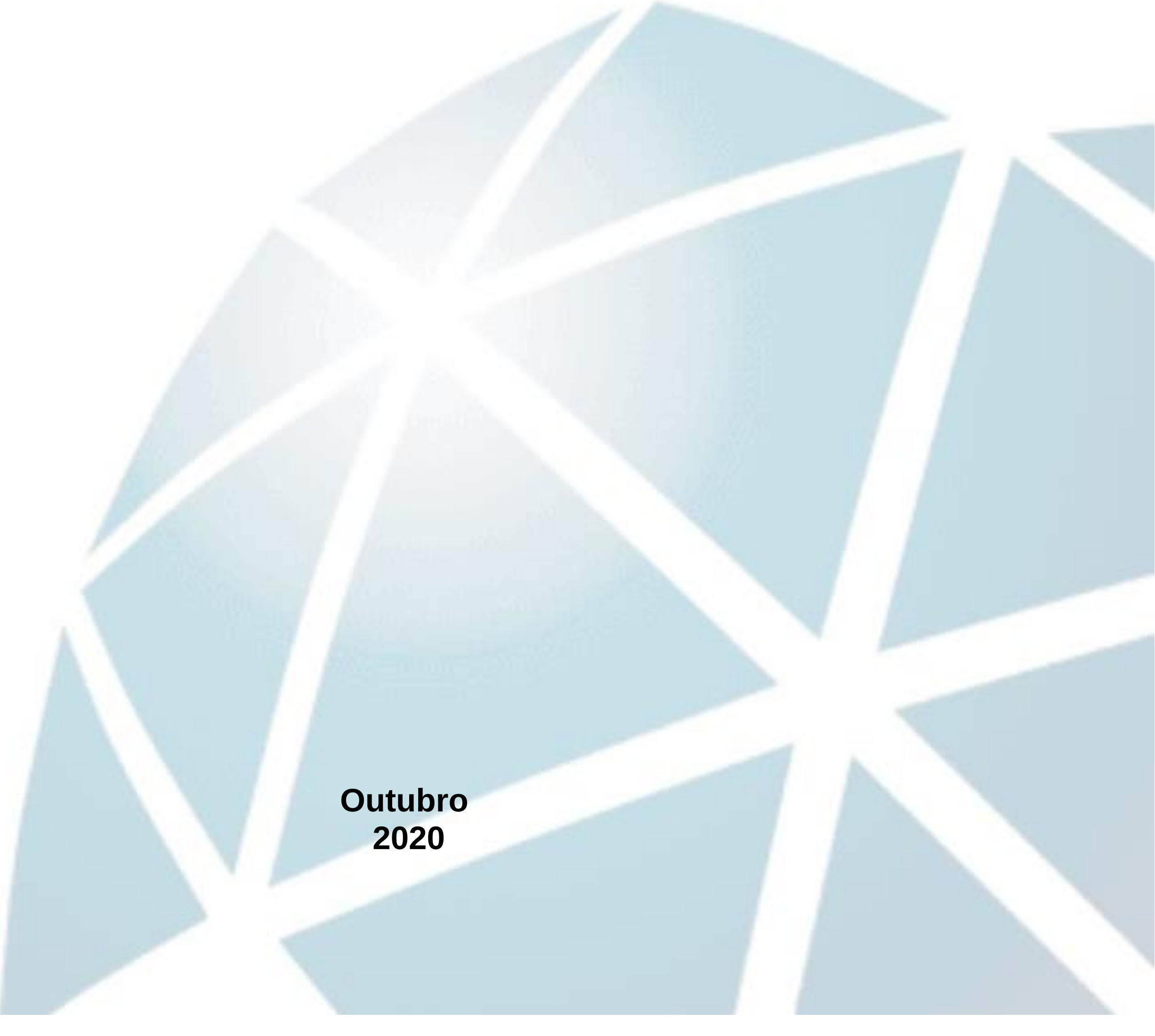
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E BIODIVERSIDADE

LIMITAÇÃO DA DISPERSÃO DE SEMENTES E SUAS CONSEQUÊNCIAS GENÉTICAS EM REMANESCENTES DA MATA ATLÂNTICA

MARÍLIA SOUZA LUCAS



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Dissertação apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Mestre em Ecologia e Biodiversidade.

Orientadora: Profª. Drª. Marina Corrêa Côrtes
Coorientadora: Drª. Carolina da Silva Carvalho

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SEED DISPERSAL LIMITATION AND ITS GENETIC CONSEQUENCES IN ATLANTIC FOREST REMNANTS

MARÍLIA SOUZA LUCAS

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RESUMO

A dispersão de sementes é o primeiro passo no recrutamento de populações naturais de plantas, é um processo importante para a manutenção do fluxo gênico e diversidade genética das populações naturais. A dispersão de sementes mediada por animais é influenciada pelo número e o comportamento de dispersores. Os frugívoros de grande porte são importantes na dispersão de sementes a longas distâncias, podendo conectar populações promovendo fluxo gênico e maior sobreposição das chuvas de sementes, fazendo com que indivíduos próximos sejam menos aparentados geneticamente. Portanto, a redução ou perda desses animais (defaunação) pode acarretar na redução da distância da dispersão. O presente trabalho investigou a limitação devido ao encurtamento da distância e a limitação devido à dispersão contagiosa de sementes geneticamente relacionadas de uma palmeira (*Euterpe edulis*) da Mata Atlântica no estado de São Paulo. Coletamos sementes dispersas em sítios de deposição estabelecidos por coletores de sementes e amostras do estipe de palmeiras reprodutivas na vizinhança desses sítios em dez remanescentes florestais com diferentes graus de defaunação. Utilizando sete marcadores moleculares de microssatélites e testes de maternidade, quantificamos a distância de dispersão de sementes que ocorre em escala local e avaliamos a relação genética (coancestria) entre sementes dispersas. Sementes sem atribuição a palmeiras-mães foram consideradas imigrantes locais e sementes atribuídas foram usadas para calcular a distância de dispersão exata. Além disso, quantificamos a coancestria entre sementes dispersas e entre os adultos dentro dos sítios de deposição. A imigração local variou de 55 a 100% entre as áreas, indicando uma baixa limitação da dispersão pela distância em todas as áreas. A variação encontrada entre os sítios de deposição não foi explicada pela perda dos frugívoros, mas foi explicada pelo número de palmitos frutificando, com imigração maior em sítios com menor abundância de adultos. Áreas com alta densidade de palmeiras frutificando atraem os animais e faz com que eles permaneçam comendo e soltando sementes na mesma área, mostrando a importância da agregação de recursos e densidade para dispersão. Além dos altos valores de imigração, encontramos uma alta porcentagem (0 a 70%) de sementes meio-irmãs e coancestria entre sementes maior do que a encontrada em adultos e a esperada ao acaso. As populações de *Euterpe edulis* são espacialmente agregadas, o que aumenta a probabilidade das sementes de meio-irmãs dispersas, e consequentemente maior será o parentesco da população. Esses resultados indicam que assembleias empobrecidas de avifauna frugívora, principalmente compostas por sabiás (*Turdus* spp.), são eficientes para dispersar sementes e genes em escala local.

Palavras-chave: análise de maternidade, análise de progênie, coancestria, diversidade genética, fragmentação florestal, frugivoria, palmito juçara, palmeira tropical.

ABSTRACT

Seed dispersal is the first step in natural plant populations recruitment and an important process for maintaining gene flow and genetic diversity in natural populations. Animal-mediated seed dispersal is influenced by the number and behavior of dispersers. Large frugivores are important in seed dispersal over long distances, being able to connect populations promoting gene flow and greater overlapping of seed rains, making close individuals less genetically related. Therefore, the reduction or loss of these animals (defaunation) can reduce the dispersal distance. The present study investigated the limitation due to the shortening of the distance and limitation due to the contagious dispersal of genetically related seeds of a palm (*Euterpe edulis*) from the Atlantic Forest in the state of São Paulo. We collected seeds dispersed in deposition sites established by seed traps and samples of stipe from the reproductive palms in the vicinity of these sites in ten forest remnants with different levels of defaunation. Using seven microsatellite molecular markers and maternity tests, we quantified the seed dispersal distance at local scales and evaluated the genetic relationship (kinship) between dispersed seeds. Seeds not assigned to maternal palms were considered local immigrants and assigned seeds were used to calculate the exact dispersal distance. In addition, we quantified kinship values between dispersed seeds and between adults within the deposition sites. Local immigration ranged from 55 to 100% between areas, indicating a low limitation of dispersal by distance in all areas. The variation found between the deposition sites was not explained by the loss of frugivores, but by the number of fruiting palms, with higher immigration in places with less abundance of adults. Areas with a high density of fruiting palms attract animals and keep them eating and dropping seeds in the same area, showing the importance of resource aggregation and density for dispersal process. In addition to the high immigration values, we found a high percentage (0 to 70%) of half-sibs seeds and higher seed coancestry than that found in adults and that expected at random. The populations of *E. edulis* are spatially aggregated, which increases the probability of the half-sib seeds dispersed, and consequently, the population will be more related. These results indicate that impoverished assemblages of frugivorous avifauna, mainly composed of thrushes (*Turdus spp*.), are efficient to disperse seeds and genes on a local scale.

Keywords: coancestry, forest fragmentation, frugivory, maternity analysis, analysis, genetic diversity, Juçara palm, progeny, rropical palm.

SUMMARY

1. INTRODUCTION

Seed dispersal is the first step in the regeneration of natural plant populations and can influence the demography, genetic structure and spatial distribution of future generations (WANG & SMITH, 2002). This process allows the seeds to be removed from the proximity of the mother plant and deposited in other sites across the landscape. The distance at which seeds are dispersed influences gene flow, genetic drift, and the genetic structure of the population (SEZEN, *et al.,* 2005; SORK *et al.,* 1999). The dispersal over greater distances can connect fragmented populations, colonize new areas, and promote gene flow between populations (GARCIA *et al*., 2007; JORDANO *et al*., 2007). For example, events over long distances can lead to the migration of new individuals, enabling new genotypes to be added to the population, which increases the effective population size and reduces drift and genetic structuring (PÉREZ-MÉNDEZ *et al.,* 2017). However, seed dispersal is a complex process influenced by several factors that may limit the amount and identity of seeds removed as well as how these seeds are distributed in space (GARCÍA & GRIVET, 2011). The limited number of effective animal dispersers and variation in their foraging behavior are some of the factors that limit the process of seed dispersal by animals (NATHAN & MUELLER-LANDAU, 2000; SCHUPP, JORDANO & GOMEZ, 2010).

In plants dispersed by animals it is common for seeds to be distributed in an aggregate manner (FRAGOSO *et al*., 2003). This may be the result of the dispersal over short distances (with many seeds deposited next to the maternal plant) or the contagious dispersal in certain places, such as under perches or resting and mating sites (GARCÍA & GRIVET, 2011; SCHUPP MILLERON & RUSSO, 2002). Distance and contagious dispersal limitation influence the seed rain and, more specifically, the composition and spatial pattern of genotypes in the environment (HAMRICK & TRAPNELLl, 2011). This pattern of dispersal usually contains nuclei of genetically related individuals, resulting in the intensification of the genetic structure at fine spatial scales in the population (HAMRICK & TRAPNELLl, 2011). The spatial genetic structure is the non-random distribution of genotypes in geographic space (VEKEMANS & HARDY, 2004). It can be influenced by the density of conspecific plants and by the pollen and seed dispersal distance kernels (NAKANISHI *et al*., 2008). So, the less dense the population and the shorter the dispersal of propagules, the greater is the spatial genetic structure (LOVELESS & HAMRICK, 1984). High fine-scale spatial genetic structure can lead to higher biparental inbreeding, smaller effective population size and, lower genetic diversity in the long term (DUMINIL *et al.*, 2016).

The dispersal at short distances is usually associated with small and medium-sized dispersers (JORDANO *et al*., 2007). Due to the body size restriction (JORDANO e SCHUPP, 2000) these animals consume few seeds per visit and can move less (JORDANO, 2017; GONZÁLEZ-VARO, 2017), increasing the chance of seeds being deposited close to the maternal plants, resulting in a higher local spatial genetic structure within populations (PÉREZ-MÉNDEZ *et al.,* 2017). Large frugivores, on the other hand, are important long-distance seed dispersers. Because of their greater mobility, they can disperse seeds hundreds of meters from the mother plant (JORDANO *et al*., 2007; HOLBROOK, 2011; JORDANO, 2017). They also generate ample seed shadows that can overlap and promote a spatial mixture of genotypes in the population, making close individuals less related (SCHUPP *et al.,* 2002). However, because these animals can stay on the same plant for a long time consuming many fruits per visit (JORDANO *et al*., 2007) and present the behavior of perching and regurgitating or defecating seeds in the same place (SCHUPP, JORDANO & GOMEZ, 2010), they are likely to disperse seeds that are half-siblings (that come from the same maternal plant), which can also strengthen the spatial genetic structure in the population.

Despite the importance of large frugivores, these animals are highly endangered mainly due to anthropogenic processes (DIRZO *et al.,* 2014). Habitat destruction and fragmentation, together with hunting, are largely responsible for the local extinction of large animals in tropical areas (WRIGHT *et al*., 2000; FORGET & JANSEN, 2007). Defaunation is a process characterized by a significant decrease in the number of animal species, and usually starts with the loss of medium and large animals (DIRZO *et al.,* 2014). As a consequence, the loss of these animals can lead to a reduction in the number of dispersed seeds and seed dispersal distances, increasing the likelihood of a seed being dispersed near the maternal plant (JORDANO *et al*., 2007).

