a flight range aligning with distance. These data show the influence of habitat fragmentation, the function of Cabruca, and the conservation strategy.

Keywords: Reproduction system; Tree Conservation; Tropical Forest; Agroforestry, mixed mating system, Conservation.

INTRODUCTION:

Human intervention caused more than three-quarter of Earth's degradation and it effects 3.2 billion people and costs 10% of annual gross product in ecosystem services and loss of biodiversity, according to, Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem, Services (IPBES) (Scholes et al. 2018). United Nations convention on biological diversity is working on agenda post-2020 global biodiversity framework to tackle these challenges by sustainable land use (CBD 2020,Marques et al. 2022).

Agroforestry system has been recognized as the sustainable way for land use. With the world facing global food security and climate change challenges, the scientific development on agroforestry could put end to these challenges (Kumar 2016). In the tropics, agroforestry system not only provide economic benefits but also benefits, environmentally and socially (Mahmud et al. 2021) as in human dominated landscapes, protected areas are not sufficient for biodiversity conservation. The agroforestry system is the strategy of land uses that combines production and biodiversity conservation (Nogué et al. 2018,Santos et al. 2019).

Agroforestry system in Brazil, accounts for 13.9 million hectares (IBGE 2017). Considering that Brazilian Atlantic Forest is a biodiversity hotspot, but has less than 11% original its forest cover, this biome is the main focus of many projects related to biodiversity conservation (Ribeiro et al. 2009, Santos et al. 2019). Agroforestry can be a promising choice to conserve biodiversity, especially for native tree species. Agroforests have a structure similar to that of primary forests, having big shady trees, but contain native species; they, therefore, conserve soil fertility and maintain diversity and many ecological processes (Cabral et al. 2020). Southern Bahia Atlantic Forest has significant percentage of native tree species and high percentage of these species have economic potential (de Oliveira and Carvalhaes 2016). In Bahia, most common agroforestry system is “cabruca” (Cacao grown under the shade of thinned Atlantic Forest). The shaded tree species in ”cabrucas” can reduce the climate change prediction of 2050s, highlighting the importance of conservation of forest in Bahia (Heming et al. 2022).

Agroforestry system as an intra-specific variation in tree species also has a value from genetic perspective. Knowledge about population genetics of trees grown in this system helps in conservation strategies (Kyndt et al. 2009). Agroforest helps in conserving local diversity by providing corridors for gene flow between forest fragments. Gene flow is the important factor that can maintain genetic diversity and major pathway of gene flow is through pollen and seed dispersal (Leal et al. 2014).

Pollination and seed dispersal studies provide valuable information about gene flow of the species. It can discuss possible limitation, distance and vectors (Danieli-Silva & Varassin 2013). Study on reproductive ecology also estimates the other aspects of species such as outcrossing, selfing, inbreeding etc. (Rogalski et al. 2017). All these topics are connected and for conservation proposal it is best that it should be supported by reproductive ecology of the species (Montagna et al. 2018). Atlantic forest produces numerous edible fruit trees and *Lecythis pisonis* Cambess, commonly known as Sapucaia is one of them, that has nutritional and economical value (Monteiro et al. 2020, Oliveira et al. 2012). It is commonly grown in forest fragments and agroforests in Bahia and Espírito Santo (Smith et al. 2016).

Tropical forest ecosystem has high species richness and most tropical trees are animal pollinated particularly bees (Khanduri 2023). Sapucaia are pollinated by bee *Xylocopa frontalis* (Monteiro et al. 2020) and its seeds are mainly dispersed by the bat *Phyllostomus hastatus*, which eats the aril and discard the seeds (Prance & Mori 1990). There is great interest in getting knowledge about the mating system of Sapucacia to understand its resilience for conservation purpose. Mating system studies, for example, can estimate the extent to which species cross to form progeny. It can estimate the extent of outcrossing, number of pollen donors and rate of inbreeding. Mating system is the one of the factors that affect genetic diversity and structure of species (Díaz-Hernández et al. 2024). So, present study was conducted to understand the reproduction pattern of the tropical tree *Lecythis pisonis* Cambess growing in agroforest system to predict survival ability and to propose viable conservation efforts for the species.

MATERIAL & METHODOLOGY:

Study area and sampling design

The research was carried out on farms located in the rural areas of the municipalities of Ilhéus and Uruçuca, in the state of Bahia, Brazil (Figure 1). The survey of the collection area was carried out by mapping the existing" cabrucas" on the property beforehand to include them in the study. This mapping was conducted with the help of property employees, with a monetary reward for each tree found (Figure 1).

Fruits from each adult tree (matrices) were collected from 12 Sapucaia in" cabrucas", along a continuous forest gradient (amount of forest present within a radius of 1 km) around each tree. From the fruits, some seeds were placed to germinate in the RPPN (Reserva Particular do Patrimônio Natural) Nova Angélica, which is located around the Una Biological Reserve. After 60 days, leaf material from 24 seedlings per family was collected in each one of the 12 families.

The 12 families sampled were identified as: F6, F60, F66, F68, F76, F87, F92, F93, F354, F361, F376, F377. The 287 descendants collected, in turn, were named with P (offspring or progeny), followed by a number, for example: P1, P2, P3... P287. In order to know the reproductive system of the Sapucaia, we used paternity analysis nuclear microsatellite markers, developed which are specific to Sapucaia (Rodrigues et al. 2015), to determine the gene flow and distance pollen travelled. For paternity analysis material was collected randomly from 40 single adult individuals from cabrucas farms from Ilhéus and Uruçuca - Bahia-Brazil. (Rodrigues et al. 2015). The collection of these 40 individuals was a part of initial mapping of cabrucas.

DNA extraction and quantification

The leaf samples from the seedlings were placed in plastic bags and sent to the Molecular Markers laboratory, located at the Center of Biotechnology and Genetics (CBG) at UESC (State University of Santa Cruz). The genomic DNA was extracted using the CTAB 2% protocol (Doyle et al. 1987). After extraction, quantification of the extracted DNA was carried out through comparative analysis with a molecular standard of known concentration (λ phage DNA) in 1.0% agarose gel, stained with Gel GreenTM (Nucleic Acid Gel Stain) diluted to 0.2% and verified in a transilluminator with a blue LED lamp (470 nm). Then, this DNA was diluted in milli-Q water to the concentration of use (2.5ng/µL).

Amplification of SSR loci and genotyping

To genotyping sampled individuals, the DNA sequence of interest was amplified via PCR, using previously developed microsatellite primers (Rodrigues et al. 2015). The amplification reaction was carried out in a solution containing: 7.5 ng of genomic DNA; 1.3µl of 10X buffer, 3.25 mM of each dNTP, 3.25 mg of bovine serum albumin (BSA), 20mM of $MgCl_2$, 1U of Taq DNA polymerase Phoneutria, Belo Horizonte, Minas Gerais,

Brazil), 3.0 μM of reverse and forward primer labeled with an M13 fluorescence tail (CACGACGTTGTAAAACGA), 0.03 μM of M13 oligonucleotide, complementary to the tail of the forward primer, labeled with fluorochromes (6-FAM, VIC, or NED, Applied Biosystems, Foster City, CA, USA) and Mili-Q water, to a final volume of 13.0 μL.

The thermocycling conditions for each polymerase chain reaction consisted of an initial denaturation step at 94 °C for 1 minute; followed by 30 cycles consisting of a denaturation step at 94 ºC for 1 minute, an annealing step at a specific temperature for each primer pair (Rodrigues et al. 2015) for 45 seconds, and an extension step at 72 ºC for 1 minute, followed by 8 cycles of M13 fluorescence annealing corresponding to 94 °C for 1 minute, 53 °C for 1 minute and 72 °C for 1 minute, plus a final extension step at 72 °C for 10 minutes.

The amplification reactions were subjected to capillary electrophoresis in the ABI 3500 automatic DNA analyzer (Applied Biosystems) in a multiload system. The Gene Mapper program version 4.1 (Applied Biosystems) was used for alleles genotyping

Analysis of the reproductive system

The parameters that were estimated we used the mixed mating model proposed by Ritland and Jain (1981), with the help of the MLTR 3.2 program (Ritland 2002). This model is based on a few assumptions: (1) each mating event is due to random mating or self-fertilization; (2) the probability of crossing is independent of the maternal genotype; (3) the pollen pool is homogeneous across all maternal trees; (4) there is no selection between fertilization and analysis time for the progeny genotype; (5) alleles at different loci segregate independently (Ritland & Jain 1981).

The estimated parameters were: matrix fixation index (maternal trees) (F_m), multilocus outcrossing rate (T_m), unilocus outcrossing rate (T_s), crossing rate between related individuals (T_m - T_s), paternity correlation (R_{pm}), and the correlation of crossing rate between loci (R_t).

A 95% confidence interval was calculated using 10,000 resamples using the bootstrap method between families. For these calculations, the algorithm used was expectation maximization (EM), which has a better convergence to meet the maximum of the parameter and the standard deviation is reduced in relation to the algorithm Newton-Raphson (NR) (Ritland 1990).

