**UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO**

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E BIODIVERSIDADE

VARIAÇÃO FENOTÍPICA, GENÉTICA E DISPERSÃO DE SEMENTES DE UMA PALMEIRA DA MATA ATLÂNTICA

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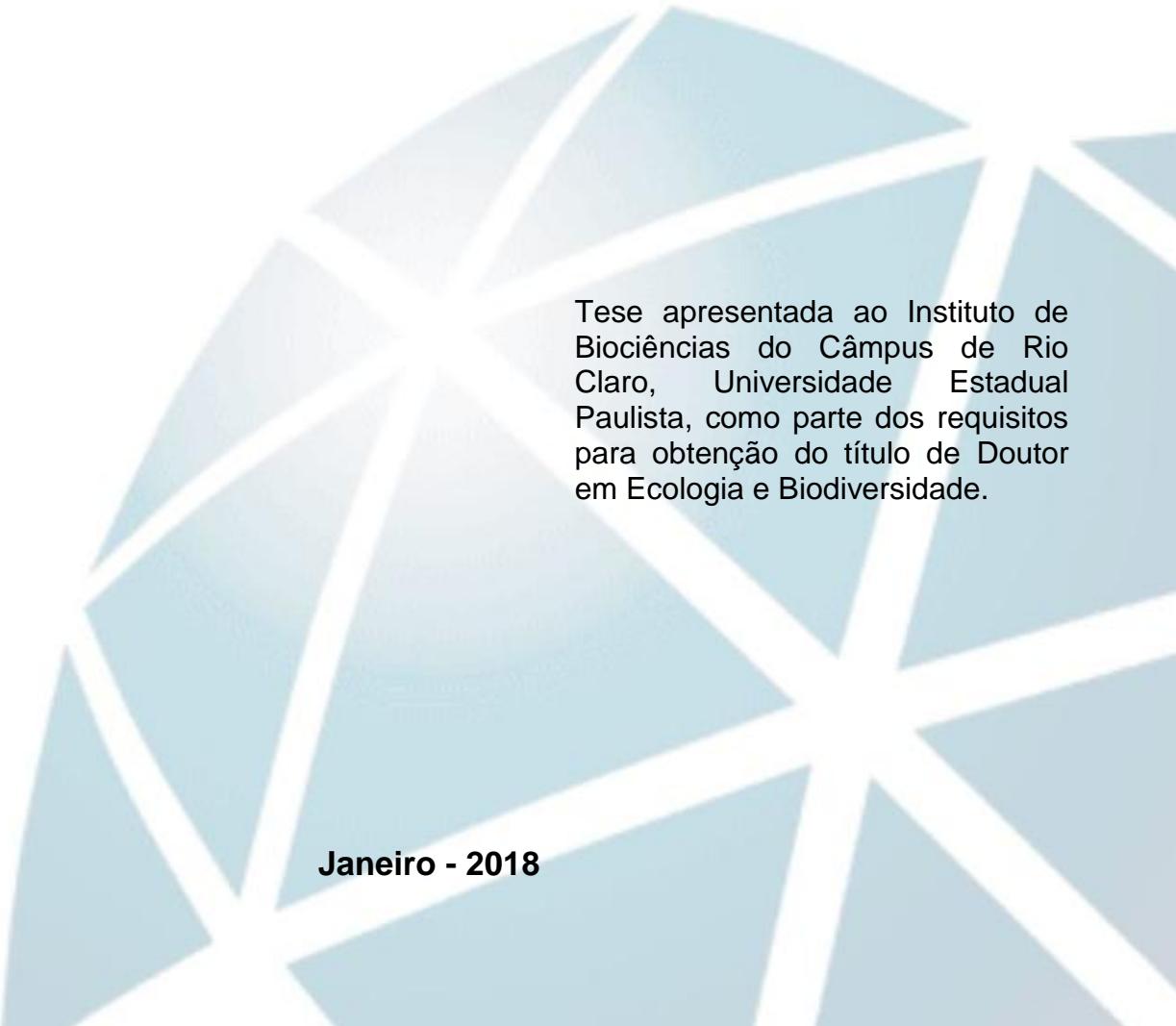
Tese apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ecologia e Biodiversidade.

Janeiro - 2018

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Rio Claro

2018

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## UNIVERSIDADE ESTADUAL PAULISTA

### Câmpus de Rio Claro

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: Variação fenotípica, genética e dispersão de sementes de uma palmeira na Mata Atlântica

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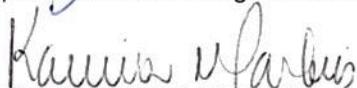
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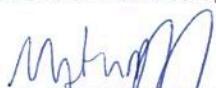
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Rio Claro, 12 de janeiro de 2018

Agradecimentos

Primeiramente, agradeço à Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2014/01029-5 e 2016/22843-8) pelas bolsas e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 401258/2012-2 e 445353/2014-7) pela bolsa e apoio financeiro para o desenvolvimento do projeto e desta tese de doutorado. Graças a esses órgãos de fomento, tive a oportunidade de realizar dois estágios no exterior por meio dos programas do Ciências sem Fronteiras-CNPq (CSIC-Espanha) e Bolsa de Estágio de Pesquisa no Exterior-FAPESP (CIBIO-Portugal) que foram essenciais para a minha formação como cientista. Também agradeço a Fundação Florestal, IBAMA e proprietários rurais pela permissão de coleta em Reservas Estaduais, Federais e propriedades particulares, respectivamente.

Gostaria de agradecer a todas as pessoas que me apoiaram tanto no campo científico como emocional para o desenvolvimento desta tese. Gostaria de agredecer meus orientadores e amigos Mauro Galetti, Marina Côrtes, Cristina García (CIBIO-Portugal) e Pedro Jordano (CSIC-Espanha). Cada um foi essencial para que eu conseguisse realizar esse trabalho. Vocês me deram todas as ferramentas para que eu pudesse seguir a carreira científica. Me ensinaram diversas análises estatísticas, a escrever um projeto e um manuscrito, a como fazer uma boa apresentação, e etc. Sou muita sortuda de ter cruzado com vocês durante a minha formação e espero um dia poder retribuir. Gostaria de agradecer especialmente a Marina, além de orientadora ela foi uma grande amiga. Muito obrigada por me consolar quando as coisas não davam certo (olha que foram muitas vezes) e não me deixar desistir.

Gostaria de agradecer todas as pessoas que me ajudaram tanto no campo quanto no laboratório. Um agradecimento especial a Marília, Giovane, Michele, Sérgio e Carlinhos, sem eles este trabalho não teria saído. Também gostaria de agradecer aos amigos que o doutorado me proporcionou e que me deram todo o apoio emocional para seguir com a tese: Gabi Beca, Rafa, Valesca, Cleber, Bárbara, Xuleta, Felipe Martello. Gostaria de agradecer também a todas as pessoas do Labic (Saldanha, Carol Bello, Nômade, Nacho, Claudinha, Fabiano, Carine, Calebe, Carlos), Labemol (Clarisse, Lo, Fer, Leles, Giu, Thabata, Isa, Vanessa, Matheus) e IEG (Nestor, Rocio, Cande, JuanPe, JuanMi, Alfredo). Foi muito bom ter compartilhado o mesmo lab com vocês. Vocês foram essenciais na discussão de artigos, trabalho do grupo, dicas de análises e etc.

Gostaria de agradecer aos meus amigos Elson, Raisa, Ju, Felipe, Cris, Carol Parga e Jac e minha família: pai Fernando, mãe Nilza, minhas irmãs Natália e Paula, meus cunhados Bruno e Tony, e especialmente o Ronaldo. Tenho muita sorte de ter vocês na minha vida, vocês sempre me apoiaram em tudo e nunca deixaram meu astral cair. Muito obrigada mesmo, sem o apoio emocional de vocês essa trajetória seria muito difícil.

Resumo

A dispersão é o primeiro estágio que dá início ao recrutamento das plantas, portanto, é crucial para a regeneração, distribuição espacial, colonização de novos ambientes e conectividade de fragmentos florestais, sendo crítico para a viabilidade a longo prazo das populações. No entanto, as atividades humanas vêm gerando pressões que alteram os processos de recrutamento de novos indivíduos. Por exemplo, vários estudos têm mostrado que a extinção de grandes frugívoros pode prejudicar os serviços de dispersão de sementes porque os vertebrados de médio e pequeno porte geralmente (i) mobilizam uma fração reduzida dos frutos produzidos por visita; (ii) não ingerem sementes grandes, portanto, apenas as de tamanho médio e pequeno são dispersadas; e (iii) não conseguem alcançar áreas distantes, o que reduz a conectividade no nível da paisagem. No entanto, nós ainda não sabemos se a defaunação também afeta a distribuição espacial das progênies maternas na chuva de sementes, o que pode comprometer o recrutamento local das plantas. Além disso, os efeitos da defaunação sobre os processos microevolutivos de plantas ainda são pouco estudados. Nós descrevemos a distribuição espacial das progênies maternas na chuva de sementes de uma palmeira tropical (*Euterpe edulis*) gerada por aves ao longo de paisagens fragmentadas e defaunadas. Além disso, testamos se a perda dos grandes dispersores de sementes pode levar a alterações microevolutivas desta palmeira tropical. No final avaliamos o potencial de recuperação fenotípica desta palmeira que apresenta redução do tamanho da semente devido à defaunação de grandes frugívoros. Nossos estudos mostraram que os frugívoros de médio e grande porte oferecem serviços complementares. Aves frugívoras de tamanho médio podem manter os serviços de dispersão de sementes, contribuindo com a manutenção da alta diversidade genética local. No entanto, a perda dos grandes frugívoros afeta a qualidade do serviço de dispersão de sementes, principalmente a dispersão a longa distância e a manutenção do tamanho de semente. Além disso, nossos resultados mostraram que a defaunação dos grandes dispersores de sementes leva a alterações microevolutivas nas populações de *E. edulis*. Portanto, a perda de grandes dispersores de sementes não está apenas levando a alterações fenotípicas, mas também genotípicas, com efeitos desconhecidos para a persistência a longo prazo de populações de plantas. Finalmente, para recuperar alterações fenotípicas é necessário restaurar os processos de dispersão de sementes por grandes frugívoros. A restauração dos processos de dispersão de sementes por grandes frugívoros beneficiará várias espécies dispersas por aves e pode ser crucial para enfrentar cenários de mudança global.

Abstract

Seed dispersal is the first template upon which plant recruitment takes place, and it is crucial to regeneration, spatial distribution, colonization of new environments, and connectivity of forest fragments, being critical for the long-term viability of populations. Human impacts, however, impose pressures that alter recruitment. For example, the extinction of large seed dispersers may impair seed dispersal services if extant medium- to small-bodied vertebrates only provide suboptimal dispersal services because: (i) they often mobilize a reduced fraction of propagules per visit; (ii) they do not ingest large-sized seeds, and therefore, only medium- to small-sized seeds get dispersed; and (iii) they fail to reach distant sites, which reduces connectivity at the landscape level. Yet, we still do not know if defaunation also leads to dispersal limitation by changing the spatial distribution of maternal progenies in the seed rain, which might imperil local plant recruitment. Moreover, the lasting effects of frugivore defaunation on microevolutionary processes of plants they disperse remain understudied. Here, we characterized the spatial distribution of the maternal progenies in a bird-generated seed rain of a tropical palm (*Euterpe edulis*) that produces medium-sized seeds. Moreover, we tested if the loss of large seed dispersers can lead to microevolutionary changes of this tropical palm. Finally, we evaluate the potential of phenotypic rescue of this bird-dispersed palm that present seed size reduction due to defaunation of large frugivores. Overall, our study highlights that medium- and large-sized frugivores provide complementary services. We found that extant medium-sized frugivorous birds can maintain the seed dispersal services in plants of medium-sized seeds, contributing the maintenance of high local genetic diversity. Nevertheless, the loss of large-sized seed dispersers impacted the quality of seed dispersal service, mainly in terms of long distance dispersal and maintenance of large seed sizes. Moreover, our results indicate that the defaunation of large seed dispersers has a distinct signal on large-scale genetic variability, potentially arising from microevolutionary changes in the palm populations. Therefore, the lack of seed dispersers leads to both phenotypic and genetic changes, with unknown effects on the long-term persistence of plant species and entire communities. Finally, to rescue large seeds in palm populations it is necessary to restore the seed dispersal processes by large frugivores. The most effective strategy to restore seed size variation is to rewild defaunated forests with large frugivores, either by connecting remnants to faunated areas or by frugivores' reintroduction. The restoration of the seed dispersal processes by large frugivores will benefit several bird-dispersed species and may be crucial to face ongoing global change scenarios.

Sumário

1. Introdução

O desmatamento, a introdução de espécies invasoras e mudanças no clima são resultados das ações antrópicas e podem acarretar na sexta grande extinção de espécies de animais e plantas (BARNOSKY et al., 2011). Para evitar a redução ainda maior da biodiversidade é necessário prever como essas mudanças globais afetarão a capacidade das espécies de se adaptar e dispersar pelas paisagens (DAWSON et al., 2011). Essa capacidade adaptativa das espécies dependerá de fatores como variação intraespecífica, plasticidade fenotípica, características da história de vida, capacidade de dispersão e colonização, e diversidade genética (BOLNICK et al., 2011; DAWSON et al., 2011).

A variabilidade intraespecífica das características dos organismos como, por exemplo, tamanho e comportamento, influencia a maneira pela qual os indivíduos interagem entre si e com o ambiente e, portanto, tem importantes implicações sobre as dinâmicas ecológicas e a estrutura de populações e comunidades (BOLNICK et al., 2011). Plantas, por exemplo, apresentam uma grande variação intra- e interespecífica no tamanho das sementes (MICHAELS et al., 1988; MOLES et al., 2005). Essa variação deve conter um alto valor adaptativo, pois o tamanho da semente é relacionado com a capacidade de dispersão e com o potencial de recrutamento de plântulas (MOLES; WESTOBY, 2004; MULLER-LANDAU et al., 2008). Para espécies de plantas dispersas por animais, o tamanho do fruto e das sementes está relacionado à guilda de animais dispersores. Isso porque a capacidade de um animal ingerir e dispersar certas sementes é limitada pelo tamanho do item alimentar relativo ao tamanho e abertura da boca e bico (LEVEY, 1987; WHEELWRIGHT, 1993). Apesar da variação do tamanho de sementes ser comum dentro e entre espécies, essa variação fenotípica pode se restringir devido à seleção diferencial dos dispersores sobre certos tamanhos de sementes (GALETTI et al., 2013).

A dispersão é o primeiro estágio que dá início ao recrutamento das plantas e, portanto, é crucial para a colonização, regeneração e conectividade de fragmentos, sendo crítico para a viabilidade a longo prazo das populações (SEGELBACHER et al., 2010; WANG; SMITH, 2002). Além disso, a dispersão aumenta o intercâmbio de genes entre populações (HAMRICK; MURAWSKI; NASON, 1993), ajudando a manter a diversidade genética dentro das mesmas e reduzir o risco de extinção por depressão endogâmica e deriva genética (HAMRICK, 2004; YOUNG, ANDREW; BOYLE; BROWN, 1996). Muitos estudos têm

contribuído para entender os processos de dispersão de sementes e o recrutamento de plantas em áreas naturais e antropizadas (MARKL et al., 2012; MCCONKEY et al., 2012; SCHLEUNING et al., 2011). Por exemplo, sabe-se que a extinção não aleatória dos grandes dispersores de sementes pode reduzir a remoção de frutos e dispersão de sementes (CORDEIRO, NORBERT J. et al., 2009; HOLBROOK; LOISELLE, 2009) e diminuir a imigração de sementes para as populações (PÉREZ-MÉNDEZ et al., 2016). Esses processos podem vir, consequentemente, a restringir a variação fenotípica dos organismos (GALETTI et al., 2013), limitar a distribuição espacial e reduzir a diversidade genética dentro das populações ao longo do tempo (GIOMBINI et al., 2017; PÉREZ-MÉNDEZ; JORDANO; VALIDO, 2015). No entanto, a alta biodiversidade, dispersão de sementes e conectividade entre populações ainda podem ser mantidas em pequenos fragmentos, dependendo da configuração e composição da paisagem e características dos animais dispersores (CÔRTES; URIARTE, 2013; SCHLEUNING et al., 2011; VERGARA et al., 2010). Portanto, investigar como os processos de dispersão de sementes e de genes variam em múltiplas escalas espaciais pode ajudar a desvendar os mecanismos que permitem a manutenção ecológica e da variabilidade genética de populações naturais (CÔRTES; URIARTE, 2013).

1.1. Dispersão de sementes e diversidade genética

Os frugívoros são os agentes geradores da chuva de sementes e genes em plantas dispersas por animais e, portanto, esses agentes têm potencial influência sobre os padrões espaciais e genéticos da população (CHOO; JUENGER; SIMPSON, 2012; CÔRTES; URIARTE, 2013; KARUBIAN et al., 2010). Os frugívoros moldam a chuva de sementes de acordo com suas características morfológicas e comportamento de forrageamento (CÔRTES; URIARTE, 2013). Por exemplo, grandes frugívoros movem e dispersam as sementes para locais distantes (HOLBROOK; LOISELLE, 2009; NATHAN, 2006), provavelmente trazendo novas progênies maternas de outras populações (GARCÍA; JORDANO; GODOY, 2007; JORDANO et al., 2007). Como resultado, a diversidade genética aumenta e a divergência genética diminui tanto em escala local em sítios de deposição de sementes quanto no nível populacional (KARUBIAN et al., 2015; SCOFIELD et al., 2012). Em contraste, as aves de pequeno e médio porte geralmente consomem poucas sementes por visita, as sementes tendem a ser de tamanho médio a pequeno e, por fim, são mais frequentemente depositadas embaixo ou próximo da planta mãe (JORDANO et al., 2007). Isso resulta em uma alta agregação de sementes da mesma planta mãe (CAUGHLIN et al., 2014), que por sua vez pode levar a uma baixa diversidade genética local (GRIVET; SMOUSE; SORK, 2005) e uma perda de

diversidade genética populacional a longo prazo (GIOMBINI et al., 2017).

Tanto aves grandes como pequenas podem apresentar dispersão de sementes contagiosa (com muitas sementes depositadas no mesmo local, por exemplo, em uma latrina) (SCHUPP; MILLERON; RUSSO, 2002) e fonte-tendenciosa (com a maioria das sementes em um sítio de deposição provenientes de uma mesma fonte) (GARCÍA et al., 2009) levando à agregação de meio-irmãos e formação de estruturas familiares. A aglomeração da progênie resulta em manchas de indivíduos altamente aparentados que provavelmente irão gerar uma forte estruturação genética na população, independente da densidade de plantas reprodutivas (HAMRICK; TRAPNELL, 2011; JONES; HUBBEL, 2006). A forte estruturação genética na população pode desencadear, a longo prazo, erosão genética com efeito detrimental para as populações. Existem muitos estudos sobre os padrões de chuva de sementes (ALCANTARA et al., 2000; HARDESTY; PARKER, 2003; JESUS et al., 2012), no entanto, pouco se sabe sobre a composição genética das sementes dispersas e a distribuição espacial das progênies maternas (GARCÍA et al., 2009).

1.2. Defaunação e diversidade genética

Populações de muitos vertebrados de grande porte estão sendo extintas ou estão em constante declínio devido à perda de habitat e à caça (CEBALLOS; EHRLICH; DIRZO, 2017; DIRZO et al., 2014; YOUNG, HILLARY S et al., 2016). A perda desses animais representa uma ameaça à interação animal-planta e à biodiversidade, já que mais de 90% das plantas tropicais são dispersas por animais (ALMEIDA-NETO et al., 2008). A defaunação dos grandes frugívoros pode prejudicar os serviços de dispersão de sementes porque os pequenos e médios vertebrados remanescentes apenas consomem uma pequena fração dos frutos produzidos (MARKL et al., 2012) e os mesmos não alcançam locais distantes e longe de conspecíficos (PÉREZ-MÉNDEZ et al., 2016). Como resultado, a perda dos grandes frugívoros tem consequências demográficas em cascata, como a redução da sobrevivência e recrutamento de plântulas (CORDEIRO, N J; HOWE, 2001; PÉREZ-MÉNDEZ; JORDANO; VALIDO, 2015).

A defaunação pode também afetar os processos microevolutivos; no entanto, estudos sobre as consequências genéticas da extinção dos grandes dispersores de sementes ainda são bastante escassos. A limitação da distância de dispersão, o aumento da deposição de sementes sob a planta mãe e a proximidade entre indivíduos aparentados devido ao processo de defaunação podem acarretar a uma possível endogamia biparental e diminuir o fluxo gênico entre as

populações de plantas (PACHECO; SIMONETTI, 2000; PÉREZ-MÉNDEZ; JORDANO; VALIDO, 2017). A longo prazo, a defaunação pode reduzir a diversidade genética e afetar a estrutura genética nas populações (CARVALHO et al., 2016; GIOMBINI et al., 2017; PACHECO; SIMONETTI, 2000), com efeitos desconhecidos para a persistência a longo prazo de espécies de plantas (HOFFMANN; WILLI, 2008).

1.3. *Euterpe edulis*

Em todos os capítulos utilizamos como espécie modelo a palmeira *Euterpe edulis* Mart. (Fig. 1). O palmito juçara *E. edulis* representa uma espécie modelo ideal para avaliar os efeitos da transformação do uso e cobertura do solo sobre os processos ecológicos e genéticos de populações naturais de plantas. É uma espécie com ampla variação na abundância populacional ao longo de sua distribuição geográfica, grande importância para a manutenção da comunidade frugívora animal devido à alta produção de frutos, frutificação longa e anual e ampla variação no tamanho dos frutos permitindo o consumo de uma gama variada de animais. Tais características permitem que os resultados encontrados para *E. edulis* sejam representativos para outras espécies de plantas dispersas por animais, que representam em torno de 75% das árvores na Mata Atlântica (ALMEIDA-NETO et al., 2008). Os resultados podem ser ainda mais relevantes para as espécies de frutos médios a grandes, que são mais suscetíveis ao impacto humano e extinção local de frugívoros.

O palmito juçara é uma árvore dominante em florestas preservadas, ocorrendo em todo o bioma Mata Atlântica e presente em diferentes tipos de vegetação, desde floresta ombrófilas até florestas sazonalmente secas, onde é restrita a microhabitats mais úmidos. *Euterpe edulis* é uma espécie monóica auto-compatível, mas com reprodução predominantemente cruzada devido à protandria (antese masculina antecede a feminina) (GAIOTTO; GRATTAPAGLIA; VENCOVSKY, 2003; MANTOVANI; MORELLATO, 2000). A polinização é realizada principalmente por abelhas de pequeno porte (por exemplo *Trigona spinipes*). Essa palmeira produz frutos carnosos arredondados de tamanho variando entre 8,3 e 14,1 mm em diâmetro (PIZO; VON ALLMEN; MORELLATO, 2006). Esses frutos constituem uma fonte de alimento importante para várias espécies de animais, entre eles mais de 20 mamíferos e 58 espécies de aves (GALETTI et al., 2013). Os dispersores de sementes de *E. edulis* englobam um conjunto diversificado de aves, incluindo grandes aves frugívoras encontradas principalmente em áreas de floresta preservada (por exemplo, tucano *Ramphastos* spp. e araponga *Procnias nudicollis*) e aves de pequeno a médio porte, principalmente os sabiás

Turdus spp., presentes em ambos ambientes preservados e perturbados (GALETTI et al., 2013) (Fig. 2). Aves frugívoras de grande porte são particularmente propensas à redução da abundância e até mesmo a extinção em fragmentos florestais, devido à diminuição das áreas de uso e extirpação direta de indivíduos devido à caça (KATTAN; ALVAREZ-LÓPEZ; GIRALDO, 1994; SODHI; LIOW; BAZZAZ, 2004). Estes grandes frugívoros desempenham um papel importante como dispersores de sementes, porque eles são os únicos capazes de engolir grandes sementes e dispersar a longas distâncias, movendo sementes para novos sítios e interligando geneticamente populações distintas (GALETTI et al., 2013; JORDANO et al., 2007). Além disso, o meristema comestível desta espécie de palmeira (ou seja, o palmito) é muito apreciado e comercializado como uma iguaria por seu valor na tradição culinária brasileira. A exploração do palmito, que é feita de maneira descontrolada e em grande escala, tem levado à redução drástica e extinção local dessa palmeira em muitos remanescentes da Mata Atlântica (GALETTI; FERNANDEZ, 1998).

Figura 1: Ilustração da palmeira *Euterpe edulis* Mart. com detalhamento das flores, frutos, plântula, jovem e adulto.

Fonte: Ilustração de autoria de Diana Carneiro.

Figura 2: Exemplos de aves dispersoras de sementes de *Euterpe edulis*. A- *Ramphastos vitellinus*, B e C- *R. dicolorus* , D- *Selenidera maculirostris* , E- *Pteroglossus bailloni*, F- *Aburria jacutinga*, G- *Procnias nudicollis*, H- *Pyroderus scutatus*, I- *Turdus flavipes*, J e K- *Turdus leucomelas*.
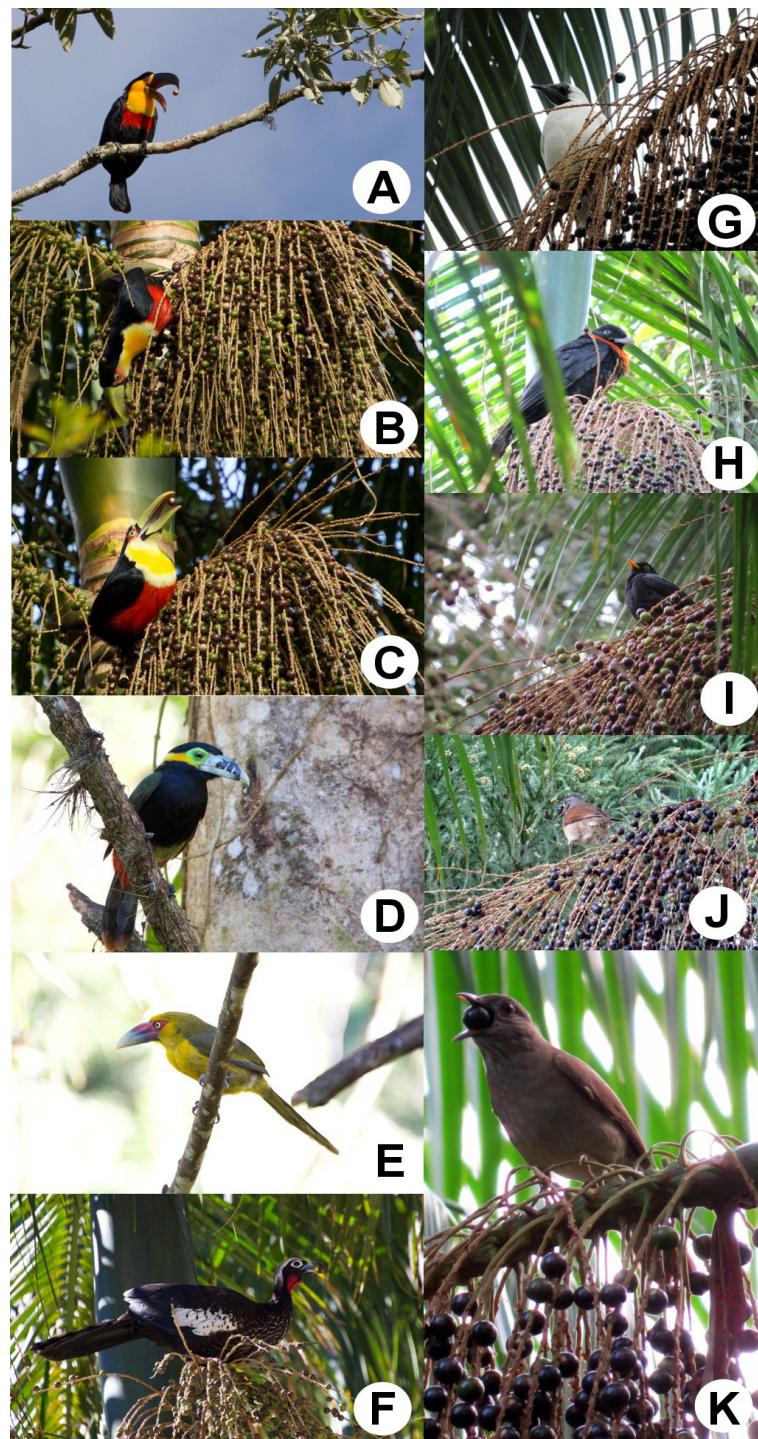
Fonte: Fotos de A-F de autoria de Pedro Jordano, G e H de Giuliana Garcia e I-K de Ana Clara Rosa Marçal.

