LUCAS FERNANDES ROCHA

IMPACTS OF SEED-TREES MANAGEMENT ON GENETIC DIVERSITY AND SPATIAL GENETIC STRUCTURE OF *Eremanthus erythropappus* (ASTERACEAE)

LAVRAS-MG
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Orientadora
Profa. Dra. Dulcinéia de Carvalho

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IMPACTOS DO MANEJO DE ÁRVORES PORTA-SEMENTES NA DIVERSIDADE GENÉTICA E ESTRUTURA GENÉTICA ESPACIAL DE *Eremanthus erythropappus* (ASTERACEAE)

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Engenharia Florestal, área de concentração em Silvicultura e Genética Florestal, para a obtenção do título de Mestre.

APROVADA, em 21 de fevereiro de 2019.
Dr. Alison Nazareno Gonçalves UFMG
Dr. Eduardo van den Berg UFLA

Profa. Dra. Dulcinéia de Carvalho
Orientadora

LAVRAS-MG
2019

“If I have seen further it is by standing on the shoulders of giants”

Isaac Newton

Aos meus pais, Ana Lúcia Rocha e
João Carlos Fernandes Rocha,
por todos os ensinamentos que fizeram de mim o que sou.

Dedico

Às minhas irmãs, Danielly, Brunna, Maria Eduarda, Maria Vitória,
ao meu irmão Felipe e
aos meus sobrinhos Juninho e Cecília

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“Só se pode alcançar um grande êxito quando nos mantemos fiéis a nós mesmos.”

Friedrich W. Nietzche

RESUMO GERAL

O conhecimento da diversidade genética e da estrutura genética espacial de espécies florestais, bem como a compreensão dos fatores ecológicos e ambientais relacionados ao seu desenvolvimento são informações essenciais para a adoção de estratégias de manejo adequadas para a conservação genética. A detecção da existência de qualquer descontinuidade genética é essencial para o aprimoramento dos atuais sistemas de manejo aplicados em espécies florestais e para a definição do ciclo de corte. O presente trabalho tem como objetivo principal analisar o efeito do manejo florestal sob sistema silvicultural de árvores porta-sementes na diversidade genética e estrutura genética espacial de *Eremanthus erythropappus*, em Itamonte, Minas Gerais. Especificamente, objetiva-se: (a) desenvolver e caracterizar nove *primers* microssatélites para a espécie *E. erythropappus*; (b) analisar a estrutura genética espacial e estrutura de parentesco dos regenerantes e dos indivíduos adultos e (c) descrever os níveis de diversidade genética dentro e entre uma área manejada e outra não manejada. Uma das principais abordagens deste estudo a detecção da existência de uma possível descontinuidade genética entre uma área manejada e outra não manejada. Para o desenvolvimento dos *primers*, foi criada uma biblioteca enriquecida de microssatélites para a espécie *E. erythropappus*. Em seguida, para a caracterização, foram coletados materiais foliares de 42 indivíduos distribuídos em dois fragmentos florestais localizados na região sul do estado de Minas Gerais. Foram identificados dezesseis *primers* dos quais nove apresentaram ser altamente polimórficos e amplificaram satisfatoriamente, produzindo um total de 38 alelos com uma média de 4,22 alelos por *primer*. O valor de conteúdo de informação polimórfica (*PIC*) variou de 0,438 a 0,943 e os valores de heterozigosidade observada (H_O) e esperada (H_E) variaram de 0,647 a 1,00; e 0,308 a 1,00; respectivamente. Em geral, os *primers* desenvolvidos demonstraram ser ferramentas eficientes para estudos de conservação genética e para o melhoramento florestal da espécie. Posteriormente, foram selecionados seis *primers* SSR para quantificar a variabilidade genética e a estrutura genética espacial de uma área sob manejo florestal e uma área natural. Dessa forma, foram coletados materiais foliares de 275 indivíduos adultos e regenerantes nas áreas manejada (213) e não manejada (62), 60 meses após o manejo. Os valores médios dos índices de diversidade genética apresentaram pequeno aumento, mas não estatisticamente significante quando comparado a área não manejada (H_E = 0,410; A = 2,67) com a não manejada (H_E = 0,437; A = 2,33). Entretanto, indivíduos regenerantes da área manejada apresentaram padrões de coancestria (SGS_{MAX} = 25), confirmados pelos baixos valores do coeficiente de *kinship* (*Sp* = 0,0070 ± 0,00354) e semelhança no valor para a primeira classe de distância ($F_{(1)}$ = 0,00690; P < 0.05). Dessa forma, este resultado indica uma possível correlação de descontinuidade genética na estrutura genética espacial para indivíduos regenerantes causada pelo manejo florestal. Por outro lado, foi evidenciada também a capacidade de manutenção dos índices de diversidade genética para as primeiras gerações após o manejo bem como um eficiente fluxo gênico entre a área natural e a área manejada.

Palavras-chave: Diversidade Genética. Marcadores Microssatélites. Manejo Florestal.

GENERAL ABSTRACT

Understanding the genetic diversity and spatial genetic structure of tree species, as well as knowing about ecological and environmental factors related to their development are essential steps for applying appropriate management strategies for the genetic conservation. The detection of any genetic discontinuity is essential for the improvement of current management systems applied to tree species and for the definition of the cutting cycle. The main objective of the present study is to analyze the effect of forest management under a silvicultural system of seed-trees on the genetic diversity and spatial genetic structure of *Eremanthus erythropappus* in Itamonte, Minas Gerais state. Specifically, we aim to: (a) develop and characterize nine microsatellite primers for *E. erythropappus*; (b) analyze the spatial genetic structure and the kinship structure of regenerating and adult individuals; (c) describe the levels of genetic diversity within and among managed and unmanaged stands. One of the main approaches used in this study is the detection of possible genetic discontinuity between managed and unmanaged stands. Firstly, for the development of the primers, we created an enriched microsatellite library for *E erythropappus*. Afterwards, leaf material was collected from 42 individuals distributed in two forest fragments located in the southern region of the state of Minas Gerais. We identified 16 primers where 9 were polymorphic and satisfactorily amplified, producing 38 alleles with a mean of 4.22 alleles per primer. The value of polymorphic information content (*PIC*) ranged from 0.438 to 0.943 and the observed (H_O) and expected (H_E) heterozygosity values ranged from 0.647 to 1.00; and 0.308 to 1.00; respectively. In general, these primers revealed as an efficient tool for studies about genetic conservation and forest breeding *E. erythropappus*. Subsequently, we used six SSR primers to quantify the genetic variability and spatial genetic structure of a stand under forest management and a natural stand. Besides, we collected leaf material of 275 adult and regenerating individuals in the managed (213) and unmanaged stands (62), 60 months after the management. Generally, we found a slight but not statistically significant increase on the mean values of genetic diversity parameters comparing the managed stand (H_E = 0.410; A = 2.67) to the unmanaged stand (H_E = 0.437; A = 2.33). However, regenerating individuals from the managed stand presented patterns of genetic coancestry (SGS_{MAX} = 25), which was confirmed by low values of the kinship coefficient (Sp = 0.0070 ± 0.00354) and genetic similarity for individuals in the first distance class ($F_{(1)}$ = 0.00690; P < 0.05). Besides, this result indicates a possible correlation of genetic discontinuity on the spatial genetic structure of regenerating individuals caused by forest management. On the other hand, we also evidenced the potential of maintenance of the genetic diversity parameters on the first generations after management as well an efficient gene flow between the managed and unmanaged stands.

Key-words: Genetic Diversity. Microsatellite Markers. Forest Management.

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LISTA DE SIGLAS

AMOVA	*Analysis of molecular variance*
ANOVA	*Analysis of variance*
CTAB	*Cetyl trimethylammonium bromide*
DBH	*Diameter at the breast height*
DNA	*Deoxyribonucleic acid*
GD	*Genetic diversity*
PCR	*Polymerase chain reaction*
PIC	*Polymorphic information content*
SFM	*Sustainable forest management*
SGS	*Spatial genetic structure*
SSR	*Simple sequences repeated*
A_E	*Effective alleles*
A_O	*Observed alleles*
A_P	*Private alleles*
A_R	*Allelic richness*
F_{ST}	*Genetic differentiation*
G_{ST}	*Genetic divergence*
H_E	*Expected heterozygosity*
H_O	*Observed heterozygosity*
I_A	*Index of association*
N	*Number of alleles*
N_A	*Number of alleles per locus*
N_E	*Number of effective alleles*

SUMÁRIO

PRIMEIRA PARTE

1. INTRODUÇÃO

As florestas tropicais são consideradas atualmente como um dos ecossistemas mais ameaçados do planeta (MITTERMEIER et al., 1998; OLSON; DINERSTEIN, 1998; BARLOW et al., 2018). A perda da biodiversidade pode comprometer a conservação de recursos naturais fundamentais para a manutenção da vida, além causar impactos irreversíveis para a produção florestal (FANIN et al., 2017; HEILPERN et al., 2018). A perda de habitats pode modificar padrões naturais de variabilidade genética como resultado das mudanças nos padrões naturais de fluxo gênico e, consequentemente, provocar o aumento dos níveis de endogamia, a perda de alelos importantes, a redução da heterozigosidade e a diferenciação genética entre populações (YOUNG et al., 1996; RAJORA, 2000; VRANCKX et al., 2012).

Os marcadores moleculares são eficientes ferramentas utilizadas para quantificar o fluxo gênico dentro e entre diferentes paisagens e permitem que pesquisadores testem hipóteses específicas usando dados genéticos espaciais e características ambientais (MANEL et al., 2003). As sequências microssatélites, também conhecidas como sequências simples repetidas (SSRs), correspondem a uma das ferramentas moleculares mais eficientes utilizadas em biologia molecular (SELKOE; TOONEN, 2006). Os marcadores SSRs são úteis para estudar a estrutura genética espacial e a diversidade genética de plantas e animais, além de serem essenciais para a promoção de uma efetiva conservação da diversidade genética florestal (VARSHNEY et al., 2005).

Eremanthus erythropappus (DC.) MacLeish é uma espécie arbórea endêmica do Cerrado e da Mata Atlântica brasileira (CARVALHO, 1994). O manejo florestal de *E. erythropappus* apresenta grande relevância econômica devido à exploração madeireira para produção de moirões de cerca e para a extração do óleo essencial α-bisobolol (OLIVEIRA et al., 2009). Dentre as diversas práticas de manejo florestal, destaca-se o sistema silvicultural de árvores porta-sementes, um método comumente utilizado que visa promover a regeneração natural das espécies exploradas (KERN et al., 2017). O uso de árvores porta-sementes baseia na manutenção de indivíduos adultos que apresentem características ecológicas satisfatórias, tais como grande resistência à pragas e doenças, além de propriedades físicas e mecânicas que favoreçam a produção de grande número de sementes saudáveis (BURNS, 1983). Apesar da aparente sustentabilidade das práticas de manejo florestal, diversos estudos utilizando espécies arbóreas já demonstraram a susceptibilidade em relação a alterações genéticas causadas pelo

corte seletivo (GLAUBITZ et al., 2003; HAWLEY et al., 2005; ORTEGO et al., 2010). Entretanto, algumas espécies também já demonstraram ser capazes de manter a variabilidade genética após o manejo florestal devido a presença de intenso fluxo gênico (AMORINI et al., 2001; LEE et al., 2002; RAJORA; PLUHAR, 2003; EL-KASSABY et al., 2003).

Vários estudos já analisaram a estrutura, variação genética, dinâmica de crescimento e aspectos econômicos de candeia (BRAGA, 2006; BARREIRA et al., 2005; CAMOLESI, 2007; PÉREZ et al., 2004; SCOLFORO et al., 2004; SILVA, 2011; SILVA et al., 2008). Entretanto, ainda são necessárias pesquisas científicas para entender as consequências genéticas do manejo florestal em populações exploradas. O presente trabalho foi estruturado em duas partes principais. A primeira parte apresenta um referencial teórico com assuntos pertinentes ao estudo, objetivando embasar a teoria científica deste estudo. A segunda parte foi dividida dois artigos científicos, os quais foram formatados de acordo com o guia de formatação de cada periódico. O primeiro artigo foi elaborado seguindo as normas da revista *Biological Research* e apresenta um protocolo de desenvolvimento de nove *primers* microssatélites para a espécie *E. erythropappus*, além da análise da diversidade genética para duas populações localizadas no sul do estado de Minas Gerais. O segundo artigo foi formatado de acordo com as normas do periódico *Forest Ecology and Management* e refere-se à avaliação do efeito do manejo florestal na diversidade genética e na estrutura genética espacial de *E. erythropappus*. A análise foi realizada em uma área submetida ao sistema silvicultural de árvores porta-sementes em comparação com uma área natural de características ecológicas semelhantes. Acredita-se que a área manejada apresenta padrões semelhantes de diversidade genética devido ao relativo curto período de tempo de análise após o manejo. Por outro lado, é possível que a estrutura genética espacial dos indivíduos regenerantes da área manejada seja influenciada pelo corte seletivo.

2. REFERENCIAL TEÓRICO

2.1 *Eremanthus erythropappus* (DC.) MacLeish

Eremanthus erythropappus (DC.) MacLeish, popularmente conhecida como candeia, é uma espécie arbórea pertencente à família Asteraceae (Compositae) (FIGURA 1). Atualmente, o gênero *Eremanthus* é composto por 22 espécies endêmicas (LOEUILLE; LOPES; PIRANI, 2012) altamente adaptadas a crescerem em solos pobres, arenosos e pedregosos. A espécie *E. erythropappus* apresenta crescimento inicial rápido que favorece a formação de populações florestais puras (RIZZINI, 1979).

Árvores adultas apresentam valores médios de altura variando entre seis e sete metros e 15 centímetros de diâmetro. Dentro do gênero *Eremanthus*, esta espécie foi a mais explorada, principalmente para a extração de óleo essencial extraído de sua madeira. O α-bisabolol é um álcool sesquiterpênico que possui propriedades antibacterianas, antimicóticas e dermatológicas, amplamente utilizado pela indústria farmacêutica (KAMATOU; VILJOEN, 2010). Além disso, *E. erythropappus* é intensamente explorada em propriedades rurais para a produção de cercas devido à alta durabilidade da madeira contra o intemperismo (SCOLFORO; OLIVEIRA; DAVIDE, 2012).

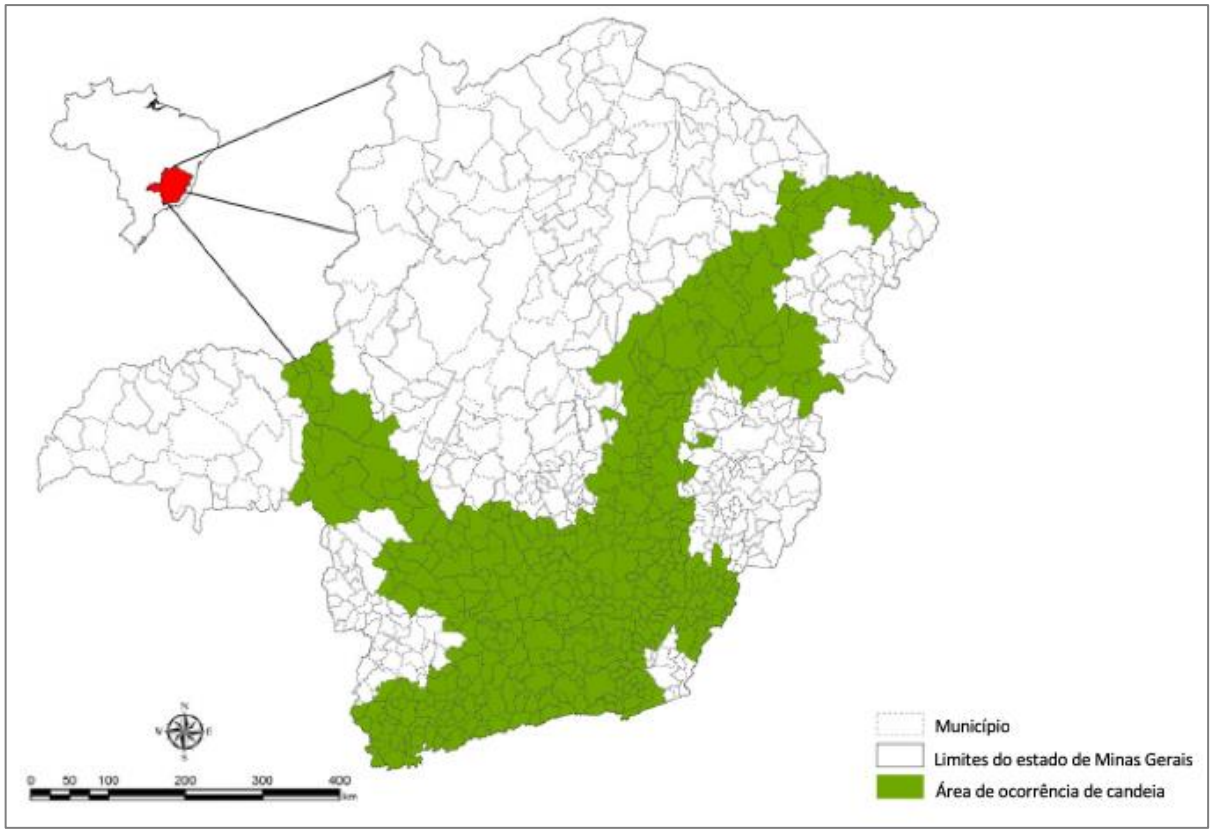
Figura 1 - Características ecológicas de árvores adultas de *Eremanthus erythropappus*. (A) Inflorescência em capítulos; (B) Desenvolvimento de árvore adulta isolada e padrão da produção de frutos; (D) e (E) Distribuição de frutos em galhos.

Fonte: Scolforo et al., (2012).

E. erythropappus é nativa das florestas sazonalmente secas da América do Sul, com ocorrência principalmente no nordeste da Argentina, norte e leste do Paraguai e no sudeste do planalto central brasileiro, geralmente ocorrendo nessa região, em altitudes que variam de 800 a 2.200 metros (CARVALHO, 1994). No Brasil, esta espécie é distribuída nas florestas sazonais semidecíduas da região Sudeste, ocorrendo principalmente nos estados de Minas Gerais, São

Paulo, Bahia, Espírito Santo e Rio de Janeiro (ARAÚJO, 1944) (Figura 2). Indivíduos adultos apresentam alta produção de sementes que são dispersas pelo vento e exigem a presença de luz para germinarem. O processo de regeneração apresenta grande número de indivíduos que se desenvolvem após um distúrbio (SCOLFORO; LOEUILLE; ALTOÉ, 2012).

Figura 2 - Área de ocorrência natural de candeia no estado de Minas Gerais.



