PPG Ecologia & Conservação

Universidade Estadual de Santa Cruz

Uma ameaça silenciosa ás populações remanescentes em paisagens antrópicas: a perda da diversidade invisível aos olhos

A silent threat to the remnant populations in anthropic landscapes: diversity loss invisible to eyes

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Coisas que só o coração pode entender
Fundamental é mesmo o amor
É impossível ser feliz sozinho"
(Tom Jobim)

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"Não é sobre chegar no topo do mundo e saber que venceu
É sobre escalar e sentir que o caminho te fortaleceu
É sobre ser abrigo e também ter morada em outros corações
E assim ter amigos contigo em todas as situações

A gente não pode ter tudo
Qual seria a graça do mundo se fosse assim?
Por isso, eu prefiro sorrisos
E os presentes que a vida trouxe pra perto de mim"
(Ana Vilela)

ÍNDICE

RESUMO

As taxas de conversão atual dos habitats naturais em paisagens antrópicas são as mais alarmantes de toda a história da humanidade. O grande corpo de informações disponível acerca dos efeitos destas modificações na biodiversidade é limitado aos padrões de composição de espécies e grupos ecológicos e são escassas as informações que esclarecem como as populações remanescentes se adaptam as perturbações na paisagem. Assim, o objetivo principal deste trabalho foi investigar o impacto dos distúrbios antropogênicos, quantificados em diferentes escalas espaciais, na diversidade genética das populações remanescentes. No primeiro capítulo, foi realizada uma meta-análise global de estudos que compararam a diversidade genética em habitats conservados e degradados. Os resultados mostraram que existe um efeito negativo consistente dos distúrbios antropogênicos na diversidade genética das espécies de plantas e animais terrestres. No entanto, existe uma variação no tamanho de efeito entre os estudos que é gerada pelas diferenças nos seguintes fatores: (i) tipo do distúrbio antropogênico; (ii) parâmetro genético estimado; (iii) especificidade do marcador microssatélite (iv) reino biológico; (v) forma de vida vegetal; (vi) modo de dispersão de sementes; (vii) modo de polinização; e (viii) compatibilidade do sistema reprodutivo. A espécie *Euterpe edulis* foi utilizada no capítulo 2 como modelo biológico para avaliar o impacto das perturbações antrópicas em um estudo de caso. A redução do habitat original e supexploração do meristema apical desta espécie são perturbações em escala de paisagem e local que culminaram na inserção desta palmeira na lista das espécies brasileiras ameaçadas de extinção. Por este motivo, o presente estudo utilizou uma abordagem multi-nível para avaliar simultaneamente o impacto causado pela redução de cobertura florestal, índice de proximidade e intensidade da exploração madeireira na diversidade e estrutura genética desta espécie. Um total de 544 indivíduos jovens de *E. edulis* pertencentes a 17 fragmentos de Mata Atlântica foram coletados e genotipados com 17 marcadores microssatélites. Os nossos resultados mostraram que embora as populações investigadas ainda conservem altos níveis de diversidade genética, um processo inicial de erosão foi desencadeado. Os fragmentos com elevado índice de extração madeireira apresentaram redução do número de alelos privativos e aumento do coeficiente de endogamia. Além disto, uma forte estrutura genética foi encontrada entre as populações remanescentes, o que pode indicar uma limitação no fluxo gênico resultante da redução do habitat em escala regional. A análise conjunta dos resultados revela que independente da escala em que as perturbações antrópicas acontecem, elas são capazes de acarretar em erosão da diversidade genética nas populações que permanecem nos habitas modificados. Os atrasos nas respostas genéticas ou amortecimento do efeito negativo das perturbações podem sem geradas tantos por características biológicas e metodológicas dos estudos como também, pelo intervalo temporal existente entre a ocorrência do distúrbio e a amostragem genética.

Palavras-chave: distúrbios antropogênicos, microssatélites, genética populacional, erosão genética.

SUMMARY

The current rates of conversion of natural habitats into anthropic landscapes are the most alarming in all humankind history. The large body of information available on the effects of these changes on biodiversity is limited to the patterns of species composition and ecological groups and few studies clarify how the remaining populations adapt to disturbances in the landscape. Thus, the main objective of this study was to investigate the impact of anthropogenic disturbances, quantified at different spatial scales, on the genetic diversity of the remaining populations. A global meta-analysis of studies comparing genetic diversity in conserved and degraded habitats was conducted in Chapter 1. The results showed that there is a consistently negative effect of anthropogenic disturbances on the genetic diversity of terrestrial plant and animal species. However, there is a variation in effect size between studies that is generated by differences in the following factors: (i) type of anthropogenic disturbance; (ii) estimated genetic parameter; (iii) microsatellite marker specificity (iv) biological kingdom; (v) plant life-form; (vi) mode of seed dispersal; (vii) pollination mode; and (viii) compatibility of the reproductive system. The *Euterpe edulis* species was used in Chapter 2 as a biological model to evaluate the impact of anthropogenic disturbances in a case study. The reduction of the original habitat and the overexploitation of the apical meristem of this species are perturbations in the scale of landscape and local that culminated in the insertion of this palm tree in the list of Brazilian species threatened with extinction. For this reason, the present study used a multi-level approach to simultaneously evaluate the impact caused by the reduction of forest cover, proximity index and intensity of logging in the diversity and genetic structure of this species. A total of 544 young individuals of E. edulis from 17 fragments of Atlantic Forest were collected and genotyped with 17 microsatellite markers. Our results show that although the populations investigated still retain high levels of genetic diversity, an initial erosion process was triggered. The fragments with high logging intensity showed a reduction in the number of private alleles and an increase in inbreeding coefficient. In addition, a strong genetic structure was found among the remaining populations, which may indicate a limitation in the gene flow resulting from habitat reduction on a regional scale. These results reveal that regardless of the scale at which anthropic disturbances occur, they are capable of eroding genetic diversity in populations that remain in the modified habitats. Delays in genetic responses or damping of the negative effect of the disturbances can be generated as much by biological and methodological characteristics of the studies as by the time interval between the occurrence of the disturbance and genetic sampling.

Key words: anthropogenic disturbances, microsatellites, population genetics, genetic erosion.

INTRODUÇÃO GERAL

Na corrente época geológica o ser humano é apontado como o principal responsável pelas modificações nos padrões de biodiversidade e biogeoquímica global (GRUNZWEIG *et al*. 2004; DIRZO *et al.* 2014; ALBERTI 2015; DORRESTEIJN *et al*. 2015) e por este motivo, alguns autores defendem a ideia de que esta era seja intitulada de Antropoceno (CRUTZEN & STOERMER 2000; RUDDIMAN 2003; STEFFEN *et al*. 2011). O rápido crescimento da população humana prevista para o ano de 2100 (7-11 bilhões no mundo UNITED NATIONS, 2015) vai demandar uma crescente modificação de paisagens florestais para suprir especialmente, as demandas relacionadas com moradia e alimentação (FOLEY *et al.* 2005; LAURANCE *et al.* 2014). Neste contexto, destaca-se a preocupação dos ecologistas com as regiões tropicais, porque além destas áreas concentrarem a maior parcela de biodiversidade de todo o planeta (GASTON 2000), são as regiões mais submetidas às pressões de desmatamento nos últimos anos (HANSEN *et al.* 2013).

A redução de florestas impulsiona o processo de extinção lento e gradual de diversos grupos taxonômicos (RANGEL 2012). Isto ocorre porque, as populações de várias espécies são severamente afetadas pelas modificações do habitat remanescente, tais como fragmentação, efeito de borda e perda de qualidade dos fragmentos, apenas em longo prazo. (HANSKI & OVASKAINEN 2002; VELLEND *et al.* 2006; METZGER *et al.* 2009; UEZU & METZGER 2016). Esta defasagem temporal na resposta da comunidade aos impactos ecológicos é gerada particularmente pelos diferentes traços nas histórias de vida das espécies (LEES & PERES 2008). Este fator determina o que se conhece na literatura como débito de extinção e os locais que possuem uma grande dívida são os que concentram uma parcela significativa de grupos potencialmente ameaçados (KUUSSAARI *et al.*2009).

O fitodomínio tropical da Mata Atlântica possui atualmente apenas 12% da sua extensão de cobertura original (RIBEIRO *et al.* 2009). No entanto comporta um grande número de espécies preditas de extinção (SILVA & TABERELLI 2000). Para criar estratégias que reduzam ou neutralizem a perda destas espécies, e em consequência, a perda de interações ecológicas e serviços ecossistêmicos (VALIENTE-BANUETE *et al.* 2015; ISBELL *et al.* 2015), são necessários estudos que avaliem a capacidade de persistência das populações dentro dos ambientes remanescentes. Neste cenário, destacam-se os estudos genéticos, pois são capazes de detectar a fragilidade das populações frente às ameaças de um habitat modificado (SPIELMAN et al. 2004).

A teoria genética prevê que as populações isoladas e com tamanho populacional reduzido estejam mais vulneráveis a limitação de fluxo gênico, aumento do cruzamento entre os indivíduos aparentados, acúmulo de alelos deletérios e perda de variantes gênicas raras em função dos efeitos da deriva genética (BROWNE & KARUBIAN 2018; STEVENS et al. 2018; ELLSTRAND & ELAM 1993; RHOADS et al. 2017). Apesar disto, não existe um consenso das evidências empíricas acerca deste quadro teórico, visto que os resultados encontrados na literatura mostram efeitos negativos e neutros das perturbações antrópicas sobre a diversidade genética das populações remanescentes (JUMP & PEÑUELAS 2006; WEI & JIANG 2012; SILVESTRINI et al., 2015). O esclarecimento destas contradições na resposta genética das espécies a degradação do habitat é essencial para o planejamento de ações de conservação mais direcionadas a especificidade de cada grupo porque possibilita a mitigação efetiva dos impactos causados pelos distúrbios quantificados em diferentes escalas espaciais.

Para aumentar a compreensão destas questões, foi realizada no **capítulo 1** desta tese uma meta-análise global de estudos que compararam a diversidade genética das espécies de plantas e animais terrestres em áreas conservadas e degradadas. Além da quantificação do efeito geral da degradação sobre a diversidade genética, foram investigadas também as causas da variação no tamanho de efeito encontrada entre os estudos. Isto considerou as diferenças metodológicas entre os estudos, tais como, o tipo de distúrbio avaliado, parâmetro genético estimado, tipo de marcador microssatélite e número de microssatélites utilizados e também considerou as diferenças nas características biológicas das espécies utilizadas como modelo biológico.

O **capítulo 2** desta tese apresenta um estudo de caso realizado com a espécie *Euterpe edulis*, nativa da Mata Atlântica (**Fig. 1**). Esta palmeira foi escolhida como modelo biológico porque que está submetida às pressões antropogênicas em escala de paisagem e local, visto que se encontra atualmente na lista das espécies brasileiras ameaçadas de extinção porque sofre com a redução do habitat que ocupa e com a exploração do meristema apical que causa a morte dos seus indivíduos (MARTINELLI & MORAES**,** 2013). Além disto, esta espécie é considerada chave na dinâmica do ambiente florestal porque a sua frutificação pode se estender por um período de seis meses (FISCH *et al*., 2000; CASTRO, 2007), abrangendo uma época de escassez de recursos (LAPS, 1996). Assim, os seus frutos, com polpa rica em lipídeos e fibras (REIS & KAGEYAMA, 2000), são utilizados como alimento por cerca de 58 espécies de aves e 21 espécies de mamíferos (GALETTI *et al*., 2013). A elaboração de planos de gestão se torna mais estratégico quando o alvo é uma espécie chave, porque a conservação desta espécie aumenta as chances de permanência nos habitats degradados de grupos taxonômicos que interagem com ela. (SOULÉ *et al.* 2005). Por este motivo, este capítulo utilizou uma abordagem em multi-nível para investigar simultaneamente o

impacto causado pela exploração dos recursos florestais e pelas modificações de composição e configuração da paisagem na diversidade e estrutura genética de *E. edulis*. A identificação das principais ameaças a diversidade genética desta espécie pode subsidiar os planos de conservação desenvolvidos para o fitodomínio da Mata Atlântica.

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Figura 1. Indivíduo adulto de *Euterpe edulis* em fragmentos da Mata Atlântica (Foto: acervo pessoal)

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CAPÍTULO 1

The effect of anthropogenic disturbances on the genetic diversity of terrestrial species: a global meta-analysis

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Abstract
Human activities are primarily responsible for habitat loss and modifications in natural environments around the world. It has been suggested that populations inhabiting human modified-landscapes are subject to reduced gene flow, inbreeding and loss of alleles by genetic drift. However, empirical evidence shows contradictory effects of anthropogenic disturbances on the genetic diversity of terrestrial species. We performed a meta-analysis of 61 studies that compared the genetic diversity of plant and/or animal populations in disturbed and relatively preserved (control) areas (374 paired comparisons) to investigate the existence of a general response trend to different kinds of habitat disturbance. We used meta-regression and sub-group analysis to understand the causes of variation in the effects detected by the studies. We found a consistent negative effect of anthropogenic disturbances on genetic diversity (effect size: -0.45). Lack of habitat connectivity has resulted in a more severe and accurate negative effect than other types of disturbances, such as loss of habitat quality We also identified that the direction and magnitude of the effects depend on the genetic parameter, so the fixation index and the number of effective alleles did not detect genetic erosion, while all other indices, especially allelic richness, were negatively affected by disturbances. Yet, only studies performed with transferred or both transferred and specific microsatellites showed negative responses to disturbance. The general effect was even more detrimental in animal than plant populations. Only plant species with biotic pollination and seed dispersal mode, self-incompatible reproductive system and shrubs had consistent negative responses to anthropogenic disturbances. Despite all heterogeneity among studies, there is an overall negative effect of habitat disturbance on genetic diversity of terrestrial populations, which suggests that the remaining populations inhabiting anthropogenic landscapes have a reduced evolutionary potential being more prone to local extinction.

Keywords: molecular marker, population genetics, genetic erosion.

1. Introduction

Human activities led to the conversion of continuous natural landscapes into fragmented and isolated areas, susceptible to edge effects and consequently reduced habitat quality (Laurance *et al.* 2014; Haddad *et al.* 2015; Cuaresma *et al.* 2017; Brinck *et al.* 2017). The remaining populations inhabiting these human-modified landscapes are subject to a reduction of gene flow (Browne & Karubian 2018; Stevens *et al*. 2018), which may lead to inbreeding depression, loss of heterozigosity, and changes in allele frequency by genetic drift (Ellstrand & Elam 1993; Rhoads *et al.* 2017). The aforementioned changes in genetic parameters tend to result in a reduced genetic diversity, making the populations more prone to extinction. This increased vulnerability to extinction may occur due to different causes such as reduced reproductive fitness and reduced flexibility to deal with the environmental changes and to adapt to the new conditions (Reed & Frankham 2003; Bijlsma & Loeschcke 2011; Fountain *et al*. 2016).

Despite all theoretical support for this prediction (Frankham et al., 2002), the detection of such patterns is not an easy task since genetic diversity loss due to anthropogenic disturbances can be extremely difficult to assess. Empirical evidence show contradictory results in which negative, and neutral effects of anthropogenic disturbances on the genetic diversity of different species has been detected by different studies (Jump & Peñuelas 2006; Wei & Jiang 2012; Silvestrini et al., 2015). Specific biological traits such as dispersion capacity and life form can influence on the species genetic responses to environmental changes (Meyer *et al.* 2009; Struebig *et al.* 2011). For example, species with limited dispersal capacity are more subject to lose genetic diversity in fragmented landscapes than those species that can easily move between fragments (Lino *et al.* 2019). Short-lived species are able to reflect the impacts of environmental disturbances faster than those with long life-cycles (Morris *et al.* 2008; Krauss *et al.* 2010; Rivera-Ortíz *et al.* 2015). A similar pattern can also be observed for different ontogenetic stages, in which early stages might reflect the impacts caused by recent disturbances while advanced ones reflect a historical genetic variation (Van Geert *et al.* 2008; Browne *et al.* 2015). In addition, it is also expected that species pollinated or dispersed by animals are more vulnerable to disturbances (Kolb & Diekmann 2005) and consequently present genetic erosion in modified environments (Giombini *et al*. 2017).

