PPG Ecologia & Conservação

Universidade Estadual de Santa Cruz

Forest Restoration Genetics: From Gene Repositories and *Ex Situ* Conservation to Practical Aspects of Maintaining Population Genetic Diversity.

Tese apresentada ao Programa de Pós-graduação em Ecologia e Conservação da Biodiversidade da Universidade Estadual de Santa Cruz, para obtenção de Título de Doutor em Ecologia e Conservação da Biodiversidade.

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Ilhéus – BA 2023

AGRADECIMENTOS

E assim vamos chegando lá...

O caminho foi tortuoso e cansativo, e só foi possível concluí-lo porque tenho vocês ao meu lado. Então, vamos aos agradecimentos...

Primeiramente, agradeço a Deus por não ter me desamparado, mesmo quando eu não sabia em que crer. Também agradeço por ter me dado a bênção de ter pais tão maravilhosos quanto os meus. Eu costumava dizer, em forma de piada, uma frase que sempre ouvi de minha mãe: "Vai nadar no mar e morrer na praia?". Tal frase sempre fora usada por ela como forma de incentivo quando eu pensava em desistir, e aqui estamos sem ter "morrido na praia". Então, sou muito grata aos dois por terem me amparado e acreditado que eu conseguiria chegar ao fim desta jornada!

Agradeço ao meu irmão, Wagner, e sua esposa, Daiane, por terem suprido minha ausência nos anos que fiquei fora. Também agradeço por terem cuidado dos meus pais e da nossa querida vó Quina.

Agradeço à minha marida, Vanessa, e às amigas, Gleice e Débora, pelos momentos de alegria e pela partilha dos momentos tristes. Agradeço também por me ajudarem nas coletas e no cuidado com minhas gatitas quando eu não pude estar presente. E às minhas xinhas, Ana, João e Eloan, pelo companheirismo e pelos 12 anos de amizade, que por mais distantes que estejamos, eu nunca estive só.

Agradeço à minha querida amiga Taise, mulher brilhante de sorriso farto que sempre tem uma palavra de conforto e um ombro amigo. Minha fotógrafa favorita, que topava os rolês mais aleatórios e programas de senhorinhas comigo. Sem sua paciência, dedicação e cuidado em me

ensinar a rotina de bancada e tirar dúvidas sobre genética, me apresentar a espécie e a Mata Atlântica e me auxiliar nas coletas de campo, esse trabalho não seria possível.

Agradeço à Zubaria pelo companheirismo nas madrugadas a caminho da UESC, nas longas jornadas no laboratório, e por cantar e rir comigo sobre coisas que só nós duas entendíamos. Só tenho mais uma coisa a dizer: "Lá, lá, lá, lá, lá, lá, lá... Do, do, do, do, do... aaaaaahhhhhhhh!!"

Agradeço à minha orientadora Fernanda pela paciência, cuidado e por todos os ensinamentos que me proporcionou. Ao Alesandro por cada palavra de incentivo e por compreender minhas limitações e me socorrer nas minhas dúvidas. Agradeço também ao Grupo de Genética da Conservação pelas trocas de conhecimento e discussões em grupo que foram cruciais para o meu crescimento pessoal e amadurecimento profissional, em especial as queridas Daniele e Geane.

E por fim, agradeço às instituições e agências de fomento que acreditaram no nosso projeto e financiaram esta jovem cientista que vos escreve: à Rufford Foundation pelo financiamento do projeto, ao CNPq (MAI DAI) e FAPESB pelo financiamento da bolsa de doutorado e à Symbiosis Investimentos e participações pela parceria e financiamento do projeto.

SUMMARY

GENERAL ABSTRACT

Ecological restoration presents a vital alternative for mitigating the escalating loss of biodiversity and fragmentation of habitats. Restoration efforts must be tailored to the specific local ecosystem and historical formation that once existed in the target area. Understanding the genetic parameters of forest remnants and the influence of restoration approaches is fundamental to achieving successful restoration outcomes. In the first chapter, I present a systematic review published in the Ecological Restoration Journal. This review summarizes the findings of empirical studies comparing genetic diversity and other genetic parameters in restored areas with those observed in natural forest remnants. We discuss the effectiveness of forest restoration in maintaining genetic diversity and perpetuating restored communities. Our review concludes that restored areas can achieve levels of genetic diversity comparable to those found in natural remnants. These findings hold significant implications for efficient restoration planning. We suggest that incorporating genetic diversity studies into restored areas can offer valuable data to corroborate the success and effectiveness of forest restoration efforts. Our analysis further underscores the critical importance of considering the specific strategies employed in restoration projects, the target species, and the source of propagules. Forest plantations established with native species possess substantial potential, as they combine the benefits of commercial forestry with reduced pressure on native forests for high-quality timber. This approach, known as "productive conservation," also presents an avenue for forest restoration. However, realizing this potential necessitates maintaining a known and traceable source of propagules, including a diverse germplasm bank for cross-breeding and genetic improvement of the target species. Our research aimed to analyze the relatedness among individuals within a germplasm bank and assess the level of genetic diversity it harbored. Additionally, we compared this germplasm bank with natural populations to evaluate whether it could be considered a genetically representative *ex situ* conservation population. Ex situ conservation refers to the strategy of preserving plant genetic resources outside their natural habitat.

RESUMO GERAL

A restauração ecológica apresenta-se como uma alternativa vital para mitigar a crescente perda de biodiversidade e a fragmentação de habitats. Os esforços de restauração devem ser adaptados ao ecossistema local específico e à formação histórica que existia anteriormente na área alvo. Compreender os parâmetros genéticos dos remanescentes florestais e a influência das abordagens de restauração é fundamental para alcançar resultados bem-sucedidos. No primeiro capítulo, apresento uma revisão sistemática publicada no Journal Ecological Restoration. Esta revisão resume os resultados de estudos empíricos que comparam a diversidade genética e outros parâmetros genéticos em áreas restauradas com aqueles observados em remanescentes naturais de florestas. Discutimos a eficácia da restauração florestal na manutenção da diversidade genética e na perpetuação das comunidades restauradas. Nossa revisão conclui que áreas restauradas podem atingir níveis de diversidade genética comparáveis aos encontrados em remanescentes naturais. Esses resultados têm implicações significativas para o planejamento eficiente da restauração. Sugerimos que a incorporação de estudos de diversidade genética em áreas restauradas pode oferecer dados valiosos para corroborar o sucesso e a eficácia dos esforços de restauração florestal. Nossa análise ressalta ainda a importância crucial de considerar as estratégias específicas empregadas em projetos de restauração, as espécies-alvo e a fonte de propágulos. Plantios florestais estabelecidos com espécies nativas possuem potencial substancial, pois combinam os benefícios da silvicultura comercial com a redução da

pressão sobre florestas nativas para madeira de alta qualidade. Essa abordagem, conhecida como "conservação produtiva", também apresenta uma via para a restauração florestal. No entanto, para se concretizar esse potencial, é necessário manter uma fonte conhecida e rastreável de propágulos, incluindo um banco de germoplasma diversificado para cruzamento e melhoramento genético das espécies-alvo. Nossa pesquisa teve como objetivo analisar a relação de parentesco entre os indivíduos dentro de um banco de germoplasma e avaliar o nível de diversidade genética que ele abrigava. Adicionalmente, comparamos este banco de germoplasma com populações naturais para avaliar se ele poderia ser considerado uma população de conservação *ex situ* geneticamente representativa.

A GENERAL INTRODUCTION

According to the Society for Restoration Ecology (SERI, 2004), ecological restoration can be understood as the process of assisting in the recovery of an ecosystem that has been lost or degraded, either by replacing the native species of this environment or by simply creating the conditions for this area to regenerate. Indeed, the United Nations (UN) declared this as the Decade of Ecosystem Restoration (2021 – 2030), proposing actions to intensify the restoration of degraded ecosystems, as a means to fight the climate crisis, improve food security, and strengthen biodiversity.

Genetic diversity is an essential component of biodiversity and must be considered in restoration strategies (Basey et al., 2015; Carnus et al., 2006; Fernandes et al., 2023) as it fosters adaptability and resistance to abiotic and biotic disturbances (Aavik & Helm, 2018). With the availability of modern genetic tools, we can monitor restored areas (Allendorf et al., 2013; Breed et al., 2019), investigate connectivity between populations in nearby areas (Cordeiro et al., 2019; Santos et al., 2016; Schwarcz et al., 2018; Sujii et al., 2021; Vanden Broeck et al., 2021), and assess whether regenerating populations retain genetic diversity to ensure viable populations by reducing genetic structure (Aavik & Helm, 2018).

Forest restoration stands as a cornerstone of ecological restoration (Brancalion et al., 2009). The origin of seeds and seedlings used for forest restoration can influence the success of this restoration effort. Fernandes et al. (2023) therefore recommend collecting them from diverse matrices and localities whenever possible. Considering the characteristics of the species, they emphasize in their review the importance of understanding the gene pool that will be implanted to maximize the genetic diversity of the restored population.

Moreover, comparable estimates of genetic diversity between populations in natural and restored remnants would be a promising outcome for endangered forest conservation (Cordeiro

et al., 2019; Fernandes et al., 2023; Schwarcz et al., 2018; Sujii et al., 2017; Viana et al., 2018; Zucchi et al., 2018). It would indicate the efficacy of forest restoration in preserving the genetic diversity of key plant species and, consequently, the potential for restored communities to self-perpetuate (Viana et al., 2018). Additionally, forest restoration facilitates the connection of remaining fragments across the landscape through gene flow (Sujii et al., 2021), contributing to the long-term resilience of tropical forests embedded in human-modified landscapes amidst the current scenario of environmental degradation.

In light of the current deforestation and fragmentation rates (Fundação SOS Mata Atlântica, 2021) and their projected future trajectories, along with the recognition of landscapes as crucial. For structuring and preserving biodiversity, implementing conservation strategies in altered and fragmented environments is imperative (Colorado Zuluaga et al., 2017). Re-establishing connectivity between isolated or poorly connected forest fragments through ecological corridors becomes particularly important at the landscape level, promoting physical and genetic connectivity for wildlife populations (Torres et al., 2022).

Considering the connectivity between forest fragments in a degraded landscape (Carnus et al., 2006), planted forests can serve as stepping-stones or corridors facilitating movement between these fragments. Planted forests perform numerous ecosystem services (Payn et al., 2015) even when surrounded by a deforested matrix. They can provide an escape and protection area for fauna, and serve as a corridor between forest remnants. This type of forest also regulates local temperatures and rainfall patterns, since it can influence precipitation at the local and regional scales, changing heat and humidity exchanges between the surface and atmosphere (van Dijk & Keenan, 2007). Additionally, a benefit of planted forests is that they reduce pressure on high-value forests for industrial use and construction purposes, helping to protect native forests

(Carle & Homgren, 2008). In recent years, while the loss of native forest continues, planted forests have been increasing significantly (Payn et al., 2015).

Bearing this in mind, I present this thesis, which comprises two chapters. The first chapter is a systematic review in which we present our findings on how forest restoration could affect the genetic diversity of plant populations. We analyzed empirical papers that compare diversity between natural and restored populations on a global scale, encompassing works from all continents and diverse biomes. In the second chapter, we evaluate the relatedness between and within families of *Plathymenia reticulata* kept in an active germplasm bank of a private company. Our findings will help to select and genetically improve the species for commercial plantations. Considering the scenario of exploitation and depletion of natural remnants, as well as the potentially low diversity remaining in these areas, this chapter also presents the germplasm bank as an *ex situ* conservation population comparing the genetic status of *P. reticulata* between ex situ conservation and natural populations.

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FIRST CHAPTER

CAN FOREST RESTORATION AFFECT THE GENETIC DIVERSITY OF PLANTS?

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ABSTRACT

Positive effects on the ecosystem can be achieved through forest restoration. Restored forests provide habitats for a wide range of plants and animals and act as corridors facilitating the movement of species between habitat fragments, thus preventing isolation and loss of genetic diversity. Understanding the genetic parameters of forest remnants and the influence of restoration approaches is crucial for successful restoration. In this review, we summarize research papers that evaluate the genetic diversity of restored areas compared to natural forest remnants and discuss the effectiveness of forest restoration for maintaining genetic diversity and perpetuating restored communities. We conclude that restored areas can attain levels of genetic diversity like those observed in natural remnants. Our findings have implications for efficient restoration planning, and we suggest that genetic diversity studies in restored areas can help corroborate the success and effectiveness of forest restoration. We conclude that it is very important to consider the strategies used for restoration projects, the species targeted, and the source of propagules.

Keywords: biodiversity; forest conservation; landscape connectivity; restoration genetics.

RESTORATION RECAP

- Restoration is key for conservation of forest, and restoration can be more successful if genetics are taken into consideration.
- Genetic tools can broaden our appreciation for maintaining connectivity between forest remnants and contribute to long-term viability of species.
- Considering the genetic parameters of restored areas and natural remnants can help guarantee the viability of species and may suggest appropriate restoration approaches.

INTRODUCTION

Restoration efforts are not merely beneficial for conserving remaining forests, but may also be the only opportunity to ensure biodiversity conservation and environmental stability over time. For example, addressing reforestation and reducing deforestation rates can lead to positive effects such as decreasing the isolation of remaining fragments (Taubert et al. 2018), while reforested areas can serve as biological corridors between conservation areas (Chazdon et al. 2017). The restoration of priority areas— especially those with high potential for regeneration—can facilitate gene flow and mitigate the decreased gene flow that inevitably follows the loss of extensive forest cover (Santos et al. 2016).