Paternity analysis

To learn about the individuals who donated the pollen grains to the germinated seedlings, paternity analysis was carried out and, for this purpose, the 12 sampled matrices were used to investigate the reproduction system, together with 31 of the 40 adult individuals used to characterize the loci developed SSR (Rodrigues et al. 2015). Paternity analysis was carried out with the help of the CERVUS 3.0.7 program (Kalinowski et al. 2007).

Firstly, the allele frequency analysis was estimated before the kinship analysis simulation. Cervus uses kinship analysis simulation to assess confidence in attributing paternity to the most likely candidate father. At this stage it is chosen algorithm (LOD or Delta), which serves as a basis for assert the paternity. The algorithm chosen was LOD.

In kinship analysis, the LOD score is obtained by taking the logarithm natural (log in base 10) of the global likelihood ratio. For the positive score of LOD was assigned kinship with 95% confidence, which means that the candidate father is the alleged father. A LOD score of zero means that the candidate parent has the same probability of being the father as of not being. A score negative LOD indicates that a candidate parent does not match the offspring in one or more locos, and that therefore, the candidate father is probably not the real father (Kalinowski et al. 2007).

Pollen flow distance analysis

We estimated the dispersal distance of pollen using the geographic coordinates of each set of individuals assigned as progenitor – progeny based on paternity test. The coordinates were obtained in field work using the GPS program TracKMaKer 13.9 (Júnior 1998).

RESULTS

Reproductive system

The multilocus and single-locus outcrossing rates indicate the proportion of mating occurring between individuals on the populations. The multilocus outcrossing rate (T_m) is the rate of outcrossing estimated using multiple genetic loci which was equal to 1.000. On the other hand, the single locus outcrossing rate (T_s) based on the average of estimations at single genetic locus was 0.960. We presented the detailed reproductive system estimates on Table 1. Our result reveals that Sapucaia reproduces only through crossbreeding which makes it an obligatory allogamous species. However, the positive difference ($T_m - T_s$) suggests some biparental inbreeding, indicating that the outcrossing is occurring between related individuals. Corroborating it we found the rate of fixation index ($F_m = 0.431$) which led us to point out some adult mating individuals are probably related. The multilocus paternity correlation (R_{pm}), which concerns the probability of two individuals chosen at random within progenies having the same pollen donor, indicated that part of the descendants shares the same father. The correlation of the crossing rate between loci (R_t), which measures the proportion of inbreeding generated by crossing between related individuals and by self-fertilization, was null (Table 1).

Paternity analysis

Among the 287 descendants, 9 (3%) had their paternity alleged. Among the 40 total adults sampled, 29% (9 trees) were pollen donors of the descendants. Trees F60 and F354 were pollen donors for 4 of the 9 seedlings that had parents alleged, each of these individuals with 2 offspring Table 2.

The offspring P11 and P20 have a family structure of full siblings, since they had the same mother plant (F6) and the same father plant (F60). Individuals P28 and P31 had a half-sibling family structure, as they only had the same mother plant (F60) with different parents. The half-sibling family structure was also observed in descendants P101 and P104, who shared the same tree maternal (F76), as well as in the P144 descendant that shared the same paternal tree (F354) with P104 (Table 2).

Pollen Flow Distance

The pollen grain from the paternal tree F354 traveled the shortest distance to carry out pollination. The greatest distance traveled by pollen found in this research was 15.26 km and produce offspring P264 (Table 2).

The average distance traveled by pollen of *L. pisonis* was approximately 5.792 km. Through Figure 2, it is possible to observe the geographic location of the maternal and paternal trees, and thus, verify the spatial distance between such trees and consequently note the distances that the pollen grains traveled in the sampled area.

DISCUSSION

Our results provide insights into the reproductive behavior of Sapucaia, emphasizing its dependence on outcrossing and the absence of inbreeding within the sampled families. Mixed-model mating system is used to determine Sapucaia exclusively relies on crossbreeding, suggesting that pollinators play an important role in reproduction of this species. Paternity analysis identifies the descendants of the trees and estimates the gene flow occurred and distance pollen grain travelled was approximately 6km, indicating the pollinator travelled over the long distances. These findings highlight the diversity in family structures among the studied individuals, with 2 in a full-sibling relationship and 4 in various half-sibling relationships. We also found that pollen travelled to great distance indicating active pollinators in the area. The study highlights some important reproductive aspects of *L. pisonis* that are necessary for its conservation in agroforestry system. Also, these findings suggest the need to maintain and enhance habitat connectivity to ensure diverse mating opportunities and facilitate gene flow to preserve genetic health of *L. pisonis.*

Reproduction system, paternity and pollen flow distance

The observed fixation index (F_m) within the matrix revealed significant kinship among maternal trees, implying a shared ancestry it is likely inbreeding occurred and pollens are originated from same source. This historical pattern of pollen dissemination elucidates the relatedness observed in the matrices. The implementation of “cabruca” involves thinning the Atlantic Forest, resulting in the removal of numerous trees and subsequently diminishing the available pollination options (Breed et al. 2013). Understanding this context provides insight into the observed genetic relationships among the trees. Similar results were obtained in *Cariniana legalis* Mart Kuntze where inbreeding occurs due to size of fragments (Tambarussi et al. 2016b).

The limit multilocus outcrossing rate and the extremely high single locus outcrossing rate found in our results lead us to believe that the mother trees in the sampled area necessarily reproduce through crossings (Zhang et al. 2019). It is, therefore, an allogamous species as most of the tropical trees (Bawa 1990, Christopher et al. 2010), as also observed in another tropical tree *Centrolobium tomentosum* Guill. ex Benth. (Fabaceae) (Sujii et al. 2021) and *Euterpe precatoria* (Perrut-Lima et al. 2023). This result confirms the assumption made by (Mori & Prance 1981), about Sapucaia in a study that lasted 6 years. In the study, the researchers report that Sapucaia has characteristics of an allogamous species. This was the only scientific research published in the past that investigated aspects related to the Sapucaia reproduction system. Therefore, the approach of this investigation is of great relevance for the knowledge about the conservation of this tree species.

Mating among related trees was also detected (Table 1), however we highlight that in this type of reproduction system, which is outcrossing, the presence of the pollinator has a fundamental influence on reproductive success. Several studies have shown that *L. pisonis* is a species dependent on the bee *Xylocopa frontalis* for fruit production (Mori et al. 1980a; Mori et al. 1980b; Mori & Prance 1981;Mori & Prance 1983; Mori 1987).

The evolutionary process of the species seems to increase and maintain genetic variability between individuals, promoting the development of an increasingly complex stamen. These characteristics lead to a reduction in the number of different species of pollinators that can penetrate the flower (Mori & Prance 1981). In general, the more specialized the stamen, the more specialized the pollinator that visits the flower (Waser & Thornhill 1993). This evolutionary process of trees favors specific pollens to fertilize them (Ismail & Kokko 2020). From this perspective, observations carried out on Sapucaia crown in southern Bahia, Brazil, indicated that *Xylocopa frontalis* is the only bee that enters its flower (Mori et al. 1980a). Since geographically closer individuals generally have a greater chance of interbreeding and consequently being related, the result obtained in the parameter "crossing rate between related individuals" (T_m-T_s) demonstrated that individuals, in general, randomly. This is positive for maintaining the genetic variability of individuals, families, and populations of the species under study, which is fundamental for the conservation of the species over generations.

Nevertheless, the value of 0.040 of difference between multilocus and unilocus outcrossing rates (T_m-T_s) is low indicating the minimal mating between related individuals. These results suggests that population engages in random mating. A possible explanation why no crossing occurs between related individuals is the fact that the pollinating bee has a wide flight radius, a common pollinator in this type of species (Tambarussi et al. 2016a;Sujii et al. 2021). It reaches a daily average of 6.7 to 12 kilometers (Winston 1990;Filho & Freitas 2003), allowing it to visit flowers of individuals distant from each other. This is beneficial for promoting genetic variability. (Khanduri 2023) discussed that *Xylocopa* bee makes frequent visits to several flowers with high foraging speed leading to pollination success.

The result of the correlation of the outcrossing rate between loci (R_t) indicates that there are no crossings between relatives nor self-fertilization in the sampled families. This result corroborates the result obtained in the parameter T_m-T_s, since it demonstrates the non-existence of crossings between relatives. Furthermore, as Sapucaia reproduces only through outcrossing, it was expected that there would be no self-fertilization. The non-occurrence of self-pollination contributes to the existence of greater genetic variability in the families studied. Beyond the anatomic and other biological aspects, the inexistence of self-pollination can also be favored by population density or size. The ecological aspects impacting on reproduction biology was observed previously on *C. legalis*, which is other tropical Atlantic forest endemic tree whose outcrossing rate can be affected by population size (Tambarussi et al. 2016b).

Self-compatibility is a self-recognition mechanism results in the rejection of pollen by the female somatic tissues of the flower itself (Haring et al. 1990). In fact, self-incompatibility is one of the mechanisms that promote allogamy and according to (Bawa 1979), it is frequently found in tropical species. Around 50% angiosperms develop self-incompatibility to favor outcrossing (Fujii et al. 2016). In fruit tree *Bertholletia excelsa* (Bonpl.) similar phenomena is observed, where tree is mostly reproduced by outcrossing due self-incompatible nature inbreeding doesn’t occur, even if the population size is small (Wadt et al., 2015). As it was not the focus of this research, it is not possible to state that *L. pisonis* is self-incompatible, however our results gave genetic evidences that it is a possibility. There are other species of same family (Lecythidaceae), such as *Bertholletia excelsa* (O'Malley et al. 1988), *Eschweilera ovata* (Gusson et al. 2006), *Cariniana legalis* (Tambarussi 2014), *Cariniana estrellensis* (Guidugli et al. 2016), and *Schima wallichii* (Khanduri 2023) whose have high rate of outcrossing and mixed mating system.