One way to accurately assess the seed dispersal distance is by employing maternity analysis (GODOY & JORDANO, 2001). Maternity analysis consists of genetically analyzing the maternal tissue present in the seeds (e.g., endocarp) and potential maternal plants in a sampling area, and then identify the most likely maternal plant based on compatibility between the adult plant and seed endocarp genotypes (GODOY & JORDANO, 2001). From the maternity assignment it is possible to estimate the dispersal distance of the propagules in relation to their mother plant. In addition, kinship coefficients can be used as measures of relatedness between individuals to evaluate the fine-scale spatial genetic structure (BINK *et al*., 2008; VEKEMANS & HARDY, 2004).

Although it is known that seed dispersal can influence the composition and genetic structure of plant populations (GARCÍA & GRIVET, 2011), there are few studies evaluating the genetic consequences of seed dispersal in frugivore impoverished areas (some examples are: PÉREZ-MÉNDEZ *et al*., 2016; CARVALHO *et al*., 2016; PÉREZ-MÉNDEZ *et al*., 2017). In this context, we investigated two components of the limitation of seed dispersal of an ecologically important palm (*Euterpe edulis*) of the Brazilian Atlantic Forest: limitation due to reduction of dispersal distance (limitation by distance) and limitation due to the contagious dispersal of genetically related seeds (limitation by genetic aggregates). Both types of dispersal limitation can result in intensification of local genetic structure. The palm tree *Euterpe edulis* has become a model species in ecological and evolutionary studies, especially pertaining seed dispersal traits and processes. In particular, the loss of large bodied birds of *E. edulis* has already been associated with microevolutionary changes in alleles frequencies (CARVALHO *et al*., 2016), reduction in seed sizes (GALETTI *et al*., 2013; CARVALHO *et al*., 2020) and seed rain composed of a greater number of maternal progenies. (CARVALHO et *al.,* in review). This present study adds to this body of knowledge by evaluating the magnitude of local seed dispersal distances and by quantifying fine-scale spatial genetic structure of seeds across forest remnants with varying levels of bird defaunation.

2. HYPOTHESIS

Our goal was to evaluate the effect of loss of frugivorous birds on seed dispersal and fine-scale genetic structure. We also tested the effect of abundance of fruiting palms and forest structure because both variables may modulate animal behavior inside forest remnants and determine spatial distribution of dispersed seeds and genotypes. They were key variables to explain the diversity of maternal genotypes in the seed rain (CARVALHO et al., 2019; CARVALHO et al., in review). We tested the following hypotheses:

1. Seed dispersal is more distance-limited in more defaunated areas.

We expected that the dispersal distance and, therefore, seed immigration rates would be higher in areas with more structured forests and less defaunated, that is, areas that still harbor large birds such as toucans and cotingas that are able to carry seeds further away and that has good structure for them to move in. Conversely, we expected that a large proportion of dispersed seeds would come from neighboring maternal plants in more frugivore impoverished fragments, as a result of more spatially restricted dispersal by smaller birds, such as thrushes (Figure 1A, Figura 2A).

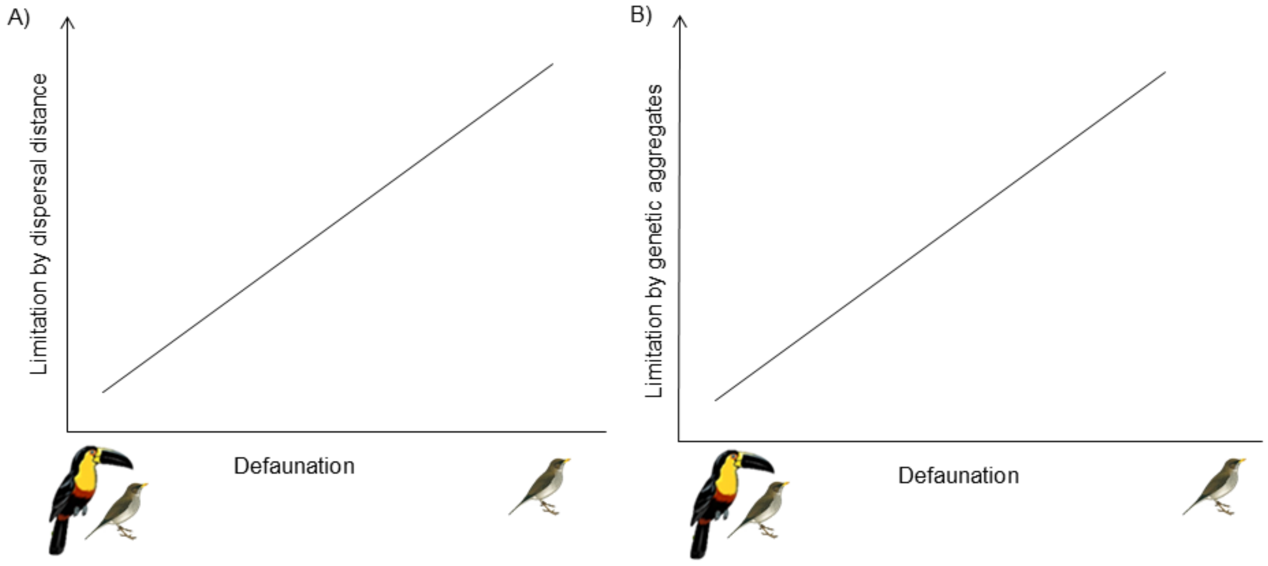

Figure 1: Graphical hypotheses **A)** Seed dispersal is more distance-limited (shorter dispersal and, therefore, lower seed immigration rates) in areas that lack large-bodied frugivores (more defaunated). **B)** Dispersed seeds are more genetically related (higher

dispersal limitation by genetic aggregates) in areas that lack large-bodied frugivores (more defaunated).

2. Dispersed seeds are more genetically related in areas that are more defaunated.

We expected that the genetic relatedness values (kinship coefficients) of dispersed seeds are higher in more defaunated areas and in deposition sites with fewer fruiting palms (Figure 1B). This is because large frugivores (present only in less defaunated areas) disperse seeds over greater distances, promoting the addition of new genotypes to the deposition sites, which would lower the kinship values between seeds (Figure 2B). Conversely, by dispersing seeds from nearby maternal plants, small frugivores generate more genetic structure when there are fewer fruiting plants around (fewer overlapping seed rains).

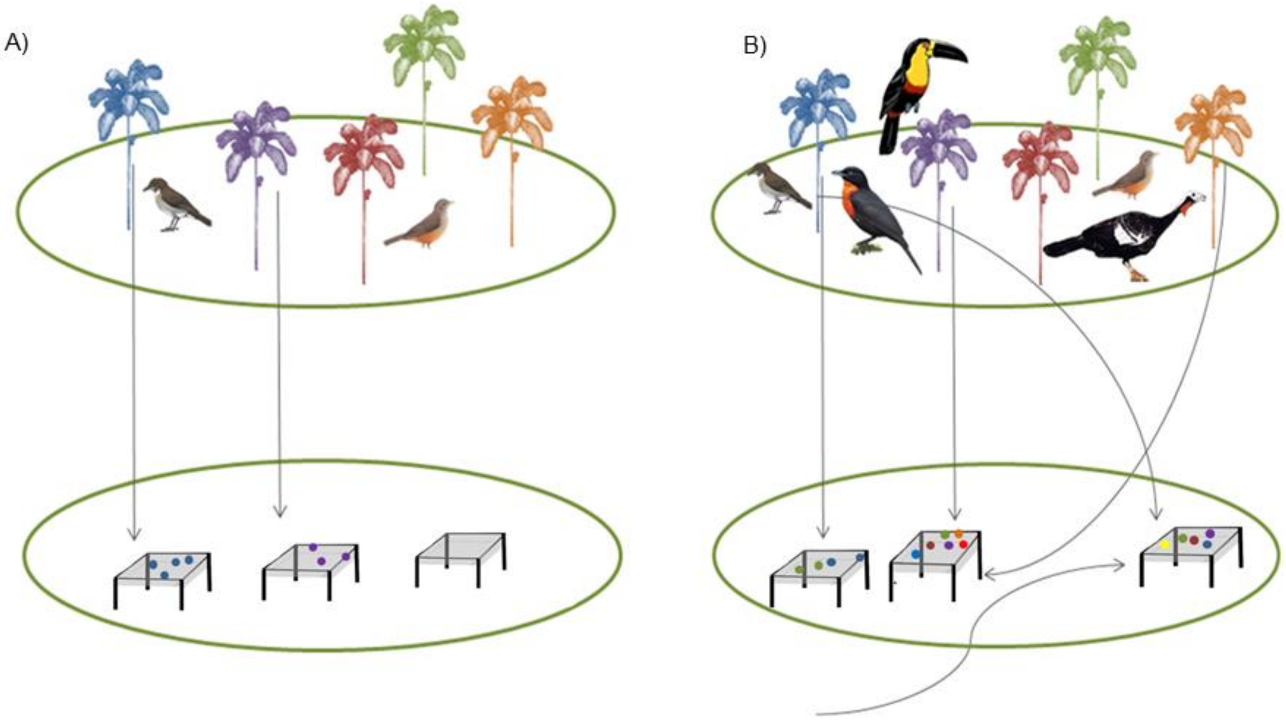

Figure 2: Representation of the seed dispersal process, where deposition sites are represented by black squares and seeds are represented by full circles (with colors matching the maternal plants). **A)** Seeds are dispersed near the maternal plants in defaunated areas that only harbor small- and medium-bodied frugivores, such as *Turdus*

spp. **B)** Areas with lower defaunation rates, which have large frugivores, such as toucans, contingas and guans, have their seeds deposited at greater distances, and some of these are considered immigrants because they come from plants located outside the sampling area.

3. MATERIAL AND METHODS

3.1 Study Species

Our study species, *Euterpe edulis* (Figure 3)*,* is one of the most abundant palms in preserved forests and considered a key species in the Atlantic Forest (GALETTI *et al*., 1999). Both pollination and seed dispersal are performed by animals. These characteristics allow the results found for *E. edulis* to be representative for other plant species that have seeds dispersed by animals, that represent around 75% of the trees in the Atlantic Forest (ALMEIDA-NETO *et al.,* 2008).

Figure 3: *Euterpe edulis* palm tree with fruits and flowers (Source: author's photo)

This palm tree has a single stem and leaves at the apex, which are alternate, composed and pinned, and have a sheath that protects the apical meristem (palm heart). It has female and male flowers constituting spike-type inflorescences with approximately 200 flowers, of which 40-50 are female (SEOANE *et al.*, 2005; LAPS, 1996,). Although it is a monoecious species, studies have found that *E. edulis* has predominantly allogamous reproductive system but with a high probability of individuals being full siblings in open pollinated progeny (GAIOTTO *et al.*, 2003). Pollination can be done by a range of insects (entomophilia), being the main: bees, wasps (both Hymenoptera), flies (Diptera), beetles (Coleoptera) and butterflies (Lepidoptera). Wind can contribute to pollination (anemophilia), although the role it plays is of less importance (SEOANE *et al*, 2005).

Due to the large fruit production, long and annual fruiting, and wide variation in fruit size, *E. edulis* is an important food source for more than 50 species of birds, from large frugivores as toucans, cotingas and guans to smaller birds such as thrushes, and some species of mammals (GALETTI *et al*., 2013). Throughout the Atlantic Forest distribution, 80% of the forest fragments are smaller than 50 ha (RIBEIRO *et al*., 2009), area that does not support large populations of large-bodied frugivorous (WILLIS, 1979; BIERREGAARD *et al*., 1992). Thus, the extirpation of these species may compromise ecological functions that directly influence the demography and genetics of the remaining palm populations in the Atlantic Forest (CARVALHO *et al.*, 2016).

3.2 Study areas

We selected ten areas across the Atlantic Forest in the state of São Paulo, with different levels of defaunation (Figure 4, Table 1). The defaunation level of each area was first calculated in a study performed by Carvalho et al. (in review), where the same areas and the same experimental design was used to evaluate if the defaunation of the large seed dispersers and the density of *E. edulis* impacted the distribution of maternal progenies in the seed rain. For each area, we obtained data from previous studies on the composition of the avian seed dispersers (BELLO *et al*., 2017; GALETTI *et al*., 2013) and additional frugivory observations (ROSA, 2016). The defaunation level for each study area was measured as the difference between the number of seed dispersers in the the most intact bird assemblage (A – State Park of Intervales, Table 1) and the number of seed dispersers found in the focal area.