Restoration can be active or passive, or a combination of both. Active restoration involves human interventions such as planting the restoration area with native tree species by direct seeding or planting seedlings, a strategy particularly valuable on sites where propagules are missing either because of a depleted soil seed bank or lack of trees for seed dispersal (Brancalion et al. 2016). Passive restoration, on the other hand, mainly involves natural regeneration by isolating the area from further anthropogenic disturbance and encouraging spontaneous seedling regeneration (Brancalion et al. 2016; Vergara et al. 2016). Both restoration approaches can yield favorable outcomes, and their complementarity can produce

ecosystem values like those found in native forests (Zeng and Fischer 2021; Crouzeilles and Curran 2016). The restoration methods used depend on many factors including availability of source populations and suitable conditions (Gastauer et al. 2021), and they can affect genetic diversity in long-term.

Genetic diversity studies are crucial to identify new and better strategies for genetic enrichment (Santini et al. 2018), and to prevent negative outcomes such as genetic bottlenecks and the founder effect due to genetic drift. These studies are essential for measuring the success of environmental restoration not only during or after restoration, but also before the project is even undertaken (Granado et al. 2018). Over time, inbreeding rates and genetic bottlenecks in reintroduced populations may lead to a reduction in the quantity and quality of seeds. These deleterious effects can be substantially aggravated under severe environmental conditions, resulting in reduced population fitness. Currently, research suggests incorporating genetic connectivity into restoration planning to increase the likelihood of success (Zeng and Fischer 2021, Proft et al. 2018). Enhancing connectivity through gene flow potentially increases the effective population size (NE) (Proft et al. 2018).

To support conservation and restoration goals, understanding genetic parameters of conserved forest remnants and the influence of various restoration approaches on these parameters is essential (Mutegi et al. 2014). Many studies have emphasized the importance of genetic diversity, although most of them have found similar values for heterozygosity in restored and native areas (Sujii et al. 2019; Zucchi et al. 2018; DeWald and Kolanoski 2017, Céspedes et al. 2003). In recent years, many studies have compared the genetic diversity of restored populations to natural populations. The results have been inconclusive due to differing factors such as the target species evaluated and restoration methods used. Generally, the genetic characteristics of the first-generation trees (planted seeds or seedlings) will determine the population's potential to adapt and reproduce for long-term survival (Aavik and Helm 2018).

Thus, restoration practioners need to ask whether using sources from different locations is advisable or whether using local seed sources yields plants that are more resilient to the conditions of these habitats. In this review, we summarize studies that have evaluated the genetic diversity of restored areas compared to natural forest remnants, and discuss the effectiveness of forest restoration in maintaining genetic diversity, depending on the restoration strategy chosen.

MATERIALS AND METHODS

LITERATURE SURVEY AND SELECTION OF STUDIES

We searched for published papers indexed in Scopus, Web of Science, Google Scholar and Scielo from 2000 to 2022. For the search, we built strings composed by the following keywords: Forest restoration, Genetic diversity, Gene flow, and their variations (i.e. “genetic variability” OR “genetic diversity” OR “genetic variation” AND “Forest restoration” OR “landscape restoration” OR “ecosystem restoration”). When our searches returned over 1000 papers, more restrictive words were added, such as: Plant OR Tree AND “tree plantation” OR “planted forest” OR “plant restoration.” These words were searched in the title, abstract, and keyword sections of the papers. In addition, we used the year of publication and the type of manuscript (i.e., research article, complete [not theoretical] paper) as search filters.

We used the tool StArt (State of the Art through Systematic Review) (Zamboni et al. 2010) to organize the selections and extract information from the papers. Our criteria for including a study in our dataset were: 1) studies performed in restored areas; 2) studies using a genetic approach, and 3) studies with forest plant species as subjects. We also focused on forest restoration projects and excluded papers dealing with aquatic and agricultural systems, and papers evaluating animal genetics. We also excluded theoretical or review papers. After completing the literature searches, we imported all the papers to the StArt to select those most

suitable for this review and searched for keywords in the title or abstract, accepting those papers that fulfilled all the inclusion criteria.

DATA EXTRACTION

From the studies selected, we proceeded to the extraction step during which we read each paper to extract the data for this review (Table 1). To answer our main question, "Can forest restoration affect the population genetic diversity of plants?" we considered the following genetic parameters as measures of genetic diversity:

- *Observed and expected heterozygosity* (H_O and H_E), where H_O is the proportion of heterozygous individuals in a population, while H_E is the proportion of heterozygous individuals expected in a population based on the frequency of alleles present in the population
- *Allelic richness* (Ar), the number of different alleles in a population
- *Average number of private alleles* (Ap), those alleles that are found in only one or a few individuals across a population—an estimate a population's genetic diversity
- *Fixation index* (F_{IS}), also known as the inbreeding coefficient, a measure of the extent to which a population is genetically different from what would be expected under random mating
- *Effective population size* (Ne), a crucial parameter in evolutionary biology because it determines the relative outcomes of genetic drift (Turner et al. 2002).

We also documented restoration site characteristics like restoration age, type (i.e., passive or active), pollination and dispersion mechanism (i.e., biotic or abiotic), and the remnant biome. Furthermore, we extracted data about the plant species and the number of individuals sampled in each paper.

RESULTS

We found 729 papers in the search stage. Of these, we screened 607 papers, yielding 39 manuscripts for potential data extraction. After reading all 39 selected papers and rejecting those that did not conform to the inclusion criteria, we ultimately identified 25 papers used to prepare this review (Table 2, Supplementary Information).

South America led the number of published papers that compared genetic parameters between natural remnants and restored areas (40%), followed by North America (24%), Asia (16%), Europe (12%), and Australia (8%) (Figure 1A). Tropical forests were the focus of a majority of the restoration projects (44%), while temperate forests and grasslands were the subjects of 20% and 12% of the papers, respectively (Figure 1E). *Centrolobium tomentosum* (Fabaceae) was one of the most cited species used as a biological model, appearing in 16% of the studies, followed by *Casearia sylvestris* (Salicaceae) and *Myroxylon peruiferum* (Fabaceae); both latter species were sampled in the Brazilian Atlantic Forest (Table 2, Supplementary Information).

In 72% of the studies, pollination occurred by biotic vectors (Figure 1B), mostly through small insects, while seed dispersal mostly occurred via abiotic vectors (54%) (Figure 1C). Among the restoration project types, 76% used active methodologies consisting of collecting seeds (local or non-local), producing seedlings, and transplanting the seedlings into the area to be restored. Twelve percent of the studies considered passively restored areas, and in 8% of the studies it was unclear what type of restoration had been implemented. Only one study described a combination of both active and passive restoration strategies.

Among the genetic parameters cited in the selected papers, expected heterozygosity (H_E) was the most evaluated (19 papers). Observed heterozygosity (H_O) was cited in 15 papers, and fixation index (F_{IS}) or inbreeding coefficient was cited in 13 papers (Figure 1D). Another

measure of diversity, allelic richness (Ar), was cited in nine papers, and private alleles (Ap) in seven. The effective population size (NE) was the least estimated parameter (Figure 1D).

DISCUSSION

RESTORATION CHARACTERISTICS

In completing our review, we found a large number of studies conducted in South America, and most were in tropical forests (Figure 1A and 1E). The substantial number of studies conducted in the Brazilian Atlantic Forest (Table 2, Supplementary Information) highlighted the importance of this biome for restoration. Tropical forests are among the ecosystems with the highest levels of biodiversity and endemism (Myers et al. 2010), yet they are rapidly declining through deforestation and degradation. Given that preserving forest remnants is essential (but unlikely in itself to maintain forest integrity), restoring degraded ecosystems offers an alternative to mitigate and curb biodiversity loss (Zemanova et al. 2017; Rosa et al. 2016).

The effect of the restoration approach on genetic diversity depends on the propagule source used (Slaymaker et al. 2015). Restoration projects that use seedlings from diverse sources (active restoration) have the advantage over natural regeneration (passive restoration) in maintaining genetic diversity in fragmented forests (Zeng and Fischer 2021; Sujii et al. 2017; Zhang et al. 2016). Thus, it is essential to determine beforehand whether local or non-local seeds will be used for restoration planning. In an active restoration, it is necessary to collect seeds for seedling establishment from a large number of mother trees or from different populations (multiple sources). This practice aims to improve heterozygosity and reduce the incidence of inbreeding (St. Clair et al. 2020). Broadhurst (2013) found that low genetic diversity found in restored populations suggests that the seeds used in restoration projects came from few mother trees. On the other hand, passive restoration requires propagules to arrive and

establish themselves; such colonization can vary widely depending on the dispersal capacity and source populations.

GENETIC DIVERSITY

Some studies show high rates of genetic diversity documented for the restored populations compared to the forest remnants (Souza et al. 2016; Schwarcz et al. 2018; Cordeiro et al. 2019; Sujii et al. 2017; Ritchie et al. 2017; Zhang et al. 2016; Pakkad et al. 2008; Dolan et al. 2008; Fant et al. 2013; St. Clair et al. 2020) highlighting that restoration actions can achieved their goal. The findings demonstrate the effectiveness of forest restoration in maintaining genetic diversity and, consequently, the in perpetuating the restored communities (Viana et al. 2018; Thongkumkoon et al. 2019). Furthermore, our review revealed when we consider all studies evaluated, similarity of genetic diversity between restored and natural areas, which is great news for forest restoration, demonstrating that it is an effective tool for biodiversity conservation (Schwarcz et al. 2018).

As discussed by Thongkumkoon et al. (2019), long-term adaptive genetic diversity should reduce inbreeding, thereby decreasing the loss of genetic diversity from genetic drift and increasing the ability of the population to adapt to future site conditions. Nevertheless, the landscape in which a population is inserted may also affect its long-term adaptative genetic potential. For instance, a decrease in allelic richness has been reported after modifications to surrounding landscape (e.g., forest cover) (DeWald and Kolanoski 2017) and loss of private alleles were registered in restored populations with the increasement of forest cover in the landscape (Schwarcz et al. 2018; Dolan et al. 2008). Sujii et al. (2017) reported that the results of genetic diversity found for juvenile trees indicate that there is no evidence of negative effects of the restoration methodology on genetic diversity in the first few generations after implementation. Moreover, Zeng and Fischer (2021) emphasize that, in comparison with natural regeneration, conservationists often view active restoration unfavorably. These authors

also highlight in their study that active restoration used for *Quercus bambusifolia* has advantages over passive regeneration by contributing to the genetic variation amount and spatial arrangement in the area within the population, thus reducing the genetic structure observed in the fragmented natural forest.

This method acts as an anthropic dispersal agent and insert a variety of seedlots from different origins, which is absent in natural regeneration, and can help improve gene flow. However, a primarily concern in active restoration is the risk of implementing genotypes with similar alleles. If this limited set of genetic diversity is inserted by active restoration, it can lead to the reduction of private allele frequency and consequences like the bottleneck effect may happen (Schwarcz et al. 2018). Nonetheless, if the genetics are taken into consideration in the restoration planning process, active restoration may act as an artificial dispersal enhancer to the populations, by approaching the work otherwise naturally done by dispersers in improving gene flow.

GENE FLOW

In a fragmented landscape, ecological corridors can help facilitate movement of propagules between degraded areas, link nearby areas and maintain gene flow (Sujii et al. 2021). Trees in a restored area can exchange genes with forest remnants, provide alleles, and increase the population's gene pool (Fotinos et al. 2015). As such, populations in restoration areas will be sources of diversity, assuming that the restored area that has been planted with high-diversity seeds or seedlings (Sujii et al. 2021, Thongkumkoon et al. 2019, Cordeiro et al. 2019, Mutegi et al. 2014).

One of the major drivers of genetic diversity is the gene flow that can occur through pollen or seed dispersion, and can be increased by the vector type. For example, plants dispersed in biotic ways can reach more distant areas depending on the capacity of the disperser to move among habitat fragments. As discussed by Sujii et al. (2021), seed dispersal for *C. tomentosum*

was limited to short distances and pollen dispersal to medium distances. Both contributed to the population genetic structure and demonstrated that pollen flow was not restricted to the populations studied, thereby highlighting the key role of pollination for the success of restoring viable populations (Thongkumkoon et al. 2019, Sujii et al. 2021, Broadhurst 2013).

Alleles present in juveniles in restored areas that were not identified in adults in the same area are an indicator of the occurrence of gene flow (Sujii et al. 2017, Neto et al. 2014). Similarly, the higher genetic diversity and lower inbreeding coefficient found by Cordeiro et al. (2019) in juveniles compared to adults from the same population, or to juveniles from other populations is another demonstration of the occurrence of gene flow. Neto et al. (2014) demonstrated a 30% increase in allelic richness in seedlings compared to adults in planted populations, a change that was not found in natural populations. Thus, striving to maximize the genetic diversity of individuals to be introduced into restoration areas should consider the species' spatial genetic structure (Thongkumkoon et al. 2019), and avoid the collection of seeds solely from neighboring individuals (Sujii et al. 2021).

Success is intrinsically linked with the area selected for restoration. The landscape composition around the restored forest can further regeneration success, either by permeability (i.e., allowing seeds to be dispersed over long distances) or by attracting pollinators that will enhance gene flow (Sujii et al. 2021, Helsen et al. 2013). In addition, forest restoration is a way of connecting remaining fragments across the landscape through gene flow (Broeck et al. 2021), which will contribute to the long-term resilience of forests remaining in human-modified landscapes.