Several methodological approaches used in the genetic studies may also influence the detectability of the disturbance effects, therefore compromising the inference power of the studies. First, the detection of changes in the genetic parameters may vary according to the selected environmental metric (Cushman et al. 212; Jackson & Fahrig 2016). Second, the sensitivity to anthropogenic disturbances should also vary according to the genetic diversity estimator used

since each one of them provides distinct clues about the ecological and evolutionary context in which the populations are inserted (Allendorf 1986). Third, codominant molecular markers have higher content information by locus than dominant markers, which makes them potentially more sensitive to detect the effects of environmental variation (Sunnucks 2000). Among the codominant markers, microsatellites represent a powerful tool for population studies because they have a biparental inheritance, a high degree of polymorphism and mutational rate. Consequently they are able to identify rapid genetic variation as a response to environmental changes (Schlötterer 2000; Ellegren 2004; Selkoe & Toonen 2006; Wang 2011). However, the number of locus used in each study and the nature of the microsatellite marker might influence the detection of the real variation in the genetic diversity parameters. Finally, since there is a reduction of polymorphism in transferred microsatellite due to the ruptures in the elements repeated in the DNA sequence (Garza et al. 1995; Primmer et al. 2005), studies using transferred microsatellites may detect less genetic variation among populations than studies using specific microsatellites (Fischer et al. 2017).

An option to overcome the constraints of individual studies and enhance our understanding on the global patterns of influence of anthropogenic disturbances on species genetic diversity is the use of a meta-analytic approach. In addition to combining unbiased results from different studies on the same topic, a meta-analytic approach also enables the understanding of the sources of heterogeneity (Borenstein *et al.* 2009; Borenstein & Higgins 2013). For this reason, the present study performed a meta-analysis on studies using microsatellite markers to answer the following questions: **(i)** how anthropogenic disturbances influence the genetic diversity of plant and terrestrial animal populations?; **(ii)** how does the methodology - such as locus number, parameter evaluated, type of microsatellite marker and the disturbance descriptor - influence the effect detected by the studies? **(iii)** how do specific traits - such as species biology, taxonomic group, life form, ontogenetic stage, pollinating mechanism, seed dispersal, and reproductive system compatibility - influence the general effect size?

2. Materials and methods

2.1. Literature survey and selection of studies

We searched the available literature considering published manuscripts that evaluated the effects of anthropogenic disturbances on plant and animal genetic diversity up to February 07th, 2019. We searched for all papers indexed to the Scopus database (https://www-scopus.ez85.periodicos.capes.gov.br/search/form.uri?display=basic) using the following term sequences: [("Genetic diversity") AND (Microsatellite OR SSR) AND ("fragmented habitat" OR "habitat fragmentation" OR deforest* OR logging OR "habitat disturbance" OR "disturbed

habitat" OR "habitat loss")]. The term sequences were searched in the title, abstract, and keyword sections of the manuscript, except for the term related to the genetic marker that was searched along the manuscript.

Initially, we identified 1263 manuscripts that were carefully assessed (Fig. S1, Supporting information 1). Our criteria for including a study in the dataset were: **I.** manuscripts published in English language; **II.** studies that used microsatellite markers; **III.** studies that evaluated the effects of anthropogenic disturbances on the genetic diversity of terrestrial plants and/or animals. We considered seven genetic descriptive parameters as a measure of genetic diversity: observed and expected heterozygosity (H_O e H_E), allelic richness (Ar), mean number of alleles (A), mean number of effective alleles (Ae), mean number of exclusive alleles (Ap), and fixation index (F_{IS}); **IV.** studies that evaluated a gradient of anthropogenic disturbances showing a clear contrast between relatively preserved (control) and disturbed (treatment) areas in the same geographical region. We considered studies that evaluated at least one of the seven aspects of habitat disturbance: local habitat quality (e.g. preserved versus disturbed area; primary versus secondary forest); landscape quality (e.g. high intensive agriculture activity versus low agriculture activity; low matrix permeability versus high matrix permeability); landscape connectivity; patch size; forest cover; edge effects; and temporal land use intensification; **V.** studies that provided the genetic data for all evaluated populations (mean value and error measure for each treatment or linear regression coefficient and the sample size) in the body of the article or in the supplementary material, and **VI**. studies with population replication in treatment and control groups. The reviews and meta-analysis identified in this initial search were used as a source of additional papers to compose our database.

This screening procedure resulted in 61 studies and 374 paired comparisons (mean ± SD = 5.2 ± 3.2 comparisons per study). For each study, we extracted information on the methodology used (e.g. nature of the microsatellite marker and the number of loci analyzed) as well as species biological characteristics (Table S1, Supporting information). Whenever the information was not available, we searched the literature to obtain species characteristics and the molecular marker. In general, we used the definitions provided by the authors, but considered plant species as self-compatible the ones with obligatorily or preferentially outcrossing.

2.2. Data analysis

To investigate the overall effect of anthropogenic disturbances on species genetic diversity we performed two-separated analysis: one for studies that used the fixation index (F_{IS}; 35 studies e 57 paired comparisons) and another for studies that used other genetic parameters (H_O, H_E, Ar, Ap

Ae e A; 61 studies e 317 paired comparisons). We opted to this division due to parameters divergence in interpretation. The expected direction of the effect is opposite in the two groups of parameters, which could prevent an indication of a trend among studies. Negative F_{IS} values indicate a positive response to anthropogenic disturbances, while for the other parameters negative values mean deleterious effects on genetic diversity (Aguilar *et al.* 2008; Lino *et al.* 2018).

For those studies that presented results as mean values and standard deviation (82.65% of the studies), we used the unbiased standardized mean difference (Hedges'g) as the effect size, following Borenstein *et al*. (2009):

$$g = J\frac{\overline{X_t}-\overline{X_c}}{S_{within}} \quad \text{(I)}$$

$$S_{within} = \sqrt{\frac{(n_t-1)S_t^2+(n_c-1)S_c^2}{n_t+n_c-2}} \quad \text{(II)}$$

$$J = 1 - \frac{3}{4(n_t+n_c-2)-1} \quad \text{(III)}$$

In which $\overline{X_t}$ e $\overline{X_c}$ represents the mean genetic diversity of the control and treatment group, S_t e S_c are the respective standard deviations, and n_t e n_c are the number of populations evaluated in each treatment group.

For those studies that provided response metrics as correlation coefficients (17.35%), we converted the coefficient value (r) into the Hedges' g value following Borenstein *et al*. (2009):

$$d = \frac{2r}{\sqrt{1-r^2}} \quad \text{(IV)}$$

$$V_d = \frac{4V_r}{(1-r^2)^3} \quad \text{(V)}$$

$$SE_d = \sqrt{V_d} \quad \text{(VI)}$$

$$J = 1 - \frac{3}{4df-1} \quad \text{(VII)}$$

$$g = J \times d \quad \text{(VIII)}$$

$$Vg = J^2 \times V_d \quad \text{(IX)}$$

$$SE_g = \sqrt{V_g} \quad \text{(X)}$$

Where r represents the correlation coefficient, Vr corresponds to the variance of this coefficient and df to the degree of freedom of the study.

We used random-effect models considering that, despite sampling error, there are real differences among studies due to multiple factors interacting on each study region (Gurevitch & Hedges 2001; Borestein *et al*. 2009). Then, we calculated the mean effect size among all studies with a confidence interval of 95%. This effect was considered significant when the CI did not include zero (Rosenberg *et al*. 2000).

Our database was composed mainly of studies encompassing multiple comparisons, which might lead to a lack of independence within data provided by the same study. For this reason, we performed an additional analysis where we used only one comparison per study with 10,000 bootstrap randomizations with repetition (Adams *et al.* 1997). Then, we calculated the mean effect size and the 95% confidence interval through all simulations.

To assess the possible determinants of heterogeneity among studies, we used information about life history and methods and performed a meta-regression (for continuous moderator variable), and sub-group analysis (for categorical moderator variables; Table 1). The meta-regression was used to evaluate the influence of the number of locus measured only by species-specific markers. We also performed linear regressions, using the effect size as the response variable and the number of locus (total and specific) as independent variables. We grouped the effect sizes in subgroups that differ according to the number of markers (total and specific) in each study. The meta-regression was performed through 10,000 randomizations for subsampling, using a single value of each subgroup in each simulation. Further we calculate the confidence interval (95%). To guarantee the statistical power of the subgroup analyses, we only included those categories which had, at least, five studies.

Table 1. Moderator variables and description of the categories used in each sub-group in the meta-analysis.

Moderator	Moderator Type	Description/Category
1. Type of anthropogenic disturbance	Categorical	Local Habitat quality Connectivity Patch size Habitat loss
2. Genetic parameter	Categorical	Observed heterozygosity Expected heterozygosity Allelic richness Mean number of alleles Mean number of effective alleles Mean number of exclusive alleles Fixation index
3. Molecular Marker Type	Categorical	Species-specific markers Transferred markers Both types

4. General number of markers	Continuous	Number of total markers evaluated in each study
5. Number of specific markers	Continuous	Number of total specific markers evaluated in each study
6. Kingdom	Categorical	Plantae Metazoa
7. Ontogenetic stage*	Categorical	Adult Progeny
8. Life-form*	Categorical	Tree Herbs Shrub
9. Pollination mode*	Categorical	Biotic Abiotic
10. Seed dispersal mode*	Categorical	Biotic Abiotic
11. Compatibility system*	Categorical	Self-compatible Self-incompatible

***** Moderators to explore heterogeneity are just a subset of the plant database.

2.3. Publication bias

Publication bias is a phenomenon of the primary literature in which significant results have a greater publication probability (Begg & Berlin 1988). Meta-analyses are sensitive to publication bias, because the strength and/or direction of the mean effect size depend on the studies included. Whether the included studies reflect (or not) the real state-of-the-art, the reliability of results and conclusions might be compromised (Moller & Jennions 2001; Lin & Chu 2018). To evaluate meta-analysis robustness and publication bias we applied two methods: (1) the Fail-Safe Number Rosenthal (Rosenthal 1991), which estimates the number of absent studies with null effects (Hedges' g = 0) required to transform a significant effect size into a non-significant result ($\alpha > 0{,}05$); and (2) Trim & Fill (Duval & Tweedie 2000), which estimates the number of absent results required to make the funnel plot symmetric (the plot in which the effect size of each study are plotted as a function of the standard error). A symmetric plot indicates an absence of bias, and calculates a new effect size considering the inclusion of these studies. For these analyses we also control for pseudo-replication performing 10,000 randomizations with bootstrap (with reposition).

2.4. Model selection

To evaluate which factor best predicts the effect size, we performed a model selection approach based on the Akaike Information Criterion corrected for small sample size (AICc; Burnham & Anderson 2002). Generalized Linear Models (GLMs) with Gaussian distribution were performed using the effect size as dependent variable (excluding outliers) and the moderators that explained part of its heterogeneity as independent variables. We performed separate GLMs for plants and animals because they do not share some moderator variables (i.e. seed dispersal and life form). All possible combinations of the independent variables for plants and animals were tested. We also evaluated a null model containing only the intercept and error. We also control for pseudo-replication using only one comparison by study in each round of the model selection, totaling 10,000 bootstrap randomizations. For each model, we calculated the percentage of simulations in which the mean Akaike weight (w), and the adjusted R^2 was selected (AICc-πi). All analyses were performed in the R software R 3.3.1. (R Core Team 2018).

3. Results

3.1 Overall effect of anthropogenic disturbances

The 61 studies evaluated the genetic response of 69 terrestrial species (38 animals and 31 plants) in 27 different countries, most of them located in Europe (**Fig. 1**). The global effect for the fixation index was not affected by anthropogenic disturbances -0.03 [95% CI: -0.09, 0.02, p = 0.2] and was homogeneous between the different studies (I^2 = 0.00%). In contrast, the other genetic parameters were negatively affected by habitat disturbances -0.45 [95% CI: -0.62, -0.29, p <0.0001], with significant heterogeneity among the effects (I^2 = 35.4%).

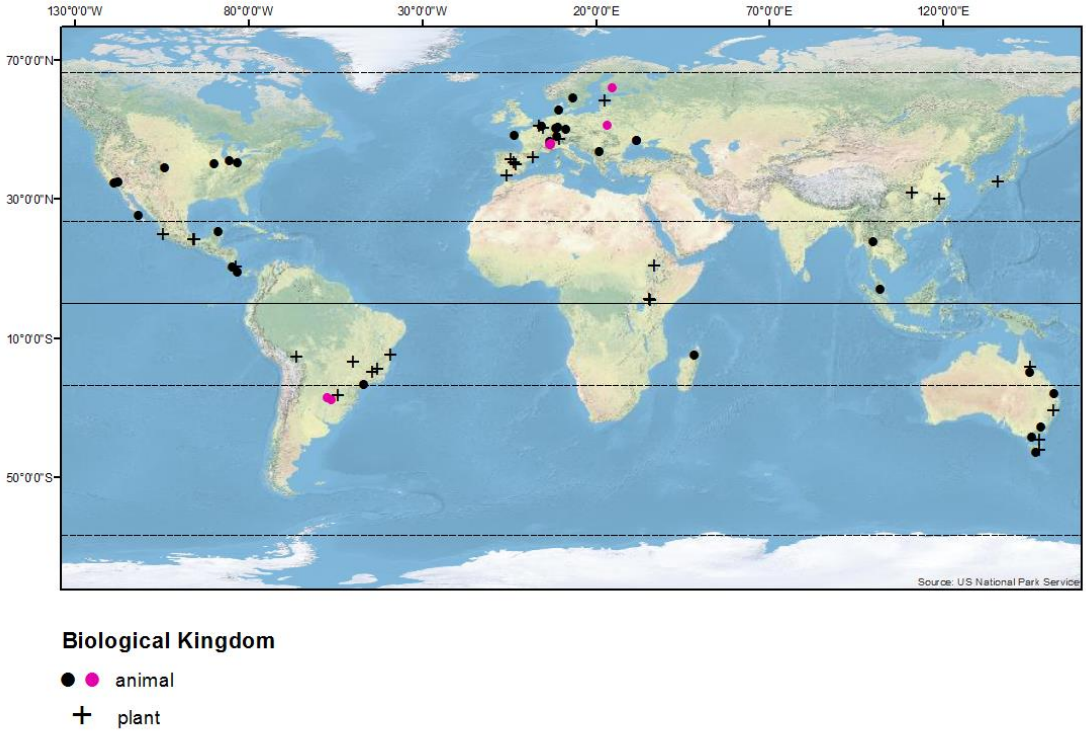

Figure 1. Geographic distribution of sites where animal and plant samples were collected from the 61 studies included in the meta-analysis. The pink dots originate from three studies that collected animal populations in more than one country.

3.2. Moderator Variables

The heterogeneity analyzes (without F_{IS} values) revealed that seven out of eleven moderators evaluated explained a significant proportion of the effect size variation among studies (Table S2, Supporting information). This variation was explained by methodology used by the studies and the species biological traits. We found different impacts and direction of effects according to the disturbance type. Habitat quality loss, measured in local scales, did not affect species genetic diversity. On the contrary, habitat loss, patch size and connectivity significantly decrease genetic diversity (**Fig. 2a**). Additionally, our results show that the direction and magnitude of the effect was also affected by the genetic parameter used, in which allelic richness was the most affected parameter while the mean number of alleles was not affected by disturbances (**Fig. 2b**). The type of microsatellite marker also reflected the divergence in magnitude of effects between the primary studies. It was observed that the genetic diversity estimated by the category of studies using specific markers was not influenced by anthropic disturbances. On the other hand, studies using only transferred markers or both types of markers

have identified a significant reduction in genetic diversity as a result of increased disturbances (**Fig. 2c**). We observed that the loss of genetic diversity due to anthropogenic disturbances was more severe in animals than plants (**Fig. 2d**). Our results show that the overall effect size for plants was masked by their biological traits, in which the mechanisms of pollination, seed dispersal, reproductive compatibility, and lifestyle explain the heterogeneity among studies. In this context, we found that species with biotic pollination and seed dispersal mechanisms, predominantly self-incompatible and those with shrub life-form were the most vulnerable to the loss of genetic diversity due to anthropogenic disturbances (**Fig. 2e, 2f, 2g** e **2h**).