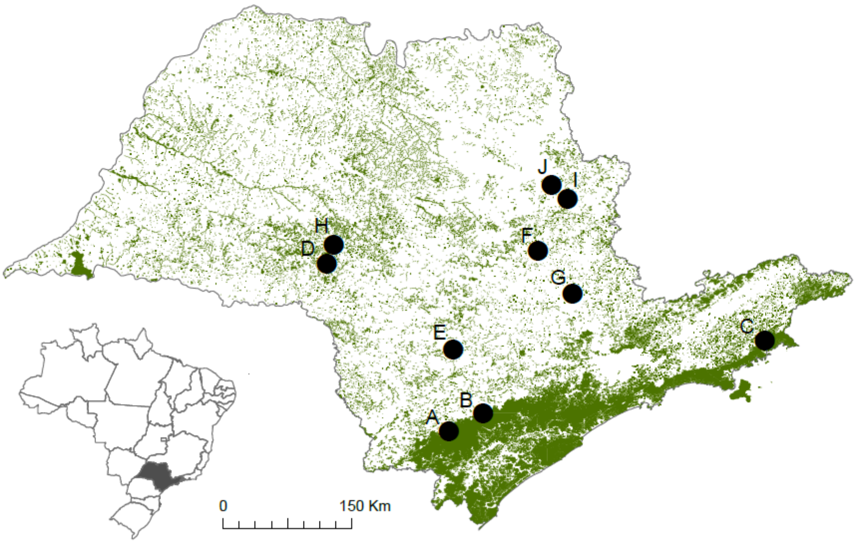

Figure 4: Location of the ten study areas in the Atlantic Forest in the state of São Paulo (Brazil's map with state boundaries in the inset). The letters indicate the areas (see Table 1). Codes are ordered according to the level of defaunation with area A harboring the most complete frugivore assemblage and J the most defaunated assemblage lacking large fruit-eating birds.

Table 1: Study areas in the Atlantic Forest in the state of São Paulo for seed and adult sampling of *Euterpe edulis* with their respective codes and defaunation values. Defaunation level was calculated as the difference between the number of frugivorous birds in the most preserved area (A) and the number of species in the focal area (B-J).

Code	**Defaunation index**	**Area**	**Forest Type**	**City**
A	0	State Park of intervales	Dense Ombrophilous Forest	Ribeirão Grande - SP
B	1	State Park of Carlos Botelho	Dense Ombrophilous Forest	São Miguel Arcanjo - SP
C	4	State Park of Serra do Mar - Núcleo Santa Virgínia	Montane Dense Ombrophilous Forest	São Luís do Paraitinga - SP
D	8	Ecological Station of Caetetus	Semideciduous Seasonal Forest	Gália - SP
E	10	Ecological Station of Angatuba	Semideciduous Seasonal Forest	Angatuba-SP

F	12	Dinamérica farm	Semideciduous Seasonal Forest	Gália - SP
G	12	Mata São José	Semideciduous Seasonal Forest	Araras - SP
H	13	Mata Santa Genebra	Semideciduous Seasonal Forest	Campinas – SP
I	15	Prudente farm	Semideciduous Seasonal Forest	Casa Branca - SP
J	17	Tambaú	Semideciduous Seasonal Forest	Tambaú - SP

Despite the loss of some large frugivorous birds, study areas A-C had very similar defaunation levels ranging from 0 to 4 (Table 1). The frugivore assemblage of these study areas were composed by 20 frugivores that included large bird families, like Ramphastidae, Trogonidae, Contigidae e Momotidae; and small-sized birds of the family Turdidae (Table 2). The study areas D-F showed intermediate defaunation values (Table 1) and still have some large birds, such as cotingas *Procnias nudicollis* and toucans *Ramphastos spp*. *Ramphastos toco* is the only large bird found in defauneted areas, but this specie is naturally found in savannas, and recently has been reported in degraded fragmented forests and urban parks. The most defaunated areas were the study areas G-J, in which frugivore assemblages included only species from the Turdidae and Tyrannidae family (except *Ramphastos toco)* (Table 2).

Table 2: Frugivorous birds dispersing seeds of *Euterpe edulis* in the ten study areas. One (1) means presence and zero (0) absence of the species. Gape width represents the mean gape size in mm. Study areas are ordered in ascending order of defaunation (A-J).

Dispersers		**Gape width**	**Areas**									
Species	**Family**	**(mm)**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Aburria jacutinga	Cracidae	19.3	1	1	0	0	0	0	0	0	0	0
Baryphthengus ruficapillus	Momotidae	17.2	1	1	1	1	0	0	0	0	0	0

Carpornis cucullata	Cotingidae	14.4	1	1	1	0	0	0	0	0	0	0
Lipaugus lanioides	Cotingidae	18.6	1	1	1	0	0	0	0	0	0	0
Myiodynastes maculatus	Tyrannidae	17	1	1	1	1	1	1	1	1	0	0
Penelope obscura	Cracidae	21.25	1	1	1	0	0	0	0	0	0	0
Pteroglossus acari	Ramphastidae	29.1	0	0	0	1	0	1	0	0	0	0
Pteroglossus bailloni	Ramphastidae	23.85	1	1	1	1	0	0	0	0	0	0
Pitangus sulphuratus	Tyrannidae	15.5	1	1	1	1	1	1	1	1	1	1
Procnias nudicollis	Cotingidae	24.5	1	1	1	1	1	0	0	0	0	0
Pyroderus scutatus	Cotingidae	26.7	1	1	1	0	1	0	0	0	0	0
Ramphastos dicolorus	Ramphastidae	30.7	1	1	1	1	0	0	0	0	0	0
Ramphastos toco	Ramphastidae	40	0	0	0	1	1	1	1	1	1	1
Ramphastos vitellinus	Ramphastidae	31	1	1	0	0	0	0	0	0	0	0
Selenidera maculirostris	Ramphastidae	25.2	1	1	0	0	0	0	0	0	0	0
Trogon surrucura	Trogonidae	18	1	1	1	1	1	1	1	0	0	0
Trogon viridis	Trogonidae	19.6	1	1	1	0	0	0	0	0	0	0
Turdus albicollis	Turdidae	11.2	1	1	1	1	1	1	1	1	1	0
Turdus amaurochalinus	Turdidae	11.4	1	1	1	1	1	1	1	1	1	0
Turdus flavipes	Turdidae	12	1	1	1	0	0	0	0	0	0	0
Turdus leucomelas	Turdidae	11.9	1	1	1	1	1	1	1	1	1	1
Turdus rufiventris	Turdidae	12	1	1	1	1	1	1	1	1	1	1
Turdus subalaris	Turdidae	12	1	0	0	0	1	0	1	1	0	0

3.3 Sampling design

To sample *E. edulis* seed rain we installed, in each area, 15 seed deposition sites. These sites are pairs of seed traps of 1 x 1 m, positioned next to each other and suspended one meter from the ground. The minimum distance between seed deposition sites was 40 m. We established a circular area with an 8 m-radius around each seed deposition site, called the neighborhood (Figure 5). All fruiting palms within the neighborhood were counted, marked and the plant tissues sampled. This radius was chosen because a previous study analyzing the spatial distribution of *E. edulis* in 12 plots of 25 x 50 m found that the maximum distance from seedlings to the nearest palm was 8 meters (VALVERDE *et al*. in press), which we assume would be adequate to sample a large fraction of seed dispersal events.

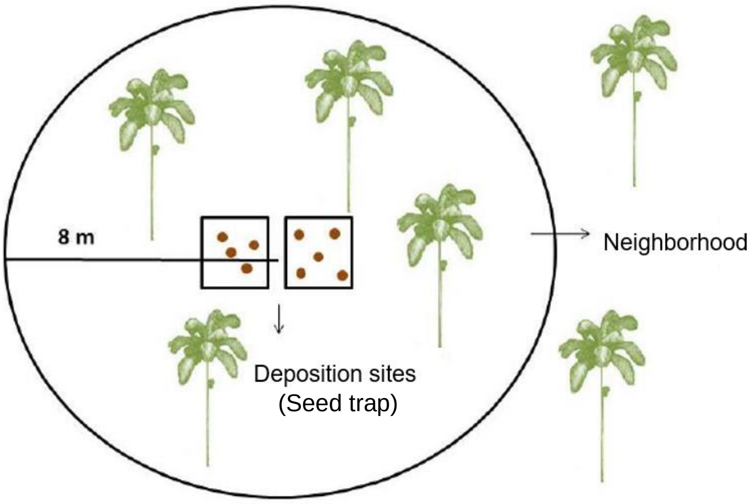

Figure 5: Representation of the seed deposition site (two seed traps for dispersed seed sampling) and respective neighborhood (surrounding area for sampling of fruiting palm and measuring forest structure).

Dispersed seeds and fruiting palms were sampled in all study areas during the fruiting period of the species between 2016 and 2018. The fruiting period varied between areas, occurring between the months of June and November. The potential maternal plants (fruiting palm trees) were sampled by collecting a stem fragment and its distance (meters) to the center of the deposition site was measured (Figure 6). The dispersed seeds (clean endocarp, without pulp, characteristic of regurgitation behavior by birds) were sampled in the seed traps every approximately 40 days during the fruiting period of *E.*

edulis. Seeds and stem tissue samples were placed in paper bags with silica gel, enclosed hermetically in a plastic bag, and stored at -20ºC for the preservation until the laboratory analysis.

One of our areas, the Mata São José (G), located in the municipality of Araras-SP, caught fire in 2016. We were able to collect seeds dispersed in one fruiting season, but due to the forest fragment and palm population destruction, we were not able to collect any stems of potential maternal plants.

Figure 6: Stem sampling from a fruiting adult palm (Source: author's photo).

The forest structure in the neighborhood was calculated previously by Carvalho *at al.* (in review). The forest structure around seed deposition sites was measured using the following variables: distance to the nearest gap opening; number of total trees with dbh > 10 cm; and dbh > 30cm; percentage of open canopy above deposition sites; leaf area index (LAI, were estimated from hemispherical photography with Gap Light Analyzer); and canopy height. Then, we applied a principal component analysis (PCA) to a matrix containing all variables per deposition site. It was found that the first axis of the PCA (PC1) explained 40% of the total variance, with positive values representing deposition sites with denser and higher forest canopy, and negative values indicating sites with open canopy (CARVALHO *et al.*, in review). The first principal component (PC1) was used as a predictor variable in our generalized mixed models (see below) to represent forest structure in the neighborhood, around each deposition site.

3.4 Genetic analysis

Genetic analysis consisted in obtaining the multilocus genotypes of at least ten seeds dispersed in each deposition site (GODOY & JORDANO, 2001). The tissue extracted from the seed's endocarp are fibrous blades inserted into the endocarp (ACCORSI & BARROS, 1974). We also analyzed the genotypes of the fruiting palms located within the neighborhood. The number of seeds with successful genotypes varied, either because there were fewer than ten seeds available in the seed traps or due to faulty DNA amplification.

The isolation of DNA was done using optimized protocols by LUCAS (2019) for DNA isolation from different tissues of the palm *E. edulis*. Briefly, the total DNA extraction from plant tissues involves three distinct steps (Rupture of the cell, DNA precipitation and purification and DNA elution). The endocarp protocol presents changes only in the first stage (rupture of the cell) in which Sorbitol, β-Mercaptoetanol and Sarkosyl were added to counteract the action of contaminants, such as polyphenol polysaccharides and other secondary metabolites. For more information, see the Supplementary material.

Microsatellites were chosen as molecular markers because they are highly polymorphic, abundant in most genomes, present in non-coding regions of DNA and codominant, allowing the identification of individuals (SELKOE & TOONEN, 2006). We used seven microsatellite molecular markers developed for *E. edulis* (GAIOTTO *et al.* 2001) marked with the fluorophores FAM, HEX e NED. PCR protocols were the same as those optimized by LUCAS *et al*. (2019) (Table 3). We genotyped the samples by fragment size analysis using the DNA sequencer ABI 3100. For this, it was identified the size of pairs of bases of DNA fragment using two primers multiplexes (Table 3) with the fluorophore FAM, HEX e NED. Alleles were assigned using GeneMapper v.4.1 software (Applied Biosystems). We have not repeated a subset of individuals to estimate the rate of genotyping error. However, we repeated all dubious genotypes (extraction, PCR and genotyping) and included only those that were scored without doubt in the analysis. In addition, we included a positive control that was unambiguously assigned to the same genotype in all fragment size analyses.

And to prevent the exclusion of incomplete multilocus genotypes, we determined from previous work (CARVALHO *et al.*, in review), with the same loci, species and study areas, the minimum number of loci needed to distinguish individuals with confidence. The complete multilocus genotypes of 296 maternal plants from five study areas were taken (two with the highest defaunation rates and three with the highest number of large frugivorous avifauna) to infer the likelihood of identity as a function of the number of loci and to determine the minimum number of loci for which two individuals do not share the same genotypes (WAITS *et al.*, 2001). It was discovered that a minimum of six fully labeled loci would be sufficient to obtain distinct multilocus genotypes for the group of 296 adults (CARVALHO *et al.*, in review). So, six loci were used in this study as a minimum number to distinguish individuals.

Table 3 – Characteristics of the microsatellite loci described by GAIOTTO et al. (2001) that were used for genetic analysis of *Euterpe edulis* seeds and adults in ten Atlantic Forest areas in the state of São Paulo, Brazil. Description contains identification of primer pairs, repeat motif, primer sequences in forward (F) and reverse (R) directions, expected size variation of the alleles in pair bases (bp), GenBank bank access number and their respective fluorescence FAM, NED and HEX.

