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# **RESTORING THE PAST, RECONSTRUCTING THE FUTURE:** HOW GENETIC PARAMETERS CAN SUPPORT FOREST RESTORATION?

# **RESTAURANDO O PASSADO, RECONSTRUINDO O FUTURO**: COMO PARÂMETROS GENÉTICOS PODEM SUBSIDIAR A RESTAURAÇÃO FLORESTAL?

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**ILHÉUS – BAHIA - BRASIL**

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## DEDICATION

To the ones who enhanced my creative imagination, building a bold, curious-mind. You will always be a part of my experiences – Jairo Cardoso and Maria Aparecida Miranda Cardoso.

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Ações antrópicas nos habitats naturais tem danificado severamente os ecosistemas, resultando em matrizes fragmentadas de florestas remanescentes. A Mata Atlântica do Brasil, bioma mundialmene conhecido pela alta biodiversidade, é um dos domínios mais impactados pelas ações humanas, e encontra-se hoje criticamente degradado. Nesse contexto, iniciativas de restauração de florestas tropicais são uma oportunidade única de reversão desse cenário, que podem garantir a persistência de populações nativas. Contudo, ações de restauração florestal usualmente não consideram os aspectos genéticos para sua implantação. Por isso, pouco se sabe sobre a efetividade da restauração florestal em reestabelecer populações geneticamente viáveis. Esse trabalho teve como objetivo compreender como florestas restauradas podem efetivamente contribuir para o restabelecimento de populações arbóreas geneticamente viáveis, usando uma espécie nativa ameaçada de extinção (*Dalbergia nigra*). Foram amostrados adultos e regenerantes, quando encontrados, de dois remanescentes de populações nativas da Mata Atlântica, e sete áreas de restauração distribuídas nas regiões de Ilhéus, Porto Seguro e Texeira de Freitas, sul da Bahia. Tais populações representam diferentes metodologias de restauração, e foram genotipadas para sete microssatelites. Estimativas genéticas populacionais foram realizadas com base em sete microssatélites , e análises estatísticas foram realizadas para melhor compreensão de padrões entre diferentes populações, tipos de área e regiões amostradas. Populações nativas e restauradas tiveram riqueza alélica similar, com alelos privativos presentes, exceto em uma população nativa. Os níveis de endogamia variam, sendo menores em nativas, e ausente em duas populações restauradas. Contudo, todos os regenerantes apresentaram evidencias de endogamia. As populações de Teixeira de Freitas tiveram constituição genética mais distinta, sendo o seu remanescente nativo o mais distinto em termos genéticos. Em contrapartida, as duas outras regiões representaram um grupo genético diverso, mas estruturado. As populações restauradas tiveram, de maneira geral, um pool gênico diverso (sete grupos genéticos), enquanto florestas nativas foram compostas por menos grupos genéticos. No entanto, observamos que cada população restaurada é composta por diferentes grupos genéticos, havendo uma tendência de ocorrência de grupos genéticos por região. Os resultados sugerem que a metodologia de restauração adotada pode influenciar os níveis de endogamia e, principalmente, o pool gênico das populações. De maneira geral, populações restauradas foram diversas e efetivas no estabelecimento de fluxo gênico com populações nativas. Ademais, as populações restauradas são fonte de novos alelos para as regiões, e podem contribuir para a redução dos níveis de estrutura genética entre as populações. Demonstramos que florestas restauradas podem funcionar como reservatórios genéticos, que promovem a base genética das populações em termos locais e regionais. Argumentamos que estratégias de restauração florestal devem ter um ponto central genético, mas associado a padrões da matriz de paisagem que compõe a região e características do local a ser restaurado, para garantir a viabilidade genética ao longo das gerações. Assim, florestas restauradas podem contribuir efetivamente para a conservação da

biodiversidade ao longo do tempo.

Palavras-chave: Mata Atlântica, recuperação de habitats, *Dalbergia nigra*, fluxo gênico, genética da restauração

## ABSTRACT

QUADROS, Taruhim Miranda Cardoso, M.Sc., Universidade Estadual de Santa Cruz, Ilhéus, February, 2020. Restoring the past, reconstructing the future: how genetic parameters can support forest restoration?. Advisor: Fernanda Amato Gaiotto. Advisor Commite Members: Daniela Custódio Talora, Ronan Côrrea Xavier.

The pervasive anthropogenic actions in natural habitats have severely damaged ecosystems, and created a fragmented matrix of remaining forests. The Brazilian Atlantic Forest, a biome worldly recognized by its unique biodiversity, is highly impacted by human interventions, and is now critically degraded. Thus, tropical forest restoration initiatives are an outstanding opportunity to reverse it and ensure the persistence of native populations. However, restoration effectiveness in reinstating genetic viable populations is not entirely addressed. Therefore, we propose a project to comprehend how restored forests can effectively contribute to the re-establishment of genetically-viable populations, using a threatened native tree species as a model (*Dalbergia nigra*). Adults and seedlings of two Atlantic Forest remnant sites and seven restored forests of three Southern Bahia region (Ilhéus, Porto Seguro and Teixeira de Freitas) were sampled. Each restored site represents a different restoration methodology. Population genetics parameters were estimated
for seven microsatellite loci, and statistical analysis were performed to better comprehend differentiation patterns between each site and the three regions. Native and restored populations had similar allelic richness, with private alleles present, except in one native population. Inbreeding levels vary, being lower in native species, and absent in two restored populations. However, all saplings showed evidence of inbreeding. Teixeira de Freitas populations were more distinct according to its genetic composition, with its native remnants being the most distinct in genetic terms. In contrast, the other two regions represented a diverse but structured genetic group. The restored populations had, overall, a diverse gene pool (seven genetic groups), while native forests were composed of fewer genetic groups (five). However, we observed that each restored population is composed of different genetic groups, with an occurrence of similar genetic groups by region. The results suggest that the adopted restoration methodology can influence levels of inbreeding and, mainly, the gene pool of populations. In general, restored populations were diverse and effective in establishing gene flow with native populations. In addition, restored populations are a source of new alleles to regions, and can contribute to the reduction of genetic structure levels among populations. We demonstrate that restored forests can function as genetic reservoirs, which support genetic basis of populations in local and regional terms. We argue that forest restoration strategies must consider genetic aspects as a central point, but associated with regional patterns of the landscape matrix and characteristics of the place to be restored, to guarantee genetic viability over generations. Once considered, restored forests can effectively contribute to the conservation of biodiversity over time.



Key-words: Atlantic Forest, habitat recovery, *Dalbergia nigra*, gene flow, restoration genetics

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## ACRONYMS

| | |
|---|---|
| AFRP | Atlantic Forest Restoration Pact |
| $A_n$ | Null allele frequency |
| $A_p$ | Number of private alleles |
| $A_r$ | Allelic richness |
| BIC | Bayesian Information Criterion |
| CITES | Convention on International Trade in Endangered Species of Wild Fauna and Flora |
| DIC | Deviance Information Criterion |
| DAPC | Discriminant Analysis of Principal Components |
| Dgenet | Genetic distance |
| Dgeo | Geographical distance |
| $F_{IS}$ | Inbreeding coefficient |
| $G_{ST}$ | Nei's fixation index |
| GR | Igrapiúna |
| $H_E$ | Expected heterozigozity |
| $H_O$ | Observed heterozigozity |
| INDC | Intended Nationally Determined Contributions |
| IUCN | International Union for Conservation of Nature |
| IL | Ilhéus |
| N | Native |
| $\bar{n}$ | Sample size |
| NGOs | Non-Governmental Organisations |
| NIL3 | Native area from Ilhéus |
| NTx3 | Native area from Teixeira de Freitas |
| Planaveg | Plano Nacional de Recuperação da Vegetação Nativa |
| PS | Porto Seguro |
| R | Restoration |
| RIL1 | Restored area 1 in Ilhéus |
| RIL2 | Restored area 2 in Ilhéus |
| RTx1 | Restored area 1 in Texeira de Freitas |
| RTx2 | Restored area 2 in Teixeira de Freitas |
| RPS1 | Restored area 1 in Porto Seguro |
| RPS2 | Restored area 2 in Porto Seguro |
| RPS3 | Restored area 3 in Porto Seguro |
| S | Saplings |
| SD | Standard deviation |
| SPS3 | Saplings in Porto Seguro restored area 3 |
| STx1 | Saplings in Teixeira de Freitas native area 1 |
| Tx | Teixeira de Freitas |
| UNESCO | United Nations Educational, Scientific and Cultural Organization |

## LIST OF UNITS

| | |
|---|---|
| ha | hectares |
| m | meters |
| Mha | Million hectares |
| mM | millimolar |
| ng | nanograms |
| µM | micromolar |
| U | unit |

## 1. INTRODUCTION

Historically, ecosystems continuously provide essential services throughout human development. Wilderness forests harboring unique biodiversity levels were often used as seed, food, and medicinal compound sources, also contributing for hydrological regulation and carbon assimilation (DAILY et al., 1997; GUO; ZHANG; LI, 2010; KAREIVA, 2013; WATSON et al., 2018a). Alongside, forested environments contribute to soil protection and are essential to crop pollination (WATSON et al., 2018a). Nonetheless, anthropogenic actions detrimentally influence ecosystems dynamics, creating a disturbed environmental scenario where progressive human intervention continues to threaten forest structure (TAUBERT et al., 2018; WATSON et al., 2018a).

In this scenario, forests connectivity has been lost due to fragmentation (TAUBERT et al., 2018). Also, biodiversity and ecosystem processes, such as carbon assimilation and weather regimes, have been negatively impacted (FOOD AND AGRICULTURE ORGANIZATION, FAO, 2015; HOUGHTON; BYERS; NASSIKAS, 2015; WATSON et al., 2018a). In Bahia, the Atlantic Forest deforestation lead to local biodiversity erosion, affecting forest remnants ecological process (DODONOV et al., 2016; MORANTE-FILHO et al., 2016, PESSOA et al., 2017). Moreover, these changes may compromise forest species viability over time (NAMKOONG et al., 1996) and affect basic evolutionary processes as gene flow and genetic diversity (SANTOS et al., 2016; SEBBENN et al., 2011).

In fact, merely 18% of the remaining 40 mi km² Earth's forest area is free of human interventions (FAO, 2015; POTAPOV et al., 2017; WATSON et al., 2018a). Fortunately, Brazil is among the top five wildest countries, and holds an area of approximately two million square kilometers of intact forests (WATSON et al., 2018b). However, this area only represents one of the five Brazilians' biomes – the Amazon. The other biomes, such as the Atlantic Forest, have faced historical loss of natural habitats, generally associated with agricultural expansion and urbanization (CARDOSO; TABARELLI et al., 2000; JOLY; METZGER; TABARELLI, 2014; RIBEIRO et al., 2009a; TABARELLI et al., 2010). Currently, the Atlantic Forest represents only remnants fragmented sites covering only 28% of its original area, all in human-modified landscapes (REZENDE et al., 2018).

As degraded areas are not capable to maintain biodiversity and its ecosystemic functions, it is crucial to recover the past unprecedented value of forests, which can be achieved using restoration strategies. Ecosystem restoration creates an exceptional opportunity to reverse nature's damage and achieve an equilibrium between human development and environment conservation (BASTIN et al., 2019; BRANCALION et al., 2018; CHAZDON; BRANCALION, 2019; HUA et al., 2016). However, it reveals an urge necessity: restoration and its effectiveness in ecosystem functionality is not yet entirely addressed. Generally, restoration actions rely on different methodologies and strategies, and a variety of aspects tend to affect its success (such as previous land use, forest region, precipitation, time since restoration started (MELI et al., 2017). A crucial point is that restoration must attempt to return an ecosystem to its previous undamaged condition, in which the forest is capable of self-maintaining (SOCIETY FOR ECOLOGICAL RESTORATION INTERNATIONAL SCIENCE & POLICY WORKING GROUP, SER, 2004).

Because ecological restoration is a very complex subject, there is an urge requirement to comprehend most reliable restoration methodologies by indicators of all ecosystem aspects. When it comes to restoration success, we need to ensure, as much as possible, the establishment of viable populations that will survive and restructure community functionality. Genetic parameters are important indicators of remaining and restored forests that are related to species capability to adapt to changing environment, also defining the forest plasticity (FAO, 2015; WATSON et al., 2018a). When implanting restoration, species must contain genetic fitness (SER, 2004), with a diversity of alleles that drives the populations to survival, reproduction, recruitment, and adaptation. Genetic parameters are also helpful in defining the rapid establishment of long-term viable populations (LESICA; ALLENDORF, 1999). For instance, population survival is frequently associated with the gene pool adopted (BASEY; FANT; KRAMER, 2015; JALONEN et al., 2017; JORDAN et al., 2019). In implanting local seeds, suitable genes that occurred naturally in the habitat are present, and individuals are historically apt to survive in a habitat condition slight degraded. Also, the adoption of genetic diversity in high levels may favor the population fitness, with a higher probability of maintenance of useful alleles to survive by an extreme environment modification event (HAVENS et al., 2015; LESICA; ALLENDORF, 1999). Populations ability to persist is also related to genetics aspects over generations, in which occurrence of disturbance in one generation tends to reflect changes in alleles frequencies in its offspring (VRANCKX et al., 2011; YOUNG; BOYLE; BROWN, 1996).

However, little is known about restoration methodologies relating to genetics. Few studies in Brazil have shown the context of genetics in restoration sites (CORDEIRO et al., 2019; PAVINATO et al., 2017; SCHWARCZ et al., 2018; SUJII et al., 2017; VIANA et al., 2018; ZUCCHI et al., 2017a). Mostly, the findings suggest a pattern of high genetic diversity among restored forest trees, however presenting some inbreeding levels and reduced effective population size (SUJII et al., 2017). A simulation approach demonstrated that the number of seeds source can influence detrimental genetic effects, such as bottleneck, and the genetic diversity implemented is likely to influence inbreeding levels over generations (SUJII et al., 2019).

The adopted seed source methodology is also determinant to the restoration outcomes, once it directly defines the genetic constitution of the founding population (HAVENS et al., 2015; LESICA; ALLENDORF, 1999). In a local approach, seeds are collected strictly from remaining populations on the site, representing alleles that would normally occur due to nearby gene flow. However, considering the regional aspect, we could use a mix of seeds from close population, as well as in a more intermediate approach, in which seeds may represent long distance gene flow. A broader approach, with admixture provenance, has a potential representation of the genetic pool throughout the natural species range (BREED et al., 2015; BUCHAROVA et al., 2019; HAVENS et al., 2015). Nonetheless, all these approaches will be strongly affected by the number of trees used in seed collecting, the distance among them, and the coancestry between the mother trees (BASEY; FANT; KRAMER, 2015). Nurseries aspects is also determinant, once survival rates are strongly associated to genetic characteristics and provenance (MICHELE BOZZANO et al., 2014; THOMAS et al., 2014).

Likewise, biological characteristics of species and habitat conditions are important in designing a restoration project, especially in a genetic perspective (KRAMER et al., 2019; MICHELE BOZZANO et al., 2014; THOMAS et al., 2017). Besides, biological characteristics of species and habitat conditions are important in designing a restoration project, especially in a genetic perspective (KRAMER et al., 2019; MICHELE BOZZANO et al., 2014; THOMAS et al., 2017). In terms of reproductive systems, pollination services may be affected in anthropic modified areas, i.e. habitat isolation negatively affected interaction between plants and bees (PFEIFFER et al 2019). Nonetheless, in natural regenerated populations of a bee pollinated species, high levels of gene flow and long-distance movement were registered (CESPEDES et al., 2003). In the Atlantic Forest landscapes that included restored areas, Pfeiffer et al. (2019) suggested bees' flights

capabilities of more than 1 km, even when restored areas were included in anthropic matrixes. Habitat conditions may also influence restoration outcomes. For instance, in recently high disturbed habitats, implanting a local provenance may result in inbreeding with subsequently reduced potential to adapt to changing environment conditions (KRAMER; HAVENS, 2009).

Practical aspects of seedling production are also essential in restoration seed sourcing. In native wood species, nurseries commonly collect seeds in natural forests, but often face problems in finding several reproductive mother trees. Planted and restored forests are also used as seed sources (JALONEN et al., 2017). Altogether, this may result in a lack of genetic diversity, whereas few reproductive trees are sampled, or even closely related individuals from fragmented native forests. Therefore, the native seed supply market is based on inconstant seedlings availability, high costs and insufficient diversity (BASEY; FANT; KRAMER, 2015; FEDRIANI et al., 2019; JALONEN et al., 2017; URZEDO et al., 2019). More important, seedlings found in market are, in some cases, improper for reestablishing self-sustainable population due to the anthropic selection of genotypes (JALONEN et al., 2017; LESICA; ALLENDORF, 1999). Commercially important native species, such as the biological model of the present study, *Dalbergia nigra*, are more intensively selected, due to the adoption of vegetative propagation which favor desirable traits, and the creation of clonal mini-gardens to supply seedling production (MALAQUIAS et al., 2015; MELLO, 1996; SANTOS, 2019).

*Dalbergia nigra*, common named as Brazilian rosewood, is an endemic Atlantic Forest species, distributed throughout five Brazilian states. Currently endangered due to its habitat fragmentation and its wood exploitation, native populations are facing restricted gene flow and, consequently, increasing patterns of genetic structuration (BARRETO, 2010; BUZATTI et al., 2012; LEITE et al., 2014; RESENDE; RIBEIRO; LOVATO, 2011; RIBEIRO et al., 2005). The species presents hermaphrodites small flowers, mainly pollinated by bees and small insects, and dispersed by wind (CARVALHO, 1994). Brazilian rosewood is recommended in ecosystem restoration, especially because is adapted to degraded terrains (LORENZI, 2002), and has been broadly adopted in restoration efforts (AMAZONAS et al., 2011; MIRANDA NETO et al., 2014, SOUZA et al., 2009). It has the potential to contribute to soil recovery, with deposition of leaves on the ground and biological nitrogen fixation (AMAZONAS et al., 2011; CARVALHO, 1994; MIRANDA NETO et al., 2014). The implanted rosewood populations represent an opportunity to the species recovery overall, and studies have registered natural recruitment occurring under restored areas

(FERNANDES & BRAGA, 2018). The uncertainties in restoration genetics topics reveal a current need to better comprehend genetic impacts in restoration effectiveness. The use of molecular markers such as microsatellites to estimate the genetic parameters are an efficient method to achieve that. For instance, the occurrence of private alleles is an indirect indicator of gene flow among populations. The estimation of this parameters help to advance the genetic understanding of different restoration methodologies, providing knowledge to trace feasible strategies and addressing restoration successfully. In this scenario, we hypothesize that: (i) the gene pool of planted trees in restored forests differs from the surrounding remnant pool; (ii) there are genetic structure in restored populations, as they are influenced by the characteristics of the implanted restoration methodology; (iii) restored areas contribute to the reestablishment of connection between fragmented forests; (iv) restored populations have reduced genetic diversity and significant inbreeding levels.