1.4. Objetivos

Nesta tese de doutorado, pretendeu-se entender como os fatores ambientais e as características intrínsecas das plantas (*i.e.*, tamanho de semente) influenciam a dispersão de sementes e a diversidade genética de uma palmeira-chave em escala local e ao longo de paisagens fragmentadas e defaunadas na Mata Atlântica. A tese foi desenvolvida em quatro capítulos, com os seguintes objetivos específicos:

1. Avaliar o processo de dispersão de uma palmeira (*Euterpe edulis*) em um fragmento florestal defaunado (Capítulo 1). Em concreto, caracterizamos i) a componente quantitativa da efetividade da dispersão de sementes; ii) a contribuição materna na chuva de sementes gerada por frugívoros; iii) a estrutura genética espacial em escala fina de plântulas e adultos para inferir distância de dispersão; iv) e a diversidade genética de plântulas e adultos para avaliar o impacto da alteração de habitat sobre ambas as classes. Este capítulo foi submetido a uma edição especial da revista espanhola *Ecosistemas* sobre Demografia e Genética.

2. Determinar como a dispersão de sementes e a distribuição das progênies maternas na chuva de semente é influenciada por fatores micro-ambientais e pela comunidade de frugívoros (Capítulo 2). Especificamente, nós analisamos o componente espacial da diversidade genética (diversidade alfa, beta e gama) na chuva de sementes de *Euterpe edulis* em paisagens heterogêneas com diferentes níveis de defaunação. Pretendemos submeter este capítulo à revista *Molecular Ecology.*

3. Investigar se há sinais da defaunação e consequentemente da redução do tamanho das sementes na estruturação genética de populações de *Euterpe edulis* (Capítulo 3). Para isso, nós testamos quatro diferentes cenários hipotéticos (defaunação, fragmentação de habitat, origem biogeográfica e desenho amostral) para determinar qual cenário melhor se ajustava ao padrão observado de variação genética. Este capítulo foi publicado na revista *Scientific Reports* (Carvalho CS, Galett M, Colevatti RG, Jordano P. 2016. Defaunation leads to microevolutionary changes in a tropical palm. *Scientific Reports* 6, article number 31957.)

4. Avaliar o potencial de recuperação fenotípica do tamanho de sementes de *Euterpe edulis* em ambientes alterados (Capítulo 4). Especificamente, avaliamos o estado atual da distribuição dos tamanhos de sementes produzidas e dispersas em populações de *E. edulis* e utilizamos modelos teóricos para simular o sucesso de duas estratégias de restauração: i)

restauração da variação fenotípica das populações adicionando fenótipos faltantes que foram originalmente encontrados na espécie; e ii) restauração da variação fenotípica das populações pela reintrodução de grandes frugívoros. No final, discutimos a inclusão da restauração fenotípica como uma nova estratégia de restauração. Pretendemos submeter este capítulo à revista *Journal of Applied Ecology*.

Para alcançar nossos objetivos, utilizamos distintas formas de obtenção e análises de dados, como coleta de dados em campo, análises genéticas e modelagem estatística. O estudo combinou esforços de escala fina - com caracterização detalhada das progênies maternais em sítios de deposição espalhados na floresta – e esforços na escala da paisagem – com caracterização dos atributos populacionais de uma área florestal e características ambientais da paisagem.

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2. Capítulo 1: Dispersão de sementes em um remanescente de floresta tropical: o papel dos sabiás na manutenção da estrutura e da diversidade genética de *Euterpe edulis*

Citação:

Carvalho CS, Valverde J, Souza M, Ribeiro T, Nazareth S, Galetti M, Côrtes MC. La dispersión de semillas en bosques remanentes tropicales: el papel de los zorzales en el mantenimiento de la estructura y diversidad genética de *Euterpe edulis*. Submitted to *Ecosistema*.

Resumo

A extinção de grandes espécies de dispersores de sementes em habitats fragmentados pode afetar a estrutura espacial, genética e demográfica de muitas espécies de plantas. Estudamos a dispersão de sementes e a diversidade genética de *Euterpe edulis* em um fragmento da Mata Atlântica. Através de observações focais, avaliamos o componente quantitativo da efetividade da dispersão de sementes. Além disso, em uma parcela de 25x50 m, genotipamos todos os adultos e uma amostra aleatória de plântulas e sementes com o objetivo de caracterizar a contribuição de diferentes plantas adultas para a chuva de sementes, a estrutura genética espacial em escala fina e a diversidade genética de plântulas e adultos. Os sabiás foram os principais dispersores desta espécie. Não observamos nenhuma estrutura genética espacial em plântulas ou adultos, embora tenhamos observado uma maior relação entre os dois estágios abaixo de seis metros. Os valores de endogamia, heterozigosidade esperada e riqueza alélica foram semelhantes entre plântulas e adultos, demonstrando estabilidade intergeracional nesses parâmetros. Nossos resultados mostram que a dispersão por sabiás ocorre principalmente a curta distância; no entanto, a sobreposição espacial na chuva de sementes elimina os padrões genéticos espaciais que esse processo pode gerar. Com este estudo, demonstramos que essas aves de tamanho médio são capazes de manter a diversidade genética entre as gerações, dando-lhes um papel crucial na manutenção da diversidade genética em paisagens fragmentadas.

2.1. Introdução

Atividades humanas vêm transformando as florestas tropicais ao redor do mundo em fragmentos isolados e empobrecidos biologicamente (Laurance et al., 2004, Nogueira et al., 2007). Esta exploração indiscriminada cria novas condições ambientais com consequências muitas vezes imprevisíveis para os organismos (Bacles e Jump, 2011). Para antecipar os potenciais impactos da fragmentação do habitat nas populações naturais, deve-se analisar como os indivíduos respondem às novas condições e seus consequentes efeitos nos processos ecológicos e padrões genéticos (Segelbacher et al., 2010). Para plantas, a dispersão de sementes é o processo que determina a distribuição espacial inicial de indivíduos e genótipos. Este processo é, portanto, um dos principais responsáveis pelo recrutamento, colonização, regeneração e conectividade de fragmentos, sendo crítico para a viabilidade a longo prazo das populações (Wang e Smith, 2002, Segelbacher et al., 2010).

Muitos estudos têm contribuído para entender os processos de dispersão de sementes e o recrutamento de plantas em áreas naturais e antropizadas (Schleuning et al., 2011; Markl et al., 2012; McConkey et al., 2012). Neste sentido, sabemos que a fragmentação afeta a dispersão de sementes por animais (Fahrig, 2007), reduzindo a taxa de consumo, de dispersão efetiva (Cordeiro et al., 2009) e diminuindo a taxa de migração entre populações (Sebbenn et al., 2011). Essas alterações podem levar a importantes consequências evolutivas para as plantas, como a restrição na variabilidade de alguns caracteres fenotípicos (Galetti et al., 2013), a limitação da distribuição espacial ou a redução da diversidade genética intrapopulacional (Aguilar et al., 2008). No entanto, certos fatores, como a configuração e a composição da paisagem, ou características dos frugívoros e plantas, podem conservar a distribuição de genótipos no espaço e a conectividade entre populações, mantendo assim a alta diversidade em pequenos fragmentos (Vergara et al., 2010; Schleuning et al., 2011; Côrtes e Uriarte, 2013)

Em plantas dispersas por animais, é comum que as sementes sejam distribuídas de maneira agregada (Schupp et al., 2002). Isso pode ser resultado de uma dispersão a curta distância (com muitas sementes depositadas perto da planta mãe) ou de uma dispersão contagiosa (com muitas sementes depositadas no mesmo local, por exemplo, em latrinas) (Schupp et al., 2002). A maneira com que as sementes são distribuídas espacialmente depende do comportamento da comunidade de frugívoros e isso, por sua vez, afeta a estrutura genética espacial (Scofield et

al., 2012). Por exemplo, os dispersores podem depositar sementes da mesma fonte materna no mesmo lugar (Jordano et al., 2007). Esse comportamento leva a agregação espacial de sementes irmãs e promove a formação de estruturas familiares. A aglomeração de indivíduos relacionados gera um padrão de estruturação genética populacional forte (Jones e Hubbel, 2006, Hamrick e Trapnell, 2011) e pode desencadear processos de erosão genética com efeitos negativos para as populações ao longo prazo (Young et al., 1996). Portanto, conectar o comportamento dos dispersores frugívoros, os movimentos das sementes e a estrutura genética resultante é importante para entender como os dispersores de sementes afetam a reprodução e a estrutura genética das plantas (Karubian et al., 2010).

A dispersão de sementes em ambientes alterados pode ser avaliada de diferentes maneiras. Uma maneira é através da caracterização da efetividade da dispersão de sementes dos diferentes dispersores (Jordano e Schupp, 2000). A efetividade está relacionado à quantidade de frutos removidos - componente quantitativo - e à qualidade do manuseio e deposição de sementes - componente qualitativo - (Jordano e Schupp, 2000). Para entender o processo de dispersão e suas consequências também devemos analisar a distribuição espacial de sementes e plântulas, bem como seus respectivos genótipos em relação à planta materna ou outros conspecíficos (Jordano e Godoy, 2002; García e Grivet, 2011). Neste trabalho, estudamos a dispersão de sementes da palmeira *Euterpe edulis*, em um fragmento florestal da Mata Atlântica brasileira. Especificamente, caracterizamos: i) o componente quantitativo da efetividade de dispersão das sementes; ii) a contribuição materna para a chuva de sementes; iii) a estrutura genética espacial em escala fina de plântulas, adultos e a interdependência de ambos para inferir a distância de dispersão; e iv) a diversidade genética das duas classes etárias para avaliar o impacto da alteração do habitat sobre esta.

2.2. Material e métodos

Espécie e área de estudo

O palmito (*Euterpe edulis* Mart., Arecaceae), é uma das espécies dominante do bioma Mata Atlântica. É uma espécie monóica e auto-compatível em que a reprodução cruzada prevalece através da participação de abelhas pequenas (por exemplo, *Trigona spinipes,* observação pessoal) e devido à maturação do androceu anterior a do gineceu (Mantovani e Morellato, 2000; Gaiotto et al., 2003). Por outro lado, a dispersão de sementes é zoocórica (mediada por

animais), com mais de 58 espécies de aves e mais de 20 mamíferos alimentando de seus frutos. Apesar desta diversidade, a dispersão ocorre principalmente por grandes aves (por exemplo, *Procnias nudicollis*, *Ramphastos* spp.) e aves de pequeno e médio porte, como os sabiás (*Turdus* spp.) (Galetti et al., 2013). Esses dispersores têm um comportamento diverso, desde grandes aves que defecam ou regurgitam grandes quantidades de sementes, até pequenas aves dispersoras que regurgitam sementes uma a uma (Laps, 1996).

O estudo foi realizado durante os anos de 2012 a 2014 na Mata São José (Rio Claro, São Paulo, Brasil), um fragmento florestal de 230 ha rodeado por cultivo de cana-de-açúcar. Neste fragmento, *E. edulis* é encontrado em uma área de 8 ha correspondente a áreas de solo úmido, onde representa 72% de todos os indivíduos de espécies arbóreas que habitam esse tipo de ambiente (Teixeira e Assis, 2005). A fauna local é composta por cerca de 268 espécies de aves (Willis e Oniki, 2002; Gussoni, 2007), dentre as quais se destacam os sabiás (*Turdus rufiventris*, *T. leucomelas* e *T. amaurochalinus*) como os principais consumidores de frutos de palmito. O fragmento é considerado defaunado devido à extinção local da avifauna frugívora de grande porte (por exemplo, Cotingidae ou Ramphastidae) e a baixa ocorrência de outros dispersores importantes para a espécie como *Penelope superciliaris* ou *Ramphastos toco* (Willis e Oniki, 2002).

Efetividade da dispersão de sementes

Quantificamos a efetividade da dispersão através do seu componente quantitativo (Jordano e Schupp, 2000) utilizando o método de observação focal (Pizo e Simão, 2001; Pizo e Galetti, 2010). Para isso, foram feitas observações diretas de 15 indivíduos de *E. edulis* com frutos maduros. O observador se posicionou a uma distância mínima de 15 metros do indivíduo para a sua presença não interferir nas visitas dos animais às plantas. No total foram realizadas 90 horas de observação focal e cada palmito foi observado, preferencialmente, no período da manhã entre 06:00h e 12:00h. Nós identificamos as espécies visitantes, o tempo de permanência na fruteira de palmito, o número de frutos ingeridos e comportamento do frugívoro (engolir, derrubar, predar) (Pizo e Galetti, 2010). Para avaliar o esforço de amostragem, construímos curvas de acumulação com base no número de plantas amostradas. Estas foram construídas aplicando o método de rarefação para estimar a riqueza esperada (índice Chao) usando o pacote *vegan* (Dixon, 2003) em R (R Core Team, 2014).

A efetividade da dispersão de cada ave frugívora foi estimada pela multiplicação dos

seguintes subcomponentes: frequência de visitas por hora, taxa de frutos manipulados por visita e probabilidade de dispersão (proporção de frutos dispersos). Foram consideradas dispersas as sementes que foram engolidas e as que foram carregadas no bico, uma vez que estes dois comportamentos garantem uma alta probabilidade de dispersão efetiva (Côrtes, 2006). Desta forma, definimos como taxa de dispersão a multiplicação da taxa de frutos manipulados por visita e a probabilidade de dispersão.

Contribuição materna para a chuva de sementes

Para quantificar a chuva de sementes na Mata São José, instalamos 15 estações de amostragem com dois coletores de sementes de 1 x 1 m na área de ocorrência de *E. edulis*. Estas estações foram distribuídas a mais de 40 metros uma da outra. Os coletores foram verificados e as sementes dispersas foram amostradas durante a época de frutificação. Além disso, contamos o número de palmitos em frutificação em um raio de oito metros ao redor de cada estação.

Pelo menos 10 sementes de cada estação de amostragem foram genotipadas com sete marcadores de microssatélites desenvolvidos para *E. edulis* (Gaiotto et al., 2001). Como estamos interessados na dispersão de sementes, a genotipagem foi realizada utilizando tecido do endocarpo, o que nos permitiu obter o genótipo materno (Godóy e Jordano, 2001). Para caracterizar a contribuição materna para a chuva de sementes, primeiro identificamos sementes da mesma planta-mãe através do pacote *AlleleMatch* (Galpern et al., 2012) do R (R Core Team, 2014). Consideramos sementes irmãs (provenientes da mesma fonte materna) aquelas com total correspondência alélica. Em seguida, estimamos a probabilidade de encontrar duas sementes irmãs dentro de cada estação de amostragem por meio de uma estimativa não enviesada da probabilidade de identidade materna (uPMI, Grivet et al., 2005). Com base nessas estimativas, caracterizamos a diversidade de fontes maternas dentro e entre estações usando medidas de diversidade clássicas (Scofield et al., 2012). Essas medidas indicam como a diversidade das progênies maternas está distribuída entre as zonas de deposição da seguinte maneira: a diversidade alfa (α), que denota a diversidade local; e diversidade delta (δ), que é análogo ao índice de diversidade β e que representa a divergência entre as zonas de deposição (Scofield et al., 2012). Para a estimativa da diversidade alfa, atribuímos valor zero às estações que não receberam nenhuma semente e o valor um para aquelas que receberam apenas uma. A partir dessas estimativas, é possível inferir muito sobre o comportamento dos dispersores. Por exemplo, valores altos de diversidade alfa e baixos de

delta podem indicar movimentos de animais a longas distâncias, com sementes dispersas de diversas fontes e atingindo muitos locais de deposição, levando à sobreposição da mancha de sementes de múltiplas árvores. Finalmente, modelamos o número de sementes dispersas e a diversidade alfa em relação ao número de palmitos em frutificação em um raio de oito metros. Para isso, usamos modelos lineares generalizados assumindo uma distribuição binomial negativa das variáveis respostas. Os modelos foram ajustados usando o pacote *MASS* (Venables e Ripley, 2002) do R (R Core Team, 2014).

Distribuição espacial e estrutura genética espacial de plântulas e adultos

Analisamos a distribuição espacial e a estrutura genética espacial (SGS) em escala fina de plântulas e adultos de *E. edulis* em uma parcela de 25 x 50 m. A localização espacial de cada indivíduo foi feita em relação à parcela de estudo dividindo-a em 1250 subparcelas 1 x 1 m. A distribuição espacial de cada classe e a associação espacial entre elas foi avaliada por análise de padrão de pontos univariados e bivariados, respectivamente (Fortin e Dale, 2005). Para isso, usamos a função K de Ripley (Ripley, 1977; Wiegand e Moloney, 2004), que considera o número acumulado de pontos dentro de raios de diâmetro crescente em torno de pontos aleatórios (Ripley, 1977; Wiegand e Moloney, 2004). O padrão observado foi comparado com o esperado sob um processo de Poisson (Wiegand e Moloney, 2004), gerado a partir de 500 simulações de Monte Carlo (Besag e Diggle, 1977; Rozas e Camarero, 2005). A visualização desta função permite caracterizar o padrão espacial (agregado, aleatório ou regular), bem como seu alcance e intensidade. As análises foram realizadas usando o pacote *spatstat* (Baddeley et al., 2005) do R (R Core Team, 2014).

Para análise da estrutura genética espacial (SGS), coletamos amostra do caule de todos os adultos. Para amostragem das plântulas, sorteamos 32 subparcelas onde coletamos folhas de todas as plântulas de *E. edulis*. Dentre essas sub-parcelas, 22 apresentaram apenas uma plântula e 10 mais do que duas plântulas. As folhas e caules coletados foram conservados em sílica em gel e, posteriormente, analisados geneticamente utilizando sete marcadores moleculares de microssatélites já desenvolvidos para *E. edulis* (Gaiotto et al., 2001). No total analisamos 48 plântulas e 34 adultos. A estrutura genética espacial em escala fina foi avaliada através de autocorrelogramas representando o decaimento da relação genética (coeficiente de coancestria, f_{ij}) entre pares de indivíduos em função da distância geográfica Euclidiana entre eles. Para a construção dos autocorrelogramas, foi plotado o valor médio de coancestria para cada classe de distância espacial. As classes de distâncias foram definidas de forma a otimizar

a proporção de indivíduos participantes em cada intervalo. Os intervalos de confiança para cada classe de distância foram estimados por *jackknife over loci*. O coeficiente de coancestria foi calculado utilizando o estimador Loiselle et al. (1995) através do software SPAGeDi (Hardy e Vekemans, 2002) e SGS foi estimada para plântulas, adultos, separadamente, e entre plântulas e adultos.

Diversidade genética de plântulas e adultos

A variabilidade genética intrapopulacional de plântulas e adultos foi estimada pela heterozigosidade esperada sob equilibrio de Hardy-Weinberg (H_e) (Nei, 1978), riqueza alélica baseada na análise de rarefação (*AR*) (El Mousadik e Petit, 1996), e coeficiente de endocruzamento (*f*) (Weir e Cockerham, 1984). As análises foram realizadas com a utilização do pacote *gstudio* (Dyer, 2012) do software R (Team, 2014).

2.3. Resultados

Efetividade da dispersão

As aves que se alimentaram dos frutos de *E. edulis* na Mata São José pertenceram a quatro espécies de sabiás (*Turdus rufiventris*, *T. leucomelas*, *T. amaurochalinus* e *T. albicollis*). Nosso esforço de amostragem foi suficiente para encontrar o número de espécies observadas dentro dos valores esperados de acordo com o índice Chao (4.47 SD 1.25, Fig. 1A). A taxa de dispersão variou pouco entre as espécies (Fig. 1B). *Turdus rufiventris*, *T. leucomelas* e *T. amaurochalinus* removeram em média 1,5 frutos/visita, enquanto *T. albicollis* apresentou uma taxa de dispersão abaixo de 1. A frequência de visitas variou entre as espécies. *T. albicolis* foi a espécie menos frequente (uma visita a cada 50 horas), enquanto *T. leucomelas* (uma visita a cada nove horas) foi a espécies que mais contribuiu para a efetividade da dispersão (Fig. 1B).
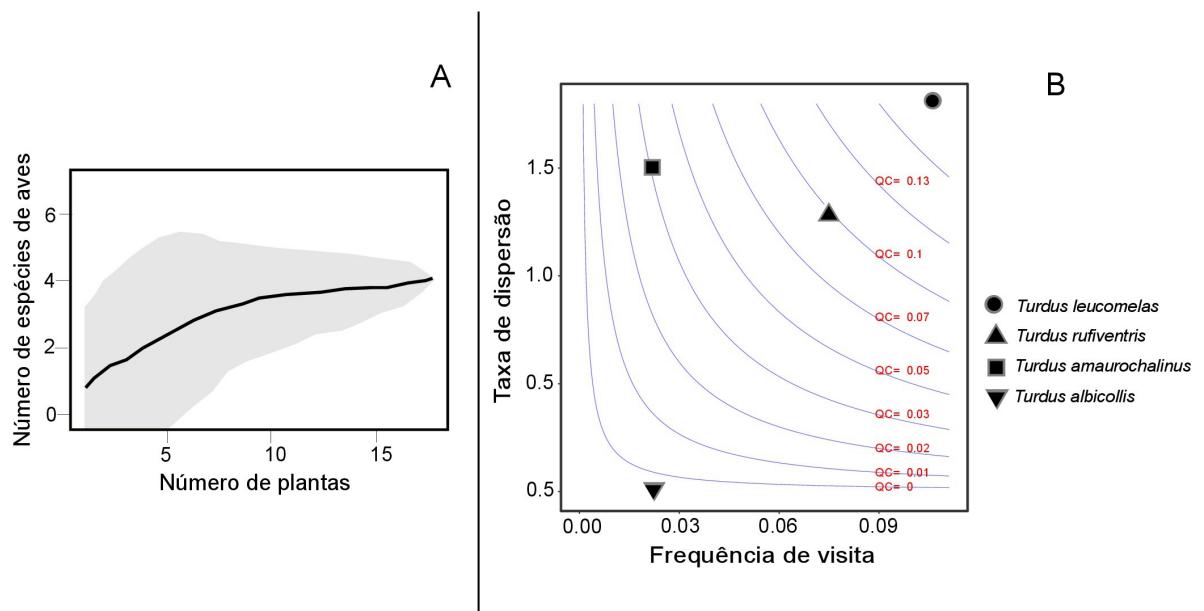
Figura 1: A – Curva de acumulação de espécies dispersoras de semente de *Euterpe edulis* na Mata São José, estado de São Paulo, Brasil. **B** – Componente quantitativo da efetividade de dispersão de semente de *E. edulis* nesta área. Isolinhas representam os intervalos de valores de componente quantitativo de efetividade de dispersão de sementes (QC) observados. Símbolos representam as espécies do gênero *Turdus* spp. que dispersaram as sementes de *E. edulis*.

Contribuição materna para a chuva de sementes

Nós amostramos 938 sementes em todas as estações amostrais, variando entre 0 a 400 sementes/estação amostral. Apenas quatro estações não obtiveram sementes (Tabela 1), das quais três não apresentaram palmeira adulta dentro do raio de oito metros. Esta variação no número de sementes correlacionou-se positivamente com o número de adultos dentro do raio de oito metros (Tabela 2). Nós amostramos 103 sementes, das quais 78 foram genotipadas com sucesso, variando esse número dentro de uma estação entre três e 13 (Tabela 2). A probabilidade média de encontrar duas sementes irmãs foi muito baixa (~ 20 [0 - 100]%). Isso refletiu no elevado número de genótipos únicos presentes na maioria das estações de amostragem (Tabela 1, Fig. 2). Encontramos uma grande variação da diversidade alfa entre as estações de amostragem (0 a 60), o que é explicado pelo número de adultos em frutificação em torno dos locais de deposição (Tabela 2). Finalmente, obtivemos o valor máximo para a diversidade delta ($\delta = 1$), indicando a ausência de genótipos idênticos entre unidades amostrais. Isso sugere a ausência de superposição de chuva de sementes entre os locais de deposição (Fig. 2).
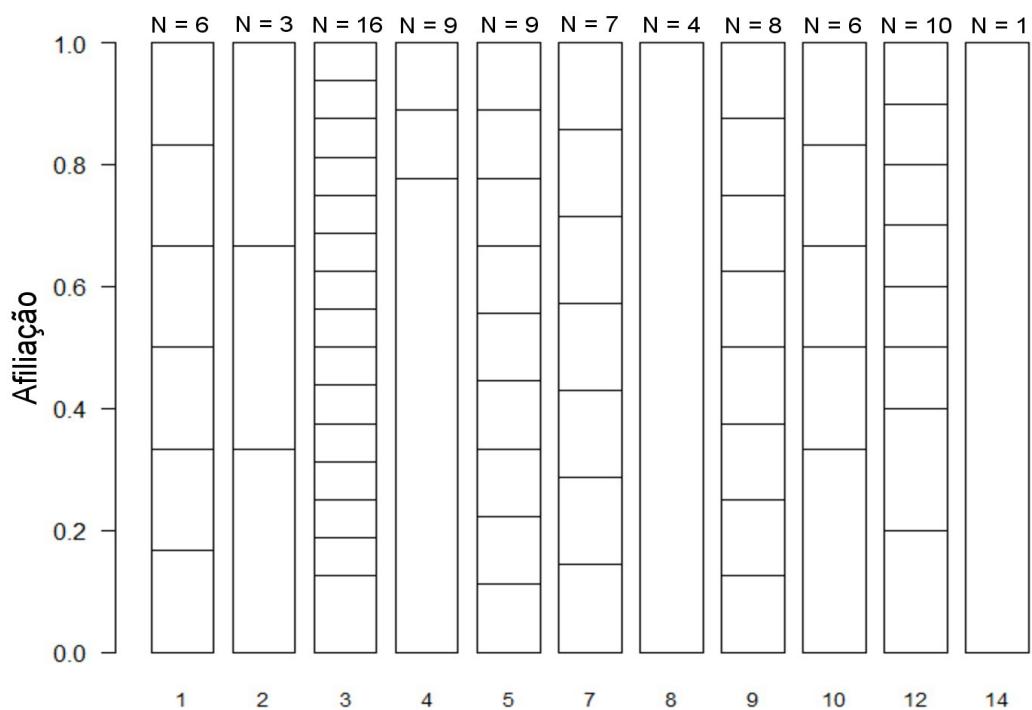
Figura 2: Representação relativa das diferentes fontes maternas das sementes de *Euterpe edulis* encontradas em 11 estações amostrais na Mata São José, São Paulo, Brasil. Cada barra branca representa a frequência relativa de fontes únicas de sementes para cada estação amostral porque não existe sobreposição entre coletores. N= número de sementes analisadas.

Tabela 1: Chuva de sementes de *Euterpe edulis* e contribuição materna em 15 estações amostrais na Mata São José, estado de São Paulo, Brasil. PMI = probabilidade de encontrar duas sementes irmãs em uma mesma estação de amostragem.

Estação de amostragem	Nº de sementes	Nº adultos frutificando	Nº de sementes analisadas	Nº genotipos únicos	Diversidade alfa	PMI
1	11	2	6	6	15	0.06
2	4	0	3	3	3	0.33
3	430	11	16	15	60.25	0.01
4	118	7	9	3	1.68	0.59
5	11	8	9	9	36	0.02
6	0	0	0	0	0	0
7	67	5	7	7	21	0.04
8	7	6	4	1	1	1
9	50	3	8	8	28	0.03
10	43	3	6	5	7.75	0.12
11	1	0	0	-	-	-
12	195	4	10	8	15.22	0.06
13	0	0	0	0	0	0
14	0	2	0	0	0	0
15	0	0	0	0	0	0

Tabela 2: Estimativas dos parâmetros dos efeitos do número de adultos de *Euterpe edulis* em um raio de oito metros sobre o número de sementes dispersas, diversidade alfa e a probabilidade de encontrar duas sementes irmãs (PMI). "*" Indica os valores das estimativas estatisticamente significativas (p <0,05) e SD indica desvio padrão.