RECOMMENDATIONS

Because the source of propagules used in restoration can have a direct influence on success, restorationists should attempt to collect propagules from a broad spectrum of the landscape and from as many different source trees as possible, while always respecting the intrinsic

characteristics of the species. We also recommended that, when possible, restorationists should conduct a study of the local and regional gene pool to maximize genetic diversity of the restored area, again, considering the characteristics of the species.

AUTHOR CONTRIBUTIONS

The study conception and design were performed by AKCF, TMCQ, TAC, ZQ, FAG and ASS. Data collection was performed by AKCF. Material preparation were performed by AKCF and TMCQ. Writing—original draft preparation was written by AKCF. Map and graphics were made by IC and AKCF. Review and editing were performed by FAG. All authors reviewed, read, and approved the final manuscript.

ACKNOWLEDGMENTS

We acknowledge the Conservation Genetics Group from UESC [https://gaiotto2.wixsite.com/geneticaconservacao] for the insights and laboratory support.

FUNDING

This work was supported by the National Council for Scientific and Technological Development with Ph.D. scholarship for AKCF (#153060/2019-0) and ZQ (#160160/2020-0), TAC was financed by the Bahia State Research Support Foundation (FAPESB #BOL0050/2018), and PosDoc fellowship for ASS by Coordination for the Improvement of Higher Education Personnel (CAPES #88887.464941/2019-00). CNPq also supports the productivity fellowship for FAG (#312065/2021-3).

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LETTER OF ACCEPTANCE

Manuscript #	020123-006R1
Title	Can Forest Restoration Affect the Genetic Diversity of Plants?
Corresponding Author	Ms. Ane Fernandes (State University of Santa Cruz)
Date:	2023-07-24 11:38:36
Last Sent:	2023-07-24 11:38:36
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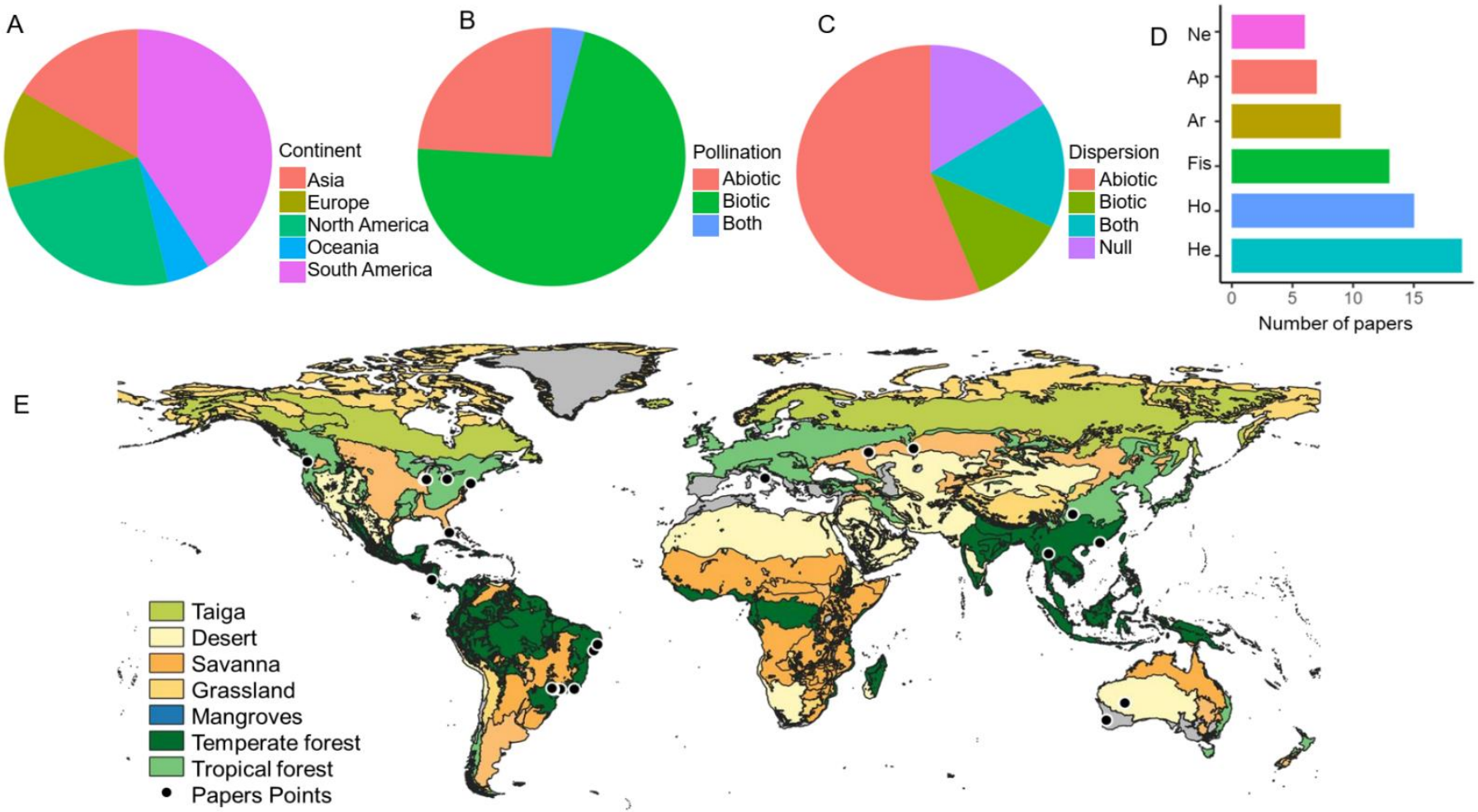
From:	erjournal@sebs.rutgers.edu
To:	fernandesak@live.com
CC:	Redacted
Subject:	Ecological Restoration - Decision on Manuscript 851
Email	July 24, 2023 Ms. Ane Karoline Campos Fernandes State University of Santa Cruz Department of Biological Sciences Rodovia Jorge Amado, km 16 Ilhéus, Bahia 45662-900 Brazil Re: Can Forest Restoration Affect the Genetic Diversity of Plants? Dear Ms. Fernandes: We are happy to inform you that your manuscript has been accepted for publication in Ecological Restoration. We feel that this work conveys an important message and will both inform and advance ecological restoration practice. Attached to this email is a License to Publish form. Please fill out, sign and email back as soon as possible. Please note that each author must sign a form. There are a few ways to do this: 1) you may send one form with all signatures 2) each author can send their own form 3) the corresponding author can sign for all authors if they have granted permission to do so. For each issue, we also solicit high quality photographs for the front and back covers of Ecological Restoration. Would you be willing to send three-five photos that convey the subject of your study? These could be photos of the study site, the study species, or people conducting research. Please provide a brief caption and image credit for any photos you submit. If your photo is selected for one of the covers, we will email you. Color Printing: Figures will be printed in black and white in the bound journals. All online figures will be in color. Please submit greyscale figures for the print version unless you wish to pay for color. There is a fee for color printing (detailed on our submission website). Fees depend on the publishing layout. Exact pricing is not finalized until the first draft proofs are available. Open Access: There is a fee for open access publication. Please contact the Managing Editor for more information. We will contact you shortly as we move through the publication process. Steven N. Handel, Ph.D., Editor Ecological Restoration Rutgers, The State University of New Jersey 93 Lipman Drive New Brunswick, NJ 08901 ERjournal@sebs.rutgers.edu 848 932-4516

FIGURES

Figure 1. Geographic, ecological, and species distribution found in the research papers included in this study that evaluated genetic estimators in restored areas and natural remnants. **A)** Number of articles per continent evaluated in the construction of this review. **B)** Pollination syndrome types of the studied plant species extracted from each article. **C)** Proportion of seed dispersal vectors, separated into biotic and abiotic. **D)** Most estimated genetic parameters in researches that evaluated genetic estimators in restored areas and natural remnants. Ne= Effective number of alleles per locus. Ap= Private alleles. AR= Allelic Richness. F_{IS}= Inbreeding coefficient. H_O= Observed heterozygosity. H_E= Expected heterozygosity. **E)** Distribution of papers by ecoregions and continents, there are points of overlap due to papers carried out in the same area.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Grassland
Mangroves
Temperate forest
Tropical forest
Papers Points

Table 1. Data extracted from selected papers in the extraction step.

Data Extraction	
Restoration characteristics	Continent Biome remnant Restoration methodology used Number of areas or populations
Biological model	Plant species Dispersion mechanism Pollination mechanism
Genetic data	Molecular marker Genetic diversity parameters

SUPPLEMENTARY INFORMATION

Table 2. Data extracted from the studies included in the systematic review, with information regarding the species, characteristics of the areas and markers used.

Authors	Continent	Remaining biome	Specie	Dispersion mechanism	Pollination mechanism	Restoration type	Gene marker	Connectivity or Gene flow
Zeng & Fischer 2021	Asia	Temperate forest	*Quercus bambusifolia*	Abiotic	Abiotic	Active	SSR markers	NA
Cordeiro et al. 2019	South America	Tropical forest (Atlantic forest)	*Centrolobium tomentosum*	Abiotic	Biotic	Active	SNPs	yes
Viana et al. 2019	South America	Tropical forest (Atlantic forest)	*Casearia sylvestris*	Biotic	Biotic	Active	SNPs	yes
Zucchi et al. 2017	South America	Tropical forest (Atlantic forest)	*Casearia sylvestris; Centrolobium tomentosum Myroxylon peruiferum; Piptadenia gonoacantha*	both	Biotic	Active	SSR markers	NA
Schwarcz et al. 2018	South America	Tropical forest (Atlantic forest)	*Myroxylon peruiferum*	Abiotic	Biotic	Active	SSR markers	yes
Sujii et al. 2017	South America	Tropical forest (Atlantic forest)	*Centrolobium tomentosum*	Abiotic	Biotic	Active	SSR markers	yes
Helsen et al. 2013	Europe	Grassland	*Origanum vulgare*	both	Biotic	Passive	SSR markers	yes
Pakkad et al. 2008	Asia	Tropical forest	*Prunus cerasoides*	Biotic	Biotic	Active	SSR markers	yes

Santini et al. 2018	Europe	Temperate forest	*Abies alba Mill*	Abiotic	Abiotic	Active	SSR markers	NA
Souza et al. 2016	South America	Tropical forest (Atlantic forest)	*Erythrina velutina*	both	Biotic	Active	ISSR	yes
Zhang et al. 2016	Asia	Temperate forest	*Pinus dabeshanensis*	Abiotic	Abiotic	Active	SSR markers	yes
Neto et al. 2014	South America	Tropical forest (Atlantic Forest)	*Inga vera* subsp. *affinis*	Biotic	Biotic	Active	SSR markers	yes
Ritchie et al. 2017	Oceania/ Australia	Grassland	*Banksia attenuata*	Abiotic	Biotic	null	SSR markers	yes
Mutegi et al. 2014	North America	Grassland	*Panicum virgatum*	Abiotic	Abiotic	Active	SSR markers	NA
Céspedes et al. 2003	South America	Tropical forest	*Swietenia macrophylla*	Abiotic	Biotic	Passive	SSR markers	yes
Granado et al. 2018	South America	Mangrove	*Laguncularia racemose; Avicennia schaueriana.*	Abiotic	Biotic	Active	ISSR	NA
Slaymaker et al. 2015	North America	Dune	*Ammophila breviligulata*	Abiotic	Abiotic	Active	ISSR	yes
Broeck et al. 2021	Europe	null	*Populus nigra*	null	null	null	null	NA
Sujii et al. 2021	South America	Tropical forests	*Centrolobium tomentosum*	Abiotic	Biotic	Active	SSR and cpSSR marker	yes
Thongkumkoon et al. 2019	Asia	Subtropical forest	*Castanopsis calathiformis; Castanopsis tribuloides; Lithocarpus polystachyus*	both	both	Passive	SSR markers	yes

Broadhurst 2013	Oceania/ Australia	Temperate forest	*Eucalyptus melliodora*	Abiotic	Biotic	Active	SSR markers	yes
Dolan et al. 2008	North America	Temperate forest (Prairies)	*Asclepias incarnata; Baptisia leucanta; Coreopsis tripteris; Zizia aurea*	Abiotic	Biotic	both	allozyme	NA
Fant et al. 2013	North America	Dune	*Cirsium pitcheri*	null	Biotic	active	SSR markers	NA
St. Clair et al. 2020	North America	Temperate forest (Prairies)	*Castilleja levisecta*	null	Biotic	Active	SSR markers	NA
Fotinos et al. 2015	North America	Temperate forest	*Pseudophoenix sargentii*	null	null	Active	SSR markers	NA

SECOND CHAPTER

EX SITU CONSERVATION FOR COMMERCIAL USE OF NATIVE TREE SPECIES AND AS A GENE REPOSITORY FOR FOREST RESTORATION IN BRAZIL.