Here, we aimed to investigate the contribution of restored forests to the re-establishment of genetically viable populations in Atlantic Forest in Southern Bahia, using as model an endangered tree native wood species, *D. nigra*. Specifically, we intend to:

(i) comprehend the genetic constitution of restored forests and conserved natural forests surrounding the restored areas;

(ii) describe structure and connectivity patterns of restored and native populations in a genetical perspective;

(iii) investigate the contribution of restored populations to the genetic aspects of an endangered native species, in a local and regional perspective;

# 2. LITERATURE REVIEW

## 2.1. The Atlantic Forest

The Atlantic Forest, a unique Brazilian biome, holds a global recognized biodiversity value (MITTERMEIER et al., 2004; MITTERMEIER; TURNER; LARSEN, 2011). It contains astonishing numbers of endemics species, and is the natural refuge of a high number of threatened species, including mammals, birds and trees (JOLY; METZGER; TABARELLI, 2014; MITTERMEIER et al., 2004). Legally, it has an extension of 130,973,638 ha, covering areas in 17 Brazilian states, that were originally covered with native vegetation (BRASIL, 2006, 2008; FUNDAÇÃO SOS MATA ATLÂNTICA, 2018; IBGE, 2012).

Several phases of human expansion, dated since the arrival of the first Portuguese have led to a stage of extremely degradation in the Atlantic Forest ecosystems (CARDOSO; TABARELLI, 2000; JOLY; METZGER; TABARELLI, 2014; TABARELLI et al., 2010). Currently, 145 million people live in the Atlantic Forest region, resulting in an anthropic area equivalent to 65% of its total extension (FUNDAÇÃO SOS MATA ATLÂNTICA, 2015). In these human-modified landscapes, deforestation rates are constantly increasing the fragmentation of remaining forests. The unfortunate outcome is a reduced native vegetation cover area of only 36 Mha (REZENDE et al., 2018). On top of that, merely 2.6% of its scarce remaining areas are protected under conservation efforts (FUNDAÇÃO SOS MATA ATLÂNTICA, 2015). Under this perspective, the remaining Atlantic Forest is considered one of the 36 recognized biodiversity global hotspot (CRITICAL ECOSYSTEM PARTNERSHIP, 2001; MITTERMEIER et al., 2004; MYERS et al., 2000).

Regardless of fragmentation, the Atlantic Forest still shelters significant threatened species (CRITICAL ECOSYSTEM PARTNERSHIP, 2001; JOLY; METZGER; TABARELLI, 2014; MITTERMEIER et al., 2004). This species diversity is distributed throughout the complex environmental variation of this domain, in which six biogeographical sub-regions have been proposed (SILVA; CASTELETI, 2003).

The Bahia bioregion cover approximately 121,000 ha, and its southern region is recognized as a center of endemism due to the astonishing high levels of unique species. The Central Corridor of Atlantic Forest represents about three quarters of the Bahia bioregion, and holds 45 Conservation Units, including areas of integral protection and areas of sustainable use (BRASIL, 2006;

CRITICAL ECOSYSTEM PARTNERSHIP, 2001; SEI, 2011).

Not surprisingly, the Atlantic Forest also holds the most extensive area of tropical rainforest classified as a restoration hotspot, contributing to almost 67% of the Brazilian hotspot restoration total area (BRANCALION et al., 2019). Also, the Bahia interior forests represent the most important ecoregion for tropical forests restoration, totalizing a restoration hotspot area of almost 20 Mha. An additional 6.6 Mha for the Bahia coastal forests consolidate the undoubtable restoration value of this region (BRANCALION et al., 2019).

### 2.2. The potential of restoration

Under the scenario of degraded ecosystem, remaining forests are noteworthy functional areas that support biodiversity, and provide resilience to environmental change (WATSON et al., 2018a). Assuredly, these forests are reference areas and primary sources of seeds (MACKEY et al., 2015). Thus, strategies to reduce pressure and enrich knowledge about these forests must be well addressed. For instance, positive effects on ecosystem, as a decrease in the isolation of remaining fragments, could be achieved by addressing reforestation and reducing deforestation rates (TAUBERT et al., 2018). Reforested areas can contribute to new forest patches and biological corridors between conservation areas (CHAZDON, 2017; CHAZDON; GUARIGUETA, 2018).

Landscape connectivity can be achieved by restoration of priority areas, especially the ones that present high potential for regeneration (intermediate resilience) (TAMBOSI et al., 2014). Studies that help to quantify patterns on these regenerated areas can subside future management decision on possible priority areas for landscape connectivity. Moreover, this interconnectivity facilitates gene flow, and might act to mitigate the decreased distance of gene flow that follows loss of forest cover, as demonstrated by Santos et al. (2016).

Restoration efforts are not merely beneficial to conservation of the remaining forests but may be the only opportunity to ensure biodiversity conservation and environment stability over time. For instance, the remaining conserved forests are not sufficient to achieve the minimum standard for biodiversity conservation proposed by the United Nation's Strategic Plan for Biodiversity – a value equivalent to 17% of the areas across the globe to be protected (MAPPIN et al., 2019; UNCBD, 2012). Basically, this target can only be achieved if at least 190 Mha of degraded land across the globe are restored (MAPPIN et al., 2019).

Reforestation adopted with sustainable development goals can not only mitigate human impacts but also provide substantial economic return over time. One financial opportunity is a system of Payments for Ecosystem Services, already active in Brazil for water, in which landowners receive a revenue for actions regarding water quality (SALZMAN et al., 2018). Another considerable economic contribution can be achieved by farming practices, in which an agricultural sustainable model confluence production and ecosystem conservation. Financial outcomes of restoration initiatives often are associated to wood and non-wood forest products, ecotourism income, agricultural products, carbon sequestration and the avoided food security costs (BENINI; ADEODATO, 2017; MOHR; METCALF, 2018; VERGARA et al., 2016).

A significant contribution of restoration regarding current environment concerns is carbon sequestration and storage. The United Nations Framework Convention on Climate Change approved in 2015 the Paris Agreement, recognized the urgent requirement of cooperation by all countries to reduce global greenhouse gas emissions (UNITED NATIONS, 2015). Brazil had pledged its main strategies through the Intended Nationally Determined Contribution (INDC): to reduce the greenhouse gas emissions by 37% until 2030 (below 2005 levels). Also, Brazil intends to restore 12 million hectares of forest areas, prioritizing the native species planting, and an additional 15 million hectares of degraded pasturelands by the same year (BRASIL, 2015, 2017). To achieve it, the government has established a set of public policies, such as a National Plan for Native Vegetation Recovery (Planaveg) (BRASIL, 2017). Planaveg main strategies include actions to promote the productive chain of native seeds and seedlings, and researches associated with native restoration efficiency (BRASIL, 2017). Additionally, Planaveg aims to intensify sustainable agriculture and protect the remaining forest vegetation.

Brazil, in its INDC, emphasizes some further measures to be adopted, such as measures to enforce and strength the new Forest Code (Law n. 12.727/2012) implementation (BRASIL, 2015). The Forest Code requires the recovery of illegally deforested and environmentally sensitive areas (Areas of Permanent Preservation), which in nowadays means restoration actions in almost 21Mha (SOARES-FILHO et al., 2014). Of this total, Bahia State contains 71,790 ha of Atlantic Forest environmental debt areas (SOARES-FILHO et al., 2014), that must be addressed to restoration purposes. When only riparian areas are considered, the Atlantic Forest vegetation could be scaled up with the recovery of 5.2 Mha of legal debt (REZENDE et al., 2018).

Recent studies have shown that Brazil is the top one country regarding restoration

opportunities to tropical rainforests – it holds a hotspot restoration area of 61.37 Mha, which represent an area of high potential to successful implant restoration and to maximize its benefits (BRANCALION et al., 2019). Accordingly, using another approach, Bastin et al. (2019) has shown that Brazil holds a potential area for restoration of 49.7 Mha. However, these data only exemplify the opportunity Brazil has to achieve its pretentious goals. To turn it into actions, effective measures to implement ecosystem restoration have to be adopted.

In order to recover this debt, the Atlantic Forest Restoration Pact (AFRP) was created, alongside several restoration efforts. It was developed as a national pact to achieve optimal results for the biodiversity conservation through the Atlantic Forest restoration. The Pact has the ambition to restore 1500 Mha of the Atlantic Forest by 2050 (MELO et al., 2013; PINTO et al., 2014). To achieve it, AFRP integrate reforestation efforts and projects of several affiliated stakeholders, including ones from public and private sector, government agencies, NGOs, companies and landowners. Mainly, the adopted strategy is to disseminate information and knowledge on restoration, thus representing an exceptional opportunity to expand local restoration outputs through all the Atlantic Forest areas (BRANCALION et al., 2013; MELO et al., 2013; PINTO et al., 2014; PREISKORN; COUTO, 2009; RODRIGUES; BRANCALION; ISERNHAGEN, 2009).

Since 2015, AFRP has committed to the Bonn Challenge, a global effort to promote restoration across the globe. An approximately value of 707,032 ha of the Atlantic Forest area has been registered as restored between the years 2011-2015, of which 84,827 ha were detected in Bahia (CROUZEILLES et al., 2019).

Figure 1. A restored Atlantic Forest area in Southern Bahia, Brazil, with adults and young individuals of *Dalbergia nigra*.

### 2.3. Restoration methodologies

A diversity of restoration methods is available (IUCN; WRI, 2014; NAVE et al., 2015, RODRIGUES; BRANCALION; ISERNHAGEN, 2009; SER, 2004). However, considering the Brazilian scenario, two approaches are mostly adopted – passive restoration, and active restoration (BRANCALION et al., 2016). Active restoration depends on human interventions, involving actions as covering the entire area with several native tree species by direct seeding or seedling plantation and is an approach essential to sites where the lack of seeds (soil seed bank or seed dispersal) is present (BRANCALION et al., 2016, VERGARA et al., 2016). On the other hand, passive restoration, which is mainly the allowance of natural regeneration, involves actions to isolate the area from anthropic disturbance and stimulate the spontaneously seedling regeneration (BRANCALION et al., 2016; VERGARA et al., 2016). A meta-analysis comparing these two

methods (CROUZEILLES et al., 2017) found that restoration success for biodiversity and vegetation structure is higher in natural regeneration than in active restoration. Indeed, biodiversity values of regenerated forests tend to be similar to the ones in old growth forest under some conditions (e.g. less intensive disturbed areas) (LATAWIEC et al., 2016). Moreover, density of species and individuals may vary in these approaches (BRANCALION et al., 2016). Nonetheless, both restoration approaches provide favorable actions to restoration, and the complementarity of these methodologies can lead to the achievement of ecosystem values similar to the ones of native forests (CROUZEILLES et al., 2017).

Essentially, no general formula can be recommended. Restoration relies on several aspects, such as plant arrangements, site conditions, ongoing management and species used (FEDRIANI et al., 2019; FUNK et al., 2012; MCCALLUM et al., 2018; RODRIGUES; BRANCALION; ISERNHAGEN, 2009; SER, 2004). All these possible influences emphasize the importance of associating restoration methods with previous knowledge of the site and the species used to adequately achieve the most valuable outcome: a restored ecosystem.

### 2.4. Genetics to recover the ecosystem

Overall, an important step to subside strategies that reach global and Brazilian government conservation and restoration purposes is to understand genetic parameters of conserved forest remnants, as well as the influence of the restored approach on these parameters. Actions helping to define patterns to better support forest restoration methodologies are also required.

A great amount of studies emphasizes the essentiality of genetic diversity, although most of them evidenced similar values for heterozygosity in restored and native areas (DEWALD; KOLANOSKI, 2017; SUJII et al., 2017; VIANA et al., 2018; ZUCCHI et al., 2017a). Nevertheless, decreases in allelic richness have been reported with the reduction of same-species trees in the surrounding landscape (DEWALD; KOLANOSKI, 2017). Losses of private alleles in restored populations were also registered (SCHWARCZ et al., 2018). Primarily, in active restoration, the risk of implementing genotypes with similar alleles is expected, and it can lead to the reduction of private alleles frequency and consequences similar of bottleneck effect (SCHWARCZ et al., 2018). It arises a worrisome question: is genetic diversity enough to ensure viable populations for environment restoration?

Schwarcz et al. (2018) accessing the intrapopulation genetic structure of an Atlantic Forest

specie's, in active restored and natural populations, found that while native forests had genetic clusters, only in near borders or in human intervened areas, restored sites had genetic structure along its area. In fact, these findings reinforce the anthropic influence in forests and the importance of studies to adequately implement restoration efforts.

In active restoration, seed source is a key factor to determine the restoration outcomes (FEDRIANI et al., 2019; MASSATTI et al., 2018; WATSON et al., 2018b). Commercial germplasm sources may represent a reduced fraction of the overall genetic diversity found in natural populations, and need to be considered alongside migration rates (MASSATTI et al., 2018). Contrastingly, the use of mixed seed sources in nurseries can result in increased values of genetic diversity, when compared to undisturbed native populations (BUCHAROVA et al., 2019). Generally, the genetic characteristics of the first-generation trees (implanted individuals) will determine two essential points for the population survival: its potential to adapt and to reproduce (AAVIK; HELM, 2018; FEDRIANI et al., 2019). Over generation, inbreeding rates and bottleneck occurrence in reintroduced populations lead to reduction of seed quantity and quality, as well as seedlings survival rates (THOMAS et al., 2015). These deleterious effects can be substantially aggravated under harsh environment conditions, as a consequence of its reduced fitness.

Currently restoration approaches suggest the incorporation of genetic connectivity in restoration planning, to increase the likelihood of restoration success (MCRAE et al., 2012; PROFT et al., 2018). Undoubtingly, connectivity with the surrounding landscape should be considered in restoration planning. Enhancing connectivity through gene flow potentially increase the effective population size (PROFT et al., 2018), also contributing for a landscape-level restoration (AAVIK; HELM, 2018). Noteworthy, landscape permeability is likely to influence the genetically effective connection (THOMAS et al., 2015; KRAMER et al., 2019).

### 2.5. Beyond restoration, recovering an endangered species

The Brazilian rosewood, also known as jacaranda-da-bahia, is an ecological and commercial highly prized Atlantic tree species occurring across five Brazilian states. *Dalbergia nigra* (Vell.) Allemao ex Benth. is an endemic specie of the coastal Atlantic Forest, and belongs to the Fabaceae family (Papilionoideae), with dry pod fruits, typical of this family. It is a perennial deciduous plant that occurs in dense primary and secondary forests, usually found in well-drained slopes (LORENZI, 2002). It is described as a pioneer species by Lorenzi (2002), and as late

secondary by Carvalho (1994) and Sambuichi et al. (2009). It is a hermaphrodite species, whose flowers are pollinated by bees (RIBEIRO et al., 2005) and other insects, during its flowering stage, between October and November. Fruits are produced from July to September, and are dispersed by the wind (SAMBUICHI et al., 2009; CARVALHO, 1994). Moreover, the species is described as ideal for plantation in degraded land, mixed with other species, due to its adaptation to dry lands (LORENZI, 2002). *D. nigra* has an aggregated distribution pattern (COSTA; SCHIAVINI; OLIVEIRA, 2011). Although the species reproduction system is not yet characterized, some authors suggest a predominantly outcrossing pattern (BUZATTI et al., 2012; RESENDE; RIBEIRO; LOVATO, 2011; RIBEIRO et al., 2005).


Figure 2. Brazilian rosewood (*Dalbergia nigra*) trees in A) a restored site and B) a native remnant Atlantic Forest domain in southern Bahia, Brazil.

Alongside habitat fragmentation, illegal logging caused drastic reduction in the species population size, now mostly reported as small fragmented populations with low genetic variability (RIBEIRO et al., 2005). *D. nigra* is considered vulnerable according to the IUCN Red List (VARTY, 1998) and is the only rosewood included in the CITES appendix I (CITES, 2017; UNDC, 2016). This attempt to limit the species trade therein demonstrates the urge need for its protection.

In Brazil, the species is also listed as vulnerable in a national status (Brazilian "Official National List of Endangered Flora Species") (BRASIL, 2014; MARTINELLI, 2013). Recently, a regional list of endangered species in one of these states updated the BR status from vulnerable to critical threatened (SEMA-SP, 2016), emphasizing the concern with the species conservation. Additionally, *D. nigra* regeneration is limited by seed predation (CARVALHO, 1994). Nonetheless, regeneration under restored areas was registered by Fernandes & Braga (2018).

Figure 3. Regenerated individual of *Dalbergia nigra* in a restored are in the Atlantic Forest domain in southern Bahia, Brazil.

Although distributed through a vast Atlantic Forest domain, *D. nigra* showed patterns of haplotype genetic differentiation during the Middle Pleistocene, resulting in three phylogeographic distinct groups. The Bahia populations were uniquely similar to the northeast Minas Gerais populations (BUZATTI et al., 2012). In considering nuclear DNA, the genetic status of *D. nigra* remaining population revealed a tendency of detrimental anthropogenic effects on genetic parameters, in which lower levels of genetic diversity were associated with higher anthropic

pressures (RESENDE; RIBEIRO; LOVATO, 2011). Additionally, spatial genetic structure was registered in well conserved and fragment remaining populations (BUZATTI et al., 2012; LEITE et al., 2014).

Hence, restoration represents a remarkably opportunity to reestablish genetic viable populations of *D. nigra*. Introduced individuals can also facilitate gene flow between previously isolated *D. nigra* populations, specially over distances higher than 89-144 m, which is the documented gene flow distance for the species (RESENDE; RIBEIRO; LOVATO, 2011).

## 3. OBJECTIVES

Here, we aimed to investigate the contribution of restored forests to the re-establishment of genetically viable populations in Atlantic Forest in Southern Bahia, using as model an endangered tree native wood species, *D. nigra*. Specifically, we intend to:

(i) comprehend the genetic constitution of restored forests and conserved natural forests surrounding the restored areas;

(ii) describe structure and connectivity patterns of restored and native populations in a genetical perspective;

(iii) investigate the contribution of restored populations to the genetic aspects of an endangered native species, in a local and regional perspective;

## 4. METHODOLOGY

### 4.1. Study area and sampling

This research was conducted in the Atlantic Forest domain located in southern Bahia, Brazil. Several sites located in the Central Atlantic Forest Ecological Corridor, across an Atlantic Forest Key Biodiversity area, were sampled. The sites included restored and native remnants areas across the regions of Teixeira de Freitas, Porto Seguro, Igrapiúna and Ilhéus at the Bahia State (Figure 4).

A previous analysis of the sites was conducted to define the areas to be sampled. Dataset provided by the companies, and satellite images were used to elaborate maps in QGIS Version 2.18.21 LTR (QGIS Development Team, 2018). Then, most suitable restored areas that have nearby land cover with native forest were filtered. The distance among these areas were

determined. Most importantly, only areas in which *D. nigra* was listed as planted in the restoration project were considered (according to the dataset). From this outcome, potential sites to be sampled were defined, then ground-checked to assess the presence of the studied species, in means of the density of adults and young individuals within a restored site.

During the field-checking, 49 previously defined potential sites were visited with a local forest worker with knowledge of the region and in identifying Atlantic's Forest species, to assist in the species recognition. The number of *D. nigra* individuals in each location was checked based on an active search within the boundaries of the site. Additionally, the land use history of each sites was investigated (vegetation type pre-restoration, and restored forest age).

In total, from the 49 checked sites, 17 restored and 5 remaining Atlantic Forest were sampled (SISBIO license for biological tissue collection #67445-1). These areas belong to four planted forest-based companies and to Conservation Units (Figure 4). We collect adult trees in each area, and to represent younger generations, saplings were collected when found. Each sampled individual was identified, geo-referenced and its steam diameter (ground height) and height were measured. Vegetal material (leaves or wood cambium) were collect and kept in identified paper bags, and stored in a -20°C freezer in the Molecular Markers lab at Centro de Biotecnologia e Genética of the Universidade Estadual de Santa Cruz.