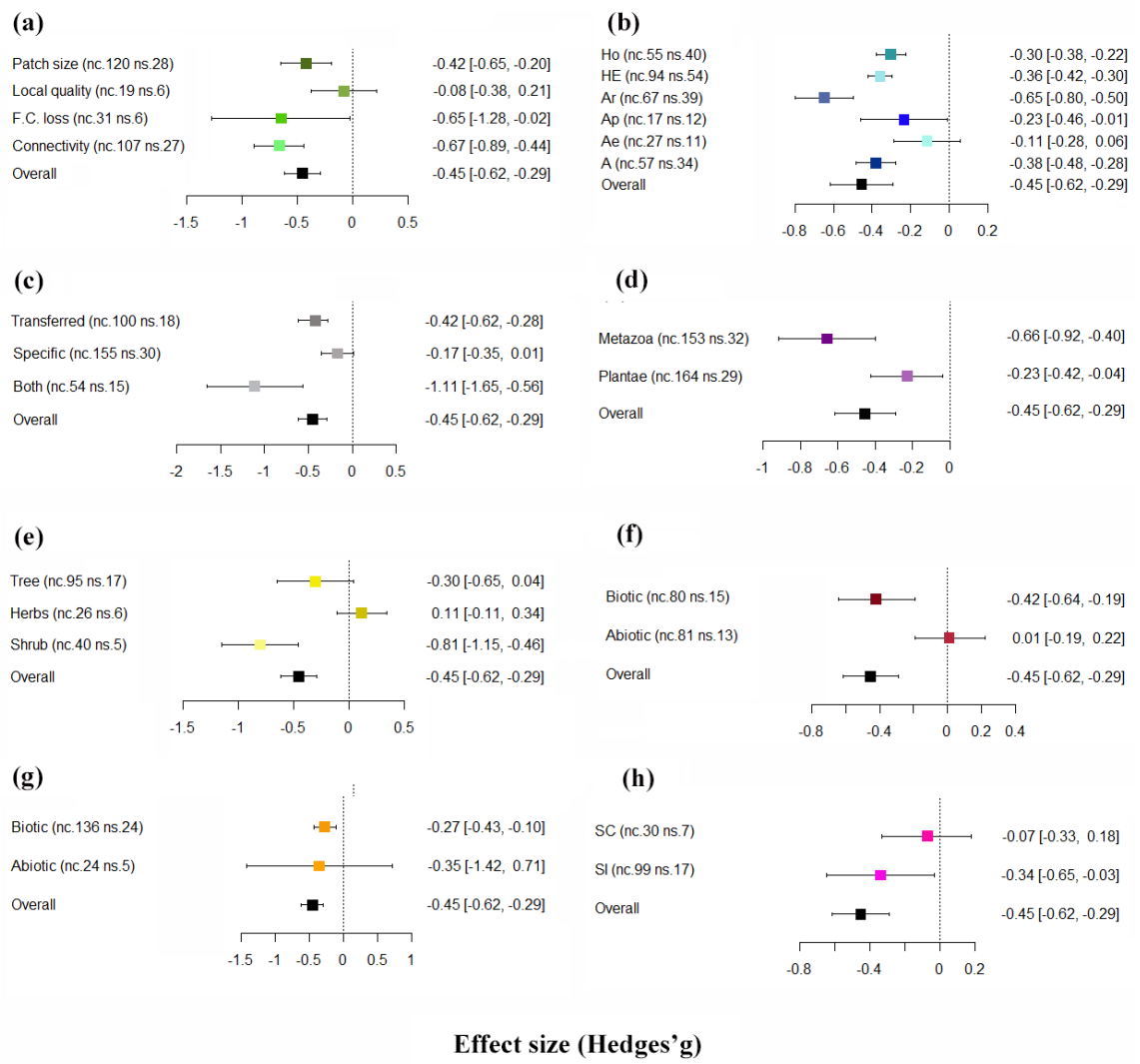

Figure 2. Mean effect size and 95% confidence interval to five subgroups **(a)** Type of anthropogenic disturbance (F.C. forest cover); **(b)** Population genetic parameters (H_O e H_E are respectively, observed and expected heterozygosity; *Ar*, allelic richness; *Ap*, private alleles; *Ae*, effective alleles; *A* mean number of alleles); **(c)** Nature of the microsatellite marker; **(d)** Biological kingdom; **(e)** Life-form; **(f)** Seed dispersal mode; **(g)** Pollination mode; **(h)** Compatibility system

(*SC* self-compatible; *SI* self-incompatible). The values in parentheses refer to the number of comparisons used in each category (nc) and the number of studies evaluated (ns).

3.3. Publication bias

The Trim & Fill analysis indicated that for the meta-analysis performed with the fixation index values it was necessary to include one additional study to make the funnel plot plot symmetrical. However, the effect size recalculated after the inclusion of this study would not change the interpretation of our results (-0.03; 95% CI: -0.08, 0.025). The Rosenthal fail-safe number test performed to the genetic parameters (excluding F_{IS}) showed that 553 no-effect studies would be required to transform the result of this meta-analysis into a non-significant global effect size. This number is almost twice as high as the value suggested by Rosenthal (1991) (5 * 61 + 10 = 315) for a study show a strong evidence of publication bias, considering sample size similar as the one used in this meta-analysis. In addition, the Trim & Fill analysis indicated that no additional studies would be require to the funnel plots presented symmetry (-0.411; 95% CI: -0.59,-0.11). These results combined indicated a high reliability of the meta-analysis results presented here.

3.4. Model selection

The model selection performed without FIS values included 127 comparisons with information from the four variables diagnosed as responsible for effect size heterogeneity in animals. For plants, we made the selection with 119 comparisons resulting from the combination of only six of the seven variables that explained the heterogeneity among the studies. We excluded the pollination variable because the lack of comparisons with plants pollinated by abiotic factors prevented the selection to be performed due to the lack of contrast of this variable. The null model was selected as the most parsimonious to explain the heterogeneity among studies for animals (AICc = 91.97; w= 0.62; AICc-πi= 62.38), and plants (AICc = 57.19; w = 0.36; AICc-πi= 44.99) (Tabela S3, Supporting information).

4. Discussion

This meta-analysis presents the first synthesis of studies on population genetics that simultaneously investigates the response patterns of several taxonomic groups to different types of anthropogenic disturbances on a global scale. Earlier studies that synthesized the effects of

anthropogenic disturbances on the genetic diversity of threatened populations have focused their efforts only on restricted groups of species such as woody plants or mammals (Vranckx et al. 2012; Lino et al. 2018) or on a specific type of disturbance such as habitat fragmentation (Honnay & Jacquemyn 2007; Aguilar et al. 2008; Rivera-Ortíz et al. 2015; Schlaepfer et al. 2018). These investigations employed as criterion of primary studies inclusion, the use of different genetic markers. We chose to focus our synthesis on studies using only a single type of genetic marker to detail the variation of effect size as a function of characteristics related to a specific methodology, since spurious results may be produced as a consequence of variations of different techniques (Fischer et al. 2017). We quantify, on a global scale, the negative effects of different anthropogenic disturbances on the genetic diversity of terrestrial species estimated with microsatellite markers. We provide a detailed quantitative assessment of response variation between studies, which was briefly explained by (i) type of disturbance (ii) population genetic parameter; (iii) microsatellite marker specificity (iv) biological kingdom; (v) plant life form; (vi) mode of seed dispersal; (vii) mode of pollination; and (viii) compatibility of the reproductive system.

We found no evidence of a genetic response pattern to local habitat quality loss, such as changes in the successional stage and conservation status of the fragment. In contrast, habitat loss at the landscape scale, patch size and connectivity negatively impact the genetic diversity of the remaining populations. We observed that the most severe impact on genetic diversity was produced by landscape scale disturbances (such as habitat and connectivity loss). These results corroborate the findings of a recent study that concluded that species abundance is mainly influence by local factors, however genetic diversity tends to be more affected by large spatial scales (Jackson & Fahrig 2014).

Previous meta-analyses have also observed negative effects of habitat loss and fragmentation in the genetic diversity of several taxonomic groups (Rivera-Ortíz *et al*. 2015; Schlaepfer *et al*. 2018; Lino *et al*. 2019). However, these investigations did not differentiate the effects of landscape composition and configuration, as we did here. Therefore, a fundamental advance of our study was the identification of loss of physical connectivity as the main driver of genetic erosion. The main assumption of structural connectivity is that animal movements are constrained by habitat, in other words habitat is equal to connectivity (Fahrig et al. in press). The identification of the role of those habitat-based connectivity metrics can help land management to reduce the deleterious effects of disturbances. For instance, increase habitat conservation is an important outcome because habitat loss is one the main threat to species diversity. In addition, our

results show a wide variation in the mean effect size of the few studies that investigated the impacts of habitat loss. This suggests the needed to more studies on the effects of habitat loss in the genetic diversity. However, increase habitat amount seems the best management strategy to curb genetic diversity erosion in anthropogenic landscapes.

Regarding the different methodologies, we found that populations subject to disturbances did not loss genetic diversity when this estimate was performed using the fixation index or the number of effective alleles. In contrast, the diversity decreased when the estimate was given by observed and expected heterozygosity, average number of alleles, number of exclusive alleles and mainly, allelic richness. The lack of effect in the studies that used the fixation index corroborates what was observed in previous meta-analyzes investigating the effects of habitat fragmentation on endogamy indices (Honnay & Jacquemyn 2007; Vranckx *et al.* 2012; Schlaepfer *et al*. 2018). This pattern was possibly generated by the mechanisms that naturally contribute to the reduction of levels of inbreeding in populations. Among them: I. selection against homozygosis, common in early plant stages (Lesica & Allendorf 1992; Hufford & Hamrick 2003), II. recognition of kinship (Parr *et al*. 2010) that prevents reproduction among closed related individuals and III. genetic purification events, which are responsible for reducing the frequency of allelic variants harmful to populations (López-Cortegano *et al*. 2018; Hedrick & Garcia-Dorado 2016; de Cara *et al*. 2013). In addition, the relatively small temporal scales between the occurrence of the disturbance and the sampling performed by most of the primary studies (i.e. Giombini *et al*. 2017; Omondi *et al*. 2016; Melo *et al*. 2014) may not have been sufficient to produce changes in the rates of current populations (Aguilar *et al*. 2008). Recent disturbance events may also be an explanation for the absence of disturbance effect on the estimates generated by effective alleles. These groups of alleles are most likely to remain in future generations because they are the most frequent in the population (Felsenstein 2016). Therefore, loss of these alleles occurs only over many generations and may characterize a scenario of severe loss of genetic diversity (Lande, 1988).

Regarding the genetic parameters affected by the anthropic disturbances, our results corroborate previous studies that demonstrated that genetic erosion is detected more easily by the allelic richness than by the measurements of heterozygosity (Allendorf 1986; Hoban *et al.* 2014; Greenbaum *et al.* 2014). This is because the richness is an infinite measure, since it considers only the allele presence, whereas the heterozygosity is based only on alleles frequency, which in the case of microsatellite markers can be very noisy due to genotyping artifacts (Fischer *et al.* 2017).

For this reason, loss of rare alleles strongly affects richness estimates, and occurs more frequently in disturbed populations due to the effects of genetic drift (Allendorf & Luikart 2007).

The type of microsatellite used in the primary studies also influenced the magnitude of effect size. Only studies that used transferred microsatellites and a combination of the two types of microsatellites (specific and transferred) detected a negative effect of anthropic disturbances on genetic diversity. Although microsatellite transfer is a widely accepted and used practice for the reduction of laboratory costs worldwide (Oliveira *et al*., 2006), our results did not corroborate our initial expectations on the accuracy of this marker for population studies. We expected that microsatellite transfer would result in less sensitivity in detecting the negative effects of disturbances since previous evidence show that this practice reduced polymorphism of species that are not the source of the projected primer (Hutter *et al*. 1998; Morin *et al.* 1998; Fischer *et al.* 2017). Microsatellite transfer between congeners species occurs due to the orthologous nature of the DNA sequence of the flanking regions (Peakall *et al.* 1998; Oliveira *et al*. 2006). The ancestral character of the orthologous sequences (Shabalina *et al.* 2001) and the characteristic of the high mutational rates of microsatellites (Ellegren 2004) probably produced a large number of rare genetic variants along evolutionary time. Therefore, an alternative explanation for the pattern found is that studies using microsatellite transfer have detected more severe effects of environmental disturbances possibly because a higher number of rare alleles has been lost as a result of genetic drift.

Our primary data set was represented by animals of different taxonomic categories, such as birds, insects, reptiles and mammals. These organisms have different reproductive strategies, mechanisms of locomotion and feeding behavior. A recent meta-analysis detected strong negative effects of habitat loss and fragmentation in mammalian genetic diversity (Lino *et al*. 2018). This meta-analysis also found that specific traits such as body size, herbivory diet and forest-dependent species are more prone to deleterious genetic effects. Here we found strong responses for animals in general, which seems to be independent of the life-history traits of the species.

Our meta-analysis also showed a significant reduction in plant genetic diversity, but this effect was less deleterious than the effect detected for animals. For this reason, we hypothesized that the descriptors related to the biology and ontogeny of plant species may have influenced the patterns of response to environmental modifications, softening the deleterious effects of disturbances (Honnay *et al*. 2005; Ghazoul 2005; Pasion *et al*. 2018). Our results show that the plant species most vulnerable to anthropogenic disturbances have a shrub life form, a self-

incompatible reproductive system, and biotic seed dispersal and pollination modes. Previous meta-analyzes have detected negative effect of habitat fragmentation on the genetic diversity of herbaceous and woody plants when these groups were evaluated individually (Aguilar *et al*. 2008; Vranckx *et al.* 2012). We previously expected that the inclusion of all these groups in a single investigation would result in a pattern governed by the differences in the generational time of these life forms since the generation number after the disturbance is considered a crucial factor in determining the genetic reduction (Lowe *et al*. 2005). Thus species with a short life cycle, such as herbaceous ones, would be most affected by anthropogenic disturbances, followed by shrubs and later by trees. On the contrary, our results did not corroborate this pattern. For this reason, we highlight that of the six herbaceous species included in our meta-analysis, four of them had abiotic mechanism of seed dispersal and a fifth species, had a self-compatible reproductive system (Table S1, Supporting information**)**. Considering that our meta-analysis has detected that these characteristics make plant genetic diversity less sensitive to environmental modifications, a possible explanation for the result found is that ecological traits are more important in determining the pattern of genetic response than life history traits. Finally, our results demonstrated a cascade effect of the loss of pollinators and seed dispersers that are commonly observed in modified landscapes (Alaniz *et al*. 2018; Taki & Kevan 2007; Pérez-Méndez *et al*. 2016). These results support empirical evidence (Calviño-Cancela *et al*., 2012, Pérez-Méndez *et al*., 2017) and expand previous knowledge about the characteristics that describe the degree of genetic vulnerability of species to environmental disturbances.

Finally, our model selection showed that when all the biological and methodological characteristics responsible for heterogeneity among the primary studies are evaluated simultaneously, there is no single variable that is more important in determining effect size. This means that irrespective of variations between studies, anthropogenic disturbances generate a consistently negative effect on the genetic diversity of terrestrial animal and plant species, which compromises the viability of the remaining populations in long-term modified landscapes.

In conclusion, among the types of disturbances evaluated, the lack of physical connectivity among the fragments was the most deleterious impact on the parameters of genetic diversity. In addition, not all parameters responded equally to environmental disturbances, so that allelic richness was identified as the best parameter to reflect the erosion of genetic variability. Contrary to all previous expectations, we showed that the transferred microsatellites were the markers that presented the greatest sensitivity to diagnose the negative impacts of the environmental

disturbances. Finally, we conclude that, in genetic terms, both animals and plants are affected by anthropogenic disturbances. However, exceptionally vulnerable organisms in the group of plants exhibit shrub life-form, mechanisms biotic of pollination and seed dispersal, and a predominantly self-incompatible reproductive system. Thus, we recommend that future conservation practices concentrate their efforts on measures that increase the structural connectivity of the fragments and monitor in the long term the species with the characteristics identified here as the most vulnerable to genetic erosion.

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Supporting information

1. Criteria for Study Inclusion

We performed a careful procedure to select the studies to be included in the meta-analysis (Figure A1). The literature survey resulted in 61 manuscripts (ver Table A1). Our dataset encompassed studies that compared the genetic diversity between a disturbed site (treatment) and an area closer to the natural condition (control) within the same study context and by data resulted from studies of correlational nature. Few studies explicitly provided mean values and standard deviation of the genetic parameters for both, treatment and control conditions. In spite of this, we obtained this information for about 82.65% of the comparisons because most studies provided row values of the individual genetic parameter for each population. The information was obtained in the body of the text or in the supplementary information section, allowing a posteriori calculations (Suárez-Montes et al., 2016). For the studies that performed simple linear regressions, we obtained the correlation values (r) of the coefficient of determination (R2). When these values were not presented in the text (for instance, when they were not significant) or when the studies used a model selection approach, but the information related to the environmental variables (eg. percentage of forest cover) and the genetic diversity by population were presented (Santos et al., 2015), we performed linear regressions after testing the normality assumptions and homoscedasticity of the variance. When at least one of these assumptions was violated, we performed Pearson correlation. In addition, we also used directly the Pearson or Spearman correlation values when presented. (Gómez-Fernández et al., 2016; Magle et al., 2010). All effect sizes were standardized by converting the r-values into the g Hedges effect size (for details see Borenstein et al., 2009).