The multilocus paternity correlation was high and different from zero (R_{pm}=0.306). This result indicated that part of the descendants shares the same father, and a possible explanation for this is the fact that some paternal trees are relatively close to the maternal trees. This justification is completely plausible when looking at the results, occurring, for example, between individuals P11 and P20 who share the father F60, and individuals P104 and P144 in which for both the father is the tree F354. Thus, although the pollinator can fly great distances, it is plausible to imagine that it can prefer to visit the flower of the nearest tree. With this behavior, the *Xylocopa frontalis* bee obtains the resource (nectar and pollen) and also saves energy. The pollinator behavior is one of the key factors effecting crossing rate as it occurs in *Anadenanthera* species (Feres et al. 2021). In *B. excelsa* studies, due to limited seed dispersal mating between related indiviuals occurs, and observed level of coancestry among parents(Baldoni et al., 2017).

On the other hand, it is important to highlight that a small number of progenies were observed sharing the same father, since for the majority it was not possible to determine the paternal tree. In turn, the non-sharing of the same father by most of the descendants is another favorable factor for the genetic variability of the species, since it can promote an increase in the number of loci in heterosis, which is interesting for both conservation and management, as well as improvement (Lobo 2002; Yu et al. 2021).

Reproduction between genetically similar individuals increases the probability of different loci reaching the homozygous state (Lobo 2002). Furthermore, the smaller number of genotypes in highly self-pollinated populations can reduce the efficiency and genetic diversity and it can restrict the ability of their populations to respond adaptively to an environmental change (Holsinger 2000;Sabadin et al. 2021). Only 3% of descendants it was attributed to paternity. This result highlights two possibilities, (i) due to the distance of the area covered, the sampling design did not include all possible individuals. And (ii) pollen immigration from the Atlantic Forest remnants occurred around the collected families.

IMPLICATIONS FOR CONSERVATION

This work showed how it is important to understand the reproductive pattern of the tree growing in agroforestry system. The land use for economical productive purposes and their biological interactions influences both the species conservation and the quality of landscape. According to (Perfecto & Vandermeer 2008), the trend over time indicates that an increasing number of forests will be converted for agricultural use, preferentially as agroforestry. From this perspective, a long-term plan for biodiversity conservation must recognize this fact, not only focusing on preserving the patches of native vegetation that remain, but also on managing a landscape that presents a possible migration system (Perfecto & Vandermeer 2008). To promote positive impact of agroforestry system, it is important that farmers actively participate in management practices to conserve species diversity (Esquivel et al. 2023). Therefore, there is an urgent need to promote ecologically sustainable agriculture (Harvey et al. 2008;Marques et al. 2022).

Considering the importance and scope of cocoa farming in southern Bahia, many studies have been carried out seeking to better understand the interaction between “cabruca” and fragments of the Atlantic Forest, as well as the fauna and flora that constitute them (Faria et al. 2006;Faria et al. 2007;Dawson et al. 2013; Sambuichi 2002; Sambuichi 2006; Uezu et al. 2008;Sambuichi et al. 2009;Tabarelli et al. 2010; Sambuichi et al. 2012;Heming et al. 2022). In this sense, knowledge about the reproductive system of a species that occurs widely in “cabruca” is, above all, extremely applicable in understanding the genetic diversity present in this agroforestry system. This information allows strategies to conserve and maintain the variability of this species in the region. Consequently, the species grown in “cabruca” can become viable models for conservation.

AUTHOR CONTRIBUTIONS STATEMENT:

The study conception and design were performed by Acácia Brasil Rodrigues; Ciro Tavares Florence; Eduardo Mariano Neto; Fernanda Amato Gaiotto. Data collection was performed by Acácia Brasil Rodrigues; Ciro Tavares Florence. Material preparation and analysis were performed by Acácia Brasil Rodrigues. Data Analysis and Writing—original draft was made by Zubaria Waqar. Review and editing were performed by F.A.G. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT:

Data is available on request to the corresponding author.

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CONFLICTS OF INTEREST:

The authors declare no conflict of interest.

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TABLES AND FIGURES:

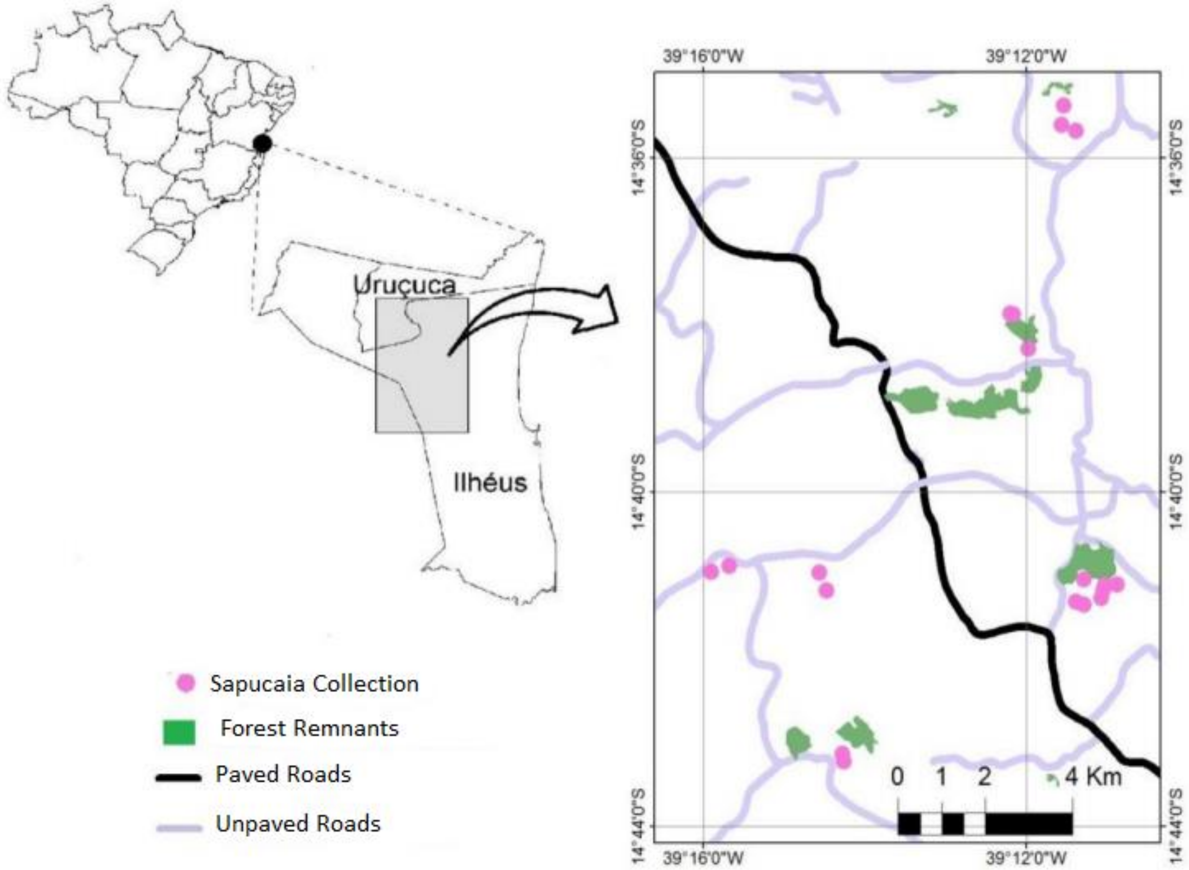

Figure 1: Map of the studied landscape with the location of sampled Sapucaia´s matrices in the Southern Bahia, Brazil.

Table 1. Estimates of the reproduction system of *Lecythis pisonis* from 10 SSR loci.

Parameters	Estimated	Standard Deviation
F_m	0.431	0.103
T_m	1.000	0.000

T_s	0.960	0.011
T_m - T_s	0.040	0.010
Rt	0.000	0.003
Rpm	0.306	0.064

F_m = matrix fixation index, T_m = multilocus outcrossing rate, T_s = unilocus outcrossing rate, T_m-T_s = mating rate between related individuals, R_{pm} = multilocus paternity correlation, R_t =correlation of crossover rate between loci 95% confidence interval using 10,000 bootstrap resamples between families

Table 2. Seedlings that had paternity assigned, with the geographic coordinates of the maternal and paternal trees and the estimated LOD values for paternity at a 95% confidence level.