Modelo	Parâmetros estimados	
	Intercepto (SD)	Número de adultos (SD)
Número de sementes dispersadas	1.42 (0.59)*	0.52 (0.12)*
Diversidade alfa	1.01 (0.50)*	0.30 (0.10)*
uPMI	0.21 (0.20)	0.00 (0.03)

<u>Distribuição espacial, estrutura genética espacial e diversidade genética de plântulas e adultos</u>

Na parcela estudada, os indivíduos adultos e plântulas apresentaram um padrão mais aleatório (Fig. 3A, B). No entanto, a análise bivariada mostra uma forte agregação de plântulas em torno de indivíduos adultos (Fig. 3C). A estrutura genética espacial (SGS) acompanhou os resultados obtidos na análise de padrão espacial dos indivíduos. Não encontramos SGS em plântulas ou adultos (Fig. 4). No entanto, o parentesco entre plântulas e adultos foi maior que o esperado ao acaso a distâncias abaixo de seis metros, demonstrando uma SGS em uma escala espacial fina. Em termos de diversidade genética, os valores da heterozigosidade esperada (H_e = 0,82 e 0,83, adultos e plântulas, respectivamente), riqueza alélica (AR = 10,43 e 10,66) e coeficiente de endogamia (f = 0,10 e 0,10) foram semelhantes entre adultos e plântulas.
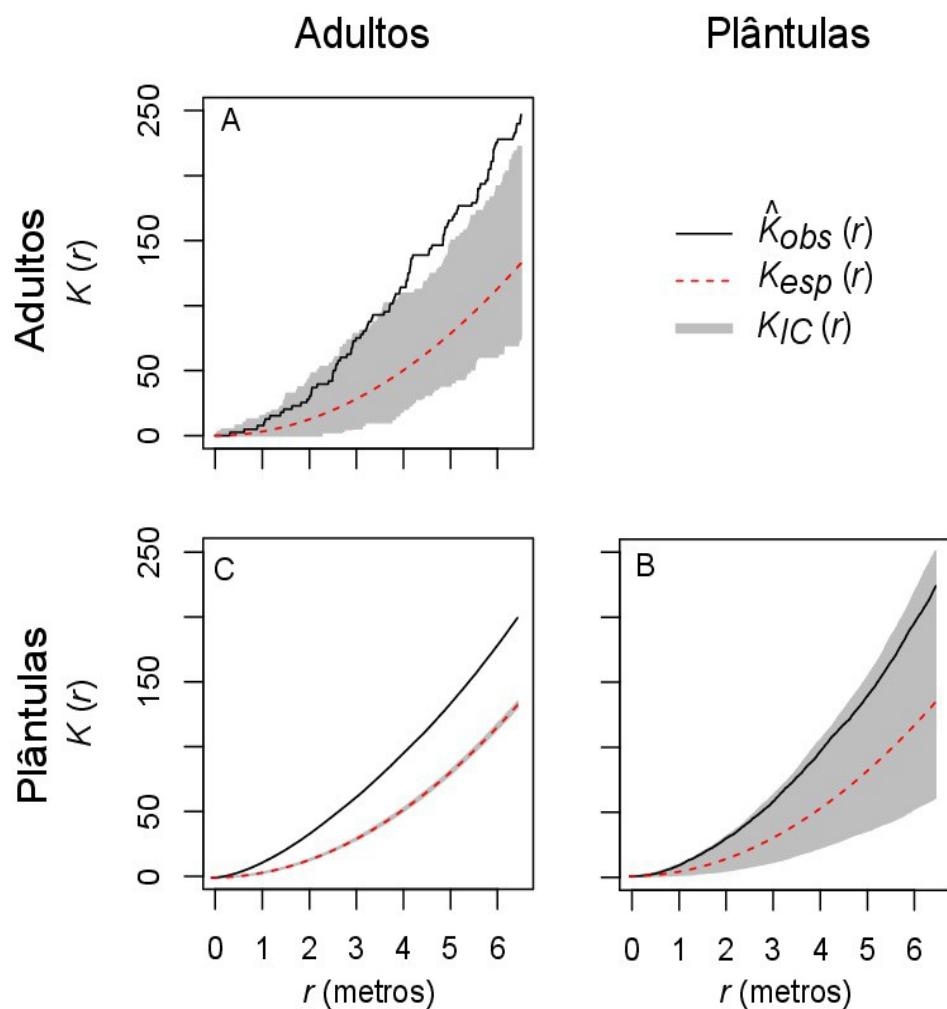
Figura 3: Função de K de Ripley para descrever a distribuição espacial de adultos, plântulas e relação entre adultos e plântulas de *Euterpe edulis* em uma parcela de 25x50 m na Mata São José, São Paulo, Brasil. K_{obs} *(r)*= padrão observado , K_{esp} *(r)* = padrão médio esperado sob um processo de Poisson , $K_{IC}(r)$= Envelope representando intervalo de confiança de padrão aleatório obtido através de 500 simulações de Monte Carlo.
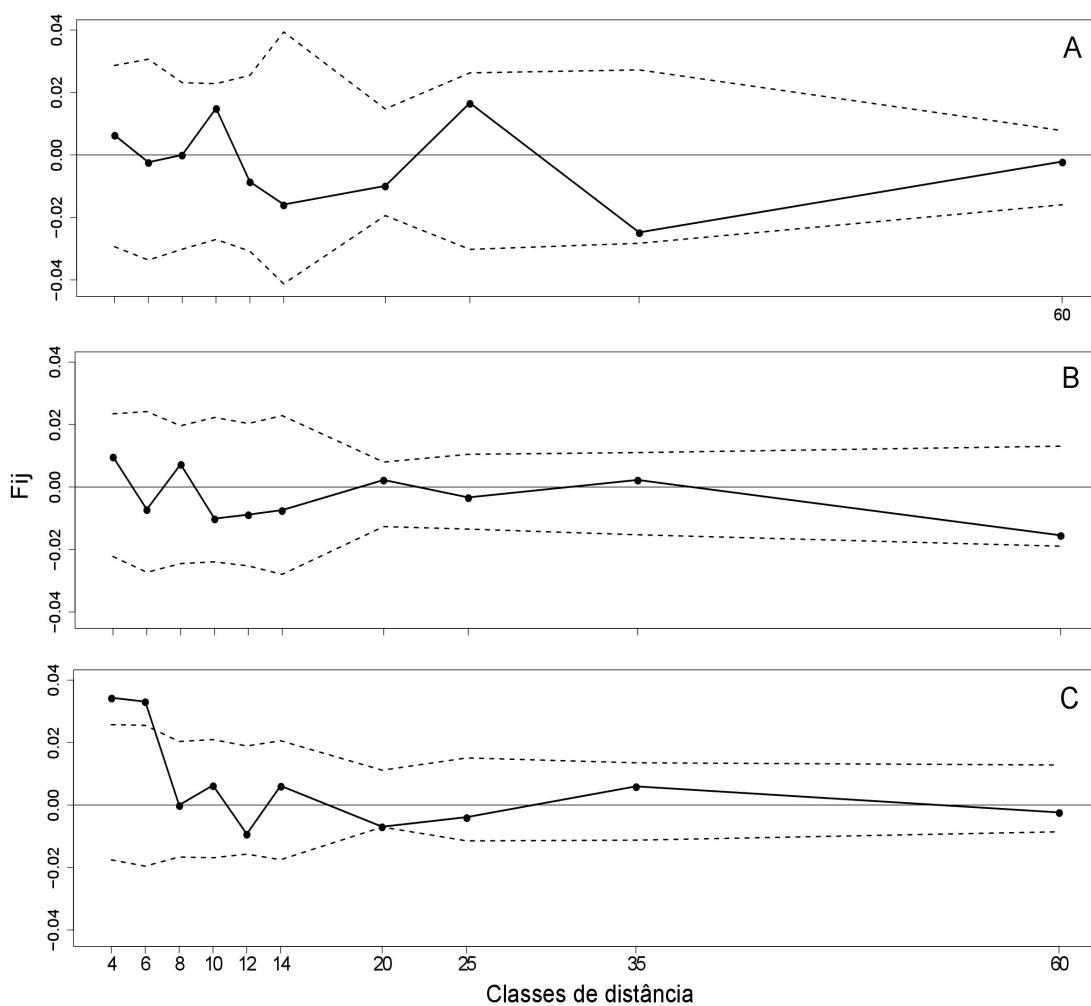
**Figura 4:** Autocorrelogramas da média do coeficiente de coancestria entre pares de adultos (A), plântulas (B) e entre adultos e plântulas (C) de *Euterpe edulis* para diferentes intervalos de distância em uma parcela de 25x50 m na Mata São José, São Paulo, Brasil. Linha contínua mostra os coeficientes de coancestria e a linha pontilhada o intervalo de confiança a 95% para um padrão de distribuição espacial aleatória de genótipos.

2.4. Discussão

Nós encontramos que os sabiás foram os únicos dispersores de semente de *Euterpe edulis* no fragmento florestal de Mata Atlântica estudado. Essas aves, ao visitarem muitas palmeiras, consumirem poucas sementes por visita e regurgitarem as mesmas uma a uma, dispersam genótipos de múltiplas plantas-mães e diminuem a probabilidade de sementes irmãs serem depositadas em um mesmo sítio. Esse comportamento se mostrou eficaz para manter a diversidade genética em recrutas e, além disso, ajudar a manter uma baixa estrutura genética espacial em plântulas e adultos. O fato de não encontrarmos plantas maternas contribuindo para diferentes sítios de deposição e identificarmos a presença de estrutura genética espacial nos primeiros intervalos de distância (~ seis metros) entre adultos e plântulas indica que a distância de dispersão de sementes gerada por *Turdus* spp. é pequena. Com esses resultados reforçamos a importância dos sabiás para a manutenção da fraca estrutura genética espacial e elevada diversidade genética de *E. edulis* em populações fragmentadas. Além disso, salientamos a importância de se manter altas densidades de *E. edulis*: a baixa distância de dispersão por *Turdus* associada a baixas densidades geraria uma baixa sobreposição de chuvas de sementes e pouca mistura de genótipos maternos, o que a curto-médio prazo aumentaria a estrutura genética espacial e eventualmente reduziria diversidade genética.

Efetividade de dispersão

Neste estudo nós registramos quatro espécies de aves dispersoras de sementes na Mata São José. Esse número é muito inferior ao número de espécies registradas em uma área conservada de Mata Atlântica (Ilha do Cardoso, n=8) (Côrtes, 2006) no qual foi utilizado o mesmo método de observação focal. Dentre os principais animais não amostrados, podemos destacar os grandes dispersores de sementes como os tucanos, os araçaris e as arapongas. A extinção local desses grandes animais pode ser devida à caça e ao alto grau de fragmentação florestal e perda de habitat (Kattan et al., 1994; Sodhi et al., 2004). A área estudada é um fragmento florestal pequeno, isolado e rodeado por matriz de cana-de-açúcar e, provavelmente, não suporta populações de grandes aves florestais. Essas aves são capazes de ingerir uma grande quantidade de frutos por visita e dispersar as sementes a longas distâncias (Nathan, 2006; Holbrook, 2011). Sua perda, portanto, pode limitar o fluxo gênico entre as populações levando ao aumento da diferenciação genética entre as populações (Carvalho et al., 2015).

Os sabiás foram os únicos dispersores de sementes encontrados na área de estudo. Nós registramos quatro espécies do gênero *Turdus* interagindo com palmitos frutificando: *T. leucomelas*, *T. amaurochalinus*, *T. rufiventris* e *T. albicollis*, sendo que a última não foi registrada removendo frutos. *Turdus leucomelas*, *T. amaurochalinus*, *T. rufiventris* não apresentaram variação em relação ao número de frutos removidos e ingeriram em média um fruto por visita. O número de frutos removidos por visita geralmente é influenciado pelas características morfológicas das aves. Por exemplo, o tamanho corpóreo e a abertura do bico tendem a estar correlacionados com o número de sementes removidas por visita (Jordano e Schupp, 2000). Aves do gênero *Turdus* apresentam largura do bico de aproximadamente 11,6 mm e peso de aproximadamente 64 gramas. Essas características limitam essas aves a consumirem poucos frutos por visita e, portanto, tendem a dispersar as sementes isoladamente em um padrão esparso (Laps, 1996; Pizo e Simão, 2001).

Mesmo em áreas conservadas, como Parques Estaduais e Estações de Pesquisa, as aves do gênero *Turdus* figuram entre os dispersores mais importantes das sementes de palmito, principalmente em relação à frequência de visitas (Zimmermann, 1999; Reis e Kageyama, 2000; Castro, 2003; Côrtes, 2006). Enquanto nas áreas preservadas as espécies florestais como *T. albicollis* e *T. flavipes* são mais abundantes (de Castro et al., 2012; Rother et al., 2016), outras espécies como *T. leucomelas*, *T. rufiventris* e *T. amaurochalinus* tendem a ser mais comuns em pequenos fragmentos florestais e vegetações sob forte influência antrópica (parques urbanos, matas secundárias, restauração, etc) (Aleixo e Vielliard, 1995; Vogel et al., 2012). Na Mata São José, essas aves apresentaram variação em relação à visitação, sendo *T. leucomelas* o dispersor com mais visitas registradas. A taxa de visita pode ser influenciada por diversos fatores, principalmente pela abundância local de frugívoros (Vázquez et al., 2005; Côrtes, 2006). As espécies de *Turdus* encontradas na Mata São José apresentam uma dieta muito similar, composta principalmente por pequenos invertebrados e frutos. Portanto, as diferenças nas taxas de visita ao palmito podem ser atribuídas principalmente a diferença na abundância entre as espécies na área de estudo. Labecca (2012) estimou o índice pontual de abundância relativa (IPA) na Mata São José e *T. leucomelas* foi a espécie de sabiá mais abundante (IPA *T. leucomelas* = 0.07, *T. amaurochalinus* = 0.03 e *T. rufiventris* = 0.04). Apesar da importância das aves do gênero *Turdus* como dispersores de sementes, principalmente em relação à frequência de visita, esses animais geralmente dispersam as sementes a curtas distâncias (Jordano et al., 2007; González-Varo et al., 2017) e necessitam de áreas florestadas para levar as sementes para além dos fragmentos florestais (González-Varo

et al., 2017). Essa combinação de fatores pode levar a uma grande chuva de sementes em uma escala local, no entanto baixo intercâmbio de sementes ao longo da paisagem.

Contribuição materna na chuva de sementes e limitação da distância de dispersão

Em plantas dispersas por animais é comum que ocorra uma contribuição desigual das árvores fontes para a dispersão das sementes na paisagem (Grivet et al., 2005; García et al., 2009). Isso ocorre devido a visitas frequentes de frugívoros às mesmas árvores ou ao grande número de frutos removidos por visita, e esse padrão é intensificado caso os frugívoros tenham o comportamento de se abrigarem, repousarem ou realizem *display* no mesmo local (Schupp et al., 2002). No entanto, nós encontramos um padrão distinto de deposição de chuva de sementes. Nós encontramos um grande número de mães contribuindo para a dispersão de sementes em uma mesma estação amostral, resultando em baixa variância reprodutiva entre plantas maternas. Este padrão reflete o comportamento dos sabiás de dispersar sementes uma a uma (Pizo e Simão, 2001), gerando uma mistura de sementes de diversas fontes e uma heterogeneização genética da chuva de sementes em escala local (i.e., sobreposição de manchas de genes). Esse padrão foi refletido em plântulas e adultos, uma vez que a maior combinação de plântulas originadas de indivíduos adultos diferentes levou à ausência de estrutura genética espacial em ambos os estágios ontogenéticos.

Apesar da alta diversidade de mães contribuindo para a chuva de sementes local e ausência de estrutura genética espacial em plântulas e adultos, a dispersão de sementes de *E. edulis* pelos *Turdus* parece ser limitada pela distância. O fato de não encontrarmos sementes oriundas da mesma planta materna em mais de uma estação amostral é uma evidência de um movimento de sementes local (Grivet et al., 2005). Além disso, nós encontramos uma autocorrelação espacial significativa da coancestria entre plântulas e adultos em intervalos curtos de distância. A análise de distribuição espacial de plântulas e adultos também mostra uma forte agregação de plântulas ao redor de adultos. Portanto, a não sobreposição de chuvas de sementes entre estações amostrais, a forte autocorrelação genética espacial nas primeiras classes de distância e a forte agregação dos indivíduos de plântulas em relação aos adultos são indícios de uma limitação da distância de dispersão. A limitação da distância de dispersão pode ser atribuída tanto à presença de apenas pequenos e médios frugívoros que depositam sementes próximo à planta-mãe (García et al., 2007; Jordano et al., 2007) como a alta densidade local de plantas conspecíficas (Carlo e Morales, 2008). Áreas com alta densidade de plantas geralmente atraem mais aves o que leva ao aumento da taxa de remoção de

sementes, no entanto, tendem a diminuir a distância de dispersão devido a menor movimentação dos frugívoros em busca de alimento (Carlo e Morales, 2008). A Mata São José, apesar de ser um fragmento florestal, apresenta uma mancha isolada com alta densidade de palmito (~ 272 palmitos/ha). Portanto, a combinação entre o comportamento de forrageamento dos sabiás e a alta densidade de adultos pode estar levando a limitação da distância de dispersão nesta espécie.

<u>Diversidade genética de adultos e plântulas</u>

Por fim, nós não encontramos uma redução da diversidade genética entre adultos e plântulas. Isso pode ser devido à baixa probabilidade de duas sementes virem da mesma planta-mãe em um mesmo metro quadrado e ao grande número de mães contribuindo para a dispersão de sementes (número efetivo de fontes de sementes para a população = 44). Esses resultados são coerentes e ajudam a explicar trabalhos anteriores sobre o efeito da fragmentação na diversidade e estruturação genética de *Euterpe edulis* (Carvalho et al., 2015; Santos et al., 2015). Nesses trabalhos foi encontrada uma maior estruturação genética em populações inseridas em paisagens fragmentadas (Carvalho et al., 2015), mas não foi encontrada uma redução da diversidade genética devido à fragmentação (Carvalho et al., 2015; Santos et al., 2015). Devido à falta de informações em escala fina, isso foi atribuído a efeitos de time-lag entre a fragmentação e mudanças genéticas. Com os nossos resultados, nós podemos sugerir que a manutenção da diversidade genética em áreas fragmentadas pode ser devida à grande densidade de palmeiras e ao comportamento dos sabiás em dispersar sementes de diferentes plantas-mães em um mesmo local. A combinação desses fatores tende a aumentar o tamanho efetivo populacional contemporâneo, o que ajuda a manter a diversidade genética. No entanto, esses animais não são eficientes em dispersar as sementes para além da borda dos fragmentos (González-Varo et al., 2017), o que pode explicar a alta estruturação genética entre populações inseridas em paisagens fragmentadas. Portanto, com a degradação, isolamento e perda de habitat, a presença apenas de espécies de pequeno porte não garante que a função e estrutura do ecossistema serão conservadas sob cenários de perturbação (Loiselle et al., 2007).

<u>Conclusão</u>

A efetividade da dispersão de sementes de uma espécie de planta, entre outros fatores, é dependente da diversidade de frugívoros, do comportamento pós-alimentação do dispersor (manipulação do fruto pelo animal - regurgitador ou defecador), padrão de movimentação do dispersor (ex. voam longas ou curtas distâncias), do lugar que essas sementes serão deixadas

(diferentes micro-habitats) e do modo como estas sementes serão dispersas no ambiente (agregadas ou esparsas) (Bleher e Böhning-Gaese, 2001). Nossos resultados mostraram que as aves remanescentes na Mata São José são eficazes para a manutenção da diversidade genética em escala local devido ao comportamento de remoção e deposição das sementes. Portanto, é importante a presença de toda a guilda de dispersores dentro da população, pois os dispersores de pequeno tamanho tem um papel importante na distribuição de plântulas pouco aparentadas e os de grande porte no fluxo gênico intra e interpopulacional (Loiselle e Blake, 2002). Além disso, nós salientamos sobre a importância de se manter populações com altas densidades de *E. edulis*. Nós não encontramos estrutura genética espacial em adultos e plântulas e isso é resultado principalmente da alta densidade de *E.edulis*. Portanto, o corte ilegal da palmeira para a extração do meristema comestível pode, a curto-médio prazo, reduzir a densidade e aumentar a estrutura genética espacial, que eventualmente pode erodir a diversidade genética, mesmo com atividade dos sabiás.

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3. Capítulo 2: Partial defaunation and density of conspecifics impact the distribution of the maternal progenies in a frugivore-generated seed rain

Citação:

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To be submitted to *Molecular Ecology*

Abstract

Populations of large-bodied vertebrates have declined worldwide driven by habitat fragmentation and poaching with a collapse of the ecosystem services they provide, such as seed dispersal services to animal-dispersed plants. Partial defaunation frequently entails the extirpation of a non-random subset of large frugivores while medium- to small-sized frugivores remain active. Yet, in spite of this remnant dispersal activity, partial defaunation reduces the frequency and extent of long distance dispersal events of endozoochorous species, compromising the connectivity among distant populations. We ignore whether partial defaunation also entails dispersal limitation by shifting the spatial distribution of maternal progenies in the seed rain which might imperil local plant recruitment. Here, we depicted the spatial distribution of the maternal progenies in a bird-generated seed rain of a tropical palm (*Euterpe edulis*) that produces medium-sized seeds. By combining molecular tools with spatial-explicit diversity measures, we partitioned the diversity of maternal progenies among and across deposition sites over ten populations that differed in the level of seed disperser impoverishment and density of adult conspecifics. We expected a spatial aggregation of maternal progenies in the seed rain that would reduce diversity across spatial scales (measured in terms of α-, δ- and γ-diversity). We found unchanged distribution patterns at the fine scale (α-diversity) because medium-sized birds provided seed dispersal services at short distances (< 10 m) promoting high levels of local genetic diversity in the seed rain. Nevertheless, the loss of large-sized seed dispersers resulted in highly aggregated seed rain patterns with reduced levels of seed shadow overlapping (low δ-diversity levels). Forest canopy structure also impacted seed deposition and genetic diversity patterns in the seed rain. Overall, our study highlights that medium- and large- sized frugivores provide complementary services, being the former important to admixture unrelated progenies at the local scale and the latter at the landscape scale.

3.1. Introduction

Populations of numerous large-bodied vertebrates have been extirpated or are in continuous decline worldwide as a result of extensive deforestation and poaching (Ceballos, Ehrlich, & Dirzo, 2017; Dirzo et al., 2014). Consequently, the persistence of endozoochorous plants that inhabit partially defaunated forests are seriously threatened because they require dispersal services provided by frugivorous vertebrates to complete their life cycle (Caughlin et al., 2014; Jordano, 2013; Kurten, 2013). Specifically, defaunation of large frugivores may impair seed dispersal services if extant medium- to small-bodied vertebrates only provide suboptimal dispersal services because: (i) they often mobilize a reduced fraction of propagules (Holbrook & Loiselle, 2009); (ii) they do not ingest large-sized seeds, and therefore, only medium- to small-sized seeds get dispersed (Galetti et al., 2013); and (iii) they fail to reach distant sites, which reduces connectivity at the landscape level (Pérez-Méndez, Jordano, García, & Valido, 2016). As a result, partial defaunation might impose different types of seed dispersal limitation to plant species they feed upon (Jordano & Godoy 2002) when disperser activity is not sufficient to disperse all seeds away from fruiting trees and/or they are dispersed in an aggregated fashion nearby parent trees. Recent studies have shown that partially defaunated areas are subjected to seed dispersal limitation in terms of reduced frequency of long distance dispersal events (Caughlin et al., 2014; Pérez-Méndez, Jordano, & Valido, 2015). Yet still ignore whether the distribution of maternal progenies in the seed rain within and among populations shifts in partially defaunated areas. This is important because the distribution of the maternal progenies in the seed rain set the genetic environment where dispersed seeds will germinate and, eventually, they will get established (Browne & Karubian, 2016; García & Grivet, 2011). Thus, the mixture of unrelated maternal progenies increases the probability of seedling survival, in turn contributing to the maintenance of high population genetic diversity (Browne & Karubian, 2016; Scofield, Smouse, Karubian, & Sork, 2012). Therefore, evaluating whether partial defaunation shifts the distribution of the maternal progenies in a frugivore-generated seed rain is crucial to forecast the probabilities of endozoochorous forest species to persist in an increasingly defaunated world.

Frugivores shape the seed rain in distinctive fashions according to their morphological and foraging behavior (Choo, Juenger, & Simpson, 2012; Côrtes & Uriarte, 2013; Karubian, Sork, Roorda, Durães, & Smith, 2010). This impacts both the quantitative (ex. the number of fruits removed) and the qualitative components (ex. the dispersal distance) of the seed dispersal

services performed by more or less complex frugivorous assemblages (Jordano & Schupp, 2000). For example, large frugivores move and disperse seeds to distant locations (Holbrook & Loiselle, 2009; Nathan, 2006), most likely bringing new maternal progenies from other populations (García, Jordano, & Godoy, 2007; Jordano, García, Godoy, & García-Castaño, 2007). As a result, genetic diversity increases and genetic divergence decreases both at the seed rain and the population level (Karubian, Ottewell, Link, & Di Fiore, 2015; Scofield et al., 2012). In contrast, small- and medium-bodied birds, such as thrushes, typically move locally and ingest few small seeds per visit (Galetti et al., 2013; Jordano et al., 2007, Carvalho et al in prep.), resulting in a high aggregation of maternal progenies (i.e., seeds dispersed from the same fruiting tree) nearby the mother tree (Caughlin et al., 2014). This foraging pattern reduces the local genetic diversity in the seed rain (Grivet, Smouse, & Sork, 2005) and may decrease population genetic diversity in the long term (Giombini, Bravo, Sica, & Tosto, 2017). Additionally, frugivore behavior is mediated by fine-scale environmental heterogeneity, which influences foraging and seed deposition patterns and imprint the spatial distribution of genetic variation in plant populations (García, Jordano, Arroyo, & Godoy, 2009). For example, sites with high density of fruiting trees typically attract more frugivores, increasing seed removal and deposition rates (Alcantara, Rey, Valera, & Sanchez-Lafuente, 2000; Carlo & Morales, 2008). At the same time, clumped distribution of fruiting trees might decrease seed dispersal distances because frugivores move short distances to forage on different maternal canopies (Carlo & Morales, 2008). This contributes to the admixture of maternal progenies at the local scale that might maintain high genetic diversity among deposition sites within a population (Giombini et al., 2017) and erases intra-population genetic structure (Jordano, 2017). Therefore, the structure of forest canopy and particularly the density of fruting conspecifics might enhance or dilute the impact of partial defaunation on the distribution of maternal progenies in the seed rain (Côrtes & Uriarte, 2013).

A spatial-explicit modification of diversity measures (α-, β- and γ-diversity) (Whittaker, 1972) provides a useful framework for assessing and comparing the impact of distinct assemblages of frugivores on the distribution of maternal progenies (Karubian et al., 2015; Scofield et al., 2012). By combining molecular tools with a thorough sampling of the frugivore-generated seed rain, we can partition the diversity of maternal progenies within and among deposition sites, as follows (Figure 1): alpha diversity (α), which represents the within-site diversity of maternal progenies; gamma (γ), which represents the total diversity of maternal progenies at the population level considering all seed deposition sites; and delta (δ), which is analogous to

the classical *β* diversity index, representing the divergence between seed deposition sites within a population (Scofield et al., 2012). These indices are suitable to evaluate whether the distribution of the maternal progenies in the seed rain shifts across spatial scales as a result of partial defaunation. For instance, high *α*- with simultaneous low *δ*-diversity indicates that multiple maternal plants contribute seeds to the same deposition site (high *α*-diversity) and that seed shadows overlap to a great extent (low *δ*-diversity) (Figure 1). This spatial diversity pattern is expected in seed rain populations generated by a complete assemblage of frugivorous where both small- and large-bodied frugivorous provide dispersal services over a wide range of fruiting trees (Schupp, Milleron, & Russo, 2002); thus, whereas small frugivorous would perform frequent short distance dispersal services, large frugivorous would reach distant sites.
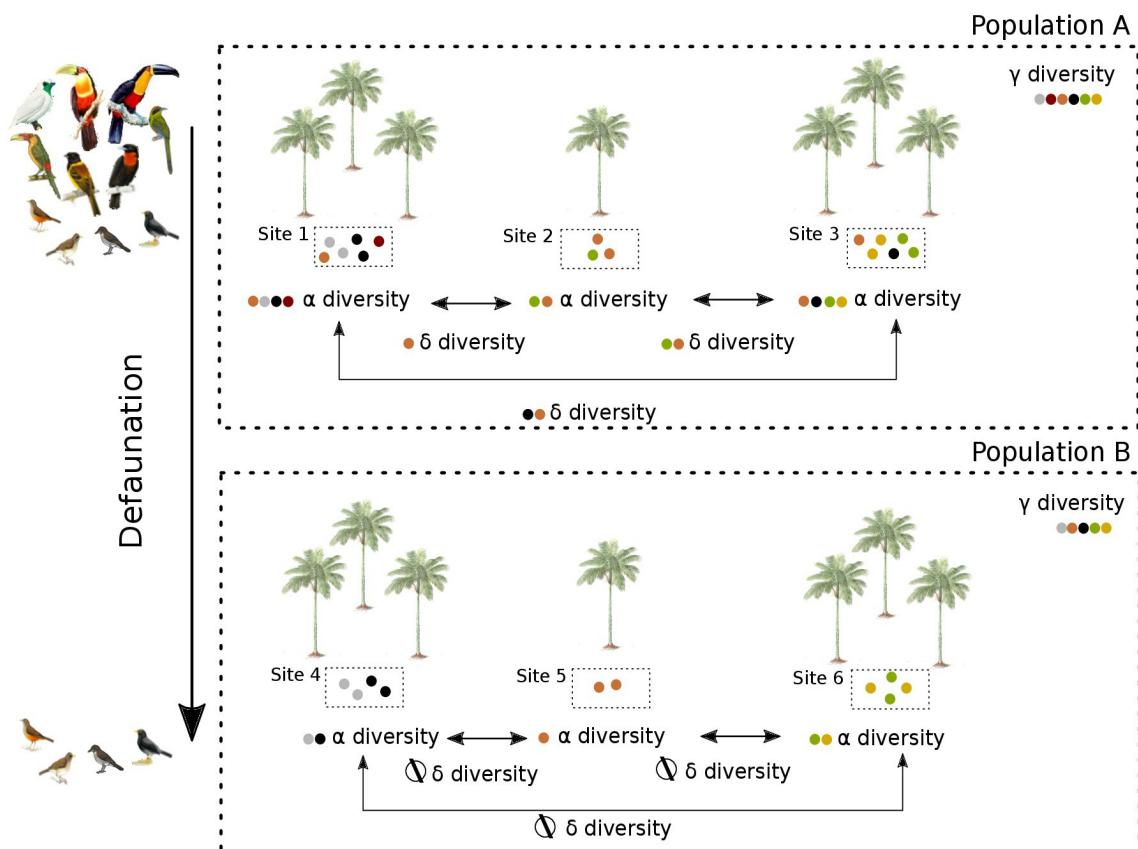
Figure 1: Scheme depicting spatial-explicit diversity measures (*α*-, *δ*- and *γ*-diversity) in frugivore-generated seed rains across populations subjected to different levels of defaunation. Based on Scofield et al. (2012), *α*-diversity (*α*) represents the within-site diversity of maternal progenies; *γ* represents the total diversity of maternal progenies at the population level considering all seed deposition sites; and *δ* represents the divergence between seed deposition sites within a population. Colourful cicles represent maternal progenies.