In the studies with multiple responses, we included more than one comparison per manuscript only when different measures of genetic diversity, categories of disturbance, species, ontogenetic stages (see details table 2), or multiple treatment conditions were assessed e.g. Taylor et al., 2007. However, even if one study evaluated the effect of anthropogenic disturbances on several ontogenetic stages, we considered only the data provided for the oldest (adult) and the most recent (progeny) ontogenetic stage.

The anthropogenic disturbances were classified according to the spatial scale of effect (local and landscape) and were subsequently subdivided into seven categories that unified characteristics of common effect among them. We define four categories of disturbance that are quantified on a local scale: habitat quality, patch size, edge effect, and temporal land use intensification. The habitat quality category included studies comparing forests at different successional stages and studies comparing conserved habitats with degraded or defaunated areas. The studies included in patch size category were all those that provided accurate information on the size of the fragment or provided qualitative information on the size these fragments (i.e. small, medium and large). The edge effect category was composed only of a single study that compared the genetic diversity of the populations present on the edge and in the interior of the analyzed habitat. The studies in the category of land use intensification were the ones considering the temporal analysis of the same scenario comparing an initial time of conserved habitat with a final time of disturbed habitat. Finally, we define three categories of disturbance that are quantified at large scales: landscape quality, connectivity, and forest cover. The studies included in the landscape quality category evaluated the environmental context of the surroundings where the populations investigated were inserted. Thus, they compared scenarios with high and low anthropic activity or distinct levels of matrix permeability. The connectivity category included studies that provided accurate information on the distance between the fragments or reported the connectivity degree of the fragments sampled (i.e. continuous, poorly isolated and very isolated habitat). Therefore this category was considered fundamentally as a measure of physical isolation

between the fragments. Finally, the category of forest cover grouped all the studies that investigated the impact of the amount of habitat in the landscape on the genetic diversity of the remaining populations. In all cases, when the studies presented two measures related to the same category of anthropogenic disturbance (i.e. connectivity measured by Euclidean distance and effective distance, Struebig et al., 2011), we used a single measure of disturbance chosen randomly to correlate with all values of the genetic parameters.

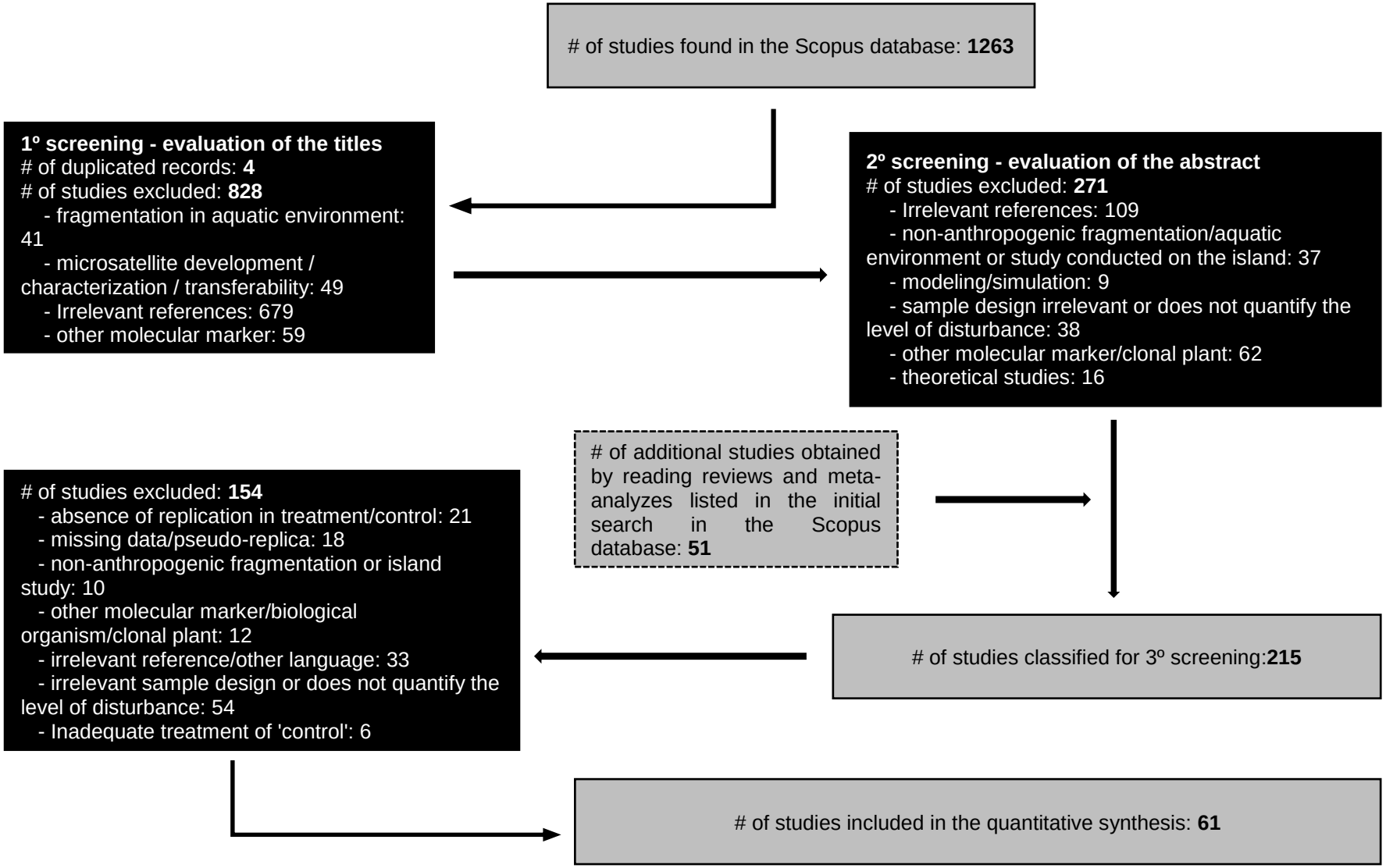

Fig. A.1. Flow diagram summarizing the meta-analysis strategy.

Table A.1. Espécies de plantas e animais investigadas nos estudos incluídos na meta-análise com informações referentes ao número de marcadores microssatélites utilizado nestes estudos e os traços biológicos das espécies vegetais. Na seção referência, os estudos que estão listados em negrito fazem parte do conjunto final dos 61 artigos selecionados na triagem dos dados. Os demais trabalhos foram utilizados para consulta de informações que não estavam claramente disponíveis nos artigos da base de dados primária.

Species	Kingdom	Total markers	Specific markers	Transferred markers	Pollination mechanism	Dispersion mechanism	Compatibility system	Life-form	References
Acacia senegal	Plantae	12	9	3	biotic	biotic	SI	tree	**Omondi et al. 2016**; Tandom et al. 2001; Odee et al. 2012
Alouatta caraya	Metazoa	10	10	0	NA	NA	NA	NA	**Oklander et al. 2017**; Oklander et al. 2007
Ambystoma texanum	Metazoa	8	7	1	NA	NA	NA	NA	**Rhoads et al. 2017**
Aphelandra aurantiaca	Plantae	11	11	0	biotic	abiotic	SC	herb	**Suárez-Montes et al. 2016**
Apis dorsata	Metazoa	4	0	4	NA	NA	NA	NA	**Rattanawannee et al. 2012**; Estoup et al. 1994, 1993; Solignac et al. 2007
Bufo calamita	Metazoa	9	9	0	NA	NA	NA	NA	**Allentoft et al. 2008**

Cabralea canjerana	Plantae	6	6	0	biotic	biotic	SI	tree	Melo et al. 2012; Pereira et al. 2011
Carabus problematicus	Metazoa	8	6	2	NA	NA	NA	NA	Gaublomme et al. 2013
Carica papaya	Plantae	6	6	0	biotic	biotic	SI	shrub	Chávez-Pesqueira et al. 2014; Suresh et al. 2008; Ocampo et al. 2006
Caryocar brasiliense	Plantae	10	10	0	biotic	biotic	SI	tree	Collevati et al. 2001
Castanopsis sclerophylla	Plantae	8	0	8	abiotic	abiotic	NA	tree	Wang et al. 2011
Centaurea hyssopifolia	Plantae	8	0	8	biotic	abiotic	SI	shrub	Matesanz et al. 2018
Cervus elaphus	Metazoa	14	0	14	NA	NA	NA	NA	Dellicour et al. 2011
Chamaea fasciata	Metazoa	7	0	7	NA	NA	NA	NA	Delaney et al. 2010
Clusia lechleri	Plantae	8	0	8	biotic	biotic	SI	tree	Quevedo et al. 2013
Clusia sphaerocarpa	Plantae	8	0	8	biotic	biotic	SI	tree	Quevedo et al. 2013
Corapipo altera	Metazoa	15	15	0	NA	NA	NA	NA	Barnett et al. 2008
Cynomys ludovicianus	Metazoa	10	10	0	NA	NA	NA	NA	Magle et al. 2010

Dalbergia nigra	Plantae	6	6	0	biotic	abiotic	NA	tree	**Resende et al. 2011**
Dipteryx panamensis	Plantae	9	0	9	biotic	biotic	SI	tree	**Hanson et al. 2008**
Eucalyptus globulus	Plantae	6	4	2	biotic	abiotic	SI	tree	**Mimura et al. 2009**; Brondani et al. 1998; Steane et al. 2001
Euglossa dilemma	Metazoa	5	0	5	NA	NA	NA	NA	**Soro et al. 2017**; Paxton et al. 2009; Souza et al. 2007
Euglossa viridissima	Metazoa	5	0	5	NA	NA	NA	NA	**Soro et al. 2017**; Paxton et al. 2009; Souza et al. 2007
Euptelea pleiospermum	Plantae	8	8	0	abiotic	abiotic	NA	tree	**Wei & Jiang 2012**; Zhang et al. 2008
Euterpe edulis	Plantae	10	10	0	biotic	biotic	SI	tree	**Santos et al. 2015**; Gaiotto et al. 2003; Conte et al. 2008;
Fagus sylvatica	Plantae	6	4	2	abiotic	biotic	SI	tree	**Jump & Peñuelas 2006**
Geum urbanum	Plantae	6	6	0	biotic	biotic	SC	herb	**Vandepitte et al. 2007**

Glis glis	Metazoa	14	11	3	NA	NA	NA	NA	**Fietz et al. 2014**; Dabert et al. 2009
Gnypetoscincus queenslandiae	Metazoa	9	9	0	NA	NA	NA	NA	**Sumner et al. 2004**; Sumner et al. 2001
Grevillea caleyi	Plantae	7	0	7	biotic	abiotic	SC	shrub	**Llorens et al. 2018**
Helianthemum squamatum	Plantae	8	8	0	biotic	abiotic	SI	shrub	**Matesanz et al. 2018**
Hyla arborea	Metazoa	6	6	0	NA	NA	NA	NA	**Dubey et al. 2009**
Kerivoula papillosa	Metazoa	15	15	0	NA	NA	NA	NA	**Struebig et al. 2011**
Lepidium subulatum	Plantae	8	8	0	biotic	abiotic	SI	shrub	**Gómez-Fernández et al. 2016**; Matesanz et al. 2018
Lychnis flos-cuculi	Plantae	7	7	0	biotic	abiotic	SI	herb	**Galeuchet et al. 2005**; Galeuchet et al. 2002
Lynx rufus	Metazoa	4	0	4	NA	NA	NA	NA	**Ruell et al. 2012**; Menotti-Raymond et al. 1999
Macadamia tetraphylla	Plantae	4	0	4	biotic	biotic	SI	tree	**Spain & Lowe 2011**; Pisanu et al. 2009

Marmosops incanus	Metazoa	12	8	4	NA	NA	NA	NA	Balkenhol et al. 2013
Metrioptera roeseli	Metazoa	6	6	0	NA	NA	NA	NA	Lange et al. 2010; Holzhauer & Wolff 2005
Microcebus tavaratra	Metazoa	15	0	15	NA	NA	NA	NA	Aleixo-Pais et al. 2018
Myrmeciza exsul	Metazoa	16	15	1	NA	NA	NA	NA	Woltmann et al. 212
Myrtus communis	Plantae	10	10	0	biotic	biotic	SC	shrub	Nora et al. 2015
Peromyscus leucopus	Metazoa	8	8	0	NA	NA	NA	NA	Mossman & Waser 2001
Petauroides volans	Metazoa	12	12	0	NA	NA	NA	NA	Taylor et al. 2007; Taylor et al. 2002
Petaurus norfolcensis	Metazoa	6	2	4	NA	NA	NA	NA	Goldingay et al. 2013
Phaius australis	Plantae	13	13	0	NA	abiotic	SC	herb	Simmons et al. 2017
Pholidoptera griseoaptera	Metazoa	8	8	0	NA	NA	NA	NA	Lange et al. 2010; Arens et al. 2005

Pinus pinaster	Plantae	6	2	4	abiotic	abiotic	NA	tree	De-Lucas et al. 2009; Mariette et al. 2001; González-Martínez et al. 2002; Chagné et al. 2004
Pistacia lentiscus	Plantae	7	7	0	abiotic	biotic	SI	shrub	Nora et al. 2015
Plestiodon skiltonianus	Metazoa	6	0	6	NA	NA	NA	NA	Delaney et al. 2010
Polyommatus coridon	Metazoa	7	7	0	NA	NA	NA	NA	Habel et al. 2014
Primula vulgaris	Plantae	3	3	0	biotic	biotic	SI	herb	Van Geert et al. 2008
Prunus africana	Plantae	7	0	7	biotic	biotic	SI	tree	Yineger et al. 2014; Nantongo et al. 2016
Pseudocheirus peregrinus	Metazoa	15	10	5	NA	NA	NA	NA	Lancaster et al. 2016
Rana temporaria	Metazoa	7	6	1	NA	NA	NA	NA	Johansson et al. 2005; Palo et al. 2003
Rattus lutreolus	Metazoa	11	0	11	NA	NA	NA	NA	Stephens et al. 2013

Rhinanthus osiliensis	Plantae	15	3	12	biotic	abiotic	SC	Hemiparasita	**Aavik et al. 2016**; Ducarme et al. 2008; Houston & Wolff 2009; Talve et al. 2014
Rhinolophus lepidus	Metazoa	10	0	10	NA	NA	NA	NA	**Struebig 2011**
Rhinolophus trifoliatus	Metazoa	8	0	8	NA	NA	NA	NA	**Struebig 2011**
Sceloporus occidentalis	Metazoa	NA	NA	NA	NA	NA	NA	NA	**Delaney et al. 2010**
Spermophilus citellus	Metazoa	12	0	12	NA	NA	NA	NA	**Ćosić et al. 2013**; Říčanová et al. 2011
Spermophilus suslicus	Metazoa	11	9	2	NA	NA	NA	NA	**Biedrzycka & Konopiński 2008**; Gondek et al. 2006
Speyeria idalia	Metazoa	4	4	0	NA	NA	NA	NA	**Williams et al. 2003**; Williams et al. 2002
Swietenia humilis	Plantae	7	5	2	biotic	abiotic	SI	tree	**Rosas et al. 2011**
Syagrus romanzoffiana	Plantae	9	0	9	biotic	biotic	NA	tree	**Giombini et al. 2017**; Guix & Ruiz 2000
Tetrao tetrix	Metazoa	14	7	6	NA	NA	NA	NA	**Caizergues et al. 2003**

Urosaurus nigricaudus	Metazoa	10	10	0	NA	NA	NA	NA	Munguia-Veja et al. 2013; Rodriguez-Estrella et al. 2006
Uta stansburiana	Metazoa	NA	NA	NA	NA	NA	NA	NA	Delaney et al. 2010
Viola grypoceras	Plantae	6	6	0	biotic	abiotic	SC	herb	Toma et al. 2015

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2. Results

Table A.2. Results of the meta-analysis using Hedges' g as effect index for as eleven moderator variables evaluated. The table shows the number of comparisons and studies in each moderator category that was used for the analysis, the mean Hedges'g and a 95% confidence intervals generated with 10.000 permutations (with replacement) considering all comparisons and only one comparison per study. Para os moderadores contínuos a tabela exibe a média de comparações em cada subconjunto da quantidade de marcadores utilizados nos estudos primários e o resultado das 10.000 simulações de regressão linear com o valor médio do R^2 e o intervalo de confiança dos slopes das regressões.