Progeny	Mother Plant	Father Plant	LOD	Distance of Pollen flow (km)
P11	F6	F60	1.86E+14	1.16
P20	F6	F60	4.94E+14	1.16
P28	F60	F69	7.41E+13	0.25
P31	F60	F66	2.18E+14	0.29
P101	F76	F376	1.06E+14	10.81
P104	F76	F354	1.08E+14	7.83
P144	F87	F354	2.46E+14	0.25
P147	F92	F48	1.01E+14	15.12

P264	F376	F361	5.22E+14	15.26

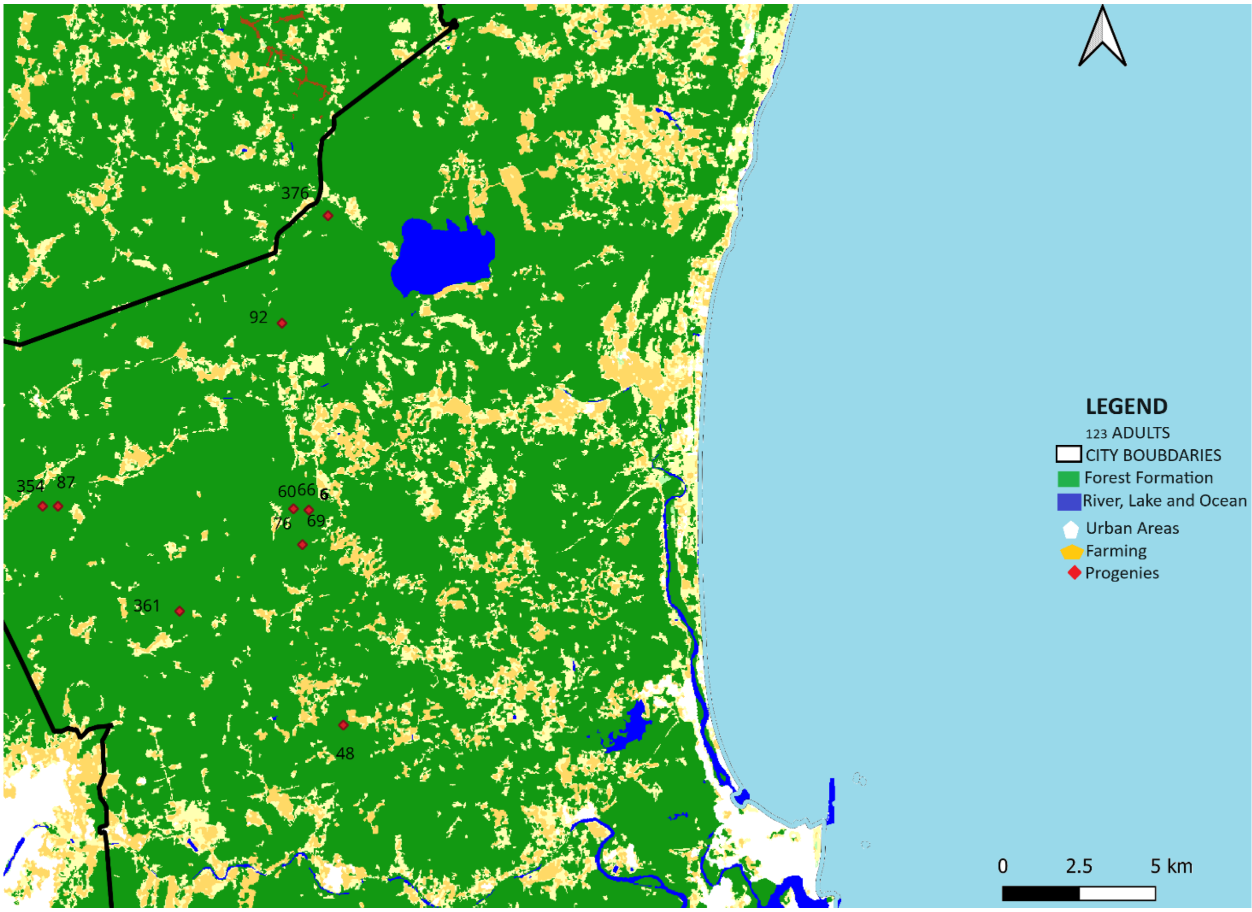

Figure 2. Land cover in studied area. Red pointer represents location of the 9 descendants of maternal and parental trees that were sampled in the rural areas, in South Bahia, Brazil.

CHAPTER 3

Genetic Diversity and Differentiation in *Plathymenia reticulata* Benth: A Comparative Study of Forest and Cocoa Agroforest Systems in the Atlantic Forest domain

ABSTRACT:

Tropical forests, housing about two-thirds of Earth's terrestrial biodiversity, face significant deforestation and fragmentation, leading to the loss of over half their area. Conservation and restoration efforts are crucial, especially for regions like the Brazilian Atlantic Forest, which suffers from reduced biomass and species diversity. Brazil’s cabrucas system in Southern Bahia, are helping to mitigate these effects by combining cacao cultivation with forest management, enhancing both socio-economic and environmental benefits. In a study focusing on *Plathymenia reticulata*, a tree species native to the Atlantic Forest, we evaluated the effectiveness of cabruca systems versus natural forests for maintaining genetic diversity and landscape connectivity. Results showed that cabruca systems, while managed, exhibit higher genetic diversity compared to natural forests, with better allele numbers and Shannon Index values. However, forest populations remain critical for their genetic reservoirs and adaptability. Cabrucas also showed higher inbreeding levels, emphasizing the need for effective management to reduce inbreeding risks.

INTRODUCTION:

Tropical forests harbor approximately two-thirds of Earth’s terrestrial biodiversity, but these ecosystems are under threat from extensive deforestation due to human activities (Shimamoto et al., 2018). While global forest conservation and restoration programs are working to change land-use practices in tropical regions, deforestation and forest fragmentation remain significant drivers of biodiversity loss and the decline of ecosystem services (Faria, Morante-Filho et al., 2023). The pervasive destruction has already led to the loss of over half of the world’s tropical forests, posing a significant conservation challenge for this century (Aronson, Goodwin et al., 2020). For instance, the Brazilian Atlantic Forest, known for its rich biodiversity, has only about ~11.7% of its original forest cover remaining, suffering significant reductions in biomass and species diversity (Ribeiro, Metzger et al., 2009).

Brazil, a country with a high level of tropical forest cover loss, is striving to counteract the destruction caused by deforestation (Ditt et al., 2010). Studies in Brazil have highlighted the socio-economic benefits of cabrucas, particularly in southern Bahia, which hosts one of the largest forested areas in the form of an agroforest-native forest mosaic (Brancalion, de Siqueira et al., 2022). Over 26% of this forested area is covered by cabrucas, spanning 600,000 hectares (Sagastuy & Krause, 2019).

Cabrucas, where cacao is cultivated under the thinned canopy of Atlantic Forest trees, can help mitigate the projected effects of climate change for the 2050s, emphasizing the critical role of forest conservation in Bahia (Heming et al., 2022). Cacao cultivation, which began in the 18th century in southern Bahia, continues to hold significant economic value for the region (Rolim & Chiarello, 2004). Moreover, this region is highly suitable for restoration projects.

Agroforest management is an effective strategy for establishing multifunctional landscapes that can simultaneously boost the economy and provide environmental benefits, including biodiversity conservation (Jose, 2009; Mahmud, Raj et al., 2021). Cabruca contribute to conservation by providing habitat and biological corridors that enhance the connectivity of plant and animal populations between habitat remnants (Casanova-Lugo et al., 2016). It helps in producing various products, and its products are called “bio-based”, that are produced naturally and renewable (Chojnacka, 2015). These bio-based helps in boosting bioeconomy by production of renewable bioresources and their conversion into bio-based products, food, and energy involve activities such as clean energy, forestry, agriculture, fishery, pulp and paper, biotechnology, and biochemistry (Bioindustries, 2011). Native forest trees are widely used for their timber, that ultimately cause illegal logging. The potential revival of silviculture practices will enhance the availability of materials, but a worldwide increase in forestry activities is essential to ensure a steady supply of resources, which would, in turn, help lower costs (De Araujo et al., 2022).

Genetic tools play a crucial role in successful restoration projects by offering valuable insights into source populations, adaptive variations, gene flow, and other important parameters (Mijangos et al., 2015). It is a fundamental element of biodiversity, is critical for maintaining the evolutionary potential of species (Mijangos et al., 2015) (Almeida-Rocha et al., 2020). Anthropogenic disturbances negatively impact genetic diversity, which is essential for population persistence and fitness (Reynolds et al., 2012). It can also provide past and present information on species, such as their expansion or contraction, connectivity, and the identification of source or mother trees (Diekmann et al., 2010). Genetic diversity, connectivity, and population size are interrelated and serve as key indicators of ecosystem health (Thomas, Jalonen et al., 2014).