Fonte: Adaptado de Gomide et al., (2012).

A floração de indivíduos adultos começa a partir do terceiro ano, com desenvolvimento das estruturas florais ocorrendo entre os meses de julho a setembro. A produção e a dispersão de sementes normalmente ocorrem a partir do mês de agosto, se desenvolvendo até os meses de outubro e novembro (SCOLFORO; OLIVEIRA; DAVIDE, 2012). As flores são roxas e apresentam as estruturas masculinas e femininas localizadas nas extremidades dos ramos (ARAÚJO, 1944). A espécie possui folhas simples e alternas que apresentam a característica de dupla coloração. Dessa forma, a face superior, ou adaxial, apresenta cor verde em razão de um aspecto glabro da folha. Por outro lado, a face inferior, ou abaxial, apresenta coloração esbranquiçada devido a presença de pequenas pilosidades. A madeira é pesada, lisa, de coloração cinza, grã escura e resistente a umidade e a putrefação. O caule possui casca grossa,

apresentando fendas embutidas menos notáveis em regenerantes (SCOLFORO; OLIVEIRA; DAVIDE, 2012).

Indivíduos regenerantes estabelecem após perturbações naturais ou antrópicas (LOEUILLE; LOPES; PIRANI, 2012). Apesar da principal forma de reprodução ocorrer via seminal (SCOLFORO; OLIVEIRA; DAVIDE, 2012), esta espécie apresenta potencial para propagação vegetativa a partir do crescimento de brotos em raízes e caules. Além disso, a reprodução clonal pode ser intensificada a partir do uso de práticas de corte raso e/ou escarificação do solo (FIGURA 3) (MELO; DAVIDE; TEXEIRA, 2012). Os efeitos da propagação clonal englobam não apenas a diversidade genética de uma espécie, mas também a diversidade genotípica, o que corresponde ao número de genótipos únicos de uma população (INGVARSSON; DAHLBERG, 2018). Sendo assim, a distribuição espacial não aleatória de genótipos dentro de uma população pode ser influenciada a partir de eventos históricos e biológicos (VEKEMANS; HARDY, 2004).

Figura 3 - Regeneração clonal de *Eremanthus erythropappus* após o corte de árvores adultas. (A) brotos em fase de crescimento; (B) Crescimento de brotos em raiz exposta após a escarificação do solo; (C) Desenvolvimento de regeneração ao longo toco de uma árvore.

Fonte: Melo et al., (2012).

2.2 Manejo florestal sustentável de candeia

O manejo florestal sustentável representa uma alternativa para a manutenção e uso adequado de coberturas florestais (HICKEY, 2008) e visa explorar a floresta de modo que provenha rendimento sustentado, sem destruir ou alterar drasticamente a composição e estrutura florestal (INNES, 2016). No estado de Minas Gerais, o manejo florestal de *E. erythropappus* foi implementado a partir da promulgação da portaria nº 01, de 5 de janeiro de 2007, autorizada pelo Instituto Estadual de Florestas, tendo como foco o manejo de maciços florestais onde apresentassem a predominância de no mínimo 70% de ocorrência das espécies *E.*

erythropappus ou *Eremanthus incanus* (MINAS GERAIS, 2007). Cruz (2006) aponta que os planos de manejo previamente aprovados no estado de Minas Gerais, contemplaram uma exploração por volta de 2.000 m^3 de madeira por ano, o que correspondia a um número muito abaixo do necessário para sustentar a demanda por produtos madeireiros no estado.

De acordo com o IMAFLORA (2007), o mercado brasileiro foi responsável pelo consumo de aproximadamente 10 toneladas por ano do óleo α-bisabolol. Além disso, o mercado estrangeiro ainda demandava em torno de 80 toneladas por ano, sendo que a maior parte da exploração era feita de forma ilegal. Visando regularizar o manejo das duas espécies de candeia no estado de Minas Gerais, foi sancionada a nova resolução de nº 1804 em 11 de fevereiro de 2013, a qual reestruturou os termos de referência para o manejo sustentável de *E. erythropappus* e *E. incanus* (MINAS GERAIS, 2013).

Os sistemas silviculturais mais utilizados para *E. erythropappus* são os cortes seletivos, o sistema de seleção em grupos e o sistema de árvores porta-sementes (SCOLFORO et al., 2008). O sistema silvicultural de árvores porta-sementes é o método mais efetivamente utilizado em populações manejadas de *E. erythropappus* e visa favorecer a regeneração natural das espécies exploradas. As práticas utilizadas baseiam-se na manutenção de indivíduos de tamanho comercial e com características apropriadas na área de exploração, para frutificação e dispersão de sementes entre os ciclos de corte (ESPADA et al., 2014). De acordo com Silva et al. (2008), o sistema de árvores porta-sementes é altamente viável para espécie *E. erythropappus* por favorecer a dispersão de sementes e no restabelecimento da regeneração, além de não apresentar uma relação de dependência espacial entre indivíduos adultos e regenerantes.

2.3 Impacto do manejo florestal na diversidade genética

O isolamento espacial e a alteração dos padrões de distribuição de espécies florestais podem restringir a estrutura genética e o fluxo gênico populacional (ELLSTRAND; ELAM, 1993; YOUNG et al., 1996; ZHANG et al., 2012). A diminuição da variabilidade genética de uma população pode ser prejudicial à evolução de indivíduos, além do consequente aumento da vulnerabilidade ao ataque de pragas e doenças (RAJORA; PLUHAR, 2003). A conservação de espécies florestais baseia-se na conservação dos níveis de diversidade genética, além da capacidade de reprodução e de gerar descendentes férteis ao longo das gerações (QUÉMÉRÉ et al., 2010).

A estrutura genética espacial de uma população pode ser influenciada por características biológicas e evolutivas como sistema reprodutivo, ciclo de vida, morfologia das flores, síndromes de polinização, dispersão de sementes, propagação vegetativa. Além disso, forças microevolutivas também podem ser impactadas, como mutação, migração, deriva genética e seleção (LOISELE et al., 1995; LOVELESS HAMRICK, 1984; SCHAPCOTT, 1995). Com o intuito de compreender a estrutura das espécies, vários modelos estatísticos foram desenvolvidos, sendo o mais utilizado proposto por Wright, utilizando as estatísticas "F" (WRIGHT, 1949; WRIGHT, 1965), "Nei" para análise da diversidade genética (NEI, 1973; NEI, 1977) e "Cockerham", pela análise do coeficiente de coancestria (WEIR; COCKERHAM, 1984). Os efeitos causados pelo manejo florestal na diversidade genética de espécies arbóreas podem variar entre diferentes espécies e entre diferentes populações de mesma espécie (TABELA 1).

Analisar os padrões naturais de diversidade genética é fundamental para garantir a sustentabilidade do manejo florestal (AVISE; HAMRICK, 1996), compreender as interações genéticas entre populações e criar estratégias efetivas para garantir a sustentabilidade do manejo (CROZIER, 1997). O corte seletivo pode resultar, em longo prazo, no aumento do nível de endogamia e na erosão do potencial evolutivo de uma espécie. Além disso, o manejo florestal pode comprometer os padrões naturais de variação genética (PAFFETTI et al., 2012) e consequentemente provocar a extinção de pequenas populações (CRNOKRAK; ROFF, 1999). Estudos específicos precisam analisar os efeitos da redução do tamanho populacional em áreas manejadas a fim de se avaliar possíveis consequências genéticas da fragmentação ao longo das gerações (YOUNG; BOYLE; BROWN, 1996).

Tabela 1 - Impactos do manejo florestal em parâmetro genéticos de espécies arbóreas.

Espécie analisada	**Marcador utilizado**	**Resultados encontrados**	**Autores**
Eremanthus erythropappus	Isoenzimas	Nenhum efeito aparente nos níveis de diversidade genética.	Barreira et al (2006)
Carapa guianensis	SSR	Níveis similares de diversidade genética antes e após o manejo florestal.	Cloutier et al., (2007)
Bagassa guianensis, Hymenaea courbaril, Manilkara huberi, Symphonia globulifera	-	Perda de alelos e aumento das distâncias genéticas; Baixo efeito no número efetivo de alelos, heterozigosidades e índice de fixação.	Sebben et al., (2008)
Swietenia macrophylla	SSR	Redução no número de alelos, heterozigosidade observada e número de genótipos multilocos.	André et al., (2008)
Hymenaea courbaril	SSR	Aumento nos níveis de heterozigosidade observada e diminuição no índice de fixação entre regenerantes e árvores adultas	Lacerda et al., (2008)
Pinus sylvestris	SSR	Nenhum efeito nos níveis de diversidade genética. Áreas manejadas demonstraram ser menos estruturadas geneticamente.	González-Díaz et al., (2017)
Nothofagus pumilio	SSR	Diferenças não significativas na diversidade genética. Possível efeito na estrutura genética espacial	Soliani et al., (2016)
Fagus sylvatica	SSR e RAPD	Nenhuma diferença em índices de diversidade genética; Alelos raros perdidos na área manejada;	Paffetti et al., (2012)
Eucalyptus sieberi	SSR e RFLP	Nenhuma redução nos parâmetros de diversidade genética ou presença de endogamia; Perda de alelos raros.	Glaubitz et al., (2003)

Fonte: Do autor (2019)

2.4 Marcadores moleculares microssatélites

Dentre várias abordagens empregadas na conservação, a genética molecular pode ser utilizada para quantificar a diversidade genética e a presença de genes deletérios causados pela depressão endogâmica (FRANKHAM; BALLOU; BRISCOE, 2002). O desenvolvimento da técnica da *Polymerase Chain Reaction* (PCR), na década de 1980, tornou possível o início dos estudos utilizando marcadores de DNA (ZANE; BARGELLONI; PATARNELLO, 2002). Marcadores moleculares utilizam genes determinantes de todo e qualquer fenótipo molecular para avaliar semelhanças e diferenças entre plantas individuais, cultivares e linhas de reprodução (YANG et al., 2015). Dentre os vários marcadores moleculares disponíveis, os microssatélites, ou sequências simples repetidas (SSR), são fragmentos de DNA que variam de 1-6 ou mais nucleotídeos repetidos, tipicamente 5 a 50 vezes. Normalmente são encontradas em grande frequência no genoma de organismos eucariotos e são ótimas ferramentas para o estudo de genética de populações (ELLEGREN, 2004; TÓTH et al., 2000).

As sequências do DNA que flanqueiam os microssatélites geralmente são muito conservadas entre os indivíduos de mesma espécie e permitem a seleção de *primers* específicos que amplificam via PCR (ZANE; BARGELLONI; PATARNELLO, 2002). Os microssatélites são normalmente encontrados em regiões não-codificadoras do genoma, ou seja, regiões não influenciadas pela seleção natural, sendo classificadas como regiões seletivamente neutras (LI et al., 2002), tornando-se ideais para estudos de genética de populações (ELLEGREN, 2004; ZANE; BARGELLONI; PATARNELLO, 2002)

Dentre as diversas vantagens relativas ao uso dos microssatélites, destaca-se a de codominância, ou seja, todos os alelos de um organismo heterozigoto são passíveis de serem identificados (CHAMBERS; MACAVOY, 2000). Além disso, os SSRs ainda apresentam importantes características como alta frequência, repetitividade e polimorfismo (VARSHNEY; GRANER; SORRELLS, 2005). O alto grau de polimorfismo dos marcadores microssatélites está principalmente relacionado com suas altas taxas de mutação por geração (GAO et al., 2013; SCHLÖTTERER, 2000). Cada microssatélite constitui um loco altamente variável e de grande conteúdo que integra uma eficiente ferramenta para estudos da estrutura genética em populações naturais de plantas (GUICHOUX et al., 2011; MANOEL et al., 2012).

A utilização de marcadores moleculares permite um melhor entendimento de como os processos microevolutivos geram diferenciação das populações no tempo e no espaço (HEDRICK, 2001; PAUTASSO, 2009). Tais informações são essenciais para a compreensão

do efeito do manejo nas características genéticas de populações. Salienta-se ainda que o conhecimento da estrutura e da diversidade genética de uma espécie, bem como a compreensão dos fatores ecológicos e ambientais relacionados é essencial para a elaboração de estratégias de manejo mais adequadas para a conservação genética de uma espécie florestal (KAGEYAMA et al., 2003).

3. CONSIDERAÇÕES FINAIS

A exploração florestal resulta em uma fragmentação intensiva dos ecossistemas naturais. O manejo florestal sustentável apresenta-se como uma alternativa para garantir a conservação e o uso adequado de produtos florestais madeireiros e não-madeireiros. A sustentabilidade do manejo está relacionada ao uso adequado de práticas objetivando conciliar a produção florestal com a conservação da natureza. A principal abordagem deste trabalho é avaliar a possível impacto do manejo de árvores porta-sementes em parâmetros de diversidade genética e estrutura genética espacial, além da intensidade do fluxo entre uma área manejada e outra não manejada.

Como resultado desta pesquisa, espera-se determinar se a intensidade das práticas de manejo florestal aplicadas é adequada para a conservação genética, bem como para gerar indicadores úteis para a proposição de conservação e manejo sustentável dos recursos genéticos florestais. A detecção de alterações nos índices de diversidade e estrutura genética em populações naturais é essencial para a melhoria dos atuais sistemas silviculturais e para a definição de uma intensidade mais adequada dos ciclos de corte. Além disso, os marcadores microssatélites aqui desenvolvidos poderão posteriormente ser utilizados em trabalhos de seleção assistida de características desejáveis para o melhoramento florestal da espécie.

4. REFERÊNCIAS BIBLIOGRÁFICAS

AMORINI, E. et al. (2001). Impact of silvicultural system on *Cryphonectria parasitica* incidence and on genetic variability in a chestnut coppice in Central Italy. **Forest Ecology and Management**, Amsterdam, v. 142, p. 19-31, 2001.

ANDRÉ, T. L. et al. Post-logging loss of genetic diversity in a mahogany (*Swietenia macrophylla* King, Meliaceae) population in Brazilian Amazonia. **Forest Ecology and Management**, Amsterdam, v. 255, n. 2, p. 340-345, 2008.

ARAÚJO, L. C. ***Vanillosmopsis erythropappa* (DC.) Sch. Bip:** sua exploração florestal. Rio de Janeiro: Escola Nacional de Agronomia, 1944. 54 p.

AVISE, J. C.; HAMRICK, J. L. **Conservation genetics:** case histories from nature. Nova York: Chapman & Hall, 1996, 511 p.

BARLOW, J. et al. The future of hyperdiverse tropical ecosystems. **Nature**, Londres, v. 559, p. 517-526, 2018.

BARREIRA, S. **Diversidade genética em população natural de *Eremanthus erythropappus (*DC.) Macleish como base para o manejo florestal**. 2005. 73 p. Tese (Doutorado em Recursos Florestais) - Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, 2005.

BRAGA, E. A. **Substratos e fertilização na produção de mudas de candeia *Eremanthus erythropappus* (DC.) McLeisch, em tubetes.** 2006. 77 p. Dissertação (Mestrado) - Universidade Federal de Lavras, Lavras, 2006

BURNS, R. M. **Silvicultural systems for the major forest types of the United States**. Washington, DC: US Department of Agriculture, Forest Service, v. 191, 1983, 445 p.

CAMOLESI, J.F. **Volumetria e teor Alfa Bisabolol para a candeia *Eremanthus erythropappus*.** 2007. 90 p. Dissertação (Mestrado em Engenharia Florestal) - Universidade Federal de Lavras, Lavras, 2007.

CARVALHO, P. E. R. **Espécies florestais brasileiras: recomendações silviculturais, potencialidade e uso da madeira**. Brasília: EMBRAPA, 1994, 640p.

CHAMBERS, G. K.; ACAVOY, E. S. Microsatellites: Consensus and controversy. **Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology**, Amsterdam, v. 126, n. 4, p. 455-476, 2000.

CLOUTIER, D. et al. Impacts of selective logging on inbreeding and gene dispersal in an Amazonian tree population of *Carapa guianensis* Aubl. **Molecular Ecology**, Vancouver, v. 16, p. 797-809, 2007.

CRNOKRAK, P.; ROFF, D. A. Inbreeding depression in the wild. **Heredity**, Washington, v. 83, n. 3, p. 260-270, 1999.

CROZIER, R. H. Preserving the information content of species: genetic diversity, phylogeny, and conservation worth. **Annual Review of Ecology and Systematics**, Nova York, v. 28, n. 1, p. 243-268, 1997.

CRUZ, E. S. **Dinâmica, modelagem do crescimento e cadeia de comercialização da candeia *Eremanthus incanus* (Less.) Less**. 2006. 165 p. Tese (Doutorado em Engenharia Florestal) - Universidade Federal de Lavras, Lavras, 2006.

EL-KASSABY, Y. A.; DUNSWORTH, B. G.; KRAKOWSKI, J. Genetic evaluation of alternative silvicultural systems in coastal montane forests: western hemlock and amabilis fir. **Theoretical and Applied Genetics**, Hohenheim, v. 107, n. 4, p. 598-610. 2003.

ELLEGREN, H. Microsatellites: Simple sequences with complex evolution. **Nature Reviews**

Genetics, Londres, v. 5, n. 6, p. 435-445, 2004.

ELLSTRAND, N. C.; ELAM, D. R. Population genetic consequences of small population size Implications for plant conservation. **Annual Review of Ecology, Evolution and Systematics**, Nova York, v. 24, p. 217-242. 1993.

ESPADA, A. L. et al. Manejo florestal e exploração de impacto reduzido em florestas naturais de proteção da Amazônia. Boletim Técnico IFT 1. **Instituto Floresta Tropical**, Belém, 2014. 32 p.

FANIN, N. et al. Consistent effects of biodiversity loss on multifunctionality across contrasting ecosystems. **Nature Ecology & Evolution**, Londres, v. 2, n. 2, 269-278. 2017.

FRANKHAM R.; BALLOU, J. D.; BRISCOE, D. A. **Introduction to Conservation Genetics**. Cambridge: Cambridge University Press, 2002, 640 p.

GAO, C. et al. Revisiting an important component of plant genomes: Microsatellites. **Functional Plant Biology**, Clayton, v. 40, n. 7, p. 645-661, 2013.

GLAUBITZ, J. C.; WU, H. X.; MORAN, G. F. Impacts of silviculture on genetic diversity in the native forest species *Eucalyptus sieberi*. **Conservation Genetics**, Durham, v. 4, n. 3, p. 275-287. 2003.

GOMIDE, L. R. et al. Sustainable forest management of native vegetation remnants in Brazil. In: MARTIN-GARCIA J. JAVIER DIEZ (Ed.). **Sustainable forest management**: case studies. Rijeka: InTech, 2012. p. 77-96.