CATEGORICAL MODERATOR						
	ALL COMPARISONS			1 COMPARISON/STUDY		
Moderator	**n comparisons**	**mean**	**95%CI**	**n study**	**mean**	**95%CI**
Antropic disturbance						
Habitat quality (local)	19	0.25	-0.81; 1.32	6	-0.08	-0.38; 0.21
Connectivity of fragment	107	-0.13	-1.15; 0.89	27	-0.66	-0.89; -0.45
Size of the fragment	120	-0.14	-0.95; 0.66	28	-0.42	-0.65; -0.20
Forest cover	31	-0.02	-1.06; 1.03	6	-0.65	-1.28; -0.03
Genetic parameters						
Heterozygosity observed	55	-0.08	-0.46; 0.31	40	-0.30	-0.38; -0.22
Heterozygosity expected	94	-0.11	-0.47; 0.25	54	-0.36	-0.42; -0.30
Allelic richness	67	-0.35	-0.73; 0.03	39	-0.65	-0.80; -0.50
Average number of alleles per locus	57	-0.09	-0.47; 0.29	34	-0.38	-0.48; -0.28
Average number of effective alleles	27	-0.19	-0.50; 0.12	11	-0.11	-0.28; 0.06
Average number of exclusive alleles	17	-0.02	-0.53; 0.49	12	-0.24	-0.46; -0.01
Inbreeding index	57	-0.04	-0.21; 0.14	35	-0.03	-0.09; 0.02

Marker molecular						
Species-specific	155	0.70	-0.91; 2.30	30	-0.17	-0.35; 0.01
Transferred	100	0.47	-1.15; 2.08	18	-0.42	-0.62; -0.28
Both	54	-0.05	-1.67; 1.57	15	-1.11	-1.65; -0.56
Kingdom						
Animalia	153	-0.42	-0.55; -0.28	32	-0.66	-0.92; -0.40
Plantae	164	0.19	-0.004; 0.38	29	-0.23	-0.42; -0.04
Ontogenetic stage						
Adult	64	-0.31	-0.47; -0.16	13	-0.27	-0.55; 0.009
Progeny	34	-0.06	-0.37; 0.25	10	-0.50	-1.04; 0.04
Life form						
Arboreal	95	2.41	0.92; 3.90	17	-0.30	-0.65; 0.05
Herbaceous	26	2.73	1.24; 4.22	6	0.12	-0.11; 0.34
Shrubs	40	2.15	0.66; 3.65	5	-0.81	-1.15; -0.46
Dispersion Mechanism						
Biotic	80	-0.23	-0.43; -0.02	15	-0.42	-0.64; -0.19
Abiotic	81	-0.1	-0.22; -0.03	13	0.01	-0.19; 0.22
Pollination Mechanism						
Biotic	136	-0.06	-0.45; 0.34	24	-0.27	-0.43; -0.10
Abiotic	24	-0.20	-0.58; 0.18	5	-0.35	-1.42; 0.71
Compatibility system						
Self-compatible	30	-0.02	-0.26; 0.22	7	-0.07	-0.33; 0.18
Self-incompatible	99	-0.31	-0.59; -0.02	17	-0.34	-0.65; -0.03

CONTINUOS MODERATOR			
Moderator	**mean comparisons / marker number**	**R^2**	**95% CI slope**
General number of markers	8.53	0.06	-0.14; 0.10
Number of specific markers	8.50	0.50	-0.04; 0.18

Table A.3. Resultados da seleção do modelo linear generalizado (GLM) para o conjunto dos estudos que avaliaram o grupo dos animais e das plantas. Estes modelos foram criados através de todas as interações possíveis entre o tamanho de efeito de cada estudo (variável dependente) e de três variáveis independentes para o grupo dos animais e sete variáveis para as plantas. Em ambos os casos de seleção incluímos o modelo nulo, contendo apenas os parâmetros de intercepção e erro. *K* is the number of parameters in the model (regression intercept, fixed-effects coefficients and residual variance), *logLik* is the log-likelihood of the model, *AICc* is the Akaike Information Criterion value corrected for small sample sizes, *AICc-πi* is the proportion of times each model was selected as the top-ranked model among all 10,000 sample fits, *w* is the Akaike weight, and R^2 is the mean goodness of fit for each model with 95% CIs at the 2.5 and 97.5 percentiles of all 10,000 sample fits.

Ranking	**Modell**	**k***	**logLik**	**AICc**	**AIC**	**Δ AICc**	**w.AICc**	**#simulation**	**AICc-π_i**	**R^2**
Metazoa										
1	null	2	-43.76	91.97	91.52	0	0.62	6238	62.38	_
2	marker specificity (MS)	4	-40.7	91	89.4	0	0.6	2949	29.49	0.22 (0.21;0.24)
3	disturbance (D)	8.5	-31.1	87.27	79.26	0	0.67	662	6.62	0.60 (0.59;0.63)
4	genetic parameter (GP)	6.5	-36.24	89.68	85.38	0	0.5	88	0.88	0.41 (0.42;0.44)
5	MS + GP	5	-40.13	92.76	90.26	0	0.52	58	0.58	0.28 (0.27;0.30)
6	GP + D	9.8	-30.66	91.75	80.82	0	0.49	2	0.02	0.61 (0.61;0.62)
7	MS +GP + D	11.7	-21.84	84.13	67	0	0.66	2	0.02	0.80 (0.80;0.81)
8	MS + D	7	-37.64	94.37	89.28	0	0.37	1	0.01	0.41 (_____)
Plantae										
1	Null	2	-26.26	57.19	56.53	0	0.36	4499	44.99	_
2	Life-form (LF)	4	-21.48	53.46	50.96	0	0.39	3272	32.72	0.35 (0.33;0.39)

3	seed dispersal (SD)	3	-23.28	53.97	52.56	0	0.35	1522	15.22	0.21 (0.19;0.24)
4	D+SD	7	-14.86	52.34	43.72	0	0.48	232	2.32	0.70 (0.69;0.74)
5	SD+LF	5	-17	48	44.01	0	0.36	142	1.42	0.53 (0.53;0.56)
6	genetic parameter (GP)	6.3	-17.41	54.04	47.33	0	0.44	98	0.98	0.62 (0.63;0.68)
7	GP+LF	8.2	-11.9	53.46	40.28	0	0.47	87	0.87	0.81 (0.81;0.86)
8	MS+LF	6	-15.83	49.66	43.66	0	0.4	57	0.57	0.63 (0.63;0.67)
9	marker specificity (MS)	4	-22.5	55.51	53.01	0	0.28	29	0.29	0.30 (0.30;0.32)
10	disturbance (D)	6	-18.14	54.29	48.29	0	0.39	16	0.16	0.57 (0.56;0.59)
11	GP+SD	6.9	-14.97	52.12	43.71	0	0.35	9	0.09	0.71 (0.72;0.73)
12	GP+CS	6.8	-16.52	54.89	46.71	0	0.28	6	0.06	0.63 (0.63;0.64)
13	D+LF	8	-12.25	52.5	40.5	0	0.36	6	0.06	0.77 (0.75;0.78)
14	MS+GP+LF	10	-7.17	56.35	34.35	0	0.55	5	0.05	0.20 (0.22;0.26)
15	Compatibility system (CS)	3	-23.94	55.3	53.89	0	0.24	5	0.05	0.16 (0.16;0.17)
16	MS+SD	5	-21.92	57.84	53.84	0	0.34	3	0.03	0.41 (0.41;0.42)
17	LF+CS+MS	7	-13.64	49.9	41.28	0	0.3	3	0.03	0.10 (0.13;0.15)
18	LF+CS+GP	9	-3.09	40.54	24.18	0	0.4	2	0.02	0.86 (0.85;0.87)
19	LF+CS	5	-17.21	48.41	44.41	0	0.36	2	0.02	0.47

										(0.47;0.49)
20	SD+LF+CS+GP	9.5	-5.32	48.82	29.64	0	0.41	2	0.02	0.25 (0.32;0.33)
21	GP+D	9	-8.53	51.43	35.06	0	0.39	1	0.01	0.98 (_____)
22	GP+D+LF	12	7.23	48.54	9.54	0	0.72	1	0.01	0.03 (_____)
23	D+SD+LF	9	-9.89	54.15	37.79	0	0.43	1	0.01	0.62 (_____)

CAPÍTULO 2

Anthropic disturbances eroding the genetic diversity of a threatened palm tree: a multi-scale approach*

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Abstract

Habitat loss and the illegal exploitation of natural resources are among the main drivers of species extinction around the world. These disturbances act at different scales, once changes in the landscape composition and configuration operate at large scales and exploitation of natural resources at local scales. Evidence suggests that both scales are capable of triggering genetic erosion in the remaining populations. However, most of the studies so far did not evaluate simultaneously the effects of these disturbances on genetic diversity and structure of plants. In this study, we used a multiple scale approach to empirically evaluate the impacts caused by local and landscape scale disturbances in the genetic diversity and structure of an endangered palm tree, *Euterpe edulis*. We sampled and genotyped with microsatellite markers 544 juveniles of *E. edulis* in 17 fragments of Atlantic Forest in Brazil. In addition, we estimated the local logging rate and the forest cover and isolation at landscape scale. We found that the palm populations have not undergone any recent bottleneck events and that only logging intensification had affected the fixation index and the number of private alleles. Additionally, we did not detect any evidence of spatial genetic structure or genetic divergence associated with environmental disturbance variables at different scales. However, we identified distinct genetic clusters, which may indicate a reduction of gene flow between fragments that were previously a continuous habitat. Our results show that local disturbances, which act directly on population size reduction, such as logging, modified the genetic diversity more rapidly, whereas genetic structure is probably more influenced by large-scale modifications. In this way, to maximize the conservation efforts of economically exploited species, we recommend increasing the inspection to reduce the illegal exploitation, and reforestation of degraded areas, in order to increase the gene flow in Atlantic Forest fragments.

Key-words: landscape genetics, tropical rainforest, conservation, threatened species, molecular marker.

INTRODUCTION

The conversion of natural environments into anthropogenic landscapes is one of the main causes of biodiversity loss worldwide (Laurance et al., 2014; Newbold et al., 2015; Miraldo et al., 2016). Mounting evidence shows the pervasive effects of habitat loss on several taxonomic groups (Mortelliti et al., 2010; Ferreira et al., 2015; Muylaert et al., 2016; Rocha-Santos et al., 2017). The remaining forest patches are being reduced and isolated with negative consequences for species diversity. In this context, the remaining populations are prone to gene flow decrease, alleles loss due to genetic drift, and inbreeding depression. The consequence of these genetic changes might be a lower ability to adapt to further environmental changes (Browne and Karubian, 2018; Rhoads et al., 2017; Fountain et al., 2016; Bijlsma and Loeschcke, 2012). Several studies have corroborated this prediction (Dixo et al., 2009; Zhang et al., 2012; Wood et al., 2017), however, the intensity and the velocity of the response to impacts vary according to life history and environmental characteristics (Vranckx et al., 2012; Lino et al., 2019).

In human-modified landscapes, species of economic value are also locally vulnerable due to (i) the direct removal of individuals or (ii) the indirect exploitation of resources, such as seeds, impairing the regeneration capacity of these species (Homma, 1992; Peres et al., 2003). Acute disturbances, such as logging, are capable of causing severe demographic depletion over generations (Richardson and Peres, 2016) and increase the risk of genetic erosion in the remaining populations (Shaanker et al., 2003, 2004; Dai et al., 2018). Theoretical and empirical studies have observed reductions in allele number and heterozygosity and an increase in spatial genetic distance in exploited populations (Cruse-Sanders et al., 2005; Sebbenn et al., 2008; Lacerda et al., 2008). However, investigations that simultaneously assessed the individual impact caused by landscape and local scale disturbances are scarce (but see Gonzalez-Fernandez et al. 2019).

The combination of anthropogenic pressures at the landscape and local scale is striking in the tropics. The tropics stand out worldwide due to the high rates of species biodiversity and endemism, but the increasing human population size and forest loss is a growing threat (Hansen et al., 2013; Lewis et al., 2015). In this scenario, understanding the individual impact of different types of disturbance on the genetic diversity of exploited species could better direct conservation efforts. For this reason, in this study,

we used a multi-level approach to investigate the effects of anthropogenic disturbances at the landscape and local scale on the genetic diversity and structure of *Euterpe edulis* populations. This species is native to the Atlantic Rainforest (Reis et al., 2000a), a biome that currently conserves only 12% of its original coverage (Ribeiro et al., 2009). Moreover, this palm is in the list of the Brazilian species threatened with extinction because of the population decline recorded in the last decades (Martinelli and Moraes**,** 2013). The species has great economic importance due to the illegal harvest to the commercialization of the apical meristem (Galetti and Fernandez, 1998; Matos and Bovi, 2002). The harvest culminates in the death of individuals, as there is no regrowth after cutting (Ferri and Cavalcante, 1997). In the tropics, changes in the landscape composition and configuration, such as forest cover loss and isolation provide more accessibility to forest resources, increasing illegal hunt and logging (Tabarelli et al., 2004). These activities might in turn, negatively affect seed dispersal by vertebrates and seedling recruitment (Peres and Palacios, 2007; Gutiérrez-Granados, 2011). Using a multi-scale inference approach, we evaluated how landscape composition and configuration, measured at several spatial scales, and local disturbances, affect the genetic diversity of *E. edulis*. We predicted that estimates of genetic diversity and structure would be strongly influenced by the synergetic effects of disturbances acting at different scales. Thus, models containing a combination of landscape and local variables would better explain the genetic diversity of the species.

MATERIALS AND METHODS

Study area and sites selection

The study region was located in the Atlantic Rainforest in Southern Bahia State, Brazil. Deforestation in the region started in the mid-1980's and was intensified in the 1990s during the cocoa crisis, the main economic product at that time (Rocha, 2006). The region presents some of the last remnants of the northeastern Atlantic Forest (Araújo et al., 1998) and still harbors a large number of flora and fauna species, including several endemics (Thomas et al., 1998). The land use history resulted in a mosaic of forest patches in different successional stages immersed in a matrix of pasture, rubber and eucalyptus plantations, and cacao agroforests (Landau, 2003; Sambuichi, 2003; Landau, 2008). The native vegetation is tropical lowland rainforest

and the climate is classified as Af, according to Köppen, hot and moist, without a distinct dry season (Gouvêa, 1969).

After an intense process of ground-truthing, we mapped the land-use of 3470 km^2 using high-resolution satellite images (i.e. RapidEye from 2009-2010, QuickBird and WorldView both from 2011; with resolutions of 5, 0.5 and 0.6 m, respectively). We avoided sampling in Montane and Restinga Forests, which resulted in a subdivision into two regions (north and south, Figure 1a) mainly due to a sandy stripe between them. The history of deforestation and land use are different between the two regions. The matrix of the north region is more heterogeneous and in the southern region the matrix is mainly dominated by pastures. However, the two regions show similar soil, topography and vegetation types (Thomas et al., 1998). The main land-use classes were classified as: forests (mature and secondary native forest), cattle pasture, and plantations of cacao, rubber tree, and *Eucalyptus* sp.) From the map, we selected 58 possible forest fragments adopting as exclusion criteria highly demanding access areas, and indigenous lands. Then, we used previous studies in the region to select a subset of 17 forest fragments, at least 2 km apart, which spanned a large range of landscape-scale forest cover (Table S1**)**, and for which the occurrence of *E. edulis* was known (Soares et al., 2015; Santos et al., 2015; Benchimol et al., 2017).

Our sampling occurred between 2014 and 2016, and we used Google Earth to evaluate substantial changes in land use cover during the period between mapping (2009 – 2011) and data collection. After finding stability in land use cover during this period, we performed all analyzes with forest cover calculations obtained by the high-resolution mapping.

Focal species and sampling

Euterpe edulis is a monoecious palm tree, with annual and predominantly crossed reproduction (Mantovani and Morellato, 2000; Castro et al., 2007; Gaiotto et al., 2003). The flowers are abundant in nectar and pollen and attract a wide variety of insects (Reis et al., 2000b). Despite this, its pollination is mainly performed by bees belonging to different taxonomic groups such as Meliponini, Euglossini, and Honeybees (Reis et al., 2000b; Dorneles et al., 2013; Santos et al., 2018 a, b). E. edulis fruits have a pulp rich in lipid and fiber (Silva et al., 2013; Da Silva et al., 2014) and are used as food by 58 bird species and 20 mammal species (Galetti et al., 2013). However, the main seed dispersers are large frugivores such as toucans and cotingas. Small birds such as

thrushes are especially important in defaunated areas (Reis and Kageyama, 2000; Galetti, et al., 2013; Santos et al., 2018a,b).