Forest restoration and agroforestry systems can help enhance connectivity among remnant forest fragments, mitigating the genetic consequences. A reduction in landscape connectivity between habitat patches can lead to increased inbreeding depression (Baguette et al., 2013; Herrera et al., 2017). It is also crucial to select suitable species and identify populations that have suffered from low genetic diversity and inbreeding (Maloney et al., 2011; Weeks et al., 2011). Therefore, genetic parameters are important indicators for landscape connectivity, influencing gene flow, population size, and genetic diversity (Proft, Jones et al., 2018). Although gene flow can occur in many species, the effects of isolation and gene flow in most tropical trees remain poorly understood (Dick, Hardy et al., 2008). Addressing these factors is essential to ensure the adaptive potential and long-term fitness of species and to determine the best approaches for restoration projects. In the present study we used the Brazilian native species *Plathymenia reticulata* Benth. to investigate the effectiveness of forest restoration and the typical cocoa agroforest called “cabruca” in landscape connectiveness. Our target species is one of the largest trees, endemic to Atlantic Forest. We selected *P. reticulata* due to its utilization in restoration efforts within the Atlantic Forest (Ribeiro et al., 2018), and its notable logging potential (De Almeida, 1998, Sambuichi et al., 2012b). Therefore, studies to better understanding the genetic diversity and gene flow of the species are a key strategy to its *in situ* or *ex situ* conservation. It is very important to have better understanding of the genetic diversity and gene flow of the species to identify the species who have a high level of genetic polymorphism (Tambarussi et al., 2017) .

The focus on genetic connectivity will help to choose appropriate statistical techniques and spatial scale for successful conservation and landscape management projects.

METHODOLOGY

Target species

Plathymenia reticulata Benth. popularly known in Brazil as “vinhático”, is a tree species of the legume family, which can reach 30 m in height (Carvalho, 2009) and 5 m in DBH (observational data). It has a stem with regular plate formation and wood with a reddish tone, which is the origin of its popular name. It is pollinated by small insects (Goulart et al., 2005), especially bees, and its seeds are dispersed by anemochory.

It stands out among the species most used in the restoration of the flora of degraded areas. It is widely distributed in Brazilian biomes, being present from the Atlantic Forest to the Caatinga, in 16 states and the Federal District (Carvalho, 2009), and is highly adaptable. This makes it an excellent candidate for implementation in the restoration of degraded areas. Furthermore, it is one of the most desired species by farmers in cocoa agroforestry systems in southern Bahia (Sambuichi et al., 2012). Due to its high-quality and durable wood, the “vinhático” is of great economic importance, being used in civil construction and the manufacture of luxury furniture, stakes, supports and fence posts (Carvalho, 2009). This makes it one of the most sought-after species in commercial plantations of native species. Thus, “vinhático” presents itself as an excellent biological model with notable ecological and economic importance.

We selected study sites based on the occurrence of *P. reticulata* in two treatments, but prioritizing areas exhibiting a great number of adult individuals (minimum of 24). Plant material from juvenile individuals was also collected. We didn’t find juveniles from all cabrucas because the cabrucas that are maintained by farmers they usually pull out the trees in juvenile stages. We collected total 96 adults from 4 different cabrucas 47 juveniles from 2 cabrucas and 74 adults from 3 different native forest. We georeferenced each individual by Garmin GPS.

Collection of plant material

Leaves were collected from the adult individuals 100 m apart and average circumference at breast height is (CBH) ≥ 25cm and juvenile individuals’ circumference at breast height is (CBH) ≤ 10 cm grown in Forest and Cabruca.

The study was conducted in southern region of the state of Bahia, Brazil specifically from the municipalities of Camacan CAM(F), Ibirapitanga IB(F) and IA(C), Amargosa AMA(F) , Ilheus CC(C), Itabuna VA(C), Una UCA(C)(Figure 1). According to the Köppen climate classification, the prevailing climate type is "Af," characterized by a tropical rainforest climate. This climate typically receives annual rainfall ranging between 1200 to 1300 mm and maintains an average temperature around 25°C. The predominant vegetation associated with this climate is the Dense Ombrophilous Forest, which thrives in such warm and consistently moist conditions.

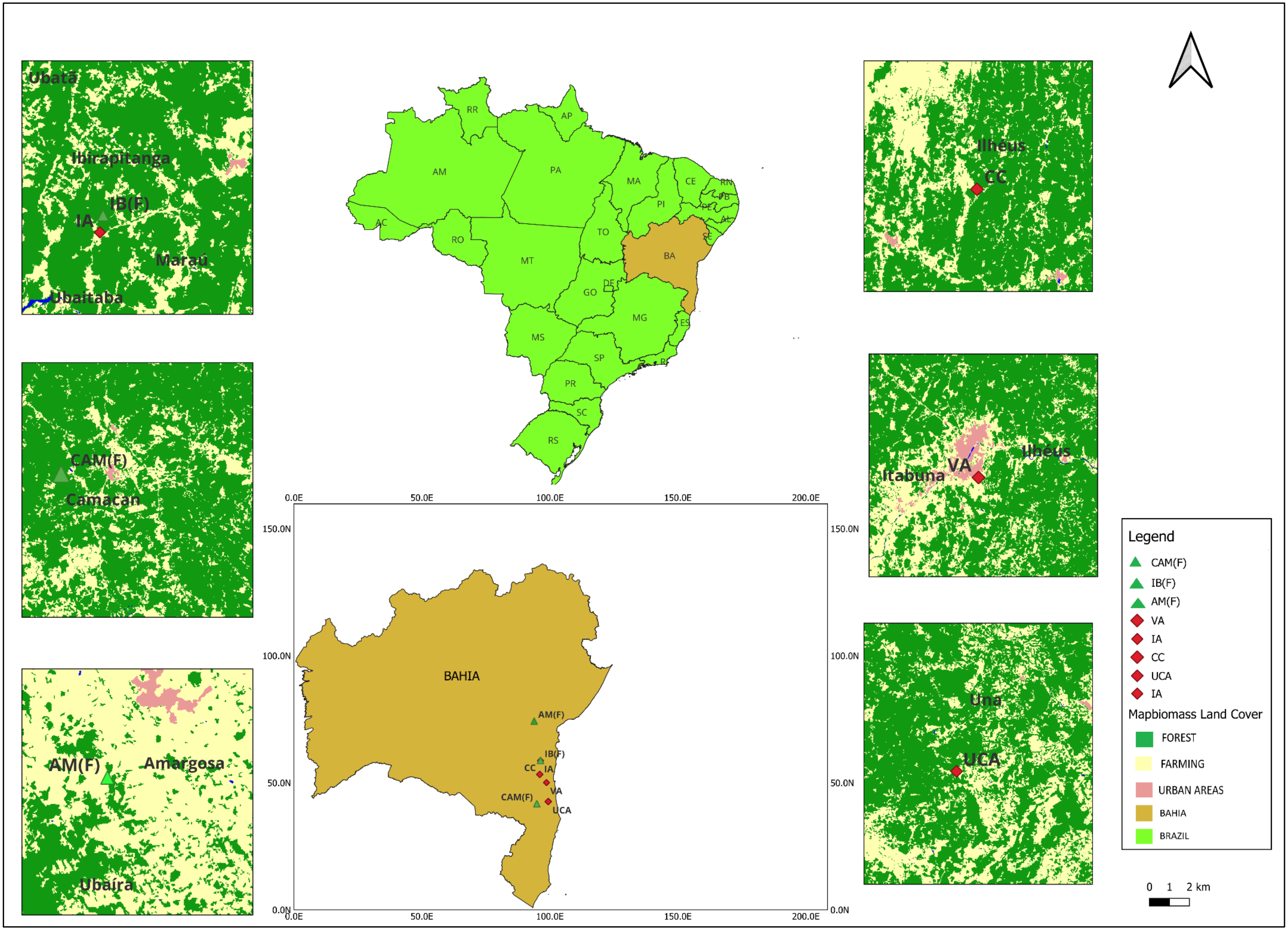
Ubatã
Ibirapitanga
IB(F)
IA
Maraú
Ubaitaba
CAM(F)
Camacan
AM(F)
Amargosa
Ubaíra
RR
AP
AM
PA
MA
CE
RN
PB
PE
AL
SE
PI
AC
RO
TO
BA
MT
DF
GO
MG
ES
MS
SP
RJ
PR
SC
RS
BAHIA
AM(F)
IB(F)
CC
IA
VA
CAM(F)
UCA
0.0E
50.0E
100.0E
150.0E
200.0E
0.0N
50.0N
100.0N
150.0N
Ilhéus
CC
Itabuna
VA
Ilhéus
Una
UCA
Legend
▲ CAM(F)
▲ IB(F)
▲ AM(F)
◆ VA
◆ IA
◆ CC
◆ UCA
◆ IA
Mapbiomass Land Cover
🟩 FOREST
🟨 FARMING
🟥 URBAN AREAS
🟫 BAHIA
🟩 BRAZIL
0 1 2 km

Figure 1: Map of Brazil, highlighting Bahia state. Red Diamonds="Cabruca", Green Triangles="Forest". Mapbiomass highlights landcovers "Forest", "Farms", "Urban Areas". Figure (A) IA=Cabruca, IB=Forest, (B)CC=Cabruca, (C) CAM=Forest , (D) VA=Cabruca , (E) AM=Forest , (F) UCA=Cabruca

DNA extraction and quantification

The DNA extraction of each sampled individual was done through the Protocol CTAB 2% (Doyle and Doyle 1990). To estimate the quality and concentration of DNA the Gel Green^{TM} was used through contrasting the samples with dilutions of bacteriophage λ DNA diluted to a known standard concentration, in an electrophoresis in 1% agarose gel.

Microsatellite Loci Amplification.