The extirpation of large-bodied avian frugivores has been previously associated with ecological (Galetti et al., 2013) and genetic (Carvalho et al. 2016) impacts in our study species, the tropical palm *Euterpe edulis*. These impacts include a reduction in mean seed size (Galetti et al., 2013) and changes in the allele frequencies across populations (Carvalho, Galetti, Colevatti, & Jordano, 2016). To elucidate the fine-scale processes that may lead to these patterns, here we depict the spatial distribution of maternal progenies in a frugivore-generated seed rain of the palm *Euterpe edulis* across sites subjected to different levels of defaunation. First, we characterized the level of defaunation in ten study sites and then analyzed seed deposition patterns as a function of defaunation and density of conspecific fruiting palms. Second, we investigated whether the spatial distribution of the maternal progenies in the seed rain changes as a function of defaunation, density of fruiting palms and forest canopy structure, all key drivers of the frugivore's foraging patterns. We additionally assessed the number of alleles and allele evenness. Small to medium-sized birds, mostly thrushes (*Turdus* spp.), visit palms more often than large birds and tend to remove seeds from multiple fruiting trees. Because these birds are abundant irrespective of the defaunation level, we expect that alpha diversity will be weakly associated with defaunation (Table 1). Instead, we hypothesize that alpha diversity will reflect the local pool of conspecific palms owing to the intense and local foraging activity of *Turdus* spp (Table 1). Conversely, we hypothesize that γ-diversity and allele diversity will be higher in non-defaunated forests due large-bodied frugivores, such as toucans and toucanets (e.g., *Ramphastos* spp.) that are able to bring maternal genotypes and new alleles from distant sites (Table 1). Finally, we hypothesize that δ diversity will be lower in non-defaunated forests and in areas where palms and specific forest structure are more homogeneously distributed (Table 1). This is because large avian birds tend to remove several seeds at once and are able to move longer distances, promoting dispersal of seeds from the same feeding bout (i.e., same maternal progeny) into different sites in the forest. Moreover, these birds will most likely move across suitable sites, with more food resources (high density of palms) and presence of specific forest structure traits (e.g., perches, canopy height).

Table 1: A priori hypotheses to explain how defaunation, adult density and forest structure may influence the effective number of mother trees (α- and γ-diversity), allele diversity and seed size of *Euterpe edulis*. (+), (-) and (=) mean positive, negative and no effect, respectively.

	Ecological theory	Response effects
Defaunation	Defaunation have effects on plant populations, such as significant reduction of fruit removal success, collapse of seed dispersal distance, and entailing rapid evolutionary changes in seed size.	(–) Effective number of mother trees (–) Allele diversity (–) Seed size
	Small birds are the most abundant. Forage one seeds per visit, but present high visit rate. However, they do not ingest large seeds due to morphological constrain	(=) Effective number of mother trees (=) Allele diversity (–) Seed size
Adult density	Areas with high plant density, that means high availability of food resource, generally attract more birds which leads to increased seed removal rates and dispersed seeds from different sources	(+) Effective number of mother trees (+) Allele diversity
Forest structure	Forest structure can determine the seed rain pattern because of avoidance of frugivores (mainly large birds) to open habitats. Dense forest canopy may receive more dispersed seeds from different sources	(+) Effective number of mother trees (+) Allele diversity

3.2. Material and Methods

Study area and species

The Brazilian Atlantic forest is an ideal system to test the effects of anthropogenic defaunation on ecological and evolutionary processes because it has been intensively disturbed by human activities for centuries; however, we can still find pristine areas along its distribution. The Atlantic forest originally covered the Atlantic coast of Brazil extending from

the Northeast to the Southern region of the continent, including parts of Argentina and Paraguay (Joly, Metzger, & Tabarelli, 2014). Today it has been reduced to 12% of its original extension, which was estimated in 150 million ha (Ribeiro, Metzger, Martensen, Ponzoni, & Hirota, 2009). Habitat fragmentation, among other drivers, has caused a sustained and alarming loss of biodiversity during the last ~500 yr that have resulted in islands of wild habitats surrounded by crops, pastures and urban matrix (Joly et al., 2014). The heart of palm (*Euterpe edulis* Mart.*,* Arecaceae) was once one of the dominant palms in the Atlantic forest but it is currently endangered and locally extinct in many areas owing to illegal harvesting of its edible meristem (Galetti & Fernandez, 1998). *Euterpe edulis* is a self-compatible monoecious species with predominantly outcrossed reproduction (Gaiotto, Grattapaglia, & Vencovsky, 2003), pollinated by small-sized bees (e.g., *Trigona spinipes*). This palm produces round fleshy fruits ranging from 8.3 to 14.1 mm in diameter (Pizo, Von Allmen, & Morellato, 2006), eaten by more than 58 birds and 20 mammalian species (Galetti et al., 2013). Yet, seeds are dispersed mostly by a reduced subset of large frugivorous birds such as cotingas (*Procnias nudicollis*), toucans (*Ramphastos* spp), and guans (*Penelope* spp. and *Aburria jacutinga*) and medium-sized avian frugivores (*Turdus* spp.) (Bello et al., 2017; Galetti et al., 2013). Historically, our study areas shared a similar assemblage of seed dispersers, but forest fragmentation and hunting have impoverished the assemblage of large frugivores in most of Atlantic forest (Galetti et al., 2013).

Sampling design

We selected 10 study sites across the Atlantic forest with available data on frugivory and abundance of avian seed dispersers (Bello et al., 2017; Galetti et al., 2013)(Ribeiro 2012; Marçal 2016) (Figure 2). In each site we set and georreferenced (with a Garmin GPS) 15 deposition sites (Figure 3A-J) that consisted of two seed traps of 1×1 m placed side by side. We assured that each deposition site was set at least 30 m apart from each other. Density of fruiting palms varied widely across deposition sites in all sites, and some deposition sites were set distant from any palm trees (hereafter, referred as isolated deposition sites). Seed traps were monitored and dispersed seeds collected every 40 days during the fruiting season of *E. edulis*, which usually spans around 3 months from April to September depending on the area (Galetti, Zipparo, & Morellato, 1999). Dispersed seeds were collected and stored in a paper bag until further work at the laboratory where we measured their diameter (mm) on an individual basis. We depicted the local environmental heterogeneity of the forest canopy within an 8 m radius from each deposition sites by recording two sets of variables. The first

set includes variables that characterize the conspecific neighborhood, including: (i) the number of fruiting palms; and (ii) the distance to the nearest fruiting palm. The second set of variables depict forest canopy structure in terms of: (i) distance to the nearest gap opening; (ii) number of total trees with dbh > 10 cm; (iii) and dbh>30cm; (iv) percentage of open canopy above deposition sites; (v) leaf area index (LAI); and (vi) canopy height. Percentage of open canopy and LAI were estimated from hemispherical photography after applying the software Gap Light Analyzer – GLA (Frazer, Canham, & Lertzman, 1999). The hemispherical photographs were taken with a Nikon Fisheye Converter FC-E8 (Nikon, Tokyo, Japan) and the camera was placed at the center of each deposition site, 1.30 m above the ground and orientated so that the top of each photograph pointed north. The number of fruiting palms and distance to the nearest fruiting palm were highly correlated, thus we retained only the first variable for further analysis. To depict the forest structure, we applied a principal component analysis (PCA) to a matrix containing all above mentioned variables (columns) per deposition site (rows). We retained the first PCA axis (PCA1) that explained 43% of the total variance. Positive values of PCA1 represented deposition sites with a dense and high forest canopy, while negative values indicate sites with open canopy (Fig SI1). To perform PCA analysis we used the *stats* package (v 3.4.0) implemented in R (v 3.4.0) (R Core Team, 2014).
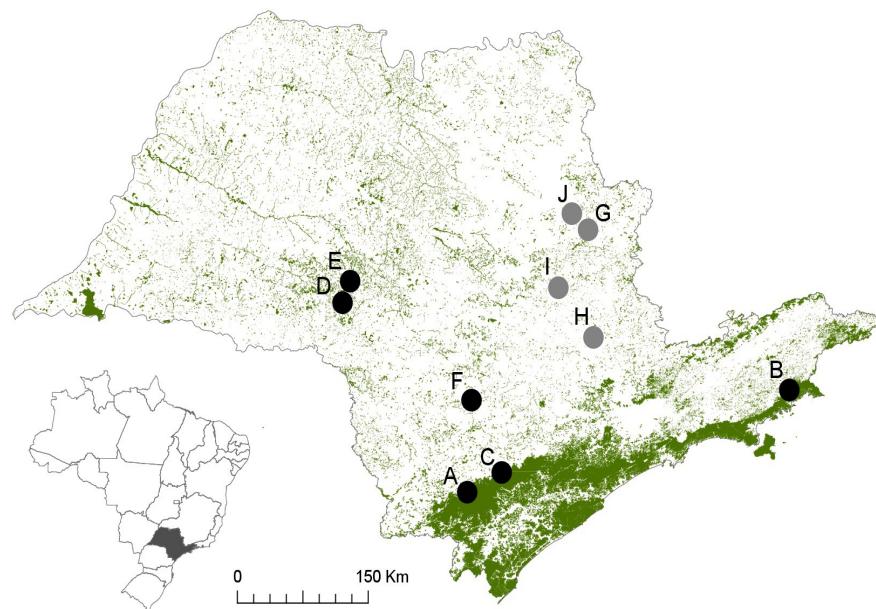
Figure 2: Distribution of 10 study sites where we characterized the diversity and spatial distribution of the maternal progenies and seed deposition patterns for *Euterpe edulis* in the Brazilian Atlantic forest. Codes indicate the level of defaunation, with A being the most faunated and J the most defaunated sites. Black and gray dots represent faunated and defaunated populations, respectively.
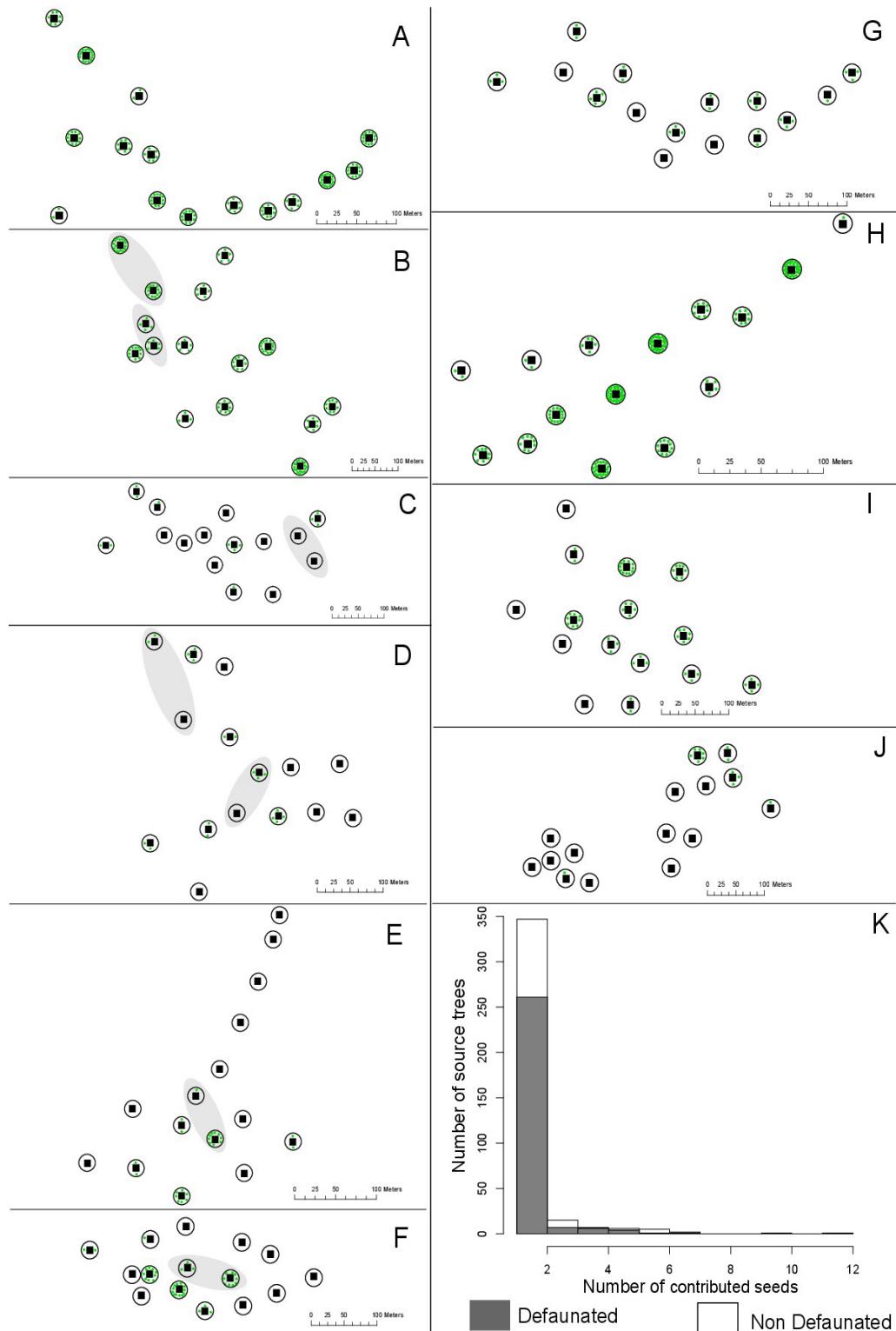
Figure 3: **A - J**. Distribution of seed deposition sites (black square) in 10 sites of *Euterpe edulis* in the Brazilian Atlantic Forest. Green dots represent fruiting palm trees in an area of 8 meters radius around deposition sites and gray shadows indicate events of shared maternal genotypes. **K**. Frequency distribution of the number of seeds dispersed from distinct maternal trees in the seed rain at defaunated and faunated forests. The population code indicates the level of defaunation, with A being the most faunated and J the most defaunated sites.

Functional defaunation index

For each site we obtained data from previous studies on the composition of the avian disperser community (Bello et al., 2017; Galetti et al., 2013) and the quantitative component of seed dispersal effectiveness of each bird species (Ribeiro 2012; Marçal 2016). Frugivory was characterized using the focal observation method on individual palms (Pizo & Galetti, 2010). The quantitative component of seed dispersal (QC) was calculated as a product of: (i) frequency of visit, (ii) number of manipulated fruits (swallowed, carried in the beak, dropped, and pecked) per visit; (iii) the probability of dispersal (proportion of fruits that were dispersed in relation to the manipulated) (Jordano & Schupp, 2000). Because several frugivorous birds were not observed during focal observations, we used the mean value of the quantitative component of seed dispersal effectiveness for each bird species, assuming that this value is fairly similar across sites. We acknowledge that this value might change across sites as a function of the bird abundance; however our approach assures that all potential dispersers are included in the estimation of the functional defaunation index in all study sites.

We adapted the defaunation index proposed by Giacomini and Galetti (2013) to measure the level of functional defaunation across frugivorous bird assemblages for each study site as:

$$D(r,f) = \sum_{k=1}^{s} \omega_k (N_{k,r} - N_{k,f}) / \sum_{k=1}^{s} \omega_k (N_{k,r} + N_{k,f})$$

Where $D(r,f)$ is the index of defaunation of focal assemblage f with respect to the reference assemblage r (i.e. most faunated site), ω_k is the species attribute of interest, in this case gape width, $N_{k,r}$ is the quantitative component of the seed dispersal effectiveness (QC) generated by species k in the reference assemblage r; $N_{k,f}$ is QC of species k in the focal assemblage f, k is the species identification ($k = 1, 2, \ldots, S$), S is the total number of species that compose the union of focal and reference assemblages (Giacomini & Galetti, 2013). The upper limit for D is 1, which indicates that all species are absent in the focal site (i.e., complete loss of species and dispersal function provided by them); $D = 0$ indicates that there is no difference between the focal and the reference assemblage (i.e., species remain in the study site and provide equal seed dispersal compared to reference site).

We sorted out all study populations by their functional defaunation index, with population A being the most faunated (D= 0) and population J the most defaunated (D= 0.73, Table 2). Despite the loss of some large-sized frugivore birds, populations A-C presented very similar

functional defaunation indexes ranging between 0 and 0.03. The frugivore assemblages of these populations were composed on average by 15 frugivores that included families of large birds such as Ramphastidae, Trogonidae, Contigidae and Momotidae; and medium-sized birds of the Turdidae family (Table SM1). Populations D-F presented intermediate functional defaunation values. Because these forests still present large birds, such as cotingas *Procnias nudicollis* and toucans *Ramphastos* spp., we considered them as faunated populations. The most defaunated sites were populations G-J whose frugivore assemblages included only species of the Turdidae family. Defaunated sites presented three times fewer frugivore species and functional defaunation indexes 30-fold higher than faunated populations (Table 2). To characterize seed deposition patterns among defaunated and partially defaunated sites (the number of dispersed seeds, number of sites that received zero seeds and number of isolated sites that received seeds), we qualitatively classified the sites between faunated (A-F) and defaunated (G-J). To investigate whether different components of the genetic diversity in the seed rain have changed as a function of the functional defaunation level, density of fruiting palms and forest canopy structure; we used functional defaunation index values.

Table 2: Characterization of the seed deposition patterns in each study site. For each site, we report the functional defaunation index; mean number of sampled dispersed seeds; average seed size per deposition site; number of identified genotypes; and number of unique genotype per population; mean effective number of mother trees per deposition sites (α diversity), effective number of mother trees per site (γ diversity), percentage of deposition site with no dispersed seeds, and percentage of deposition site with dispersed seeds but in isolated sites. SD, standard deviations. Nc= non calculated

Study site	Functional defaunation Index	Mean # of dispersed seeds (SD)	Mean seed size (SD) (mm)	# of identified genotypes	# of unique genotype	Mean α diversity (SD)	γ diversity	% of empty deposition sites (n=15)	% of isolated deposition sites with seeds
A	0	13.17 (9.31)	11.44 (0.25)	150	101	12.05 (11.16)	63.92	0	nc
B	0.01	5.06 (3.33)	11.28 (0.42)	166	117	18.73 (11.16)	64.99	0	nc
C	0.03	2.75 (4.22)	11.94 (1.04)	79	49	4.25 (6.80)	21.30	20	66
D	0.33	2.07 (4.23)	11.05 (0.61)	64	45	4.93 (6.03)	33.57	26.7	50
E	0.30	4.99 (5.85)	10.82 (0.63)	67	45	3.79 (4.13)	29.72	20	50
F	0.20	7.19 (9.69)	10.53 (0.53)	34	26	4.37 (4.65)	22.23	6.7	87
G	0.65	4.31 (4.13)	10.03 (0.30)	95	70	7.03 (6.53)	48.26	6.7	50
H	0.68	22.32 (21.67)	10.40 (0.45)	192	136	18.86 (24.16)	97.01	0	nc
I	0.68	31.26 (57.83)	9.43 (0.65)	79	66	13.56 (17.42)	44.26	20	25
J	0.69	1.15 (2.39)	9.95 (0.79)	10	9	0.81 (2.04)	8.33	60	30
Faunated	0.14	14	11.20	560	383	8.19	39.29	12.2	63
Defaunated	0.67	36	9.98	376	281	10.01	49.46	21.7	35

DNA extraction and genotyping

We identified the mother tree of each dispersed seed by genotyping its endocarp (Godoy & Jordano, 2001). We attempted to genotype at least 10 seeds from each deposition site, but we failed to reach this number at some deposition sites because fewer seeds arrived to the seed traps or due to poor DNA amplification. Overall, we successfully extracted DNA from 1330 endocarps following the protocol described by Souza et al. (in prep). All endocarps were genotyped using seven highly polymorphic microsatellite loci (EE3, EE23, EE25, EE45, EE47, EE52 and EE54) (Gaiotto et al. 2001), following PCR protocol described by Souza et al. (in prep). DNA fragments were sized in ABI Prism 3100 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA) using GeneScan Rox 500 size standard (Applied Biosystems), and they were scored using GeneMaper v.4.1 software (Applied Biosystems).

We recorded a high rate of amplification failure possibly due to DNA degradation, contamination from fungal or microbial DNA or the presence of polymerase inhibitor, such as humic acid. These recurring failures lead to a high number of incomplete multilocus genotypes. To avoid excluding incomplete multilocus genotypes we defined the minimum number of loci necessary to distinguish individuals. For that we used the genotypes of 296 adult palms from five study sites (two defaunated and three faunated) to measure the identity probability of different set of loci (Supplementary Information, Fig. SI2). We found that a minimum of three scored loci were sufficient to obtain distinct multilocus genotype for the set of 296 adults. Based on that, 936 endocarps out of 1330 (70%) were successfully genotyped for at least three loci. For this set of endocarps, all microsatellite loci showed polymorphism ranging from 22 to 27 alleles (Table SI2). We used Microchecker v 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004) to search for genotyping errors and we applied PopGenReport v 3.0.0 (Gruber, Adamack, & Gruber, 2017) to infer the frequency of null alleles that varied between 0.03 and 0.08 across loci (Table SI2). The overall missing-data load of our final data set was 15%.

Dispersal and allelic diversity analysis

We identified maternal progenies (seeds from the same mother tree) by matching the multilocus genotypes of dispersed seeds using the R package *allelematch* v 2.5 (Galpern, Manseau, Hettinga, Smith, & Wilson, 2012) that aims at identifying repeated genotypes. We assumed that they come all from the same mother tree when they for at least 3 loci. To characterize the diversity of mother trees within and across deposition sites, we estimated the

α and γ diversity of mother trees using the unbiased probability of maternal identity estimator (uPMI) that is less biased for small sample sizes (Scofield, Sork, & Smouse, 2010). uPMI estimates the probability that two seeds randomly collected from the same deposition site come from the same source tree (Grivet et al., 2005), and $uPMI^{-1}$ is the effective number of mother trees contributing to the seed rain at the deposition site (α) and study site level (γ) (Scofield et al., 2012). We calculate the δ-diversity by estimating the probability that two seeds randomly sampled from different deposition sites come from the same source tree (Scofield et al., 2010). Therefore, δ-diversity quantifies the amount of overlap in the composition of mother trees between deposition sites, where $\delta = 1$ entails no sharing of seed sources, indicating total divergence between sites, and $\delta = 0$ entails total overlap, indicating that the same set of mother trees contribute equally to both deposition sites (Scofield et al., 2012). All diversity indexes based on the uPMI estimator were obtained in https://github.com/douglasgscofield/dispersal. Finally, we inferred allele diversity in terms of: (i) allelic richness (*AR*) and (ii) alleles evenness (*Ae*) using the *vegan* v 2.4-3 R package (Dixon, 2003).

Influence of forest environmental heterogeneity and defaunation on seed rain genetic diversity
Structural equation models (SEM) evaluate multivariate direct and indirect causal relationships among different ecological factors that impact a response variable (Fan et al., 2016). Piecewise SEM combines information from multiple separate linear models into a single causal network, which allows fitting generalized linear models to a range of distributions (Lefcheck 2016). Piecewise SEM also account for hierarchy in the data by incorporating nested variables in a mixed model framework (Lefcheck, 2016). Because piecewise SEM can incorporate random structures and non-normal distributions, it is a powerful and flexible alternative to traditional variance-covariance based SEM (Lefcheck & Duffy, 2015). We set *a priori* piecewise SEM to evaluate how the number of fruiting palm trees forest canopy structure and the defaunation level impact four response variables (see Figure 4A and Table 1): (i) the effective number of contributing maternal trees (α-diversity), (ii) alleles diversity components (*AR* and *Ae*), and (iii) seed size (Figure 4A). Negative binomial GLMM and LMM were used within our SEM analysis, and we used seed deposition sites nested within site as a random effect factor. Overall fit was assessed using Shipley's test of d-separation, which yields a Fisher's C statistic and $p > 0.05$ represents a good fit (Lefcheck, 2016). Coefficients reported in the diagram (Figure 4B) are scaled by means and standard deviations so that comparisons can be made across responses (Lefcheck & Duffy,

2015). To perform piecewise SEM we applied the *piecewiseSEM* package v 1.2.1 (Lefcheck, 2016) implemented in R (R Core Team, 2014).
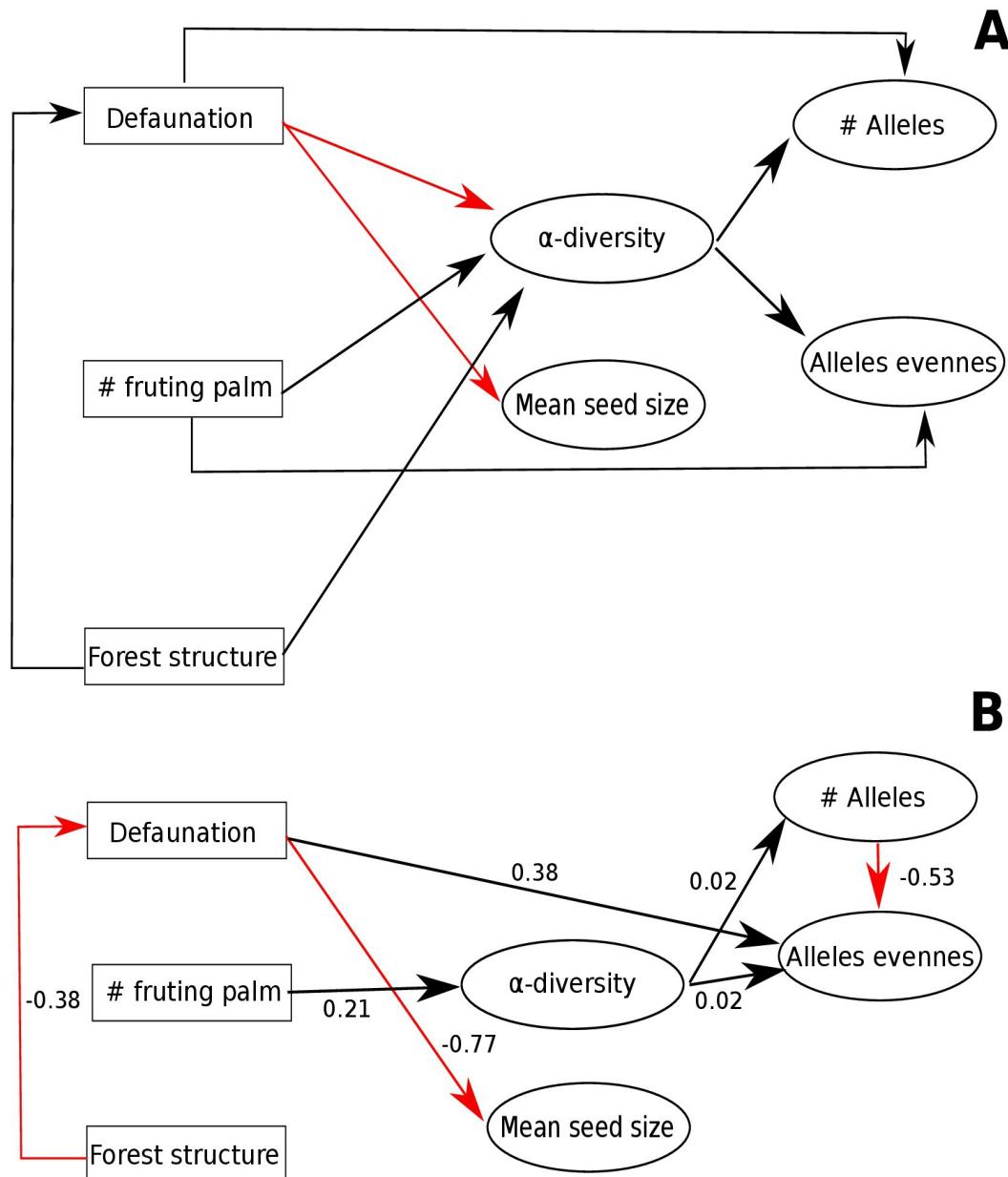
Figure 4: **A.** Structural equation model applied a priori to test for the effect of defaunation and local heterogeneity on diversity of mother trees in seed rain. **B.** Structural equation model of the effect of defaunation and local heterogeneity on diversity of maternal progenies and seed dispersal of key species palm (*Euterpe edulis*) across landscapes in Atlantic forest. Black arrows represent positive paths (P < 0.05), and red arrows represent negative paths (P < 0.05). Overall fit of piecewise SEM was evaluated using Shipley's test of d-separation: Fisher's C statistic (P>0.05, showing that no paths are missing and the model is a good fit).