GONZÁLEZ-DÍAZ, P. et al.. Ecology and management history drive spatial genetic structure in Scots pine. **Forest Ecology and Management**, v. 400, p. 68-76. 2017

GUICHOUX, E. et al. Current trends in microsatellite genotyping. **Molecular Ecology Resources**, Vancouver, v. 11, n. 4, p. 591-611, 2011.

HAWLEY, G. J. et al. Silviculture alters the genetic structure of an eastern hemlock forest in Maine, USA. **Canadian Journal of Forest Research**, Ottawa, v. 35, n. 1, p. 143-150, 2005.

HEDRICK, P.W. Conservation genetics: where are we now. **Trends in Ecology and Evolution**, Cambridge, v. 16, p. 629-638, 2001.

HEILPERN, S. A.; WEEKS, B. C.; NAEEM, S. Predicting ecosystem vulnerability to biodiversity loss from community composition. **Ecology**, Washington, v. 99, n. 5, p. 1099-1107, 2018.

HICKEY, G. M. Evaluating sustainable forest management - Preface. **Ecological Indicators**, Amsterdam, v. 8, n. 2, p. 109-114, 2008.

IMAFLORA - Instituto de Manejo e Certificação Florestal e Agrícola. **Certificação FSC, um diferencial para a indústria cosmética e farmacêutica**. 2007 (Boletim, 19).

INGVARSSON, P. K.; DAHLBERG, H. The effects of clonal forestry on genetic diversity in wild and domesticated stands of forest trees. **Scandinavian Journal of Forest Research**, Alnarp, p. 1-10. 2018.

INNES, J. L. **Sustainable Forest Management:** From Concept to Practice. Abingdon: Routledge, 2016. v. 1, 396 p.

KAGEYAMA, P. Y. et al. Diversidade genética em espécies arbóreas tropicais de diferentes estágios sucessionais por marcadores genéticos. **Scientia Forestalis**, Piracicaba, n. 64, p. 93-107, 2003.

KAMATOU, G. P. P.; VILJOEN, A. M. A review of the application and pharmacological properties of α-bisabolol and α-bisabolol-rich oils. **Journal of the American Oil Chemists' Society**, Nova York, v. 87, n. 1, p. 1-7, 2010.

KERN, C. C. et al. Challenges facing gap-based silviculture and possible solutions for mesic northern forests in North America. **International Journal of Forestry Research**. Londres, v. 90, p. 4-17, 2017.

LACERDA, A.E.B.; KANASHIRO, M.; SEBBENN, A.M.; 2008. Effects of reduced impact logging on genetic diversity and spatial genetic structure of a *Hymenaea courbaril* population in the Brazilian Amazon Forest. **Forest Ecology and Management**, Amsterdam, v. 255, p. 1034-1043.

LEE, C. T. et al. Maintenance of genetic diversity in *Parkia speciosa* in logged-over forests. **Journal of Tropical Forest Science**, Nova York, v. 14, n. 2, p. 163-178, 2002.

LI, Y. et al. Microsatellites: genomic distribution, putativa functions, and mutational mechanism: a review. **Molecular Ecology**, Vancouver, v. 11, p. 253-256, 2002.

LOEUILLE, B.; LOPES, J. C.; PIRANI, J. R. Taxonomic novelties in *Eremanthus* (Compositae: Vernonieae) from Brazil. **Kew Bulletin**, Londres, v. 67, n. 1, p. 1-9, 2012.

LOISELLE, B. A. et al. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). **American Journal of Botany**, St. Louis, v. 82, n.11, p. 1420-1425. 1995.

LOVELESS, M. D., HAMRICK, J. L. Ecological determinants of genetic structure in plant populations. **Annual Review of Ecology and Systematics**, Nova York, v. 15, n. 1, p. 65-95. 1984.

MACLEISH, N. F. F. Revision of *Eremanthus* (Compositae: Vernonieae). **Annals of the Missouri Botanical Garden**, St. Louis, v. 74, n. 2, p. 265-290, 1987.

MANEL, S., et al. Landscape genetics: combining landscape ecology and population genetics. **Trends in Ecology & Evolution**, Cambridge, v. 18, p. 4, p. 189-197. 2003.

MANOEL, R. O. et al. Contemporary pollen flow, mating patterns and effective population size inferred from paternity analysis in a small fragmented population of the Neotropical tree *Copaifera langsdorffii* Desf. (Leguminosae-Caesalpinioideae). **Conservation Genetics**, Durham, v. 13, n. 3, p. 613-623, 2012.

MELO, L. A.; DAVIDE, A. C.; TEIXEIRA L. A. F. Metodologia para resgate de matrizes e enraizamento de estacas de *Eremanthus erythropappus*. **Cerne**, Lavras, v. 18, n. 4, 2012.

MINAS GERAIS. **Portaria nº 01**, de 5 janeiro de 2007. Dispõe sobre normas para elaboração e execução do Plano de Manejo para Produção Sustentada da Candeia - *Eremanthus erythropappus* e *Eremanthus incanus* no Estado de Minas Gerais e dá outras providências. Belo Horizonte, 2007. Disponível em: <http://www.siam.mg.gov.br/sla/download.pdf?idNorma=6692>. Acesso em: 10 out. 2017.

MINAS GERAIS. Secretária de Estado de Meio Ambiente e Desenvolvimento Sustentável. Resolução Conjunta SEMAD/IEF nº 1804, de 11 de janeiro de 2013. Dispõe sobre os procedimentos para autorização da intervenção ambiental no Estado de Minas Gerais e dá outras providências. **Diário do Executivo de Minas Gerais**, Belo Horizonte, ano 121, n. 23, p. 23-25, 2 fev. 2013.

MITTERMEIER, R. A. et al. Biodiversity hotspots and major tropical wilderness areas: approaches to setting conservation priorities. **Conservation Biology**, Londres, v. 12, n. 3, p. 516-520, 1998.

NEI, M. Analysis of gene diversity in subdivided populations. **Proceedings of the National Academy of Sciences**, Washington, v. 70, n. 12, p. 3321-3323. 1973.

NEI, M. F-statistics and analysis of gene diversity in subdivided populations. **Annals of human genetics**, Londres, v. 41, n. 2, p. 225-233. 1977.

OLIVEIRA, A. D. D. et al. Market chain analysis of candeia timber (*Eremanthus erythropappus*). **Cerne**, Lavras, v. 15, n. 3, 2009.

OLSON, D. M.; DINERSTEIN, E. The Global 200: a representation approach to conserving the Earth's most biologically valuable ecoregions. **Conservation Biology**, Londres, v. 12, n. 3, p. 502-515, 1998.

ORTEGO, J.; BONAL, R.; MUÑOZ, A. Genetic consequences of habitat fragmentation in long-lived tree species: the case of the Mediterranean holm oak (*Quercus ilex*, L.). **Journal of Heredity**, Oxford, v. 101, n. 6, p. 717-726, 2010.

PAFFETTI, D et al. The influence of forest management on beech (*Fagus sylvatica* L.) stand structure and genetic diversity. **Forest Ecology and Management**, Amsterdam, v. 284, p. 34-44, 2012.

PAUTASSO, M. Geographical genetics and the conservation of forest trees. **Perspectives in Plant Ecology, Evolution and Systematics,** Amsterdam, v. 11, n. 3, p. 157-189, 2009.

PÉREZ, J. F. M. et al. Sistema de manejo para a candeia *Eremanthus erythropappus* (DC.) MacLeish, a opção do sistema de corte seletivo. **Cerne**, Lavras, v. 10, n. 2, p. 257-273, 2004.

QUÉMÉRÉ, E. et al. Landscape genetics of an endangered lemur (*Propithecus tattersalli*) within its entire fragmented range. **Molecular Ecology**, Vancouver, v. 19, n. 8, p. 1606-1621, 2010.

RAJORA, O. P.; PLUHAR, S. A. Genetic diversity impacts of forest fires, forest harvesting, and alternative reforestation practices in black spruce (*Picea mariana*). **Theoretical and Applied Genetics**, Hohenheim, v. 106, n. 7, p. 1203-1212, 2003.

RAJORA, O. et al. Microsatellite DNA analysis of genetic effects of harvesting in old-growth eastern white pine (*Pinus strobus*) in Ontario, Canada. **Molecular Ecology**, Vancouver, v. 9, p. 339–348. 2000.

RIZZINI, C. T. **Árvores e madeiras úteis do Brasil**: manual de dendrologia brasileira. São Paulo: Edgard Blücher, 1979. 296p.

SCHLÖTTERER, C. Evolutionary dynamics of microsatellite DNA. **Chromosoma**, Berlin, v. 109, n. 6, p. 365-371, 2000.

SCOLFORO, J. R. S. Estimativas de volume, peso seco, peso de óleo e quantidade de moirões para a Candeia (*Eremanthus erythropappus* (DC.) MacLeish). **Cerne**, Lavras, MG, v.10, n.1, p.87-102, 2004.

SCOLFORO, J. R. S. et al. **Zoneamento ecológico econômico de Minas Gerais**: componentes geofísico e biótico. Lavras: UFLA, 2008. p. 89-101.

SCOLFORO, J. R. S.; LOEUILLE, B.; ALTOÉ, T. F. Caracterização da candeia. In: SCOLFORO, J. R. S.; OLIVEIRA, A. D.; DAVIDE, A. C. (Ed.). **Manejo sustentável da candeia**: o caminhar de uma nova experiência em Minas Gerais. Lavras: Ed. UFLA, 2012. p. 19-27.

SCOLFORO, J. R. S.; OLIVEIRA, A. D.; DAVIDE, A. C. **O manejo sustentável da candeia:** o caminhar de uma nova experiência florestal em Minas Gerais. Lavras: UFLA, 2012, 329 p.

SEBBEN, A.M. et al. Modelling the long-term impact of selective logging on genetic diversity and demographic structure of four tropical tree species in the Amazon forest. **Forest Ecology and Management**, Amsterdam, v. 254, p. 335-349, 2008.

SELKOE, K. A.; TOONEN, R. J. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. **Ecology letters**, Hoboken, v. 9, n. 5, p. 615-629, 2006.
SHAPCOTT, A. The spatial genetic structure in natural populations of the Australian temperate rainforest tree *Atherosperma moschatum* (Labill.) (Monimiaceae). **Heredity**, Oxford, v. 74, n. 1, p. 28-38, 1995.

SILVA, C. S. J. **Análise econômica da produção de madeira de Candeia (*Eremanthus erythropappus*) em plantios.** 2011. 135 p. Dissertação (Mestrado em Engenharia Florestal) - Universidade Federal de Lavras, Lavras, 2011.

SILVA, M. A. et al. Análise da distribuição espacial da candeia (*Eremanthus erythropappus* (DC.) MacLeish) sujeita ao sistema de manejo porta-sementes. **Cerne**, Lavras, v. 14, n. 4, p. 311-316, 2008.

SOLIANI, C., et al. Logging by selective extraction of best trees: Does it change patterns of genetic diversity? The case of *Nothofagus pumilio*. **Forest Ecology and Management**, Amsterdam, v. 373, p. 81-92, 2016.

TÓTH, G.; GÁSPÁRI, Z.; JURKA, J. Microsatellites in different eukaryotic genomes: survey and analysis. **Genome Research**, Nova York, v. 10, n. 7, p. 967-981, 2000.

VARSHNEY, R. K.; GRANER, A.; SORRELLS, M. E. Genic microsatellite markers in plants: features and applications. **Trends in Biotechnology**, Amsterdam, v. 23, n. 1, p. 48-55, 2005.

VEKEMANS, X.; HARDY, O. J. New insights from fine-scale spatial genetic structure analyses in plant populations. **Molecular Ecology**, Vancouver, v. 13, n. 4, p. 921-935, 2004.

VRANCKX, G. U. Y. et al. Meta-analysis of susceptibility of woody plants to loss of genetic diversity through habitat fragmentation. **Conservation Biology**, Londres, v. 26, n. 2, p. 228-237, 2012.

WEIR, B. S.; COCKERHAM, C. C. Estimating F-statistics for the analysis of population structure. **Evolution**, Hoboken, v. 38, n. 6, p. 1358-1370,1984.

WRIGHT, S. The genetical structure of populations. **Annals of Eugenics**, Hoboken, v. 15, n. 1, p. 323-354, 1949.

WRIGHT, S. The interpretation of population structure by F-statistics with special regard to systems of mating. **Evolution**, Hoboken, v. 19, n. 3, p. 395-420, 1965.

YANG, H. B. et al., Methods for Developing Molecular Markers. In: KOH H. J., KWON, S. Y., THOMSON M. (eds.). **Current Technologies in Plant Molecular Breeding**. Dordrecht, Springer, p. 15-50, 2015.

YOUNG A.; BOYLE, T.; BROWN, T. The population genetic consequences of habitat fragmentation for plants. **Trends in Ecology and Evolution**, Cambridge, v. 11, p. 413-418, 1996.

ZANE, L.; BARGELLONI, L.; PATARNELLO, T. Strategies for microsatellite isoloation: a review. **Molecular Ecology**, Vancouver, v. 11, n. 1, p. 1-16, 2002.

ZHANG, X.; SHI, M. M.; SHEN, D. W.; CHEN, X. Y. Habitat loss other than fragmentation per se decreased nuclear and chloroplast genetic diversity in a monoecious tree. **PLoS ONE**, San Francisco, v. 7, n. 6, 2012.

SEGUNDA PARTE

ARTIGO 1

DEVELOPMENT AND CHARACTERIZATION OF NUCLEAR MICROSATELLITE MARKERS FOR *Eremanthus erythropappus* (DC.) MacLeish (ASTERACEAE) AND THEIR TRANSFERABILITY ACROSS RELATED SPECIES

(Preparado de acordo com as normas da revista *Biological Research*. Esta é uma versão preliminar, o conselho editorial do periódico poderá sugerir alterações para adequá-lo ao seu próprio estilo)

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1. Abstract

Background: We developed new microsatellite markers (SSR) for *Eremanthus erythropappus* (DC.) MacLeish, an endangered and endemic tree species from the Brazilian savanna and Atlantic forest. We also tested their transferability in two closely related *Eremanthus* species.

Results: Using a genomic library enriched with tandem repeat motifs, we identified 16 primers sets, characterized for two populations. Nine primers amplified satisfactorily and seven microsatellite markers were polymorphic, providing an average of 38 alleles and 4.222 alleles per marker. The polymorphic information content value (PIC) ranged from 0.438 to 0.943 with an average of 0.650. The average observed heterozygosity across all loci varied from 0.606 to 1.000. The observed (H_O) and expected (H_E) heterozygosity within the two populations varied from 0.647 to 1.000 and from 0.308 to 1.000, respectively. The majority of these primers could be amplified in the two *Eremanthus* species.

Conclusions: These newly SSR markers are powerful tools for population genetic analyses and may be useful to understand ecological, evolutionary, and taxonomic concerns.

Keywords: *Eremanthus erythropappus*; simple sequence repeats; genetic diversity

2. Background

Eremanthus erythropappus (DC.) MacLeish is an endemic tree species native from the high mountains of the Brazilian Savanna and Atlantic Forest (MacLeish, 1987; Loeuille, 2012). The species normally occurs in shallow, rocky and low fertility soils (Pérez et al. 2004). Regenerating individuals are light demanding as is common in pioneer species (Carvalho, 1994; Scolforo et al. 2012). In Brazil, *E. erythropappus* has been historically overexploited for the construction of rural fences because of the high durability of the wood against weathering (Scolforo et al. 2008; Silva et al. 2008), and for the extraction of a sesquiterpene alcohol. The α-bisabolol is extracted from the wood and it is highly used by pharmacy industries for manufacturing because of the anti-inflammatory, antibacterial, skin-smoothing and wound healing properties (Sousa et al., 2008; Kamatou and Viljoen, 2010).

Microsatellite markers also known as simple sequence repeats (SSR) are intensely used for studying the genetic diversity of animals and plants. SSR sequences are distributed throughout euchromatin and eukaryotes genome (Katti et al. 2011; Phumichai et al. 2015) and normally present high polymorphism, frequency and repeatability (Vieira et al. 2016). Microsatellite loci present the highest information content among all classes of molecular markers (Varshney et al., 2005) and can be analyzed using polymerase chain reaction (PCR), allowing a highly informative evaluation of a large number of loci, and different effects on species population genetics, breeding programs and germplasm conservation (Powell et al., 1996). The objective of this study was to develop a set of microsatellite markers to be used as a tool to assess the genetic diversity and structure of *E. erythropappus* species and other species from the *Eremanthus* genera.

3. Methods

Leaf tissues from adult trees of *E. erythropappus* were collected and preserved in silica gel. Total genomic DNA was extracted using CTAB method according to Doyle and Doyle (1990) and 30 ng of genomic DNA were digested using the *Rsa*I restriction enzyme. The fragments were connected to adapters (*Rsa*21 5'-CTCTTGCTTACGCGTGGACTA-3' and *Rsa*25 5'-TAGTCCACGCGTAAGCAAGAGCACA-3'). For the enrichment of dinucleotide sequences, we used the biotinylated oligonucleotides $(CT)_8$, $(GT)_8$ and $(CTT)_8$. Furthermore, streptavidin-coated paramagnetic bands and biding biotin were used to recover the fragments containing the microsatellites. The *Rsa*21 adapter sequences were used as a primer-template for the amplification of fragments and the microsatellite fragments were connected to a pGEM-T

Easy vector (Promega Corporation, Madison, Wisconsin, USA). The plasmids were introduced into *Escherichia coli* XL1-Blue strains. Transformed cells were inoculated on a Petri dishes containing ampicillin (100 μg ml^{-1}) and X-galactosidase (5-bromo-4-chloro-indolyl- β-D-galactoside) (50 μg ml^{-1}). The obtained recombinant colonies were sequenced using ABI 377 automated sequencer and the Big Dye Thermator Kit (Applied Biosystems, Vienna, Austria). We found 16 positive clones that presented microsatellite sequences with at least five tandem repeats. Primer pairs were designed using the software Primer 3 (Rozen and Skaletsky, 2000) applying the product size from 100 to 300 base pairs (bp), primer size from 18 to 22 bp, GC% from 40 to 60, and annealing temperature from 57 to 60°C.

PCR reactions were performed in order to validate the primers. The final volume of each reaction was 15.0 μl using 30 ng of template DNA added to 12.0 μl reaction mix containing 3,33 mM IB Phoneutria buffer (consisting of 100 mM Tris-HCl pH 8.4; 500 mM KCl, 1% Triton X-100, 15 mM $MgCl_2$), 1.5 mM $MgCl_2$, 0.28 mM of each dNTP, 1 U Taq polymerase, 0.22 mM each primer (Forward and Reverse). The temperature regime was assessed separately for each primer pair. In total, we tested 17 temperatures (from 46ºC to 62ºC) in six individuals from two different populations using MJ Mini™ Thermal Cycler. The optimal PCR profile used for the amplification of each microsatellite consisted of 3 minutes initiation at 94°C, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing temperature (Table 1) for 30 seconds, extension at 72 °C for 1 minute and a final extent at 72 °C, for 7 minutes. Amplifications were performed using a MJ Mini™ Thermal Cycler (Bio-Rad, Singapore).