ABSTRACT

Forest plantations established with native species offer significant potential by combining the benefits of commercial forestry with reduced pressure for high-quality timber on natural forests. This approach, known as "productive conservation," also presents an avenue for forest restoration. However, it necessitates maintaining a known source of propagules, including a diverse germplasm bank for cross-breeding and genetic improvement of the target species. Our study aimed to analyze the relatedness between individuals within a germplasm bank and the level of genetic diversity it harbored. Additionally, we compared this germplasm bank with natural populations to assess whether it could be considered a genetically representative *ex situ* conservation population. *Ex situ* conservation refers to the strategy of preserving plant genetic resources outside their natural habitat. *Plathymenia reticulata*, a key timber species, was chosen as the biological model for our investigation. This ecologically significant forest species is found in two Brazilian biodiversity hotspots: the Cerrado and the Atlantic Forest. Our findings revealed an unexpectedly high degree of relatedness among certain individuals belonging to different families, despite the matrices originating from distinct locations. Furthermore, the germplasm bank exhibited highest Shannon diversity index than the natural populations used for comparison.

INTRODUCTION

Habitat loss and fragmentation are the leading causes of global biodiversity decline (Gardner et al., 2009; Giam, 2017; Horváth et al., 2019; Wright & Muller-landau, 2006; Zemanova et al., 2017). As forest loss increases, the amount of habitat available to local species decreases, and the connectivity and movement of fauna between fragments is reduced (Zemanova et al., 2017). Recent studies have shown that after a certain threshold, forest loss leads to a drastic decrease in local biodiversity (Horváth et al., 2019; Vallejos et al., 2020). This is because the reduction in the quality and availability of habitats makes it difficult for local animals to persist. They must either adapt, leave, or perish (Vallejos et al., 2020; Zemanova et al., 2017).

Large commercially planted areas are of great importance to fauna, providing shelter and/or refuge in the face of a deforested matrix. Planted forests serve as an alternative escape and protection for fauna, also functioning as a corridor between remaining areas (Payn et al., 2015). They regulate temperatures and rainfall regimes, as planted forests can influence precipitation patterns at local and regional scales, altering the exchange of heat and moisture between the surface and atmosphere (van Dijk & Keenan, 2007). Additionally, planted forests reduce the pressure for the extraction of high-quality wood for industrial and construction purposes from native forests (Carle & Homgren, 2018). Forest plantations with native species have great potential because they combine the benefits of commercial plantations (such as reducing pressure on native forests) with the potential to contribute to forest restoration using species with a "productive conservation" model.

Commercial plantations of native trees are the best alternative to the extensive exploitation of native forests. To start this type of enterprise, it is necessary to have a source of propagules, such as a seed bank. However, some species, especially tropical ones, cannot be stored, refrigerated, or dried because they may lose their germination viability (Piovesan et al., 2022).

In this case, an alternative is to use an active germplasm bank (Dawson et al., 2013), which is a way to maintain a base population from which seedlings can be taken for planting in new commercial plots (Wani et al., 2019). The base population is usually composed of individuals of known origin, derived from a few elite matrices (Lebedev et al., 2020). The same principle can be applied to *ex situ* conservation, as long as the population maintained in the germplasm bank is genetically representative of the species.

Ex situ conservation is a strategy for preservation of plant genetic resources outside their natural habitat (Dawson et al., 2013), which can be done in various ways, including seed banks, tissue culture, and living collections (Hay, 2021). This strategy is an important tool for the conservation of tree species, providing an alternative for protecting species from extinction and supporting research and forest recovery efforts (Abeli et al., 2020). We highlight that *ex situ* conservation is not a substitute for in situ conservation measures, however *ex situ* populations play a supporting role in the recovery of biodiversity. In a review evaluating the role of herbaria as populations in *ex situ* conservation, Abeli et al. (2020) emphasize the importance of maintaining genetically representative populations, as such populations can act to promote and rescue plant species, especially those that are locally extinct

We selected *Plathymenia reticulata*, a key timber species, as a biological model for our study. Although the International Union for Conservation of Nature (IUCN) globally classifies the species as Least Concern (LC), it has suffered severe threats from residential and commercial development, as well as forest conversion to agriculture. In Brazil specifically, *P. reticulata* is of great economic importance due to its high-quality and durable wood, which is used in construction and the manufacture of luxury furniture, stakes, pillars, posts, and other timber products (Carvalho, 2009). However, there is a lack of local studies to assess whether the species is threatened locally.

P. reticulata is a forest species of great ecological importance that is found in two Brazilian hotspots (the Cerrado savanna and the Atlantic Forest). It has a wide distribution in Brazilian biomes present in the Amazon, Caatinga, Cerrado and Atlantic Forest phytogeographic domains (Morim, 2020), occurring in 16 states (Carvalho, 2009). Its high adaptability makes it an excellent biological model for this study. Evaluating the growth and survival of *P. reticulata* in an Atlantic Forest area with different phosphorus dosages, (Araujo et al., 2021) found that it showed high survival and rapid initial growth rate, indicating that it has high potential for use in reforestation programs in the Atlantic Forest. *P. reticulata* is one of the most used species for flora restoration in degraded areas, and it is also one of the most desired species by farmers in cocoa agroforestry systems in southern Bahia (Sambuichi et al., 2012). It has a botanical synonym, *Plathymenia foliolosa* Benth (Warwick & Lewis, 2003), which is listed as Vulnerable (VU) by the IUCN (World Conservation Monitoring Centre, 1998). This information highlights the importance of conducting studies to understand the current conservation and genetic status of this species.

The choice of *P. reticulata* as the biological model is a strategic attempt to contribute to forest restoration and conservation practices. Through knowledge of the aspects already described for this species of relevant ecological role, this work will also make contributions to the use of the species in commercial plantations and restoration systems. Supporting economic, social, and environmental development, given the economic potential of the species.

METHODS

Sampling plant material in the ex-situ conservation area

Leaves were collected from juvenile individuals of *P. reticulata* kept in the base population (Germplasm Bank) of Symbiosis Investimentos e Participações S.A, located in the Trancoso district, Porto Seguro - BA. The individuals in this area are divided into 30 families composed

of a maximum of 10 individuals each. The original matrices that gave rise to the families in the germplasm bank were collected in conserved Atlantic Forest fragments in four Brazilian states (Bahia, Espírito Santo, Minas Gerais and Rio de Janeiro) (fig.1) belonging to the central corridor of the Atlantic Forest. The company aims to produce high-quality native wood to supply the timber market, thereby reducing the demand for this wood from remaining natural forests.

Additionally, as the company is located in the heart of the southern Bahian Atlantic rainforest, these "planted forests" will serve as a refuge and corridor connecting fragmented forest areas. Consequently, science becomes a powerful ally when we consider future scenarios facing the constant loss of forests and the vulnerable status of *P. foliolosa* (*P. reticulata* synonym) in the Atlantic Forest. A genetically representative population will be crucial when we think about forest restoration and rescuing the species. After all, restoring areas in the near future may necessitate utilizing populations in *ex situ* conservation. Therefore, it is strategically crucial to maintain a population that accurately represents the species' gene pool. Genotyping individuals from *ex situ* conservation areas will provide valuable information for restoration efforts, enabling the selection of propagules from unrelated individuals. This approach reduces the inbreeding coefficient, expands the gene pool of the populations to be planted, and maximizes the likelihood of success for the restored area (Basey et al., 2015).

Sampling plant material in natural remnant area

We selected three populations of *P. reticulata* in natural forest remnants, these areas belonging to the Atlantic Forest in a considerable state of preservation in the south of Bahia, located in Amargosa (AMA), Ibirapitanga (IBI) and Una (UNA). Leaves were collected from at least 22 randomly selected individuals in each population.

DNA extraction, quantification and amplification

In the laboratory, the DNA extraction of each individual sampled was done through the CTAB 2% protocol (Doyle & Doyle, 1990) with a few changes where necessary. To estimate the quality and concentration of the DNA, comparisons were made with phage λ DNA standards and GelGreenTM staining. The quality of the extraction was assessed using 1% agarose gel electrophoresis.

The DNA extracted from each individual was amplified in a Life Pro thermal cycler (Bioneer Technology Co., China), using the amplification program indicated for each primer, 14 microsatellite marker loci (also known as SSR - simple sequence repeats) developed for *P. reticulada* previously (Cruz et al., 2012; Oliveira et al., 2012) were tested. After a previous screaning, eight of them were used in our analysis. The quality of the amplification was assessed using 2% agarose gel electrophoresis for a part of the amplified samples, which were then subjected to capillary electrophoresis in an ABI3500 automatic sequencer (Applied Biosystems, USA) to separate the SSR fragments using a multiload strategy to save resources and time. Genotyping was carried out using GeneMarker software (SoftGenetics, USA).

Data analysis

To verify the number of genetic clusters, Bayesian simulations were implemented by Structure software version 2.3.4. and the results were analyzed to find the Delta K based on Evano methodology at Harvest website (Earl & vonHoldt, 2012). For further tests the assignment of individuals, discriminant principal component analysis (DAPC) was performed using the Adegenet package (Jombart & Collins, 2015).

A relatedness analysis was conducted within and between families of the germplasm bank. We estimate the pairwise relatedness with all individuals, like a single population, and the same analysis for each family separately. We selected one individual per family, to represent a germplasm bank like one single population, and estimated the relatedness between these

individuals. The relatedness analysis was performed with the Demerelate 0.9.3 package (Kraemer & Gerlach, 2017) using the Loiselle´s estimator (Loiselle et al., 1995), which was the most suitable for our dataset. The Demerelate package calculates relatedness from the average number of alleles shared between individuals (Kraemer & Gerlach, 2017), while the Loiselle estimator calculates relatedness between individuals, taking into account the correction for sample size effects (Loiselle et al., 1995).

The Loiselle relatedness coefficient can vary from 0 to 1. The value 0 indicates that the two individuals are unrelated, while a value of 1 indicates that the two individuals are clones. Values between 0 and 1 indicate that the pair of individuals have some relatedness. A negative value of this estimator also indicates that the two individuals are unrelated. Indeed, the Loiselle relatedness estimator is based on genetic distance, and negative genetic distance values indicate that the two individuals are genetically different. The observed frequencies of half siblings (HS) and full siblings (FS) are compared to those expected in a random population of non-related individuals using a chi-squared test (Kraemer & Gerlach, 2017). The data is then compared using a t-test, these analyses were performed in R version 4.0.1 (R Core Team, 2020).

To answer the question "Is there a difference in the genetic diversity of *P. reticulata* in ex-situ populations and forest remnants?", we used the selected germplasm bank individuals as a hypothetical *ex situ* population (BAG2) and estimated the following standard genetic parameters for the three natural populations and our ex-situ population: average number of alleles per locus (A), allele richness (AR), number of private alleles (AP), the effective number of alleles (Ne), observed (HO) and expected heterozygosity (HE) under Hardy-Weinberg equilibrium, and fixation index (F_{IS}). We used GenAlEx version 6.5 software to calculate these parameters. Genetic indices and parameters were compared using the t-test and ANOVA in R version 4.0.1 (R Core Team, 2020).

RESULTS

Relatedness

Relatedness analysis of the 24 germplasm bank families revealed that, for example, family 5 stands out with an observed frequency of 28 full siblings (FS), 2 half siblings (HS), and 15 non-related siblings (NON). This was unexpected, as family 5 is theoretically expected to have at least half siblings.

When we evaluated the pairwise relatedness between the representatives of each family (BAG2), we found the highest values between representatives of families 11-13, 10-18, and 3-4 (0.457, 0.408, and 0.365, respectively). A histogram of relatedness among all individuals, with the corresponding thresholds for full siblings and half siblings, for all loci matched, is represented in Figure 3. The histogram was created by transforming the similarities of the relatedness metrics (S) to distances (D = 1 - S) (Kraemer & Gerlach, 2017). The calculations made for BAG2 population showed the Kinship thresholds for half (0.085) and full (0.209) siblings. The observed frequencies of full siblings (FS), half siblings (HS) and unrelated pairs (NON) were respectively 8, 54 and 238. The expected frequencies FS (11), HS (65) and NON (224). However, the chi-squared statistic showed no statistical significance with $p>0.05$ (tab.1).

Genetic structure and diversity of germplasm bank population

The structure analysis carried out on Structure 2.3.4 showed that, although we analyzed 24 families, the germplasm bank is divided into 3 probable groups (DeltaK=3), (Earl & vonHoldt, 2012). The DAPC analysis revealed that the families are genetically related, except for family 5, which is more distant from the main group.

The highest number of effective alleles (NE) was found in families F5, F3 and F1 (2.945, 2.906, and 2.870 respectively), with 2.247 being the average NE value for the germplasm bank as a single population. The Shannon (I) index varied from 1.107 (family 5) to 0.518 (family 4) with

192 0.841 as average value. The observed heterozygosity (H_O) ranged from 0.641 (family 21) to
193 0.281 (family 16) and the mean value over family and loci was 0.473. At least the expected
194 heterozygosity (H_E) ranged from 0.573 at family 5 to 0.337 at family 26, with 0.475 as the mean
195 value over loci and families.

196 *Genetic diversity in germplasm bank ex situ population and forest remnants*

197 The four populations analyzed had a maximum number of 27 individuals (AMA: 23, BAG2:
198 25, IBI: 27 and UNA: 27), with a total of 72 individuals. The BAG2 showed a higher value for
199 all genetic parameters estimated (tab. 3). The Ne ranged from 2.294 (UNA) to 2.842 (BAG2),
200 The H_E and H_O ranged from 0.485 and 0.613 (AMA and BAG2) to 0.449 and 0.523 (UNA and
201 BAG2) respectively. The Shannon diversity index (I) varied from 1.279 (BAG2) to 0.952
202 (UNA) and the Fixation index (F) ranged from -0.0216 (IBI) to 0.1475 (BAG2). We used the
203 ANOVA analysis to compare the variation of diversity index between populations and the result
204 was not statistically significant ($p = 0.443$).