3.3. Results

<u>Influence of defaunation on seeds deposition patterns</u>

The number of dispersed seeds varied across deposition sites from zero (19 % of deposition sites) to a maximum of 430. Overall, 40% of the deposition sites received between one and 10 seeds and 41% collected more than 11 seeds (Table 2). When we compared according to their defaunation level, the percentage of sites that received zero seeds was 21.7% *vs.* 12.2% in defaunated and faunated populations ($\chi^2 = 1.90$, df = 1, p = 0.11), respectively. We observed that 63% of the isolated deposition sites (those distant more than 8 m from a fruiting palm tree) contained dispersed seeds in faunated populations. In contrast, dispersed seeds reached only 35% of isolated deposition sites in defaunated populations ($\chi^2 = 3.36$, df = 1, p = 0.04). We found a mean and median of 14 and 6 seeds/deposition site, respectively, in faunated populations, varying from 0 to 93 seeds, whereas deposition sites in partially defaunated population received a mean and median of 36 and 10 seeds per site, respectively, varying from 0 to 430, suggesting a highly aggregated deposition pattern.

<u>The contribution of mother trees and alleles to the seed rain: α-, γ- and δ-diversity</u>

In total, we identified 664 mother trees (unique genotypes) contributing to the sampled seed rain composed of 936 seeds. The number of unique genotypes across the deposition sites varied from 0 to 23. We observed that the majority of the sampled maternal trees contributed with a single seed dispersal event to the seed rain. Source trees contributing with more than one seed were found in both faunated and partially defaunated sites but they were more frequent in faunated sites ($\chi^2 = 5.32$, df = 1, p = 0.01), where some trees contributed with up to 12 seeds (Figure 3K). The effective number of mother trees contributing to the seed rain varied widely among deposition sites (*α*-diversity) and across populations (*γ*-diversity) (Table 2). Some deposition sites showed a low effective number of mother trees either because few mothers contributed to the deposition site, or because mothers contributed progeny disproportionally to a given site. High numbers of dispersed seeds tended to be associated with high effective numbers of mother trees and high number of alleles, which ranged from 0 to 15 alleles/deposition site. In general, each deposition site contained seeds from a distinctive pool of mother trees (high *δ*-diversity), suggesting a highly structured seed rain in both faunated and partially defaunated populations. We only found seven instances of shared maternal genotypes between deposition sites, all of them in faunated populations (Figure 3). For these seven examples, the degree of maternal overlap is low (high *δ*-diversity) and ranged

between 0.76 to 0.90.

Influence of forest environmental heterogeneity and defaunation on genetic diversity in the seed rain

The piecewise SEM showed an acceptable global fit to the data and the Fisher's C statistic indicated that there were no missing pathways (C = 14.76, df = 16, p = 0.542). The final significant model included seven significant pathways (Figure 4B). As expected, the effective number of mother trees/deposition site (α-diversity) was positively affected by the density of fruiting palms and not associated with defaunation. Apart from the number of palms, forest canopy structure did not exert any direct influence on α-diversity. In turn, α-diversity had a significant, but weak, positive effect on the allele richness (*AR*) and allele evenness (*Ae*). Forest canopy structure was negatively associated with the functional defaunation index, with dense and high forest canopy harboring high diversity of frugivores at the study site level. Contrary to the expected, defaunation did not influence *AR*. Conversely, defaunation had a positive effect on *Ae* and a negative effect on the seed size, as found in our previous studies. Thus, high values of the functional defaunation index were associated with lower mean seed size but with higher *Ae*.

3.4. Discussion

By sampling along a defaunation gradient, we found that extant medium-sized frugivorous birds assemblages still provide dispersal services to a tropical palm species, *Euterpe edulis*. Seed deposition patterns in defaunated forests were highly aggregated and the distribution of maternal progenies did not vary at the deposition site level (similar values of α-diversity). The seed rain generated in faunated and partially defaunated populations mainly differed in four important features: (1) the percentage of seeds that reached distant seed traps; (2) the level of seed shadow overlaping; (3) the size of dispersed seeds; and (4) the allele evenness values. Our results showed that seeds reached isolated seed traps more frequently in faunated locations, where seed rains tend to overlap more than in partially defaunated populations. Furthermore, most mother trees contributed with a single seed dispersal event to a given deposition site, but trees contributing with more than one seed were more frequent in faunated populations. As a result, allele evenness values were lower in faunated populations. The effective number of mother trees also varies among deposition sites (α-diversity) and across populations (γ-diversity), and both were positively affected by the density of fruiting palms.

Overall, our results showed that medium-sized birds have an important role in providing dispersal service to *Euterpe edulis*, because even in the absence of large seed dispersers, they disperse a sizeable number of seeds from a high number of fruiting trees. However, this study confirmed that medium-sized frugivores failed to disperse large seeds and that they generated highly aggregated seed rains composed of non-overlapping seed shadows around fruiting trees, which suggest seed dispersal limitation in distance (Jordano & Godoy, 2002).

Influence of defaunation on seed deposition patterns

We found that plant populations lacking large-sized dispersers still receive quantitatively efficient dispersal services (i.e. the number of dispersed seeds) when they retain an active set of medium-sized frugivores. This pattern was found in other studies that document medium-sized seeded plants still receive dispersal service of extant small- and medium-bodied frugivores in degraded habitats (Farwig, Schabo, & Albrecht, 2017; Pérez-Méndez et al., 2015). As a result, seed removal rates or the number of dispersed seeds remain high even in partially defaunated habitats (Farwig et al., 2017; Kurten, 2013; Pérez-Méndez et al., 2015). Previous studies have attributed this unexpected result to competition release caused by the extirpation of the large seed dispersers, which results in augmented densities of small- and medium-sized frugivores (Farwig et al., 2017; Kurten, 2013). In contrast to quantitative aspects, our results showed that partial defaunation reduced some qualitative aspects of dispersal services. Hence, we show that dispersed seeds from partially defaunated populations failed to reach distant deposition sites, increasing seed aggregation around conspecific fruiting trees. Furthermore, dispersed seeds in partially defaunated populations were smaller than those dispersed in faunated populations, as expected based in our previous work (Galetti et al. 2013). Based on these results, we anticipate cascading effects of defaunation into the recruitment cycle of *Euterpe edulis:* on the one hand, the aggregation of propagules near conspecifics decreases their probability of survival (Janzen-Connell model); on the other hand, small-sized seeds (those mainly dispersed in partially defaunated populations) do not survive drying or freezing (Wyse & Dickie, 2017). Consequently, partial defaunation and increased arid conditions could interact to reduce the average population fitness and their recruitment rate under prospective warming conditions (Dybzinski & Tilman, 2012; Galetti et al., 2013).

Forest environmental heterogeneity and defaunation influence genetic diversity in the seed rain

Variation in different components of the spatially explicit genetic diversity measures (α-, γ- and δ-diversity) in the seed rain can be explained by both differences of foraging behavior between medium- and large-sized frugivores, and forest environmental heterogeneity. Thrushes (*Turdus* spp.) are the main seed dispersers in defaunated and faunated populations (Galetti et al., 2013) because they occur in pristine and degraded habitats. Their small body and gape sizes allow thrushes to consume and disperse only one or two seeds per visit when they forage on *E. edulis* (Galetti et al., 2013; Rother, Pizo, & Jordano, 2016), which results in substantial local dispersal patterns (Jordano, 2017). As a result, thrushes generate seed rains with high number of mother trees and high α- and γ-diversity both in faunated and partially defaunated populations. In contrast, large-sized frugivores, only found in faunated populations, consume large quantity of fruits per visit (Galetti et al., 2013; Rother et al., 2016) and, therefore, they disperse numerous half-sibs propagules to the same deposition site (Jordano, 2017). Consequently, fruiting trees tend to contribute with more than one seed to deposition sites in faunated locations, which increases the dominance of some alleles (low *Ae*) in the deposition sites. These results show that local genetic diversity can be still maintained in populations subjected to different levels of human-driven perturbation depending on the composition and animal life history traits of remnant frugivorous. Our results also showed a positive effect of fruiting palm density on α-diversity. This effect can be a result of an overlapping seed shadows due to high densities and short distances among conspecifics (Alcantara et al., 2000) or the attraction of seed dispersers of *E. edulis* by sites with high availability of fruit resources (Carlo & Morales, 2008). We also showed that aggregation of fruiting palm decreased dispersal distances (high δ-diversity) because frugivores tend to move shorter distances to forage (Carlo & Morales, 2008). These results show that frugivore-generated seed rains are usually clustered due to either distance restricted dispersal or contagious seed dispersal (with many seeds deposited in the same location) (Schupp et al., 2002). Despite this, our results suggest that large frugivores are able to move and disperse seeds of *E. edulis* to distant locations, once we found occasional observations events of shared maternal genotypes between deposition sites only in faunated populations (i.e. overlapped seed shadows). Partially defaunated populations do not present any overlapping seed shadows between deposition sites, which suggests that defaunation of large-bodied frugivores lead to the collapse of long-distance dispersal events. Long distance seed dispersal is usually rare but extremely important to plant survival (Jordano, 2017), because: (i) it moves propagules away from the location of close relatives, increasing seedling survival (Jansen-Connell hypothesis), (ii) it can connect disparate populations, allowing for genetic connectivity; and iii) it increases

the probability of colonizing distant and vacant habitats, helping plants to escape global warming (González-Varo, López-Bao, & Guitián, 2017). Thus, although medium-sized frugivores are able to maintain local genetic diversity in fragmented and defaunated landscapes (Carvalho et al., 2016), the loss of large frugivores impose a dispersal limitation in distance for this species, which have lasting effects on gene flow and genetic structure (Carvalho et al., 2016; Santos, Cazetta, Dodonov, Faria, & Gaiotto, 2016).

Overall, we found that extant medium-sized frugivorous birds can maintain the seed dispersal services in plants of medium-sized seeds, showing unchanged patterns of short dispersal distances and contributing the maintenance of high local genetic diversity. The admixture of maternal progenies provided by these frugivores may be crucial to the local demographic cycle of *E. edulis* because experimental studies have documented influences of the genetic relatedness among neighbor seeds on germination and seedling survival rates (Browne & Karubian, 2016; Scofield et al., 2012). Nevertheless, the loss of large-sized seed dispersers impacted the quality of seed dispersal service, mainly in terms of long distance dispersal and seed size, with anticipated negative effects in the long term for the persistence of a key palm tropical tree *E. edulis*. Our results are consistent with previous findings on the effect of defaunation on genetic diversity and differentiation of *E. edulis* populations (Carvalho et al., 2016; Carvalho, Ribeiro, Cortes, Galetti, & Collevatti, 2015). Previously, we reported that the functional loss of the large-seeded dispersers resulted in genetic differentiation among *E. edulis* populations; however, unexpectedly, we did not find evidences that defaunation would reduce genetic diversity or increased fine-spatial genetic structure at seedlings stages (Carvalho et al., 2016, 2015). Because of the lack of fine-scale information, it was attributed to time-lag effects between defaunation and genetic changes. The present result suggests that the maintenance of genetic diversity in defaunated populations is driven by the dispersal activity of remnant medium-sized frugivores in dispersing seeds of different maternal sources in the same deposition site. Thus, our study highlights that medium- and large- sized frugivores promote complementary services, by maintaining diversity by the admixture of unrelated seeds at local scale and landscape scale. In this study, we focused on the seed pool structure approach to study the distribution of genetic diversity in a seed rain in faunated and partially defaunated populations. Our next step will be to integrate information on the location of the maternal sources to estimate distance of seed dispersal and quantify how different frugivores contribute to connect highly fragmented landscapes, such as the Brazilian Atlantic forest.

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Supplementary Information

Partial defaunation and density of conspecifics impact the distribution of the maternal progenies in a frugivore-generated seed rain
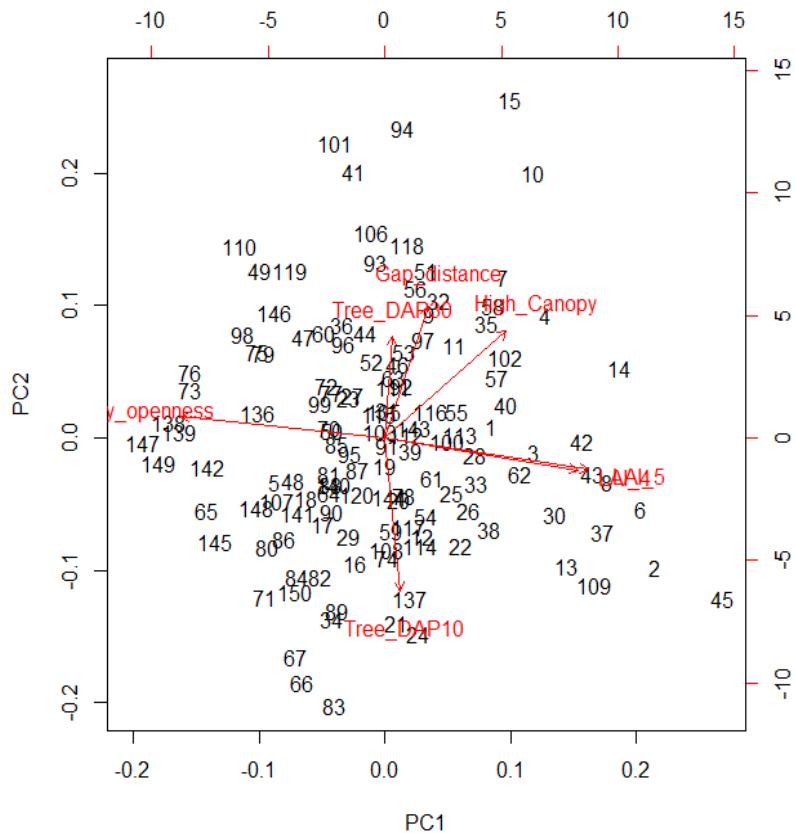
**Figure SI1** Biplot of Principal Component Analysis (PCA) to depict forest canopy structure. The x-axis explains 43 % of the variation, and the y-axis explains 18%. Gap_distance = distance to the nearest gap opening; Tree_DAP10 and Tree_DAP30 = number of total trees with dbh > 10 cm and dbh>30cm, repectively; Canopy_openess = percentage of canopy opening above deposition sites, LAI = leaf area index, High_Canopy = canopy height.

Identity probability of different set of loci

We genotyped 296 adults from five populations (two defaunated and three faunated) to evaluate the minimum number of loci required to obtain a unique genotype on an individual basis. Using the software IDENTITY v.4.0 (Wagner & Sefc, 1999), we estimated the probability of identity combining from one to seven loci. The smaller the probability of identity, the fewer the probability of identifying two random individuals displaying the same genotype at multiple loci. This analyses showed that from three loci the probability of identity is remarkably similar with the addition of new microsatellite loci set (Figure SI2). We estimated a mean probability of identity for three loci of 0.0000042, indicating that three loci are sufficient to obtain distinct multilocus genotypes for this set of 296 adults. Thus, we included this result in further analyses with endocarps and we consider all endocarps successfully genotyped for at least three loci.
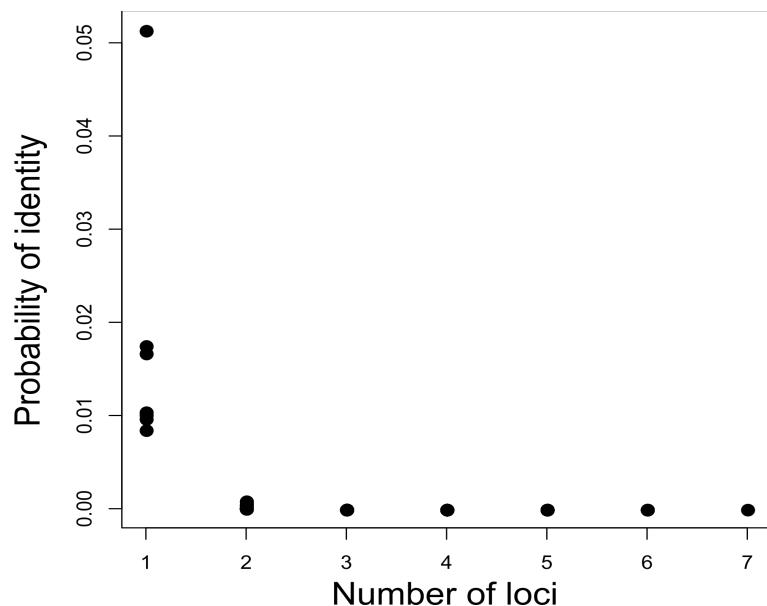

Figure SI2 Probability of individual identity estimated for adult individuals of *Euterpe edulis* using a combination from one to seven microsatellite loci.

Table SI2 Genetic characterization of the seven microsatellite loci used in this study, based on 296 adults and 936 dispersed seeds of *Euterpe edulis* from 10 sites in Atlantic Rainforest remnants in São Paulo state, Southeast Brazil. *A*, number of alleles; *Null alleles*, frequency of null alleles.

Locus	Adults (n= 296)		Endocarps (n= 936)	
	A	Null alleles	*A*	Null alleles
EE47	22	0.03	22	0.07
EE45	23	0.05	25	0.05
EE52	24	0.02	27	0.07
EE25	21	0.01	23	0.03
EE23	24	0.07	27	0.08
EE3	18	0.03	22	0.06
EE54	24	0.01	24	0.06

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4. Capítulo 3: Defaunation leads to microevolutionary changes in a tropical palm

Citação:

Carvalho CS, Galetti M, Colevatti RG, Jordano P. 2016. Defaunation leads to microevolutionary changes in a tropical palm. *Scientific Reports* 6, article number 31957.

Abstract

Many large species have declined worldwide due to habitat fragmentation and poaching. The defaunation of large frugivores and the consequent reductions of seed dispersal services may have immediate effects on plant demography. Yet, the lasting effects of frugivore defaunation on microevolutionary processes of the plants they disperse remain understudied. We tested if the loss of large seed dispersers can lead to microevolutionary changes of a tropical palm. We show that frugivore defaunation is the main driver of changes in allelic frequency among populations. Turnover of alleles accounted for 100% of dissimilarity in allelic frequencies of individuals between defaunated and non-defaunated forests; and individuals from defaunated sites are 1.5 times more similar genetically than those found in pristine sites. Given that sizeable fractions of the palm fruit crops remain undispersed in defaunated sites due to lack of large-bodied frugivores, this distinct pattern of gene pool composition of early recruits may reveal strong dispersal limitation for specific genotypes, or collapses of gene flow between fragmented areas, or both. Because most of tropical tree species rely on seed dispersal by vertebrates, our results show that defaunation has a lasting effect on microevolutionary processes, with potential consequences for persistence under scenarios of environmental change.

4.1. Introduction

Many species and populations of animals are facing a dramatic decline worldwide, a phenomenon named "Anthropocene defaunation" [1]. The decline of animals may have serious negative ecological and evolutionary consequences, especially if these animals play important roles in mutualistic processes, such as pollination and seed dispersal [2–4]. Vertebrate defaunation frequently targets large-bodied species [1] and several studies have found that the extinction of seed dispersers may have immediate effects on plant populations, such as significant reductions of fruit removal success [5], collapse of seed dispersal distance [6,7] and recruitment [8]. Size-biased defaunation of seed dispersers may also impose marked changes in the selective pressures on plants because smaller species cannot provide the same dispersal services, entailing rapid evolutionary changes in seed size [9]. A lasting consequence of these contemporary, human-driven, effects would be changes in the genetic pool caused by the loss of connectivity among fragmented populations due to the reduction of long-distance dispersal (LDD) events [6,10,11] and by filtering-out specific genotypes from the gene pool due to the selective foraging behavior of dispersal agents [12].

Given the negative effects of defaunation on plant populations, we tested the hypothesis that the functional loss of large seed dispersers may lead to microevolutionary changes of the plants that rely on their dispersal. In addition to anthropogenic impacts, the distribution of genetic variability among plant populations over large spatial scales may be also due to a combination of historic and ongoing landscape effects that ultimately influence the joint action of gene flow, selection and genetic drift [13–15]. Therefore, we explored alternative hypotheses (Table 1) defined from specific landscape attributes and used replicated areas in a diverse array of landscape conditions to test the relative importance of alternative drivers of genetic variability distribution [16].

Here, we analyzed recruits (seedlings) of the palm *Euterpe edulis* in 19 populations in the Brazilian Atlantic forest. This palm is a dominant species distributed across the Atlantic forest of South America [17]. *Euterpe edulis* produces round fleshy fruits ranging from 8.3 to 14.1 mm in diameter [18] and they are dispersed by large (e.g., cotingas *Procnias nudicolli*s, toucans *Ramphasto*s spp. and guans *Penelope* spp. and *Aburria jacutinga*) and medium-sized avian frugivores *(Turdu*s spp.) [9]. We found previously that the functional loss of the large seed dispersers due to defaunation resulted in phenotypic differentiation in seed traits among *E.*

*eduli*s populations and has been a driver for a rapid evolutionary reduction of seed size in defaunated palm populations [9].

We tested four different hypothetical scenarios to assess variation in local patterns of genetic variability, i.e., to determine which scenario best fits the observed pattern of genetic variation. We thus contrasted genetic variability patterns across replicated local palm populations in different situations according with these scenarios.

Defaunation hypothesis. Our working hypothesis is framed on the effects of loss of large-bodied frugivores and the lasting effects of the associated loss of long-distance seed dispersal and reduced recruitment success in defaunated areas [6,9,10]. Several studies have pointed out the role of seed dispersers in determining the spatial genetic structure of animal-dispersed plants [7,13,19–22]. These empirical studies provide convincing evidence of negative impacts of defaunation on tree populations, showing that the foraging behavior and movement pattern of the seed dispersers may affect seed dispersal distance, and consequently, have a lasting signal on the spatial genetic structure of plants at local scales. Large frugivores consume many fruits and a wide range of seed sizes, while smaller birds eat fewer fruits and smaller seeds (< 12 mm wide) [9]. Consistent directional selection against large seed size in defaunated areas [9] may also drive changes in allelic frequencies, with the gene pools of the populations that lost the large seed dispersers being more similar when contrasted with "control" stands in pristine areas, where the full assemblage of mutualistic avian frugivore species is present. Thus, the loss of large seed dispersers may drive microevolutionary changes among *E. edulis* stands due to a selection against large seed size and to the collapse of the long-distance seed dispersal events, impairing effective gene flow (see e.g., Karubian et al. [22], documenting small-scale genetic signatures of variable foraging patterns by frugivores). Simulation models demonstrate that it is possible to find effects of divergent selection on neutral markers, mainly with the combined action of strong selection and very low migration rate [23]. We expect this selection to be strong in defaunated landscapes where the local extinction of large frugivores leads to dramatic decrease in fruit removal rates and seed dispersal effectiveness [10,24].

Forest fragmentation. Neotropical forests have been severely affected by habitat loss and fragmentation, resulting in biologically impoverished patches [25,26]. Forest fragmentation may lead to drastic reductions in population size and may increase the spatial isolation of populations [27]. Population isolation may raise inbreeding levels by increasing the probability

of mating between closely related individuals and self-pollination due to, for example, changes in the composition and behavior of pollinators [28]. Isolation may also limit the dispersal among populations, reducing gene flow and population connectivity [28]. Therefore, we predicted that forest fragmentation, measured by percentage of forest cover, would lead to microevolutionary changes among *E. edulis* populations.

Biogeographical origin*.* The Atlantic forest of South America is heterogeneous in climate, relief and vegetation type [29] and can be divided into several biogeographical regions [30]. Biogeographical region may also influence the distribution of genetic variability through evolutionary time (phylogeographic effects) and impose distinct selective pressures leading to microevolutionary changes among populations with distinct biogeographical origins. For example, at the community level, plant species composition differs between biogeographical regions composed by rain forest and by semideciduous forest, and plant composition may further include species that are able to cope with a longer dry season [31]. Thus, individuals from the same biogeographical region (here rain forest or semideciduous forest) may share similar gene pool features due to dispersal limitation combined to local adaptation, and we would thus expect microevolutionary changes among individuals from rain forest to semideciduous forest.

Sampling design. We sampled the *E. edulis* individuals in delimited and isolated populations with adequate replication across sample groups (strata) defined by the previous hypotheses. We also tested the effects of sampling design as a potential influence for the observed genetic variability distribution, i.e., a situation where differences would emerge by chance effects related to the specific populations sampled.

Table 1 Correct assignment statistics and *β* diversity analysis of individuals of *Euterpe edulis* in Atlantic forest in Southeast Brazil, into groups according to different hypotheses driving genetic differentiation. Hypothesis - hypothesis for main drivers of microevolutionary changes; Predictions - predictions for main drivers of microevolutionary changes; Kappa - kappa estimator for correct assignment, the larger is the kappa estimator, the greater is the support of the data for a hypothesis; CI - 95% Confidence Interval; *β*-allelic diversity - *β*-allelic diversity using Bray-Curtis's dissimilarity index; SD - Standard Deviation

Hypothesis	Predictions	Kappa [CI]	*β*-allelic diversity [SD]
Defaunation: The loss of large seed dispersers may lead to microevolutionary changes among populations due to a selection against large seed size and to the collapse of the long-distance seed dispersal events.	High correct assignment (Kappa) and higher *β*-allelic diversity among non-defaunated than defaunated sites	0.92 [0.88 - 0.95]	Defaunated sites: 0.49 [0.10] — Non defaunated sites: 0.64 [0.09]
Biogeographical regions: Rain forest and semideciduous forest may lead to microevolutionary changes among populations due to their influence on the distribution of genetic variability through evolutionary time, and due to the distinct selective regimes imposed by biogeographical differences between the two regions.	High correct assignment (Kappa) and *β*-allelic diversity among rain forest sites similar to semideciduous forest sites	0.92 [0.88 - 0.96]	Rain forest sites: 0.53 [0.11] — Semideciduous forest sites: 0.58 [0.10]
Forest Fragmentation: Forest fragmentation may led to microevolutionary changes among populations because of the associated drastic reductions in population size, and may also increase the effect of spatial isolation among populations.	High correct assignment (Kappa)	0.75 [0.69 - 0.80]	-
Sampling design: Given that individuals were sampled in delimited and isolated populations, the sampling design may generate differences in the gene pool among populations.	High correct assignment (Kappa)	0.58 [0.54 - 0.62]	-

4.2. Results

We found that the hypotheses based on defaunation and biogeographic regions lead to significant microevolutionary changes among populations of *Euterpe edulis* (Table 1, Fig. 1). Both hypotheses had the greatest discrimination power and correct assignment of individual palm genotypes to the *a priori* groupings defined by the hypotheses (represented by kappa index), followed by forest fragmentation and sampling design (Table 1 and Fig. 1). As expected, adscription to biogeographic areas had a significant effect on the differentiation of the genetic pools (kappa estimator of correct assignment, 0.92 [0.88 - 0.96]). However, the kappa value for the defaunation hypothesis was very similar (0.92 [0.88 - 0.95]) despite the fact of including populations from either biogeographic origin in each of the groups being compared. The cluster comprising individuals from defaunated populations showed more variability in discriminant function scores when compared to the cluster including individuals from non-defaunated sites and to the clusters related to the hypothesis of biogeographical regions effects (Supplementary Information Fig. S1 and S2).