Additionally, we used 42 individuals from the population 1 - Itamonte (22°16'45.00"S 44°46'25.60"W) and the population 2 - Lavras (21°19'51.85"S 44°57'54.76"W) in order to perform the characterization of the polymorphism. Voucher specimens were deposited in the ESAL herbarium of the Federal University of Lavras (UFLA), Brazil. The amplification was done using the thermocycler GeneAmp PCR System 9700. We applied the same reaction components and PCR thermal cycle used on the validation process. We separated the PCR products using a 3% high-resolution agarose MetaPhor™ (Lonza, Rockland, Maine, USA) and it was colored using GelRed®. Allele sizes were estimated by comparison to a 10-bp DNA Ladder standard (Invitrogen, Carlsbad, California, USA). Individuals showing amplification fails for three or more loci were excluded.

We applied the Brookfield 1 method (Brookfield, 1996) using 1000 permutations in order to analyze genotyping errors due to the presence of null alleles, stuttering, and allele dropout using the software Micro-Checker 2.2.3 (Van Oosterhout et al., 2004). We estimated

the allele richness using MSA software (Dieringer; Schlötterer, 2003). The number of alleles per locus (N_A), observed heterozygosity (H_O), and expected heterozygosity (H_E) for each population and locus according to the Hardy-Weinberg equilibrium were calculated using the GenAlEx 6.4 (Peakall and Smouse 2006). The within-population inbreeding coefficients (F_{IS}) were determined using FSTAT 2.9.3.2 (Goudet, 1995), applying the Bonferroni correction for multiple comparisons. The probability of non-exclusion for each locus and the combined probability of paternity exclusion and the polymorphic information content (PIC) were calculated using the CERVUS 3.0 (Kalinowski; Taper; Marshall, 2007).

Table 1 Characteristics of nine microsatellite loci developed for *Eremanthus erythropappus* from Minas Gerais state. Locus name, primer sequence (F: forward, R: reverse), repeat motif, fragment size (base pair), melting temperature (Tm), and GenBank accession numbers are shown.

Locus	Primer sequences (5'-3')	Repeat motif	Size	Ta (ºC)	GenBank
ERE02	F: TCTTGCTTACGCGTGTGACT R: TGCATCCACTCCAATCACTT	(GA)21	119	53	MK075833
ERE03	F: GAAGGGAGACATCGGAAGAA R: ACGGAACGGAGAAGAAGAAA	(CTT)5; (CTT)9; (CTT)10; (CTT)8	232	53	MK075834
ERE04	F: CAGTGAGGGGAAGGGAGAAT R: CCTCCACTATAGGGCGGAAT	(CTT)37	398	53	MK075835
ERE07	F: GCGTGGGACTAACCCATTC R: ACCTGTTGGTGAAAGGATGC	(CTT)9	120	53	MK075836
ERE08	F: GAGCCTTCCATGGGAGTAGG R: TGGGAGGGAGAAATTGAACA	(AGC)5	238	53	MK075837
ERE09	F: GCTTACGCGTGGGACTAACT R: GCGTGGACTAGGAAAACGAA	(CA)3; (GA)8; (GTA)6	269	53	MK075838
ERE10	F: GATCATCGCCATGAAGGAAT R: CAGTGAGGGGAAGGGAGAAT	(GAA)3; ATA; (GAA)27	244	53	MK075839
ERE13	F: GAGACCCTGGCTGTCTTCAT R: GCGTTGAGTTTCGGAGAAGT	(CT)6; (CA)6	378	53	MK075840
ERE14	F: CATCGATTTGGAGGCTTCAT R: TGCTTACGTGTGCTCTTGCT	(CT)11; (AT)8; (GT)18	207	53	MK075841

For each locus, prime's name, sequence, repeat motif, product size, annealing temperature (Ta) and GenBank accession numbers.

We also tested the cross-amplification in two other species, *Eremanthus incanus* and *Eremanthus glomerulatus*. PCR reactions and electrophoresis were performed according to the conditions described above.

4. Results

From the initial 16 primers pairs, nine amplified the expected size fragment while the others seven showed no amplification, patterns of multibands or stuttering. Among the nine amplified primers, seven were polymorphic (Ere02; Ere03; Ere07; Ere08; Ere10; Ere13 and Ere14) and two were monomorphic (Ere04 and Ere09). The observed and expected heterozygosities ranged from 0.308 to 1.000 and from 0.379 to 0.909, respectively. The mean number of alleles ranged from 1 to 18 (Table 2). The mean within-population inbreeding coefficient (F_{IS}) was -0,227. The polymorphic information values ranged from 0,438 to 0,943. The combined paternity exclusion probability was high enough to perform a paternity/maternity exclusion analysis in breeding populations using these seven loci. So, for the first parent the paternity exclusion probability reached 0,985657 and for the second parent 0,998918. All loci did not significantly differ from Hardy-Weinberg equilibrium, and any of them showed presence of null alleles or linkage disequilibrium ($\bar{r}_d < 0.5$) (P > 0.05).

Table 2. Genetic characterization of the newly developed nine microsatellites for *Eremanthus erythropappus* [a] and its transferability for *Eremanthus incanus* and *Eremanthus glomerulatus.*

		Itamonte (n = 21)				Lavras (n = 21)				Transferability	
Locus	***PIC***	***Na***	***H_O***	***H_E***	***F_{IS}***	***Na***	***H_O***	***H_E***	***F_{IS}***	***Eremanthus incanus***	***Eremanthus glomerulatus***
Ere02	0.607	3	1.000	0.561	-0.782	3	1.000	0.614	-0.629	-	-
Ere03	0.683	5	1.000	0.600	-0.667	9	0.650	0.558	-0.165	+	+
Ere04	0.438	1	0.000	0.000	0.000	1	0.000	0.000	0.000	-	-
Ere07	0.532	2	0.854	0.491	-0.739	2	0.833	0.486	-0.714	-	+
Ere08	0.674	2	0.950	0.495	-0.919	3	0.308	0.379	0.187	+	+
Ere09	0.509	1	0.000	0.000	0.000	1	0.000	0.000	0.000	+	+
Ere10	0.943	11	0.836	0.836	0.226	18	0.810	0.909	0.109	-	-
Ere13	0.792	3	0.480	0.480	1.000	5	0.000	0.639	1.000	+	+
Ere14	0.670	3	0.585	0.585	-0.538	3	0.625	0.430	-0.453	+	+

Note: PIC = Polymorphic content information, *Na* = Number of alleles, H_O = observed heterozygosity; *He* = expected heterozygosity, F_{IS} = Endogamy coefficient; +, successful PCR amplification; -, unsuccessful PCR amplification.

[a]Geographic coordinates for the populations are Itamonte = 22°16'45.00"S 44°46'25.60"W and Lavras = 21°19'51.85"S 44°57'54.76"W; All two populations are located in the Minas Gerais state, Brazil.

According to Nazareno and Reis (2011), monomorphic microsatellites may also be used as a powerful tool for studying genetic population and phylogenetic analyses in plant species as it presents polymorphic sites in its flanking regions. Besides, the two monomorphic microsatellites developed in addition to the seven polymorphic markers may be useful for studying genetic parameters of *E. erythropappus* since there is evidence of possible use of these primers due to the high level of information found in SSR markers. Additionally, we found that five microsatellites showed cross amplification for *E. incanus* (Ere03, Ere08, Ere09, Ere13 and

Ere14) and six also were transferred for *E. glomerulatus* (Ere03, Ere07, Ere08, Ere09, Ere13 and Ere14). Therefore, these primers could be used for genetic diversity analysis of other species of the genus *Eremanthus*.

5. Conclusions

We developed nine microsatellite markers which seven were polymorphic and two monomorphic in two populations of *E. erythropappus*. These markers may be useful for further research on population genetics, genetic diversity, spatial genetic distribution and help to understand the management and conservation practices applied to this species. Additionally, these primers may be used on breeding programs already started for this species. The cross-amplification confirms the possible application on *E. incanus* and *E. glomerulatus* species.

6. Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LFR and DC performed the laboratory work; DC, LFR and AGN analyzed the raw data and contributed with the statistical and genetical analysis. DC also administered the funds of this research. All authors read and approved the final manuscript.

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7. References

Brookfield J. A simple new method for estimating null allele frequency from heterozygote deficiency. Mol Ecol. 1996;5:453-455.

Brown AHD, Feldman MW, Nevo E. Multilocus structure of natural populations of *Hordeum spontaneum*. Genetics. 1980;96:523-36.

Carvalho PER. Espécies florestais brasileiras: recomendações silviculturais, potencialidade e uso da madeira. Brasília: EMBRAPA, 1994.

Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Geog. Genet.1987; 12(1):13-15.

Dieringer D, Schlötterer C. Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. Mol Ecol Notes. 2003:167-9.

Goudet, JFSTAT. FSTAT (version 1.2): a computer program to calculate F-statistics. J Hered. 1995;86(6):485-6.

Kalinowski ST, Taper ML, Marshall TC. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 2007;16(5):1099-106.

Kamatou GP, Viljoen AM. A review of the application and pharmacological properties of α-Bisabolol and α-Bisabolol-rich oils. J. Am. Oil Chem.' Soc. 2010;87(1):1-7.
Katti MV, Ranjekar PK, Gupta VS. Differential distribution of simple sequence repeats in eukaryotic genome sequences. Mol. Biol. Evol. 2001;18(7):1161-7.

Loeuille B, Lopes JC, Pirani JR. Taxonomic novelties in *Eremanthus* (Compositae: Vernonieae) from Brazil. Kew Bull. 2012;67(1):1-9.

MacLeish NF. Revision of *Eremanthus* (Compositae: Vernonieae). Ann. Missouri Bot. Gard. 1987:265-90.

Nazareno AG, Reis MS. The same but different: Monomorphic microsatellite markers as a new tool for genetic analysis. Am J Bot. 2011;98(10):265-267.

Peakall RO, Smouse PE. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes. 2006;6(1):288-95.

Pérez FMJ, Scolforo SJR, Mello JM, Borges RLF, Camolesi JF. Sistema de manejo para a candeia-*Eremanthus erythropappus* (DC.) MacLeish a opção do sistema de corte seletivo. Cerne. 2004;10(2).

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mole Breed. 1996;2(3):225-38.

Phumichai C, Phumichai T, Wongkaew A. Novel chloroplast microsatellite (cpSSR) markers for genetic diversity assessment of cultivated and wild *Hevea rubber*. Plant Mol Biol. 2015;33(5):1486-98.

Rozen S, Skaletsky H. Primer3 on the WWW for General Users and for Biologist Programmers. In: Misener S, Krawetz SA, editors. Methods in Molecular Biology™. Humana Press: Totowa; 2000;132:365-386.

Scolforo JRS, Loeuille BFP, Altoé TF. Caracterização da candeia. In: Scolforo JRS, Oliveira AD, Davide AC, editors. O manejo sustentável da candeia: o caminhar de uma nova experiência florestal em Minas Gerais. Lavras: UFLA; 2012. p. 19-27.

Scolforo JRS, Oliveira AD, Acerbi Junior FW. Inventário Florestal de Minas Gerais: equações de volume, peso de matéria seca e carbono para diferentes Fitofisionomias da Flora Nativa. Lavras: Editora UFLA, 2008.

Silva MA et al. Análise da distribuição espacial da candeia (*Eremanthus erythropappus* (DC.) MacLeish) sujeita ao sistema de manejo porta-sementes. Cerne. 2008;14(4).

Sousa OV et al. Antinociceptive and anti-inflammatory effects of the essential oil from *Eremanthus erythropappus* leaves. J. Pharm. Pharmacol. 2008;60(6):771-7.

Van Oosterhout C et al. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes. 2004;4(3):535-8.

Varshney RK, Graner A, Sorrells ME. Genic microsatellite markers in plants: features and applications. TRENDS in Biotechnology. 2005;23(1):48-55.

Vieira ML, Santini L, Diniz AL, Munhoz CD. Microsatellite markers: what they mean and why they are so useful. Genet Mol Biol. 2016;39(3):312-328

ARTIGO 2

IMPACTS OF FOREST MANAGEMENT ON GENETIC DIVERSITY AND SPATIAL GENETIC STRUCTURE OF *Eremanthus erythropappus* (ASTERACEAE)

(Preparado de acordo com as normas da *Forest Ecology and Management.* Esta é uma versão preliminar, o conselho editorial do periódico poderá sugerir alterações para adequá-lo ao seu próprio estilo)

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1. Abstract

Forest management may cause severe effects forest connectivity and effective population size in natural populations. Nevertheless, harvesting old-growth individuals may also change natural patterns of genetic variation and spatial genetic structure as a result of the alteration on tree density and age class structure at different stages during a stand rotation. The present study evaluated the impacts of forest management using a silvicultural system of seed trees on the genetic diversity and spatial genetic structure (SGS) of *Eremanthus erythropappus* (DC.) MacLeish, an endemic tree that naturally occurs in the high mountains of the Brazilian Savanna and Atlantic Forest. *E. erythropappus* has been historically overexploited due to the high durability of the timber and extraction of the essential oil α-bisabolol from its wood. Nevertheless, we applied a total census of living trees within four plots to compare the genetic variation of a managed stand with an unmanaged stand, totalizing 275 samples. The study site is located in Itamonte, southern Minas Gerais state, Brazil. We genotyped all adult and juvenile individuals using six polymorphic microsatellite loci 60 months after the management. We compared the genetic diversity and the spatial genetic structure parameters among the managed and unmanaged stands. Overall, the genetic diversity was considered high because of an efficient gene flow between stands. We found a slight but not statistically significant increase on allelic and genetic variation on the managed stand and a slight present of private alleles on the unmanaged stand. In general, we found no genetic differentiation between stands and a not evident endogamy. Genetic clustering identified a single population (K = 1), indicating no genetic differentiation between managed and unmanaged stands. Adult and juvenile individuals of the unmanaged stand appeared to be more geographically structured than individuals from the managed one. Besides, we found a tendency of coancestry among juveniles at the first class of distance of the managed stand, suggesting a drift on the genetic structure probably caused by forest management. Therefore, understanding early responses of forest management on genetic diversity and stand structure is a primordial step to ensure the effectiveness of conservation practices of tree species. The sustainability of forest management of *E. erythropappus* on genetic diversity and more accurately on the SGS needs to be evaluated over the time to promote effective conservation of the population size and genetic variability.

Keywords: Genetic diversity; microsatellite markers; gene flow; silvicultural management; Brazilian Cerrado

2. Introduction

Sustainable forest management uses appropriate silvicultural techniques to produce different goods and services and simultaneously promotes biodiversity conservation (Petrokofsky et al. 2015). The reduction in tree-density inherent to forest management practices may result in increased levels of inbreeding and erosion of the evolutionary potential (Crnokrak and Roff, 1999; Frankham et al. 2002; Quemeré et al. 2010). Among the main effects, silvicultural management may affect species selection and spatial genetic structure (Hawley et al. 2005; Lacerda et al. 2008). However, although natural regeneration allows the conservation of genetic information to the next generation, it does not avoid adaptive and non-adaptive changes in genetic structure (Rajora and Pluhar, 2003).

Tropical forest regeneration is a natural process that is quite important for the conservation of tropical ecosystems (Chazdon, 2014). The maintenance of the regeneration potential is essential to ensure the sustainability of silvicultural management, promote forest succession dynamics, and consequently preserve the natural patterns of resilience in disturbed environments (Ackzell, 1993; Folke, 2006). Due to the high fragility of tropical biomes, it is fundamental to analyze its genetic diversity in order to define strategies of management and conservation, especially those determined by high altitudes, that normally shows an intense risk of extinction (Avise and Hamrick, 1996; Brown et al. 2001).

Overall, forest management practices can modify different stages of tree density and age class structure in forest populations (Bergeron et al. 2017). Changes in the frequency and distribution of tree species may alter the genetic structure of forest populations (Sjolund and Jump, 2015; González-Diaz et al. 2017). Several patterns of genetic effects of forest management have been found as a response of forest management practices. The main impacts of forest thinning on natural populations are related to frequency changes and loss of alleles (Schaberg et al. 2008; Ortego, Bonal and Muñoz, 2010). Commonly, forest management processes may also cause loss of alleles between old-growth individuals and juveniles (El-Kassaby and Benowicz 2000; Rajora et al. 2000; Solani et al. 2016) but can also be responsible for enhancing the genetic diversity parameters by increasing gene flow (Cloutier et al. 2007; Young et al., 1996; Wickneswari, 2011). Nevertheless, many studies of forest management have not detected any significant influence on genetic diversity (Borrel et al. 2018; Lee et al. 2002; Martins et al., 2016; Robledo-Arnuncio et al. 2004). The lack of response may be an effect of the long generation process of tree species, which takes many generations for the

appearance of initial genetic effects (Lowe et al. 2015). Therefore, it is important to detect early genetic responses in different categories of succession to evaluate management sustainability (García-Gil et al. 2015).

Genetic diversity provides the elementary condition for the evolution of tree species (Cavers and Cottrell, 2014). The maintenance of natural genetic variation is essential to the continuity and evolution of populations through selective forces (Schaberg et al. 2008). Nevertheless, anthropogenic impacts can disrupt the frequency and distribution of forest genetic resources and may generate severe impacts for species genetic conservation (Dixo et a. 2009; Young et al. 1996). The most common consequences of habitat change induced by human activities are related to variations in natural pollen and seed dispersal, which may influence its genetic diversity and spatial genetic structure (Ghanzoul, 2005). Additionally, temporal environmental variation may decrease reproductive fitness, disease resistance, population adaptation to new environments and protect the forest against threats (Telford et al. 2014).

The spatial genetic structure is mainly influenced by gene flow, genetic drift, and selection (Loiselle et al. 1995). Some studies have already found significant effects of silvicultural management on spatial genetic structure within populations, even when overall levels of variation are maintained, allowing us to explore the genetic legacy of forest management (Gonzáles-Diaz et al. 2017; Sjölund and Jump, 2015). According to Smouse and Peakall (1999), changes in genetic structure may alter local breeding and evolution of species. In addition, clonal propagation can also have an intense effect on the SGS by improving the density of regenerating individuals and consequently raising the incidence of clonal individuals (Sjölund and Jump, 2013). Pollen and seed dispersal are the main factors responsible for driving gene flow among populations and consequently driving the spatial genetic structure (Vekemans and Hardy, 2004; Cavers et al. 2005). Therefore, sustainable forest management should proactively evaluate the impacts of harvesting large trees on tree genetic diversity and the spatial genetic structure of the regenerating individuals (Aravanopoulos, 2018).