205 **DISCUSSION**

206 With the threat of extinction of many native tree species due to logging, forest fragmentation,
207 and climate change, it is necessary to implement long-term programs that use population
208 genetics and forest management to understand the genetic patterns of target species and
209 implement measures that favor their maintenance (Wheeler et al., 2015). With this objective we
210 obtained genetic data for *P. reticulata* natural and *ex situ* conserved populations in order to
211 make some recommendations for management and restoration in the near future.

212 *Relatedness within and between families*

213 The results of our study contradict what would be expected. As this base population consisted
214 of individuals belonging to different families, with the matrices originating from different

locations, it was expected that pairwise relatedness would be higher within families than between individuals from different families. We observed a high degree of kinship between individuals from different families (tab. 4). This fact leads us to question the reasons for this outcome. One possibility that we raise was families seed "contamination" with seeds from the environment or from different family groups. This could have happened through carelessness when selecting the seeds from the matrices and separating them for planting in the tubes, or when the seedlings were transplanted into the field. This kind of seed contamination is very common, mainly when the plant has seeds of small size (Cossu et al., 2020; Wilson et al., 2016), like *P. reticulata* (Orestes et al., 2020).

Another point we question is the origin of the matrices and how isolated these individuals were from their original population. For example, family 5 has the highest average degree of relatedness among all the populations (fig. 2). However, this family appears to be the furthest from the main group in the discriminant analysis of the main components (fig. 3). This is consistent with the pairwise result obtained for our hypothetical population, which showed a stronger relationship between individuals from family 3-4, whose matrices came from the same location (Supplementary information). Looking more closely at the graph of means (fig. 2), one of the families with the lowest mean relatedness was family 1, but if we evaluate the specific family cluster, we can verify that the individuals are intrinsically related (Supplementary information: Fig 4). The same occurs with other families, with the major number of individuals being full siblings. When a mother plant is fully isolated from other individuals of the same species and lacks mechanisms to inhibit self-fertilization (Eaves et al., 2014), the relatedness values observed in the analysis will be akin to those of full siblings.

Therefore, relatedness analysis can help identify and avoid crossing related individuals, generating populations with a low inbreeding rate (Basey et al., 2015). Considering the

germplasm banks a source of propagules for commercial plantations, estimating relatedness is fundamental to ensure a plantation with the most genetically diverse individuals. Crossing related individuals is undesirable, as a higher rate of inbreeding can favor the prevalence of deleterious traits, and in a plant breeding program, it is important to separate genetically similar individuals to ensure that the propagules are healthy.

BAG population structure and genetic diversity: applications for genetic improvement.

The basis of any breeding program is detecting the desired traits and promoting admixture among selected individual parents in order to produce offspring with better commercial performance (Hill, 2013). On the other hand, Wheeler et al. (2015), suggest integrating existing tools with forest genetics to achieve a sustainable approach to forest management aimed not only at financial gain but also at conservation. They point out that forest breeding and forest genetics have made significant contributions to forestry and the timber trade in the US, for example. Unfortunately, in Brazil we are not in the same stage despite the huge potential of our tropical forests for tree genetic resource uses as for commercial timber exploitation as for conservation.

After selecting matrices from natural populations, propagating them for the base population, and selecting propagules for planting, loss of genetic diversity can put the productivity and resilience of the overall population at risk due to inbreeding depression (Cortés et al., 2020). To reduce this risk, a breeding population is established to increase genetic variability (Wani et al., 2019). With this purpose, we can indicate, for example, a breeding between families 6 and 10 (fig.2) as they have higher genetic distance and no relatedness, since they are the best families for phenotypic traits.

Another important aspect is the effective size (Ne) of the initial population, which has a significant impact on the rate of loss of genetic diversity (Isik & McKeand, 2019). When we

examine Table 1, we see that the families with the highest effective population size (Ne) also have the highest diversity index Shannon. By cross-referencing this information with kinship data, we can select breeding pairs within the germplasm bank.

Breeding individuals with economically desirable characteristics, such as better growth, straight trunks, and higher biomass yield, that have been selected using tools of quantitative genetics, must be confirmed by molecular data. Li et al., (2020), in their review of the genetic improvement of *Pinus koraiensis* in China, concluded that conventional breeding should be combined with molecular marker-assisted breeding to accelerate the breeding cycle. Traditional breeding based on phenotypic evaluation is carried out at the adult stage of development, which makes phenotypic selection long and costly for species with a long-life cycle (Isik & McKeand, 2019). In contrast, selection based on genetic analysis, as we can recommend with our data, can significantly shorten the breeding cycle, as this marker-based evaluation can be carried out in the early stages of tree development, once the DNA can be isolated without damaging the plant (Lebedev et al., 2020).

Molecular genetic evaluation is a key component in the genetic improvement of commercially valuable species (Li et al., 2020; Sharma et al., 2016), with genetic diversity being the focus of this characterization. The main aim of genetic improvement is to increase the frequency of desirable genes in the base population (Wani et al., 2019). In the case of commercially important trees, such as *P. reticulata*, these desirable traits are known, but the genes that favor them or their distribution in the native population are unknown. Therefore, breeding programs must maintain genetic variability to allow for continuous genetic gains over generations (REF). In the case of the populations analyzed, we recommend that crossing be done considering the results of pairwise relatedness. Crosses between the most closely related pairs should be

avoided, as our findings indicate that even individuals belonging to different families exhibit a high degree of relatedness.

*"Is there a difference in the genetic diversity of **P. reticulata** in ex situ populations and forest remnants?"*

Contrary to what has been reported (Wei & Jiang, 2021), our *ex situ* population shows higher levels of genetic diversity. High values of Ne, H_E, H_O, and the Shannon index indicate that the germplasm bank population has the highest genetic diversity and a more complex genetic background. This is essential for forests to withstand stress and survive long-term climate change (Ivetić et al., 2016). Evaluating the genetic diversity and structure of Plathymenia populations in the Atlantic Forest, Cerrado, and ecotone between the two biomes, Muniz et al. (2022) found lower diversity in the Atlantic Forest populations than in the other two biomes. They argue that the Atlantic Forest populations may have lower adaptive potential and be more affected by the fragmentation and habitat loss that the Atlantic Forest is currently experiencing.

Degraded areas are, for the most part, a hostile habitat for the establishment and growth of seedlings, generating greater selection pressure on propagules (Thomas et al., 2014). On the other hand, Wei & Jiang (2021) argued that the lower genetic diversity in *ex situ* plant populations can occur mainly due to weak sampling strategies that failed to retain genetic variation from natural populations during population establishment. To ensure the success of *ex situ* conservation, the *ex situ* population must be representative of the in-situ population. Hoban (2019) showed that the *in situ* sampling individuals should represent 95% of the alleles of the species, with 5 copies of each allele. This will ensure that the *ex situ* population remains viable in the long term. Our results indicate that the germplasm bank population was formed with a good level of genetic diversity, which could be useful information for breeding and conservation strategies.

Forest plantations with native species have great potential, as they combine the benefits of commercial plantations (Ivetić et al., 2016; Lebedev et al., 2020) and reduce pressure on native forests. This kind of investment in forest plantation also brings the perspective of restoration using species with a "productive conservation" model. Therefore, it is essential to ensure that this base population genetically represents the natural populations of the same species (REF). When we compare the genetic diversity between germplasm bank and the natural population, we can assume that the germplasm bank is fulfilling its role as an *ex situ* conservation area.

Since our colonization, Brazil has experienced an intense scenario of exploitation of its native trees, especially in the south of Bahia State, in the northeast of the country. This state was the arrival locality where the colonizers first docked, and it has suffered high levels of deforestation since then. Currently, the state is experiencing a second great wave of exploitation, with the most desired species already extinct. Successful forest restoration using native species requires attention to the selection and sourcing of seeds, which includes the application of effective indicators of correspondence between provenance and genetic diversity (Thomas et al., 2014). To ensure the self-sustainability of restored ecosystems, the genetic diversity implemented must be considered.

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Table 1. Observed and expected frequencies of full siblings and full + half siblings for our BAG2 population.

	Observed	Expected	Chi^2	d.f.	p-value	0.95 Lower CI	0.95 Upper CI
Full Siblings	0.027	0.037	0.489	1	0.484	-0.038	0.018
Full+Half Siblings	0.207	0.253	1.845	1	0.174	-0.114	0.021

Table 2. Means of genetic parameters for each family and the overall mean for the BAG as a single population. Estimated parameters: Na = number of alleles; Ne = Effective number of alleles per locus; I = Shannon diversity index; H_O = Observed heterozygosity; H_E = Expected heterozygosity and F = Fixation Index.

Mean over Loci for each Pop						
Family	**Na**	**Ne**	**I**	**H_O**	**H_E**	**F**
family_1	3.667	2.870	0.996	0.587	0.530	-0.149
family_2	3.111	2.228	0.832	0.456	0.476	0.034
family_3	4.333	2.906	1.078	0.517	0.549	0.109
family_4	2.222	1.560	0.518	0.307	0.318	0.044
family_5	4.444	2.945	1.107	0.520	0.573	0.082
family_6	3.889	2.471	0.981	0.369	0.531	0.248
family_7	3.556	2.365	0.941	0.464	0.522	0.177
family_8	4.111	2.611	1.044	0.476	0.548	0.113
family_9	2.778	2.308	0.892	0.620	0.548	-0.113
family_10	4.000	2.563	1.064	0.605	0.568	-0.072
family_11	2.889	2.030	0.791	0.492	0.466	-0.064
family_12	2.556	1.970	0.690	0.293	0.404	0.286
family_13	3.333	2.501	0.937	0.466	0.523	0.158
family_14	3.222	2.330	0.888	0.538	0.501	0.004
family_15	2.556	1.908	0.655	0.484	0.389	-0.266
family_16	2.667	2.059	0.701	0.281	0.406	0.248
family_17	2.222	1.657	0.584	0.393	0.362	-0.097
family_18	2.333	1.645	0.583	0.400	0.342	-0.137
family_19	3.556	2.537	0.958	0.584	0.530	-0.023
family_20	3.778	2.300	0.956	0.514	0.538	0.054
family_21	3.000	2.333	0.873	0.641	0.504	-0.267
family_22	3.222	2.248	0.890	0.539	0.513	-0.033
family_23	2.222	2.059	0.664	0.500	0.417	-0.200
family_24	3.000	2.172	0.845	0.461	0.485	0.075
family_26	2.222	1.597	0.567	0.309	0.337	0.094
Grand Mean over Loci and Pops						
	Na	**Ne**	**I**	**Ho**	**He**	**F**
Total	3.156	2.247	0.841	0.473	0.475	0.012

Table 3. Mean values over loci of each genetic parameter per population: Ibirapitanga (IBI). Una (UNA). Amargosa (AMA) and Germplasm Bank population (BAG2). Mean value of effective number of alleles per locus (Ne); Observed heterozygosity (H_O); Expected heterozygosity (H_E). Shannon diversity index (I) and Fixation Index (F).

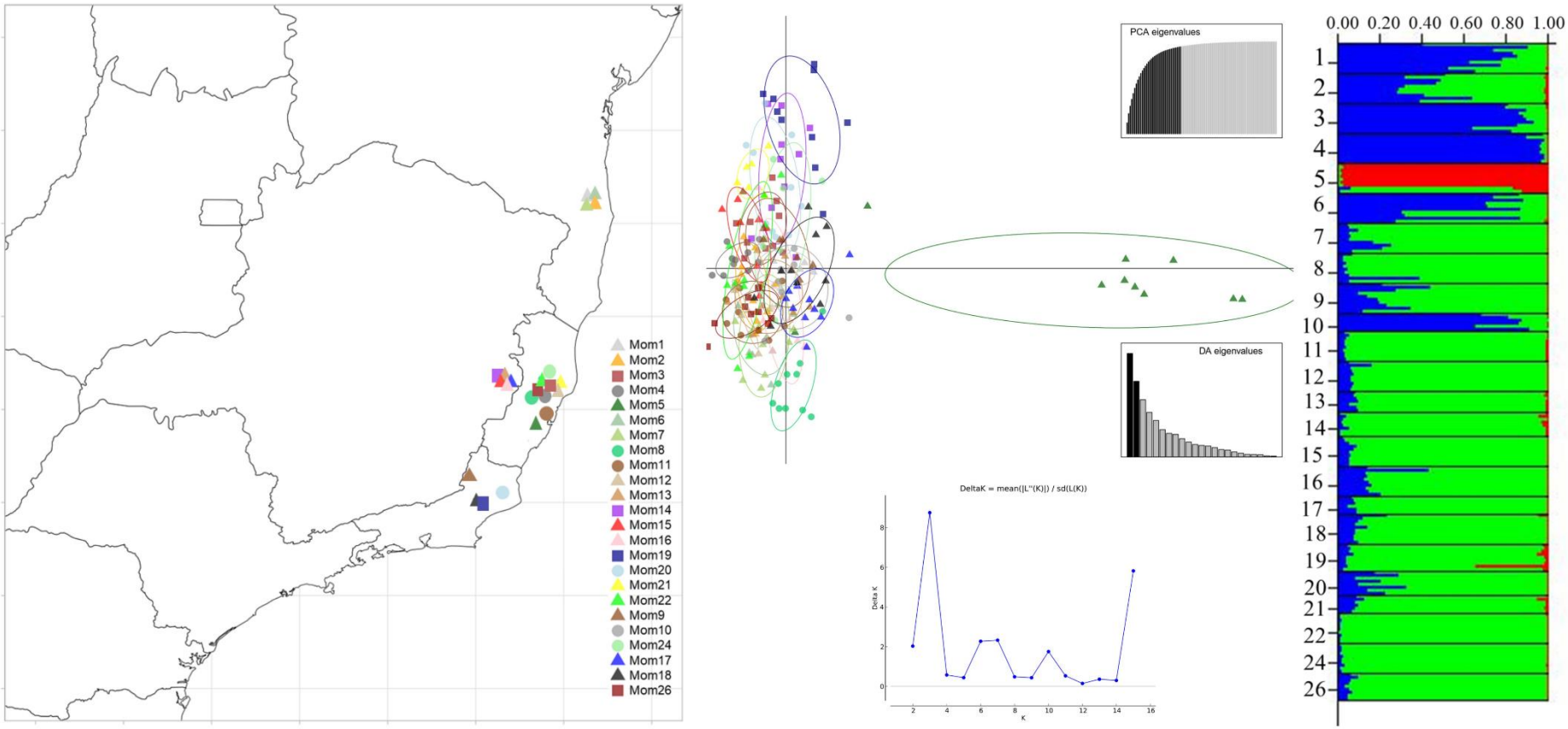
Pop	N	Na	Ne	I	Ho	H_E	F
IBI	21.778	4.222	2.385	0.964	0.507	0.515	-0.022
UNA	26.778	4.778	2.295	0.953	0.449	0.504	0.077
AMA	22.333	4.889	2.440	0.981	0.475	0.497	0.010
BAG2	24.222	6.556	2.843	1.280	0.523	0.627	0.148

Table 4. Greater and Lower Loiselle relatedness index calculations for pairwise individuals from the germplasm bank. The first number before the dot refers to a family, and the number after the dot is the individual number within each family.