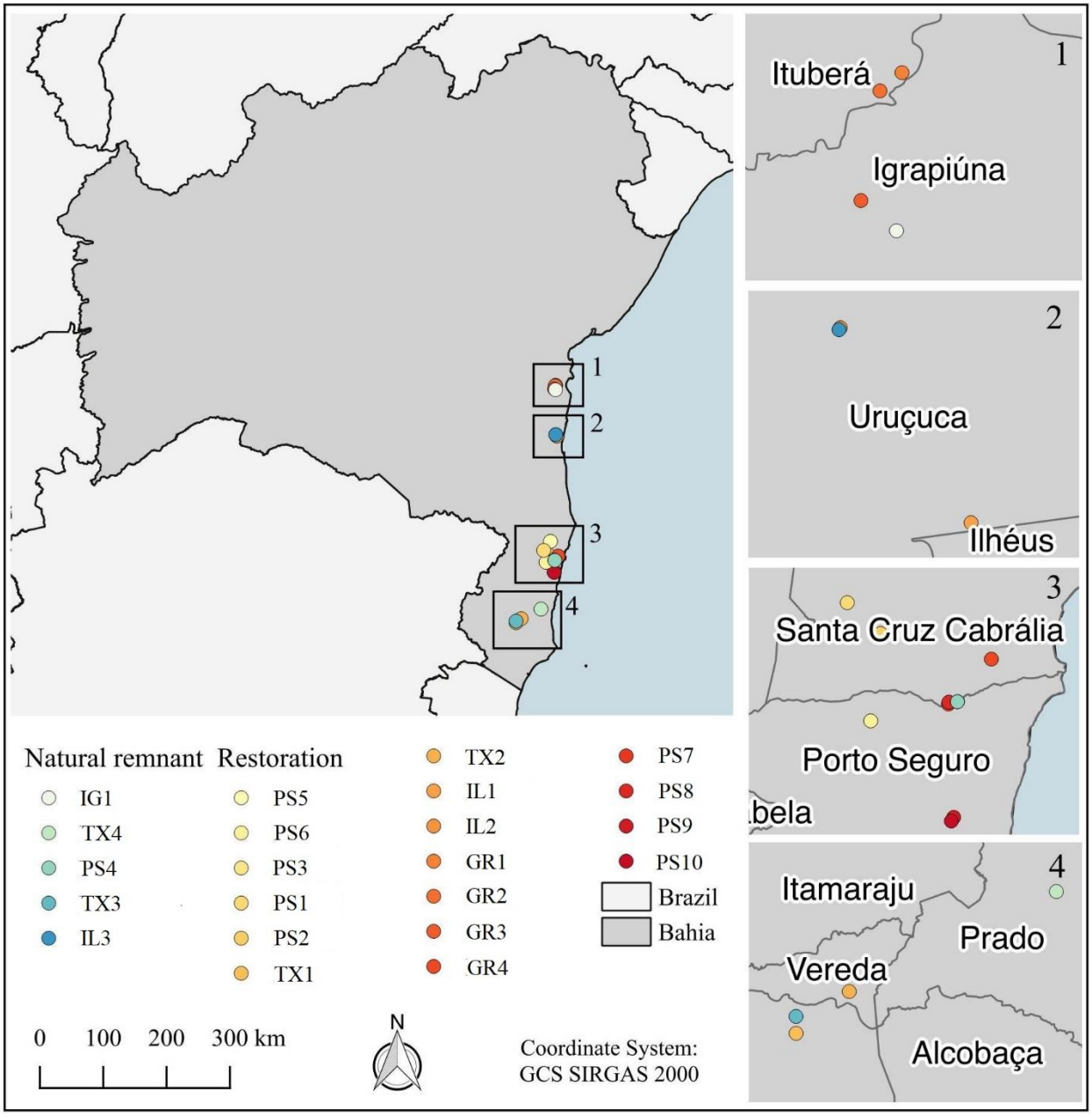



Figure 4. Location *D. nigra* sampled sites across the Atlantic Forest Biome. Rectangle represents the zoomed-in sub-region, where 1) Igrapiúna region (GR); 2) Ilhéus region (IL); 3) Porto Seguro region (PS); 4) Teixeira de Freitas region (TX). Green-blue scale represents the Natural Remnant Atlantic Forest populations, while yellow-red scale represents the restored sites.

Due to laboratory unforeseen events as cited on results below (4.1. Optimization of procedures for *D. nigra*), this study does not include genetic analysis for all sampled sites. Thus, further analyzes were conducted for nine sampled areas. These nine sites are distributed across three regions in southern Bahia - Ilhéus (IL), Porto Seguro (PS) and Teixeira de Freitas (Tx). The

Ilhéus region, located at the southern coast, include two restoration sites and a natural remnant, all within the boundaries of Conservation Unit (Serra do Conduru State Park). Porto Seguro included a World Heritage Natural Site recognized by UNESCO – the Veracel Station Private Natural Heritage Reserve, and two restored sites. Teixeira de Freitas region has two restoration areas and a native remnant experiencing initial succession. In total, 12 populations were analyzed, because some sites had adults and saplings evaluated (Table 1). Here, the term "population" is used to describe individuals that share a common evolutionary history or identical historical restoration conditions.

Table 1. Description of study sites across the Atlantic Forest Biome for *Dalbergia nigra*

| *Study area* | *Region* | *Coordinates (UTM, WGS84)* | *Type* | *Implantation year* | *Sample size* | | *Description* | *Partner* |
|---|---|---|---|---|---|---|---|---|
| | | | | | *Adults* | *Saplings* | | |
| IL1 | Ilhéus, BA | -14.529626, -39.137937 | Restoration | 2010 | 26 | 0 | Mixed native species from local source, nucleation method, previous anthropic area, protected since 1998, 22 ha | Instituto Floresta Viva/ Serra do Conduru State Park |
| IL2 | Ilhéus, BA | -14.506608, -39.153126 | Restoration | 2012 | 22 | 0 | Mixed native species from local source, abandoned sugar-cane field, with formerly anthropic area surrounding | Instituto Floresta Viva/ Serra do Conduru State Park |
| PS1 | Porto Seguro, BA | -16.239799, -39.334807 | Restoration | 1994 | 30 | 0 | Mixed native species with seedlings from a conserved Atlantic Forest remnant from ES, 11.06 ha | Veracel Celulose S/A |
| PS2 | Porto Seguro, BA | -16.286175, -39.284092 | Restoration | 1994 | 25 | 30 | Mixed native species with seedlings from a conserved Atlantic Forest remnant from ES, 21.8 ha | Veracel Celulose S/A |
| PS3 | Porto Seguro, BA | -16.388863, -39.168336 | Restoration | 1997 | 26 | 0 | Mixed native species using regional seedlings, with recurrent enrichment, 2.25 ha, inside a RPPN | Veracel Celulose S/A |
| Tx1 | Teixeira de Freitas, BA | -17.323036, -39.746638 | Restoration | 2010 | 28 | 38 | Mixed native species in between remaining native trees, with subsequent enrichment | Suzano Papel e Celulose |
| Tx2 | Teixeira de Freitas, BA | -17.258869, -39.671261 | Restoration | 2010 | 27 | 19 | Mixed native species, subsequent enrichment | Suzano Papel e Celulose |
| IL3 | Ilhéus, BA | -14.506335, -39.153349 | Native remnant | - | 24 | 32 | Serra do Conduru State Park, protected since 1997. Formerly anthropic disturbances | Instituto Floresta Viva/ Serra do Conduru State Park |
| Tx3 | Teixeira de Freitas, BA | -17.29675, -39.747705 | Native remnant | - | 23 | 0 | Conservation area in initial successional stage | Suzano Papel e Celulose |

### 4.2. Laboratory procedures

Genomic DNA extraction followed Doyle & Doyle (1987) CTAB protocol. Previous results showed a sufficient quantity of DNA, although with an unstable quality – dark DNA. When tested for DNA amplification, no products were obtained. To avoid this problem, two DNA extraction were tested, a modified CTAB method recommend for Leguminosae trees steam bark (NOVAES; RODRIGUES; LOVATO, 2009) and a pre-washing method for species with high levels of secondary compounds (INGLIS; PAPPAS; GRATTAPAGLIA, 2016).

We tested ten microsatellite markers previously developed for *D. nigra* (RIBEIRO et al., 2009b). Several tests had to be conducted in order to determine the most adequate protocol for each primer. Nuclear DNA was amplified using 0.25 mM dNTPs, 1.5 mM $MgCl_2$, 1X PCR Buffer (pHneutria I0 - 100 mM $(NH_4)_2SO_4$, 100 mM KCl, 100 mM Tris-HCl pH 8.4, 1% Triton X-100), 0.25 mM BSA, 0.06 µM of forward primer and 0.12 µM of reverse, 1 U of Taq DNA polymerase, 0.4 µM of M13 fluorescent-labeled primer and 7.5ng of nuclear DNA. PCR reactions were realized in Life Pro thermocycler (Bioneer Technology Co., China), with a specific amplification temperature for each primer. To conclude, we perform the separation of SSR fragments through capillary electrophoresis by automated DNA analyzer ABI3500 Genetic Analyzer (Applied Byosystems, USA). Genotyping was performed with the help of GeneMarker software (SoftGenetics, USA).

### 4.3. Genetic and statistical analyses

Random genotyping failure and null alleles may influence genetic estimations depending on its intensity (CHYBICKI; BURCZYK, 2009; DABROWSKI et al., 2014; DEWOODY; NASON; HIPKINS, 2006; POMPANON et al., 2005). Hence, we characterized the possible biases in our dataset due to its influences. The presence of null alleles and genotyping failure was checked in a Bayesian model approach, following the Vogl et al. (2002) procedure in the INEst 2.2 software (CHYBICKI, BURCZYK, 2009). The model accounts for null allele presence, random genotyping failure and inbreeding effects influencing the dataset. For each population, the model with smallest Deviance Information Criterion (DIC) was adopted as the better fit to the population data (Supplementary table 2). INEst also provided estimation of unbiased $F_{IS}$ (corrected for null alleles). For all Bayesian analyses 1,000,000 Marcov Chain Monte Carlo iterations, thinning of 100,000 cycles and 100,000 burn-in. The corrected allele frequencies were used to subsequent analysis.

Null allele frequencies were also estimated by Van Oosterhout estimator in the program Microchecker (VAN OOSTERHOUT et al., 2004), and the corrected genotype dataset was adopted for individual analysis. A random sampling of genotypes versus loci was produced to determine the minimum number of multilocus genotypes necessary to distinct between individuals (KAMVAR et al., 2014).

All genetic and statistical analyses were conducted in the R software version 3.6.1 using the packages *adegenet* (JOMBART, 2008), *pegas* (PARADIS, 2010) and *poppr* (KAMVAR et al., 2014). To characterize the genetic diversity and genetic pool of all study areas, we estimated the following standard genetic parameters: average number of alleles per locus, allele richness, number of private alleles, observed and expected heterozygosity, and fixation index according to Nei's statistics. Adherence to Hardy-Weinberg equilibrium proportions was estimated in a bootstrap approach with *pegas* package (PARADIS, 2010). Nei's fixation index ($G_{ST}$) and genetic distance were also calculated following Nei (1973) in *hierfstat* package (GOUDET, 2005). Statistical comparison was performed with Kruskal-Wallis Test in the *rstatix* package (ALBOUKADEL, 2019) in R.

The genetic structure between populations in a broad-scale was investigate by means of a Discriminant Analysis of Principal Components (DAPC) in the *adegenet* package in R. The number of principal components to retain was determined by the optimum a-score. In order to detect differences in ancestry among restored population, and differences in ancestry among ontogenetic samples within restored versus nearby native forest remnant we conducted a second DAPC, to characterize the actual number of clusters of all populations, according to its genetic component. By using a membership probability approach for this DAPC result, individuals were grouped in each pre-defined population and the total admixture of each population was computed. The ideal k was determined by a Bayesian Information Criterion (BIC) method implemented in adegenet.

In order to comprehend the cited parameters in the restoration context, two main approaches were adopted to conduct data analysis: (i) the uncover of the populations past genetic characteristics, which represents introduced individuals for restored sites and previous non-fragmented populations in native sites; (ii) unravel the genetic characteristics of saplings, which represent the current genetic patterns of the populations. Then, the genetic parameters of

ontogenetic stages within populations were compared, as well as between populations in the same ontogenetic stage, by a Welch t-test in R version 3.5.0 (R CORE TEAM, 2018).

The influence of the distance between areas was investigated by an isolation by distance approach in *adegenet*, in which a matrix of individual geographic distance was compared with a matrix of genetic distance using Mantel test.

## 5. RESULTS

### 5.1. Optimization of laboratory procedures for *Dalbergia nigra*

Genomic DNA extracted with different protocols showed contrasting patterns for DNA quality, although with sufficient quantity of DNA. Doyle & Doyle protocol resulted in unstable DNA, in a dark colour, showing no products of DNA amplification when tested. The DNA extraction protocol of Inglis et al. (2016) resulted in DNA of quality, with no degradation in agarose gel, with a mean 260/280 ratio of 1.89.

From the ten *D. nigra* microsatellite markers (Ribeiro et al., 2008) tested for the original protocol, eight showed amplification products, all with a percentage of PCR fails. Thus, the primers were tested for several times changing variables that could influence the PCR performance as temperature amplification, primer concentration, $MgCl_2$, and DNA concentration. After the set of tests, seven primers satisfactorily amplified the *D. nigra* DNA, resulting in a mean amplification rate of 51.4%.

General recommendations to minimize statistical errors in microsatellite analyses includes PCR replication (BONIN et al., 2004; GAGNEUX, 1997; HOFFMAN; AMOS, 2005; POMPANON et al., 2005). Thus, in order to ensure data quality and eliminate missing data, the PCR procedure was repeated for 164 samples that showed genotyping information for less than five primers. This steep resulted in some locus showing a higher amount of PCR fails than others: primers *Dnig1*, *Dnig3* and *Dnig5*, with a PCR fail frequency of 0.387, 0.534 and 0.375, respectively. Published studies have shown that some loci may present higher probabilities of amplification fails, due to problems in the binding-steep and in the primer annealing efficiency (BUCHAN et al., 2005; WANG; SCHROEDER; ROSENBERG, 2012). These failed samples and locus were amplified once more, resulting in a dataset of 231 genotyped individuals for seven loci, with an amplification rate over all loci of 68.9%.

Besides, we checked for the influence of missing genotypes and null alleles occurrence. An approach broadly adopted to correct errors in allele amplification is the estimation of allele frequencies by statistical adjustments using computer simulations (CHAPUIS; ESTOUP, 2004; SÉRÉ et al., 2017; WANG; SCHROEDER; ROSENBERG, 2012). By means of a Bayesian analysis and a Monte Carlo simulation, we ensured that the allele copies that fail to amplified (for both alleles – missing genotypes or for one allele – null alleles) were accounted, quantified and corrected. It resulted in a databased of 193 individuals, with 12 populations, each individually corrected for null alleles, with a mean amplification rate over all loci of 76.68% (Supplementary Figure 1), genotyped for seven loci. All loci that showed a percentage of missing data equal or higher than 70% in a population were excluded. Thus, the saplings from PS region (SPS2) were evaluated for 6 primers, RIL1 and RIL2 for 5 primers and RIL2 for four.

Null alleles were present in all loci, with frequencies varying in each site (Table 2). The mean null allele frequency across loci ranged from 0.14 to 0.4, and *Dnig1* and *Dnig3* showed the highest values (0.28 and 0.27, respectively). Our models suggest small frequencies of estimated genotyping failure (RTx1=0.203, RTx2=0.014, NTx3=0.143, STx1=0.319, RPS1=0, RPS2=0.022, RPS3=0.24, SPS3=0.312, RIL1=0.217, RIL2=0.235, NIL3=0.177, SIL3=0.29)

Regardless of the missing data, an ideal number of locus to effectively discriminate between unique individuals is achieved with six loci (Figure 5).

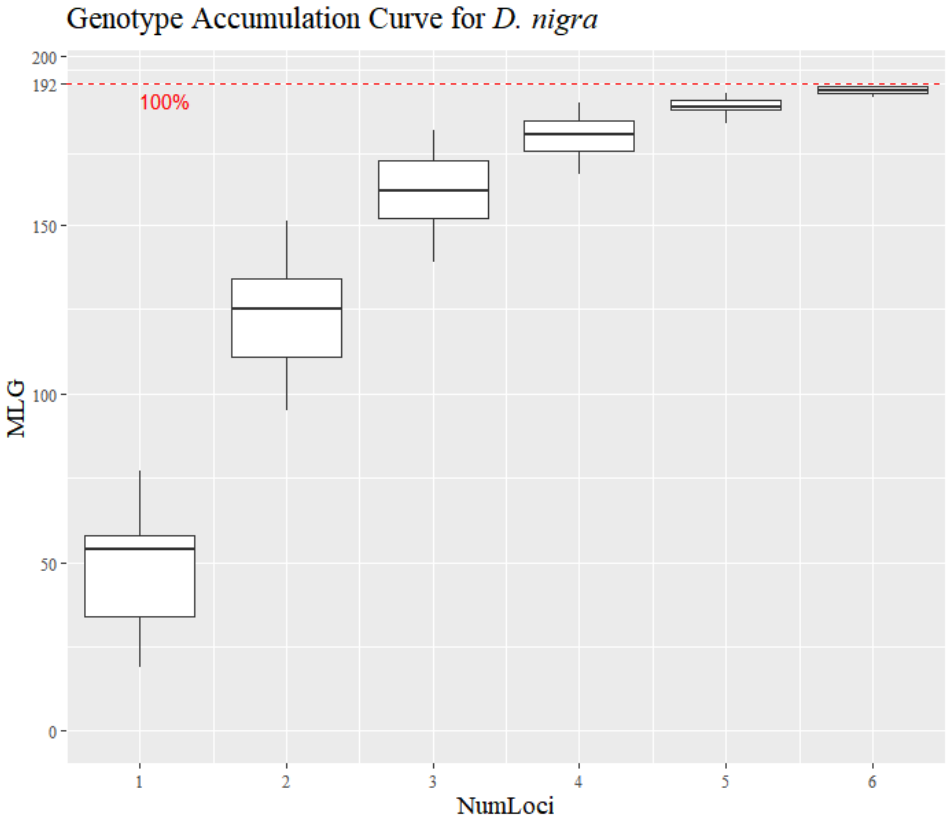



Figure 5. Estimation of the minimum number of loci to discriminate a unique individuals given a random sample of n loci. Horizontal axis: number of loci randomly sampled without replacement; vertical axis: unique multilocus genotypes observed for n loci.

### 5.2. Genetic differentiation

The reassemblance DAPC followed an α–score optimization, with 20 informative principal components retained (Supplementary Figure 4). It revealed that eight genetic clusters ideally represent all sampled individuals (k=8, Supplementary Figure 6). These clusters characterize different groups of gene pool that compose the whole genetic diversity of the sampled populations. Native areas, overall, were composed of five main gene pools, with one in a low percentage (Figure 6b). Restoration was the most diverse, containing seven out of the eight gene pool found. One group was exclusive present in this type of forest (k4). Younger generations mainly represent a mix of all gene pools, having two groups that were found only in adults, one of restored areas (k4), and another found exclusively in native areas (k8).