Moreover, forest harvesting can have strong effects on genetic diversity, connectivity and effective population size (Dixo et al., 2002). *Eremanthus erythropappus* and *Eremanthus incanus* are two managed tree species in Minas Gerais state, Brazil (Scolforo et al. 2012). These species are commonly used for fence production, because of the high durability of the wood, and for extraction of α-bisabolol, an important essential oil that is highly demanded by the pharmaceutical and cosmetic industries (Oliveira et al., 2009). Several studies on the structure (Scolforo et al. 2012), growth dynamics (Scolforo et al. 2015; Silva et al. 2008), economic

aspects (Andrade, 2009; Camolesi, 2007; Cruz, 2006; Santos et al. 2017; Scolforo et al. 2004), silviculture (Braga, 2006; Tonetti, 2004) and genetic analysis (Rocha et al. 2019; Pádua et al. 2016) of the genus *Eremanthus* have already been carried out.

Further analyses should be performed to evaluate the genetic sustainability of forest management in exploited populations. In this paper, we analyze the effect of a silvicultural system of seed trees on the genetic diversity and spatial genetic structure of a managed stand of *E. erythropappus* in southern Minas Gerais State, Brazil. We aim to obtain information that can be used to maintain the genetic variability of the species to evaluate the following hypotheses: (1) the managed stand presents similar levels of genetic diversity in comparison to the unmanaged stand; (2) the spatial genetic structure of the regenerating individuals may be affected by forest thinning. We estimated the correlation between the frequency of alleles and the forest management strategy applied. Additionally, we analyzed the effects of possible changes on genetic structure, as well as the importance of gene flow for maintaining the genetic variability in the managed stand.

3. Material and Methods

3.1 Study species

The genus *Eremanthus* naturally grows throughout the mountains of the Brazilian Cerrado. *Eremanthus erythropappus* (DC.) MacLeish, commonly known as “candeia”, is an endemic tree species mostly found in southern Minas Gerais state. This species normally develops in rocky, shallow and low fertility soils at altitudes up to 1,000 m (3280 ft), forming pure stands (Pérez et al. 2004). Fertilization is predominantly via outcrossing, with flowers frequently pollinated by small bees, such as *Apis mellifera* and *Trigona* sp. (Vieira et al. 2012), and seeds are wind-dispersed (anemochory) reaching long distances (Scolforo et al. 2012).

3.2 Study site

The study site was located in Itamonte, Southern Minas Gerais state, Brazil. We selected two stands located 300 meters from each other and designated them as managed (22°16’45’’S; 22°16’44.6’’S) and unmanaged (44°46’24’’W; 44°46’25.6’’W). Before harvesting, the managed stand was composed only of *E. erythropappus* individuals. The sampling sites are located inside the Serra da Mantiqueira Environmental Protected Area (APA Serra da

Mantiqueira), a mountain range that reaches 2,798 meters (9180.79 ft) at the top. The control stand was selected considering its proximity, relatedness and structural similarity to the managed stand, and it had no tree exploitation of any kind.

Table 1. Characterization of the managed and unmanaged stands. Mean values of annual rainfall (precipitation); annual temperature; initial predominance of *Eremanthus erythropappus*; density of individuals per hectare; total volume quantified in the managed stand; volume of trees removed after the management.

Stand	Managed	Unmanaged
Latitude	22° 16’ 45’’ S	22° 16’ 44.6’’ W
Longitude	44° 46’ 24’’ S	44° 46’ 25.6’’ W
Area (hectare)	2,44	-
Predominance (%)	100	100
Altitude (meters)	1750	1738
Precipitation (millimeters)	1700	1700
Temperature (°C)	16	16
Soil Type	Cambisol	Cambisol
Density of individuals	3197	2425
Total volume	165,09	-
Volume explored	99,05	-

Edaphoclimatic classification of the sampling sites revealed a predominance of humic Cambisols, presenting extensive rates of soil compaction with low patterns of organic matter decomposition and little vulnerability to environmental contamination (Curi et al. 2008). The climate is described as a temperate humid climate (Cwa) with dry winters and hot summers (Köppen and Geiger, 1928). The mean precipitation and temperature per year are approximately 1,700 millimeters and 16°C, respectively. The forest management in this area occurred in 2009 and extracted over 1,918 adult individuals, leaving the number of remaining individuals at approximately 3,197 trees ha^{-1}. The standing timber volume before management was approximately 165.09 m^3 ha^{-1}, with 99.05 m^3 ha^{-1} remaining after management (Table 1). The managed stand received soil scarification immediately after management (circles with 60 cm radius distributed throughout the area). Finally, the regeneration was thinned 40 months after the management to reduce the competition between plants.

3.2 Data collection

The minimum sampling intensity comprised 1.5% of the total area in each stand. Ribeiro (2009), studying the natural regeneration of a managed population of *E. erythropappus*, also employed a silvicultural system of seed trees and found 5,364 individuals per hectare 48 months after the management. Moreover, we systematically arranged four plots of 100 m^2 each (10 m × 10 m) inside the managed and unmanaged stands with at least 50 meters of distance from

each other (Figure s1). We centered each plot on a seed tree to analyze the occurrence of clonal individuals. However, we defined a maximum distance of 5 meters from the original point to maintain the original position between plots (50 m). Afterward, each plot was subdivided into four other subplots (5 m × 5 m) to facilitate the sampling (Figure 1).

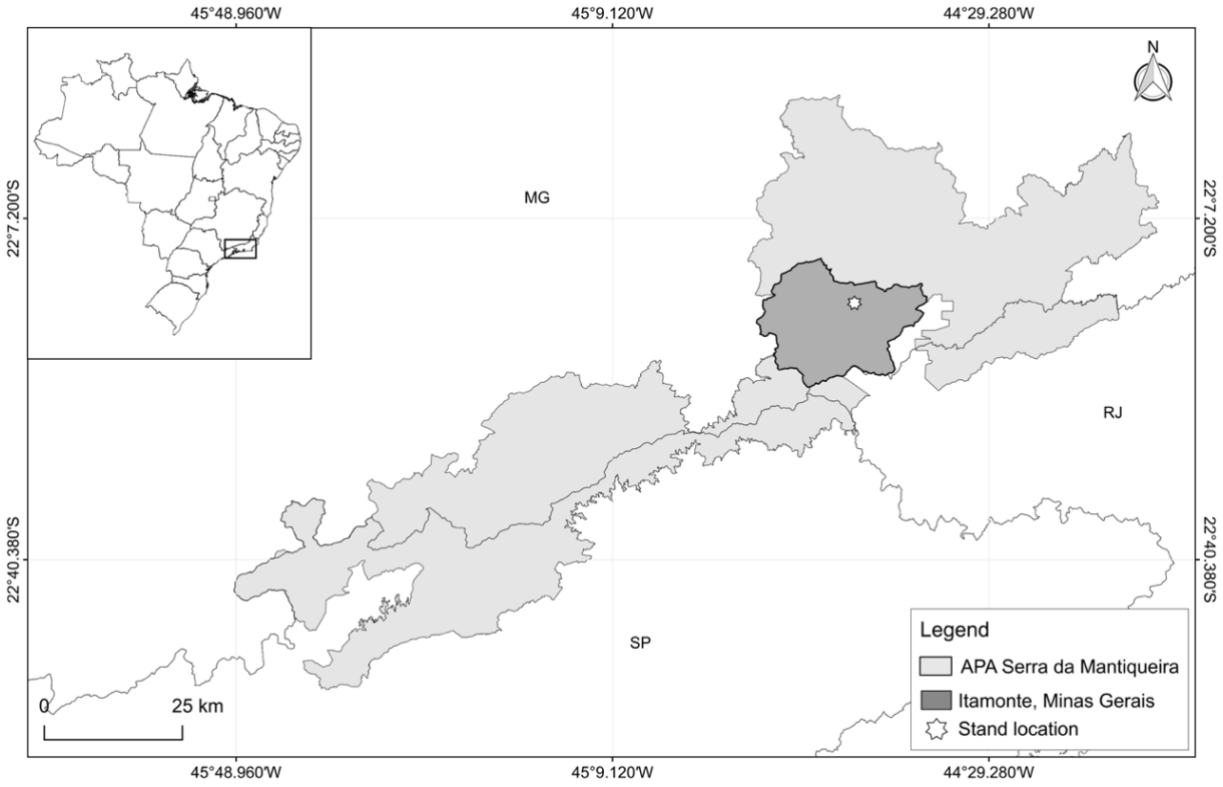

Figure 1. Location of the managed stand in Itamonte, Minas Gerais, Brazil. Squares with dots represent the plot allocation.

The sampling was performed in January 2014, 60 months post-management. We collected leaf tissues from juvenile cohorts and old-growth adult trees (seed trees) found in the managed and unmanaged stands. We classified the sampled individuals as adults and juveniles to analyze the genetic diversity parameters. Tree individuals taller than 1.5 m and presenting more than 5 cm of diameter at the breast height (DBH) were classified as adults. Consequently, those individuals less than 1.5 m in height and 5 cm in DBH were classified as juveniles.

Afterwards, also based on DBH and height, we divided the two previous life stages (adults and juveniles) into four different ontogenetic stages to analyze the SGS as follows: juveniles (seedlings), large juveniles (nonreproductive trees), adults (reproductive adults), and large (reproductive) adults.

3.3 Microsatellite analysis

DNA extraction was carried out using the CTAB protocol (Doyle and Doyle, 1987). After extraction, the DNA was quantified using a NanoVue Plus™ spectrophotometer, and its purity was checked using the A_{260}/A_{280} ratio. The PCR process used noncorrelated microsatellite primers (Ere02, Ere03, Ere04, Ere07, Ere08 and Ere14) developed for *E. erythropappus* (Rocha et al., 2019 - unpublished data) according to specific conditions (Table A5). The total volume of each sample was 15.0 µl: approximately 30 ng of DNA was added to 12.0 µl of reaction mixture (3.33 mM IB Phoneutria buffer (consisting of 100 mM Tris-HCl pH 8.4; 500 mM KCl, 1% Triton X-100, 15 mM $MgCl_2$), 1.5 mM $MgCl_2$, 0.28 mM of each dNTP, 1 U Taq polymerase, 0.22 mM each primer (Forward and Reverse)) and the final volume was achieved with ultrapure water.

The PCR conditions consisted of 3 minutes of initiation at 94°C, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing temperature for 30 seconds, extension at 72°C for 1 minute and a final extension at 72°C for 7 minutes (Table 2). PCR products were subjected to capillary electrophoresis in an ABI 3730 Automatic Sequencer (Life Technologies) with the GS500 LIZ size standard reference.

4. Data analysis

4.1 Genetic diversity

Fragment analysis was scored using GeneMarker 1.9 V (AppliedBiosystems). We analyzed the genetic diversity parameters in the managed and unmanaged stands to obtain the average number of observed (A_O), the average of exclusive alleles (A), the allelic richness (A_R), effective (A_E) and private (A_P) alleles, and the expected (H_E) and observed (H_O) heterozygosities using GenAlEx 6.501 (Peakall and Smouse, 2006, 2012). The frequency of null alleles and genotyping errors were analyzed using Micro-Checker v. 2.2.3. (Van Oosterhout et al. 2004). The inbreeding coefficient (F_{IS}), defined as [1 - (H_O / H_E)], was obtained using FSTAT 2.9.3.2 (Goudet, 2002) with Bonferroni correction for multiple comparisons.

We estimated the differentiation index (F_{ST}) between stands and life stages using ARLEQUIN v 3.5 (Excoffier and Lischer, 2010) to analyze the differences in allele frequencies among stands. Nevertheless, we assumed that adult and regenerating individuals had the same pattern of mating (Nei, 1978) using a distance method and performed an analysis locus-by-

locus with pairwise differences for the polymorphic loci with 1,000 permutations. The gene flow among populations was obtained according to the equation proposed by Crow and Aoki (1984): $N_M = [(1/F_{ST}) - 1] / 4\ \alpha$, where $\alpha = [n / (n - 1)^2]$ and "n" is the number of populations.

4.2 Spatial genetic structure

We firstly implemented the model-based clustering algorithm (Pritchard et al. 2000; Falush et al. 2003) and the empirical statistic *K* to determine the number of subpopulations in each stand for both juvenile and adult life stages using the software STRUCTURE v.2.3.3. We used default model parameters with *K* varying from 1 to 5. Each run consisted of 20 replicates of 250,000 burn-in repetitions and 750,000 data collection iterations. The number of representative populations was identified according to the ΔK method (Evanno et al. 2005), considering the log probability among successive *K* values (ΔK) and the mean of the log-likelihoods of 20 runs of each *K*. We used the STRUCTURE HARVESTER (Earl, 2012) to visualize likelihood parameters across the *K* values and visualize the number of genetic groups that best represent the data.

The old-growth adults and juvenile cohorts in both stands were georeferenced to evaluate the spatial genetic structure (SGS) between life stages. Afterward, we correlated position coordinates with genetic data to create a spatial autocorrelation analysis. The presence of inbreeding was analyzed using the kinship coefficient (Ritland et al. 1996). In addition, we calculated the SGS_{MAX} (Jump et al., 2012), which is defined as the greatest distance at which the mean kinship coefficient (F_d) is significant at $p < 0.05$. We assumed a minimum proportion of 50% for each individual present at least once in each distance class, and the coefficient of variation for the number of times that each individual was expressed in each class was defined as less than or equal to one (Hardy and Vekemans, 2002). SGS was carried out using 10,000 permutations for each life stage on both stands for the full distance range. We plotted the average pairwise estimates of the genetic relatedness as a function of distance to generate spatial autocorrelograms.

The kinship statistic, also known as coancestry, analyzes the probability of identifying two sampled alleles from homologous genes. Particularly, it is the probability of identifying a descending allele by gene comparison. The kinship coefficient is defined as $Fij = (Qij - Qm) / (1- Qm)$, where *Qij* is the probability that the random samples of individuals *i* and *j* are identical by ancestry and *Qm* is the probability that random samples taken in the population are identical

by descent. The kinship coefficient was estimated using SPAGeDi, version 1.1 (Hardy and Vekemans, 2002). The mean standard error was obtained by a resampling jackknife test. The confidence intervals were analyzed using 95% probability for each class of distance.

We also calculated the S_p statistic (Vekemans and Hardy, 2004) to provide a comparison of the SGS between stands. We used the following formula: $-b_F$ / (1 - F_1), where b_F is the regression slope curve of the kinship coefficient (F_{ij}) on distance classes and F_1 is the mean kinship coefficient of the first class of distance (0 - 10 m). Finally, the analysis of molecular variance (AMOVA) among and within stands were also calculated using GenAlEx 6.501 (Peakall and Smouse, 2006)

5. *Results*

Canopy gaps in tropical forests normally induce the development of the newly recolonizing saplings (Brokaw, 1985). As expected, most of the individuals sampled in the managed stand were classified as juveniles (97) and large juveniles (44) as a result of gap-phase regeneration. We also sampled adult (42) and large adult (30) trees, which represent the seed trees that remained post-management (Figure 2). On the other hand, the effects of high canopy volume of adult individuals may make the development of regenerating individuals difficult in species that require a high incidence of sunlight for development. Furthermore, the unmanaged stand showed a lower number of individuals sampled. We found a more evident presence of large adults (26) and adults (26) compared to a low incidence of juveniles (01) and large juveniles (09) as a result of tree shade.

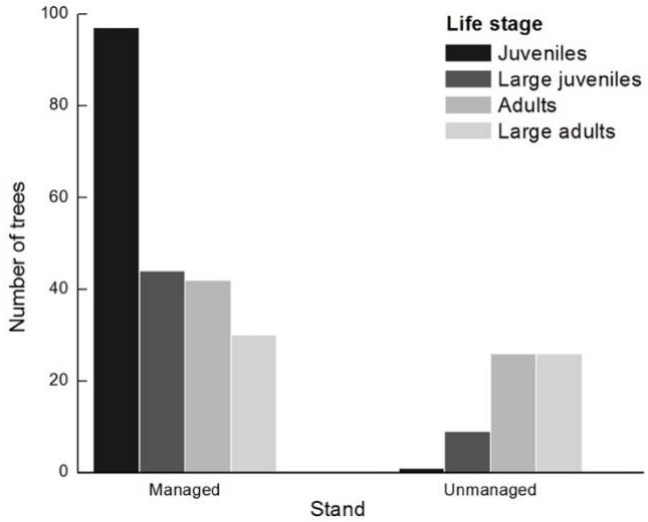

Figure 2. Number of trees sampled for each life stage in the managed and unmanaged stands of *Eremanthus erythropappus*. Different colors represent different life stages for both stands.

5.1 Genetic diversity

Genetic diversity parameters were considerably high but not statistically different between the managed and unmanaged stands (Table 3). Overall, the total number of alleles was 18, considering all six microsatellite loci. The number of alleles per locus (A_O) ranged from 1 to 5, with an average of alleles (A) equal to 2.667 and 2.334 for the managed and unmanaged stands, respectively. Allele richness (A_R) ranged from 1.000 to 4.733 and 1.000 to 4.239 for the managed and unmanaged stands, respectively. The number of effective alleles (A_E) was 1.972 and 1.879 for the managed and unmanaged stands, respectively. We found a tendency for private alleles in the managed stands (A_P = 0.500) and unmanaged stands (A_P = 0.167). Expected heterozygosity (H_E) did not significantly differ between life stages and presented a value of 0.437 for the managed stand for both life stages. For the unmanaged stand, H_E was 0.402 and 0.418 for the juveniles and adults, respectively. The observed heterozygosity (H_O) values reached 0.675 and 0.700 in the managed area and 0.622 and 0.631 in the unmanaged area for the juveniles and adults, respectively (Table 3). F_{IS} did not statistically differ ($p < 0.05$) for managed and unmanaged stands. We did not find any presence of null alleles, linkage disequilibrium, stuttering or large allele dropout. The F_{IS} values ranged -0.588 and -0.540 in the managed stand and -0.506 to -0.503 in the unmanaged stand, both for juvenile and adults, respectively. This pattern indicates an excess of heterozygotes within both stands. Additionally, F_{ST} values revealed low differentiation between the managed and unmanaged stands (F_{ST} = 0.01051; p-value ± 0.0001) and among different life stages for the managed (F_{ST} = 0.00797; p-value = 0.04985 ± 0.00682) and the unmanaged (F_{ST} = 0.00307; p-value = 0.04985 ± 0.00682) stands. The mean number of migrants per generation (N_M) was 5.88, in the managed and unmanaged stands.