We assessed how defaunation and biogeographical regions have influenced genetic variability and contemporary effective population sizes across the sites. We found that microevolutionary changes between defaunated and non-defaunated sites and between rain forest and semideciduous forest were not due to changes in genetic variability or contemporary effective population size (see Table S4 for genetic variability of each site). Models containing defaunation and biogeographical regions as explanatory variables did not predict variation in genetic diversity (Defaunation: df= 1, F= 0.02, p-value= 0.90; Biogeographical regions: df= 1, F= 1.00, p-value= 0.33), allelic richness (Defaunation: df= 1, F= 2.29, p-value= 0.15; Biogeographical regions: df= 1, F= 0.05, p-value= 0.82), inbreeding coefficient (Defaunation: df= 1, F= 1.14, p-value= 0.30; Biogeographical regions: df= 1, F= 0.57, p-value= 0.46) and contemporary effective population size (Defaunation: df= 1, F= 1.60, p-value= 0.22; Biogeographical regions: df= 1, F= 0.48, p-value= 0.50).

We analyzed whether differences in allelic richness (*β-allelic diversity*) among defaunated and non defaunated sites or among sites from distinct biogeographic regions is due to alleles replacement (turnover, *βsim*) or richness differences driven to passive allelic loss (nestedness-resultant dissimilarity, *βsne*) [32]. The turnover component of allelic richness measures the

replacement of alleles in one site by different ones in other site. On the other hand, the nestedness component measures allelic gain or loss; for instance, the alleles of one site with low number of alleles are a subset of alleles of other site containing a high number of alleles [32]. A nested pattern may thus represent two main situations: the alleles missing in populations with lower allelic richness were lost by drift, or the population with higher allelic richness presents ancient alleles that were spread in all populations and new mutations which have not yet widespread. We found that 100% of dissimilarity in allelic richness among palm recruits in defaunated and non defaunated sites (Mean *βdiversity* = 0.64, 95% CI = 0.63 to 0.64; Mean *βsim* = 0.64, 95% CI = 0.63 to 0.64; Mean *βsne* = 0.00, 95% CI = 0.00 to 0.00, Supplementary Information Table S5) and among rain forest and semideciduous forest (Mean *βdiversity* = 0.61, 95% CI = 0.60 to 0.62; Mean *βsim* = 0.61, 95% CI = 0.60 to 0.62; Mean *βsne* = 0.00, 95% CI = 0.00 to 0.00, Supplementary Information Table S5) is due to allele turnover. Moreover, we found that palm recruits from defaunated sites are genetically more similar than those from non-defaunated sites; and recruits from rain forests sites are genetically more similar than those from semideciduous forests. The *β*-allelic diversity and its components are lower among palm recruits in defaunated sites (Mean *βsim* = 0.49, SD = 0.10, Mean *βsne* = 0.00, SD = 0.00, Table 1) compared to non defaunated sites (Mean *βsim* = 0.64, SD = 0.09, Mean *βsne* = 0.00, SD = 0.00, Table 1) and lower among individuals in rain forest (Mean *βsim* = 0.53, SD = 0.11, Mean *βsne* = 0.00, SD = 0.00, Table 1) compared to semideciduous forest (Mean *βsim* = 0.58, SD = 0.10, Mean *βsne* = 0.00, SD = 0.00, Table 1).

Finally, we estimated the contribution of spatial effects on genetic differentiation of *E. edulis*. We performed Redundancy Analysis (RDA) using the forward selection method and found that the model including the effects of defaunation and biogeographical regions, but not spatial effects, was the best to explain variation in allele frequencies among sites (R^2adj = 0.12, p-value = 0.01).
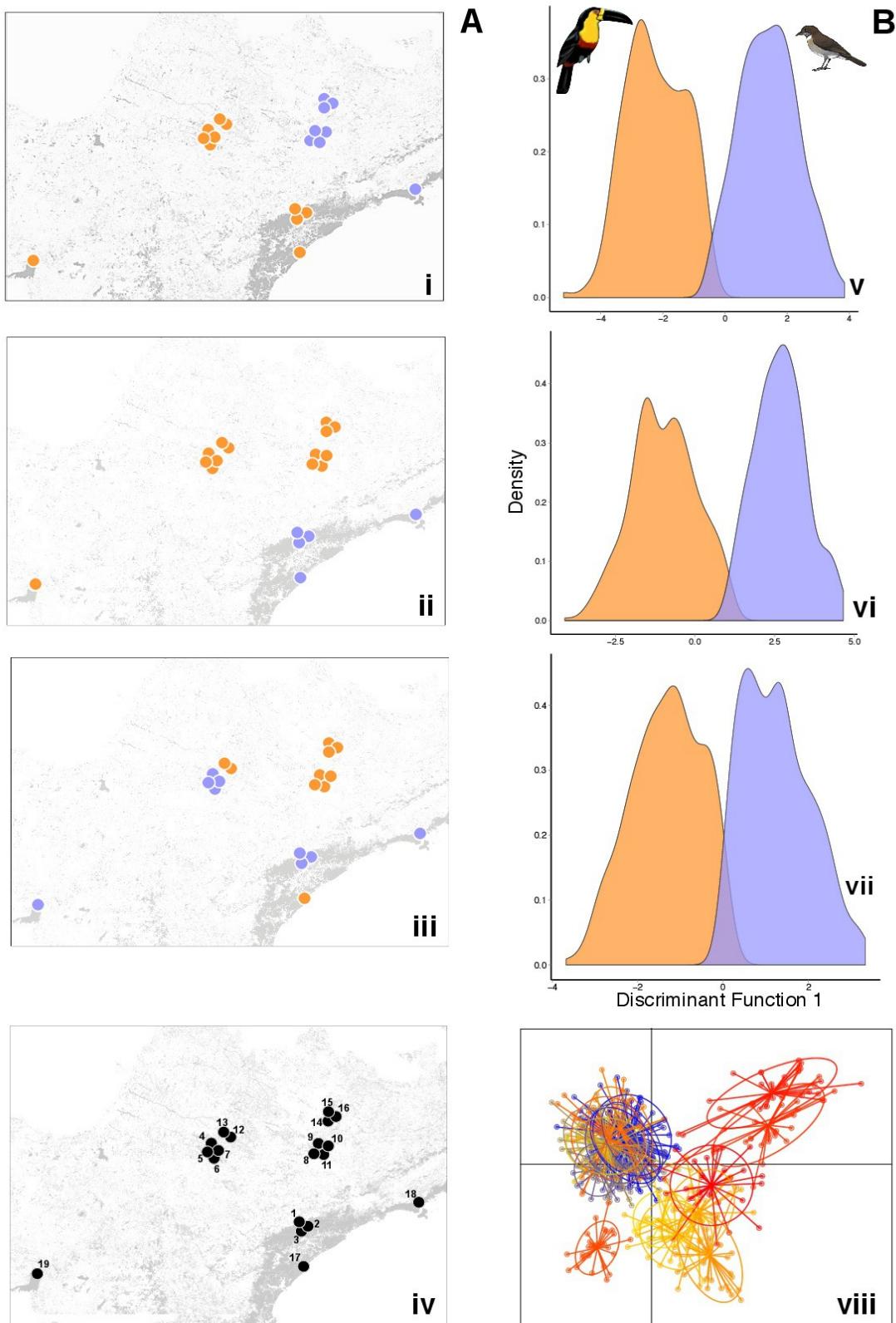
Figure 1: A) Arrangement of sampled sites into groups according to the present-day complexity patterns of the landscape that led to formulation of alternative genetic differentiation hypotheses for *Euterpe edulis* within the Atlantic forest hotspot in Southeast Brazil. Blue and orange colors indicate the groupings of samples being compared under each hypothesis tested: i) Defaunation hypothesis: light blue circles represent sites with functional extinction of large seed dispersers and light orange circles represent sites with the full

assemblage of mutualistic avian frugivore species. ii) Biogeographical regions hypothesis: light orange circles represent sites that are in semideciduous forest and light blue circles represent sites that are in rain forest. iii) forest fragmentation hypothesis: light orange circles represent small size sites and light blue circles represent large size sites. iv) Sampling design hypothesis: numbers represent distinct sampling sites. **B)** Frequency distribution of scores on the first discriminant function for individual genotypes of 545 *Euterpe edulis* seedlings in the Atlantic forest in Southeast Brazil. v) Defaunation hypothesis, vi) Biogeographical regions hypothesis, vii) Forest fragmentation hypothesis, viii) Sampling design hypothesis. The maps were generated using QGIS (www.qgis.org/en/site/) based on a map from SOS Mata Atlântica/INPE (http://mapas.sosma.org.br/). The birds were drawn by Carl Buell.

4.3. Discussion

Our results indicate that the defaunation of large seed dispersers has a distinct signal on large-scale genetic variability, potentially arising from microevolutionary changes in the palm populations. This supports and extends previous findings [9] providing evidence for a deeper far-reaching consequence than previously thought. There has been multiple recent evidence demonstrating that the extinction of seed dispersers leads to a decline in recruitment success [8,33] and an increase in plant extinction risk [34]. In addition, previous research has associated seed dispersal limitation with genetic consequences consistent with the loss of gene flow among populations [11]. By assessing realized dispersal (early seedlings established) our study demonstrates for the first time that defaunation may lead to changes in the frequency of alleles among populations, potentially driving long-term homogenization of genetic pools within plant populations from defaunated sites.

We found that defaunation leads to genetic changes in *Euterpe edulis* populations. This could be a synergistic effect from a higher frequency of mating among closely-related individuals, a reduction of effective population size, limited long distance dispersal among populations [11,15,35], and rapid evolutionary changes in the seed size of *E. edulis* populations in defaunated areas [9]. Our results suggest that the observed genetic changes were not due to a higher frequency of mating among closely-related individuals or reduction of population size. Instead, the results suggest that genetic changes among *E. edulis* populations were consistently associated with scenarios of strong seed dispersal limitation [9] and/or collapse of gene flow among fragmented areas [11]. We found that the allelic richness differences among defaunated and non-defaunated sites are largely attributable to allele replacement. Moreover, we found that palm recruits from defaunated sites are genetically more similar than recruits from non-defaunated sites. The altered seed rain composition due to reduction of fruit

removal success in defaunated sites [9] might be determined by distinct combinations of recruited genotypes in these disturbed populations, creating a lasting signal in the composition of the gene pool [12,36]. This suggests that strong selection pressure imposed by defaunation could result in changes of allelic frequencies among defaunated and non defaunated sites. Moreover, our results suggest that a similar selective pressure (lack of large-bodied seed dispersers) has led to a homogenization in terms of allelic frequency across palm populations in defaunated sites [37].

Our results also showed that biogeographical origin has a signature on the genetic structure of *E. edulis* populations, with allele replacement being the main driver of allelic richness differences between rainforest and semideciduous forests. These two biogeographical regions show marked differences in annual rainfall and predominant soil types [38]. There are currently few studies that compare differences in biodiversity composition (genotype, phenotype and species level diversities) between these two forest types [31,39,40]. Nevertheless, the few available studies show ample differences in plant community composition [31] among these biogeographical regions. For example, species in semidecidous forest are able to cope with longer dry seasons [31]. Thus, we would expect differences in genetic composition among plant population between rain and semidecidous forest, given the differences among plant community composition between the two biogeographical regions and the potential for a marked phylogeographic signature on genetic diversity. These signals of biogeographical regions on genetic variability distribution can be a result of dispersal limitation within each of the two forest types, genetic drift, and local adaptation of *E. edulis* to these different domains of the Atlantic forest [35]. These historical influences would be "overlaid" on top of the defaunation effects: the DAPC directly contrasting the replicated populations in defaunated and non-defaunated scenarios (i.e., ignoring biogeographic region) indicated that the loss of large dispersers had a distinct signal on genetic structure of *E. edulis* populations, remarkably differentiating the two scenarios despite the variation due to forest physiognomy. Remarkably, the magnitude of this effect appears as large as the magnitude of differentiation when comparing populations from the two biogeographic regions. The large-scale fragmentation and defaunation in Atlantic forest is a recent anthropogenic impact. Despite that, our results reveal that the strong selective pressure imposed by defaunation on the genetic pool of *E. edulis* can be comparable in terms of its effects size with signals left by biogeographical process. If we assume that smaller values of *β*-allelic diversity represent a higher homogenization of genetic pools among populations, ongoing defaunation is driving a

stronger genetic homogenization than we would expect just from historical, phylogeographic dynamics.

Our results provide a rigorous test, within an adequately replicated setup of distinct landscape types, of the hypothesis that contemporary defaunation is affecting the diversity of genetic pools of animal-dispersed tropical plants. This supports previous findings [10,19,33,41,42] and strengthens the evidence for pervasive alterations of forest recruitment in disturbed tropical landscapes. Moreover, our results contradict predictions [43] that rapid genetic changes are not as important as ecological degradation in human-disturbed habitats [15]. Our previous studies showed that the loss of large-bodied frugivores and different biogeographical origin are causing subtle changes in the selection regimes that drive rapid phenotypic evolution in *E. edulis* [9]. We found that this rapid evolutionary change in seed size and limited long distance seed dispersal due to the functional extinction of large frugivores are strong enough to have imprinted the gene pool characteristics of plant populations. These results have broad implications because most tree species are animal dispersed in tropical forests [44] and defaunation of large frugivores is becoming omnipresent in fragmented and non-fragmented forests [9]. Therefore, the lack of seed dispersers is not only leading to phenotypic changes but also genotypic diversity and gene pool structure, with unknown effects on the long-term persistence of plant species and entire communities. This genetic erosion due to defaunation may be crucial to plants facing ongoing climate change scenarios.

4.4. Material and Methods

Study sites and species

The Brazilian Atlantic forest is an ideal system to test the effects of anthropocene defaunation on ecological and evolutionary processes. The Atlantic forest originally covered from northeast to south of the Brazilian territory and parts of Argentina and Paraguay [45], but today it has been reduced to 12% of its original 150 million ha [46]. This reduction entailed a sustained and dramatic process of habitat fragmentation during the last ~ 500 yr resulting in islands of wild habitat surrounded by crops, pastures and urban matrix and an alarming loss of biodiversity [45]. The Brazilian Atlantic forest can be divided into several biogeographical regions based on climate, altitude and biodiversity composition [30]. Our study covers two of these biogeographical regions: Serra do Mar (here after rain forest) and Interior (here after semideciduous forest). Rain forests occur along the Brazilian Atlantic coast and receive high

annual rainfall, whereas semideciduous forests have marked rainfall seasonality and occur in the inland Atlantic forest [47].

The heart of palm (*Euterpe edulis,* Arecaceae), a threatened Atlantic forest species, was once one of the dominant palms in this ecosystem [17]. The species is present in rain forests and semideciduous forests, where it is restricted to wet microhabitats. Although once abundant, this palm species is currently endangered and locally extinct in many areas owing to illegal harvesting of the edible meristem (heart of palm; [48]). *E. edulis* is a self-compatible monoecious species, but with predominant outcrossed reproduction [49] and pollination performed mainly by small-sized bees (e.g., *Trigona spinipes*). Their fruits are eaten by more than 58 birds and 20 mammalian species but are dispersed mostly by a reduced subset of large frugivorous birds and thrushes (*Turdus* spp.; [9,50]).

Sampling design and hypotheses for genetic differentiation

We sampled 19 sites from two different biogeographical regions, rain forest and semideciduous forest. The sites had a distinct percentage of forest cover (Supplementary Information Table S1) and well documented bird community composition (Supplementary Information Table S6). Historically, all sites shared a similar assemblage of seed dispersers, but forest fragmentation and hunting have impoverished the assemblage of large frugivores in many sites [9]. We sampled 30 seedlings (total of 545 individuals) of *E. edulis* in each site. To avoid or minimize the effects of spatial genetic autocorrelation, we spaced the sampled seedlings at least 10 m apart. This sampling design allowed us to test distinct hypotheses, once we sampled small and large patches sizes with distinct bird compositions and biogeographical regions (Fig. 1A, see details in Supplementary Information Table S1).

Genetic analysis

We isolated genomic DNA by using the CTAB extraction procedure and genotyped all individuals using eight microsatellite loci (EE5, EE8, EE25, EE43, EE45, EE47, EE52, EE63), following PCR protocol described by Gaiotto et al. [51]. We sized DNA fragments on an ABI Prism 3100 automated DNA sequencer (Applied Biosystems, CA) with the GeneScan ROX 500 size standard (Applied Biosystems, CA), and scored alleles with GeneMapper v4.1 software (Applied Biosystems, CA). We genotyped ten percent of all individuals two times in independent PCR amplifications to check for genotyping error and, we also checked for alleles dropout and null alleles (see details on Supplementary Information Table S2).

Testing hypotheses for genetic differentiation

We used Discriminant Analysis of Principal Component (DAPC [52]) to find out which hypothesis (defined as *a priori* groupings of the local palm populations according to defaunation status, biogegraphical region, forest fragmentation status and sampling) maximized the differences among groups (clusters). The sampled palm populations were defined according to a replicated design across the different strata defined by each hypothesis (see Fig. 1A). DAPC relies on data transformation using Principal Component Analysis as an *a priori* step to a Discriminant Analysis [52]. DAPC optimizes the separation of individuals into the pre-defined groups [52]. We defined these groups of sampled genotypes according to each of the different hypotheses and ran separate DAPC analyses for each. We used the DAPC to estimate the percentage of correct assignment (proportion of genotypes correctly assigned to their actual sample group) to test how well each hypothesis could discriminate among palm recruits based on grouped genotypes [52]. Thus, we are not using DAPC as an exploratory analysis to find k-clusters, but as a statistical method to test alternative hypotheses about differences among pre-defined groups of samples. To carry out the analysis, we first classified the individuals into groups according to each hypothesis (e.g., samples coming from defaunated sites *vs.* samples from pristine sites). Then, we conducted a DAPC and calculated the proportion of correct assignment of individuals into each hypothesis (defaunation, biogegraphical regions, forest fragmentation and sample design). To avoid over-fitting, we retained 50 principal components, corresponding to 82% of the total genetic information. We carried out the DAPC using the R package *adegenet* [53]. To verify whether the proportion of assignment of each hypothesis differed from a random distribution, we constructed a null model with 1000 permutation and a 95% confidence interval. We also calculated the Kappa estimator, which estimates the mean proportion of corrected assignment after removing correct assignment by chance [54]. We interpreted the hypothesis that had highest proportion of corrected assignment as the most likely hypothesis for distribution of genetic variation.

Genetic variability and contemporary effective population size

We assessed the influence of defaunation and biogegraphical regions on genetic variability and contemporary effective population size across the study sites. We estimated genetic diversity (H_e - expected heterozygosity under Hardy-Weinberg equilibrium, following Nei [55]), allelic richness based on rarefaction analysis (AR [56]) and inbreeding coefficient (f, obtained from analysis of variance of allelic frequency [57]) using the software FSTAT 2.9.3.2 [58]. We also

estimated contemporary effective population size (Ne), using the software NeEstimator [59]. To test whether defaunation and biogegraphical regions are correlated with variation in genetic variability and contemporary effective population size, we conducted a generalized linear model (GLM), after testing for spatial autocorrelation (see details on Supplementary Information Table S3).

Turnover component of allelic richness

The difference in allelic richness among sites may be mainly due to allele replacement (turnover) or richness differences (nestedness-resultant dissimilarity [32]). We estimated each component using the R package *betapar* [60], which partitions allelic richness dissimilarity into turnover and nestedness components. In contrast to Diniz-Filho et al. [32] that estimated these components using presence/absence of alleles, we carried out the analysis using allelic frequency. We used the Bray-Curtis's dissimilarity index to estimate β-allelic diversity and its components, and we also calculated the relative importance of the turnover component for the overall allelic richness dissimilarity. We carried out the β-allelic diversity analysis in two ways. First, we randomly sampled all palm recruits from one site of each group of each hypothesis (i.e. one from defaunated and other from non-defaunated sites or one from rain forest and other from semideciduous forest) and estimate the β-allelic diversity and its components among these groups (i.e. β-allelic diversity between defaunated and non-defaunated sites or between rain forest and semideciduous forest). This procedure were carried out 499 times (bootstrapping) and we obtained the mean, standard deviation and 95% confidence interval for each β diversity component. We constructed a null model with 1000 permutation and a 95% confidence interval to verify whether the results of the β diversity analysis for each hypothesis differed from random. With this analysis we could estimate the importance of each β-allelic diversity component for the overall allelic richness dissimilarity between defaunated and non-defaunated sites or between rain forest and semideciduous forest. Second, we randomly sampled all palm recruits from two sites of each group (i.e. two sites from defaunated sites) and estimate the β-allelic diversity and its components among the sites from each group. We carried out this procedure 499 times (bootstrapping) and obtained the mean, standard deviation and 95% confidence interval for each β diversity component. The same was done for all groups of all hypotheses (defaunated, non-defaunated, rain forest and semideciduous forest). With this analysis we could compare and contrast the β-allelic diversity results between defaunated and non-defaunated sites or between rain forest and semideciduous forest. For example, we expected that β diversity might be lower among

individuals in defaunated sites compared to non-defaunated sites.

Spatial component of genetic differentiation

The spatial distribution of individuals and populations are also important factors that affect genetic differentiation patterns. For example, some populations can follow an isolation by distance model while others may follow an island model. To deal with spatial effects on genetic differentiation, we conducted a Redundancy Analysis (RDA). RDA is a multivariate method that assesses the influence of a matrix of independent variables (e.g. geographic coordinates) on a matrix of dependent variables (e.g. alleles frequency) [61]. RDA also allows the use of environmental data as independent variables [61]. We performed a RDA using an allele frequency matrix as the response variable and the spatial variable, defaunation and biogeographical regions as the explanatory variables. For RDA model selection, we performed RDA using the forward selection method with double stop criteria of Blanchet [62]. To reduce the incorporation of too many variables into the model and to not inflate type I error, Blanchet et al. [62] proposed a forward selection based on two criterion: the forward selection is stopped if either the significance level alpha is reached or the global adjusted coefficient of multiple determination (R^2_{adj}) is exceeded. We used the *adegenet* R package [53] to calculate allele frequencies for each site and *packfor* [63] and *vegan* [64] R package to carry out RDA and forward selection.

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Supplementary Information

Defaunation leads to microevolutionary changes in a tropical palm

<u>Sampling design and hypotheses for genetic differentiation</u>

We sampled 19 sites from different biogeographical regions (rain forest and semideciduous forest sites), with distinct forest cover and well known bird community composition. We subdivided the sites into small and large patch size. To be considered in the small patch size category, sites should have less than 200 ha and a percentage of forest cover within a radius of 500 m lower than 30% (threshold in forest amount to severe species loss, low genetic diversity and high genetic differentiation). On the other hand, to be included into the category of large patch size, sites should have more than 500 ha and percentage of forest cover above 40%. Patch size was measured using the mapping with visual digitalization and classification at the scale of 1:5,000 using high-resolution 1x1 m images available at Google Earth (http://earth.google.com). QGIS (www.qgis.org) software was used to access the images on the fly with the OpenLayer plugin (OpenLayer 2013).

We classified sites into defaunated and non-defaunated categories. We considered as defaunated sites those which present functional loss of large frugivores (mean gape width > 12 mm following Galetti et al. 2013). The data on frugivore community composition were obtained from Galetti et al. 2013, the WikiAves dataset (www.wikiaves.com) and personal observation. We used the classification of Veloso to separate the sites into rain forest and semideciduous forest. Table S1 shows the characteristics of each site.

Table S1 Sampling location for the 19 sites of *Euterpe edulis* in Atlantic Forest in Southeast Brazil. N, number of individuals sampled. Defaunation status – Defaunated = fuctional loss of large frugivores. Habitat loss status – small patch = Patch size < 200 ha and landscape forest cover < 30%, large patch = Patch size > 500 ha and landscape forest cover > 40%. Biogeographical regions – Rain forest, Semideciduous forest.

Sites	N	Defaunation status	Fragmentation status	Biogeographical regions
1	30	Non-Defaunated	Large patch	Rain forest
2	30	Non-Defaunated	Large patch	Rain forest
3	30	Non-Defaunated	Large patch	Rain forest
4	30	Non-Defaunated	Large patch	Semideciduous forest
5	30	Non-Defaunated	Large patch	Semideciduous forest
6	30	Non-Defaunated	Large patch	Semideciduous forest
7	31	Non-Defaunated	Large patch	Semideciduous forest
8	14	Defaunated	Small patch	Semideciduous forest
9	28	Defaunated	Small patch	Semideciduous forest
10	28	Defaunated	Small patch	Semideciduous forest
11	30	Defaunated	Small patch	Semideciduous forest
12	30	Non-Defaunated	Small patch	Semideciduous forest
13	30	Non-Defaunated	Small patch	Semideciduous forest
14	31	Defaunated	Small patch	Semideciduous forest
15	31	Defaunated	Small patch	Semideciduous forest
16	30	Defaunated	Small patch	Semideciduous forest
17	28	Non-Defaunated	Small patch	Rain forest
18	28	Defaunated	Large patch	Rain forest
19	26	Non-Defaunated	Large patch	Semideciduous forest

Genetic analysis

All pairs of microsatellites loci were in linkage equilibrium (all p > 0.05) and there was no evidence of genotyping errors or null alleles (results not shown). All loci presented high genetic variability, but the observed heterozygosity differed from the expectation under Hardy-Weinberg equilibrium for all loci (all p < 0.001, Table S2).

Table S2 Genetic characterization of the eight microsatellite loci used in this study, based on 545 individuals of *Euterpe edulis* from 19 sites in Atlantic Forest remnants in Southeast Brazil. *A*, number of alleles; H_e, expected heterozygosity; H_o, observed heterozygosity; F_{IS}, inbreeding coefficient (* significant, p < 0.001); SD, standard deviation.

Locus	*A*	H_e	H_o	F_{IS}(SD)
EE5	25	0.668	0.527	0.218 (0.047)*
EE8	22	0.728	0.615	0.161 (0.037)*
EE43	13	0.737	0.564	0.226 (0.057)*
EE52	25	0.874	0.661	0.254 (0.052)*
EE63	19	0.738	0.643	0.118 (0.041)*
EE25	23	0.853	0.678	0.218 (0.048)*
EE47	24	0.750	0.684	0.102 (0.032)*
EE45	19	0.745	0.509	0.327 (0.048)*
Overall loci		0.762	0.610	0.197 (0.025)*

Genetic variability and contemporary effective population size

To account for the presence of autocorrelation, we tested models with distinct spatial covariance structures (Gaussian, Exponential and Spherical) using the restricted maximum likelihood (REML) method in a GLM and compared with a model without spatial covariance structure. These models contained defaunation and biogeographical regions classification as explanatory covariates. To find the best spatial covariance structure, we compared the models using Akaike Information Criteria (AIC). Models with ΔAICc< 2 were considered as equally plausible to explain the observed pattern.

Table S3 Model selection of model with distinct spatial covariance structures (Gaussian, Exponential and Spherical) and a model without spatial covariance structure for H_e (expected heterozygosity – genetic diversity), AR (allelic richness), F_{IS} (inbreeding coefficient) and Ne (contemporary effective population size) in 19 sites of *Euterpe edulis* in Atlantic forest remnants in Southeast Brazil. These models contained defaunation and biogeographical regions classification as explanatory variables. K, number of parameters estimated for each model; ΔAICc, Akaike corrected for small samples

	H_e		AR		f		Ne	
	K	ΔAICc	K	ΔAICc	K	ΔAICc	K	ΔAICc
Without spatial covariance structure	5	0.0	5	0.0	5	0.0	5	0.0
Exponential	6	4.4	6	4.4	6	4.4	6	4.9
Gaussian	6	4.4	6	4.4	6	4.4	7	4.9
Spherical	6	4.4	6	4.4	6	4.4	7	4.9

Table S4 Genetic variability in 19 sites of *Euterpe edulis* in Atlantic forest remnants in Southeast Brazil estimated with 8 loci. N – number of sampled individuals; H_e - expected heterozygosity; H_o - observed heterozygosity; AR - allelic richness; F_{IS} - inbreeding coefficient; Ne - contemporary effective population size.