In a regional perspective, IL, PS and Tx demonstrated different patterns of genetic constitution, in which restored populations are more similar to native ones in the same region

(figure 6). Nevertheless, all regions shared at least one gene pool. Tx is the most genetic distinct region, characterized by the presence of one gene pool (k7) within all populations. Also, the two pools found only in adults are exclusive from this region, one present in both restored populations (k4) and another in the native (k8). PS and IL share a genetic background (k6) and are less structured. In PS, all populations are constituted, at least partially, by k3 and k6. IL is predominantly represented by one gene pools, k5, also having a considerable percentage of k6 in all adults populations. Our results also indicate local patterns of structure. In Tx, restored populations were diverse, with a mix of five gene pools representing RTx1, and 6 the RTx2, while the native population were composed of only two pools. Also, restored populations had a pool found only in restored sites of PS implanted with seeds from ES. In Porto Seguro, the two restoration implanted with seeds from ES (RPS1 and RPS2) shared a similar percentage of the same four pools (k1, k2, k3 and k6), while RPS3 had an only two of those gene pools (k3 and k6). Saplings had similar genetic constitution than the same-site adults, except for the Tx region. At this region, saplings had a gene pool found exclusively in the Tx native area, also having a percentage of a gene pool that was present in nine other restored areas (k6), such as RTx2, but not in its original adults RTx1. In IL, saplings presented a gene pool that was not observed in adults of this region – k2.

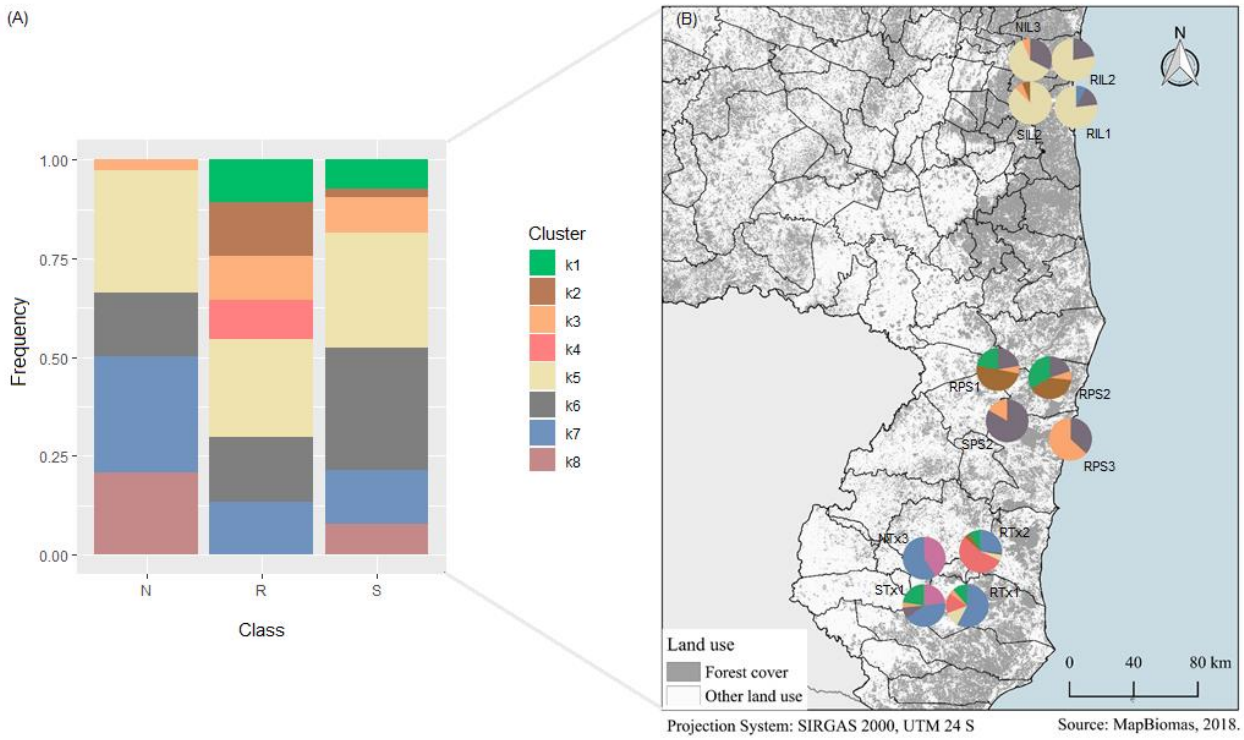



Figure 6. Membership probability of each sampled site according to the genetic clusters obtained in a Discriminant Analysis of Principal Components (DAPC) of 196 *Dalbergia nigra* genotypes in Southern Bahia. Colors represent the eight different gene pools found (k) (clusters). (A) Frequency of the gene pool for the forest type; native (N), restored (R) and saplings (S) groups are composed of all population in the designated category.(B) Gene pool mixture of each population plotted in its geographical localization in Bahia - BR. RTx1 – Restored population from Teixeira de Freitas; RTx2 – Restored population for Teixeira de Freitas; NTx3 – Native population from Teixeira de Freitas; STx1 – Sapling population from Tx1 site; RPS1 – Restored population from Porto Seguro; RPS2 – Restored population from Porto Seguro; RPS3 – Restored population from Porto Seguro; SPS2 – sapling population from the PS2 site; RIL1 – Restored population from Ilhéus; RIL2 – Restored population from Ilheús; NIL3 – Native population from Ilhéus; SIL3 – sapling population from Ilhéus native population. Grey areas within Bahia limits indicates forest cover according to Land Use classification from MapBiomas (2018).

Population structure was demonstrated in a broad-scale by the minimization of variances within groups while maximizing variance between groups by a DAPC analysis, plotted in a scatterplot (Figure 7). The DAPC a priori approach defined clusters according to sampling site (colored ellipses), plotted in a scatterplot that represents 80% of the dataset variance. The overlapping points revealed an aggregation patterns of three different groups (Figure 7A). One clearly separated the native population NTx3 from all other regions. Another group is composed of individuals from the Tx region, that are more genetic distinct when compared to the other regions (Figure 7A - gray scale dots). The differentiation of the Tx region is evident in the individual

density distribution of the first retained function, as well as in the density distribution of the first discriminant function (Figure 7B). PS and IL regions share a genetic component, evidenced by the overlapping distribution groups, with all population sharing a genetic component (Figure 7B, C). The first discriminant component (x axis on Figure 7A) showed that saplings (dotted points) have a most evident association with adults from their original region. For IL and PS areas, for example, sapling individuals were ploted overlaping ellipses of restored areas. Tx showed a particular distribution, in which saplings collected in the RTx1 area were exclusivelly related to its adults and non grouped with other Tx populations. Interestingly, some Tx1 saplings were grouped within restored populations of PS region (green discs). However, no sapling from PS showed a component related to Tx region. Second discriminant component separates the Tx native site and the restored site RTx2 (Figure 7C), suggesting that all other areas share a strong genetic component, representing one diverse group.

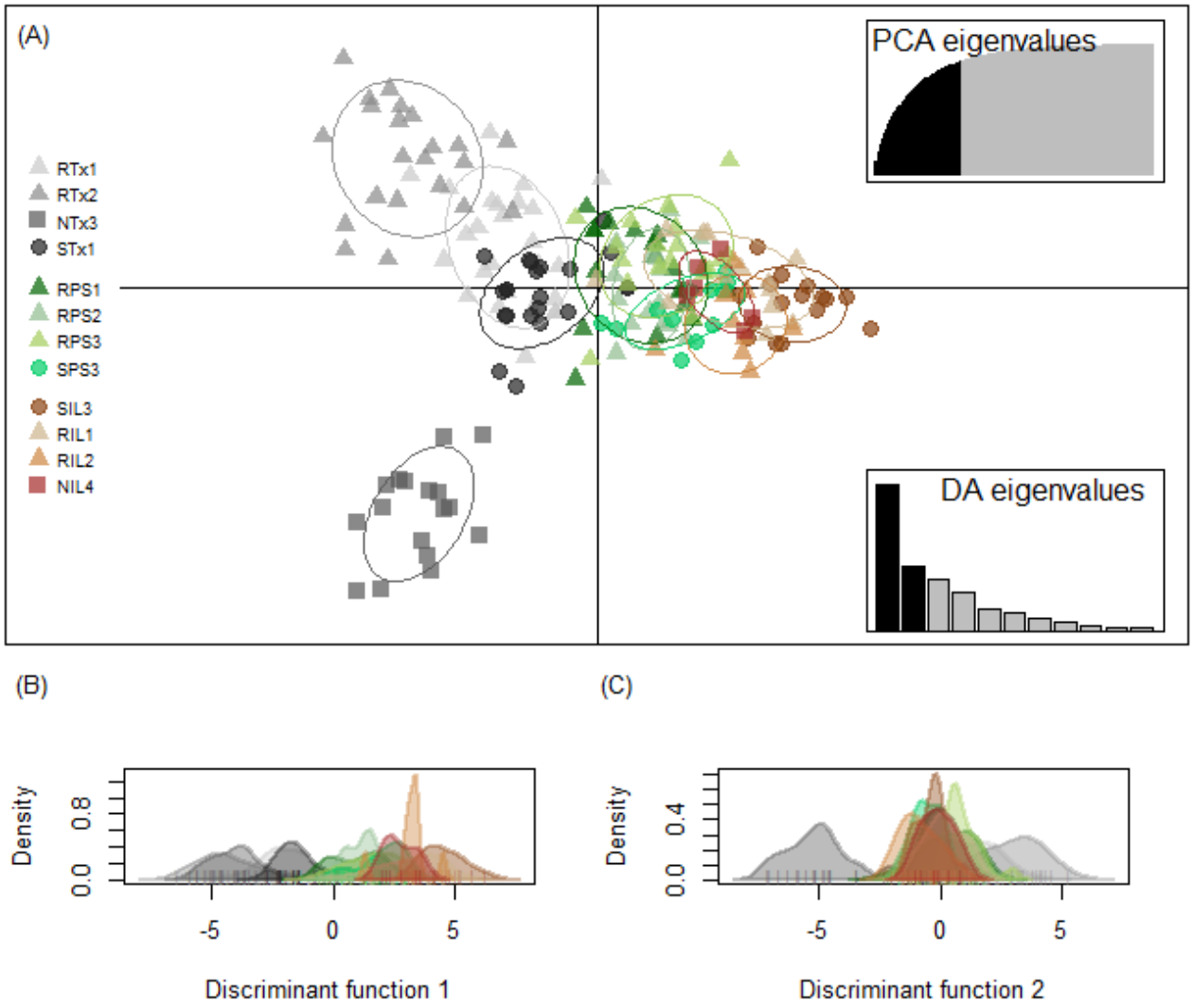



Figure 7. Discriminant Analysis of Principal Components (DAPC) scatter plot of 196 *Dalbergia nigra* sampled genotypes. (A) Scatterplot of the first two principal components discriminating a priori defined populations by regions; (B) individual density plot on the first discriminant function. Points represent individuals, and inertia ellipses represents inclusion of 88%. Colors represent sampling original region: grey scale – Teixeira de Freitas (Tx), green scale – Porto Seguro (PS) and brown scale – Ilhéus (IL). Different symbols represent the class of the site: dots represent saplings, triangle represents restored areas and squares, native areas.

Paired $G_{ST}$ between populations ranged from a minimum of -0.135 to 0.147. Most of pairwised populations showed $G_{ST}$ values significant different from zero (dark shades of gray in Figure 8), thus demonstrating an overall tendency of structuration in *D. nigra* populations. Only five paired populations had no significant structure. In a regional perspective, higher values of $G_{ST}$ were found for the Ilhéus region when compared to other regions (darker shading in Figure 8). Also, $G_{ST}$ showed that adults and saplings from the same area had different genetic composition in

two regions (Teixeira and Porto Seguro), but not in Ilhéus. Although small, the paired fixation index ($G_{ST}$) between these adults and saplings demonstrates a reduction in heterozygosities between these ontogenetic stages for a panmictic expectations (Figure 8). It demonstrates an increase in genetic differentiation between generations.

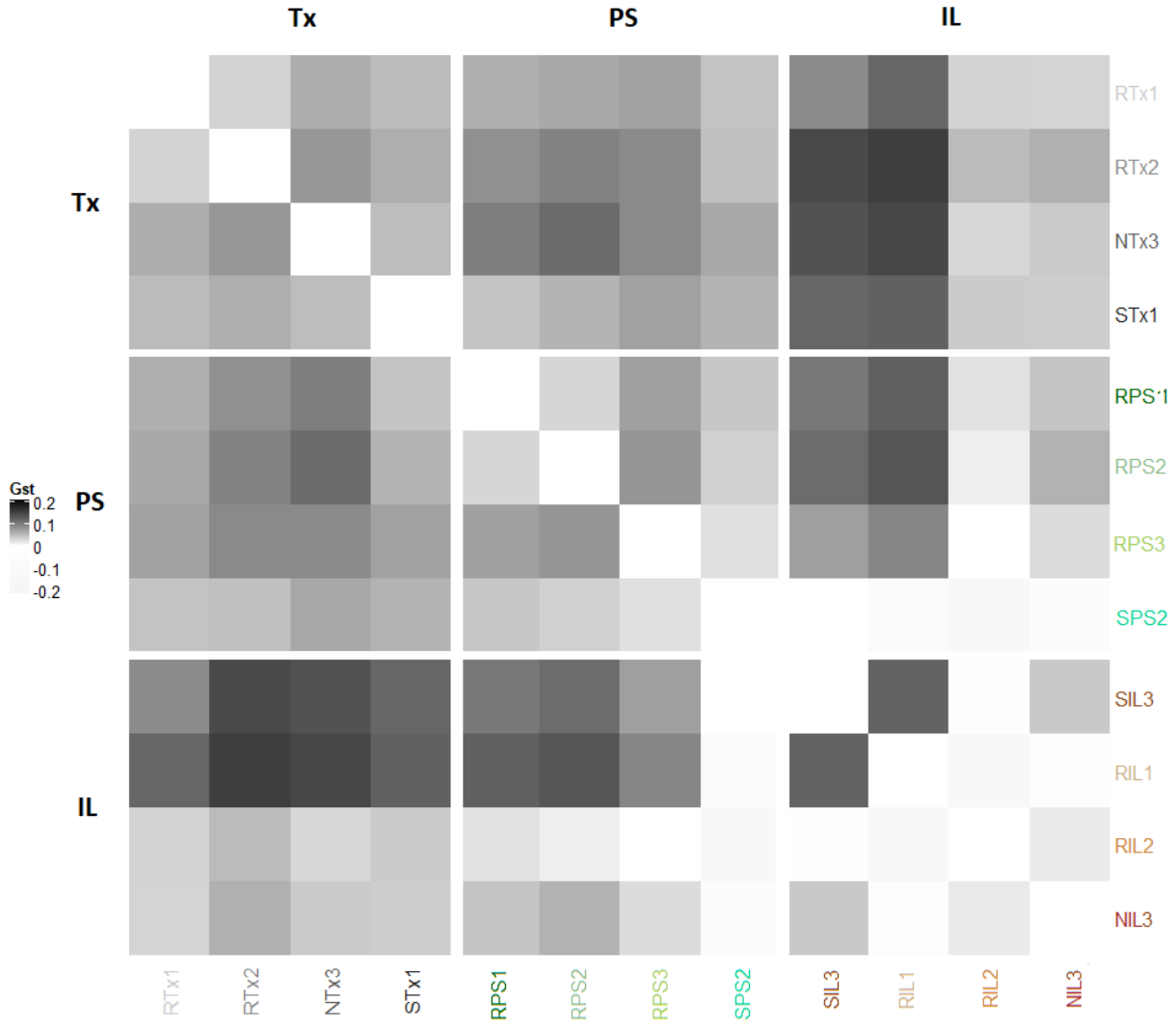



Figure 8. Pairwised Nei's Genetic Distance of *D. nigra* populations. Darker shading indicates greater values, measured as $G_{ST}$. Colors in legend represent regions: grey scale – Teixeira de Freitas (Tx), green scale – Porto Seguro (PS) and brown scale – Ilhéus (IL).

### 5.3.Genetic diversity

The estimated nuclear genetic diversity ($A_r$, $H_O$, $H_E$ and $F_{IS}$) for restored sites was variable (Figure 9, Table 2). Our dataset composed of 196 individuals (12 populations) had a mean of 13.71 alleles per loci (Supplementary table 1). The overall number of alleles per marker ranged from 11 to 18, except for *Dnig7*, that had five alleles (Supplementary table 1). Expected heterozygosity under Hardy-Weinberg Equilibrium ($H_E$) was lower in native areas. Several loci showed evidence of departures from Hardy-Weinberg Equilibrium proportions (Supplementary Figure 2). Here, it is essential to highlight that SPS2 and RIL1 mean values represent five loci, and for RIL2 and NIL4, four loci.

In terms of allele richness, no significant differences were found between forest types (Figure 9A). The mean multi-locus allelic richness was similar for saplings (5.682 ±0.476), restoration (5.547 ±0.971) and native areas (4.198 ±1.064). Regardless of differences in restoration methodology, we did not detected differences for mean $A_r$ across locus in any population (Kruskal-Wallis test, *p-value*=0.267).

Table 2.Estimated population genetics parameters based on seven microsatellite loci of *D. nigra*. Sample size ($\bar{n}$), null allele frequency ($A_n$), number of private alleles ($A_p$), observed heterozigozity ($H_o$) and expected heterozigozity ($H_E$)

| Population | $\bar{n}$ | $A_n$ | $A_p$ | $H_O$ | $H_E$ |
|---|---|---|---|---|---|
| RTx1 | 21.57 | 0.000 | 15.000 | 0.500 | 0.796 |
| RTx2 | 20.43 | 0.231 | 9.000 | 0.616 | 0.770 |
| RPS1 | 15.14 | 0.268 | 9.000 | 0.619 | 0.714 |
| RPS2 | 12.57 | 0.289 | 5.000 | 0.678 | 0.662 |
| RPS3 | 14.43 | 0.000 | 3.000 | 0.340 | 0.720 |
| RIL1 | 10.86 | 0.429 | 6.000 | 0.607 | 0.609 |
| RIL2 | 6.14 | 0.194 | 2.000 | 0.748 | 0.691 |
| **Mean (SD)** | 14.15 (5.32) | 0.202 (0.156) | 7.000 (4.435) | 0.587 (0.132) | 0.709 (0.063) |
| NTx3 | 14.86 | 0.199 | 5.000 | 0.466 | 0.665 |
| NIL3 | 5.71 | 0.144 | 0.000 | 0.700 | 0.659 |
| **Mean (SD)** | 10.28 (6.47) | 0.172 (0.039) | 2.500 (3.536) | 0.583 (0.165) | 0.662 (0.004) |
| STx1 | 14.57 | 0.227 | 11.000 | 0.513 | 0.749 |
| SPS2 | 8.33 | 0.300 | 9.000 | 0.345 | 0.675 |
| SIL3 | 15.29 | 0.180 | 14.000 | 0.498 | 0.727 |
| **Mean (SD)** | 12.73 (3.82) | 0.236 (0.060) | 11.333 (2.517) | 0.452 (0.093) | 0.717 (0.038) |

Private alleles were found in all population expect in NIL2 (Table 2), and RTx1 had the highest value. The occurrence of private alleles over loci were higher in saplings when compared to native populations (Kruskal-Wallis test, *p-value* <0.01). IL and PS had higher values of private alleles in saplings than in adults of the same site. Tx had contrary patterns, still with a high number of private alleles.