Primer	**Repeat Matif**	**Primer Sequence (5´3´)**	**Allele Size Variation (pb)**	**N° GenBank access**	**Fluorescence**
EE3	(AG)11(AG)16	F: TTCgCgCACACTgAgAg R: ggTAgCgTTgATTggTCC	194-210	AF328881	HEX
EE 23	(A)14(AG)23	F: gTTCTgCgATTCATACTCCTg R: TACgAACCAAgATggAgCAA	100-132	AF328877	FAM
EE25	(AG) 26	F:CggATCCTgAgACTgAATTg R:CACACAgATTCAgAgCACA	156-190	AF 328878	NED
EE45	(AG)28	F: AAAgAAATTggCgTgACATC R: AACCAgTCTTCTCCCTCTCg	70-154	AF328887	HEX
EE47	(AG) 20	F:CgAAATCAATggTTTCAgTg R:AATTATTgTTgTgggCAgC	214-246	AF 328874	FAM
EE52	(AG) 22	F:TTCTgTggAgAgTCAATCATC R:AATCTgACAAggCCTCAAC	230-260	AF 328888	FAM

EE54	(AG)25	F: CATgTATCTAAggAACAAgg R: CTgTgCTCTCTCATTCTCA	140-160	AF328876	HEX

3.5 Statistical analysis

3.5.1 Maternity analysis

We performed maternity analysis using the package *Allelematch* in R software (GALPERN *et al*., 2012). Maternity was attributed to a fruiting palm when the multilocus genotype of the seed matched the genotype of a fruiting palm within the respective neighborhood. To ensure that the true mother plant was not excluded due to errors in genotyping, we only excluded a fruiting palm if two alleles or more were not compatible with the seed's genotype (KARUBIAN *et al*., 2010).

The dispersal distances were characterized in two ways. First, we calculated the percentage of seeds considered local immigrants. The seeds were treated as local immigrants when they were not assigned to a maternal palm within the neighborhood and, therefore, they originated from unknown plants located outside the neighborhood. In these cases, we considered that the seeds were dispersed at distances greater than 8 m from the mother plant (radius of the circle that defines the neighborhood). Based on this information, we calculated the seed immigration rate for each deposition site as the proportion of seeds considered immigrants in relation to the total genotyped dispersed seeds. This metric was obtained for each deposition site. Second, for those seeds that were attributed maternity to palm individuals within the immediate neighborhood, we measured the exact dispersal distance. Posteriorly, we calculated the average local dispersal distance for each deposition site and then we compared these values with the average distances of the first, second and third closest fruiting palms. We conducted this comparison to investigate if dispersal distances could be predicted from proximity with adult palms in the population. Lower local immigration rates and shorter distances indicate a high limitation of dispersal by distance.

We modeled the proportion of local immigration and the average of local dispersal distance in the neighborhood using generalized linear mixed models (GLMM). We fitted full models using R package *lme4* (Bates *et al.*, 2015), using binomial (proportion of local immigration) and normal (average of local dispersal distance) error distributions, and the study area as the random effect. The covariates used were: defaunation level of each study area, the number of fruiting palms and forest structure at the neighborhood scale (values of the first principal component, see section 3.3). We built all possible nested models alternating the covariates and used the model selection approach based on the Akaike selection criterion (∆AICc ≤ 2) to select the best models.

We also conducted fraternity analysis to quantify the number of siblings among dispersed seeds. Seeds in the same deposition site with similar maternal genotypes (less than two alleles mismatch) were considered half-sibs (originating from the same maternal palm). We calculated the percentage of half-sibs for each deposition site as the number of siblings in relation to all the genotyped seeds in the deposition site. Then to see if these half-sibs seeds were coming from maternal plants inside or outside the neighborhood, we calculated the percentage of immigrant half-sibs seeds for each deposition site as the number of immigrants in the neighborhood in relation to all half-sibs seeds. We performed a GLMM to model the percentage of half-sibs and the percentage of immigrants half-sibs as a function of the same covariates (defaunation, fruiting palm abundance, and forest structure). We fitted full models using R package *lme4* (Bates *et al.*, 2015), using binomial error distribution. The study area was treated as random effect. After building the full models we built nested models alternating the covariates and then we used the model selection approach based on the Akaike selection criterion (ΔAIC ≤ 2) and significant p-values (p ≤ 0,05) to see the significance of the coefficient of variables within the model to select the best models. Finally, the results of best models were plotted using the *effects* R package (FOX, 2003).

3.5.2 Kinship Analysis

In addition to maternity analysis, we evaluated if the average genetic similarity (i.e. kinship) between seeds within the deposition sites were greater than expected at random and whether it was explained by the defaunation level, the forest structure (PC1) and the abundance (number) of palms. Kinship is the probability that two alleles from homologous chromosomes, randomly taken from two individuals, are identical by descent, that is, they are copies of the same allele of a recent ancestor (LYNCH, 1988; VEKEMANS & HARDY, 2004). Higher mean kinship values were interpreted as seed limitation by genetic aggregates.

The kinship was estimated using the Loiselle estimator (Loiselle *et al*. 1995). Average of pairwise kinship was estimated for seeds and adults, separately, in each deposition site using as allele frequency the total population (multilocus genotypes of seeds and fruiting palms). For that we used the *Demerelate* package (KRAEMER, 2016) on R software. We simulated random dispersal of seeds across space by randomly sampling (with replacement) ten genotypes per deposition site from all genotypes sampled in the population (seeds and fruiting palms). This sampling process was repeated 999 times and mean pairwise kinship was estimated for each iteration. For each area, we depicted the distributions of mean simulated kinship values for seeds and adults and compared with the mean observed kinship values.

We also modeled the values of observed pairwise kinship for seeds as a function of the covariates defaunation, number of fruiting palms and forest structure (PC1) using generalized linear mixed models (GLMM). We fitted full models using normal error distribution, on R package *lme4* (Bates *et al.*, 2015), with the study area treated as random effect. After building the full models we built nested models alternating the covariates and then we used the model selection ($\Delta AIC \leq 2$, and p value $\leq 0{,}05$) approach based on the Akaike selection criterion to select the best models. Finally, the results of best models were plotted using the *effects* R package (FOX, 2003).

4. RESULTS

4.1 Maternity

We extracted DNA from 936 seeds and 529 adults, of which 705 seeds (endocarp) and 497 adults (stems) were successfully genotyped and used in the statistical analysis. The proportion of genotyped seeds in relation to the number of seeds sampled varied from 40 to 85.3%; and the proportion of adults genotyped in relation to the number of adults collected ranged from 73.7 to 100% (Table 4). Among the total genotyped seeds, 130 (18,4%) were attributed to maternal palms and among the genotyped adults, 65 (13,07%) were considered mother-plants (Table 4). The proportion of seeds with assigned maternity ranged from 0 to 45.4% (Figure 7 A); and the proportion of adults which were identified as mother plants ranged from 0 to 22.7% (Figure 7 B) across study areas. The study area G was removed from this analysis because adults were not sampled because of the fire that destroyed the forest fragment.

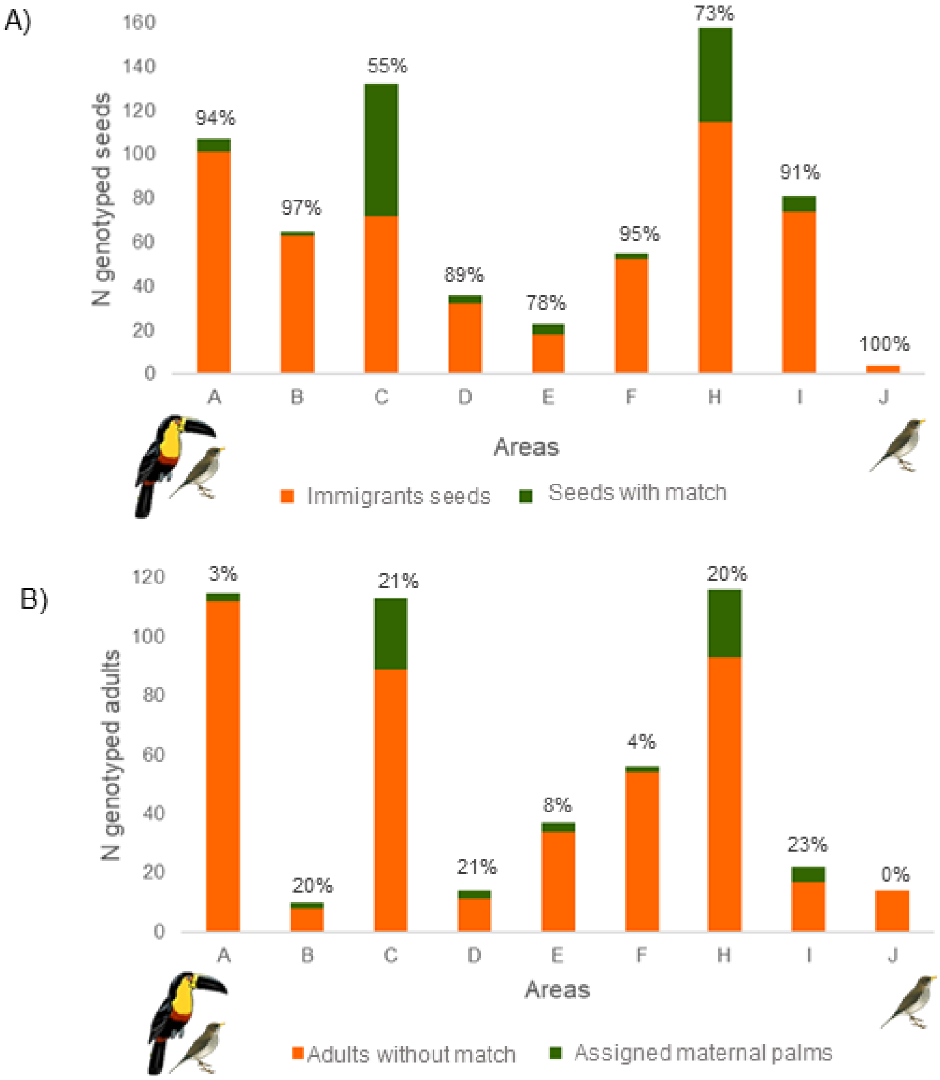

Figure 7: **A)** Number and percentage (%) of seeds that are immigrant (orange) and that originated locally (green) (A-J, ordered in an increasing defaunation index). **B)** Number and percentage (%) of adults that were assigned as maternal palms (green) or not (orange) in each study area (A-J, ordered in an increasing defaunation index). Area G was removed from the analysis (see session 3.3)

Table 4: Number of seeds and adults sampled and genotyped, number of adults and seeds assigned maternity inside the neighborhood area, number of half-sibs seeds and immigrant half-sibs seeds in each study area and its corresponding proportion calculated from genotyped samples (between parentheses).

Area	N sampled seeds	N genotyped seeds	N seeds with identified mother	N half-sibs seeds		N adults sampled	N sampled adults	N adults assigned as mother
				Total	Immigrants			
A	150	107 (71,3%)	6 (5,6%)	68 (63,5%)	68	122	115 (94,3%)	3 (2,6%)
B	79	65 (82,3%)	2 (3,1%)	37 (56,9%)	37	10	10 (100%)	2 (20%)
C	166	132 (79,5%)	60 (45,4%)	72 (54,4%)	27	120	113 (94,2%)	24 (21,2%)
D	64	36 (55,4%)	4 (11,1%)	7 (19,4%)	7	19	14 (73,7%)	3 (21,4%)
E	34	23 (67,6%)	5 (21,7%)	16 (69,6%)	11	38	37 (91,4%)	3 (8,1%)
F	67	55 (82,1%)	3 (5,4%)	32 (58,2%)	30	59	56 (94,9%)	2 (3,6%)
G	79	44 (55,7%)	NA	19 (43,2%)	NA	NA	NA	NA
H	192	158 (82,3%)	43 (27,2%)	97 (61,4%)	69	123	116 (94,3%)	23 (19,8%)
I	95	81 (85,3%)	7 (8,6%)	40 (49,4%)	36	24	22 (91,7%)	5 (22,7%)
J	10	4 (40%)	0	0 (0%)	0	14	14 (100%)	0
Total	936	705 (75,3%)	130 (18,4%)	388 (55%)	285	529	497 (93,9%)	65 (13,07%)

From the seeds that were assigned maternity, it was possible to calculate the mean distance of seed dispersal in each study area. Distances ranged from 2.6 to 8 meters (Figure 8), and the mean dispersal distance was higher than the first nearest palm in all populations except B and J (in this area all seeds were immigrant). Only in two study areas (C and F), we found four (tree and one, respectively) seeds which multilocus genotypes matched adult palms in other deposition sites. These potential dispersal events were removed from the analysis to not overestimate dispersal distance.