Parwise	Higher loiselleindex	Parwise	Lower loiselleindex
1.8_11.8	0.882	**19.2_21.6**	-0.0001
15.5_16.1	0.877	**20.5_10.2**	-0.0001
5.9_11.8	0.839	**13.3_22.5**	-0.0001
5.9_11.5	0.834	**4.5_16.1**	-0.0001
1.9_24.8	0.813	**2.4_20.9**	-0.0001
3.1_11.8	0.786	**13.6_24.10**	-0.0001
1.8_5.9	0.781	**19.6_10.6b**	-0.0001
1.8_11.6	0.778	**11.5_15.3**	-0.0001
9.9_24.4	0.770	**4.1_14.4**	-0.0001
5.9_24.4	0.767	**19.9_18.5**	-0.0001
1.8_24.4	0.763	**6.4_20.5**	-0.0001
11.8_12.5	0.763	**22.4_26.2**	-0.0001
15.8_24.4	0.746	**5.7_20.3**	-0.0001
5.9_17.10	0.745	**13.6_10.7**	-0.0001
11.5_24.4	0.724	**8.9_10.6a**	-0.0001
3.3_5.9	0.722	**3.4_26.9**	-0.0001
15.8_9.9	0.721	**1.5_16.2**	-0.0001
4.7_11.8	0.720	**1.8_22.8**	-0.0001
11.6_15.8	0.715	**6.4_22.10**	-0.0003
12.5_17.10	0.714	**15.9_18.4**	-0.0003
15.1_24.4	0.713	**21.1_17.4**	-0.0003
5.9_12.5	0.709	**14.10_16.8**	-0.0003
1.8_4.7	0.708	**12.6_16.2**	-0.0003
13.9_17.10	0.706	**10.6b_24.8**	-0.0003
5.9_13.5	0.701	**22.2_24.5**	-0.0003
1.9_13.6	0.699	**21.3a_17.2**	-0.0003
3.3_11.5	0.679	**8.10_20.8**	-0.0003
11.6_24.4	0.672	**1.3_21.8**	-0.0003
4.7_5.9	0.666	**14.7_9.2**	-0.0003
3.1_4.7	0.658	**6.7_7.3**	-0.0003
11.6_15.5	0.654	**8.10_20.9**	-0.0003
3.3_4.7	0.649	**9.1_10.6b**	-0.0003
11.5_16.1	0.640	**20.9_10.6a**	-0.0003
1.8_3.1	0.636	**4.2_12.8**	-0.0003
1.8_2.2	0.630	**2.9_19.5**	-0.0003
12.5_13.9	0.624	**19.3_18.5**	-0.0003
1.8_15.8	0.623	**5.2_14.3**	-0.0003
4.2_13.10	0.615	**8.6_18.1**	-0.0003
11.5_15.5	0.614	**16.6_17.4**	-0.0005
1.8_12.5	0.614	**1.4_11.4**	-0.0005
2.2_15.8	0.613	**2.6_20.5**	-0.0005
5.9_15.5	0.611	**22.8_26.10**	-0.0005
5.9_13.9	0.608	**1.7_19.5**	-0.0005
1.8_11.5	0.601	**15.9_20.6**	-0.0005
3.1_11.5	0.597	**4.1_17.6**	-0.0005

478 **FIGURES**

479



480 **Figure 1: A)** Geographic representation of the origin of each BAG family matrix. **B)** Discriminant analysis. **C)** Graphic representation of Delta
481 K. based on Evano methodology. **D)** Structure of BAG population.

482

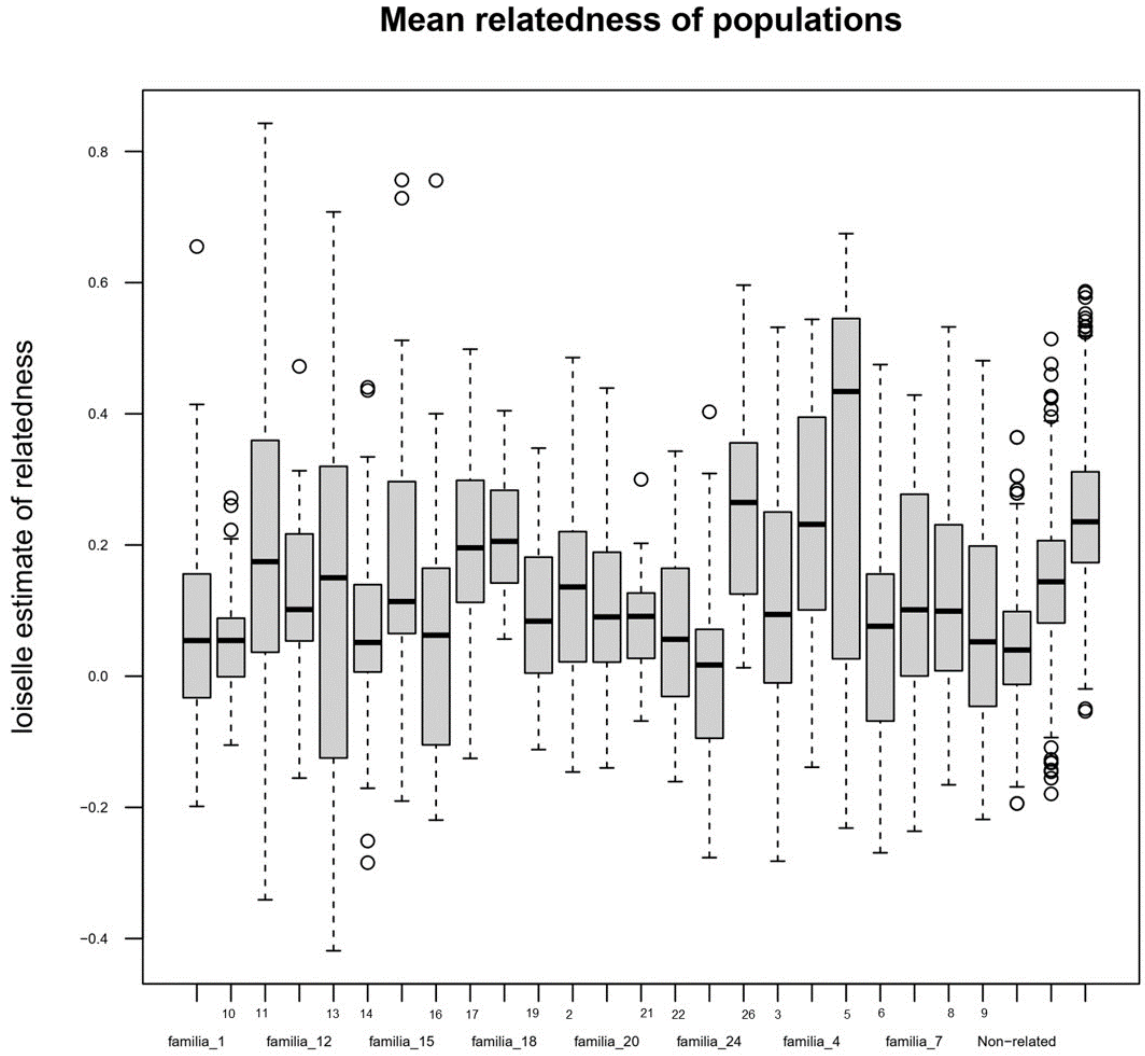
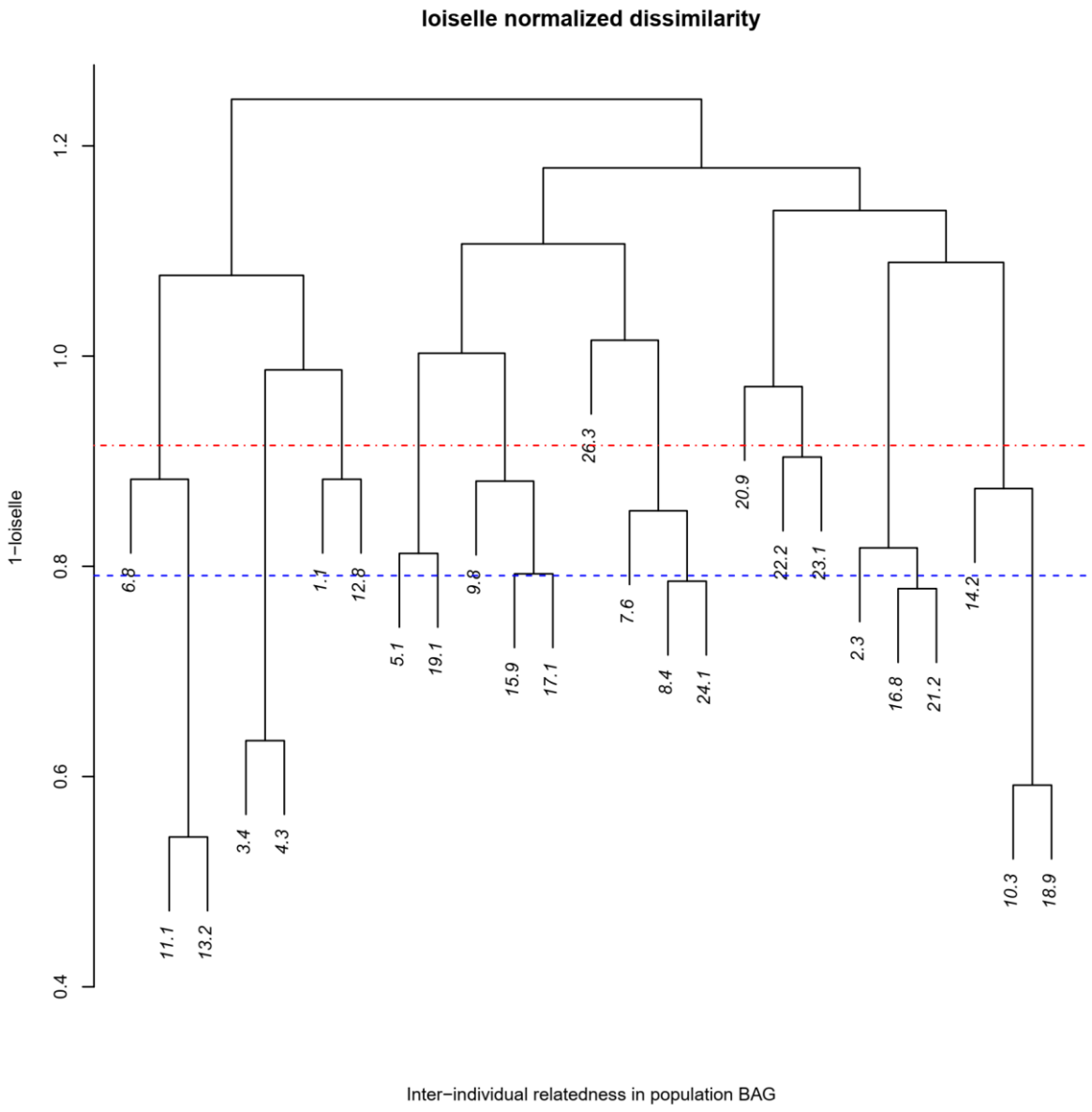


Figure 2: Graphic representation of mean relatedness of each family as population.