Inbreeding levels corrected for null alleles (Figure 9B) demonstrated that, overall, native populations statically differ from saplings evaluated. Mean values across forest types were 0.0736 (±0.0343) for natives, 0.1271 (±0.0376) for restored sites and 0.1801 (±0.0412) for saplings. $F_{IS}$ was present in four restored sites, in both native populations and in all saplings populations (Figure 9B). Native forests had the lowest values, $F_{IS}$=0.095 (0.0005-0.2005) and 0.04 (0.0003-0.2723) for NTx3 and NIL3, respectively. Notably, each restored forest represents a unique pattern, with significant differences in inbreeding levels, that demands a more detailed approach. Both restored populations in Tx region had significant levels of inbreeding. In Ilhéus, the population restored in a nucleation methodology had a $F_{IS}$ statisticantly different from zero, while the RIL2 did not. For PS region, RPS1 and RPS3 had inbreeding levels significantly differing from zero, while RPS2 showed no inbreeding levels (*p-value* = 0.624). Only RPS2 and RIL2, did not had $F_{IS}$.

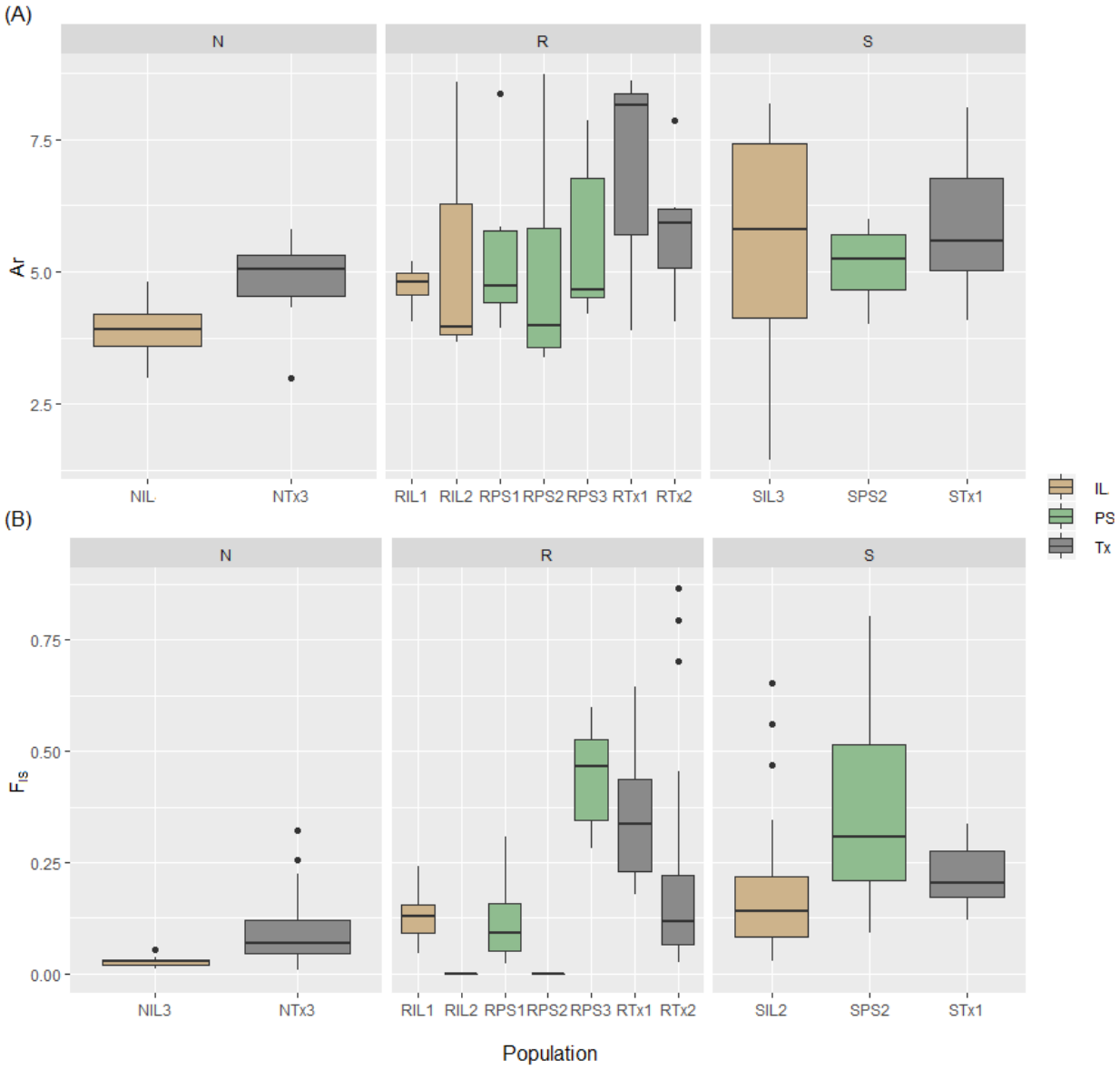



Figure 9. Genetic diversity and inbreeding in native (N), restored (R) and saplings (S) populations of *D. nigra*. (A) Estimated allele richness (based on a minimum sample size of 18 individuals) for each population and (B) inbreeding coefficient corrected for null alleles for each population. IL – Ilhéus, PS – Porto Seguro and Tx – Teixeira de Freitas.

### 5.4. Genetic and geographic distance

Nei's genetic distance revealed that individuals tend to be grouped by region. The RTx1 population is an exception, in which saplings were genetically closer to adult individuals from PS, especially from restored sites RPS1 and RPS2 (Supplementary Figure 5). Isolation by distance was significant (*p-value* = 0.073), and positive across all populations (Supplementary Figure 6). The

correlation was small, with a value of 0.2 was found between genetic and geographic distances (Figure 10).

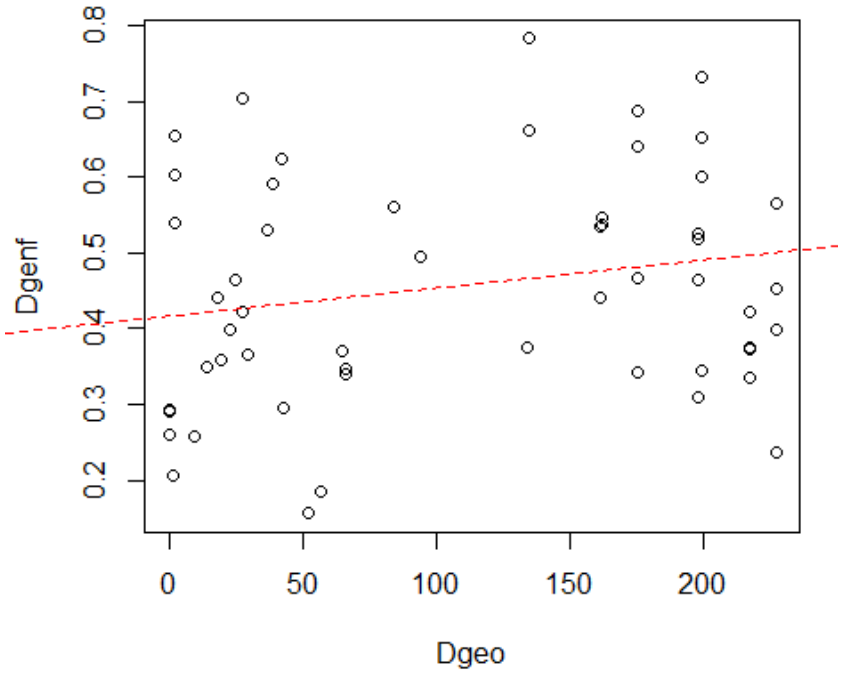



Figure 10. Correlation by genetic (Dgenet) and geographical distance (Dgeo) of 196 *Dalbergia nigra* individuals sampled in restored and native areas of Atlantic Forest.

## 6. DISCUSSION

Genetics aspects of restoration are an emerging tendency, and novels approaches have been developed to modeling and predicting patterns related to restauration genetics (SUJII et al., 2019). Nonetheless, there is still a necessity to better comprehend the genetics aspects under the perspective of restoration methodologies in practical terms. For instance, it is essential to investigate the effects of the adopted methodology in the restored population gene pool, and how it can influence restoration success. This study represents a pioneer approach for the Atlantic Forest restoration, as it contributes to the comprehension of restoration in several methodologies and conditions, and also overall *versus* regional patterns in genetic terms. Besides, our study showed that restored and native forest effectively contribute to new generations, which indicates the establishment of connectivity and gene flow among populations.

The DNA of poor quality obtained with Doyle & Doyle (1987) protocol was, probably, a consequence of the secondary metabolites occurrence in Dalbergia species, as suggested by Ribeiro & Lovato (2007). In fact, *D. nigra* is registered as one species with dalbergiones and isoflavones, natural compounds that are usually reported as bio-products acting in stress-related responses (EYTON et al., 1966; KITE et al., 2010; MATHIAS et al., 1998). To avoid problems related to these secondary compounds, Ribeiro & Lovato (2007) suggests the adoption of Jobes et al. (1995) protocol as the most efficient for *D. nigra* DNA extraction. Nonetheless, our results for Inglis et al. (2016) protocol showed a DNA of even higher molecular concentration and quality, and successful amplification. The adoption of Inglis et al. (2016) also provided results similar to the ones found for *D. nigra* leaves in an efficient protocol proposed by Novaes et al. (2009), with the advantage of being less labor intensive.

Our models demonstrated that, even though some levels of random genotyping failure have influenced our data, it occurred in a small frequency (lower than 0.2) in five populations, and were only higher than 0.3 in two populations (with a maximum value of 0.319). Moreover, the simultaneously estimation of null alleles, genotype failure and inbreeding demonstrated that the increase of homozygotes found in several populations is due to the occurrence of significant inbreeding, and not a consequence of null alleles and missing genotypes. The genotype accumulation curve evidences the power of our analysis with seven loci. It demonstrates a decrease in variance achieved with five markers, reinforcing that the observed diversity in our data will not increase with the addition of new markers.

Contrary to our hypothesis, the introduced genotypes in restored sites represent, overall, a highly diverse gene pool, more diverse than the native one, as suggested by the genetic groups found in our cluster analysis (Figure 4). Although this heterogeneous restored gene pool mostly represented the native, it omitted part of the *D. nigra* natural genetic composition, the k8 pool. In a context of fragmented landscapes and small populations, remaining local forest patches likely represent a disturbed genetic status (Lander, Boshier, 2015; McKay et al., 2005, Thomas et al,, 2014). We conjuncture this is possible the case of the sampled native remnants, in which the number of native pools here found represented a depleted set of alleles. Nonetheless, due to the existence of the exclusive pool in native populations, we suggest that these remaining forests are still a significant representation of the species natural genetic composition. Conservation efforts

that include anthropogenic impacted forests are also key to safeguard the natural genetic composition of endangered species (Lander & Boshier, 2015).

The genetic constitution found in *D. nigra* populations (Figure 4) revealed particularities related to each region, site and methodology adopted. In a regional perspective, same geographic distributed populations had patterns of predominant gene pools, with a partial mix of different regions pools. For native populations, environment geographical attributes likely shape genetic variation, creating different patterns in distinct regions (Brown, Hardner, 2000; REF). Nonetheless, we believe this is not the case for *D. nigra* southern Bahia populations. Ribeiro et al. (2011) demonstrated that *D. nigra* native population distributed across Bahia (including our sampled regions) had a pattern of extensive gene flow, thus constituted a unique group of similar cpDNA haplotype composition. It suggests that different genetic compositions between regions here found probably reflects the impact of anthropic-mediated actions in Atlantic Forest, in which remaining populations were isolated and genetic depleted, which lead to genetic differences over time (Ribeiro et al., 2011). Yet, it is also possible that the different gene pools found in each native remnant are due to large distance between patches, as already suggested in other species (Zucchi et al., 2017). Notwithstanding, restoration is especially beneficial in this degraded scenario, because re-introduced plants inserted pools that represented distinct geographic regions of Southern Bahia. This human-caused gene flow facilitates the recovery of a previously common genetic composition, and underpin the overall genetic diversity enhancement (Thomas et al., 2014; Aavik et al., 2018; Jordan et al., 2019).

Our findings also support the important potential of restored population in reestablish functional connection between patches. We found that the gene pool of implanted trees influenced the characteristics of future generations, as suggested by previous results (Thomas et al., 2014; Mckay et al., 2005). Nonetheless, the surrounding populations may also contribute to saplings genetic pool, as saplings represented a partial mix of native and restored pools. Interestingly, the unique genetic component of native areas was present in regenerating plants of restored population. This is in agreement with other study (Sujii et al., 2017), and strongly indicates the occurrence of gene flow between restored and native population.

The structure patterns found in all populations (Figure 5) re-inforce our findings on genetic composition. *D. nigra* populations had weak differentiation in a local perspective, but stronger between regions. Considering a scenario without forest restoration, both native populations of *D.*

*nigra* here sampled represented a completely different set of alleles, as suggested by the two DAPC analysis. Nonetheless, weak population differentiation for these native remnants, demonstrated by $G_{ST}$ (Figure 6), indicates the occurrence of historical gene flow in high rates. This implies that, possibly, remaining native forests sampled were strong structured more recently, probably due to jeopardized gene flow before restoration took place. It supports the hypothesis that current differentiation found in Southern Bahia reflects effects of anthropic activities, as already suggested for *D. nigra* populations in Minas Gerais state (RIBEIRO et al., 2011). Once trees implanted in restoration were included in the perspective, a set of local alleles were covered, maintaining a level of regional characteristics, but also incorporating alleles of other regions. This build-up of similar genetic composition between regions can favor the re-establishment of widespread alleles, a pattern that used to occur in Bahia.

In a regional pattern, the similarity of all Ilhéus populations demonstrates an efficient seed sourcing, in which local genetic pools were implanted in restoration. Nonetheless, the $G_{ST}$ value between Ilhéus restored population one (RIL1) and saplings from the native population (SIL3) suggest that new generations are more structured, probably a reflection of a currently reduced gene flow, even though these population are only 3km distant. We predict that small structure levels naturally occur in *D. nigra*, likely reflecting seed dispersal patterns of wind dispersed species (CARVALHO, 1994), that is usually limited. However, $G_{ST}$ measures are more sensitive to historical gene flow, and restoration recent effect may not yet be detected (AAVIK; HELM, 2018; PROFT et al., 2018).

Although weak, the existence of geographical influence in the genetic distance supports the hypothesis of landscape-level structure for restored and native populations of a wind-dispersed native Atlantic Forest species. Overall, the genetic aspects of restored and native remnants *D. nigra* populations suggest the occurrence of gene flow. Although we did not estimate directly gene flow patterns, we indirectly demonstrated that restored population act as new forest patches that can increase functional connection between previous isolated and structured populations.

Restored populations in Tx were more differentiated, characterizing Tx as the most divergent region. Alternatively, all populations of IL and PS were aggregated in the same group, suggesting low levels of divergence between these two regions. Most importantly, it indicated genetic similarity in restored and native populations in IL and PS. In PS, RPS1 and RPS2 share a strong genetic component, as expected due to its similar restoration methodology. The seedlings

implanted in these restorations forest were collected in a well-conserved native reserve in Linhares - Espírito Santo (D. S. Almeida 2018, personal communication, technical report). Interestingly, we observed a shared genetic component between this populations and populations restored with seeds from Bahia local nurseries (such as RPS3) and native populations from Ilhéus. We believe that the shared genetic component between IL and PS most likely reflects a historically common genetic background, as indirectly inferred by genetic distances (PROFT et al., 2018), with historical extensive gene flow between *D. nigra* populations across Atlantic Forest domain. In analyzing seedlings from Linhares (Espírito Santo) and Minas Gerais nurseries with ISSR markers, Santos (2019) revealed moderate genetic differentiation and historical occurrence of gene flow between these Brazilian states. It suggests a past-condition in which these populations were connected by continuous forests or even considered a metapopulation. Accordingly, our results suggest a similar pattern between forests of Espírito Santo and Bahia. It also demonstrated that, in active restoration, seeds from another state can represent a gene pool similar to native ones. Human-defined geographical limit does not necessarily define the extend of local and non-local sources for native species, and geographical distance is a poor predictor of the species historical pattern. Thus, we suggest a seed sourcing strategy in agreement with the regional admixed provenance proposed by Bucharova et al. (2019).

Taking a local approach, we showed that restored areas can differ even when inserted in the same region and executed by the same stakeholder. It reinforces the influence of the adopted methodology, as well as the characteristics of the site to be restored (REF). For instance, our results for IL populations were consistent with the expected for the adopted seed collection practice, in which local seed sources successfully captured the native genetic composition.

Our results reveals that restored populations had, overall, similar genetic diversity, even though implanted in different contexts and methodologies. Opposed to our expectation, absence of variability between restoration suggests no influences of the seed collection methodology adopted, nether effects of the site characteristics in this genetic parameter. Sujii et al. (2017) found similar pattern for other Atlantic Forest native tree. It surprisingly contradicts the theoretical assumption of context dependence in genetic diversity of restored populations (ZUCCHI et al., 2017).

When comparing native and restored population, similar genetic diversity was found. Also, our sampled native remnants demonstrated smaller diversity levels than the ones found in continuous conserved forest for the species (RESENDE et al., 2011). A reduced genetic diversity

was expected due to the characteristics of these remnant populations, which had suffered with anthropic disturbance, and are currently inserted in a fragmented landscape. For instance, NTx3 is a secondary forest undergoing early ecological succession, and NIL3 is a remnant impacted by logging, that occurs near a previous anthropic area. We hypothesize that the currently genetic diversity of the sampled remnants reflects a negative effect of the habitat disturbance, in which a decrease in genetic variation is expected (YOUNG; BOYLE; BROWN, 1996). Thus, careful consideration must be taken when using native population as references, as proposed in some restoration approaches (BUCHAROVA et al., 2019; KRAMER et al., 2019; THOMAS et al., 2014). We recommend that, when inserted in a fragmented landscape, native populations genetic patterns should not be considered as ideal for restoration. Instead, these remaining fragmented population could be registered as the species currently genetic status, as an initial point to be monitored alongside restoration outcomes over time.

Despite similar genetic diversity results obtained by $A_R$, several populations showed evidence of inbreeding. Our results demonstrated that *D. nigra* native populations had small inbreeding, even though our sampled populations were historically highly impacted by anthropic interferences. We believe this reflects the complexity in detect negative effects of recent fragmentation in long-lived trees, once adults represent the population pre-fragmented past (KRAMER et al., 2008; YOUNG; BOYLE; BROWN, 1996). Even for saplings, inbreeding evidence may be detected only after several generations (APARECIDA et al., 2019; KRAMER et al., 2008; MARTINS et al., 2016). In our study, multiple-locus showed panmixy departures in both native populations, thus suggesting restrictions in interbreeding. An appropriate interpretation of these values also involves the breeding system of the species. Even though there are no direct studies on it, D. nigra demonstrates a predominantly outcrossing pattern (BUZATTI et al., 2012; RESENDE et al., 2011; RIBEIRO et al., 2005). Nonetheless, this species is mainly pollinated by small insects such as bees, thus theoretically presenting a limited pollen movement. Indeed, D. nigra historical gene dispersal has been registered as varying from 89 to 144 meters in a recently fragmented forest (BUZATTI et al., 2012). A highly reliable hypothesis is that small rates of self-fertilization may have played a role in the inbreeding levels, as already suggested for tropical trees have (Dick et al., 2008). Also, its congeneric species Dalbergia miscolobium has a self-incompatibility system, which suggest a similar system to D. nigra (GIBBS; SASSAKI, 1998). Hence, we believe that D. nigra natural populations generally have some levels of inbreeding, as

those found in the NTx3 population, due to its aggregated spatial pattern of distribution, in which native populations have genetic-related individuals up to 80m (BUZATTI et al., 2012; LEITE et al., 2014). This physical closely distribution may have favor mating between relatives, as suggested by Leite et al. (2014).