We randomly established three 50x10 forest-plots in each of the 17 forest fragments. Plots were located at least 50 m apart from each other and from the nearest edge. In each forest plot, we sampled all *E. edulis* juvenile (i.e. individuals with pinnate leaves, height ≤ 0.15 and > 1.00m) based on the categories proposed by Silva et al. (2009). Subsequently, all individuals were georeferenced and had leaf tissue sampled. We chose the juvenile ontogenetic stage because the genetic parameters of the early developmental stages better represent the effects of recent environmental disturbances (Vranckx et al., 2012). In addition, juveniles might show more consistent responses to anthropogenic disturbances because they exhibit higher numerical stability in terms of population fluctuation than seedlings (Conte et al., 2000). All collected individuals were numbered and later, 32 individuals were drawn in each of the 17 population for genotyping. This procedure resulted in a total sampling of 544 individuals. The determination of the number of individuals per population was based on a previous study that detected little benefit on the accuracy of allele frequencies and diversity estimates for microsatellite loci above 30 individuals (Hale et al., 2012).

DNA extraction and genotyping

DNA extraction followed the CTAB protocol (Doyle and Doyle, 1987). We genotyped all *E. edulis* individuals using 17 nuclear microsatellite markers developed for the species (Gaiotto et al., 2001). Multiplex PCR reactions were performed in a Veriti™ Thermal Cycler (Applied Biosystems, Foster City, CA) with two triplex combinations (EE43, EE45, EE52 and EE47, EE59, EE63) and two duplex combinations (EE2, EE32 and EE8, EE23). We also performed single locus PCRs to the following markers: EE3; EE5; EE9; EE15; EE25; EE48 and EE54. Subsequently, we submitted only the EE5 marker product to the individual electrophoresis system, while the PCR products of the other markers were submitted to the multiload electrophoresis system adapted from Gaiotto et al. (2003) and were organized with the following combinations: (**i**) Pentaload I, (EE2, EE32, EE8, EE23, EE3); (**ii**) Pentaload II (EE43, EE45, EE52, EE48, EE54); (**iii**) Tetraload (EE47, EE59, EE63, EE25) and (**iv**) Biload (EE9 and EE15). The mix submitted to the electrophoresis was composed of 2 µl of the PCR product (or of the mix of the PCR products for the cases of the multiload system), 0.2 µl of GeneScan™ 500 Liz™ (Applied Biosystems, Thermo-Fisher Scientific, Inc.,

Waltham, MA, EUA) and 7,8 µl of deionized formamide (AppliedBiosystems). Later, we performed genotyping on the ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). The sizing of fragments was obtained with GeneMarker® software 2.2 (SoftGenetics, State College, PA, USA).

Population genetics

We evaluated the Hardy-Weinberg equilibrium within each sample population with the package DiveRsity (Keenan et al., 2013) on the R environment 3.5.2 (R Core Team 2018) and tested the linkage disequilibrium between all pairs of loci through FSTAT 2.9.3 (Goudet, 2001). The null alleles were corrected using the method of Oosterhout (Oosterhout et al., 2006), in MICRO-CHECKER software v. 2.2.3 (Van Oosterhout et al., 2004) after the results of 999 bootstrap simulations with a confidence interval of 95%. After null alleles correction, we perform the estimations of all genetic parameters. The DiveRsity package was used to calculate observed and expected heterozygosity (H_O and H_E, respectively), allelic richness and fixation index with a 95% confidence interval estimated by 999 bootstrap simulations. In addition, we used the GENALEX 6.5 program (Peakall and Smouse, 2012) to calculate the number of private alleles in each population. Considering that detection of private alleles may be biased by sample insufficiency, we acknowledge that this estimate might include alleles that occur exclusively in a single population but also those presented in low frequencies (<0.05) that could not be detected. Finally, we used the software BOTTLENECK v.1.2.02 (Cornuet and Luikart, 1996; Piry et al., 1999) to investigate the occurrence of recent genetic bottlenecks in the populations sampled. We chose the two-phase mutation model (TPM), due to it is the most recommended for microsatellite data (Piry et al., 1999; Williamson-Natesan, 2005). We fitted this model with 95% of single-step mutations and 5% of mutations of multiple steps, as suggested by Piry et al. (1999). Subsequently, we used the Wilcoxon test with 5,000 iterations to evaluate the occurrence of excess heterozygosity.

Landscape metrics and local variable

To evaluate whether the genetic diversity of *E. edulis* is influenced by anthropogenic disturbances, we related the genetic estimates with landscape and local attributes. At the landscape scale, we evaluated metrics of landscape composition and configuration that might affect genetic diversity and structure (Balkenhol et al., 2013;

Jackson and Fahrig, 2016). These landscape attributes were calculated using the program FRAGSTATS v4.2.1.603 (McGarigal et al., 2002) for six buffers of different radii sizes, ranging from 0.5 to 2 km, each 250 m, from the central plot in each forest fragment (Figure 1b). The highest radius was chosen because it covers the foraging distances of important large seed dispersers of *E. edulis*, such as toucans, which have a medium-sized seed dispersal distance ranging from 269 to 449m (Holbrook, 2011). In addition, it also includes different foraging distances reported for potential pollinators of *E. edulis*, such as *Plebeia doryana* (maximum 540m) and *Apis mellifera* that can reach distances of hundreds of meters of foraging (Zurbuchen et al., 2010). We evaluated (i) forest cover (a proxy of habitat amount) as landscape composition descriptor and (ii) mean patch size, proximity index, and edge density as landscape configuration descriptors. For all scales, forest cover was calculated as the area occupied by the sum of mature and secondary native forests divided by the total landscape area. We used mature and secondary forests as a proxy of habitat because natural populations of *E. edulis* occurred in both types of forests. The calculation of all landscape configuration metrics was performed at the class level and was based only on the areas occupied by forest remnants within each landscape. Then, we evaluated the Pearson correlation among all landscape variables. We excluded from the subsequent analyses mean patch size and edge density due to the high correlation with forest cover amount in more than one spatial scale ($r > 0.4$) (Table S2).

At the local scale, we estimated logging activity as a disturbance variable related to *E. edulis* harvest. We used logging as a proxy of harvesting because both activities are intensified in anthropogenic landscapes and close to urban centers where they become one of the main income sources for local populations (Shaanker et al., 2003; Tabarelli et al., 2004). We also opt to estimate logging because the record of the illegal palm harvest is very scarce due to the rapid stipe decomposition when compared with hardwood trees (personal observation). The logging estimative in each fragment was made in 2014 when we evaluated a 100 x 8 m plot, distant at least 50 m from the closest edge. In all plots, we counted the wood stumps with the diameter at ground level ≥ 20 cm.

Data analysis

Genetic diversity

To evaluate the spatial scale to which the environmental context influenced the genetic diversity (scale of effect) we used the multifit function (Huais, 2018) and generalized additive models (GAM: Hastie and Tibshirani, 1990). We related simultaneously the genetic attributes to the forest cover and proximity index quantified on six spatial scales (buffers with 0.5, 0.75, 1.0, 1.5, 1.75, 2.0 km radius). In addition, we evaluated the spatial autocorrelation of the residual models using the selected scale by applying a Moran I index (Fortin and Dale, 2005) (Table S3). We did not find any spatial autocorrelation in our data (supplementary information), hence it was not necessary to consider the spatial structure in further analyses.

We performed GAM to capture linear and non-linear relationships between variables. All possible additive combination of independent variables (landscape and local attributes) and the genetic parameters were tested, totaling 7 models. We tested for the concurvity among predictors i.e. the nonparametric counterpart of multicolinearity in linear regressions. We also included a null model containing only the intercept. Finally, we performed a model selection approach using the Akaike Information Criterion corrected for small sample sizes (AICc; Burnham and Anderson, 2002) for each one of the genetic parameters. Models with ΔAIC values ≤ 2.00 were considered to be equally plausible. But we chose the most parsimonious model, using the criterion of at least weight among plausible models.

Population structure

The genetic differentiation among populations of *E. edulis* was investigated through three distinct approaches: we used the DiveRsity package to calculate the Gst-statistics (Hedrick, 2005) and Wright's F_{ST} The statistical significance of these two analyses was evaluated by 1000 randomizations with a confidence interval of 95%. The genetic differentiation measures estimated by Gst values are more suitable for microsatellite locus than those generated by Fst because they standardize the bias generated by the polymorphic character of these markers (Hedrick, 2005). However, Meirmans and Hedrick (2011) recommend that Fst values be presented in current studies to enable comparisons with older studies. The third approach of the genetic differentiation used in our study was the discriminant analysis of principal components

(DAPC: Jombart et al., 2010) performed by the Adegenet package in R (Jombart, 2008). This analysis creates a model in which the genetic variation is partitioned between groups and within groups with the objective of maximizing variation between groups (Jombart and Collins, 2015). We initially used the K-means algorithm of the 'find cluster' function and retained all the major components to detect evidence of genetic clusters in our populations (Supplementary Figure 1A). We then applied the Bayesian Information Criterion (BIC) to infer the best number of clusters by identifying the lowest BIC value (Supplementary Figure 1B). Subsequently, we used the cross-validation function (Xval.Dapc) to identify the ideal number of principal component (PCs) to be retained based on the least mean square error after 1000 stratified random simulations (Supplementary Figure 1C). After obtaining this result, we performed the DAPC, which in the first moment transformed the genetic data using principal component analysis (PCA) and then submitted the number of retained PCs to linear DAPC.

RESULTS

Genetic diversity within populations

Our results did not show evidence of genotypic linkage disequilibrium in any of the pairs of loci. In addition, we detected the presence of null alleles in all loci with frequency varying from 0.5 to 16%. The average of genetic parameters estimated with original or corrected data for null alleles leads to similar results (data not shown). However, we opted to show all results based on the corrected data for null alleles (Table 1). We detected a total number of 347 alleles in the 17 loci by the 544 juvenile individuals from all 17 *E. edulis* populations evaluated. We used highly polymorphic microsatellites and the number of alleles generated for each marker ranged from 7 (EE43) to 33 (EE52), with a mean of 20.4 alleles for loci.

We did not detect a signal of a bottleneck in any population since the unicaudal probability for the excess of heterozygotes between microsatellite markers was not significant ($P > 0.7$) and the proportion of alleles in the frequency intervals followed an L distribution format (Table 1).

Environmental landscape and local variable

The scale of the effect that strongly influences the genetic parameters varied in relation to the response variable and also to the landscape metric (Table S1). The observed and expected allelic richness and heterozygosity were not affected by any landscape or local variable (null model selected; Table 2). In contrast, the number of private alleles and the coefficient of inbreeding were significantly affected by logging intensity within the fragment (Figure 2). The concurvity measures were very small in the selected models, suggesting negligible concurvity (estimate < 0.0005)

Genetic structure

Paired G_{ST} ranged from 0.077 to 0.563 (0.292 ± 0.095). All estimates of genetic differentiation given by this parameter were significantly different from zero (Figure 3). Estimated values for paired F_{ST} ranged from 0.022 to 0.19 (0.089 ± 0.032) and although genetic differentiation between populations was less pronounced than that presented by G_{ST} (Table S4), we also found a significant genetic structure pattern through this analyze. The ideal number of genetic groupings indicated by the lowest BIC in the DAPC analysis was 9 (K9= 1048.768) (Supplementary Figure 1B). However, only three clusters were formed by a clear genetic differentiation (populations 6, 15 e 16) (Figure 4). The other groups were composed of different proportion of individuals that belonged to distinct geographic populations, which indicates a great mixture of the gene pool of those populations.

DISCUSSION

Multi-level approach studies have been increasing in landscape ecology literature in recent years (Graham and Blake, 2001; Buskirk, 2005; Calamari et al., 2018), but are still scarce in the genetic field (Gonzalez-Fernandez et al. 2019). Our study represents a step forward in filling this gap since it simultaneously evaluated the impacts of human activities at landscape and local scales on the genetic diversity of *E. edulis* populations in the Atlantic Forest. Despite the demographic decline recorded in many localities of *E. edulis* occurrence (Galetti and Fernandez, 1998; Silva and Bovi, 2002), we did not find evidence of a genetic bottleneck in any of the populations sampled. However, we found that the number of effective alleles and the fixation index were influenced by logging activity. In addition, we found evidence of genetic structure

among the populations investigated suggests a limitation on gene flow that operates at larger scales.

The geographic distribution of *E. edulis* was originally recorded in almost all Atlantic Forest biome, with high densities in sub-canopy of dense ombrophilous forests (Reis et al., 2000a). The current threat status of *E. edulis* is vulnerable to extinction, mainly due to the 30% population decline registered in the last six decades (Martinelle and Moraes, 2013). In spite of that, our study revealed that the populations investigated showed no signs of recent bottlenecks and still retain high genetic diversity. This result is compatible with previous studies (Conte et al., 2008; Carvalho et al., 2015; Santos et al., 2015), and also corroborates the hypothesis that recent population declines are not sufficient to produce changes in genetic diversity levels (Klank et al., 2012; Montes et al., 2016). This is because although genetic bottleneck events result commonly in the loss of rare alleles - frequency < 0.05 - (Allendorf, 1986), heterozygosity levels are poorly influenced since these alleles contribute little to the formation of heterozygous individuals (Hartl and Clark, 1997). Therefore, our results suggest that *E. edulis* populations reflect a historical condition of the original species distribution since it corresponds to the expected diversity pattern for populations with a broad geographic distribution (Hamrick et al., 1992).

Contrary to our initial expectations, we found that only local scale disturbances affect some estimates of genetic diversity. Previous studies suggested the importance of landscape scale effects on genetic diversity. For instance, a simulation study by Jackson and Fahrig (2014) found that while species abundance is influenced by local scale disturbances, genetic diversity tends to be more affected by landscape composition and configuration at large spatial scales. In addition, Carvalho et al. (2015) assessing the effects of recent landscape changes on genetic diversity of the same species, *E. edulis*, found the resistance of the matrix as one of the main factors affecting the allelic richness of these populations. These authors conducted the study in the southeastern Atlantic Forest, thus a possible explanation for the lack of landscape effects on our study may be attributed to differences in land-use history between regions. The fragmentation of the southeastern Atlantic Forest began with coffee plantations in the 19th century (Dean, 1996). In contrast, intensive deforestation in our region began only during the 1980s (Rocha, 2006). A meta-analysis by Schlaepfer et al. (2018) identified

that the age of anthropogenic fragmentation is a determining factor to detect genetic diversity loss. Thus, we believe that the recent landscape modification in the southern Bahia still did not trigger a genetic diversity erosion in *E. edulis* populations.

On the other hand, we observed a faster negative effect of logging activity, on the number of private alleles and fixation index. The inverted U-shaped relationship between logging and the number of private alleles showed that both areas with low and high logging activity had few alleles, which are unshared among populations. One possible explanation for this pattern is that the small number of private alleles in the poorly explored areas reflected the historical condition of the geographic distribution of *E. edulis*, with great populations and high level of gene flow (Gaiotto et al., 2003, Santos et al., 2015). In contrast, the low number of private alleles in intensively exploited areas was probably the result of genetic drift, which is a major cause of random loss of alleles in reduced populations (Frankham et al., 2002).

The relationship between logging and fixation index was expressed by a non-linear relationship, evidencing an increasing inbreeding with logging intensification. The results obtained are in agreement with the expected pattern for the traditional harvest system of this species that compromises the demographic structure of the populations by leaving only a few reproductive individuals in the fragments. (Reis et al., 2000a). In these scenarios, an inbred pattern is common because the natural regeneration of populations is associated with the presence of few matrices (Murawski et al., 1994). On the other hand, at efficient forest restoration practices in which several matrices of distinct geographical origins is used the inbred patterns can be diluted (Zucchi et al. 2018). The genetic structure of *E. edulis* populations showed a significant difference in the gene pool of all populations compared. In addition, we found that 3 out of 17 populations investigated had completely distinct gene pools due to the low probability of sharing common alleles among them. This result is in agreement with Santos et al. (2015, 2016) who investigated the effect of habitat loss on the genetic structure of *E. edulis*, and recorded that deforestation caused a decline in the distance of gene flow of juvenile individuals. In addition, a recent study with the *Oenocarpus bataua* palm tree found that habitat loss and fragmentation were mainly responsible for increasing structure and reducing genetic diversity in female gametes of this species (Browne and Karubian, 2018). Thus, the limitation of pollen exchange and especially

in processes involving long-distance seed dispersal should be the main reason for the strong genetic structure found in our populations, since currently there are only 12% of the original habitat for this species (Ribeiro et al., 2009).