Site	N	H_e	H_o	AR	F_{IS}	Ne
1	30	0.766	0.564	8.8	0.264	310.0
2	30	0.808	0.571	8.6	0.295	209.2
3	30	0.787	0.591	8.6	0.248	Inf
4	30	0.815	0.695	9.0	0.148	40.3
5	30	0.781	0.702	7.5	0.101	35.8
6	30	0.786	0.631	9.2	0.197	767.3
7	31	0.799	0.651	7.4	0.186	20.7
8	14	0.864	0.661	9.0	0.235	143.0
9	28	0.736	0.631	6.9	0.143	48.5
10	28	0.799	0.627	8.3	0.216	61.4
11	30	0.729	0.597	6.3	0.182	3.1
12	30	0.794	0.633	8.6	0.203	18.4
13	30	0.724	0.629	7.5	0.131	17.8
14	31	0.716	0.551	6.2	0.229	19.5
15	31	0.724	0.524	6.4	0.275	6.9
16	30	0.816	0.581	8.3	0.288	22.6
17	28	0.654	0.602	7.0	0.079	Inf
18	28	0.691	0.585	6.7	0.153	50.4
19	26	0.677	0.560	6.0	0.172	25.5

Testing distinct hypotheses for genetic differentiation

R code:

1. Calculate proportion of observed/predicted assingment

```
Hypothesis1 <- dapc(data, paste(groups$M1), n.pca=50, n.da=2)

Hypothesis1.summary <- summary(Hypothesis1)

Hypothesis1.assign <- Hypothesis1.summary$assign.prop
```

2. Calculate proportion of random assingment using 1000 permutations

```
Hypothesis1.aleat<-numeric(1000)

for(i in 1:1000){

  groups.aleat<-sample(groups$M1)

  Hypothesis1.aleat <- dapc(data, grupo.aleat, n.pca=50, n.da=2)

  Hypothesis1.aleat.summary <- summary(Hypothesis1.aleat)

  Hypothesis1.aleat.assign <- Hypothesis1.aleat.summary$assign.prop

  Hypothesis1.aleat[i] <- Hypothesis1.aleat.assign

}
```

3. Calculate 95% CI

```
mean.Hypothesis1.aleat <- mean(Hypothesis1.aleat)

sd.Hypothesis1.aleat <- sd(Hypothesis1.aleat)

CIinf.Hypothesis1.aleat <- mean.Hypothesis1.aleat-(1.96*(dp.Hypothesis1.aleat /sqrt(1000)))

CISup.Hypothesis1.aleat <- mean.Hypothesis1.aleat+(1.96*(dp.Hypothesis1.aleat /sqrt(1000)))
```

Table S5 Correct assignment of individuals of *Euterpe edulis* in Atlantic Forest in Southeast Brazil, into different hypothesis driving genetic differentiation. % Correct assignment, percentage of corrected assignment using Discriminant analysis of Principal Components; % Random assignment, percentage of corrected assignment of null model using Discriminant analysis of Principal Components; Bray-Curtis Null model, Bray-Curtis's dissimilarity index of null model; Turnover Null model, turnover dissimilarity component of null model; Nestedness Null model, nestedness-resulting dissimilarity component of null model; CI, Confidence Interval.

| Hypothesis	% Correct assignment	% Random assignment [CI]	Bray-Curtis Null model [CI]	Turnover Null model [CI]	Nestedness Null model [CI]
Defaunation	0.96	0.65 [0.65 - 0.65]	0.59 [0.58 – 0.60]	0.59 [0.58 – 0.60]	0.00 [0.00 – 0.00]
Biogeographical regions	0.97	0.74 [0.74 - 0.74]	0.59 [0.58 – 0.60]	0.59 [0.58 – 0.60]	0.00 [0.00 – 0.00]
Defaunation + Biogeographical regions	0.95	0.50 [0.49 – 0.50]	-	-	-
Fragmentation	0.87	0.62 [0.62 - 0.63]	-	-	-
Population	0.60	0.21 [0.21 - 0.21]	-	-	-
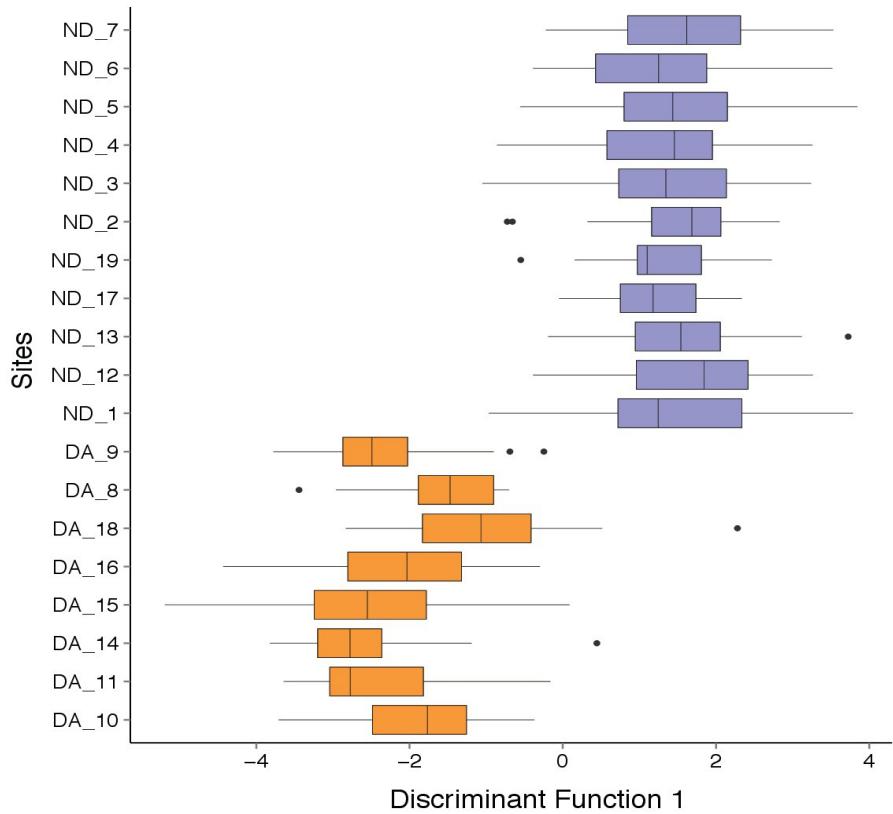
Figure S1. First discriminant function scores of individual genotypes from 545 seedlings of *Euterpe edulis* in Atlantic Forest in Southeast Brazil. Light orange boxplots represent individuals into sites with functional extinction of large seed dispersers and light blue boxplots represent individuals into sites with the full assemblage of mutualistic avian frugivore species. The center vertical line is the median of the sample. The top of the box above the median shows the 75th percentile and the bottom of the box bellow the median shows the 25th percentile. The whiskers show the maximum and minimum values of the sample.
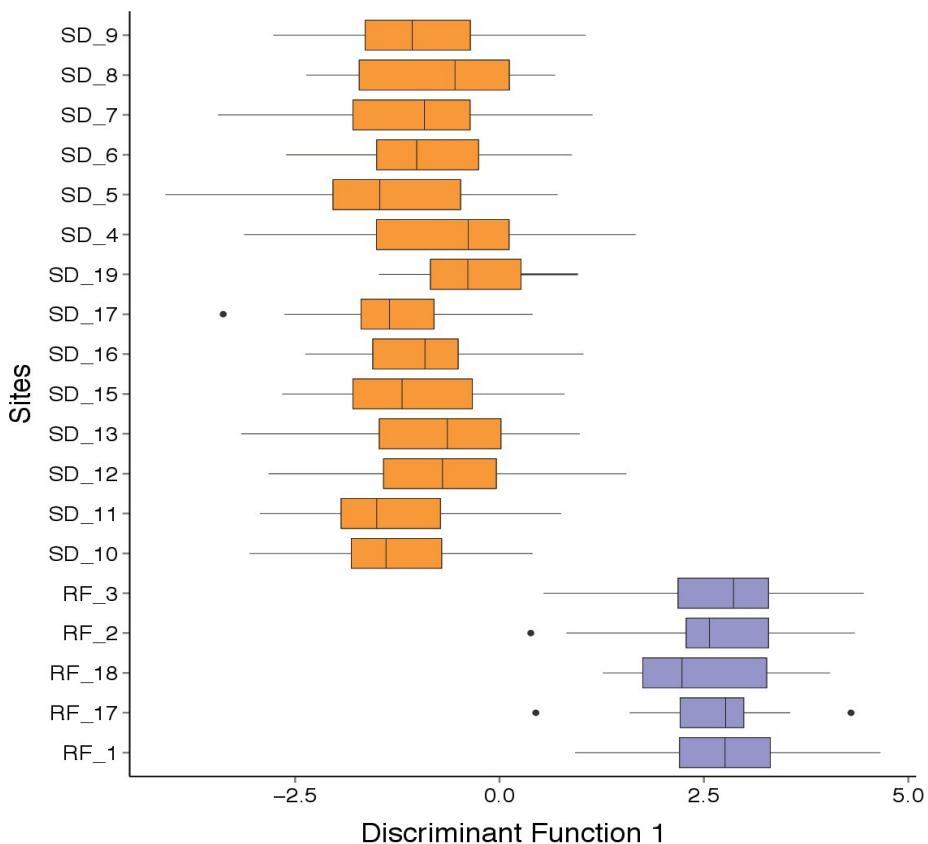
Figure S2. First discriminant function of individuals genotypes of 545 seedling of *Euterpe edulis* in Atlantic Forest in Southeast Brazil. Light orange boxplots represent individuals into sites that are in semideciduous forest and light blue boxplots represent individuals into sites that are in rain forest. The center vertical line is the median of the sample. The top of the box above the median shows the 75th percentile and the bottom of the box bellow the median shows the 25th percentile. The whiskers show the maximum and minimum values of the sample.

5. Capítulo 4: Rescuing intraspecific variation in human impacted environments

Citação

Carvalho CS, Souza M, Côrtes MC.
To be submitted to *Journal of Applied Ecology*

Abstract

1. Anthropogenic activities influence ongoing selective regimes leading to changes in phenotypic variation of plants and animals. The reduction of phenotypic variation may decrease populations' ability to cope with environmental changes. To counteract the increasing risk of extinction of affected populations, it is important to rescue intraspecific variability, assuring higher success of establishment and persistence under global changes.

2. We evaluate whether it is possible to revert phenotypic changes caused by humans using as study case a bird-dispersed palm (*Euterpe edulis*) that present seed size reduction due to defaunation of large-gaped frugivores. First we investigated how defaunation changes the seed size profile of each population by evaluating the coefficient of variation, mean and frequency of extreme phenotypes of produced and dispersed seed sizes. Then, we used simple theoretical models to simulate the success of two restoration strategies: i) direct reintroduction of missing phenotypes that were originally found in the species; or ii) reintroduction of large-bodied frugivores to restore the ecological function of large-seed dispersal. At the end, we discuss the importance of rescuing phenotype variation and states in restoration strategies.

3. We found that by only adding missing phenotypes, mean seed size values of the palm population decreased back to phenotypically depauperated scenarios in only two generations. Conversely, large seed sizes could be rescued in approximately five generations after seed dispersal processes generated by large seed dispersers were reestablished.

4. Thus, to rescue extreme phenotypes such as large seeds in palm populations is necessary to restore the seed dispersal processes by large frugivores. The most effective strategy to restore seed size variation is to rewild defaunated forests with large frugivores, either by connecting remnants to faunated areas or by frugivores reintroduction. The restoration of the seed dispersal processes by large frugivores will benefit several bird-dispersed species and may be crucial to face ongoing global change scenarios.

5. *Synthesis and applications:* Phenotypic changes are likely to degrade ecological processes within decades, having impact on populations, species and ecosystems. Thus, to prevent species extinction is paramount that phenotype variation is preserved. We proposed the inclusion of phenotype restoration of wild populations as a new goal for restoration framework.

5.1. Introduction

Anthropogenic activities inducing phenotypic changes in wild animals and plants is a worldwide phenomenon (Fenberg & Roy 2008; Palkovacs et al. 2012). More than one hundred marine and terrestrial species have been reported to suffer from size-selective harvesting (Fenberg & Roy 2008; Sullivan, Bird & Perry 2017). New selective regimes have been shown to result in smaller plant height and seed size (Hall et al. 2003; Law & Salick 2005; Galetti et al. 2013). These phenotypic changes often go in the opposite direction from those driven by natural selection (Stenseth & Dunlop 2009), increasing the proportion of poorly adapted phenotypes (Hendry, Farrugia & Kinnison 2008). Consequently, changes in key phenotypic traits lead to the reduction in fitness components, such as fecundity and population growth rates (O'Dea et al. 2014; Sullivan, Bird & Perry 2017), and, foremost, to the loss of ecological functions, with impacts on the interacting community (Kuparinen & Merilä 2007; Fenberg & Roy 2008). The selection pressure imposed by anthropogenic activities is frequently directional and may also lead to genetic changes given that the target phenotype is often partially genetically determined (Allendorf et al. 2008). These changes may (i) reduce and change the frequency of desirable genotypes; (ii) intensify genetic structure among populations; and (iii) erode genetic diversity (Allendorf et al. 2008; Bijlsma & Loeschcke 2012; Carvalho et al. 2016). Recent studies have shown that phenotypic and genetic changes due to anthropogenic activities may have serious negative ecological and evolutionary consequences for the persistence of species under scenarios of environmental change (Fenberg & Roy 2008; Galetti et al. 2013). Yet, we still ignore whether it is possible to revert phenotypic changes caused by humans in natural populations (Conover, Munch & Arnott 2009).

Phenotypic changes due to anthropogenic activities can occur in few generations (Galetti et al. 2013; O'Dea et al. 2014) depending on the selective force and the heritability of the selected trait (Allendorf et al. 2008). Phenotypic and genotypic changes may be irreversible when the alleles that code for such traits are lost after anthropogenic selection (Stenseth & Dunlop 2009). Conversely, if strong differential selection is interrupted (i.e. selection relaxation), the distribution of phenotype values can be restored, although time for restoration can be much longer given the mild nature of selection in the reverse direction (de Roos, Boukal & Persson 2006; Allendorf et al. 2008). Although theoretical models suggest that phenotype and genotype changes may be impossible to reverse, there is scant rigorous empirical evidence

(Conover, Munch & Arnott 2009) and modelling efforts to predict population resilience for phenotype rescue (Allendorf et al. 2008). Moreover, despite the importance of genetic and phenotypic variation for species to respond to environmental changes (Watters, Lema & Nevitt 2003; Bijlsma & Loeschcke 2012) and the several examples in the literature of phenotypic changes caused by human, little effort has been made to manage and rescue phenotypic variation in natural populations under conditions of low resilience (Watters, Lema & Nevitt 2003; Allendorf & Hard 2009; Conover, Munch & Arnott 2009). Rescuing phenotypic variation may be important to restore different functions in an ecosystem and, therefore, increases the probability of ecosystem to become self-sustaining in the future.

Here we evaluate whether it is possible to revert phenotypic changes caused by humans in natural populations, using as study case a bird-dispersed palm (*Euterpe edulis*) that present seed size reduction due to defaunation of large gaped frugivores (Galetti et al. 2013). Previously, we found that the functional loss of large seed dispersers due to habitat loss and hunting led to phenotypic differentiation in seed traits among *E. edulis* populations and has been a driver of rapid evolutionary reduction of seed size in defaunated palm populations (Galetti et al. 2013). This happens because large frugivores consume a wide range of seed sizes, while smaller birds are only able to swallow fruits with smaller seeds (< 12 mm wide) (Galetti et al. 2013). Consistent directional selection of smaller seed sizes in defaunated areas and the collapse of the long seed dispersal events can also lead to changes in allelic frequencies among populations, potentially driving long-term homogenization of genetic pools within plant populations from defaunated sites (Carvalho et al. 2016). Having larger seeds provide several advantages, mainly in relation to recruitment and population dynamics (Moles et al. 2005; Dybzinski & Tilman 2012). In the case of *E. edulis,* larger seed sizes result in greater total, shoot, and root biomass of 1-year-old seedlings (Pizo & Simão 2001; Pizo, Von Allmen & Morellato 2006), which may result in an adaptive advantage over smaller seeds sizes. Moreover, *E. edulis* seeds do not survive desiccation, and the seed size reduction results in the significantly increased vulnerability of seeds to desiccation in defaunated areas (Galetti et al. 2013), mainly during extended and intensified periods of drought. Thus, seed size reduction may increase seed mortality in drier conditions and result in smaller seedlings, thereby tending to reduce the average fitness of the population (Dybzinski & Tilman 2012; Galetti et al. 2013). If regeneration in defaunated areas becomes critically dependent on small seeds, extended and intensified periods of drought induced by ongoing climate change may be particularly harmful to the seedling establishment of this threatened palm species (Galetti et

al. 2013).

We evaluate the potential of reversibility of seed size reduction by comparing the coefficient of variation, mean, frequency of extreme phenotypes and distribution of seed sizes between produced and dispersed seeds of *E. edulis* across sites with different degrees of defaunation of avian seed disperser communities. Based on these data we categorized populations in three seed size scenarios that could have different impacts on restoration strategies: *I*. Faunated populations in which seed size distributions are well-preserved so that sizes of dispersed seeds are similar to the ones produced locally and globally when pooling all palm populations that have not suffered phenotypic changes (hereafter global seed sizes) (Fig. 1I); *II*. Populations that only recently lost large frugivores and therefore are functionally impaired. In this case, dispersed seeds are smaller than the ones produced locally, but produced seeds still do not differ from global seed sizes (Fig. 1II); and *III*. The worst case scenario in which the effect of frugivore loss is already perceived in adult palms so that dispersed and produced seeds are smaller than global seed sizes, indicating that certain phenotypes (large seeds) have been lost (Fig. 1III). Moreover, we applied simple theoretical models to test how **frugivore reintroduction** - the reintroduction of large-bodied frugivores that are able to disperse large seeds - affects the restoration of populations' phenotypic average over time in populations subjected to different seed size scenarios (Fig. 1). As a cheaper and logically more feasible strategy, we also tested the success of restoration by means of **seed introduction -** restoration of populations' phenotypic average by directly adding missing phenotypes (large seeds) that were originally found in the species (global seed sizes). Because seed size reduction is driven by loss of large gaped frugivores, we hypothesize that frugivore reintroduction is the best restoration strategy to revert phenotypic change, mainly in *scenario II* where seeds produced still do not differ from global seed sizes. Yet, the combination of both strategies - seed introduction and frugivore reintroduction – will be paramount in populations where extreme phenotypes have already been lost (*scenario III*). Finally, we discuss the importance of including phenotype rescue in restoration strategies and provide a simple framework/check list to help identify whether a population can be phenotypically restored.

I. Dispersed = Produced = Global
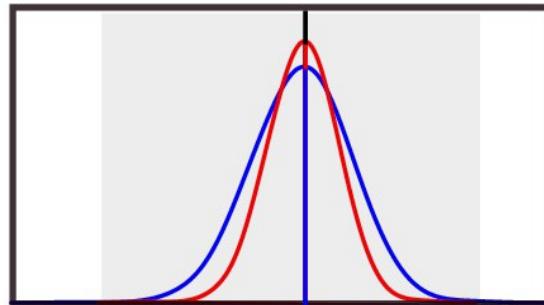
II. Dispersed < Produced = Global
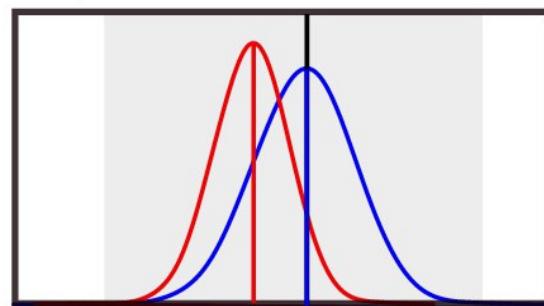
## III. Dispersed = Produced < Global
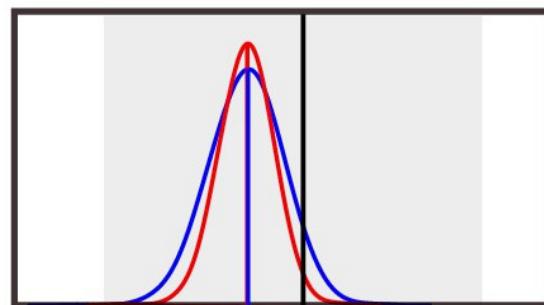
Figure 1: Scenarios of produced (blue lines) and dispersed (red lines) seed size distributions in local populations in relation to global seed sizes (gray box): I. populations in which seed size distributions are well preserved so that size of dispersed seeds are similar to the ones produced in the local population and globally when pooling all palm populations; II. Populations that only recently lost large frugivores and therefore are functionally impaired. In this case, dispersed seeds are smaller than the ones produced, which still do not differ from global seed sizes; and III. scenario in which the effect of frugivores loss is already perceived in adult palms so that dispersed and produced seeds are smaller than global seed sizes, indicating that certain phenotypes (large seeds) are lost.

5.2. Methods

<u>Study system</u>

Euterpe edulis is a dominant species distributed across the Atlantic forest of South America (Henderson, Galeano-Garces & Bernal 1997). Although once abundant, this palm species is currently endangered and locally extinct in many areas owing to illegal harvesting of the edible meristem (heart of palm) (Galetti & Fernandez 1998). *Euterpe eduli*s produces round fleshy fruits ranging from 8.3 to 14.1 mm in diameter (Pizo, Von Allmen & Morellato 2006) and they are dispersed by large (e.g., cotingas *Procnia*s *nudicolli*s, toucans *Ramphasto*s spp. and guans *Penelop*e spp. and *Aburria jacuting*a) and medium-sized avian frugivores *(Turdu*s spp.) (Galetti et al. 2013). The large seed dispersers of *E. edulis* are found mainly in pristine forests and the medium size frugivores are present in both preserved and degraded environments (Galetti et al. 2013). Habitat loss and hunting are the main drivers of abundance reduction and extirpation of large size frugivores birds in forest fragments (Kattan, Alvarez-López & Giraldo 1994; Sodhi, Liow & Bazzaz 2004). These large frugivores have an important role as seed dispersers, because they are the only ones able to ingest large seeds and disperse at large distances, moving the seeds to new sites and genetically linking distinct populations (Jordano et al. 2007; Galetti et al. 2013).

<u>Degree of defaunation of avian seed dispersers communities</u>

We sampled 10 sites with a well-documented bird community composition (Fig. 2). Historically, all sites shared a similar assemblage of seed dispersers, but forest fragmentation and hunting have impoverished the assemblage of large frugivores in many sites (Galetti et al. 2013). We adapted the defaunation index proposed by Giacomini and Galetti (2013) to estimate the defaunation degree of avian seed dispersers communities:

$$D(r,f) = \sum_{k=1}^{s} \varpi_k(N_{k,r} - N_{k,f})/\sum_{k=1}^{s} \varpi_k(N_{k,r} + N_{k,f}).$$

Where *D(r,f)* is the index of defaunation of focal assemblage *f* with respect to the reference assemblage *r* (i.e. most faunated population), ϖ_k is the importance of species *k* for the attribute being measured by defaunation (gape width), $N_{k,r}$ is the quantitative component of seed dispersal effectiveness (Schupp, Jordano & Gómez 2010) of species *k* in the reference assemblage *r,* $N_{k,f}$ is the quantitative component of species *k* in the focal assemblage *f, k* is the species identification (*k* = 1, 2, . . . , *S*), *S* is the total number of species that compose the

union of focal and reference assemblages, r is the reference assemblage, i.e. the collection of species with which the focal assemblage is being compared to assess its defaunation, f is the focal assemblage, i.e. the collection of species in the local of interest (Giacomini & Galetti 2013). The upper limit for D is 1, which indicates that all species are absent in the focal site (complete defaunation); $D = 0$ indicates that there is no difference between the focal and the reference assemblage (no defaunation has occurred). The quantitative component of seed dispersal effectiveness is a standardized way of measuring the quantity of fruits that are removed by each disperser and thus is a useful tool to evaluate the effectiveness of seed dispersal of different frugivores dispersers (Jordano & Schupp 2000).

The data on avian disperser community composition in each population were obtained from Galetti *et al.* (2013) and Labecca (2012). And the data on the quantitative component of seed dispersal effectiveness of different frugivore species in each population were obtained from Ribeiro (2012) and Marçal (2016). Both authors used focal observation methods (Pizo & Galetti 2010) to calculate the quantitative component of seed dispersal effectiveness using the following sub-components: frequency of visit, number of fruits that were manipulated per visit and probability of dispersal (proportion of fruits that were dispersed in relation that were manipulated) (Schupp 1993; Schupp, Jordano & Gómez 2010). Many birds that were recorded in Galetti *et al.* (2013) and Labecca (2012) did not appear during the focal observations. Thus, we used the mean of the quantitative component of seed dispersal effectiveness of all seed dispersers that visited palms in all populations. We acknowledge that the quantitative component may vary across areas, mainly because of differences in birds abundance, however with this approach we assure that all potential dispersers are included in the estimation of the defaunation index in all populations.
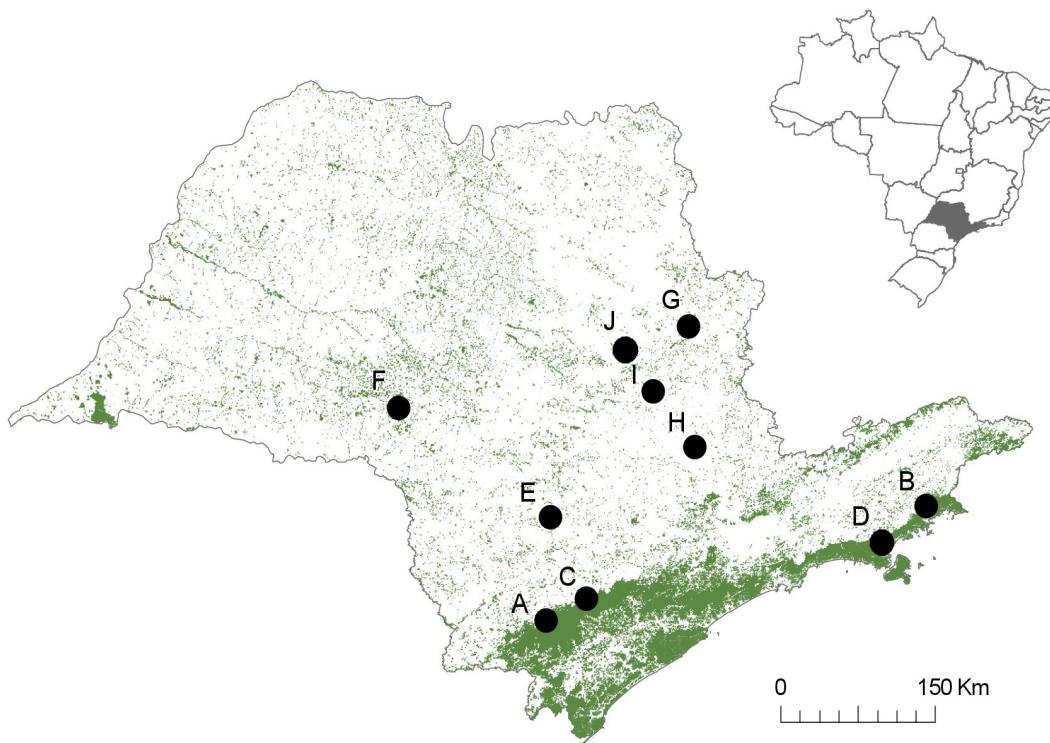
Figure 2: Location of the 10 populations with produced and dispersed seed size distributions information for *Euterpe edulis* in the Brazilian Atlantic forest biome in Brazil. Population codes correspond from the most faunated (A) to the most defaunated sites (J).

The potential for rescuing large seed size

We sampled 30 fruits directly from 15 fruiting palms (~ 450 seeds) and dispersed seeds from 30 seed traps of 1m² in each of the ten populations. The fruits sampled directly from adult palms represent the produced seed size variation, and the seeds collected in the seed traps provide information on seed size variation that is being successfully dispersed and passed to the next generation. Fruits from palms were manually defleshed and all seeds were measured in relation to diameter. Seed traps were monitored and clean seeds (i.e. regurgitated by birds) were sampled throughout the fruiting season.

We characterized the seed size profile of each population by evaluating the coefficient of variation, mean, frequency of extreme phenotypes and distribution of diameters of produced and dispersed seed. First, we tested if the coefficients of variation differed between produced and dispersed seed sizes in each population. For that we used the *asymptotic_test* function of the *cvequality* (Marwick & Krishnamoorthy 2016) R package (Team 2014). This function tests the equality of coefficients of variation from two or more samples. Second, we compared the mean size between produced and dispersed seeds using the *t*-student test in each population. Third, we calculated the percentage and frequency of extreme phenotypes - seed

sizes above 12 mm. We chose 12 mm as a threshold because the seed dispersed by thrushes are < 12mm in diameter while large birds disperse a broader range of seed sizes (Galetti et al. 2013). Fourth, we evaluated if the mean seed size, coefficients of variation and frequency of extreme phenotypes of produced and dispersed seeds were associated with the degree of defaunation using a linear regression in the software R.