Although possible, we disregarded selfing because *D. nigra* demonstrates a predominantly outcrossing pattern (BUZATTI et al., 2012; RESENDE; RIBEIRO; LOVATO, 2011; RIBEIRO et al., 2005). Also, its congeneric species *Dalbergia miscolobium* has a self-incompatibility system, which suggest a similar system to *D. nigra* (GIBBS; SASSAKI, 1998). We believe that *D. nigra* natural populations generally have some levels of inbreeding, as those found in the NTx3 population, due to its aggregated spatial pattern of distribution, in which native populations have genetic-related individuals up to 80m (BUZATTI et al., 2012; LEITE et al., 2014). This physical closely distribution may favor mating between relatives, as suggested by Leite et al. (2014). Notwithstanding, the low levels of inbreeding suggests that even small, impacted remaining populations can contribute to the species conservation, as a valuable source of local alleles.

Previous studies demonstrated that, when present, inbreeding levels of restored populations is generally similar to native populations (SCHWARCZ et al., 2018; SUJII et al., 2017; ZUCCHI et al., 2018). In contrast, our results evidences that, for most of the restored population, a high fixation index value is found, although $F_{IS}$ was absent in two restored populations – RIL2 and RPS2. RIL2, one of the sampled restored areas in Ilhéus, is extremely close to the native population sampled in this region (NIL3). The mean distance between individuals of this two areas is 15 meters. Moreover, the native population is abundant and dense, with a high number of individuals, and is highly reproductive, with occurrence of seeds and young, saplings and seedlings individuals (personal observation). Although we ensured that trees sampled in RIL2 were within the restored area delimitation, we believe that *D. nigra* adults sampled in this area may include native trees that previously existed or germinated from native seeds and grew alongside actively implanted seedlings. Therefore, we suppose, based on our genetic structure results, that these two populations are actually one panmitic population composed of native remaining and human introduced trees. The non-occurrence of inbreeding in NIL3 reinforces this hypothesis

Inbreeding patterns are affected by the genetic composition of the initial introduced trees (SUJII et al., 2019), and we suggest that, in forest restoration, inbreeding must be considered according to each site characteristics. For instance, RPS2 population was planted in a similar

approach to the RPS1 population, with the same methodology, period and, specially, with the same seedling source. The main difference is the area size and, consequently, the number of *D. nigra* seedlings planted (RPS2 area is almost two times greater, Table 1). RPS2 showed no evidence of inbreeding, while RPS1 had a high level ($F_{IS}$ = 0.113). This result corroborates with a model proposed by Sujii (2019), in which a tendency of $F_{IS}$ increase is related to small areas (in our case, 20 to 10 ha). Schwarcz et al. (2018) suggested that a small number of seedlings implanted in Atlantic Forest restoration also influenced another genetic parameter, the loss of rare alleles. Another possible explanation is that the RPS2 soil had a better seedbank composition (according to Almeida[1] - report of the areas under recovery). Thus, the seedbank may have contributed to the gene pool present in adults currently, as suggested in other restoration study (SILVA et al., 2019).

The adopted restoration methodology can also influence populations inbreeding. We observed that two different methodologies resulted in higher levels of fixation index, when compared to other restored populations. In Porto Seguro region, RPS3 was implanted with frequently introduction of new seedlings in the following years (according to Almeida[1] – verbal information). Intriguingly, this population showed the highest fixation index ($F_{IS}$ = 0.451), even though implanted in a Protected Area with high forest cover. The deficit of heterozygotes relative to the expected Hardy-Weinberg proportions may be due to Wahlund effect, in which separated demes were created in each enrichment of this area. A similar effect can explain the inbreeding coefficient of RIL1. In this restoration, seedlings were implanted in a nucleation methodology, creating "microhabitats", dense patches of seedlings, possibly with divergent allele frequencies. An alternative explanation for RIL1 is that these microhabitats were composed of *D. nigra* clusters, with related individuals closely planted. This could create a favorable scenario for inbreeding occurrence. Notably, results on genetic parameters could be influenced by the plant arrangement in the restored site, as suggested by previous studies (McCallum et al., 2018; Millar et al., 2020). This demonstrates the potential to use genetic knowledge to elaborate restoration projects, in which planning seedlings arrangements can beneficially contribute to the population fitness and, consequently, the ecosystem recovery in the long-term.

Private alleles (Table 2) also indicate that, in each restored site, implanted seedlings can act as new sources of genetic variance, containing a gene pool that, at least partially, did not occur

[1] ALMEIDA, D. S. de. Suçuarana Meio Ambiente e Florestas director.

previously in those areas. We believe that, taken together, the seed sources used in restoration projects in southern Bahia represent a mixture of genotypes, in which seeds are transferred between regions and represent an exchange of genetic information. These restored populations can re-establish forest patches and enhance physical connectivity in the fragmented Atlantic Forest matrix, increasing the likelihood of pollination and dispersal process with a considerable genetic diversity, as proposed by Aavik & Helm (2018).

In terms of the following generation, a significant increase of fixation index in saplings when compared to adults occurred only for the Ilhéus region (NIL3 – SIL3). The other two saplings populations also had inbreeding significantly different from zero, although not different from the inbreeding found in adults of the same local. A similar pattern has been found for restored population of *C. tomentosum*, an Atlantic Forest tree also pollinated by bees and wind dispersed (Sujii et al. 2017), in which adults and seedlings of restored sites showed significant and similar levels of inbreeding (SUJII, 2017). Inbreeding depression is a concern for outcrossing species, and has been suggested as a main driver of genetic variation in restored populations (McKay et al., 2005, Thomas et al., 2014). We suggest that the occurrence of $F_{IS}$ is likely a consequence of populations small size. It is noteworthy that restored populations here sampled occur in fragmented landscapes, thus we would expect, initially, impacted pollination patterns as the ones of native fragmented habitat (Pfeiffer et al., 2019). Breed et al. (2015) revealed that reduced forest density in the surrounding area will lead to a reduction in small-insects-pollination for woody species. That could be the case for the early ontogenetic stage regenerating in restored areas, in which the increased homozygosity demonstrated by high fixation indexes reflects limited capacity of pollination and seed dispersion for *D. nigra*. In fact, bee pollen dispersal has been registered as restricted among individuals that are spatially aggregated in restoration areas (Millar et al., 2020). In contrast, other studies in the Atlantic Forest context have found an enhancement of bee functionality alongside restoration, and that appears to be the case for our saplings populations, as suggested by the private alleles results. Thus, we believe that gene flow indeed increased, but in local perspective, as a consequence of the reduced pollen neighborhoods. Moreover, restored areas tend to be small, and in active restoration, several species are used, resulting in a reduced number of individuals per species as cited above. It may raise concerns for the long-term plant persistence and require special attention, because even though our results indeed suggested the occurrence of gene flow, the subsequent generations still presented higher levels of inbreeding. Nonetheless, the

restored areas can effectively contribute to gene flow patterns in the long-term, and thus support conservation (ZUCCHI et al., 2018).

Other genetic parameters, such as $A_R$ and $A_P$ , demonstrated no significant reduction across adults and saplings. Indeed, the occurrence of $A_P$ in saplings indicate the occurrence of external allele sources, supporting the assumption of gene flow existence. Gene flow from surrounding populations was also found for other restored sites (SCHWARCZ et al., 2018; SUJII et al., 2017). It suggests that reproduction events in restored areas can shift mating patterns of fragmented population in the long-term, due to the provision of more diverse pollen. We propose that contemporary gene flow studies should be conducted, such as paternity analysis (AAVIK et al., 2013), to search for such evidences.

To effectively restore an ecosystem, forest restoration methodologies should consider genetic diversity, as broadly suggested in previous studies (BASEY; FANT; KRAMER, 2015; SUJII et al., 2019; THOMAS et al., 2014; ZUCCHI et al., 2017), alongside genetic constitution (gene pools) and gene flow patterns. Although diverse, the genetic pool found in restored populations of *D. nigra* have particularities related to each site and methodology adopted. Thus, the potential contribution of restoration to the ecosystem in the long-term could be maximized when genetic aspects are incorporated in restoration planning.

In a broad perspective, our results showed that the heterogeneous gene pool of restored populations partially represented the native gene pool. Native areas had a unique genetic component that was, however, present in regenerating plants of restored population. This is a strong evidence of gene flow between restored and native population. Our findings also suggest that founder individuals in restored sites represent a key option to reduce the structure between remnants. For instance, we demonstrated that restored population from Tx region had at least a partial common pool with the native population from IL region. In occurrence of gene flow, recruitment in natural remnant may include admixed individuals, in which these alleles may be introduced. This gene pool is present in reproduction, as they appear in saplings from Tx1. Altogether, this reinforce the important potential of restored population in reestablish functional connection between patches.

Finally, we emphasize that there is no general formula to effectively restore forests. Local conditions may differ from regional conditions, but an overall benefit is always achieved. In practical terms, restored sites represent an extremely valuable *ex-situ* conservation strategy.

Furthermore, restoration should aim not only in achieving a similar pattern as the ones found in native populations, but mainly be designed as an opportunity to genetically rescue those disturbed populations. Parentage analysis in nurseries may be and efficient method to avoid inbreeding. We propose that, to measure the effectivity of restoration, a more detailed approach than the usual should be adopted, number of seedlings and survival rates are not enough.

## 6. CONCLUSION

Restore an ecosystem is a long process, that need to be monitored and evaluated. Genetic tolls are ideal to better comprehend it in the long-term, and predict possible opportunities of improvement. Importantly, our results provided evidence for the use of genetic knowledge in restoration efforts. We demonstrated that genetic parameters are informative and can be used as a valuable source in monitoring restoration outcomes for any biological models.

Overall, restoration contributed to broad genetic diversity of *D. nigra* in Southern Bahia. A combination of genetic variation patterns, genetic structure, gene flow and genetic drift defined the genetic scenario of restored populations. Each region presented a pattern of predominant gene pool constitution. In this scenario, we demonstrated that restored areas are beneficial to the re-establishment of connectivity between forest patches. Thus, restoration facilitated a reduction of structuration between the southern Bahia regions trough human assisted migration. Nonetheless, the diversity of methodology adopted provided a variation of genetic outcomes in a regional perspective. More importantly, the adopted strategy in implanting restoration may negatively impact subsequent generations, and we further discuss some alternatives to mitigate it. Mainly, restored forests were beneficial to the re-establishment of functional connection in the landscape. Restored populations of *D. nigra* are genetic reservoirs, and promote the conservation of this endangered species.

In practical terms, we urge that seed collector and nurseries keep register of seed source adopted, and restoration executors to keep data on the characteristics of the restoration project. We suggest a seed sourcing that balances local seed collection from several populations, with the possible mix of seeds from different regions. Also, we reinforce that the history of the native forest used as seed source need to be accounted – if highly fragmented, a viable option is to collect seeds in more distant forests but that are historically conserved. Lastly, our recommendation is that same species seedlings should be planted distributed throughout the area to be restored, avoiding spatial aggregation.

## 7. REFERENCES


AAVIK T., HOLDEREGGER R., EDWARDS P.J., BILLETER R. Patterns of contemporary gene flow suggest low functional connectivity of grasslands in a fragmented agricultural landscape. **Journal of Applied Ecology**, v. 50, p.395–403, 2013.

AAVIK, T.; HELM, A. Restoration of plant species and genetic diversity depends on landscape-scale dispersal. **Restoration Ecology**, v. 26, n. Ser 2004, p. S92–S102, 2018.

AMAZONAS, N. T., MARTINELLI, L. A., PICCOLO, M. C. & RODRIGUES, R. R. Nitrogen dynamics during ecosystem development in tropical forest restoration. **For. Ecol. Manage**. v. 262, p. 1551–1557, 2011.

APARECIDA, L. et al. Anthropogenic Disturbances Eroding the Genetic Diversity of a Threatened Palm Tree : A Multiscale Approach. v. 10, n. November, p. 1–12, 2019.

BARRETO, M. A. **Diversidade genética de quatro populações de jacarandá-da-bahia (*Dalbergia nigra* - *Fabaceae*) por meio de marcadores microssatélites**. Dissertação (Doutorado em Genética e Biologia Molecular) – Universidade Estadual de Santa Cruz, Ilhéus, 2010.

BASEY, A. C.; FANT, J. B.; KRAMER, A. T. Producing native plant materials for restoration: 10 rules to collect and maintain genetic diversity. **Native Plants Journal**, v. 16, n. 1, p. 37–52, 2015.

BASTIN, J.-F. et al. The global tree restoration potential. **Science**, v. 79, n. July, p. 76–79, 2019.

BENINI, R. DE M.; ADEODATO, S. **Economia da restauração florestal**. The Nature Conservancy, São Paulo, 2017. 136 p.

BONIN, A. et al. How to track and assess genotyping errors in population genetics studies. **Molecular Ecology**, v. 13, n. 11, p. 3261–3273, 2004.

BRANCALION, P. H. S. et al. How to Organize a Large-Scale Ecological Restoration Program? The Framework Developed by the Atlantic Forest Restoration Pact in Brazil. **Journal of Sustainable Forestry**, v. 32, n.7, p.728-744, 2013.

BRANCALION, P. H. S. et al. Balancing economic costs and ecological outcomes of passive and active restoration in agricultural landscapes: the case of Brazil. **Biotropica**, v. 48, n. 6, p. 856–867, 2016.

BRANCALION, P. H. S. et al. Maximizing biodiversity conservation and carbon stocking in restored tropical forests. **Conservation Letters**, n. July 2017, p. 1–9, 2018.

BRANCALION, P. H. S. et al. Global restoration opportunities in tropical rainforest landscapes. **Science Advances**, v. 5, n. 7, p. eaav3223, 2019.

BRASIL. LEI Nº 11.428, DE 22 DE DEZEMBRO DE 2006. **Diário Oficial da União**, 2006.

BRASIL. DECRETO Nº 6.660, DE 21 DE NOVEMBRO DE 2008. **Diário Oficial da União**, 2008.

BRASIL; AMBIENTE, M. DO M. **O Corredor Central da Mata Atlântica - uma nova escala de conservação da biodiversidade**. Ministério do Meio Ambiente, Conservação Internacional e Fundação SOS Mata Atlântica, Brasília, 2006. 46 p.

BREED, M. F. et al. Mating patterns and pollinator mobility are critical traits in forest fragmentation genetics. **Heredity**, v. 115, December 2012, p. 108–114, 2015.

BUCHAN JC, ARCHIE EA, VAN HORN RC, MOSS CJ, ALBERTS SC. Locus effects and sources of error in noninvasive genotyping. **Molecular Ecology Notes**, v. 5, p. 680–683, 2005.

BUCHAROVA, A. et al. Mix and match : regional admixture provenancing strikes a balance among different seed-sourcing strategies for ecological restoration. **Conservation Genetics**, v. 20, n. 1, p. 7–17, 2019.

BUZATTI, R. S. DE O. et al. Fine-scale spatial genetic structure of *Dalbergia nigra* (Fabaceae), a threatened and endemic tree of the Brazilian Atlantic Forest. **Genetics and Molecular Biology**, v. 35, p. 838–846, 2012.

CARDOSO, J. M.; TABARELLI, M. Tree species impoverishment and the future flora of the Atlantic forest of northeast Brazil. **Letters to nature**, v. 404, March, p. 72–74, 2000.

CARVALHO, P. E. R. **Espécies florestais brasileiras**: recomendações silviculturais potencialidades e uso da madeira. Colombo: Embrapa/CNPF; Brasília, DF: Embrapa/SPI, 1994. 640 p.

CHAPUIS, M.; ESTOUP, A. Microsatellite Null Alleles and Estimation of Population Differentiation. **Molecular Ecology and Evolution**, v. 23, n.3, 2004.

CHAZDON, R.; BRANCALION, P. Restoring forests as a means to many ends. **Science**, v. 365, n. 6448, p. 24–25, 2019.

CHAZDON, R. L. Landscape Restoration, Natural Regeneration, and the Forests of the Future. **Annals of the Missouri Botanical Garden**, v. 102, n. 2, p. 251–257, 2017.

CHAZDON, R. L.; GUARIGUETA, M. R. **Decision Support Tools for Forest Landscape Restoration: current status and future outlook**. Occasional Paper 183. Bogor, Indonesia: CIFOR, 2018.

CHYBICKI, I. J.; BURCZYK, J. Simultaneous estimation of null alleles and inbreeding coefficients. **Journal of Heredity**, v. 100, n. 1, p. 106–113, 2009.

CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES OF WILD FAUNA AND FLORA, CITES. The CITES Appendices: Appendices I, II and III. Cites.org. 2017.

CORDEIRO, E. M. G. et al. Diversity , genetic structure , and population genomics of the tropical tree Centrolobium tomentosum in remnant and restored Atlantic forests. **Conservation Genetics**, n. 0123456789, 2019.

COSTA, R. O.; SCHIAVINI, I.; OLIVEIRA, A. P. DE. Ecologia populacional e germinação de *Dalbergia nigra* (Vell.) Alemão ex benth (Fabaceae) em um fragmento de Floresta Estacional Semidecidual no Parque Municipal do Sabiá, Uberlândia, MG. **Horizonte Científico**, v. 5, n. 1, p. 1–24, 2011.

CRITICAL ECOSYSTEM PARTNERSHIP, F. **Ecosystem Profile: Atlantic Forest Biodiversity Hotspot - Brazil**. Report - Final Version, Critical Ecosystem Partnership Fund, December, 2001.

CROUZEILLES, R. et al. There is hope for achieving ambitious Atlantic Forest restoration commitments. **Perspectives in Ecology and Conservation**, v. 17, n. 2, p. 80–83, 2019.

DABROWSKI, M. J. et al. Reliability assessment of null allele detection: Inconsistencies between and within different methods. **Molecular Ecology Resources**, v. 14, n. 2, p. 361–373, 2014.

DAILY, G. C. et al. Ecosystem Services: Benefits Supplied to Human Societies by Natural Ecosystems. **Issues in Ecology**, n. 2, p. 16, 1997.

DEWALD, L. E.; KOLANOSKI, K. M. Conserving genetic diversity in ecological restoration: a case study with ponderosa pine in northern Arizona, USA. **New Forests**, v. 48, n. 2, p. 337–361, 2017.

DEWOODY, J.; NASON, J. D.; HIPKINS, V. D. Mitigating scoring errors in microsatellite data from wild populations. **Molecular Ecology Notes**, v. 6, n. 4, p. 951–957, 2006.

DODONOV, P., MORANTE-FILHO, J.C., MARIANO-NETO, E., CAZETTA, E., ANDRADE, E.R., ROCHA-SANTOS, L.R., INFORZATO, I., GOMES, F.S. AND FARIA, D. Forest Loss Increases Insect Herbivory Levels in Human-Altered Landscapes. **Acta Oecologica**, v. 77, p.136-143, 2016.

DOYLE, J. J.; DOYLE, J.L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. **Phytoc**. Bul., v.19, 11-15, 1987.

EYTON, W. B. et al. The Neoflavanoid Group Products-I of Natural New Class of Quinones. **Tetrahedron**, v. 21, p.2683-2696, 1966.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, FAO. **Global Forest Resources Assessment 2015: How are the world's forest changing?**. FAO:Forestry Department; Rome, 2016. 54 p.

FEDRIANI, J. M. et al. Combined effects of seed provenance , plant facilitation and restoration site on revegetation success. **Journal of Applied Ecology**, v. 56, n. December 2018, p. 996–1006, 2019.