CONCLUSIONS AND CONSERVATION IMPLICATIONS

To conclude, our study revealed that the assessment of local and landscape-scale anthropogenic pressures may provide different information about the genetic vulnerability of a species. Our findings showed that intensification of logging activities affected *E. edulis* genetic diversity more rapidly than recent landscape modifications. However, the strong genetic structure found in all populations in our study suggests a limitation of gene flow resulting from habitat loss on a regional scale. Although current populations of *E. edulis* still maintain high levels of genetic diversity, this situation tends not to be maintained if local and landscape-scale anthropogenic pressures are not attenuated. This is because the loss of private alleles and increased inbreeding, as found in our results, can affect the suitability and adaptability of *E. edulis* populations if kept small and isolated for many generations. For this reason, to maximize conservation efforts for species of long-term economic interest, we recommend increasing inspection to reduce illegal exploitation of these species. In addition, we also suggest investments in reforestation of degraded areas in order to increase gene flow among Atlantic Forest fragments and rebalance inbreeding levels in other populations.

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TABLES AND FIGURES

Table 1. Genetic estimates and genetic bottleneck signature of 17 *E. edulis* populations. Ap number of private alleles, **Ar** allelic richness, $\mathbf{H_O}$ e $\mathbf{H_E}$ observed and expected heterozygosity, ***f*** fixation index. **TPM** unicaudal probability of excess heterozygotes between microsatellites following the two-phase mutation model.

Population	Ap	Ar	H_O	H_E	*f* (CI 95%)	TPM	Mode-shift
Pop1	1	8.19	0.66	0.68	0.022 (-0.034; 0.072)	1	L-shaped
Pop2	6	8.4	0.67	0.71	0.05 (-0.01; 0.105)	0.983	L-shaped
Pop3	7	8.38	0.69	0.72	0.036 (-0.015; 0.085)	0.997	L-shaped
Pop4	1	8.11	0.68	0.7	0.027 (-0.02; 0.069)	0.998	L-shaped
Pop5	3	7.58	0.6	0.65	0.08 (0.016; 0.135)	0.999	L-shaped
Pop6	11	7.35	0.63	0.65	0.041 (-0.025; 0.100)	0.996	L-shaped
Pop7	9	8.24	0.67	0.71	0.049 (-0.009; 0.096)	0.964	L-shaped
Pop8	4	8.66	0.74	0.76	0.027 (-0.018; 0.072)	0.993	L-shaped
Pop9	10	8.79	0.69	0.75	0.079 (0.025; 0.127)	0.998	L-shaped
Pop10	0	8.55	0.7	0.73	0.043 (-0.011; 0.096)	0.993	L-shaped
Pop11	3	8.72	0.7	0.75	0.072 (0.02; 0.122)	0.96	L-shaped
Pop12	5	8.44	0.72	0.76	0.057 (0.011; 0.100)	0.991	L-shaped
Pop13	3	9.11	0.74	0.76	0.036 (-0.03; 0.101)	0.997	L-shaped
Pop14	2	7.71	0.6	0.65	0.066 (0.014; 0.116)	1	L-shaped
Pop15	4	6.41	0.71	0.7	-0.014 (-0.075; 0.046)	0.742	L-shaped
Pop16	3	7.38	0.69	0.68	-0.019 (-0.073; 0.033)	0.996	L-shaped
Pop17	5	8.26	0.68	0.74	0.075 (0.022; 0.126)	0.868	L-shaped
Mean	4.5 (±3.2)	8.13 (±0.66)	0.68 (±0.04)	0.71 (±0.04)	0.04 (0.03)	–	–

Mean: mean estimation of the genetic diversity parameters and standard deviation (between parenthesis).

Table 2. Result of the multi-model inference using generalized additive models (GAM) for the different parameters of genetic diversity. In all cases, we include the null model, containing only the intercept and error parameters. **ΔAICc** value of the Akaike Information Criterion corrected for small sample size, **df** number of parameters of the models and **Wi** Akaike's weight. **Ar** allelic richness, **Ap** number of private alleles, $\mathbf{H_O}$ and $\mathbf{H_E}$ observed and expected heterozygosity, ***f*** fixation index. **F.C**. forest cover, **P.I.** proximity index, and **Log** logging intensity.

	Model	**ΔAICc**	**Df**	***Wi***
Ar	**Null**	**0**	**2**	**0.418**
	P.I.	1.6	3.37	0.186
	Log	1.9	3	0.159
	F.C.	2.7	3	0.109
	P.I. + log	3.8	4.01	0.061
	F.C. + log	4.9	4	0.036
	F.C. + P.I.	5.2	4.38	0.031
	Full	7.9	5.01	0.008
Ap	**Log**	**0**	**4.16**	**0.686**
	F.C. + log	3.8	5.09	0.103
	P.I. + log	4.1	5.11	0.09
	Null	5.1	2	0.054
	P.I.	6.1	3	0.032
	F.C.	6.7	3	0.024
	F.C. + P.I.	8.4	4	0.01
	Full	20.7	9.7	0.001
H_O	F.C.	0	3.95	0.296
	Null	**0.2**	**2**	**0.262**
	P.I.	1.1	3	0.173
	Log	2.4	3	0.091
	F.C. + P.I.	2.5	4.90	0.085
	P.I. + log	3	4	0.066
	F.C. + log	4.8	5.95	0.027
	Full	9.2	7.45	0.003
H_E	**Null**	**0**	**2**	**0.342**
	P.I.	0.3	4.74	0.293
	F.C. + P.I.	2.1	4	0.122
	F.C.	2.6	3	0.095
	Log	2.9	3	0.081
	P.I. + log	3.5	5.78	0.058
	Full	6.5	6.74	0.013
	F.C. + log	7.1	5.73	0.010
f	**Log**	**0**	**3.42**	**0.582**
	Null	2.8	2	0.145
	F.C. + log	3.5	4.32	0.103

	P.I. + log	3.6	4.36	0.097
	P.I.	5.7	3	0.034
	F.C.	5.8	3	0.033
	Full	7.7	5.27	0.012
	F.C. + P.I.	9.2	4	0.006

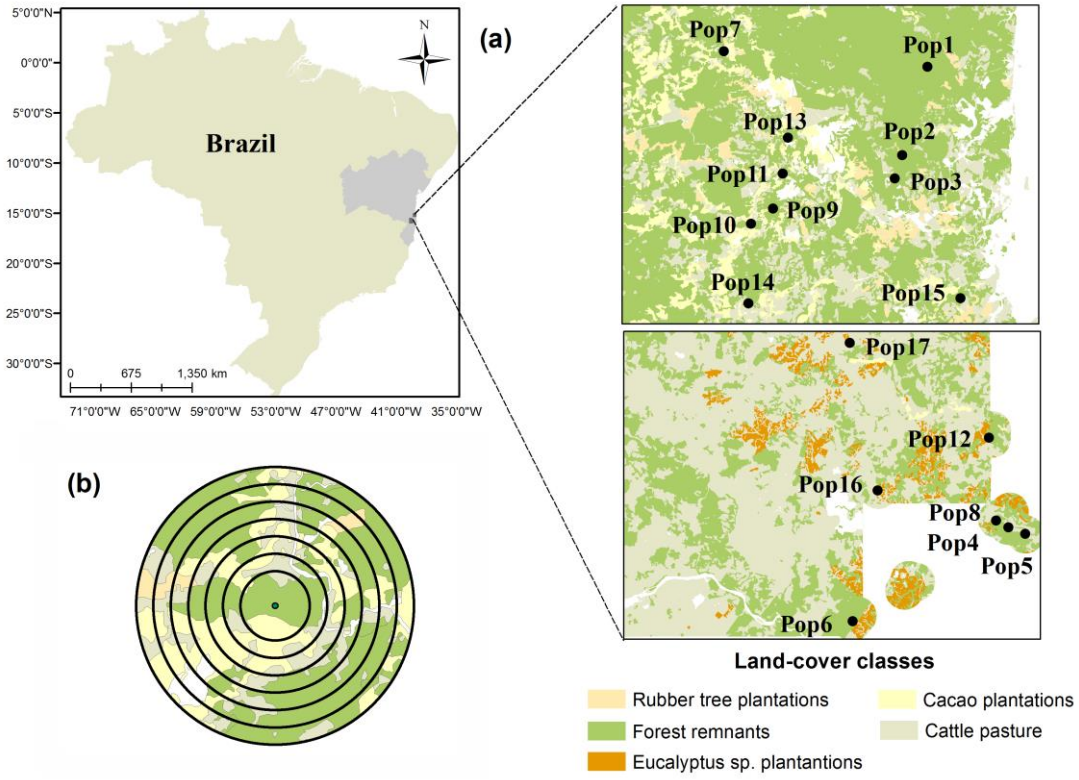


Figure 1. **Location of the 17 forest fragments in which the populations of *Euterpe edulis* were sampled in the Atlantic Forest of southern Bahia. (A)** Distribution of fragment center points (circle) and characterization of the main forms of land use in the region; **(B)** Example of the six buffers created around the fragment's center point for landscape metric calculations.

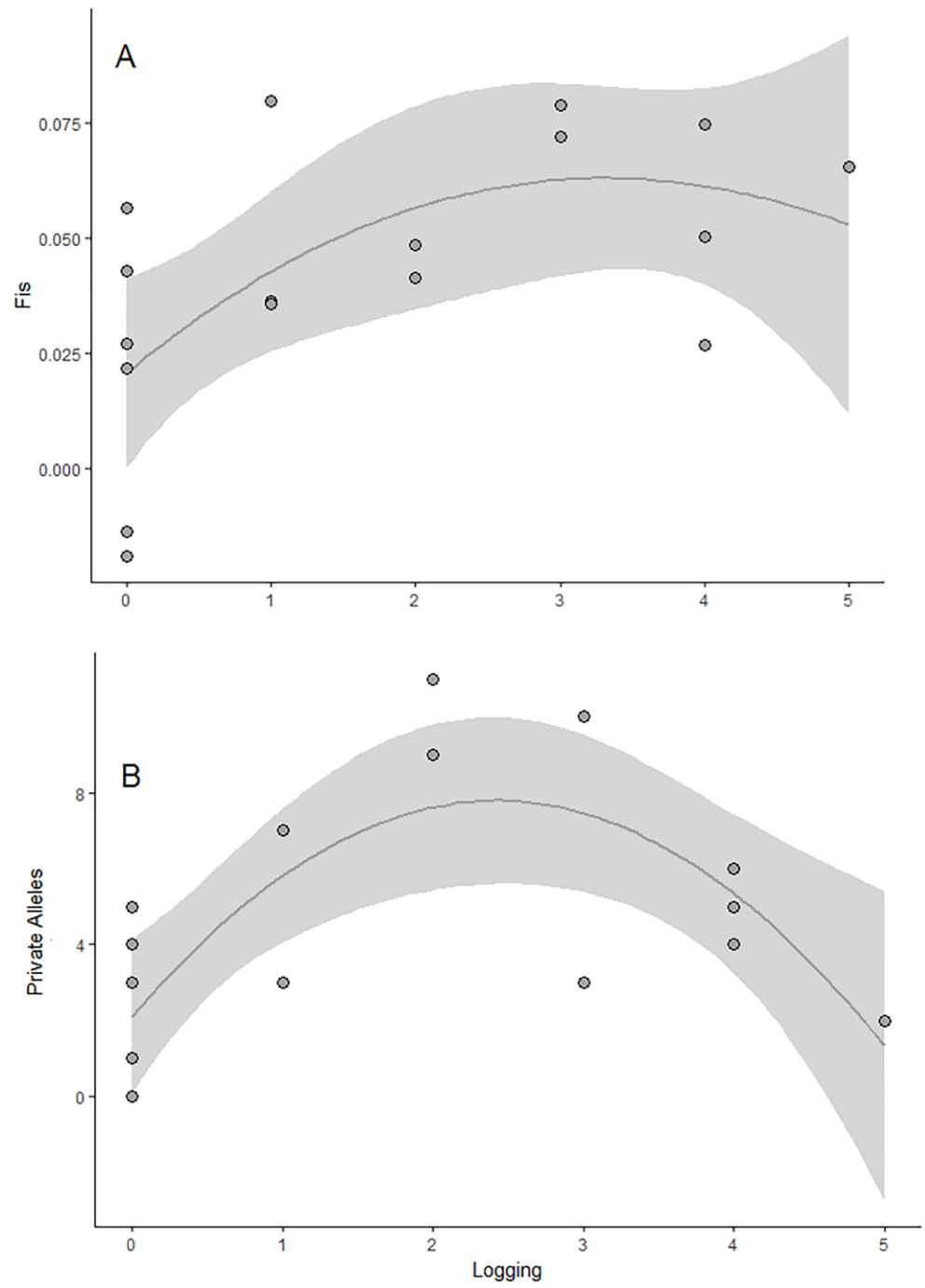


Figure 2. Effects of logging (number of stumps) on the genetic diversity of *E. edulis* populations. (A) private alleles; **(B)** fixation index (*f*). The gray area corresponds to the confidence interval of the generalized additive models.

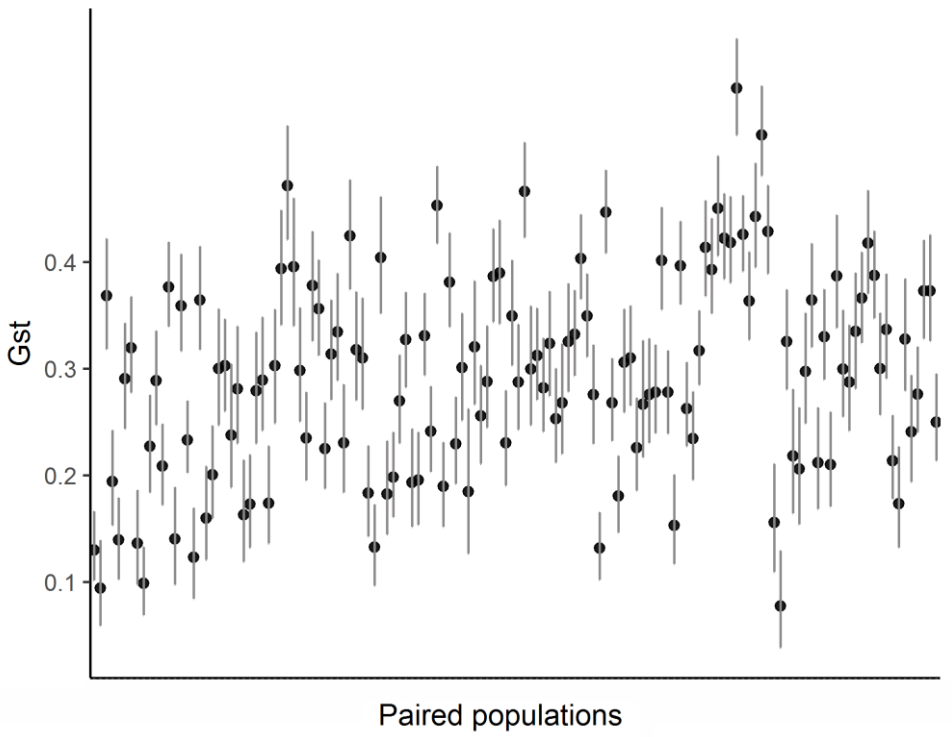


Figure 3. The G_{ST} values paired among the 17 populations of *E. edulis* located in the Atlantic Forest of Southern Bahia.