The DNA of each sampled individual was amplified by 11 microsatellite marker loci (also known as SSR - simple sequence repeats) developed specifically for *P. reticulata*. (Oliveira et al., 2012, Cruz et al., 2012). For DNA amplifications, PCR mix (final volume 13 µL) containing 4.8 µL of H_2O, 1.3 µL of Buffer(10X), 1.3 µL of DNTPs(2.5 mM), 1.3 µL of BSA(2.5 mg/ml), 0.4 µL of $MgCl_2$(25 mM), 0.4 µL of coloring M13(10 mM), 0.3 µL of Primer(10 mM), 0.2 µL of Taq polymerase(1 U) (Phoneutria, Belo Horizonte, Minas Gerais, Brasil) and 3.0 µL of genomic DNA in Life Pro thermocycler (Bioneer Technology Co., China), with the amplification program according (Oliveira et al., 2012;Cruz et al., 2012). The amplificons was submitted to capillary electrophoresis in an ABI3500 automatic sequencer (Applied Biosystems, USA) to separate the SSR fragments in a multiload strategy. A mixture containing 0.3 µL of GeneScan™, 500 µL of

Liz™ (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA), and 7.7 µL of formamide (Applied Biosystems) was prepared. Genotyping was performed with GeneMarker software (SoftGenetics, USA).

Genetic parameters estimate:

To characterize the genetic diversity of all study areas, we estimated the following standard genetic parameters: Number of Alleles (Na), Effective Number of Alleles (Ne), Shannon's Information Index (I), Observed Heterozygosity (H_o), Expected Heterozygosity (H_E), and Fixation Index (F) using the GenAlEx version 6.5 software. We compared f-coefficient such as F_{IS} (Inbreeding coefficient within subpopulations), F_{IT} (Inbreeding coefficient within individuals relative to the total population), F_{ST} (Genetic differentiation among subpopulations) and pairwise F_{ST} between forest and cabruca using GenAIEx version 6.5 software. Genetic indices and parameters cited above was compared by clustering analysis and principal component analysis in R4.0.1 environment.

Paternity Analysis:

We did paternity analysis on 47 juveniles with 96 adult trees in four cabrucas using CERVUS.

RESULTS

The average number of different alleles (Na) was 5.00 for forest and 7.44 for Cabruca indicating higher allelic diversity in cabruca. CAM(F) show significant allele diversity among forest populations (Na=5.889), while CC(C) was 9.00 shows high allelic diversity in cabruca population. Effective number of alleles (Ne) is higher in cabruca populations mean 3.618, with

CC(C) was 4.444 indication of higher genetic variation. In forest population Shannon Index(I) is highest for CAM(F) population I=1.247, and for cabruca population IA(C) has I=1.569, it is highest among both forest and cabruca indicating rich and well-distributed variety of alleles. We observed that IB(F) has negative value of fixation index (F=-0.022), caused by slightly higher observed heterozygosity (H_O=0.507) than expected heterozygosity (H_E=0.503), indicating a healthy genetic variation with little or no inbreeding. Other forest population of interest is AM(F), which is close to the Hardy-Weinberg equilibrium with slight difference between Ho and HE (0.475,0.486) and F close to zero (0.010). Between the Cabruca populations we find a high fixation index, with VA(C) showing the highest value (F=0.792), indicating low genetic diversity and high levels of inbreeding. On average, heterozygosity among forest is higher than in cabrucas with low fixation index, indicating that forest population has maintained genetic diversity with lower levels of inbreeding (Table 1).

Table 1: Estimation of genetic parameters of *P.reticulata* in forest and cabruca populations

Forest	**N**	**Na**	**Ne**	**I**	**Ho**	**H_E**	**F**
CAM	28.222	5.889	2.944	1.247	0.451	0.610	0.261
IB	21.778	4.222	2.385	0.964	0.507	0.503	-0.022
AM	22.333	4.889	2.440	0.981	0.475	0.486	0.010
Mean	**22.333**	**4.889**	**2.440**	**0.981**	**0.475**	**0.486**	**0.010**
Cabrucas	**N**	**Na**	**Ne**	**I**	**Ho**	**H_E**	**F**
VA	23.667	7.778	3.728	1.374	0.108	0.597	0.792

IA	22.444	8.889	4.103	1.569	0.431	0.670	0.403
CC	24.778	9.000	4.444	1.534	0.451	0.645	0.249
UCA	23.222	4.111	2.195	0.864	0.334	0.454	0.253
Mean	**23.528**	**7.444**	**3.618**	**1.335**	**0.331**	**0.591**	**0.424**

Number of Alleles (Na), Effective Number of Alleles (Ne), Shannon's Information Index (I), Observed Heterozygosity (Ho), Expected Heterozygosity (H_E), and Fixation Index (F).

The F_{IS} value was higher in the cabruca (0.475) than in the forest (0.114). These results corroborate the estimation of genetic parameters and show the effects of crosses between relatives within cabruca subpopulations. Similarly, cabruca populations demonstrate a significantly higher inbreeding coefficient (0.544) compared to forest populations (0.210). The F_{ST} value indicates genetic differentiation among cabruca subpopulations (0.150), while the value for forest populations (0.116) remains similar (Table 2).

Table 2: F-coefficient (Inbreeding coefficients within populations (F_{IS}), Inbreeding coefficients relative to the total population (F_{IT}), Genetic differentiation among populations (F_{ST}) estimation for Three Forest and Four Cabruca populations.

Populations	**F_{IS}**	**F_{IT}**	**F_{ST}**
Forest	0.114	0.210	0.116
Cabruca	0.475	0.544	0.150

The differentiation between populations was accessed using a pairwise F_{ST} matrix. The lowest F_{ST} value (0.014) was found between AM(F) and IB(F), suggesting minimal genetic differentiation. Conversely, the highest F_{ST} value (0.202) was observed between UCA(C) and CAM(F), indicating a significant degree of genetic differentiation (Table 3).

Table 3: Pairwise F_{ST} values among seven populations

	VA(C)	**IA(C)**	**CC(C)**	**UCA(C)**	**AM(F)**	**CAM(F)**	**IB(F)**
VA*(C)*	-						
IA*(C)*	0.101	-					
CC*(C)*	0.121	0.036	-				
UCA*(C)*	0.087	0.138	0.118	-			
AM(F)	0.119	0.160	0.139	0.112	-		
CAM(F)	0.155	0.171	0.181	0.202	0.115	-	
IB(F)	0.121	0.158	0.144	0.119	0.014	0.111	-

Low Differentiation ($F_{ST} < 0.05$) = frequent gene flow. Moderate Differentiation ($0.05 \leq F_{ST} < 0.15$) = restricted gene flow. High Differentiation ($F_{ST} \geq 0.15$) = limited gene flow.

Populations UCA(C) and VA(C) have less genetic distance, as they branch off from a common node before diverging from the rest of the tree. AM(F) population is somewhat similar to UCA(C) and VA(C) but diverges from them before connecting to the main tree. IB(F) branches off next, indicating a similarity with AM(F), while CAM(F) shows a significant genetic distance from IB(F) and the rest of the populations, indicating it is the most genetically distinct among the listed groups. CC(C) and IA(C) populations are grouped together, with IA(C) showing a close genetic relationship to CC(C) (Figure 2).

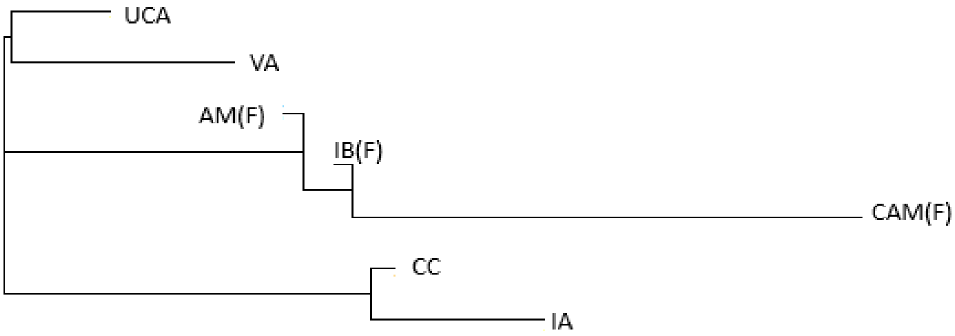

Figure 2. Graphic representation of the clustering analysis using R based on Nei's unbiased minimum genetic distance (1978) for seven *P. reticulata* populations both forest and cabruca based on microsatellite markers.

Dim1 (67.4%): The first principal component, which explains 67.4% of the total variance in the data. Dim2 (18.9%): The second principal component, which explains 18.9% of the total variance in the data. According to Dim1 two cabruca populations (CC(C) and IA(C)) (green triangle and teal diamond respectively), shows positive score indicating they have similar genetic score, while one forest (AM(F) (red square) population and one cabruca (IB(C) (light blue circle) population shows negative score showing different set of genetic characteristics. Forest (CAM(F) (brown circle) also shows positive score but it far apart from CC(C) and IA(C) indicated difference in genetic diversity. According to Dim2 VA(C) (pink triangle with a hollow center) and UCA(C) (purple square with a hollow center) has negative score indicating lower genetic diversity indices (Figure 3).