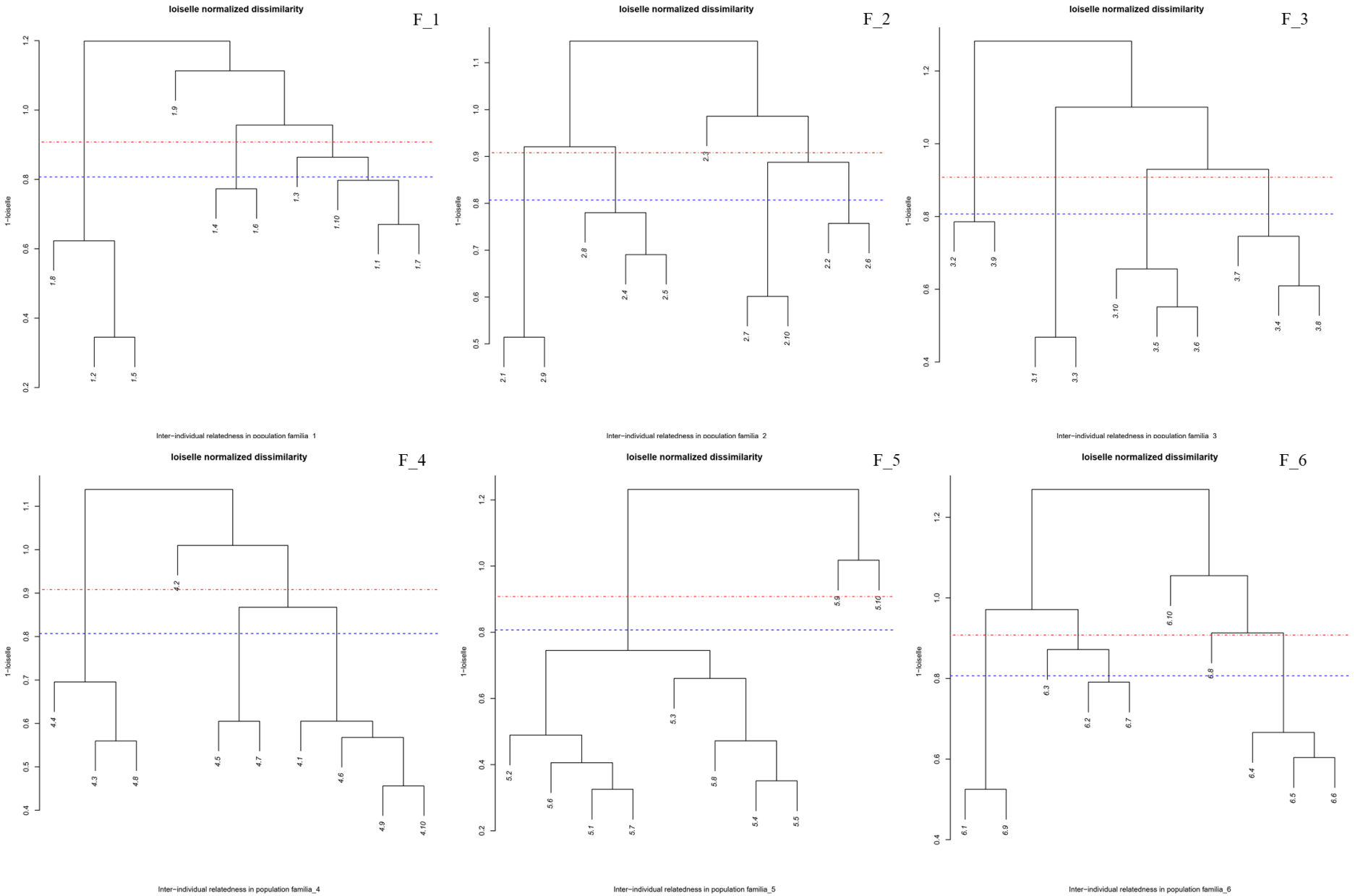


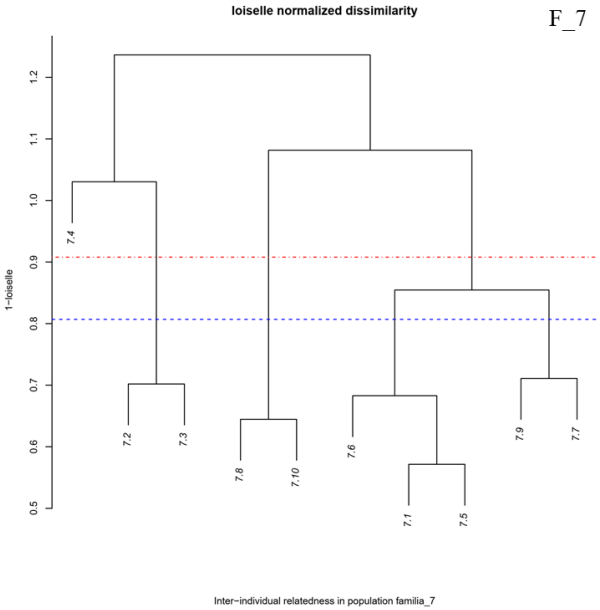
485

486 **Figure 3:** Genetic relatedness cluster for our hypothetical population BAG2. The graph is based
487 on Loiselle values converted into dissimilarities. The lines represent relatedness levels
488 calculated by logistic regression (blue dashed line: full siblings [FS]; red dotted line: half
489 siblings [HS]).

SUPPLEMENTARY INFORMATION

Figure 4. Genetic relatedness cluster for each family of BAG. The graph is based on Loiselle values converted into dissimilarities using the formula D = 1 - relatedness among pairs. The lines represent relatedness levels calculated by logistic regression (blue dashed line. full siblings [FS]; red dotted line. half siblings [HS]).





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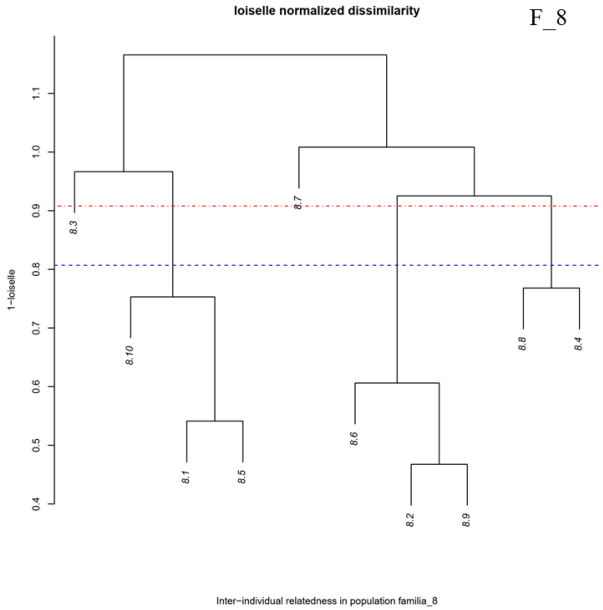

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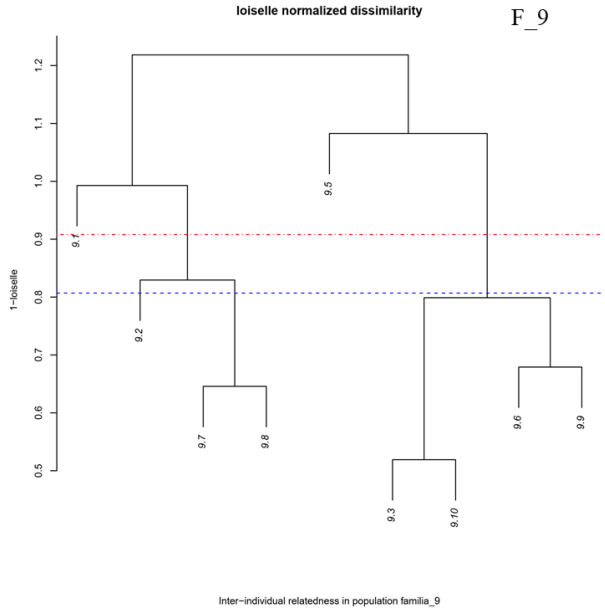

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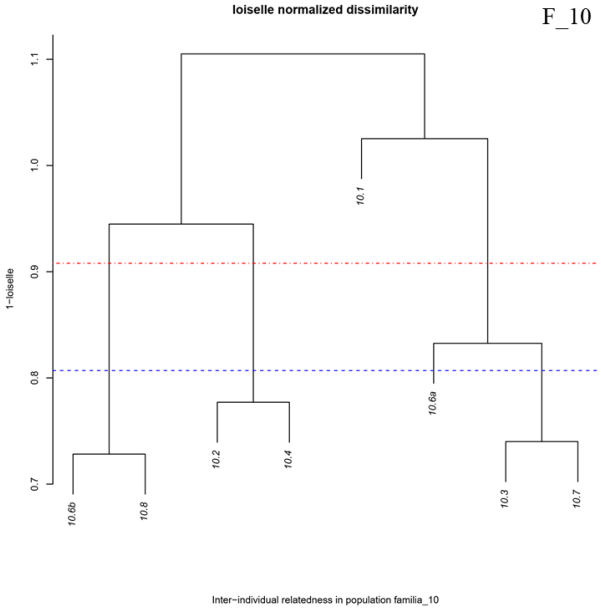

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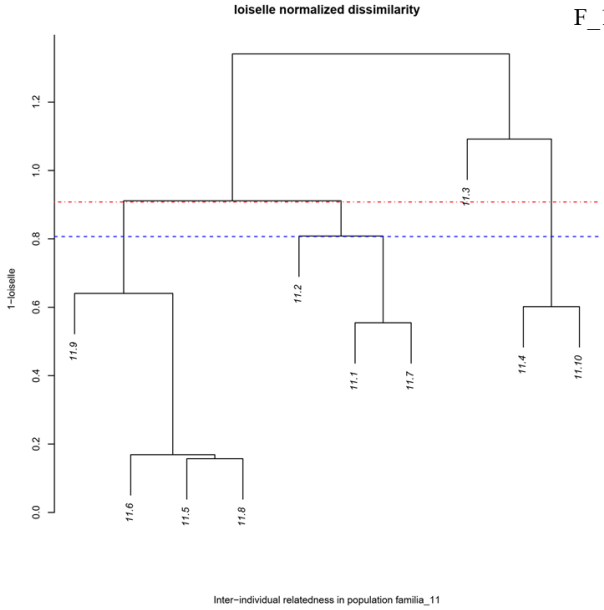

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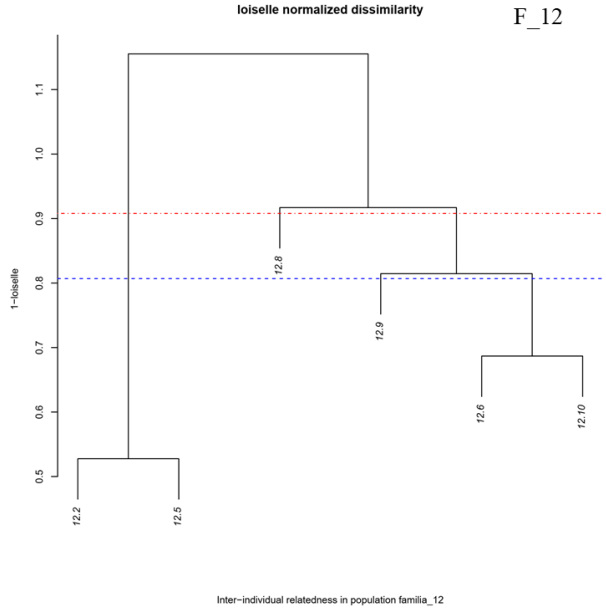

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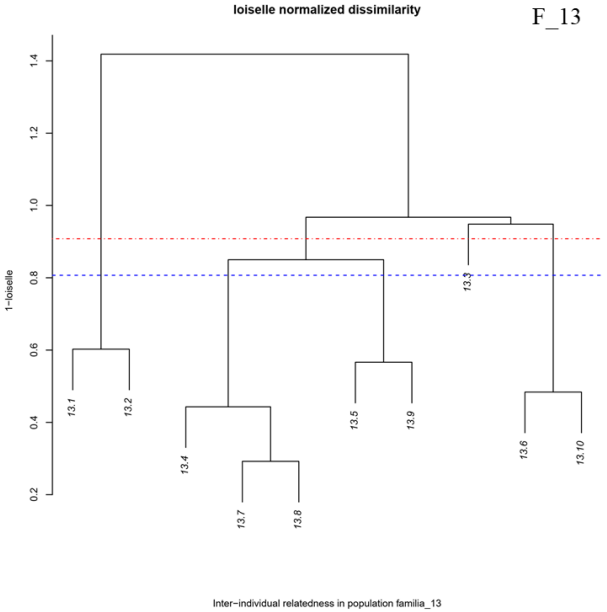

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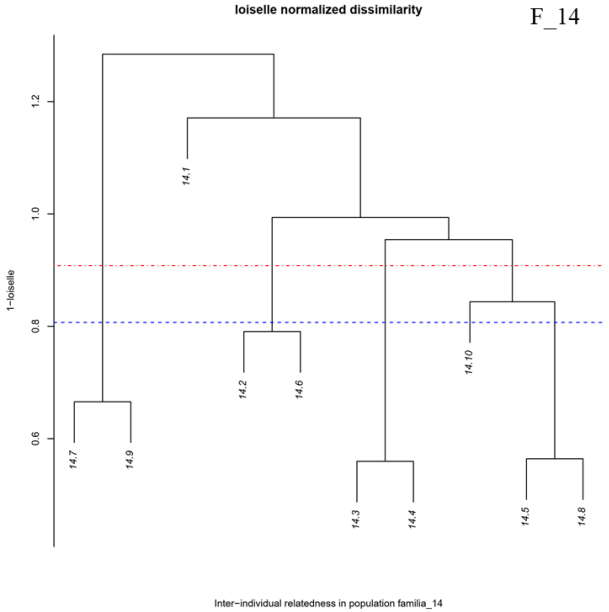