FERNANDES, G. S. F.; BRAGA de A., Avaliação da Regeneração Natural como indicativo da Consolidação da Restauração Florestal em área de Reflorestamento Misto. IX Congresso Brasileiro de Gestão Ambiental São Bernardo do Campo/SP, 2018.

FUNDAÇÃO SOS MATA ATLÂNTICA. Cartilha: **Aqui Tem Mata?**. SOS Mata Atlântica, São Paulo, 2015, 24 p.

FUNDAÇÃO SOS MATA ATLÂNTICA, INSTITUTO NACIONAL DE PESQUISAS ESPACIAIS. **Atlas dos Remanescentes Florestais da Mata Atlântica - Relatório técnico, 2017-2018**. São Paulo, 2019.

FUNK, W. C. et al. Harnessing genomics for delineating conservation units. **Trends in Ecology and Evolution**, v. 27, n. 9, p. 489–496, 2012.

GAGNEUX, P. ; BOESCH, C.; WOODRUFF, D.S. Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. **Molecular Ecology**, v. 6, p. 861–868, 1997.

GIBBS, P.; SASSAKI, R. Reproductive Biology of *Dalbergia miscolobium* Benth . (Leguminosae- Papilionoideae) in SE Brazil: The Effects of Pistillate Sorting on Fruit-set. **Annals of Botany**, v. 81, p. 735–740, 1998.

GOUDET, J. Hierfstat, a package for R to compute and test hierarchical F-statistics. **Molecular Ecology Notes**, v. 5, p.184-186, 2005.

GUO, Z.; ZHANG, L.; LI, Y. Increased Dependence of Humans on Ecosystem Services and Biodiversity. **PLoS ONE**, v. 5, n. 10, e13113, 2010.

HAVENS, K. et al. Seed Sourcing for Restoration in an Era of Climate Change Seed Sourcing for Restoration in an Era of Climate Change. **Natural Areas Journal**, v. 35, n. 1, p. 122–133, 2015.

HOFFMAN, J. I.; AMOS, W. Microsatellite genotyping errors: Detection approaches, common sources and consequences for paternal exclusion. **Molecular Ecology**, v. 14, n. 2, p. 599–612, 2005.

HUA, F. et al. Opportunities for biodiversity gains under the world's largest reforestation programme. **Nature Communications**, v. 7, p. 1–11, 2016.

IBGE, I. B. DE G. E E. **Mapa da Área de Aplicação da Lei nº11.428 de 2006**. n. 2ª edição, 2012.

INGLIS, P. W.; PAPPAS, M. DE C. R.; GRATTAPAGLIA, D. Protocolo de Extração de DNA e RNA de Alta Qualidade para Espécies Ricas em Compostos Secundários. **Embrapa - Comunicado técnico**, v. 204, p. 2–4, 2016.

JALONEN, R. et al. Forest and landscape restoration severely constrained by a lack of attention to the quantity and quality of tree seed: Insights from a global survey. **Conservation Letters**, n. October, p. 1–9, 2017.

JEROME, A.; JOMBART, T.; GOUDET, M. J. Package ‘ hierfstat ’. 2015.

JOBES, D. V., D. L. HURLEY, and L. B. THIEN. Plant DNA isolation: a method to efficiently remove polyphenolics, polysaccharides, and RNA. **Taxon**, v. 44, p.349– 386, 1995.

JOLY, C. A.; METZGER, J. P.; TABARELLI, M. Experiences from the Brazilian Atlantic Forest : ecological findings and conservation initiatives. **New Phytologist**, v. 204, p. 459–473, 2014.

JOMBART, T. adegenet : a R package for the multivariate analysis of genetic markers. **Bioinformatics**, v. 24, n. 11, p. 1403–1405, 2008.

JORDAN, R. et al. How well do revegetation plantings capture genetic diversity? **Biology Letters**, v. 15, 2019.

KAMVAR ZN, TABIMA JF, GRU¨NWALD NJ. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. **PeerJ**, v.2, e28, 2014.

KAREIVA, P. Domesticated Nature: Shaping Landscapes and Ecosystems for Human Welfare. **Science**, v. 1866, n. 2007, 2013.

KITE, G. C. et al. Phytochemistry Dalnigrin , a neoflavonoid marker for the identification of Brazilian rosewood ( Dalbergia nigra ) in CITES enforcement. **Phytochemistry**, v. 71, n. 10, p. 1122–1131, 2010.

KRAMER, A. T. et al. The paradox of forest fragmentation genetics. **Conservation Biology**, v. 22, n. 4, p. 878–885, 2008.

KRAMER, A. T. et al. Sourcing native plants to support ecosystem function in different planting contexts. **Restoration Ecology**, v. 27, n. 3, p. 470–476, 2019.

KRAMER, A. T.; HAVENS, K. Plant conservation genetics in a changing world. **Trends in Plant Science**, v. 14, September, p. 599–607, 2009.

LATAWIEC, A. E. et al. Natural regeneration and biodiversity: a global meta-analysis and implications for spatial planning. **Biotropica**, v. 48, n. 6, p. 844–855, 2016.

LEITE, F. A. B. et al. Fine-scale genetic structure of the threatened rosewood *Dalbergia nigra* from the Atlantic Forest : comparing saplings versus adults and small fragment versus continuous forest. **Tree**, v. 10, p. 307–316, 2014.

LESICA, P.; ALLENDORF, F. W. Ecological genetics and the restoration of plant communities: Mix or match? **Restoration Ecology**, v. 7, n. 1, p. 42–50, 1999.

LORENZI, H. **Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas do Brasil**. Vol. 1, 4. Ed, Nova Odessa:SP, Instituto Plantarum, 2002.

MACKEY, B. et al. Policy Options for the World's Primary Forests in Multilateral Environmental Agreements. **Conservation Letters**, v. 8, n. 2, p. 139–147, 2015.

MALAQUIAS, J. O. DA S. et al. **Germinação In Vitro de *Dalbergia Nigra* Vell. Fr. All. Ex Benth**. Educação e ciência para a cidadania global. **Anais**... INICEPG, 2015

MAPPIN, B. et al. Restoration priorities to achieve the global protected area target. **Conservtion Letter**, May, p. 1–9, 2019.

MARTINELLI, G., MORAES, M. V. **Livro vermelho da flora do Brasil**. 1.ed., Rio de Janeiro: Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, 2013.

MARTINS, K. et al. The role of very small fragments in conserving genetic diversity of a common tree in a hyper fragmented Brazilian Atlantic forest landscape. **Conservation Genetics**, v. 17, n. 3, p. 509–520, 2016.

MASSATTI, R. et al. Population history provides foundational knowledge for utilizing and developing native plant restoration materials. **Evolutionary Applications**, v. 11, March, p. 2025–2039, 2018.

MATHIAS, L. et al. A New Isoflavone Glycoside from *Dalbergia nigra*. **Journal of Natural Products**, v. 61, n. 9, p. 1158–1161, 1998.

MCCALLUM, K. P. et al. Spatially designed revegetation — why the spatial arrangement of plants should be as important to revegetation as they are to natural systems. **Restoration Ecology**, v. 26, n. 3, p. 446–455, 2018.

MCRAE, B. H. et al. Where to Restore Ecological Connectivity? Detecting Barriers and Quantifying Restoration Benefits. **PLoS ONE**, 2012.

MELI, P. et al. A global review of past land use, climate, and active vs. passive restoration effects on forest recovery. **PLoS ONE**, v. 12, n. 2, p. 1–17, 2017.

MELLO, L. N. DO C. **MICROPROPAGAÇÃO DE *Dalbergia nigra* (Fr. Aliem) A PARTIR DE SEMENTES GERMINADAS "IN VITRO"**. Dissertação (Mestrado em Ciências Florestais) - Universidade Federal do Paraná, 1996. 77 p.

MELO, F. P. L. et al. Priority setting for scaling-up tropical forest restoration projects : Early lessons from the Atlantic Forest Restoration Pact. **Environmental Science and Policy**, v. 33, p. 395–404, 2013.

MICHELE BOZZANO et al. **Genetic considerations in ecosystem restoration using native tree species. State of the World's Forest Genetic Resources – Thematic Study**. Rome: FAO and Bioversity International, 2014.

NETO, M. et al. Natural regeneration in a restored bauxite mine in southeast Brazil. **Bosque**, v. 35, n. 3, p. 377-389, 2014.

MITTERMEIER, R. A. et al. **Hotspots revisited: Earth's biologically richest and most endangered ecoregions**. 1st. ed. Mexico City, Mexico: CEMEX, 2004.

MITTERMEIER, R. A.; TURNER, W. R.; LARSEN, F. W. Global Biodiversity Conservation : The Critical Role of Hotspots. In: ZACHOS, F.E.; HABEL, J.C. **Biodiversity Hotspots**, August, 2011.

MOHR, J. J.; METCALF, E. C. The business perspective in ecological restoration: issues and challenges. **Restoration Ecology**, v. 26, n. 2, p. 381–390, 2018.

MORANTE-FILHO, J.C., ARROYO-RODRÍGUEZ, V., LOHBECK, M, TSCHARNTKE, T. AND FARIA, D. Tropical forest loss and its multitrophic effects on insect herbivory. **Ecology**, v. 97, 3315–3325, 2016.

MYERS, N. et al. Biodiversity hotspots for conservation priorities. **Nature**, v. 403, February, p. 853–858, 2000.

NAMKOONG, G. et al. Testing Criteria and Indicators for Assessing the Sustainability of Forest Management : Genetic Criteria and Indicators. **CIFOR:Working paper**, v. 62, n. 10, p. 15, 1996.

NEI, M. Analysis of gene diversity in subdivided populations. **Proc Natl Acad Sci**, v. 70, p.3321-3323, 1973.

NOVAES, R. M.; RODRIGUES, J. G.; LOVATO, M. B. An efficient protocol for tissue sampling and DNA isolation from the stem bark of Leguminosae trees. **Genetics and Molecular Research**, v. 8, June 2014, 2009.

PARADIS, E. pegas : an R package for population genetics with an integrated – modular approach. **Bionformatics**, v. 26, n. 3, p. 419–420, 2010.

PAVINATO, V. A. C. et al. Restriction site associated DNA (RAD) for de novo sequencing and marker discovery in sugarcane borer, *Diatraea saccharalis* Fab. (Lepidoptera: Crambidae). **Molecular Ecology Resources**, v. 17, n. 3, p. 454–465, 2017.

PESSOA, M.S., ROCHA-SANTOS, L., TALORA, D.C., FARIA, D., MARIANO-NETO, E., HAMBUCKERS, A. AND CAZETTA, E. Fruit biomass availability along a forest cover gradient. **Biotropica**, v. 49, p. 45–55, 2017.

PINTO, S. R. et al. Governing and Delivering a Biome-Wide Restoration Initiative: The Case of Atlantic Forest Restoration Pact in Brazil. **Forests**, v. 5, September, p. 2212–2229, 2014.

POMPANON, F. et al. Genotyping errors: Causes, consequences and solutions. **Nature Reviews Genetics**, v. 6, n. 11, p. 847–859, 2005.

POTAPOV, P. et al. The last frontiers of wilderness: Tracking loss of intactforest landscapes from 2000 to 2013. **Science Advances**, v. 3, n. e1600821, p. 1–14, 2017.

PREISKORN, G. M.; COUTO, H. T. Z. Metodologia de restauração para fins de aproveitamento econômico (Reserva LEgal e Áreas Agrícolas). In: **Pacto pela Restauração da Mata Atlântica - referencial dos conceitos e ações de restauração florestal**. Ricardo R. Rodrigues, Pedro Henrique S. Brancalion, LERF/ESALQ: Instituto BioAtlântica, 2009, 256 p.

PROFT, K. M. et al. Making the connection : expanding the role of restoration genetics in restoring and evaluating connectivity. **Restoration Ecology**, v. 26, n. 3, p. 411–418, 2018.

QGIS DEVELOPMENT TEAM. **QGIS Geographic Information System**. Open Spurce Geospatial Foundation Project, 2018.

R CORE TEAM. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria, 2018

RESENDE, L. C.; RIBEIRO, R. A.; LOVATO, M. B. Diversity and genetic connectivity among populations of a threatened tree (*Dalbergia nigra*) in a recently fragmented landscape of the Brazilian Atlantic Forest. **Genetica**, v. 139, p. 1159–1168, 2011.

REZENDE, C. L. et al. From hotspot to hopespot : An opportunity for the Brazilian Atlantic Forest. **Perspectives in Ecology and Conservation**, v. 16, n. 4, p. 208–214, 2018.

RIBEIRO, M. C. et al. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. **Biological Conservation**, v. 142, n. 6, p. 1141–1153, 2009a.

RIBEIRO, R. A. et al. Genetic Variation in Remnant Populations of *Dalbergia nigra* (Papilionoideae), an Endangered Tree from the Brazilian Atlantic Forest. **Annals of Botany**, v. 95, p. 1171–1177, 2005.

RIBEIRO, R. A. et al. Development of polymorphic microsatellite markers for *Dalbergia nigra* (Papilionoideae), an endangered tree from the Brazilian Atlantic Forest. **Molecular Ecology Resources**, v. 9, p. 203–206, 2009b.

RODRIGUES, R. R.; BRANCALION, P. H. S.; ISERNHAGEN, I. **Pacto pela restauração da mata atlântica: referencial dos conceitos e ações de restauração florestal**. São Paulo, LERF/ESALQ: Instituto BioAtlântica, 2009, 256 p.

SALZMAN, J. et al. The global status and trends of Payments for Ecosystem Services. **Nature Sustainability**, v. 1, n. 3, p. 136–144, 2018.

SAMBUICHI, R. H. R.; MIELKE, M.S.; PEREIRA, C. E. **Nossas árvores: conservação, uso e manejo de árvores nativas no sul da Bahia**. Editus, Ilhéus – BA. 2009, 296 p.

SANTOS, A. S. et al. Landscape-scale deforestation decreases gene flow distance of a keystone tropical palm, *Euterpe edulis* Mart (Arecaceae). **Ecology and Evolution**, v. 6, n. 18, p. 6586–

6598, 2016.

SANTOS, A. R. DOS. **Propagação Sexuada, Vegetativa e Diversidade Genética de *Dalbergia Nigr*a em Minijardim Clonal**. 2019. 136 f. Dissertação (Ciências Florestais) - Universidade Federal do Espírito Santo, 2019.

SCHWARCZ, K. D. et al. Shelter from the storm: Restored populations of the neotropical tree *Myroxylon peruiferum* are as genetically diverse as those from conserved remnants. **Forest Ecology and Management**, v. 410, December 2017, p. 95–103, 2018.

SEBBENN, A. M. et al. Low levels of realized seed and pollen gene flow and strong spatial genetic structure in a small, isolated and fragmented population of the tropical tree *Copaifera langsdorffii* Desf. **Heredity**, v. 106, n. 1, p. 134–145, 2011.

SECRETARIA DO MEIO AMBIENTE DO ESTADO DE SÃO PAULO, SEMA. Resolução SMA nº 57, de 05 de junho de 2016.

SEI. Unidades de Conservação - Estado da Bahia. 2011.

SÉRÉ, M. et al. Comparison of different genetic distances to test isolation by distance between populations. **Heredity**, v. 119, November, p. 55–63, 2017.

SILVA, J.M.C., CASTELETI, C.H.. Status of the biodiversity of the Atlantic Forest of Brazil. In: Galindo-Leal, C., Câmara, I.G. (Eds.), **The Atlantic Forest of South America: Biodiversity Status, Threats, and Outlook**. Island Press, Washington, DC, pp. 43-59, 2003.

SILVA, K. D. A. et al. Soil Seed Banks in a Forest Under Restoration and in a Reference Ecosystem in Southeastern Brazil. **Floresta e Ambiente**. v. 26, n. 4, 2019.

SOCIETY FOR ECOLOGICAL RESTORATION INTERNATIONAL SCIENCE & POLICY WORKING GROUP. The SER International primer on ecological restoration. **Ecological Restoration**, v. 2, n. 2, p. 206–207, 2004.

SOUZA, A. M.; CARBONI, M.; VIEIRA, A. S. **Riqueza em espécies arbóreas utilizadas no Projeto Mata Ciliar na Microbacia do Santo Antonio no Município de Jaú/SP**. Jaú: Instituto Pró-Terra, 2009.

SUJII, P. S. et al. Recovery of genetic diversity levels of a Neotropical tree in Atlantic Forest restoration plantations. **Biological Conservation**, v. 211, November 2016, p. 110–116, 2017.

SUJII, P. S. et al. A genetic approach for simulating persistence of reintroduced tree species populations in restored forests. **Ecological Modelling**, v. 403, n. April, p. 35–43, 2019.

TABARELLI, M. et al. Prospects for biodiversity conservation in the Atlantic Forest : Lessons from aging human-modified landscapes. **Biological Conservation**, v. 143, n. 10, p. 2328–2340, 2010.

TAMBOSI, L. R. et al. A framework to optimize biodiversity restoration efforts based on habitat

amount and landscape connectivity. **Restoration Ecology**, v. 22, n. 2, p. 169–177, 2014.

TAUBERT, F. et al. Global patterns of tropical forest fragmentation. **Nature**, v. 554, p. 519-522, 2018.

THOMAS, E. et al. Genetic considerations in ecosystem restoration using native tree species. **Forest Ecology and Management**, v. 333, p. 66-75, 2014.

THOMAS, E. et al. The Importance of Species Selection and Seed Sourcing in Forest Restoration for Enhancing Adaptive Capacity To Climate Change: Colombian Tropical Dry Forest As a Model. **Convention on Biological Diversity Technical Series**, n. 89, 2017.

UNCBD, U. N. C. ON B. D. Decision Adopted by The Conference of the Parties to the Convention on Biological Diversity at its Tenth Meeting X/29. Marine and coastal biodiversity. **Strateg. Plan Biodivers. 2011–2020**, n. October 2010, 2012.

UNITED NATIONS OFFICE ON DRUGS AND CRIME, UNDC. **World Wildlife Crime Report: trafficking in protected species**. United Nations, 2016.

URZEDO, D. I. DE et al. How policies constrain native seed supply for restoration in Brazil. **Restoration Ecology**, v. 27, n.4, 2019.

VARTY, N. 1998. *Dalbergia nigra*. **The IUCN Red List of Threatened Species 1998**. International Union for Conservation of Nature and Natural Resources. Available at: http://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T32985A9741135.en.

VERGARA, W. et al. **The Economic Case for Landscape Restoration in Latin America**. World Resource Institute, Creative Commons, p. 1–62, 2016.

VIANA, J. P. G. et al. Genomic diversity is similar between Atlantic Forest restorations and natural remnants for the native tree *Casearia sylvestris* Sw. **PLoS ONE**, v. 13, n. 3, p. 1–14, 2018.

VRANCKX, G. U. Y. et al. Meta-Analysis of Susceptibility of Woody Plants to Loss of Genetic Diversity through Habitat Fragmentation. **Conservation biology**, v. 26, n. 2, p. 228–237, 2011.

VOGL C, KARHU A, MORAN G, SAVOLAINEN O. High resolution analysis of mating systems: inbreeding in natural populations of *Pinus radiata*. J **Evol Biol**., v. 15,433–439, 2002.

WANG, C.; SCHROEDER, K. B.; ROSENBERG, N. A. A Maximum-Likelihood Method to Correct for Allelic Dropout in Microsatellite Data with No Replicate Genotypes. **Genetics**, v. 192, n. October, p. 651–669, 2012.