To categorize all ten populations into the three seed sizes scenarios (Fig. 1), we compared the mean seed size produced and dispersed in each population in relation to the global mean seed size. The global mean seed size was estimated by pooling seeds from all palm populations considered as faunated, that is, that present low defaunation index values (population A-D, Fig. 2). Seed size *scenario I* includes populations in which mean size and frequency of extreme phenotypes of dispersed seeds are similar than the ones produced locally and globally (dispersed ~ produced ~ global seed size). We do not expect that mean size of dispersed and produced seeds are exactly the same, but similar, because in faunated populations medium-sized birds also contribute heavily to seed removal (Côrtes 2006), dispersing a great amount of small-sized seeds. Seed size *scenario II* includes populations in which mean size of dispersed seeds are smaller and present fewer seeds with extreme phenotypes than the ones produced locally, but the latter still do not differ from global mean seed sizes (dispersed < produced ~ global seed size). Seed size *scenario III* includes populations in which mean size of dispersed seeds frequency of extreme phenotypes are similar to the ones produced locally, but they are smaller than global seed sizes (dispersed ~ produced < global seed size).

<u>Theoretical models to simulate the success of two restoration strategies</u>
We used simplified theoretical models to simulate the success of two phenotypic restoration strategies: A) **Seed introduction**: direct addition of extreme phenotypes that were originally found in populations before defaunation. B) **Frugivore reintroduction**: addition of large seed dispersers to reestablish the quality of seed dispersal process. For simulating both strategies we adapted the model of evolutionary changes of seed size (Galetti et al. 2013):

$$Z_{t+1} = Z_t + h^2 (P - Z_t)$$

Where Z_t is the mean seed size in the generation t, h^2 is the heritability value of seed size ($h^2 = 0.35$ - 95% CI 0.289-0.499, Galetti *et al.* 2013), and P is the mean seed size of dispersed seeds by the assemblage of frugivores. For the strategy *A*, Z_t corresponds to the mean seed size of

E. edulis in each population after the direct addition of extreme phenotypes and *P* corresponds to the mean seed size dispersed by frugivores in the focal population (local assemblage). To calculate the mean seed size we simulated a data set using the empirical data on produced seeds in each population and added extreme seed sizes values (>12 mm) until the percentage of extreme phenotypes was similar to non-defaunated sites. In this case we simulate a restoration strategy that relies on a one-time event (time zero) of palms transplantation or seed addition, without restoring the quality of seed dispersal process itself. For the strategy *B*, Z_t corresponds the mean seed size found in the focal population and *P* corresponds to the mean seed size dispersed by the complete community of frugivores (intact assemblage). Here, we simulate a strategy that aims at restoring phenotypic variation by improving the quality of the seed dispersal processes, and not by the reintroduction of seeds with extreme phenotypes. Because large gaped dispersers are needed to maintain extreme phenotypes in a population, we expected that reestablishing the integrity of the dispersal process would be successful over time. Finally, we simulate a situation where both strategies are applied - seed introduction and frugivore reintroduction. This strategy may be the only alternative for cases in which extreme phenotypes are no longer produced (scenario III). All statistical analysis and simulations were carried out in R software (Team 2014).

5.3. Results

Estimating defaunation degree of frugivores dispersers communities

We classified the sites from the most faunated (population A, D=0, Table S1) to the most defaunated (J, D=0.73, Table S1). Despite the loss of some species, populations A-D presented very similar defaunation indexes (range from 0 to 0,04, Table S1). The most defaunated sites were populations G-J. These sites presented three times fewer seed dispersers than faunated sites and defaunation indexes were, on average, 30-fold of that faunated sites.

The potential for rescuing large seeds

Size distribution of produced and dispersed seeds in *E. edulis* varied across populations depending on the defaunation index (Fig. 3). Size distributions of both produced and dispersed seeds overlapped nicely to the distribution of global seed sizes in more faunated sites (Fig. 3A-D). As the defaunation increases, the distributions of produced and dispersed seed sizes are displaced to the left of the global distribution (Fig. 3E-J). The coefficients of variation differed between produced and dispersed seeds in most populations, being the

coefficient of variation of produced seeds always larger than dispersed ones. Coefficient of variation of produced and dispersed seeds did not differ in the most defaunated sites (populations I and J, Table S1), suggesting that variation of produced seeds has diminished. Despite that, the coefficients of variation of produced and dispersed seed size were not related to defaunation (Fig. 4B). The mean size and frequency of extreme phenotypes were different between produced and dispersed seeds in all populations (Table S1) and they were negatively associated with defaunation (R^2 = 0.76 and 0.82, for mean size of produced and dispersed seeds, respectively, Fig. 4A; and R^2 = 0.72 and 0.73, for percentage of extreme phenotypes of produced and dispersed seeds, respectively, Fig. 4C). All populations had at least 4% of produced seeds larger than 12 mm. The most faunated sites had on average 44% of produced seeds larger than 12 mm. Conversely, the most defaunated sites did not present any dispersed seeds larger than 12 mm, being 11.80 mm the largest seed size dispersed in these sites.

Based on these data we classified populations in the following scenarios: *scenario I* - populations A-D (mean size of dispersed, produced and global seeds were similar, and they produced and dispersed high proportion of extreme phenotypes); and *scenario III* – populations E – J (dispersed and produced seeds were similar and both smaller than global seed sizes). Because the proportion of dispersed and produced extreme phenotypes differed in these populations, we subdivided *scenario III* in three phases. Populations E-H were categorized as *scenario III initial phase* – in which populations produce and disperse low proportions of extreme phenotypes. Populations I and J were categorized as *scenario III intermediate phase* - these populations produce but do not disperse any seeds larger than 12 mm. *Scenario III advanced phase* entails populations that would not produce and disperse extreme phenotypes. None of the sampled populations were categorized as *scenario II* and *scenario III advanced phase.*
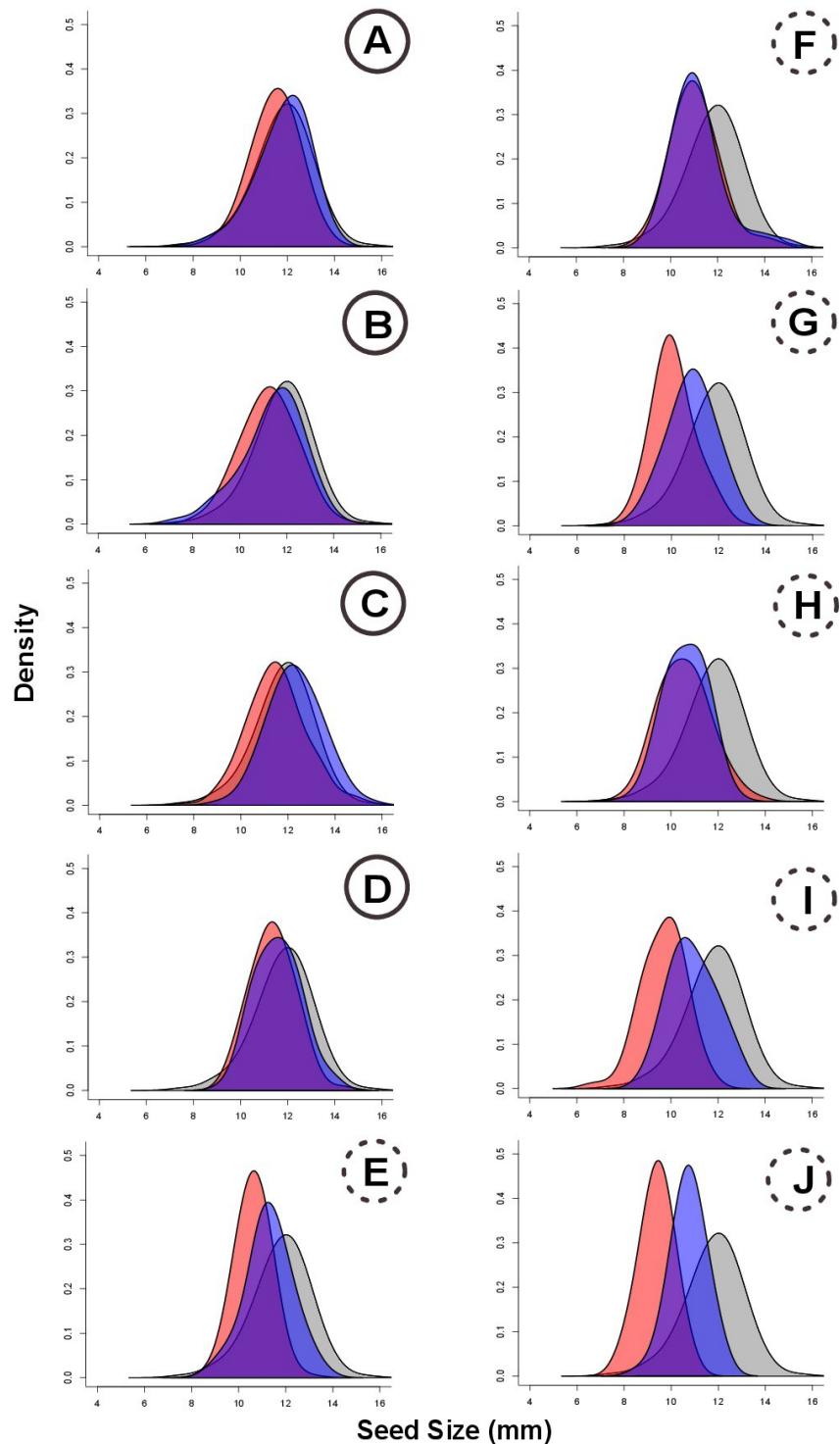
Figure 3: Density curves for produced (blue) and dispersed (red) seed size variation in 10 sites of *Euterpe edulis* in Brazilian Atlantic forest remnants. Gray is the distribution of produced seed size in all faunated populations of *E. edulis*. The density curves are plotted from most faunated (A) to most defaunated sites (J). The circles around each letter correspond to the seed size scenario each population was classified *a posteriori*: continuous line circles – scenario I of figure 1; dashed line circles – scenario III of figure 1
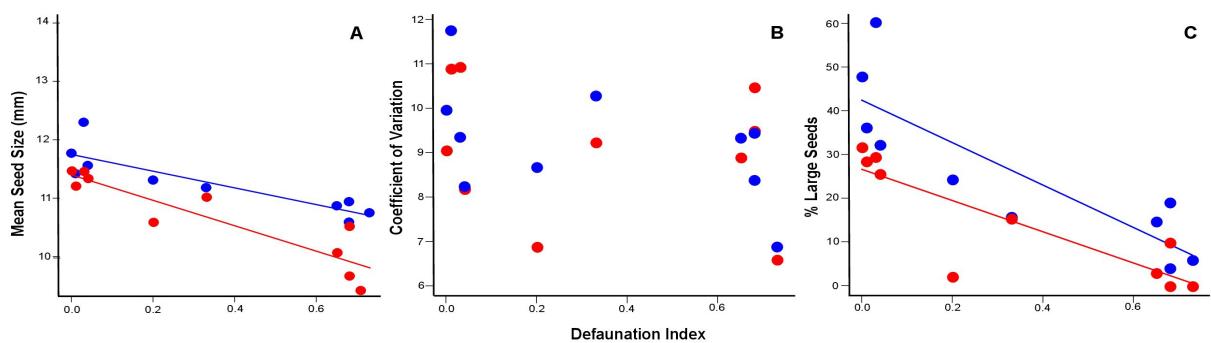
Figure 4: A) Relationship of mean (A), coefficient of variation (B) and percentage of extreme phenotype (C) of produced (blue) and dispersed (red) seed size and defaunation index for 10 sites of *Euterpe edulis* in Brazilian Atlantic forest remnants. Defaunation index = 1 indicates that all species are absent in the focal site (complete defaunation); Defaunation index = 0 indicates that there is no difference between the focal and the reference assemblage (no defaunation has occurred).

Theoretical models to simulate the success of two restoration strategies

By adopting the strategy of seeds introduction, the mean population seed size returned to the pre-restoration scenarios in two to six generations depending on the population (from 36 to 100 years, Figure 5A). On the other hand, in the strategy of frugivores reintroduction and maintenance, population mean seed sizes would reach the global seed size in about five generations (90 years, Figure 5B). The populations in the *scenario III intermediate phase* (populations G and J) took longer to reach the global mean seed size, while the population E with higher proportion of extreme phenotypes took only two generations. We did not sample any population where extreme phenotypes were completely lost, but in this case, direct addition of missing extreme phenotypes would be required in consortium with fruvigore reintroduction.
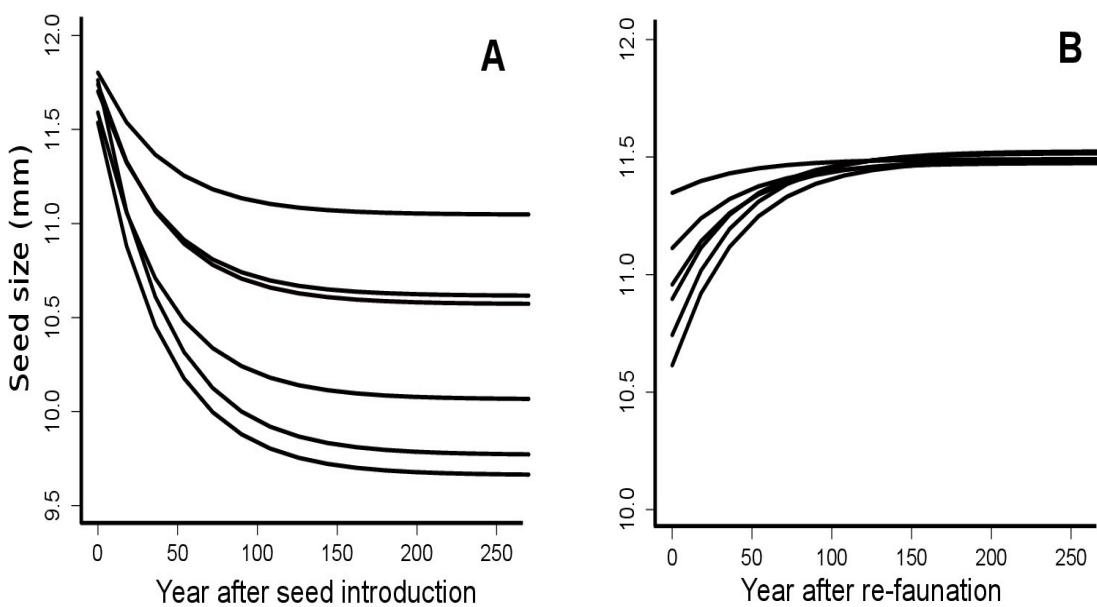
Figure 5: Simulated seed size trends after restoration strategies in each (A) direct addition of extreme phenotype that were originally found in populations before defaunation and (B) restoring the seed dispersal process of large seed dispersers. Expected trajectories over time were estimated from the phenotypic selection model. We considering *E. edulis* generation time of 18 years (Franco & Silvertown 2004).

5.4. Discussion

We found that seed size distribution and mean, and the percentage of extreme phenotypes varied with the defaunation degree; confirming that medium-sized frugivores failed to disperse large seeds and indicating that phenotypic restoration is imperative in defaunated populations. Nevertheless, the mean seed size and percentage of extreme phenotypes were higher in produced than dispersed seeds and extreme phenotypes were produced in faunated and defaunated populations, indicating that all populations have an intrinsic potential to revert phenotypic changes, even without the addition of extreme phenotypes. Conducting a restoration project without the introduction of new phenotypes is important because the mixing of individuals from different populations during reintroduction or translocation may cause outbreeding depression and thus reduce the fitness and the capacity to adapt to a new habitat (Moritz 1999; Edmands 2007). In relation to restoration strategy, we showed that by only adding missing phenotypes the mean seed size would return to the one in pre-restoration scenarios in about two generations. In contrast, if the seed dispersal processes of large seed dispersers are to be reestablished, seed size profiles could be restored. Although our simulations show that all populations were phenotypically restored after the reintroduction of frugivores, the trajectories over time differed among them. Populations with lower percentage

of extreme phenotypes were the ones that took longer to reach the global mean seed size. Thus, combining both strategies might accelerate the phenotypic rescue in populations that produce few extreme phenotypes and it is also important in cases where these phenotypes were already lost.

Restoration strategies for phenotypic rescue

An ecological system that has an intrinsic potential to revert phenotypic changes is expected to restore the distribution of phenotypic values after differential selection is interrupted (de Roos, Boukal & Persson 2006; Allendorf *et al.* 2008). However, the time for restoration can be longer than the time of phenotype changes given the mild nature of selection in the reverse direction (de Roos, Boukal & Persson 2006; Allendorf et al. 2008) For example, Conover and collaborators (2009) showed that fish populations evolving smaller body size when subjected to size-selective fishing recovered the full size in about 12 generations when fishing ceased. Previously we found that a period of less than 75 years after a severe defaunation would be sufficient to cause seed size reduction in palm populations in defaunated sites (Galetti et al. 2013). However, our simulations show that will be necessary at least 90 years to rescue the same mean seed size found in faunated populations. We also show that when we attempt to rescue phenotypes we have to manage the drivers of such selection. In the case of the fishes study, the phenotypic changes were driven by the selective pressure of fishing activities, thus when fishing ceased the fish sizes were rescued. In the case of *E. edulis,* the lack of large seed disperser leads to phenotypic changes because the extant medium- to small-bodied frugivores do not ingest large-sized seeds, and therefore, only medium- to small-sized seeds get dispersed. Thus, to rescue the seed size of *E. edulis* it is required not only stop defaunation, but also refaunate forests with large-bodied frugivores and restore ecological processes in defaunated populations.

The great majority of the restoration projects focuses on reintroducing plant communities (Young 2000), and there are only few that focus on rewilding empty forests (Galetti et al. 2017) to restore ecological functions. This occurs mainly because of a widespread hypothesis called "field of dream" that settle that if we restore the vegetation animals would come (Hilderbrand, Watts & Randle 2005). However, Brazilian Atlantic forest is a very fragmented biome with an average distance of 1440 m between patches (Ribeiro et al. 2009). Moreover, large-bodied species are absent in forest remnants (Jorge et al. 2013) which compromises the recolonization of animals in restored sites and the self-sustaining of restoration project. In the

last decades, there has been an increased interest in refaunating empty forests, aiming to restore ecological processes and ecosystem functions by reintroducing and translocating a set of recently extirpated animal species (Fernandez et al. 2017). Nevertheless, refaunating empty forest has been challenging because the success of restablishing viable populations requires planning long-term programs that monitor the reintroduced population regarding demography, spatial patterns, diet and their effect on ecological interactions (Fernandez et al. 2017; Zamboni, Martino & Jiménez-Pérez 2017). For example, to garantee the success of a reintroduction program, it is pivotal to consider how biotic interaction affect the dynamics of managed populations, once many programs fail due to high population mortality by predation, pathogen attack, competition and starvation (Pires 2017). Furthermore, large-bodied animals usually require large areas to reproduce and forage; thus, together with a reintroduction program, it is also necessary focus on building ecological corridors to connect distinct forest fragments and improve habitat quality. Although challenging, the restoration of ecological interactions by refaunation has been proven essential to the effectiveness of restoration projects. In Brazilian Atlantic Rainforest (Cid et al. 2014) and in Gorongosa National Park (Mozambique) (Correia et al. 2017), for example, the reintroduction of medium and large mammals was important to recover seed dispersal processes and enhance recruitment in a disperser-impoverished forest. Our study case also shows that without restoration of ecological interactions, the goals of phenotype restoration would be unsuccessful and highlights the urgency of reverse defaunation to rescue phenotype variation. Rewilding Brazilian Atlantic forest will bring benefits not only to *E. edulis* populations but also to other endozoochorous plants that require dispersal services provided by frugivorous vertebrates (> 75% of trees in Atlantic forest) (Almeida-Neto et al. 2008).

Rescue of phenotypic variation as a novel goal in the restoration ecology framework

Restoration ecology focuses on restoring ecosystem functioning and resilience (Heller & Hobbs 2014; Mijangos et al. 2015). The ultimate objective of any restoration project is the establishment of a self-sustaining population; however, outcomes have indicated low success rate (< 23%) (Seddon et al. 2014). The success of a restoration project depends on the ability of individuals to survive and reproduce (Watters, Lema & Nevitt 2003). This ability may be affected by phenotypic variation because different phenotypes respond to environment changes in different ways (Watters, Lema & Nevitt 2003). Thus, we propose the inclusion of *phenotype restoration* as a novel goal in the restoration ecology framework. Rescuing the natural phenotype variation of a population may increase the probability that some individuals

will survive under drastic environment changes, which will allow an ecosystem to become self-sustaining (Hobbs & Cramer 2008). Although there is a worldwide concern about the phenotypic changes caused by anthropogenic activities, few restoration projects have been focused on rescuing phenotypic changes (Watters, Lema & Nevitt 2003; Allendorf & Hard 2009; Conover, Munch & Arnott 2009); and the few has focused mainly on harvested populations of animals (Kuparinen & Merilä 2007; Jørgensen et al. 2007; Allendorf et al. 2008). To help managers identify whether a population needs to be phenotypically restored, we propose some questions to be evaluated before undertaking a phenotype restoration: a*) Is the population showing phenotypic changes? b*) Are the phenotypic changes caused by human factors?* c) *Are the phenotypic changes causing fitness reduction?* d) *How to rescue the phenotypes?*

First of all, we have to identify whether populations are showing phenotypic changes (*question a*). This can be identified by several methods such as comparing the phenotype traits over time (Coltman et al. 2003; Carlson et al. 2007) or replicated field experiment that compare pristine vs. human impacted population suffering anthropogenic selection (Galetti et al. 2013; de Amorim et al. 2017). Secondly, it is extremely important identify whether the phenotypic changes are caused by human factors (*question b*) and, third, whether these changes are causing fitness reduction (*question c*). Phenotypic changes are natural processes driven by natural selection due to environmental changes that can be human-lead or not. There are several examples showing that anthropogenic factors cause phenotypic changes by natural selection (Sullivan, Bird & Perry 2017). For example, a recent study has documented that songbirds changes the wings shape in response to habitat fragmentation, mitigating negative consequences of habitat loss caused by humans (Desrochers 2010). Other study shows that gecko species have larger heads in populations inhabiting artificial islands formed by the construction of a reservoir than populations in nearby mainland, resulting in an increase in dietary niche breadth (de Amorim et al. 2017). Both examples show that populations and species can evolve in response to anthropogenic factors, thus there is no need for restoration interference for these species. Phenotype restoration focuses on situations where human interfer in the natural selection (so called unnatural selection, Stenseth & Dunlop 2009), causing fitness reduction and threatening the persistence of population and species under scenarios of environmental changes.

Finally, several intervention procedures are applied in restoration projects to recover animal

and plant communities and ecological processes, such as seed addition, weed control, prescribed burning, translocation and rewilding (Perring et al. 2015). Such interventions can also be applied in phenotype restoration to manage the drivers of anthropogenic selection and reverse the phenotypic changes (*question d*). For example, we showed that rewilding and, consequently, restoring ecological functions is the best alternative to reverse phenotype changes in plants that face seed size reduction due to extinction of large frugivores. Allendorf and Hard (2009) propose the "*sustainable harvest*", a concept applied mainly for harvested animals, which suggest an adequate monitoring of phenotypes that are sensitive to anthropogenic selection and may influence the individuals' viability. They also propose a management that maintains breeding populations that are large and diverse enough to foster the full range of phenotypes (Allendorf & Hard 2009). Futhermore, Watters and collaborators (2003) propose the "*phenotype management*", in which restorers entail restoring the resources in a habitat to manage phenotypic diversity. To decide which intervention type is required to successfully reverse phenotype changes demands a deep knowledge of the system. This knowledge is important to clearly understand what the problem is, how to fix it, and what the end result should be (Hobbs & Cramer 2008).

Conclusions

Many species and populations of animals and plants are facing a dramatic decline worldwide and phenotypic changes caused by anthropogenic selection may prevent those species to cope with environmental changes. Phenotypic changes are likely to degrade ecological processes within decades, having impact on populations, species and ecosystems (Jørgensen et al. 2007). Thus, a successful management preventing species to go extinct is preserving the phenotype variation. In this study we proposed the inclusion of phenotype restoration of wild populations as a new goal for restoration framework. We also show that to phenotypically restore a population demands a deep knowledge of the target species and the causes of phenotypic changes. We highlighted the importance of modeling to predict when reversal is expected to be possible under various strategies. In our study case, to reverse phenotype changes in natural populations of *E. edulis* would be necessary to restore seed dispersal processes by large frugivores. Rewilding empty forest has been proposed for several researchers (Galetti et al. 2017) and now we show that without this strategy, the phenotype restoration of *E. edulis* would be unsuccessful. Most tree species in tropical forest are animal dispersed (Jordano 2013) and defaunation of large frugivores is becoming omnipresent in fragmented and non-fragmented forests. Thus, reversing defaunation in order to rescue

phenotype changes of *E. edulis* populations will benefit several plant species and may be crucial to facing ongoing environmental change scenarios.

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Supplementary Material

Rescuing intraspecific variation in human impacted environments

Table SM1: Mean, coefficient of variation (cv) of produced and dispersed seed size, percentage of large seeds and defaunation index for 10 sites of *Euterpe edulis* in Brazilian Atlantic forest remnants. Defaunation index = 1 indicates that all species are absent in the focal site (complete defaunation); Defaunation index = 0 indicates that there is no difference between the focal and the reference assemblage (no defaunation has occurred).

Sites	Defaunation Index	Mean produced seed size	Mean dispersed seed size	CV produced seed size (%)	CV dispersed seed size (%)	% large seeds produced	% large seeds dispersed
A	0.00	11.77	11.49	9.98	9.05	48	31.8
B	0.01	11.42	11.23	11.77	10.89	36.32	28.56
C	0.03	12.30	11.48	9.37	10.93	60.44	29.56
D	0.04	11.56	11.36	8.26	8.18	32.36	25.68
E	0.20	11.31	10.61	8.69	6.88	24.44	2.16
F	0.33	11.18	11.04	10.30	9.23	15.88	15.44
G	0.68	10.87	10.09	9.35	8.89	14.8	3
H	0.67	10.59	10.54	8.40	10.47	4.12	9.96
I	0.68	10.94	9.69	9.46	9.49	19.16	0
J	0.73	10.75	9.37	6.90	6.59	6	0

6. Conclusão

Nós encontramos que os sabiás são importantes dispersores de semente e de genes de *Euterpe edulis*. Essas aves, ao visitarem muitas palmeiras, consumirem poucas sementes por visita e regurgitarem as mesmas uma a uma, dispersam genótipos de múltiplas plantas-mães e diminuem a probabilidade de sementes irmãs serem depositadas em um mesmo sítio. Esse comportamento se mostrou eficaz para manter a diversidade genética em plântulas e, além disso, ajudou a manter uma baixa estrutura genética espacial em plântulas e adultos.

Apesar da importância dos sabiás para a manutenção da estrutura genética espacial e diversidade genética em populações fragmentadas, a perda dos grandes frugívoros afeta a qualidade do serviço de dispersão de sementes, principalmente em termos de dispersão a longa distância e tamanho das sementes dispersadas. Esses resultados em escala fina são consistentes com os resultados encontrados em escala de paisagem sobre o efeito da defaunação na diversidade e diferenciação genética de populações de *E. edulis*. Nós encontramos que a perda funcional dos grandes dispersores de sementes resulta na diferenciação genética entre as populações de *E. edulis*; e demonstramos, pela primeira vez, que a defaunação pode levar a mudanças na frequência de alelos entre as populações. No entanto, não encontramos evidências de que o defaunação reduz a diversidade genética.

Portanto, nossos resultados sugerem que a manutenção da diversidade genética em populações defaunadas e fragmentadas é impulsionada pela atividade de dispersão de frugívoros de tamanho médio. Assim, nosso estudo destaca que os frugivores de médio e grande porte promovem serviços complementares, mantendo a diversidade através da mistura de sementes não geneticamente relacionadas em escalas local e de paisagem, respectivamente. Além disso, salientamos sobre a importância de se manter altas densidades de *E. edulis*: a baixa distância de dispersão por *Turdus* em áreas defaunadas associada a baixas densidades gera uma baixa sobreposição de chuvas de sementes e pouca mistura de genótipos maternos, o que a curto-médio prazo aumentaria a estrutura genética espacial e eventualmente reduziria diversidade genética.

Finalmente, nós encontramos que para recuperar a variabilidade fenotípica e fenótipos extremos, como grandes sementes em populações de *E. edulis*, é necessário restaurar os processos de dispersão de sementes por grandes frugívoros. A estratégia mais eficaz para

restaurar a variação do tamanho da semente é refaunar florestas defaunadas com grandes frugívoros, seja conectando remanescentes a áreas faunadas e/ou por reintrodução de animais. A restauração dos processos de dispersão de sementes por grandes frugívoros beneficiará várias espécies dispersas por aves e pode ser crucial para enfrentar cenários de mudança global.