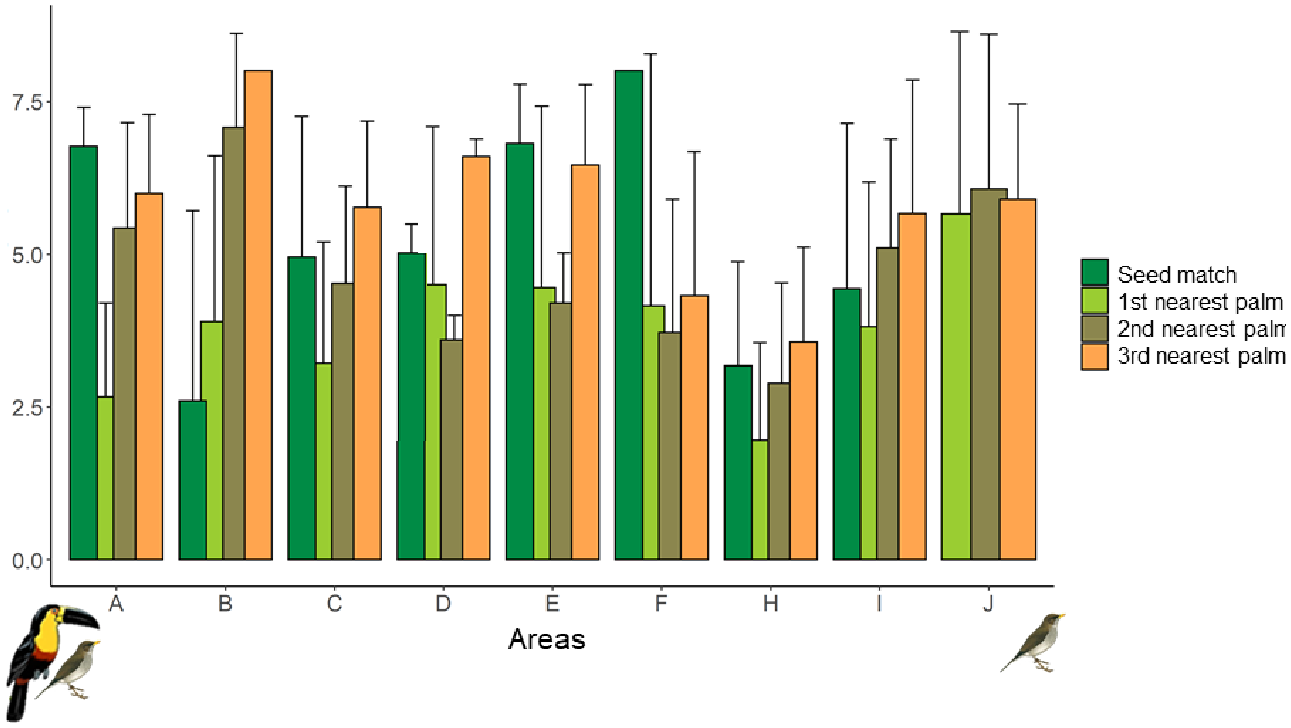

Figure 8: Mean seed dispersal distances based on maternity analysis of *Euterpe edulis* inside the neighborhood area and the mean distances between the first, second and third nearest palm in each study area in (A-J, ordered in an increasing defaunation index). Area G was removed from the analysis (see session 3.3). In area J all seeds were immigrants, so exact seed dispersal distances at the neighborhood scale was not calculated.

Immigration rates were high, varying from 55 to 100% across areas (Figure 7 A). A total of 388 (55%) half-sibs seeds were found in all areas (Table 4). The proportion of half-sibs ranged from 0 to 63.5% (Figure 9). We categorized the half-sibs seeds into two types, those who had mothers inside the neighborhood and those who had mothers outside (half-sibs immigrants). A total of 285 (73,5%) were immigrant half-sib seeds (Table 4).

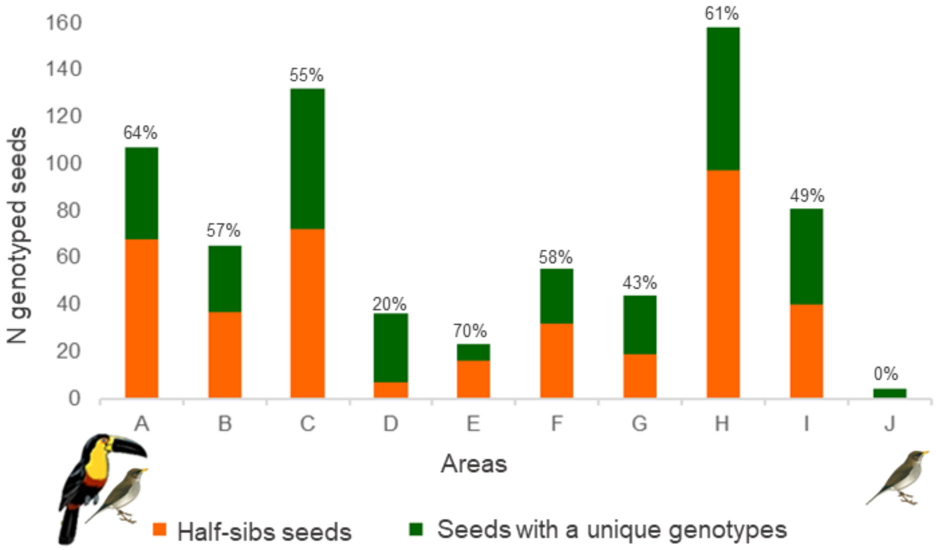

Figure 9: Number and percentage (%) of half-sib seeds (orange) and single seeds with unique genotypes (green) (A-J, ordered in an increasing defaunation index).

The mean local dispersal distance was not influenced by any of the tested covariates given that the null model was among the plausible candidate models (Table S4). The immigration rate, however, was best explained by the model containing the number of fruiting palms ($\Delta AIC \leq 2$, and p-value ≤ 0,05, Table S4). Specifically, the local immigration was negatively influenced by the number of fruiting palms (Figure 10 A). The percentage of half-sib seeds was also best explained by the model with the number of fruiting palms (only variable with significant values, $\Delta AIC \leq 2$, and p-value ≤ 0,05,) (Table S3), so that the higher the number of fruiting palms, the higher the percentage of half-sibs seeds in the seed deposition site (Figure 10 B).

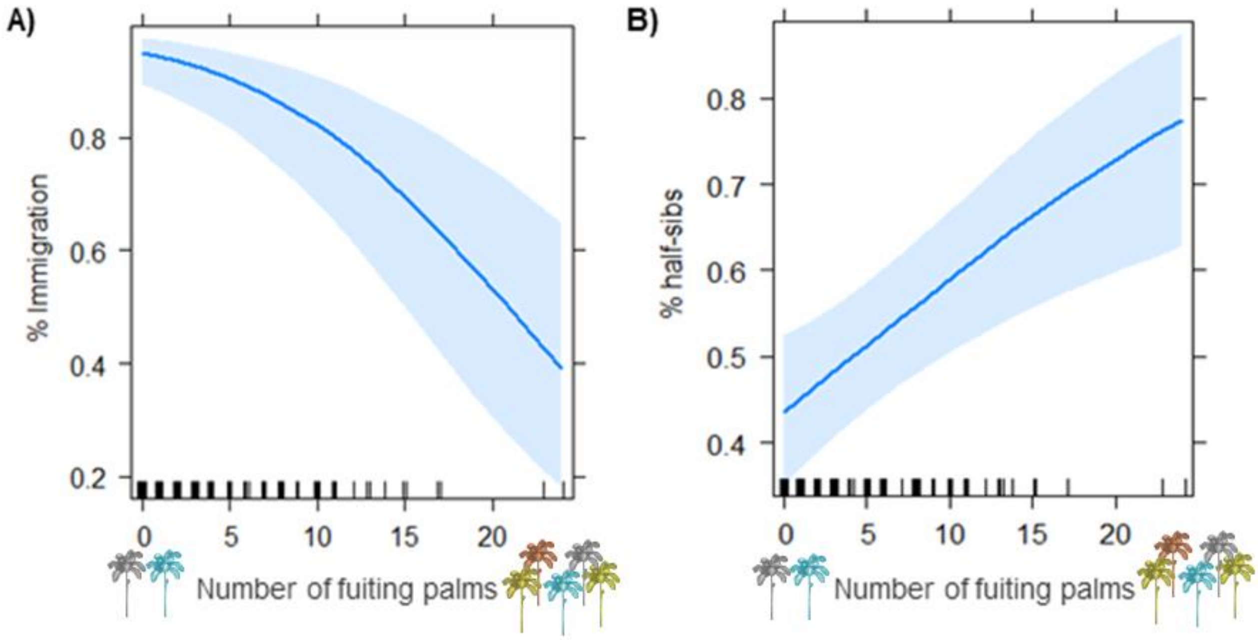

Figure 10: Relationship between number of fruiting palms and rate of seed immigration (A) and % of half-sibs seeds (B) predicted by the top-ranked models.

Finally, the percentage of immigrant half-sibs (% of half-sib seeds without mother-palms within the neighborhood in relation to all half-sib seeds) was best explained by the model with the number of fruiting palms and the forest structure (PCA). The percentage of immigrant half-sib seeds was negatively influenced by the number of fruiting palms, and lower under more closed canopy (higher PC1 values) (Figure 11).

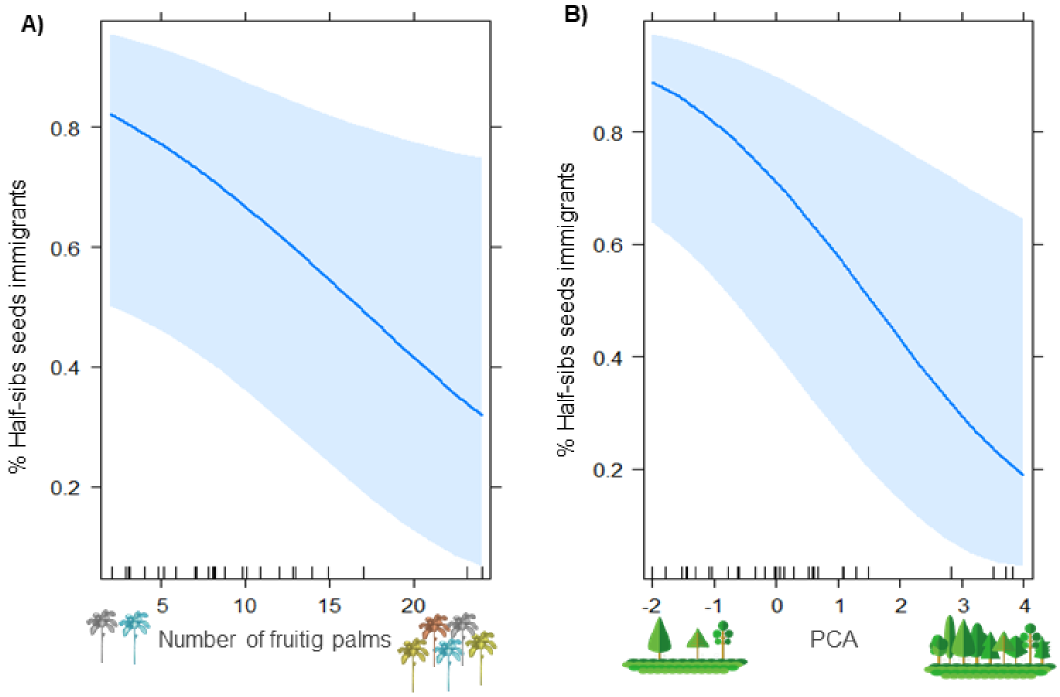

Figure 11: Relationship between % of immigrants half-sib seeds and the number of fruiting palms (A) and the forest structure (PC1) (B) predicted by the top-ranked models.

4.2 Kinship

As expected, we found that the mean observed kinship values of seeds and adults (Figure 1S and 2S) are consistently higher than those expected by chance in all areas despite of defaunation levels (Figure 12). By comparing the density distributions of mean pairwise kinship between seeds and adults we can see that the mean kinship of seeds is significantly higher than adults in seven out of nine populations, (five with p value significant, two with p value marginally significant) with the exception of areas I and J (t-test, Figure 13).

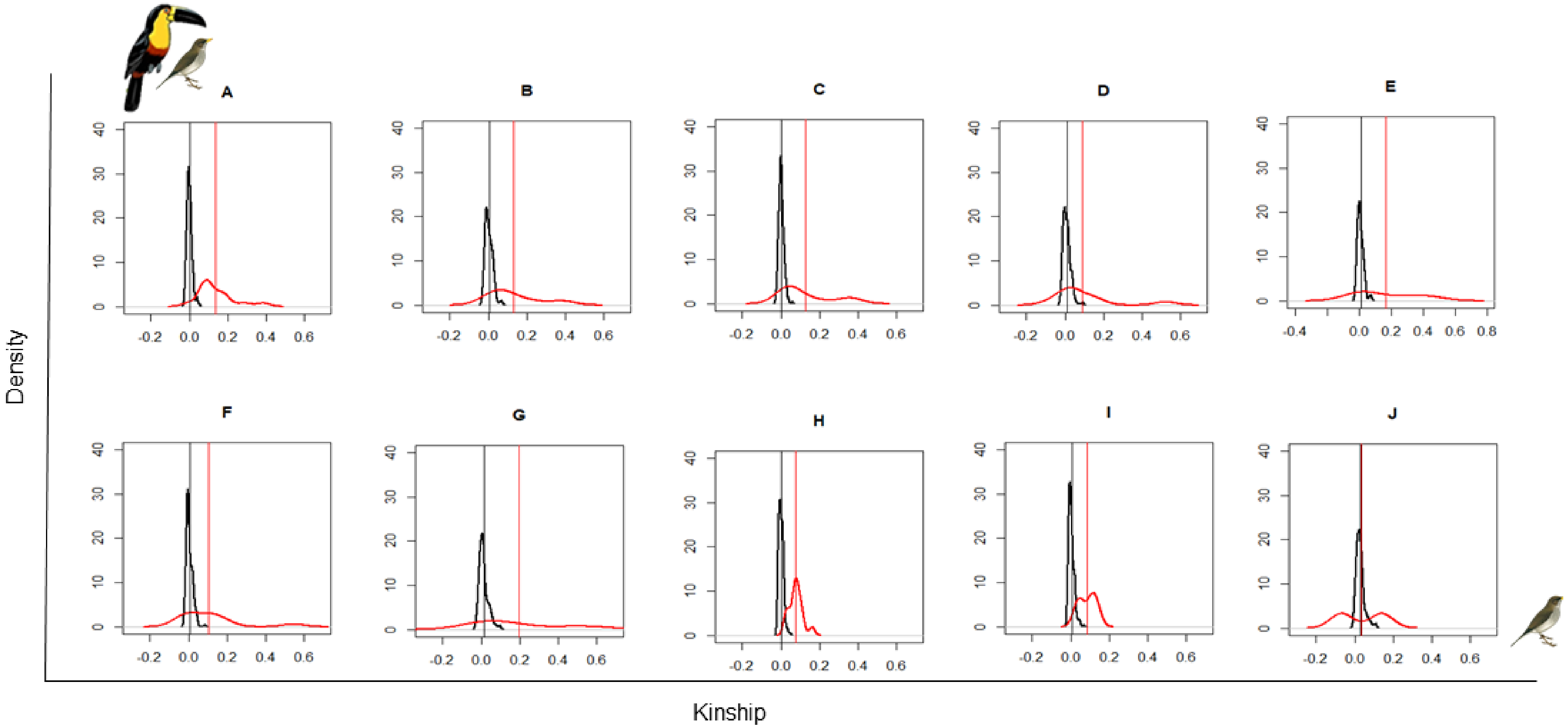

Figure 12: Density distributions of observed mean kinship of *Euterpe edulis* seeds (red) and mean kinship expected at random (black) in the ten study areas (A-J, ordered according to increasing defaunation levels). Their respective means are indicated by the vertical lines