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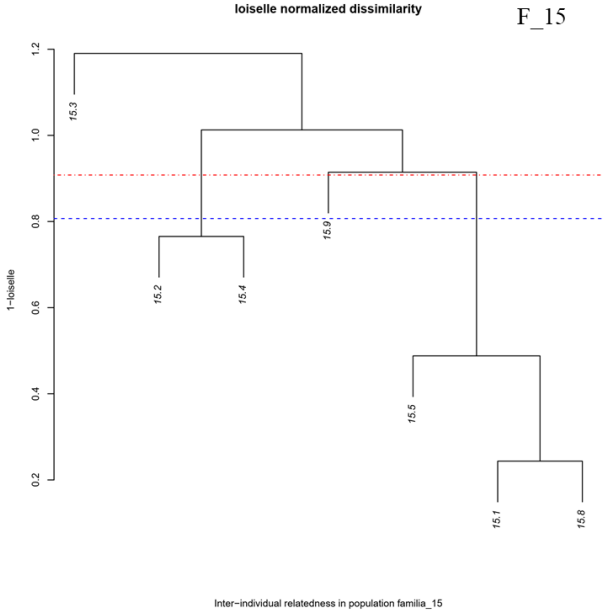

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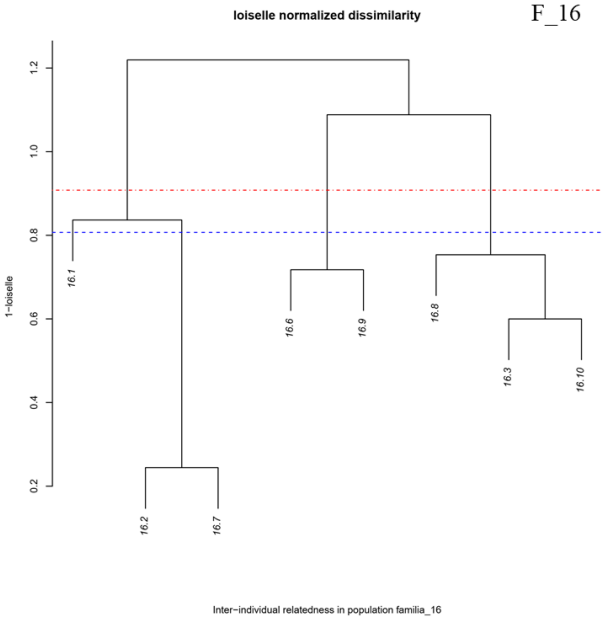

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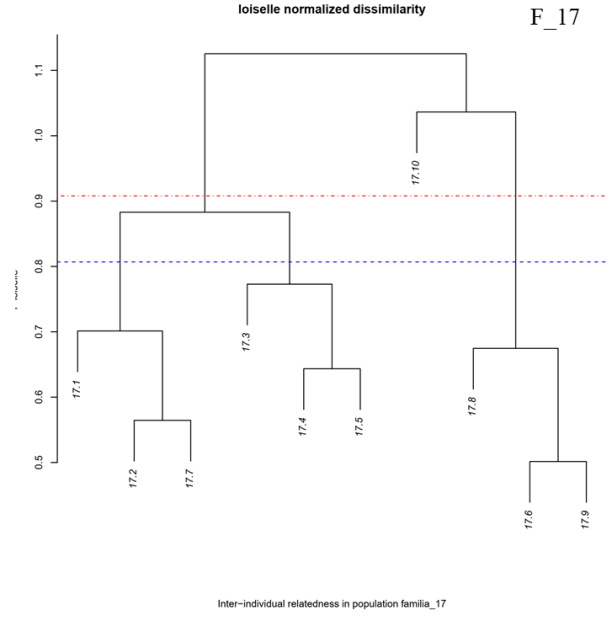

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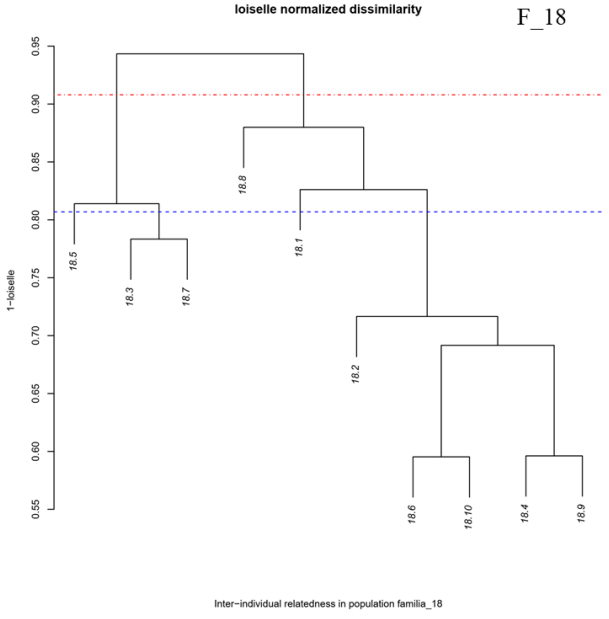

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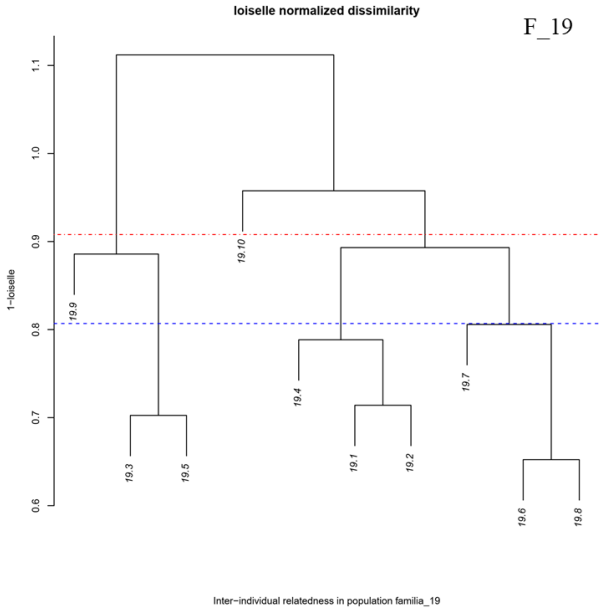
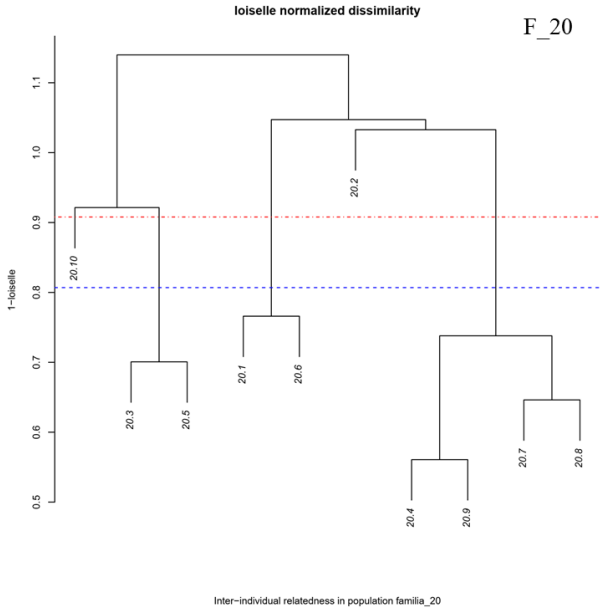


F_19

Inter−individual relatedness in population familia_19



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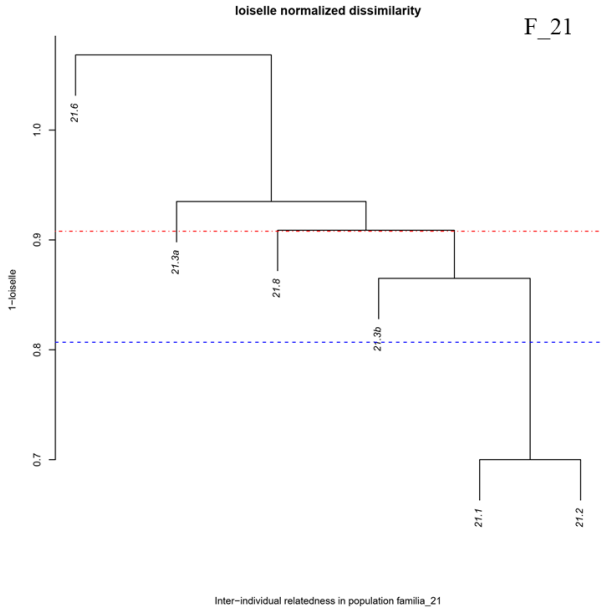
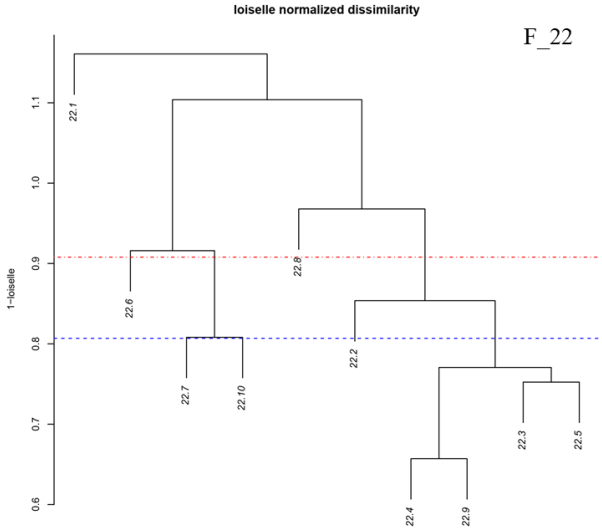
Inter−individual relatedness in population familia_20



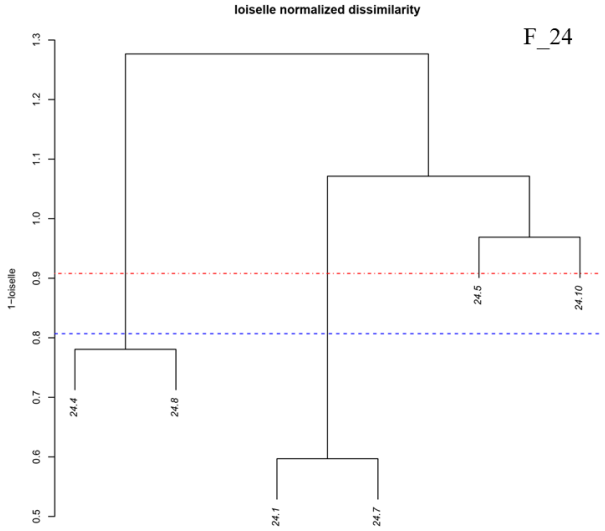
F_21

Inter−individual relatedness in population familia_21



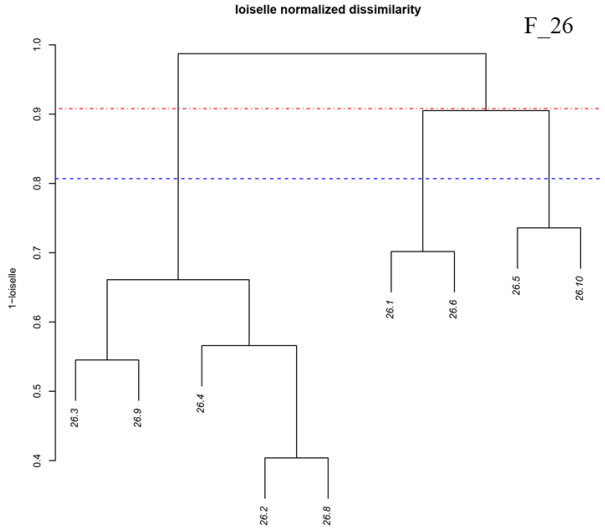
F_22

Inter−individual relatedness in population familia_22



F_24

Inter−individual relatedness in population familia_24



F_26

Inter−individual relatedness in population familia_26

GENERAL CONCLUSIONS

Guaranteeing the survival of the transplanted population is fundamental for ecological restoration. This necessitates knowing the origin of the propagules. To perpetuate restored populations, insights into the genetic status of the species and the gene pool being transplanted are crucial. A more genetically varied population will have a better chance of surviving adverse events, such as diseases or changing environmental conditions. Additionally, this population can connect to other populations in a fragmented landscape through gene flow, thus acting as a lever for regional genetic diversity.

Genetically improving forest trees requires maintaining a propagule source sufficiently large to sustain and select for desired characteristics. As new traits of interest emerge, the number of individuals must be expanded, recognizing that some low-frequency genes may not be present in the initial breeding population (Johnson et al., 2001). Therefore, those engaged in forest species breeding should observe the existing in situ reserves to establish, enrich or maintain ex situ populations when necessary, ensuring they represent the species' gene pool.

The origin of germplasm holds significant importance from a conservation perspective (Dawson et al., 2013). Knowing the origin of the matrices allows for allocating their propagules to their region of occurrence, which aligns with the practice of restoring with local propagules (Zeng & Fischer, 2021). In our case, we possess information on both the matrix origin and the genetic diversity of their offspring. This enables us to select unrelated individuals from matrices within the same region for crossing, thereby producing seedlings suitable for both commercial and restoration plantings.

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