5.2 Spatial genetic structure

Bayesian clustering suggested an optimum genetic cluster based on delta K (ΔK) equal to one (K = 1) (Figure A2). The inferred cluster did not show a contrasting pattern among managed and unmanaged stands, indicating an evident genetic similarity and sufficient gene flow among stands. We found a significant fine-scale SGS in the managed and unmanaged stands between life stages. SGS_{MAX} revealed a significant structuring of adult individuals in the unmanaged stand, showing a more intense genetic similarity than the expected range from 16 to 25 m but also less similarity for larger distances ranging from 76 to 83 m (Figure 3a). The

kinship coefficient for the first class of distance (F_1) was statistically significant for this life stage. We found a positive SGS for juvenile individuals ranging from 19 to 27 m and less genetic similarity with an SGS ranging from 115 to 137 m in the managed stand (Figure 3b). Additionally, the kinship coefficient $F_{(1)}$ and the *Sp* statistic also suggested a positive association between SGS and the management of juveniles in the managed stand (Table 2).

Comparing SGS between life stages, we found that *Sp* value was higher for adults in both managed and unmanaged stands. In addition, the $F_{(1)}$ value was higher for the juvenile individuals in the managed stand. On the other hand, juvenile individuals from the unmanaged stand presented a negative value of $F_{(1)}$, indicating the absence of endogamy for this life stage (Table 2). The hierarchical AMOVA also supported the hypothesis of a single genetic cluster (94%, $P < 0.001$), since the component of variance between the two population groups was statistically equal to zero (Table A4).

Table 2. Summary of genetic diversity and SGS estimators for each study site (Managed and Unmanaged) and life stage (adult and juvenile) for *Eremanthus erythropappus*.

		Genetic diversity parameters						Spatial genetic structure parameters		
Stand	Life Stage	*N*	*A*	A_R	H_E	H_O	F_{IS}	$F_{(1)}$	SGS_{MAX}	*Sp*±SE
Managed	Adult	72	2.67	2.81	0.437	0.700	-0.588	0.00481	0	0.0111±0.00472
	Juvenile	141	2.67	2.66	0.437	0.675	-0.540	0.00690*	25	0.0070±0.00354
Unmanaged	Adult	52	2.50	2.46	0.418	0.631	-0.503	0.01467*	21	0.0156±0.00433
	Juvenile	10	2.16	1.96	0.402	0.622	-0.506	-0.03492	0	0.0128±0.02407

N, sample size; *A*, mean number of alleles per locus; *AR*, rarefied allelic richness; *HE*, expected heterozygosity; *Ho*, observed heterozygosity; F_{IS}, inbreeding coefficient. $F_{(1)}$, kinship coefficient for first distance class (0-10 m); SGS_{MAX}, greatest distance at which the kinship coefficient of a given distance class *F(d)* is significant at $p < 0.05$; bF ± SE, regression slope of the kinship coefficient F_{ij} computed among all individuals against geographical distances ± standard error; *Sp* ± SE, *Sp* statistic ± standard error. Significant p-values are indicated as *$p < 0.05$.

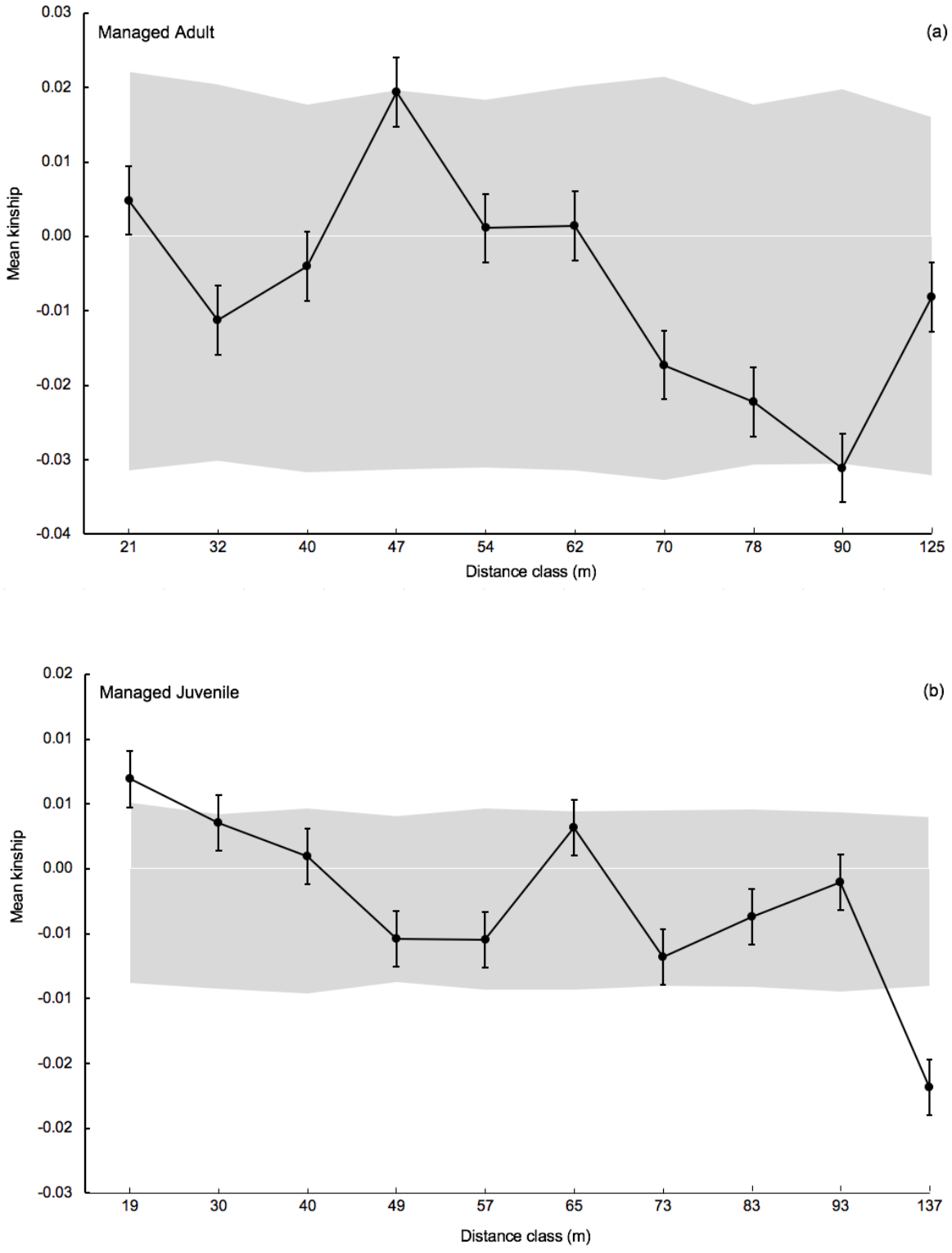

Figure 3. Spatial genetic structure autocorrelograms for *Eremanthus erythropappus* managed stand: Adult (a) and Juvenile (b) based on the kinship coefficient *Fij*, estimated from six microsatellite loci. Shaded areas represent 95% confident intervals obtained from 10,000 permutations of genotypes among locations. Black bars around mean kinship (*Fij*) values represent standard errors derived through jackknifing over loci.

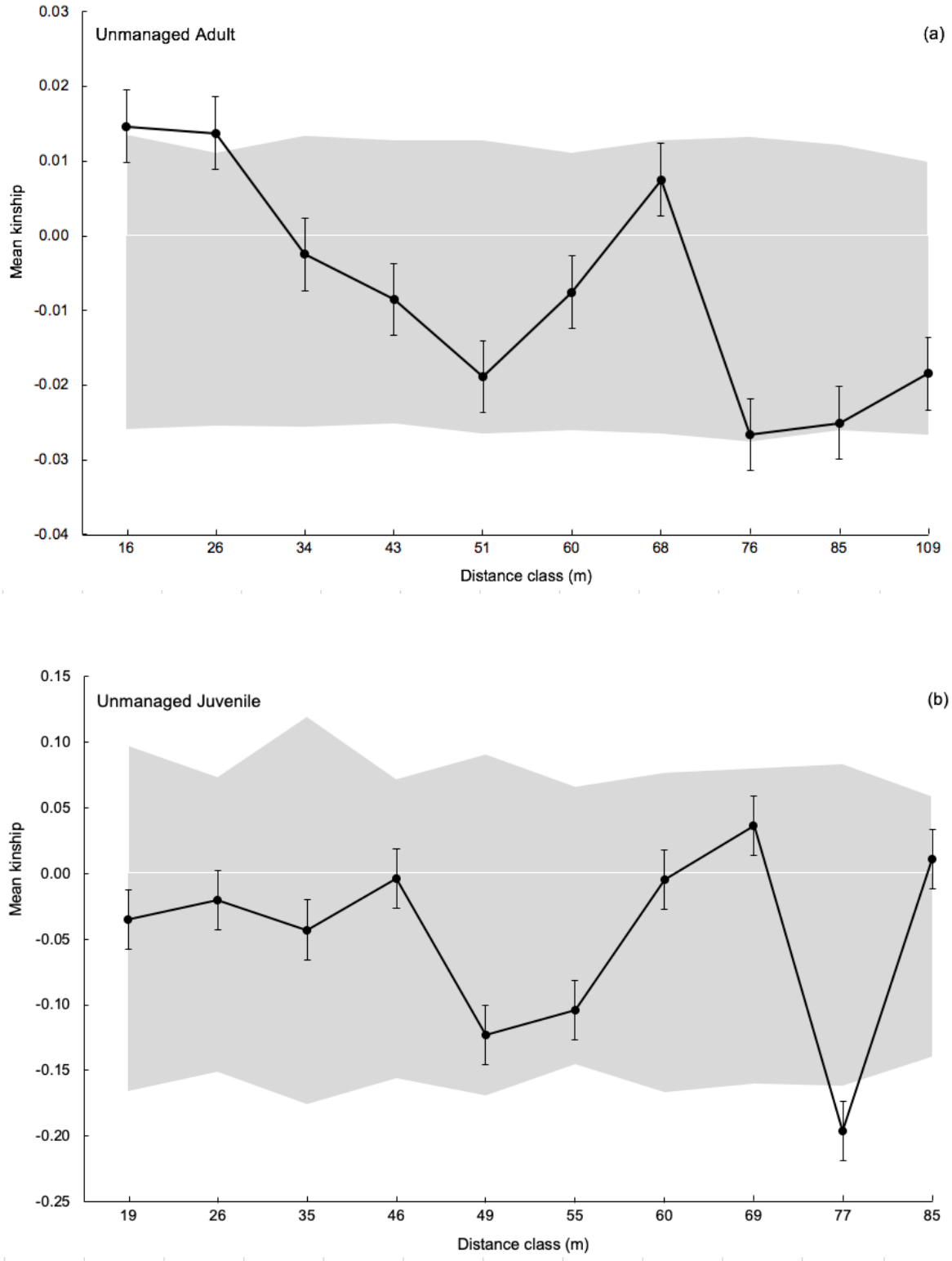

Figure 4. Spatial genetic structure autocorrelograms for *Eremanthus erythropappus* unmanaged stand: Adult (a) and Juvenile (b) based on the kinship coefficient *Fij*, estimated from six microsatellite loci. Shaded areas represent 95% confident intervals obtained from 10,000 permutations of genotypes among locations. Black bars around mean kinship (*Fij*) values represent standard errors derived through jackknifing over loci.

6. Discussion

Herein, we first used microsatellite markers to analyze the effects of forest management on the genetic diversity and SGS of *E. erythropappus*, an overexploited tree species from the Brazilian Savanna and Atlantic Forest. This approach may be very important for exhibiting possible impacts of forest structure alteration on genetic diversity parameters. Although the mean levels of genetic diversity were quite high but not significantly different, we found a positive relationship among SGS and forest management for juvenile cohorts, indicating an effect of forest management on spatial genetic structure. Moreover, we also found a tendency for increasing allelic richness in both life stages in the managed stand. The high patterns of genetic diversity may be an effect of increasing pollen/seed flow among different populations. Further analysis should be performed in different approaches to better understand the complexity of the genetic interactions after management.

6.1 Impact of forest management on genetic diversity parameters

Genetic diversity parameters were generally high, suggesting that the population remnants are enough to inhibit the effects of gene drift resulting of forest management. As expected, we found higher values of expected (H_E = 0.437) and observed (H_O = 0,700 and 0.675) heterozygosity in the managed stand for juveniles and adults, respectively. Regardless, according to Scolforo et al. (2012), *E. erythropappus* intensively produces small seeds that can travel long distances. Therefore, effective seed distribution may intensify gene flow and prevent the effects of genetic drift. In addition, these results may be a response of an intensive incidence of sunlight. The canopy opening that generally intensifies the growth of regenerating individuals of *E. erythropappus*. In addition, the presence of canopy gaps may also allow the migration of seeds from different populations and accelerate the colonization process. The effective gene flow on the managed stand was confirmed by the high number of migrants found (N_M = 5.88). According to Slatkin and Barton (1989), N_M values higher than 1.0 indicate gene flow sufficient for preventing genetic drift.

In general, early successional species exhibit higher genetic divergence among their populations than late-successional species (Nybom and Bartish, 2000; Nybom, 2004). In outcrossing species that predominantly belong to advanced successional stages, a higher genetic divergence is expected within rather than among populations due to intense gene flow. On the other hand, species that present self-fertilization, belonging to initial successional stages or

annual species are expected to exhibit low diversity within populations and high divergence among populations. The longevity of individuals, mating systems, pollination strategies, seed dispersal, and clonal reproduction are the main factors involved in the maintenance and distribution of genetic variation levels within and between populations of plant species (Loveless and Hamrick, 1984).

The main seed dispersal method of *E. eryhtropappus* is anemochory (Scolforo et al. 2012). According to Vieira et al. (2012) this species is mainly pollinated by *Apis mellifera* and *Trigona* sp. (Hymenoptera, Apidae) and presents hermaphroditic flowers with capitula at the end of the branches, a special characteristic of the Asteraceae family. Therefore, the high patterns of genetic diversity patterns may be due to the high production of seeds presented by this species, that maintain genetic cohesion among populations.

Cloutier et al. (2007) used microsatellite markers to analyze the effects of selective logging on genetic diversity in populations of *Carapa guianensis,* a high-density and fast-growing climax species from the Brazilian Amazon. These authors found no evidence of negative effects in the managed stands and overall high levels of pollen flow and similar levels of genetic diversity and allelic richness pre- and post-logging. Similarly, Soliani et al. (2016) also used microsatellite markers to analyze the effect of forest management of *Nothofagus pumilio,* a deciduous tree in the southern Andes. High genetic variation but nonsignificant differences in genetic diversity parameters were found between managed and unmanaged stands. Nonetheless, these authors also found a relatively high genetic heterozygosity, with a slight increase in the values observed for regenerating individuals in the three managed stands (H_O = 0.454; 0.578 and 0.483) but not differing significantly from the other three unmanaged stands (H_O = 0.458; 0.473 and 0.458).

Although forest fragmentation can modify natural patterns of genetic diversity, gene flow through pollen or seed dispersion may reduce the effects of biodiversity loss and prevent gene drift (Couvet, 2002). Barreira et al. (2006) used isoenzyme markers to analyze the effects of forest management in *E. erythropappus* populations. The mating system found was predominantly allogamous, with high rates of multi-locus outcrossing (t_m=0.963). Similarly, forest management also caused a positive but not statistically significant increase in genetic diversity by comparison among the unmanaged (H_O = 0.357; H_E = 0.403) and managed (H_O = 0.423; H_E = 0.425) stands as a result of the increase of outcrossing fertilization among genetically different individuals. According to Hamrick and Godt (1990), tree species with a long cycle, efficient pollen and seed flow, a mixed reproduction system and a wide geographic

distribution may present high levels of genetic diversity. Normally, those high levels of genetic diversity in tree species are due to a combination of many factors, such as widespread pollination by animals, high out-crossing rates and complex breeding systems, allowing their persistence even if their populations undergo an abrupt decline (Cascante et al. 2002; Hamrick et al. 1992).

Genetic diversity parameters within tropical tree species may be influenced by different characteristics, such as tree size, longevity, fecundity, breeding systems and geographic distribution (Hamrick and Godt, 1990). We found a relatively low number of alleles (N = 18) when compared with different microsatellite analyses for tropical tree species, indicating a low polymorphism rate and high genetic similarity among the six microsatellite loci. In fact, both stands analyzed in this study are located at a high elevation mountain range, and therefore, this species could be influenced by topographic isolation as a response of the restricted geographic distribution. Furthermore, Castilla et al. (2015) found a positive influence caused by elevation in *Miconia affinis*, a pioneer tropical tree species from the high mountains of South America, where geographic distance significantly increased genetic differentiation between populations.

Considering that the six microsatellite loci were distributed throughout the genome of *E. erythropappus*, genetic interactions revealed by our analysis within the investigated population are representative and very important for the genetic conservation of *E. erythropappus*. Similarly, Rocha et al. (2019) used RFLP markers to analyze the chloroplast diversity and phylogeographic patterns of two *Eremanthus* species (*E. erythropappus* and *E. incanus*) since the Quaternary climate (last glacial maximum ~ 21 kyr BP and Mid-Holocene ~ 6 kyr BP). These authors found a total of three haplotypes shared by both species with relatively low intensity and total genetic diversity, indicating that these species may be very susceptible to habitat fragmentation and future climate change.

The relatively low allelic richness may be an intrinsic characteristic of the analyzed species. Additionally, the isolation by distance may also cause the occurrence of a low number of alleles in segregated populations (Wright, 1943; 1946). In addition, considering that *E. erythropappus* is an endemic tree species that occurs in isolated mountains, even with the high seed and pollen dispersal, the species may not be able to overcome the gaps, isolating effectively the populations. Furthermore, even when there is little exchange of migrants or alleles, gene flow is able to contribute to the cohesion among populations (Wright, 1931; Morjan and Rieseberg, 2004). Nevertheless, further analyses should integrate landscape genetics to analyze possible patterns of isolation among nearby populations, considering that

possible effects of forest management practices in isolated populations may induce evolutionary pressure.

The impacts of forest management on tree genetic diversity may differ even when adopting the same type or intensity of harvesting. Therefore, similar silvicultural harvesting has different genetic effects among and within populations. The main factors influencing different responses in forest management are biological and ecological attributes intrinsic to each species and population (Ratnan et al. 2014). In general, we found very similar genetic diversity parameters between stands. Nevertheless, the averages of genetic diversity parameters found in our study were considerably higher than those found in other studies for *E. erythropappus* populations (Estopa et al. 2006; Pádua et al. 2016; Freitas et al. 2008). This pattern may be related to the higher levels of polymorphism normally found in microsatellite markers (Varshney et al. 2005). The high genetic diversity may be inherent to intrinsic ecological characteristics, such as natural occurrence in environments presenting unfavorable conditions, which normally hinder the development of interspecific competition. According to Kisdi and Geritz (1999), populations adapted to extreme conditions may have a higher probability of incorporating new alleles by mutation than small populations from homogeneous environments.