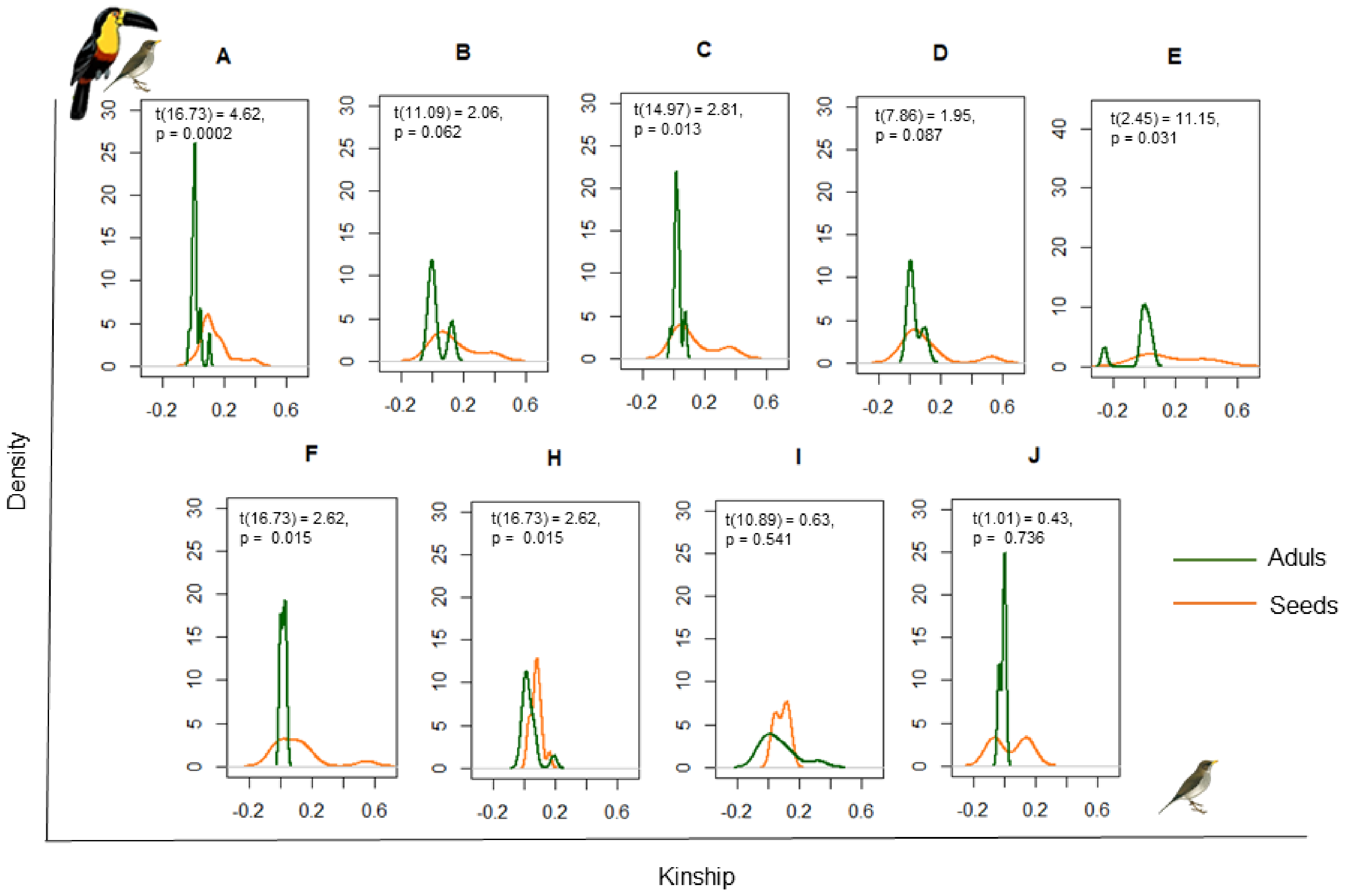

Figure 13: Density distributions of mean kinship values per seed deposition sites for seeds (orange lines) and adults (green lines) of *Euterpe edulis* in the nine study areas in the Atlantic Forest (A-J, ordered according to increasing defaunation levels). Area G was removed from the analysis (see session 3.3). The statistical terms in the upper left corner correspond to: t(degrees of freedom) = t value, p = p-value. We use p value with marginally significant values.

We found that for the observed mean seed kinship the null model was among the plausible models indicating that genetic structure of dispersed seed was not influenced by any of the tested covariates (Table S4). But as expected, the observed kinship was positively influenced by the number half-sibs seeds (Figure 14).

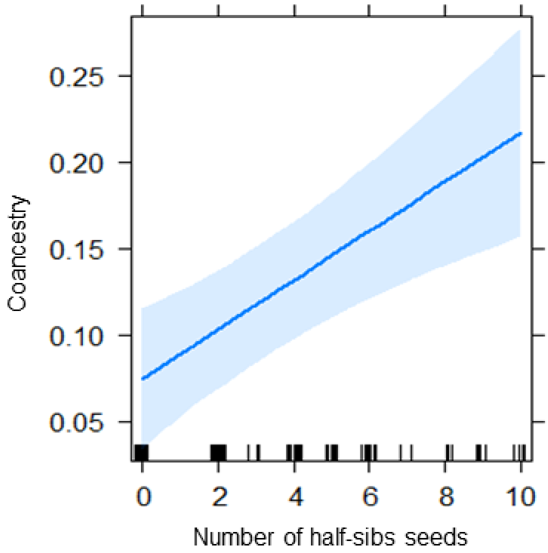

Figure 14: Relationship between number of half-sib seeds and mean seed kinship of *Euterpe edulis* predicted.

5. DISCUSSION

Our hypothesis that defaunation would lead to both limitation due to reduction of dispersal distances (limitation by distance) and due to the contagious dispersal of genetically related seeds (limitation by genetic aggregates) were rejected. Conversely, we found high seed immigration rates at local scales (beyond the 8 m neighborhood radius) in all areas, indicating that the seed dispersers that remain in more defaunated areas are as efficient as a rich avian assemblages in moving seeds beyond the immediate maternal vicinity. Despite the lack of effect of defaunation, populations presented high kinship values at the seed stages. The number of fruiting palms in the neighborhood emerged as the main factor affecting immigration rates negatively and the number of sibling seeds positively.

5.1 Maternity

The mismatches between the genotypes of seeds and adults (potential mother plants) indicate that a large part of the dispersed seeds originate from palms located outside the immediate neighborhood (8m-radius circular area). We expected that most of the dispersal events would occur at short distances so that seeds would be assigned to local mother palms, especially in more defaunated areas. However, our results did not corroborate the hypothesis that assemblages composed mainly by small-medium frugivores scatter seeds close to mother plants (GARCÍA & GRIVET, 2011; SCHUPP *et al*. 2002). Our results showed that the mean dispersal distance was higher than the mean distance to the nearest fruiting palm in most areas. In fact, from 130 dispersal events, only 38 (29,2%) seeds came from the nearest fruiting palm. Therefore, as reported by Sezen *et al*. 2009 in a study also performed with a neotropical palm with seed dispersal by animals, the proximity is a bad proxy for seed dispersal. They found that more than 70 per cent of seedlings were dispersed at least 50 m from parent trees. These results are important because they expose simplistic predictions and allow for model refinements: Due to the difficulty in identifying the mother plant of young plants and, therefore, reconstructing individual seed dispersal events, many studies that attempt to characterize the seed dispersal distance assume that the closest conspecific is the potential mother plants of seeds and seedlings, mainly in studies that use inverse modeling to estimate dispersal curves (URIARTE *et al*., 2005, NATHAN & MUELLER-LANDAU, 2000). However, as we show in our study, the proximity between seeds and the closest adult is not always synonymous with maternity (SEZEN, *et al.,* 2009), so the dispersal distance is often underestimated (HARDESTY *et al*., 2006; WEHNCKE *et al*., 2003).

Our results emphasizes that small frugivores are efficient in carrying seeds away from the vicinity of reproductive plants (CARVALHO *et al.*, in review). Studies with species of *Turdus spp*. show that these animals move well in vegetated areas (GONZÁLEZ-VARO *et al.*, 2017), but they tend to stay close and move more slowly near the edges of the forest which increases the probability of seed deposition within the fragment (DA SILVEIRA *et al*., 2016). Our results are evidence that these smaller frugivores are moving seeds inside the forest remnants, but it is still unclear if they are able to move and disperse seeds beyond fragment edges and between isolated populations.

Despite the lower-than-expected maternity assignment, many seeds shared the same multilocus genotype, resulting in large numbers of sibling seeds arriving at the same deposition site. The strong spatial aggregation of seeds from a single mother tree may be linked to the behavioral patterns of frugivores. Two main frugivore-generated patterns may occur, the first when we refer to Small-medium birds. They may move repeatedly to the same trees to feed and then disperse seeds nearby (GALETTI *et al.*, 2013; ROTHER *et al.*, 2016), generating a distance-limited dispersal pattern (TORIMARU *et al*., 2007). This pattern may be pronounced in areas with high density of fruiting conspecifics so that animals remain eating and dropping seeds in the same area, showing the importance of resource aggregation and density for dispersal (CARLO & MORALES, 2008). The second one is about the large birds, they may stay longer and consume many seeds at once in the same tree, and disperse clumps of related seeds under perches located further from the maternal plant, but still generating a contagious seed dispersal structure. Using the same plants as food resources can leading to a contagious seed dispersal limitation (SCHUPP et al., 2002). As a consequence of both patterns, the subsequent recruitment stages in any population may exhibit strong spatial genetic patterns, regardless of the frugivore assemblage composition and defaunation status.

Our results also showed a negative effect of the number of fruiting palms on the percentage of immigration of the seeds and a positive effect on the percentage of half-sib seeds. This half-sib effect can be a result of the attraction of seed dispersers to sites with high fruit resources availability (CARLO & MORALES, 2008). With a higher density of plants and, consequently, of resources, frugivorous move less, which reduces the arrival of immigrant seeds (seeds coming from palm trees outside the neighborhood). And this result corroborates Carlo and Morales (2008) study, which shows that bird local movement are shorter when there is high density of fruiting plants in the neighborhood.

Finally, we found a negative effect of the number of fruiting palms and closed canopy structure (higher PCA1 values) on the percentage of immigrant half-sib seeds. That is, the higher the number of fruiting palms and the more closed is the canopy at the neighborhood the lower is the chance of having sister seeds coming from mother plants outside the neighborhood. That again be explained by the fact that avian frugivores probably stay longer in sites with high availability of fruit resources and with a more structured and closed forest, which reduces mobility and the influx of immigrant seeds.

As we can see the number of palm trees seems to mediate many dispersal processes of *Euterpe edulis*. However, this is an endangered palm species (GALETTI *et al*., 2013) and the density can vary a lot naturally and also due to the illegal palm heart extraction (MARTINELLI & MORAES, 2013). The human induced reduction in palm abundance may have more immediate effects for the population demography and genetics than defaunation, at least at this local scale. It would be important to assess the interplay between aggregation and abundance in these populations. Less spatial aggregation can increase local immigration, but if this weaker aggregation is a result of lower population abundance, a collapse in dispersal may take place.

5.2 Kinship

Although the seeds were dispersed beyond the neighborhood of the mother plants, we found seeds in the same deposition site to be more genetically related than expected by chance, indicating that frugivores activity resulted in a spatial aggregation of related progenies at the deposition sites in all study areas. The high kinship may be due to two non-excluding processes: 1) birds may disperse many sibling seeds to the same site (multiple visits or few longer visits to the same tree); and 2) or because birds consume fruits from related maternal trees, which is more likely to occur in patches with high density of fruiting conspecifics. That is because plant populations naturally present fine-scale spatial genetic structure, with pairwise genetic similarity decaying with distance (DICK *et al.*, 2008). In fact, it seems that the first behavioral process is more likely to explain our results. This is because the kinship values are consistent with the fraternity analysis that we carried out in the study, in which deposition sites with high numbers of sibling seeds were those with high mean pairwise kinship coefficients. Studies carried out by García et al (2009) involving seed maternity indicated that seeds deposited in the same collectors tend to be maternally correlated (that is, they have a high probability of being maternal half-sibs). The populations of Euterpe edulis in many of these areas are spatially aggregated, usually nearby water, which increased the probability of the dispersed half-sib seeds, and consequently the kinship of the population. As a consequence of high kinship and non-random seed dispersal, future plant populations may develop substantial genetic structure (AGUILAR & GALETTO, 2004).