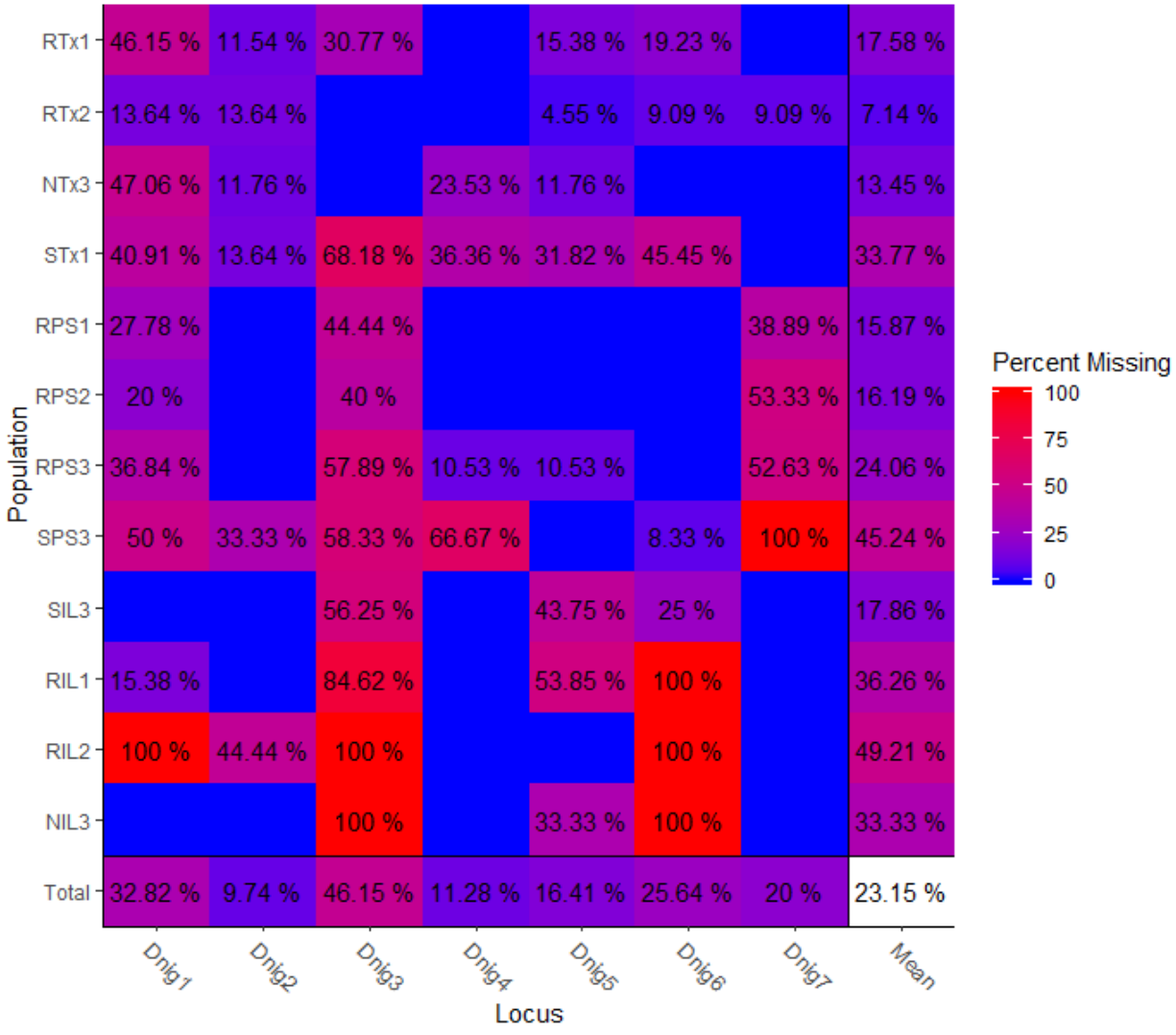
WATSON, J. E. M. et al. The exceptional value of intact forest ecosystems. **Nature Ecology & Evolution**, v. 2, p.599-610, 2018a.

WATSON, J. E. M. et al. Protect the last of the wild. **Nature**, v. 563, p. 27–30, 2018b.

YOUNG, A.; BOYLE, T.; BROWN, T. The population genetic consequences of habitat fragmentation for plants. **Tree**, v. 11, n. 10, p. 413–418, 1996.

ZUCCHI, M. I. et al. Genetic diversity of reintroduced tree populations in restoration plantations of the Brazilian Atlantic Forest. **Restoration Ecology**, v. 26, n. 4, p. 694–701, 2018.

## SUPPLEMENTARY MATERIAL



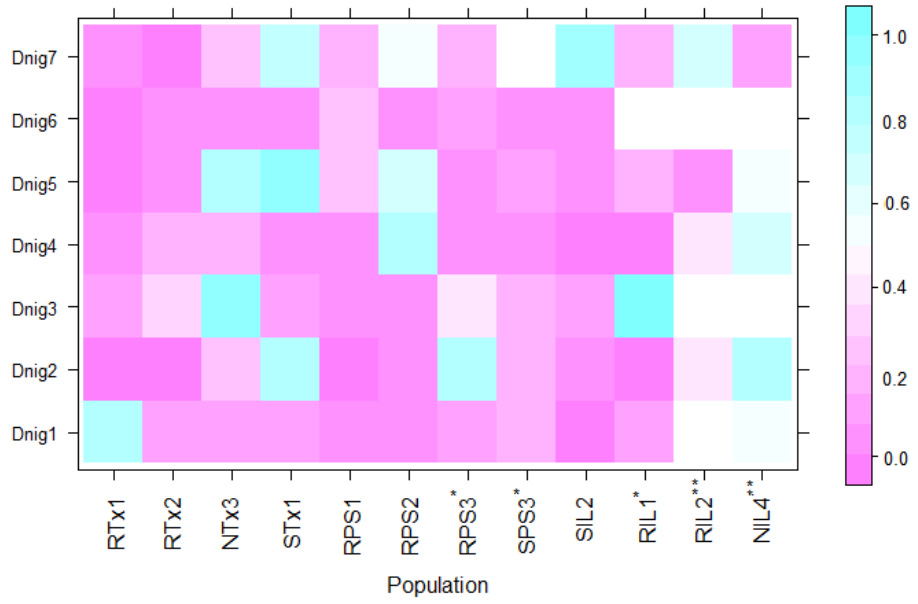

**Supplementary Figure 1**. Percentage of missing genotypes per locus per population of *D. nigra*. Red shadings indicate higher percentages of missing genotypes, while blue shadings smaller percentages.

**Supplementary Table 1**. Number of alleles per locus in each *D. nigra* population

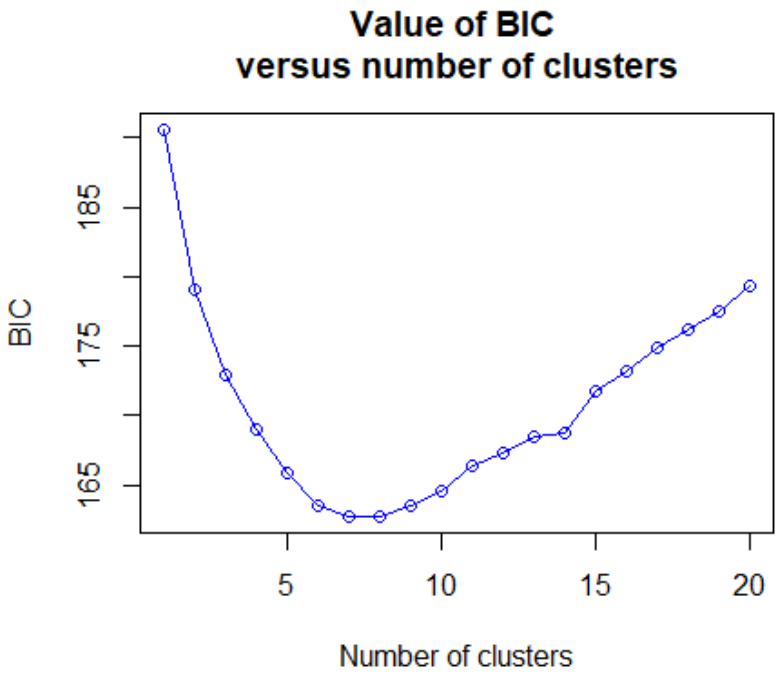
| Locus | RTx1 | RTx2 | NTx3 | STx1 | RPS1 | RPS2 | RPS3 | SPS2 | RIL1 | RIL2 | NIL3 | SIL3 | Overall |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Dnig1* | 6 | 9 | 5 | 5 | 4 | 3 | 5 | 4 | 5 | - | 4 | 6 | 14 |
| *Dnig2* | 6 | 7 | 5 | 6 | 8 | 4 | 7 | 6 | 6 | 4 | 5 | 6 | 11 |
| *Dnig3* | 10 | 7 | 6 | 4 | 9 | 9 | 9 | 5 | 1 | - | - | 9 | 16 |
| *Dnig4* | 11 | 5 | 6 | 7 | 7 | 4 | 8 | 5 | 6 | 4 | 5 | 9 | 15 |
| *Dnig5* | 11 | 7 | 8 | 9 | 8 | 7 | 7 | 6 | 5 | 9 | 4 | 7 | 18 |
| *Dnig6* | 12 | 11 | 6 | 10 | 4 | 7 | 5 | 3 | - | - | - | 10 | 17 |
| *Dnig7* | 6 | 6 | 3 | 4 | 3 | 3 | 4 | - | 3 | 2 | 2 | 2 | 5 |
| **Mean** | **8.86** | **7.43** | **5.57** | **6.43** | **6.14** | **5.29** | **6.43** | **4.83** | **4.33** | **4.75** | **4.00** | **7.00** | **13.71** |

**Supplementary Table 2**. Deviance Information Criterion for each model estimated in INEst. Models accounts for null allele presence (n), random genotyping failure (b) and inbreeding effects (f) influencing the dataset.

| | DIC | | | | | |
|---|---|---|---|---|---|---|
| Population | *nfb* | *nf* | *nb* | *fb* | *n* | *b* |
| RTx1 | 1174.365 | 1171.149 | 1179.916 | 1170.431 | 1190.051 | 1259.235 |
| RTx2 | 870.107 | 871.503 | 879.605 | 883.962 | 881.153 | 962.474 |
| NTx3 | 632.336 | 645.413 | 635.434 | 647.016 | 654.055 | 669.415 |
| STx1 | 758.885 | 776.838 | 764.786 | 765.893 | 785.302 | 811.735 |
| RPS1 | 627.720 | 623.035 | 628.253 | 640.147 | 630.761 | 664.069 |
| RPS2 | 463.597 | 458.081 | 456.722 | 479.858 | 458.329 | 517.517 |
| RPS3 | 669.651 | 658.134 | 652.637 | 645.499 | 656.881 | 715.110 |
| SPS3 | 353.290 | 361.154 | 359.886 | 356.609 | 362.468 | 388.217 |
| RIL1 | 410.110 | 420.591 | 410.695 | 411.557 | 418.290 | 434.685 |
| RIL2 | 235.160 | 246.513 | 234.970 | 251.406 | 247.061 | 254.331 |
| NIL3 | 195.888 | 206.532 | 197.693 | 207.195 | 215.911 | 202.747 |
| SIL3 | 680.437 | 688.822 | 696.442 | 735.144 | 696.699 | 728.870 |




**Supplementary Figure 2**. Heatmap of significant departures from Hardy-Weinberg Equilibrium proportions ($p \leq 0.05$) per locus in 12 populations of *D. nigra*. X axis indicates populations and Y axis indicates loci. Pink shadings indicate smaller values, while blue shadings higher values. *Population genotyped for six locus; **Population genotyped for four locus

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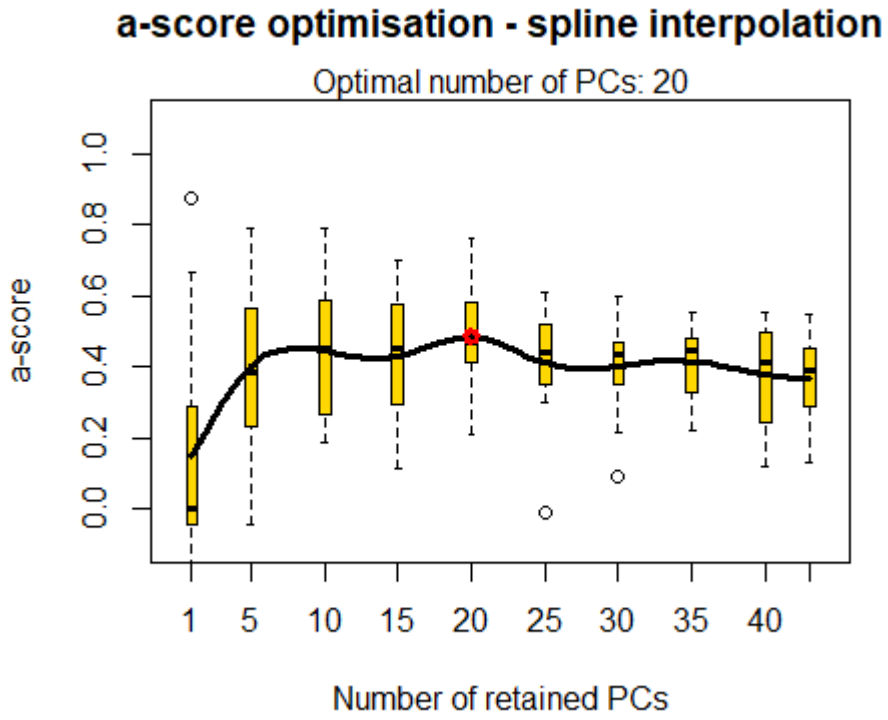
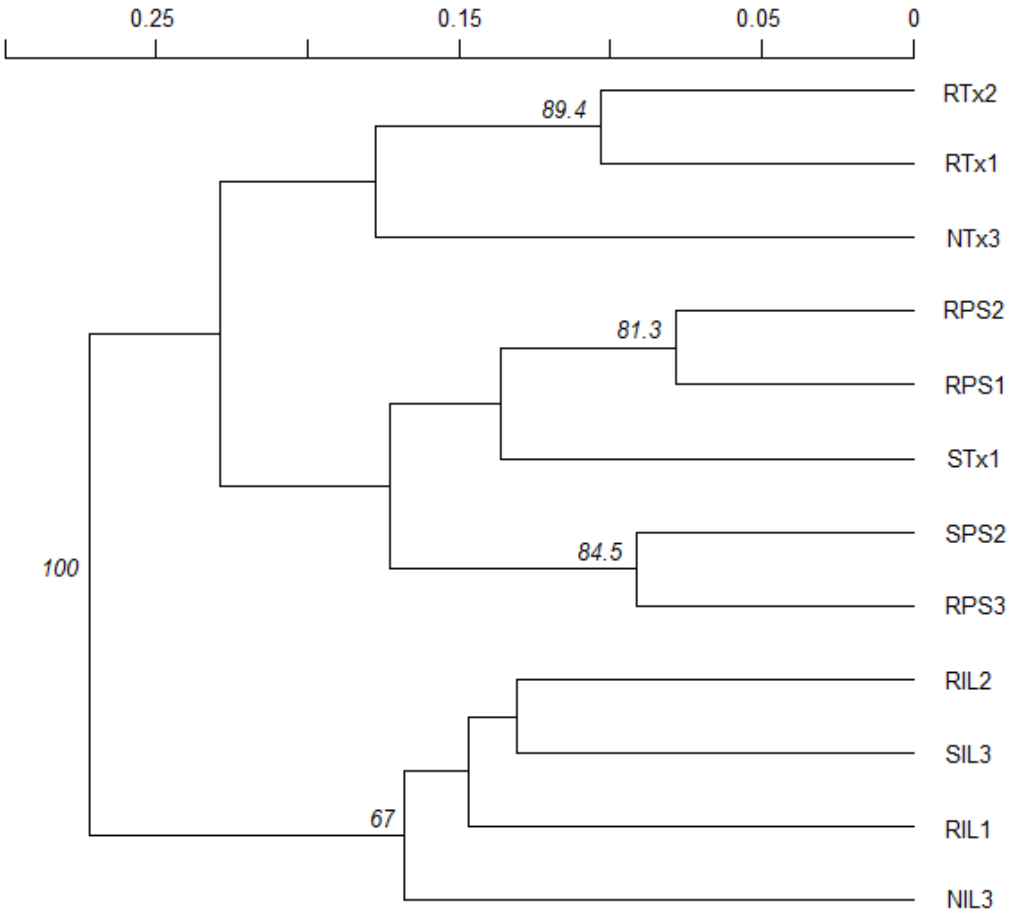
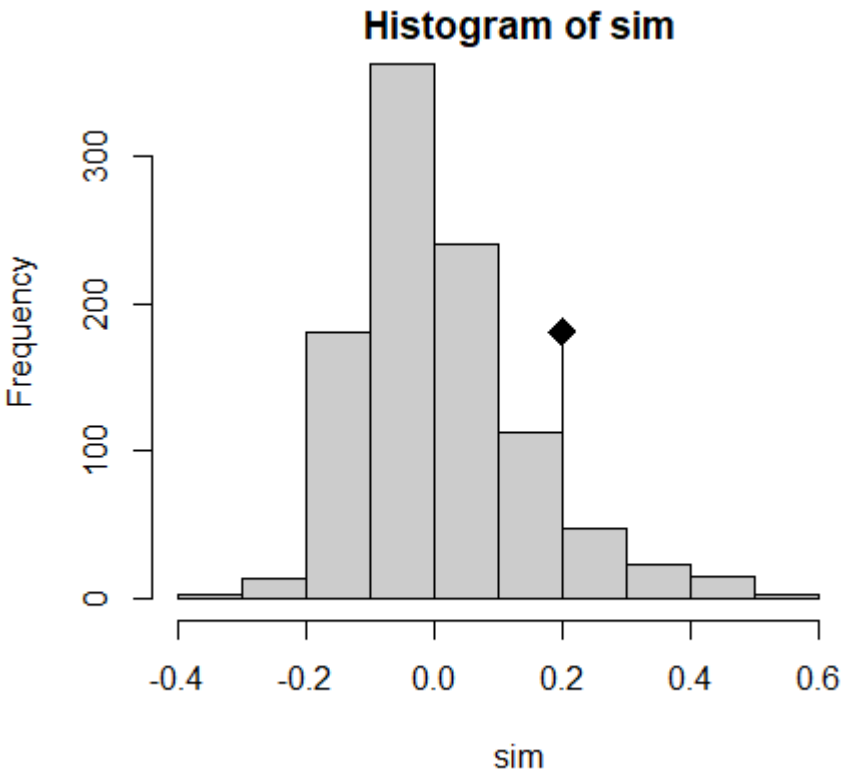
**Supplementary Figure 3**. Compared optimal number of cluster using Bayesian Information Criterion (BIC) to define genetic groups that best describe the 12 populations of *D. nigra*. Ideally, the best descriptor is the lowest BIC (k=8).




**Supplementary Figure 4**. Number of retained Principal Components (PCs) according to optimization of the power of discrimination and over-fitting (α-score) for the reassignment DAPC analysis of 196 genotypes of *D. Nigra*.




**Supplementary Figure 5**. UPGMA dendrogram generated from Nei's genetic distance on 12 populations of *D. nigra*. Node labels represent bootstrap support (>50%) out of 1000 bootstrap replicates.




**Supplementary Figure 6**. Isolation by distance of 196 individuals of *D. nigra*, measured as correlation between Edwards' distances and Euclidean geographic distances. Histograms represent permuted values.