Furthermore, the overall number of alleles per locus (*A*) and rarefied allelic richness (A_R) were not significantly different in the managed stand. However, adult individuals from the managed stand showed a tendency of higher (A_R = 0.500) than juvenile individuals (A_R = 0.167). Although forest management may lead to a range of losses in the total number of alleles in tropical trees (Carneiro et al. 2011, Silva et al. 2008; Vinson, 2009), surrounding populations may mitigate the effects of forest harvesting by the reintroduction of the lost alleles from regenerating individuals from surrounding populations. Barreira et al. (2006) found a number of effective alleles per locus (*A*) ranging from 1.67 to 1.93. Nevertheless, Pádua et al. (2006) used ISSR markers in natural populations of *E. erythropappus* in Minas Gerais state and found the number of alleles per locus ranging from 1.46 to 1.68.

Finally, the observed heterozygosity presented higher levels compared to the expected heterozygosity, suggesting an excess of heterozygotes according to the Hardy-Weinberg equilibrium. The maintenance and even a slight increase in natural levels of genetic diversity in the managed stand of *E. erythropappus* indicates that this species presents efficient systems of gene flow and seed dispersion to maintain the levels of natural genetic diversity when subjected to this silvicultural intervention. Furthermore, endemic and rare plant species may have developed strategies that allow them to survive and promote their perpetuation even under

self-fertilization, low genetic diversity and high inbreeding rates (Oostermeijer et al. 1994; Lande and Schemske, 1985).

Long-lived and outcrossing species present most of the genetic variability within populations (Hamrick and Godt, 1996; Loveless and Hamrich, 1987; Nybom, 2004). In fact, several studies have found a likelihood for high genetic variation within the populations in tropical outbreeding tree species (Brito et al. 2016; Dias et al. 2017; Duarte et al. 2015). AMOVA results showed that most of the genetic variance is retained within populations (96%) (Table 4). In accordance, the low differentiation indexes (F_{ST}) found corroborate to the hypothesis of high gene flow among the stands. These results confirm the theory of effective gene flow among populations because the genetic divergence is considerably low (6%) among stands. Moura (2005), studying the genetic diversity of *E. erythropappus* populations using isoenzyme and RAPD markers, found a similar pattern of variation (96.5%) within populations. Additionally, Castilla et al. (2015) used highly polymorphic microsatellite markers to study the genetic diversity parameters of *Miconia affinis*. Nevertheless, these authors also found a low molecular variance (8%) among populations. Therefore, molecular variance analysis indicates that pollen and seed flow are effective at reducing genetic relatedness between *E. erythropappus* stands and consequently prevents genetic drift. Although forest management can often reduce gene flow (Ratnam et al. 2014), moderate and relatively low pollen or seed flows between disrupted populations can significantly mitigate the negative effects on the genetic diversity of trees and prevent genetic drift (Aguilar et al. 2008; Couvet, 2002). Because both stands are located only 300 meters apart from each other, our results indicate that pollen and seed flow among the stands is sufficient to maintain the genetic diversity at this point.

6.2 Impacts of forest management on spatial genetic structure

Tropical forests commonly present contrasting patterns of regeneration based on the sources available at the site, such as the seed bank, stem and root sprouts and remnant vegetation, and on the colonization process from seeds outside the site (Guariguata and Ostertag, 2001). Our results from Bayesian clustering confirm that the managed and unmanaged stands are composed of a single genetic population, presenting a single genetic cluster structure (K = 1). These results confirm that the managed and unmanaged stands are controlled by intense pollen and/or seed flow among stands and life stages. Consequently, the influences of forest

thinning were not able to distinguish the stands analyzed as two genetic populations due to the relatively small distance between them.

On the other hand, we detected an influence on the SGS of the managed stand, indicating the presence of coancestry in the first class of distance for juvenile individuals (SGS_{MAX} = 25 m; 100 m) from the managed stand and for adult individuals (SGS_{MAX} = 21 m) from the unmanaged stand. These results indicate the presence of more genetically similar individuals in the first class of distance. We did not find a pattern of SGS (except for some scatter) for the other classes, suggesting that individuals may have a random genetic distribution. Similarly, *Sp* values were similar to those of previous studies for outcrossing, wind-dispersed tropical tree species (Vekemans and Hardy, 2004).

The positive SGS for juvenile individuals as an impact of forest management is confirmed by the lowest value of *Sp* (0.0070) and by a relatively high and statistically significant value of $F_{(1)}$ (0.00690; $p < 0.05$), indicating that the juveniles are less structured than adults. Additionally, the SGS for adult individuals from the managed stand was also confirmed by $F_{(1)}$ (0.01467), which was the highest and was statistically significant. In addition, the *Sp* value for adult individuals in the managed stand was the highest (0.0156), indicating a more structured SGS. Generally, *Sp* values were slightly higher in the unmanaged stand. We found an increase in *Sp* values from juveniles to adults within both stands, which means that distant pairs of juvenile individuals from both stands are more genetically similar that they used to be by chance. The absence of any effect on SGS for adult trees in the unmanaged stand may be related to the old growth of the seed trees sampled, which might not be genetically related.

Barreira et al. (2006) also found an effect of forest management on the SGS of *E. erythropappus* within 180 m of distance. Positive results for coancestry may be a result of vegetative propagation but also a direct effect of inefficient seed dispersion and the regeneration near the mother trees, creating a nonrandom distribution of alleles and genotypes among populations (Loveless and Hamrick, 1984). The magnitude of gene flow and seed dispersal are the main factors responsible for determining the extent of gene dispersion in tropical rain forests (Dick et al. 2008). According to Melo et al. (2012), *E. erythropappus* tends to sprout from roots and stems after harvest of adult trees, which may induce the growth of clonal individuals. Because the stands analyzed here appeared to have efficient patterns of gene flow, confirmed by the similar levels of genetic diversity between populations, vegetative propagation may be a trend influencing the SGS of *E. erythropappus*. Pádua et al. (2016) used inter simple sequence repeat markers (ISSR) in nine populations of *E. erythropappus* throughout Minas Gerais state.

In addition, these authors also identified an SGS for reproductive trees. Moreover, five populations showed a random spatial distribution of the genotypes, and another four populations had significant genetic structuring ($p > 0.05$), indicating a positive and significant coancestry.

The effects of forest logging have been found on the SGS of several tropical tree species. Lacerda et al. (2008) used microsatellite markers to analyze the effects of forest management at small classes of distance of *Hymenaea courbaril*, a tree species from the Brazilian Amazon. In addition, these authors found that forest management reduced the SGS from approximately 800 to 200 m. Similarly, Azevedo et al. (2007) used seven microsatellite loci aiming to analyze the genetic parameters of a heavily logged and endangered tree species *Manilkara huberi*. These authors found SGS of 300 m caused mainly due to limited seed and pollen flow. González-Díaz et al. (2017), analyzing the effects of forest management on the genetic parameters of *Pinus sylvestris* L. found a positive impact in the spatial genetic structure of regenerating individuals in managed stands. Furthermore, for *E. erythropappus*, juvenile individuals were less spatially structured than adults in the managed stands. Although *E. erythropappus* is native to tropical climates, our results also showed that forest harvesting might have influenced the spatial genetic structure.

Genetic structuring may be an induced response to changes in seed dispersion methods and the pioneering behavior of regenerating individuals. According to Aldrich et al. (1998), the intrinsic conditions of a population can promote changes in the type and abundance of seed dispersers that can affect dispersal distances and the probability of the establishment of maternal families and their spatial distribution within a population. Although there was no evident effect on genetic diversity, we emphasize that the managed stand was relatively recently exploited, and there was not enough time to perform a complete analysis. Therefore, the effects of forest management on the genetic structure may be more evident in the subsequent generations.

7. Conclusions

We carried out a comparison between a managed stand using a silvicultural system of seed trees and an unmanaged stand, 300 meters distant from each other at the Serra da Mantiqueira Environmental Protected Area (APA Serra da Mantiqueira), Itamonte, Minas Gerais State, Brazil. Although old-growth adults and juvenile cohorts from both stands presented low allelic richness, overall, we found high levels of genetic diversity. Furthermore, both life stages from

the managed stand showed a not statistically significant increase in genetic variability. In addition, outcrossing tree species normally present a high genetic diversity that can be maintained by long-distance pollen flow in fragmented landscapes. Previous studies of *E. erythropappus* populations found high patterns of genetic diversity (Estopa et al. 2006; Mori et al. 2009; Carvalho et al. 2012; Moura, 2005), and genetic variability may not be influenced by forest management (Barreira et al. 2006). Nevertheless, our results indicate that forest management using a silvicultural system of seed trees may have affected the fine-scale spatial genetic structure in juveniles from the managed stand and may increase the genetic relatedness of individuals in the long term.

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9. References

Ackzell, L., 1993. A comparison of planting, sowing and natural regeneration for *Pinus sylvestris* (L.) in boreal Sweden. For Ecol Manage. 61, 229-245. doi: https://doi.org/10.1016/0378-1127(93)90204-Z

Aguilar, R., Quesada, M., Ashworth, L., Herrerias-Diego, Y.V.O.N.N.E., Lobo, J., 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Mol Ecol, 17(24), 5177-5188. doi: 10.1111/j.1365-294X.2008.03971.x.

Aldrich, P.R., Hamrick, J.L., Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. 1998. Science 281.5373, 103-105. doi: 10.1126/science.281.5373.103

Andrade, I.S., 2009. Avaliação técnica e econômica de sistemas de manejo de candeais nativos. 2009. Unpublished D. Phil. Thesis, Universidade Federal de Lavras: Lavras. Brazil.

Aravanopoulos, F., Do Silviculture and Forest Management Affect the Genetic Diversity and Structure of Long-Impacted Forest Tree Populations? 2018. Forests 9.6, 355. doi: doi.org/10.3390/f9060355

Avise, J.C., Hamrick, J.L., Conservation genetics, case histories from nature**.** Chapman & Hall, 1996. 512 p.

Azevedo, V.C.R., Kanashiro, M., Ciampi, A.Y., Grattapaglia, D., 2007. Genetic structure and mating system of *Manilkara huberi* (Ducke) A. Chev., a heavily logged Amazonian timber species. J. Hered, 98, 646-654. doi:10.1093/jhered/esm074

Barreira, S., Sebbenn, A. M., Scolforo, J. R. S., Kageyama, P. Y. 2006. Diversidade genética e sistema de reprodução em população nativa de *Eremanthus erythropappus* (DC.) MacLeish sob exploração. Sci For, 71, 119-130.

Bergeron, Y., Irulappa Pillai Vijayakumar, D. B., Ouzennou, H., Raulier, F., Leduc, A., Gauthier, S. 2017. Projections of future forest age class structure under the influence of fire and harvesting: implications for forest management in the boreal forest of eastern Canada. For: Int J For Research, 90(4), 485-495. doi:10.1093/forestry/cpx022

Braga, E.A., Substratos e fertilização na produção de mudas de candeia *Eremanthus erythropappus* (DC.) McLeisch, em tubetes**.** 2006. 77 p. Dissertação (Mestrado em Engenharia Florestal) - Universidade Federal de Lavras, 2006.

Brown, A.D., Kappelle, M. Introdducción a los bosques nublados del neotrópico: una síntesis regional. In: Kappell, M., Brown, A.D., (Ed.). Bosques nublados del neotrópico. Santo Domingo de Heredia: Instituto Nacional de Biodiversidad, p. 25-40, 2001

Brokaw, N.V. 1985. Gap-phase regeneration in a tropical forest. Ecology, 66(3), 682-687. doi:10.2307/1940529

Brito, V.L., Mori, G.M., Vigna, B.B., Azevedo-Silva, M., Souza, A.P. and Sazima, M., 2016. Genetic structure and diversity of populations of polyploid *Tibouchina pulchra* Cogn.

(Melastomataceae) under different environmental conditions in extremes of an elevational gradient. Tree Genet Genomes, 12(6), 101. doi:10.1007/s11295-016-1059-y

Borrell, J.S., Wang, N., Nichols, R. A., Buggs, R. J. 2018. Genetic diversity maintained among fragmented populations of a tree undergoing range contraction. Heredity, 121(4), 304. doi:10.1038/s41437-018-0132-8

Camolesi, J.F., 2007. Volumetria e teor Alfa Bisabolol para a candeia *Eremanthus erythropappus*. Unpublished Msc Thesis, Universidade Federal de Lavras, Lavras, Brazil.

Carneiro, F.S., Lacerda, A.E.B., Leme, M.R., Gribel, R., Kanashiro, M., Wadt, L.H.O., Sebbenn, A.M., 2011. Effects of selective logging on the mating system and pollen dispersal of *Hymenaea courbaril* L. (leguminosae) in the Eastern Amazon as revealed by microsatellite analysis. For. Ecol. Manage. 262, 1758–1765. doi:10.1016/j.foreco.2011.07.023

Cascante, A., Quesada, M., Lobo, J.J., Fuchs, E.A. 2002. Effects of dry tropical forest fragmentation on the reproductive success and genetic structure of the tree *Samanea saman*. Conserv Biol, 16(1), 137-147. doi:10.1046/j.1523-1739.2002.00317.x

Castilla, A.R., Pope, N., Jha, S. 2015. Positive density-dependent reproduction regulated by local kinship and size in an understory tropical tree. Ann Bot, 117(2), 319-329. doi:10.1093/aob/mcv170

Cavers, S., Degen, B., Caron, H., Lemes, M. R., Margis, R., Salgueiro, F., Lowe, A.J., 2005. Optimal sampling strategy for estimation of spatial genetic structure in tree populations. Heredity, 95(4), 281-289. doi:10.1038/sj.hdy.6800709

Cavers, S., Cottrell, J.E., The basis of resilience in forest tree species and its use in adaptive forest management in Britain. 2014. For: Int J For Res, 88(1), 13-26. doi:10.1093/forestry/cpu027

Chazdon, R.L., 2014. Second growth: the promise of tropical forest regeneration in an age of deforestation. University of Chicago Press.

Cloutier, D., Kanashiro, M., Ciampi, A.Y., Schoen, D.J., 2007. Impacts of selective logging on inbreeding and gene dispersal in an Amazonian tree population of *Carapa guianensis* Aubl. Mol Ecol, 16, 797-809. doi:10.1111/j.1365-294x.2006.03193.x

Crnokrak, P., Roff, D.A., 1999. Inbreeding depression in the wild. Heredity, 83, 260-270.

Crow J.F., Aoki K. 1984. Group selection for a polygenic behavioral trait: estimating the degree of population subdivision. Proc Natl Acad Sci, 81(19), 6073-7. doi: 10.1073/pnas.81.19.6073

Couvet, D., 2002. Deleterious effects of restricted gene flow in fragmented populations. Conserv. Biol, 16(2), 369-376. doi: doi:10.1046/j.1523-1739.2002.99518.x

Cruz, E. S., 2006. Dinâmica, modelagem do crescimento e cadeia de comercialização da candeia *Eremanthus incanus* (Less.) Less. Unpublished D. Phil. Thesis, Universidade Federal de Lavras, Lavras, Brazil.

Curi, N., Marques, J.J.G., Marques, A.F.S.M., Fernandes, E.I., 2008. Solos, relevo, geologia e mineração, in: Scolforo, J.R., Carvalho, L.M.T., Oliveira, A.D., (Eds.) Zoneamento Ecológico Econômico de Minas Gerais: componentes geofísico e biótico. Editora UFLA, Lavras, pp. 73-88.

Dias, A.C.C., Serra, A.C., Sampaio, D.S., Borba, E.L., Bonetti, A.M., Oliveira, P.E., 2017. Unexpectedly high genetic diversity and divergence among populations of the apomictic Neotropical tree *Miconia albicans*. Plant Biol, 20(2), 244-251. doi:10.1111/plb.12654

Dick, C.W., Hardy, O.J., Jones, F.A., Petit, R.J. 2008. Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. Trop. Plant Biol, 1(1), 20-33. doi:10.1007/s12042-007-9006-6

Dixo, M., Metzger, J.P., Morgante, J.S., Zamudio, K.R., 2009. Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal. Forest Biol Conserv, 142(8), 1560-1569. doi:10.1016/j.biocon.2008.11.016

Doyle, J. J., Doyle, J.L. 1987. Isolation of plant DNA from fresh tissue. Geogr Genet, 12(1), 13-15.

Duarte, J. F., Carvalho, D., Vieira, F.A., 2015. Genetic conservation of *Ficus bonijesulapensis* in a dry forest on limestone outcrops. Biochem Syst Ecol, 59, 54-62. doi:10.1016/j.bse.2015.01.008

Earl, D.A., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour, 4(2), 359-361.

El-Kassaby, Y.A., Dunsworth, B.G., Krakowski, J., 2003. Genetic evaluation of alternative silvicultural systems in coastal montane forests: western hemlock and amabilis fir. Theor Appl Genet, 107, 598-610. doi: doi:10.1007/s00122-003-1291-3

Estopa, R.A., Souza, A.D., Moura, M.C.O., Botrel, M.C.G., Mendonça, E.G., Carvalho, D. 2006. Diversidade genética em populações naturais de candeia (*Eremanthus erythropappus* (DC.) MacLeish). Sci Forestalis, 70, 97-106.

Excoffier, L., Lischer, H.E., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour, 10(3). 564-567.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol, 14, 2611-2620. doi:10.1111/j.1365-294x.2005.02553.x

Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics. 164, 1567-1587.

Frankham, R., Ballou, J., Briscoe, D., 2002. An Introduction to Conservation Genetics. Cambridge University Press, Cambridge, UK.

Freitas, V.L.O., Lemos-Filho, J.P., Lovato, M.B. 2008. Contrasting genetic diversity and differentiation of populations of two successional stages in a neotropical pioneer tree (*Eremanthus erythropappus*, Asteraceae). Genet Mol Res, 7(2), 388-398. doi:10.4238/vol7-2gmr429

Folke, C., 2006. Resilience: The emergence of a perspective for social–ecological systems analyses. Global Environ Chang, 16(3), 253-267. doi:10.1016/j.gloenvcha.2006.02.003

García-Gil, M.R., Floran, V., Östlund, L., Gull, B.A. 2015, Genetic diversity and inbreeding in natural and managed populations of Scots pine. Tree Genet Genomes, 11(2), 1-12. doi:10.1007/s11295-015-0850-5

Ghanzoul, J., 2005. Pollen and seed dispersal among dispersed plants. Biol Rev, 80, 413-443. doi:10.1017/S1464793105006731

González-Díaz, P., Jump, A.S., Perry, A., Wachowiak, W., Lapshina, E., Cavers, S., 2017. Ecology and management history drive spatial genetic structure in Scots pine. For Ecol Manag, 400, 68-76. doi:10.1016/j.foreco.2017.05.035

Goudet, J., 2002. Fstat Vision (1.2) A Computer Program to Calculate F-Statistics. J Hered, 86, 485-486.