Though without a pattern found in relation to the defaunation levels of the areas, when we compare the kinship values between seeds and adults we found that the mean pairwise kinship values of adults in the neighborhoods were often lower than that found in seeds. This is expected because for a seed to reach adulthood several environmental filters will occur. This can lead to a decrease in kinship due to the fact that many of these seeds are not recruited. (FRAAIJE *et al.,* 2015). And this shows us that these populations are not yet in a stage of genetic structuring.

Seed dispersal is responsible for the recruitment and spatial distribution of genotypes resulting in future populations (HOWE & MIRITI, 2004). Even though pollen dispersal can compensate for genetic drift and neutralize genetic differentiation between populations (HOWE & MIRITI, 2004), seed dispersal is generally considered to have a stronger effect on small-scale genetic patterns (HAMILTON, 1999; GELMI-CANDUSSO *et al*., 2017). Seeds carry male and female gametes and, since it carries twice the number of genes than pollen, it is expected to have a comparatively greater impact on the patterns of genetic structure of populations (PAREJOS-FARNES *et al*., 2017; HAMILTON, 1999; GARCÍA E GRIVET, 2011; SORK *et al*., 2015). Knowing this, although we have not studied the pollination process, the pattern of genetic structuring found in this study should probably reflect the pattern of seedlings recruited in the future.

In general, our results showed that small-medium sized birds may play an important role in providing dispersal services for *Euterpe edulis*, and that populations are not yet spatially genetic structured. But we saw that the dispersal does not occur randomly, since we found many seeds with a high degree of kinship. And knowing that small and medium sized birds are not able to ingest large seeds and, therefore, only medium to small seeds are dispersed (GALETTI *et al*., 2013; CARVALHO *et al*., in review). The seed size variation along with the dispersal of genetics aggregates can affect in the future several aspects dispersal process and the establishment and, consequently population structure (CARVALHO *et al*. in press; MOLES, 2005; SNELL *et al*., 2019).

6. CONCLUSION

Care should be taken to infer seed dispersal patterns and kinship by proximity to individuals without knowledge of the ongoing dispersal processes of the species under study. We provide evidence that clumps of progenies from the same maternal plant are not necessarily located around the mother tree and the nearest plants are not always the maternal plant. Therefore, the distribution of individuals within a population and the distance of seed dispersal away from the parent plant may reflect the movement pattern of each disperser (HARDESTY et al. 2005) which directly interacts with the landscape features and distribution of food resources.

We did not find evidence that defaunation would reduce the distance of dispersal at short scales or increase fine-spatial genetic structure at patch level. The present results corroborate other studies that indicate that the seed dispersal can be maintained in frugivore impoverished areas by the dispersal activity of remnant small-sized frugivores (SANTOS *et al*.,2018). However, further analysis should be done to investigate the decay of genetic relatedness with distance to evaluate the magnitude of fine-scale spatial genetic structure beyond the neighborhood scale. We use the local distance of eight meters as a criterion for neighborhood, which did not allow us to find a difference in the dispersal distance. Thus, it is possible that this limit of 8 meters can be insufficient to measure longer-distance dispersal at the local scale in a strict sense (JORDANO, 2017). We know that for *Euterpe edulis* the break in the dispersal distance exists in defaunated areas, since studies show that sites in defaunated areas are less likely to receive seeds compared to more preserved ones (CARVALHO, 2018; CARVALHO *et al*., in press).

Our study species is long-lived and can take decades for changes in seed and pollen dispersal patterns to translate into changes in the genetic structure of adults (SOARES *et al*., 2019; YOUNG *et al*., 1996; AGUILAR *et al*., 2008). And knowing that the populations of *Euterpe edulis* are vulnerable to extinction, mainly due to the 30% decline in population recorded over the past six decades due to illegal logging (MARTINELLI & MORAES, 2013), lower effective numbers of mothers can result in lower effective parental numbers too, what can increase local genetic structure in the next generation (PAREJOS-FARNES *et al*., 2017). And since apparently the density of palm trees seems to mediate dispersal processes, the decrease of density can generate immediate effects locally for the population.

7. REFERENCES

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Supplementary material

DNA EXTRATION

We isolated DNA from two the different tissues (stem and endocarp of dispersal seeds) using a Cetyl trimethylammonium bromide (CTAB) protocol for isolation of plant DNA optimized by LUCAS *at al.* (2019). The general protocol (see description below) was used to extract DNA from the stems and, additionally, we customized a protocol that provided better DNA quality for endocarps of dispersed seeds (procedure called as endocarp protocol) (LUCAS et al., 2019).

In general, the extraction of total genomic DNA comprises of three distinct steps. The endocarp protocol differs only in the first step (*I. Cell rupture*).

Extraction Protocol

I. Cell rupture: the tissue maceration is the first step in the DNA extraction from plant cells. The reagents contained in the extraction buffer are responsible for breaking the membranes and releasing the cellular content (DNA, proteins, etc). The proteinase K enzyme is added to denature the proteins, keeping the DNA intact.

General Protocol - Step 1

Place approximately 100 mg of plant tissue (*i.e.*, leaf without the veins, root, endocarp, stem or embryo) in 2 mL tubes. Add 7 mm beads for maceration. Macerate for 1 minute and 30 seconds in the macerator Mini-Beadbeater™ (depending on the lignification of the sample another maceration cycle is needed). Add 800 µL of 1% CTAB buffer solution preheated at 65 °C to each tube. Incubate the tubes at 65° C for 1-2 hours, preferably with gentle shaking.

Endocarp Protocol - Step 1

Place approximately 100 mg of dispersed seed endocarp tissue in 2 mL tubes. Add 7 mm beads for maceration. Macerate the tissue for 1 minute and 30 seconds in the macerator Mini-Beadbeater™. Add 1 mL of cold Sorbitol extraction buffer (4 °C) and 2 µL of β-Mercaptoethanol to each tube and mix in a vortex for five seconds to neutralize the action of contaminants, such as polysaccharides polyphenols and other secondary metabolites (Tel- Zur *et al.* 1999). After resting for 20 minutes in a 4 °C refrigerator, centrifuge the tubes for 10 minutes at 10000 rpm at 4 °C. Dispose of the supernatant. If necessary, the adding of Sorbitol extraction buffer and β-Mercaptoethanol should be repeated until the supernatant is clear. Add a mix of 800 µL of 3% CTAB extraction buffer preheated at 65 °C, 30 µL of sarkosyl and 2.5 µL of proteinase K to each tube. Incubate the tubes at 65 °C for 1-2 hours with gentle shaking.

General and Endocarp Protocol - Step 2

After the incubation period, centrifuge the tubes at 13000 rpm for 15 minutes, under a temperature of 22 °C. Add 600 µL of CIA (Chloroform and isoamyl alcohol, 24: 1) to each tube. Manually homogenize the samples and centrifuge at 13000 rpm for 10 minutes at 6 °C. Transfer the supernatant to a new 1.5 mL tube and add 600 µL of CIA. Manually homogenize the samples and centrifuge at 13000 rpm for 10 minutes at 6 °C. Transfer the supernatant once again to a new 1.5 mL tube.

<u>II. DNA precipitation and purification:</u> isopropanol is used in this protocol to precipitate the DNA from the solution. The DNA pellet is washed to purify it to the maximum.

Add a mix of 5M NaCl (or sodium acetate) and isopropanol to each tube at a volume equivalent to 10% and 70% of the recovered supernatant, respectively. Manually homogenize the samples and place the tubes in the freezer at -14 ºC for, at least, 2 hours (preferably overnight) for the precipitation of DNA. Centrifuge the tubes for 20-30 minutes at 14000 rpm at 16 °C. At this point the DNA pellet should be attached to the tube wall in the bottom, carefully dispose of the liquid. To improve the washing of the pellet, add 1000 µL of 70% ethanol and manually and gently agitate until the pellet peels off the wall. Rest the tubes for 10 minutes and then centrifuge at 14000 rpm at room temperature for 10 minutes. Repeat again the 70% ethanol wash after careful disposal of the liquid. The third and final washing step should be done using 1000 µL of absolute ethanol.

<u>III. DNA elution:</u> at this stage the DNA pellet is dried and re-suspended in aqueous solution.

Dispose of all liquid and leave the tubes open at room temperature for air drying the DNA. Once the pellet is completely dry and free from ethanol, solubilize the DNA with 50 µL of purified water or TE buffer (EDTA pH 8.0-0.5M e Tris-Cl pH 8.0-1M) and 1 µL of RNase 10 mg/mL. In order for the RNase to degrade the RNA molecules still present in the sample, incubate the tubes at 37 °C for 1 hour. Tubes can stand overnight in a fridge (at 4 °C) before DNA extraction evaluation and finally store in a freezer (-20 °C) for preservation.

Evaluation of DNA extraction

The DNA quantification was performed by applying a mix of 1 µL of DNA and 1 µL of running buffer (xylene cyanol, bromophenol blue, sucrose and the fluorescent GelRed) per sample in each well of 1.5% agarose gel, using 0.5%TBE buffer. One (1) µL of Low Mass ladder (Invitrogen Inc.) with 1 µL GelRed dye was also added in one well of each race line to assign amount of DNA from each sample. The electrophoresis was set at 110 V/cm for 40 minutes. The electrophoresis gel was visualized under UV light and registered using the gel documentation system MiniBis Pro (Bio-Imaging Systems).

The DNA extraction success was evaluated by quantifying the DNA of each sample in the agarose gel according to the bands determined by the Low Mass Ladder, which are100 ng/µL, 60 ng/µL, 40 ng/µL, 20 ng/µL, 10 ng/µL and 5 ng/µL respectively (figure 2). The isolation of DNA was considered successful when a clear band of at least 10 ng/µL was visualized.

PCR (Polymerase Chain Reaction)

The PCR technique was used to amplify seven microsatellite loci (Table S1). We optimized PCR protocols (LUCAS et al., 2019) for dispersed seed endocarp (*Endocarp*) and the strem (*General*) (Table S1) based on Gaiotto *et al*. (2001).

DNA amplification (for both tissues types) was performed using a touchdown program in the thermal cycler as follows: Initial denaturation at 95 °C during 3 minutes followed by 10 cycles comprising denaturation at 94 °C during 30 seconds, annealing of primers starting at the highest temperature (table 1) for 30 seconds followed by 1 °C decrease for every subsequent round until the lowest primer temperature is reached, and extension at 72 °C during 30 seconds. After these 10 cycles other 30 succeed, comprised of denaturation at 94 °C for 30 seconds, primer annealing at the lowest T °C and extension at 72 °C for 30 seconds. And finally, a final extension at 72 °C for 7 minutes and cooling at 4 °C indefinitely.

Evaluation of microsatellite loci amplification

A mix of 2 µL of the PCR product and 2 µL of running buffer (xylene cyanol, bromophenol blue, sucrose and the fluorescent GelRed) was loaded into wells of 1.5% agarose gel and sized with the aid of the DNA ladder with the fluorescent GelRed. Electrophoresis was set at 110 V/cm for 40 minutes. Gels were visualized using the gel documentation system. PCR amplification was considered successful when a band appeared on the agarose gel at the expected size. Even faint bands were considered as successful amplification because fragment size analysis in first generation sequencers (Sanger or capillary electrophoresis) is sensitive to low amounts of PCR products.

Table S1: Amount of reagents and annealing temperature for optimized PCR of seven microsatellite loci for dispersed seed endocarps (*Endocarp*) and stem, leaf, root, non-dispersed seed endocarp and embryo (*General)* of *Euterpe edulis*. The values next to each reagent correspond to its stocked concentration. The amount of all reagents is given in microliters (µL). The reactions were carried out in a final total volume of 13 µL (Adapted from LUCAS et al., 2019).

Reagent	**EE3**		**EE23**		**EE25**		**EE45**		**EE47**		**EE52**		**EE54**	
	Endocarp	*General*	*Endocarp*	*General*	*Endocarp*	*General*	*Endocarp*	*General*	*Endocarp*	*General*	*Endocarp*	*General*	*Endocarp*	*General*
DNA (2,5 ng)	3	3	3	3	3	2	3	3	3	3	3	3	3	3
Buffer (10x)	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
DNTP (10mM)	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Primer F (0.9 uM)	2.88	2.88	2.88	2.88	2.88	1.8	2.88	2.88	2.88	2.88	2.88	2.88	2.88	2.88
Primer R (0.9 uM)	2.88	2.88	2.88	2.88	2.88	1.8	2.88	2.88	2.88	2.88	2.88	2.88	2.88	2.88
Taq (5u)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
BSA (62.5 mg/mL)	0.65	0.65	0.65	0.65	0.65	0.52	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
MgCl^2 (50mg/mL)	0.91	0.78	0.78	0.78	0.78	0.65	0.78	0.78	0.91	0.78	0.91	0.78	0.78	0.78
H2O	0.86	0.99	0.99	0.99	0.99	4.41	0.99	0.99	0.86	0.99	0.86	0.99	0.99	0.99
Annealing temperature (ºC)	64-54	66-56	66-56	66-58	68-58	60-68	64-54	64-54	60-50	66-56	64-54	66-56	66-56	66-56

COANCESTRY

We calculate the coancestry values (Loiselle kinship values) of the dispersed seeds in relation to the total population (seeds and adults) for each area (Figure S1).