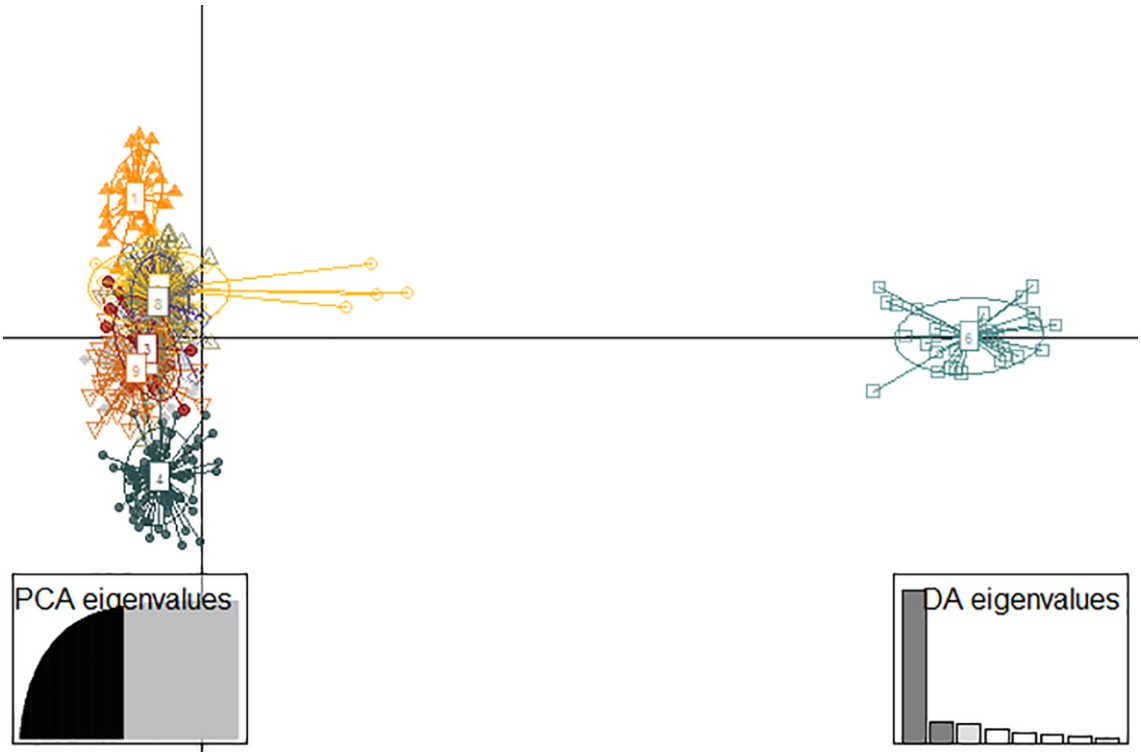
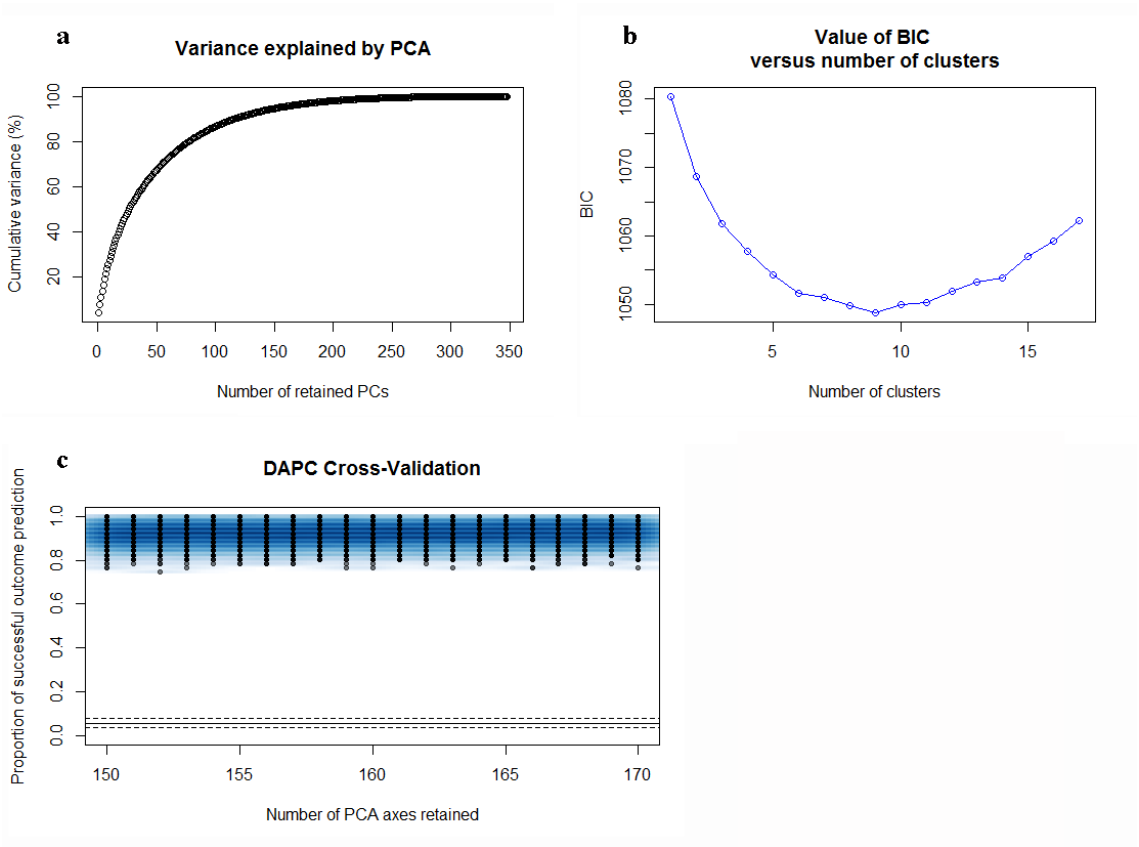


Figure 4. Discriminant analysis of principal components (DAPC) for 17 populations of *E. edulis* from the Atlantic Forest of Southern Bahia, Brazil. The dots symbolize individuals within the population and the circles represent the genetic clusters defined by the DAPC

Supplementary Material



Supplementary Figure 1. Initial stages of the Discriminant analysis of principal components (DAPC). **(A)** cumulative variance explained by the principal component analysis relative to the number of components (PCs) retained; **(B)** selection of the optimal number of clusters in the DAPC using the lowest value of the Bayesian information criterion (BIC); **(C)** cross-validation procedure to choose the optimal number of principal components for DAPC.

Table S1. Characterization of the composition (forest cover) and configuration (proximity index) of the landscape in the six spatial scales analyzed in this study (0.5 - 2.0 km) and quantification of logging intensity for the 17 Atlantic Forest fragments sampled in southern Bahia.

Fragments	**Landscape-scale: measures quantified in 6 buffers with different radius size (km)**														**Local scale**
	Mean Proximity index							Forest cover (%)							Logging Intensity
	0.5	0.75	1	1.25	1.5	1.75	2	0.5	0.75	1	1.25	1.5	1.75	2	
Frag 1	0	0	0	0	0	0	734.7	100	100	100	100	100	99.5	95.8	0
Frag 2	0	0	820.5	0	2266.5	434.9	3312.5	97.8	97.8	97.0	96.0	92.4	88.6	85.4	4
Frag 3	0	0	3340.3	0	0	551.3	4588.2	89.8	84.0	78.5	78.7	80.0	80.9	79.5	1
Frag 4	0	16.3	80.1	168.9	286.0	630.9	1606.7	77.4	65.6	66.5	71.3	73.7	73.4	71.0	0
Frag 5	0	4.5	17.4	119.3	516.4	362.7	456.2	73.9	61.0	59.9	61.0	60.5	62.6	63.7	1
Frag 6	0	0	0	393.1	351.4	261.8	253.1	97.1	84.6	76.7	72.1	68.4	66.1	64.0	2
Frag 7	27.9	4.3	33.2	721.5	242.1	487.2	1723.6	45.5	47.9	49.2	52.2	53.4	56.3	61.2	2
Frag 8	0	0	95.6	361.5	1725.5	246.9	358.3	85.1	72.6	60.4	58.0	55.7	52.5	51.9	4
Frag 9	0	0	1215.5	1986.2	1523.3	1333.1	1016.8	93.9	90.3	80.4	69.1	59.5	53.7	51.4	3
Frag 10	0	127.4	187.8	68.8	157.4	232.4	471.0	61.4	50.1	40.8	37.9	43.2	46.6	49.8	0
Frag 11	784.1	1663.8	1158.1	1725.5	1460.1	1169.9	1293.1	79.9	62.1	53.3	51.2	48.3	44.8	45.6	3
Frag 12	7.3	12.1	19.7	796.8	19.5	37.0	92.2	61.3	55.8	51.0	48.1	46.1	42.3	41.8	0
Frag 13	0	266.8	830.5	1195.5	1030.9	1056.7	1033.6	65.4	64.6	58.7	54.7	48.3	45.0	43.0	1
Frag 14	1.4	99.8	15.7	219.8	93.1	75.0	110.0	3.5	52.1	21.6	44.4	31.3	43.1	42.5	5
Frag 15	0	310.6	433.2	554.2	455.3	342.8	175.7	78.4	63.3	52.8	46.1	40.4	38.4	37.0	0
Frag 16	10.6	13.4	46.0	49.5	18.8	53.1	57.0	39.4	22.1	20.1	25.9	28.5	26.9	27.1	0
Frag 17	0	42.8	10.8	0	57.2	0.5	2.6	28.7	18.2	17.7	21.5	22.7	21.7	18.6	4

Table S2. Valores da correlação de Pearson entre as variáveis descritoras da composição e configuração da paisagem quantificadas em seis escalas espaciais. F.C. forest cover, E.D. edge density, P.S. mean patch size and P.I. proximity index,

Scale (km)	Correlação de Pearson (r)					
	F.C./ E.D	F.C./P.S.	F.C./M.P.I.	E.D/P.S.	E.D/M.P.I.	P.S./M.P.I
0.5	-0.32	0.92	0.087	-0.45	0.47	-0.2
0.75	-0.34	0.87	-0.063	-0.64	0.66	-0.31
1	-0.26	0.73	0.35	-0.63	0.32	-0.004
1.25	-0.33	0.8	-0.053	-0.61	0.72	-0.42
1500	-0.41	0.82	0.26	-0.59	0.35	-0.1
1.75	-0.43	0.8	0.059	-0.63	0.72	-0.24
2	-0.33	0.75	0.62	-0.62	0.11	0.22

Table S3. Scale selection reflecting the strongest interaction between genetic parameters and landscape metrics (effect scale) with significant values of the spatial autocorrelation test by Moran's index. **Ar** allelic richness, **Ap** number of private alleles, $\mathbf{H_O}$ e $\mathbf{H_E}$ observed and expected heterozygosity, ***f*** fixation index.

Model	Genetic parameter	Landscape metrics	Scale of effect (km)	Moran
GAM	Ar	forest cover	1.75	0.314
GAM	Ap	forest cover	0.75	0.626
GAM	H_O	forest cover	0.5	0.457
GAM	H_E	forest cover	1.5	0.861
GAM	*f*	forest cover	2	0.816
GAM	Ar	proximity index	1	0.643
GAM	Ap	proximity index	1.25	0.934
GAM	H_O	proximity index	1.25	0.316
GAM	H_E	proximity index	1.5	0.569
GAM	*f*	proximity index	1	0.777

Table S4. Genetic differentiation estimated by G_{ST} (dark grey) and F_{ST} (light gray) values for all pairs of the 17 *E. edulis* populations sampled in southern Bahia.

Populations	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6	Pop 7	Pop 8	Pop 9	Pop 10	Pop 11	Pop 12	Pop 13	Pop 14	Pop 15	Pop 16	Pop 17
Pop 1		0.033	0.074	0.097	0.077	0.135	0.047	0.107	0.070	0.042	0.029	0.108	0.058	0.052	0.098	0.112	0.043
Pop 2	0.099		0.060	0.103	0.083	0.152	0.059	0.105	0.064	0.055	0.037	0.110	0.050	0.069	0.085	0.107	0.056
Pop 3	0.227	0.196		0.107	0.098	0.154	0.091	0.086	0.077	0.088	0.052	0.087	0.069	0.099	0.118	0.125	0.066
Pop 4	0.289	0.331	0.349		0.047	0.152	0.085	0.052	0.087	0.097	0.073	0.075	0.091	0.117	0.127	0.116	0.081
Pop 5	0.208	0.241	0.287	0.132		0.149	0.091	0.074	0.100	0.103	0.072	0.083	0.086	0.107	0.139	0.102	0.050
Pop 6	0.377	0.453	0.466	0.447	0.397		0.149	0.158	0.132	0.135	0.123	0.137	0.128	0.157	0.190	0.152	0.117
Pop 7	0.140	0.190	0.299	0.268	0.262	0.443		0.092	0.059	0.047	0.022	0.090	0.061	0.073	0.094	0.119	0.062
Pop 8	0.359	0.381	0.312	0.181	0.234	0.519	0.330		0.084	0.103	0.076	0.071	0.082	0.115	0.117	0.115	0.078
Pop 9	0.233	0.229	0.282	0.306	0.317	0.429	0.210	0.337		0.058	0.044	0.081	0.059	0.088	0.106	0.111	0.065
Pop 10	0.130	0.183	0.301	0.324	0.310	0.414	0.156	0.387	0.214		0.033	0.096	0.042	0.068	0.091	0.096	0.066
Pop 11	0.094	0.132	0.185	0.253	0.226	0.393	0.077	0.300	0.173	0.123		0.070	0.041	0.056	0.080	0.088	0.046
Pop 12	0.368	0.404	0.320	0.268	0.267	0.450	0.326	0.287	0.328	0.364	0.281		0.073	0.122	0.130	0.116	0.077
Pop 13	0.194	0.182	0.256	0.325	0.275	0.423	0.218	0.335	0.241	0.160	0.163	0.303		0.074	0.105	0.104	0.058
Pop 14	0.139	0.198	0.288	0.332	0.278	0.418	0.206	0.366	0.276	0.201	0.173	0.394	0.235		0.110	0.123	0.076
Pop 15	0.290	0.270	0.386	0.403	0.402	0.563	0.297	0.418	0.373	0.300	0.279	0.472	0.378	0.313		0.140	0.094
Pop 16	0.320	0.327	0.390	0.350	0.278	0.426	0.364	0.387	0.373	0.303	0.289	0.396	0.356	0.335	0.425		0.096
Pop 17	0.136	0.193	0.230	0.275	0.153	0.364	0.212	0.300	0.250	0.238	0.174	0.298	0.225	0.230	0.318	0.310	

CONCLUSÕES GERAIS

Os resultados apresentados nesta tese contribuem para o avanço no conhecimento dos impactos causados pelas atividades humanas sobre a diversidade genética das populações remanescentes. A meta-análise realizada no capítulo 1 ajudou a esclarecer os resultados controversos encontrados na literatura ao se concentrar apenas nas estimativas dadas por uma única metodologia (microssatélites). As principais conclusões obtidas neste capítulo foram:

1. De maneira geral, os distúrbios antropogênicos em escala de paisagem são os principais responsáveis pela erosão da diversidade genética. No entanto, a ruptura da conectividade estrutural entre os fragmentos se destaca com o efeito negativo mais severo entre eles;
2. A detectabilidade de erosão genética é sensível ao tipo de parâmetro utilizado. A riqueza alélica é a estimativa que sofre a maior redução quando se compara áreas conservadas com degradadas, enquanto o índice de fixação e o número de alelos efetivos não são significativamente alterados pelos distúrbios;
3. Marcadores microssatélites transferidos são mais sensíveis para detectar perda de diversidade genética do que os microssatélites específicos;
4. Os animais são afetados mais severamente pela degradação antrópica do habitat do que as plantas;
5. O impacto dos distúrbios antropogênicos na diversidade genética das plantas varia de acordo com as características biológicas deste grupo, sendo que as espécies mais vulneráveis apresentam forma de vida arbustiva, mecanismos bióticos de polinização e dispersão de sementes e sistema de reprodução predominantemente auto-incompatível;
6. E por fim, apesar de toda a heterogeneidade existente entre os estudos, existe um efeito negativo consistente dos distúrbios antrópicos sobre a diversidade genética das populações de plantas e animais terrestres.

O capítulo 2 desta tese trouxe a abordagem de multi-nível, que é pouco utilizada nos estudos de genética populacional, para investigar simultaneamente o impacto de

pressões antrópicas em diferentes escalas (de paisagem e local) sobre a diversidade genética de uma espécie explorada comercialmente e ameaçada de extinção. As principais conclusões deste capítulo foram:

7. Apesar do declínio populacional significativo enfrentado pela espécie *Euterpe edulis* nos últimos 60 anos, não existe sinais de gargalo genético recente e a espécie ainda conserva altos níveis de diversidade genética.
8. Embora a diversidade genética permaneça alta nas populações investigadas, detectamos o início do processo de erosão desta diversidade. As populações sujeitas à intensificação do corte de madeira (escala local de perturbação) apresentaram redução no número de alelos privativos e um aumento no coeficiente de endogamia.
9. Existe uma forte estrutura genética entre as populações remanescentes, o que indica uma limitação de fluxo gênico nesta espécie resultante da redução do habitat em escala regional.

Finalmente, o agrupamento de todos estes resultados demonstra que independente da escala em que as perturbações antrópicas acontecem, elas são capazes de acarretar em erosão da diversidade genética nas populações que permanecem nos habitas modificados. Esta redução na diversidade genética aumenta à vulnerabilidade destas populações as modificações ambientais e pode agravar o débito de extinção dos habitas remanescentes. Por este motivo, o planejamento de conservação das espécies deve adotar medidas que sirvam para aumentar o fluxo gênico entre as populações isoladas através, por exemplo, da restauração ecológica em áreas que ficam entre fragmentos pequenos e desconectados. Assim, os futuros estudos devem buscar entender as consequências genéticas deste processo de restauração e encontrar as melhores estratégias de manejo para garantir a persistência genética das populações em longo prazo.