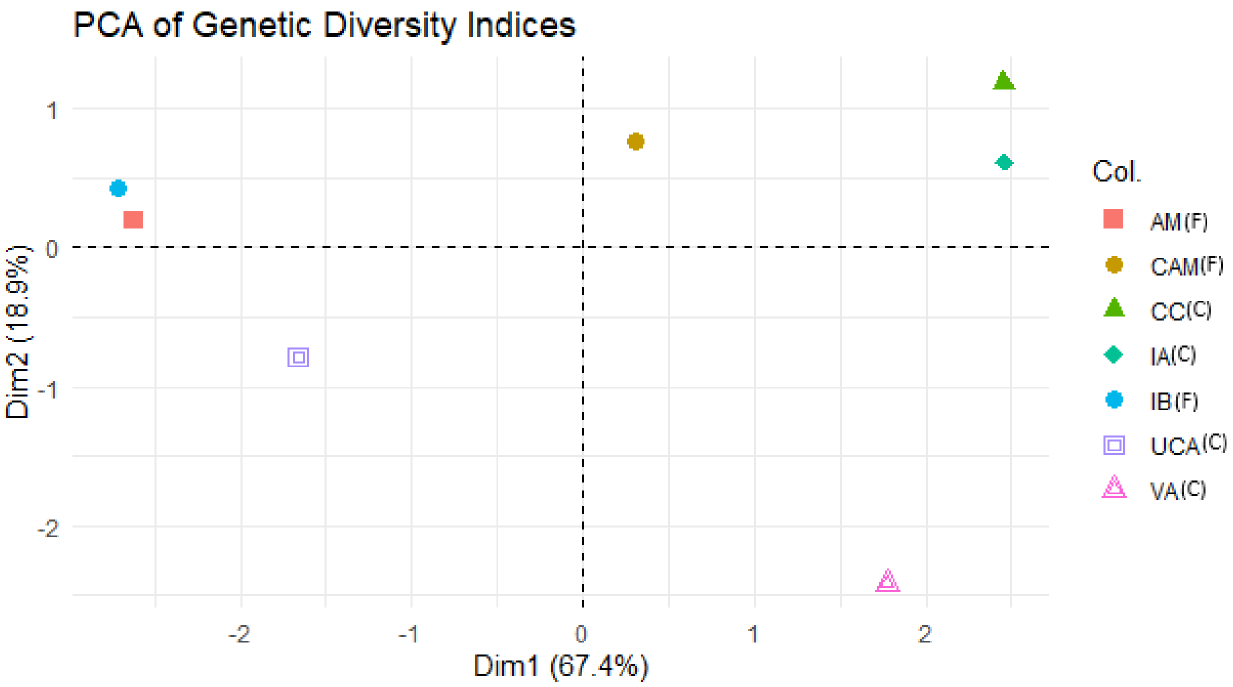

Figure 3: Principal Component Analysis (PCA) of genetic diversity indices for two treatments: Native Florest and Cabruca Agroforest Systems labeled as Forest(F)= “AM, CAM, IB”, Cabruca(C)= “CC, IA, UCA, and VA”. Each point represents a population with colors and shapes indicating different populations as shown in the legend on the right.

Paternity

Paternity analysis revealed that 22 Juveniles whose parents are detected with 95% confidence has positive LOD score. The remaining has low probability of paternity. We also observed average distance between individuals of the same areas is 82m. Maximum distance between juveniles and adults of different areas is 45km (Table 4).

Table 4: Paternity analysis of *P. reticulata* considering all sampled individuals in four cabruca in southern Bahia, Brazil.

Offsprings	**Mother ID**	**Candidate father ID**	**Trio LOD score**	**Trio confidence**	Distance(m)
J-UC6	A-UC20	A-UC20	5.99E+00	*	165
J-UC4	A-UC20	A-UC20	5.41E+00	*	84
J-UC18	A-UC2	A-UC2	5.74E+00	*	60
J-UC1	A-UC16	A-UC15	4.45E+00	*	47
J-UC5	A-UC20	A-UC20	2.12E+00	*	56
J-I18	A-I36	A-I36	6.62E+00	*	9
J-UC3	A-UC23	A-UC15	3.83E+00	*	95
J-I20	A-I35	A-I35	2.53E+00	*	150
J-UC20	A-UC2	A-UC2	5.78E+00	*	67
J-UC22	A-UC2	A-UC2	5.78E+00	*	77
J-UC9	A-UC2	A-UC2	5.78E+00	*	82
J-UC17	A-UC2	A-UC2	5.06E+00	*	62
J-UC13	A-UC18	A-UC18	4.86E+00	*	144
J-UC29	A-UC11	A-UC11	4.53E+00	*	38
J-UC7	A-UC13	A-UC13	4.42E+00	*	29
J-UC10	A-UC16	A-UC16	3.73E+00	*	13
J-I24	A-I33	A-I33	6.31E+00	*	130
J-UC11	A-UC16	A-UC16	2.27E+00	*	13
J-I23	A-CC10	A-CC10	5.31E+00	*	45000

J-I31	A-I33	A-I33	2.08E+00	*	125
J-I26	A-I35	A-I35	4.71E+00	*	152
J-UC24	A-UC18	A-UC18	4.18E+00	*	134

An asterisk (*) indicates that the confidence level is 95% using the “strict” criterion implemented in CERVUS. LOD = logarithm of odds. A = Adults and J = Juveniles.

DISCUSSION

In this study we used genetic estimates on one of the threatened species in Atlantic Forest *Plathymenia reticulata* to reveal significant insights into biodiversity of forest and cabruca. The results highlight the future of this species in agroforest system in comparison to forest. Cabruca despite being an agroforest system maintain a high level of genetic diversity, compared to natural forest environment. Moreover, PCA reveals genetic differentiation between studied populations of cabruca and forest. The separation between components explains the influence of different environmental conditions and management practices on genetic structure of populations. Finally, paternity analysis revealed that almost all juveniles whose parents are identified belong to the same cabruca, indicating that gene flow occurs mainly over the small distance. These findings are significant as they highlight the importance of cabruca system in conserving genetic diversity of *P. reticulata*, mitigate the impact of climate change and helps with bioeconomic future of the species. By prioritizing bio-products produce by timber tree such as *P. reticulata*, but also doing research and making policies to ensure no illegal logging occurs, providing incentives to farmers for conservation, and well-managed mixed cropping and increase the development of studies and research on the native forest to forecast sustainability of trees.

With regard to specific genetic diversity indices, the mean number of alleles, effective number of alleles and Shannon Index are observed to be higher in *P. reticulata* cultivated in cabruca than in species in a natural forest environment. These indices provide an accurate reflection of the genetic variation of species grown in different landscapes and environmental conditions. These findings are corroborated by previous studies on Southern Bahia cabruca, which demonstrate its capacity to sustain species richness and demographic health (Sambuichi et al., 2012a). Research on another tropical species, Inga edulis, indicated that agroforestry systems with a high level of allelic richness may be influenced by a number of factors, including population size and the contribution of neighboring natural remnants (Hollingsworth et al., 2005). The observed variation between agroforestry systems is dependent on the management practices employed by the farmer (Molla and Asfaw, 2014).

P. reticulata exhibits considerable genetic diversity when cultivated in forest and agroforestry settings, particularly with regard to heterozygosity. This is of critical importance for the adaptability and resilience of the species to environmental stresses. Both study areas are affected by fragmentation, yet natural forest remains a crucial reserve for *P. reticulata*, in comparison to agroforest. These findings lend support to the hypothesis that human intervention affects the genetic diversity of species (Hollingsworth et al., 2005). The elevated heterozygosity levels are linked to cross-pollination facilitated by wasps and bees, as well as wind-dispersed seeds (Lacerda et al., 2002; de Souza et al., 2017; Conceição et al., 2024). The geographical proximity of cabruca to forest can be a factor in maintaining high heterozygosity found in our study. The isolation from the forest is associated with a reduction in heterozygosity in VA(C) cabruca. A reduction in heterozygosity in trees grown in an agroforestry system is also associated with a decrease in population size due to selective logging, which is a common practice in agroforestry systems (Leal et al., 2014). Leal (2014) explained the similar phenomena in another forest tree whose hardwood is used for construction purpose i.e. *C.legalis* trees occurred in small fragments and cabrucas as it suffered logging in cacao areas and ultimately loss of heterozygosity as number of adults decreases due to fragmentation and also expansion of cacao in 1960s and 1970s.

We also observed low fixation index(f) in forest population. The fixation index explains patterns of inbreeding in population. The low levels of inbreeding and high value of heterozygosity is expected as the species is hermaphrodite, and cross-pollinated that helps its adaptability (Liu et al., 2023). The pollinators also help in gene flow over the long-distances (de Souza et al., 2017) making inbreeding close to zero or negative in continuous forest. Inbreeding coefficient is high in agroforest due to isolation. Trees are in low density in agroforestry system (Lowe et al., 2005) and like in case of cabruca VA(C) that has highest value of inbreeding coefficient, it is vulnerable in the absence of near-by forest that could help in pollination (Ian et al., 2014).