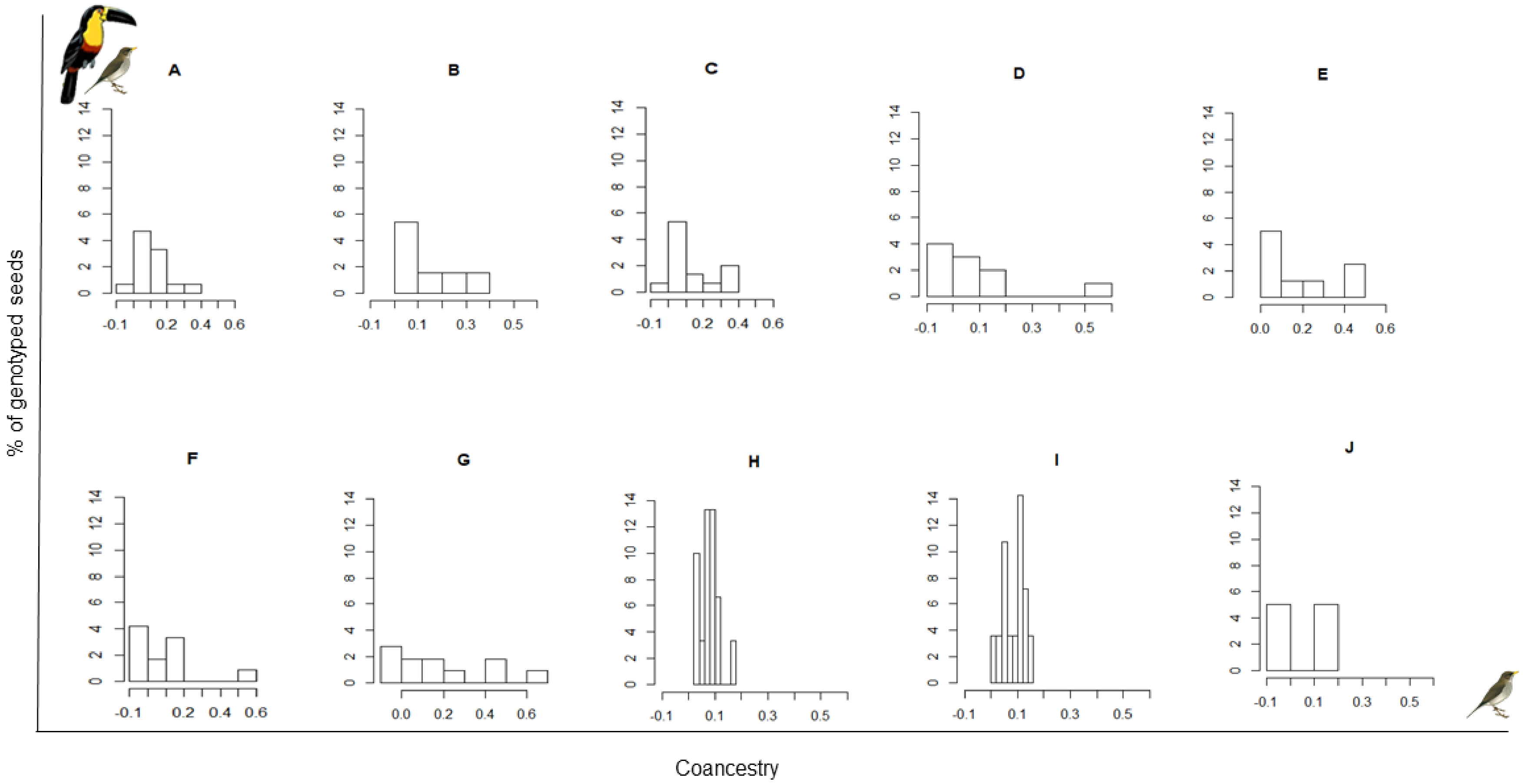

Figure S1: Representation of the coancestry values (Loiselle values) of *Euterpe edulis* seeds in the ten study areas (A-J, ordered in an increasing defaunation index).

And we also calculate the coancestry values (Loiselle kinship values) of the adults (mother plants) in relation to the total population (seeds and adults) for each area (Figure S2).

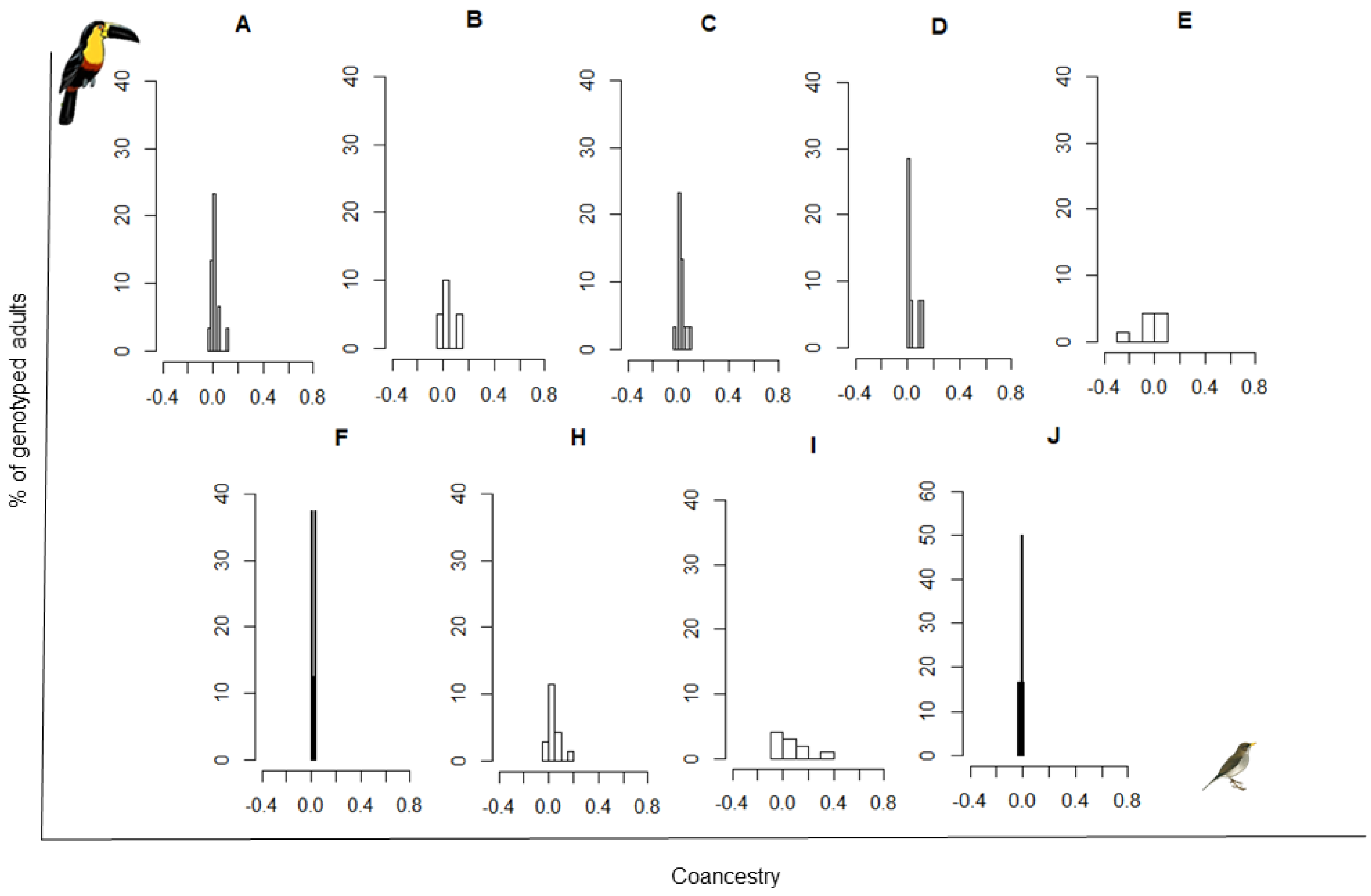

Figure S2: Representation of the coancestry values (Loiselle values) of *Euterpe edulis* aduts in the ten study areas (A-J, ordered in an increasing defaunation index). Area G was has been removed from the analysis (see session 3.3)

GLMM

Table S3: Statistical estimates for the of covariates contained in the best models (ΔAIC ≤ 2) of seed dispersal distance (immigration rate and distance) and kinship (half-sibs and kinship values) in 140 (excluding Mata São José sites, area G) deposition sites across nine study areas with *Euterpe edulis* populations in the Brazilian Atlantic forest. Parameter estimates for each predictor variable are shown followed by standard errors (SE), t-values and p-values.

	Model	Model ID	Error distribution	Predictors	Estimate	SE	t-value	p-value
Distance Models	Immigration rate	1	Binomial	N fruiting palm	-0.67	0.11	-6.07	< 0.001
		2	Binomial	N fruiting palms +	-0.66	0.11	-5.1	<0.001
				PC1	-0.17	0.14	-1.18	0.238
Kinship Models	Proportion of half-sibs seeds	1	Binomial	N fruiting palm	0.29	0.84	3.57	<0.001
		2	Binomial	Defaunation index +	-0.16	0.14	-1.09	0.27
				N fruiting palms	0.29	0.83	3.51	<0.001
Half-sibs seeds Models	Proportion of half-sibs seeds Immigrants	1		N Fruiting palms +	-0.57	0.28	-2.02	0.042
				PC1	-0.96	0.33	-2.81	0.004
		2		PC1	-1.14	0.31	-3.17	<0.001

Table S4: Summary of distance and coancestry models in 140 deposition sites (exclusion from Mata São José sites, area G) in ten study areas with *Euterpe edulis* populations in the São Paulo state Atlantic forest. Response variables are: number of immigrant seeds, mean dispersal distance. The predictors differ among models and may include number of fruiting palms, defaunation index, PC1 (first component of the principal component analysis indicating closed canopy forest structure) and number of half-sibs seeds. The null model represents the absence of an effect; K, the number of estimated parameters; AICc, Akaike corrected for small samples and wAICc, Akaike's weight of evidence. Models highlighted in bold are considered equally plausible (ΔAICc<2).

	Response	Error distribution	Model	K	AICc	ΔAICc	wAICc
Distance Models	Immigration rate	Binomial	**1. N fruiting palms**	**3**	**281.7**	**0.00**	**0.42**
			2. N fruiting palms + PC1	**4**	**282.5**	**0.73**	**0.29**
			N fruiting palms + Defaunation	4	283.8	2.05	0.15
			N fruiting palms + Defaunation + PC1	5	284.3	2.56	0.11
			PC1	3	316.9	35.11	0.00
			Defaunation + PC1	4	318.3	36.59	0.00
			Null model	2	320.7	38.93	0.00
			Defaunation	3	322.7	40.97	0.00
			Null model	**3**	**183.9**	**0.00**	**0.40**

Exact seed dispersal distance	Normal	Defaunation	4	185.3	1.39	0.20
		PC1	4	186.2	2.26	0.13
		N fruiting palms	4	186.7	2.82	0.09
		Defaunation + PC1	5	187.6	3.64	0.06
		Defaunation + N fruiting palms	5	188.1	4.20	0.05
		N fruiting palms+ PC1	5	189.0	5.07	0.03
		Defaunation +N fruiting palms + PC1	6	190.4	6.48	0.01
Proportion of half-sibs seeds	Binomial	**1. Fruiting palms**	**3**	**490.2**	**0.00**	**0.44**
		2. Defaunation + Fruiting palms	**4**	**491.0**	**0.88**	**0.28**
		Fruiting palms + PC1	4	492.3	2.11	0.15
		Defaunation + N fruiting palms + PC1	5	493.1	2.91	0.10
		Null model	2	500.7	10.55	0.02
		Defaunation	3	501.6	11.44	0.01
		PC1	3	502.8	12.61	0.01
		Defaunation + PC1	4	503.7	13.51	0.01

	Proportion of half-sibs Immigrants seeds	Binomial	**1. N fruiting palms + PC1**	**4**	**132.9**	**0.00**	**0.52**
			3. PC1	**3**	**134.7**	**1.81**	**0.21**
			Defaunation + N fruiting palms + PC1	5	135.3	2.42	0.15
			Defaunation + PC1	4	136.6	3.78	0.07
			N fruiting palms	3	139.6	6.70	0.01
			Defaunation + N fruiting palms	4	142.0	9.13	0.00
			Null model	2	149.7	16.86	0.00
			Defaunation	3	151.6	18.73	0.00
Kinship models	Observed kinship	Normal	**Null model**	**3**	**-119.6**	**0.00**	**0.94**
			PC1	4	-112.5	7.10	0.02
			Defaunation index	4	-111,6	8.01	0.01
			N fruiting palms	4	-110.5	9.07	0.01
			Defaunation index + PC1	5	-103.9	15.73	0.00

N fruiting palms + PC1	5	-103.4	16.24	0.00
Defaunation index + N fruiting palms	5	-102.2	17.14	0.00
Defaunation index + N fruiting palms =PC1	6	-94.7	24.89	0.00

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