We also observed other genetic diversity metrics for two types of populations: Forest and Cabruca. F_{IS} values suggested that there is high inbreeding within each cabruca population as compare to forest which has more genetic diversity. F_{IT} values suggested that forest population as a whole somewhat does show inbreeding but total cabruca population shows homogeneity with less genetic variation. F_{ST} values showed moderate genetic differentiation among forest population. It means populations are somewhat distinct but still some genetic exchange occurs, while gene flow in cabruca populations are limited with greater genetic differentiation. Forest Populations shows lower F_{IS} and F_{IT} values indicate better genetic health and diversity. Moderate F_{ST} suggests reasonable genetic exchange among populations, maintaining overall genetic diversity. Cabruca Populations shows higher F_{IS} and F_{IT} values point to significant inbreeding, potentially leading to reduced fitness and adaptability. The higher F_{ST} value indicates distinct genetic structuring, possibly due to isolated or management practices. The higher differentiation is due to limited gene flow, genetic drift and inbreeding (Barreto et al., 2023). Inbreeding depression can be prevented by maintaining an effective number of populations of native trees in the area and cacao agroforest system can act as a stepping stone or corridors to improve connectivity with natural forest fragment to improve pollen-mediated gene flow (Dawson et al., 2013). An example for this is a timber tree mahogany (*Swietenia humilis*), grown as a lone individual in pastureland participated in gene flow with forest trees for more than 4.5km (White et al., 2002).

We also performed pairwise F_{ST} analysis to measure genetic differentiation between seven populations of forest and cabruca. F_{ST} values are from 0 to 1, where 0 is no genetic differentiation while 1 indicates complete differentiation. Cam(F)shows greatest differentiation from other populations. These results are also consistent with phylogenetic tree base on Nei genetic distance. Lowest values of pairwise F_{ST} is between IB(F) and AM(F), they are also close distance in Nei genetic distance. CAM(F) is the only forest population in our results that shows heterozygosity and inbreeding values close to cabruca population. This population is isolated with greater differentiation, but it maintained genetic diversity. While VA(C) cabruca has highest value of inbreeding and lowest heterozygosity it does not have highest genetic differentiation or Nei distance from other populations. It could be established from population with small number of individuals (limited population), However, if these founders came from a genetically similar source population, the differentiation (F_{ST}) between them and other populations might not be high (Klimova et al., 2023). Population size, habitat diversity helps gene flow in forest (Ratnam et al., 2014) as compare to agroforest which is highly influenced by human activities. One forest population with highest genetic differentiation could be due to small and fragmented population which can limit gene flow (Ksiazek-Mikenas et al., 2019, Duminil et al., 2009).

Cluster analysis based on pairwise genetic distances revealed that CAM(F) is the most distinct population. This clustering pattern was consistent with the results from principal coordinate analysis, which was used to supplement the cluster analysis data (Figure 2,3). Cluster analysis tends to be more sensitive to closely related individuals, while PCA provides better insights into the distances between major groups (Hauser and Crovello, 1982). Although the dendrogram did not strictly organize provenances by the geographical origins of the accessions, it offered valuable insights into the evolutionary processes shaped by colonization and isolation patterns. Principal component analysis (PCA) was done on genetic diversity metrices to compare variation between cabruca and forest. It helps to understand similarities and differences between populations of two landscapes. It maximizes the observed variance derived from the original variable (Tabachnick and Fidell, 2018). These populations have genetic diversity indices values are similar to each other while two populations of forest AM(F) and IB(F) has similar values especially of fixation index that is negative or close to zero. The results suggested that value of these populations is in Hardy-Weinberg equilibrium. UCA(C) shows negative score in both PCs, it is less contributor towards genetic diversity. VA(C) is too far from other population shows its unique genetic diversity profile. This may be due to significant barriers to gene flow related to dispersal and pollination, which were associated with isolation (Kithure et al., 2015).

Paternity analysis revealed that inbreeding occurred in population grown in cabruca as most identified juveniles are descended from adults grown in the same cabruca. Anthropogenic activities lead to reduction of population and limited gene flow (Bem et al., 2015). Small and isolated population can also lead to limited gene flow (Wang et al., 2011). Also, in agroforest trees distribution is over small distances and low population density, both of these factors reduce connectivity between forest and agroforest, especially when connectivity depends on bees as pollinator in case of *P. reticulata*, which can only travel to short distances (Kindt et al., 2006, Dawson et al., 2013, Ward et al., 2005). Only one juvenile's parent has been identified over the long distance maybe due to in the past the Atlantic Forest was continuous (Waqar et al., 2021) and juvenile was originated from the parent grown in other cabruca. Another reason for long distance gene flow could be in tropical forests, many tree species rely on animals as their secondary seed dispersers, capable of transporting seeds over moderate to long distances (Sujii et al., 2021). These can help *P. reticulata* to sustain its genetic diversity in human-modified landscape.

CONCLUSION

In conclusion, this study plays the critical role of cabruca agroforestry systems in maintaining the genetic diversity of *Plathymenia reticulata*, a key species in the Atlantic Forest. Despite being a modified landscape, cabruca retains a genetic diversity level comparable to natural forests, indicating its potential as a conservation strategy. The genetic differentiation observed between forest and cabruca populations suggests that varying environmental conditions and management practices influence the genetic structure of *P. reticulata*. Additionally, the high level of heterozygosity in forest populations highlights the importance of continuous forest areas in preserving genetic diversity and reducing inbreeding risks. The findings emphasize the need to conserve both natural forests and agroforestry systems to ensure the long-term survival of *P.*

reticulata and other species, thereby contributing to biodiversity conservation and climate change mitigation efforts.

GENERAL CONCLUSION

Our studies emphasize the critical role of maintaining genetic diversity in tree species across various landscapes. *Manilkara multifida* populations in protected areas show considerable allelic richness, but anthropogenic factors such as deforestation had caused considerable heterozygosity loss in juveniles. This threatens long-term genetic diversity of this tree, making habitat protection and restoration crucial. For *Lecythis pisonis* tree, outcrossing and active pollination as mediums of gene flow are required; therefore, habitat connectivity may become significant in maintaining the genetic health. The restoration projects in cabruca agroforest system can help to improve landscape connectivity. For example, in our research on *Plathymenia reticulata* cabruca agroforestry systems retain genetic diversity similar to that of natural forests. Cabruca agroforestry system can conserve much of the genetic diversity present in the native forests. Overall, protection of natural forests and agroforestry systems alike is important for the conservation of genetic diversity and biodiversity and, by extension, climate resilience.

The Atlantic Forest is an especially complex tropical system, with high biodiversity, while the choice of the best practices regarding restoration methods creates hard challenges. Developing strategies that are appropriate to the global and Brazilian goals concerning nature conservation conceives important takes on genetic parameters. Our research results bring important implications for conservation strategies aimed at maintaining genetic diversity and the long-term viability of keystone species in the Atlantic Forest, especially in a protected-areas, natural-forest, and agroforestry-system framework. Information about the genetic parameters of native species supports decision-making, making sure that their full potential will be realized in appropriate conservational interventions that are to minimize habitat degradation and promote the persistence of genetic diversity.

Here is the list of insight revealed during my research:

1. **Impacts of habitat degradation**: Habitat degradation is very dangerous for the persistence of species. Targeted conservation actions to reduce these impacts on species shall be pursued.

2. **Conservation as a Restoration Strategy**: Proven in restoring lost or diminished biodiversity and ecosystem services. It also aids in combating climate change through the restoration of carbon storage and minimizing distress on primary forests.

3. **Issues on Restoration Techniques:** Due to the complexity of the Atlantic Forests biodiversity a restoration technique is not clear and, therefore, cannot be determined. A more subtle technique that caters to the actual characteristic fundamentals of the eco-system is needed.

4. **Global Commitments to Local Actions:** Global platforms such as the Bonn Challenge can play a critical role in developing restoration goals. But the attainment of it will be done through specific ecosystem local actions, such as the Atlantic Forest.

5. **Multifaceted Conservation Approach:** Success in conservation could be underneath protection of habitat, restoration, and land-use methods that are sustainable. Multiple causes of biodiversity loss and ecosystem resilience need a multi-faceted approach.

6. **Climate Change Mitigation Link of Conservation:** Genetic diversity and ecosystem restorational conservation strategy has a linked contribution to climate change mitigation. These strategies promote the increased ability of the ecosystem in carbon sequestration and thus more adaptive capacity in respect to the climate variation.

7**. Importance of Genetic Diversity:** Genetic diversity is one of the pertinent issues relevant to the long-term viability and adaptability of the key species found in the Atlantic Forest. The conservation of genetic diversity favors species with an increase in resistance against changes and threats that may eventually turn harmful for their existence.

8. **Role of Genetic Parameters in Conservation:** Genetic parameters hold extreme importance in shaping appropriate conservation policies. These provide indications on the potential of the species for sustainability and give restoration as well as conservation efforts a proper direction.

9. **The Relevance of Protected Areas:** While protected areas play a very important role in the maintenance of genetic resource diversity and protection of key species, this would only be effective if well managed and, at the same time, well connected with the adjacent landscapes that house them, including agroforestry systems.

10. **The Potential of Agroforestry Systems**: Agroforestry systems in a general way—cabruca in specific—hold promises for supportive ways of biodiversity conservation activities. Hence, they would even help in maintaining or increasing genetic resource diversity while supporting livelihood activities at the local levels.

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