Guariguata, M.R., Ostertag, R. 2001. Neotropical secondary forest succession: changes in structural and functional characteristics. For Ecol Manag, 148(1-3), 185-206. doi:10.1016/S0378-1127(00)00535-1

Hamrick, J.L., Godt, M.J.W., 1990. Allozyme diversity in plant species, in: Brown, A.H. D., Clegg, M.T., Kahler, A.L., Weir, B. S. (Eds.), Plant Population Genetics, Breeding and Genetic Resources. Sinauer., Sunderland, pp. 44-64.

Hamrick J.L., Godt M.J.W., 1996. Effects of life history traits on genetic diversity in plant species. Philos. Trans. R. Soc. Lond. B Biol Sci, 351, 1291-1298. doi:10.1098/rstb.1996.0112

Hamrick, J.L., Godt, J.W.M. Sherman-Broyles, S.L., 1992. Factors influencing levels of genetic diversity in woody plant species. Popul Genet Forest Trees, 95-124. doi:10.1007/BF00120641

Hardy, O.J., Vekemans, X., 2002. SPAGeDi: a versatile computer program to analyze spatial genetic structure at the individual or population levels. Mol Ecol Notes, 2, 618-620.

Hawley, G.J., Schaberg, P.G., DeHayes, D.H., Brissette, J.C., 2005. Silviculture alters the genetic structure of an eastern hemlock forest in Maine. USA. Can J For Res, 35, 143–150. doi:10.1139/x04-148

Jump, A.S., Rico, L., Coll, M., Penuelas, J., 2012. Wide variation in spatial genetic structure between natural populations of the European beech (*Fagus sylvatica*) and its implications for SGS comparability. Heredity, 108(6), 633-639. doi:10.1038/hdy.2012.1.

Kisdi, E., Stefan, A.H.G., 1999. Adaptive dynamics in allele space: evolution of genetic polymorphism by small mutations in a heterogeneous environment. Evolution, 53(4), 993-1008. doi: 10.1111/j.1558-5646.1999.tb04515.x.

Koppen, W., Geiger, R., 1928. Klimate der Erde. Gotha: Verlag Justus Perthes. Wall-map 150cm x 200cm.

Lacerda, A.E.B., Kanashiro, M., Sebbenn, A.M., 2008. Effects of reduced impact logging on genetic diversity and spatial genetic structure of a *Hymenaea courbaril* population in the Brazilian Amazon Forest. For Ecol Manage, 255, 1034-1043. doi: 10.1016/j.foreco.2007.10.009

Lande, R., Schemske, D.W., 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution, 39(1), 24-40. doi: 10.1111/j.1558-5646.1985.tb04077.x.

Lee, C.T., Wickneswari, R., Mahani, M.C., Zakri, A.H., 2002. Maintenance of genetic diversity in Parkia speciosa in logged-over forests. J Trop For Sci, 14, 163-178.

Loiselle, B. A., Sork, V. L., Nason, J., Graham, C., 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). Am J Bot, 82(11), 1420-1425. doi:10.2307/2445869

Loveless, M.D., Hamrick, J.L., 1987. Distribuicion de la variacion en especies de arboles tropicales. Rev Biol Trop, 35(1), 165-175.

Loveless, M.D., Hamrick, J.L., 1984. Ecological determinants of genetic-structure in plant populations. Annual Rev Ecol Syst, 15, 65-95. doi:10.1146/annurev.es.15.110184.000433

Lowe, A.J., Cavers, S., Boshier, D., Breed, M.F., Hollingsworth, P.M., 2015. The resilience of forest fragmentation genetics no longer a paradox, we were just looking in the wrong place. Heredity. 115, 97-99. doi:10.1038/hdy.2015.86

Martins, K., Kimura, R.K., Francisconi, A.F., Gezan, S., Kainer, K., Chris- tianini, A.V., 2016. The role of very small fragments in conserving genetic diversity of a common tree in a hyper fragmented Brazilian Atlantic forest landscape. Conserv Genet, 17, 509-520. doi: 10.1007/s10592-015-0800-7

Melo, L.A., Davide, A.C., Teixeira, L.A.T., 2012. Metodologia para resgate de matrizes e enraizamento de estacas de *Eremanthus erythropappus*. Cerne. 18(4), 631-638. doi: 10.1590/S0104-77602012000400013.

Moura, M.C.O., 2005. Distribuição da variabilidade genética em populações naturais de *Eremanthus erythropappus* (DC) MacLeish por isoenzimas e RAPD. 2005. Unpublished D. Phil Thesis, Universidade Federal de Lavras, Lavras, Brazil.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89(3), 583-590.

Nybom, H., Bartish, I.V., 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspect Plant Ecol Syst, 3(2), 93-114. doi: 10.1078/1433-8319-00006

Nybom, H., 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol, 13, 1143-1155. doi: 10.1111/j.1365-294X.2004.02141.x

Morjan, C.L., Rieseberg, L.H., 2004. How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. Mol Ecol, 13, 1341-1356. doi: 10.1111/j.1365-294X.2004.02164.x

Oliveira, A. D. D., Silva Andrade Ribeiro, I., Soares Scolforo, J. R., Mello, J. M. D., Acerbi Junior, F. W., Camolesi, J. F., 2009. Market chain analysis of candeia timber (*Eremanthus erythropappus*). Cerne, 15(3), 257-264.

Oostermeijer, J.G.B., Van Eijck, M.W., Den Nijs, H.C.M., 1994. Offspring fitness in relation to population size and genetic variation in the rare perennial plant species *Gentiana pneumonanthe* (Gentianaceae). Oecologia, 97, 289-296. doi:10.1007/BF00317317

Ortego, J., Bonal, R., Muñoz, A., 2010. Genetic consequences of habitat fragmentation in long-lived tree species: The case of the mediterranean holm oak (*Quercus ilex*, L.). J. Hered. 101(6) 717–726. doi: 10.1093/jhered/esq081

Pádua, J.A.R., Brandão, M.M., Carvalho, D., 2016. Spatial genetic structure in natural populations of the overexploited tree *Eremanthus erythropappus* (DC.) MacLeish (Asteraceae). Biochem. Syst. Ecol. 66, 307-311. doi: 10.1016/j.bse.2016.04.015

Peakall, R.O.D., Smouse, P.E., 2006. "GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes, 6(1), 288-295.

Petrokofsky, G., Sist, P., Blanc, L., Doucet, J.L., Finegan, B., Gourlet-Fleury, S., Healey, J.R., Livoreil, B., Nasi, R., Peña-Claros, M., Putz, F.E. 2015. Comparative effectiveness of silvicultural interventions for increasing timber production and sustaining conservation values in natural tropical production forests. A systematic review protocol. Environ Evid. 4(8). doi: 10.1186/s13750-015-0034-7

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics, 155, 945–959. doi: 10.1111/j.1471-8286.2007.01758.x

Quéméré, E., Crouau-Roy, B., Rabarivola, C.; Louis JR, E.E., Chikhi, L. 2010. Landscape genetics of an endangered lemur (*Propithecus tattersalli*) within its entire fragmented range. Conserv Genet, 19, 1606-1621. doi: 10.1111/j.1365-294X.2010.04581.x

Rajora, O.P., S.A. Pluhar. 2003. Genetic diversity impacts of forest fires, forest harvesting, and alternative reforestation practices in black spruce (*Picea mariana*). Theor Appl Genet, 106(7), 1203-1212. doi:10.1007/s00122-002-1169-9

Rajora, O., Rahman, M., Buchert, G., Dancik, B., 2000. Microsatellite DNA analysis of genetic effects of harvesting in old-growth eastern white pine (*Pinus strobus*) in Ontario, Canada. Mol Ecol, 9, 339–348. doi:10.1046/j.1365-294x.2000.00886.x

Ratnam, W., Rajora, O. P., Finkeldey, R., Aravanopoulos, F., Bouvet, J. M., Vaillancourt, R. E., ..., Vinson, C. 2014. Genetic effects of forest management practices: global synthesis and perspectives. For Ecol Manag, 333, 52-65. doi: 10.1016/j.foreco.2014.06.008

Ritland, K. 1996. Estimators for pairwise relatedness and individual inbreeding coefficients. Genet Res, 67(2), 175-185. doi:10.1017/S0016672300033620

Ribeiro, I.S.A., 2009. Avaliação técnica e econômica de sistemas de manejo de candeais nativos. Unpublished D. Phil Thesis, Universidade Federal de Lavras, Lavras, Brazil.

Robledo-Arnuncio, J.J., Alia, R., Gil, L., 2004. Increased selfing and correlated paternity in a small population of a predominantly outcrossing conifer, *Pinus sylvestris*. Mol Ecol, 13(9), 2567–2577. doi: 10.1111/j.1365-294X.2004.02251.x

Rocha, L.F., Carmo, I.E.P., Póvoa, J.R.S., Carvalho, D., (in press), 2019. Effects of climate change on the distribution of two endemic tree species in Brazil: Phylogeography of *Eremanthus erythropappus* and *Eremanthus incanus* (Asteraceae). J. Forest Res.

Santos, K. A., Frohlich, P.C., Hoscheid, J., Tiuman, T.S., Gonçalves, J.E., Cardozo-Filho, L., Silva, E.A., 2017. Candeia (*Eremanthus erythroppapus*) oil extraction using supercritical CO_2 with ethanol and ethyl acetate cosolvents. J Supercrit Fluids, 128, 323-330. doi: 10.1016/j.supflu.2017.03.029

Schaberg, P.G., DeHayes, D.H., Hawley, G.J., Nijensohn, S.E. 2008. Anthropogenic alterations of genetic diversity within tree populations: Implications for forest ecosystem resilience. For Ecol Manag, 256(5), 855-862. doi:10.1016/j.foreco.2008.06.038

Scolforo, H.F., et al. 2015. Autoregressive spatial analysis and individual tree modeling as strategies for the management of *Eremanthus erythropappus*. J Forest Res, 27(3), 595-603. doi: 10.1007/s11676-015-0185-y

Scolforo, J.R.S., et al. 2004. Estimativas de volume, peso seco, peso de óleo e quantidade de moirões para a candeia (*Eremanthus erythropappus* (DC.) MacLeish). Cerne, 10(1), 87-102.

Scolforo, J.R.S., Loeuille, B.F.P., Altoé, T.F., 2012. O manejo sustentável da candeia: o caminhar de uma nova experiência florestal em Minas Gerais. Editora UFLA, Lavras.

Silva, M.B., Kanashiro, M., Ciampi, A.Y., Thompson, I., Sebbenn, A.M., 2008. Genetic effects of selective logging and pollen gene flow in a low-density population of the dioecious tropical tree Bagassa guianensis in the Brazilian Amazon. For Ecol Manag, 255, 1548-1558. doi: 10.1016/j.foreco.2007.11.012

Sjölund, M.J., Jump, A.S., 2013. The benefits and hazards of exploiting vegetative regeneration for forest conservation management in a warming world. Forestry, 86(5), 503-513. doi: 10.1093/forestry/cpt030

Sjölund, M.J., Jump, A.S., 2015. Coppice management of forests impacts spatial genetic structure but not genetic diversity in European beech (*Fagus sylvatica* L.). For Ecol Manag, 336, 65-71. doi: 10.1016/j.foreco.2014.10.015

Slatkin, M., Barton, N.H., 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution, 43(7), 1349-1368. doi:10.1111/j.1558-5646.1989.tb02587.x.

Smouse, P.E., Peakall, R., 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity, 82(5), 561-573. doi: 10.1046/j.1365-2540.1999.00518.x

Soliani, C., Vendramin, G.G., Gallo, L.A., Marchelli, P. 2016. Logging by selective extraction of best trees: Does it change patterns of genetic diversity? The case of *Nothofagus pumilio*. For. Ecol. Manag. 373, 81-92. doi: 10.1016/j.foreco.2016.04.032

Telford, A., Cavers, S., Ennos, R.A., Cottrell, J.E. 2014. Can we protect forests by harnessing variation in resistance to pests and pathogens? For: Int J For Res, 88(1), 3-12. https://doi.org/10.1093/forestry/cpu012

Tonetti, O.A.O., 2004. Melhoria da qualidade física e estudo da germinação de sementes de candeia (*Eremanthus incanus* (Less.) Less. e *Eremanthus erythropapus* (DC.) Mac Leish). Unpublished Msc. Phil Thesis, Universidade Federal de Lavras, Lavras, Brazil.

Van Oosterhout, C., Hutchinson, W.F., Wills, D. P., Shipley, P., 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes, 4(3), 535-538.

Varshney, R.K., Graner, A., Sorrells, M.E. 2005. Genic microsatellite markers in plants: features and applications. Trends Biotechnol, 23(1), 48-55. doi:10.1016/j.tibtech.2004.11.005

Vekemans, X., Hardy, O.J. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. Mol Ecol, 13(4), 921-935. doi: 10.1046/j.1365-294X.2004.02076.x

Vieira, F.A., Fajardo, C.G., Carvalho, D., 2012. Biologia Floral de candeia (*Eremanthus erythropappus*, Asteraceae). Pesqui Florest Bras, 32(72), 477-481. doi: 10.4336/2012.pfb.32.72.477

Vinson, C.C., 2009. The impact of selective logging on inbreeding and gene flow in two Amazonian timber species with contrasting ecological and reproductive characteristics. Unpublished D. Phil. Thesis, University of Oxford: Oxford. U.K.

Wickneswari, R., 2011. Threats to genetic viability of southeast Asian forest species, in: Wickneswari, R., Cannon, C.H. (Eds.), Managing the Future of Southeast Asia's Valuable Tropical Rainforests, A Practitioner's Guide to Forest Genetics. Springer Dordrecht Heidelberg, London, New York, pp. 69-82.

Wright, S., 1931. Evolution in mendelian populations. Genetics, 16, 97-159.

Wright, S. 1946. Isolation by distance under diverse systems of mating. Genetics, 31(1).

Young, A., Boyle, T., Brown, T. 1996. The population genetic consequences of habitat fragmentation for plants. Trends Ecol Evol, 11(10), 413-418. doi: 10.1016/0169-5347(96)10045-8

10. Appendices

Table A1 - Genetic diversity parameters for the six loci analyzed. Exp. Het.: Expected heterozygosity; Tot. Het.: Total heterozygosity; Obs. Het.: Observed heterozygosity.

Locus	Exp. Het.	Tot. Het	Obs. Het.
Ere02	0.61479	0.61479	0.99119
Ere03	0.60381	0.60381	0.84016
Ere04	0.00000	0.00000	0.00000
Ere07	0.48708	0.48708	0.83333
Ere08	0.47121	0.47121	0.74797
Ere14	0.45002	0.45002	0.61134
Mean	0.43782	0.52538	0.
s. d.	0.22550	0.07782	0.07782

Table A2. Expected heterozygosity for the managed and unmanaged stands considering each locus. s.d.: standard deviation; Tot. Het.: Total heterozygosity.

Locus	Managed	Unmanaged	Mean	s.d.	Tot. Het.
Ere02	0.60555	0.63049	0.61802	0.01763	0.67443
Ere03	0.61954	0.54104	0.58029	0.05551	0.64454
Ere04	0.00000	0.00000	0.00000	0.00000	0.14522
Ere07	0.48811	0.48774	0.48792	0.00026	0.52695
Ere08	0.48413	0.40739	0.44576	0.05427	0.52337
Ere14	0.44781	0.45876	0.45329	0.00774	0.50252
Mean	0.44086	0.42090	0.43088	0.01411	0.50284
s. d.	0.22696	0.21982	0.22339	0.00505	0.18890

Table A3. Genetic diversity indexes for each life stage on the managed and unmanaged stands. Na: Number of alleles, Na Freq.: Frequency of alleles; Ne: Number of effective alleles; I: Shannon index; He: Heterozygosity, uHe: Mean heterozygosity.

Population	Managed Adults	Managed Juveniles	Unmanaged adults	Unmanaged juveniles
Na	2,667	2,667	2,500	2,167
Na Freq. >= 5%	2,167	2,333	2,333	2,167
Ne	1,943	1,968	1,880	1,825
I	0,686	0,699	0,668	0,619
No. Private Alleles	0,167	0,167	0,167	0,000
No. LComm Alleles (<=25%)	0,000	0,000	0,000	0,000
No. LComm Alleles (<=50%)	0,167	0,167	0,167	0,000
He	0,437	0,437	0,418	0,402
uHe	0,441	0,439	0,422	0,425

Table A4. Summary of analysis of molecular variance for 275 individuals of two stands (managed and unmanaged) of *Eremanthus erythropappus* based on SSR markers contrasting all populations, successional pairs, and successional stages. d.f. = degrees of freedom, V.C. = Variance components, Est. Var. = Estimated variance.

Source	**d.f.**	**Sum of squares**	**V.C.**	**Est. Var.**	**% of variation**	**P-value**
Among stands	1	8.699	8.699	0.078	6%	< 0.001
Within stands	273	318.498	1.167	1.167	94%	< 0.001
Total	274	327.196		1.245	100%	< 0.001

Table A5. Characteristics of the six microsatellite markers used on the amplification. Locus name, primer sequence (F: forward, R: reverse), repeat motif, fragment size in base pair (bp), melting temperature (*Tm*), and GenBank accession numbers are shown.

SSR	Primer sequences (5'-3')	Repeat motif	Size range	*Tm* (ºC)	GenBank No.
ERE02	F: TCTTGCTTACGCGTGTGACT R: TGCATCCACTCCAATCACTT	(GA)21	119	53	MK075833
ERE03	F: GAAGGGAGACATCGGAAGAA R: ACGGAACGGAGAAGAAGAAA	(CTT)5; (CTT)9; (CTT)10; (CTT)8	232	53	MK075834
ERE04	F: CAGTGAGGGGAAGGGAGAAT R: CCTCCACTATAGGGCGGAAT	(CTT)37	398	53	MK075835
ERE07	F: GCGTGGGACTAACCCATTC R: ACCTGTTGGTGAAAGGATGC	(CTT)9	120	53	MK075836
ERE08	F: GAGCCTTCCATGGGAGTAGG R: TGGGAGGGAGAAATTGAACA	(AGC)5	238	53	MK075837
ERE14	F: CATCGATTTGGAGGCTTCAT R: TGCTTACGTGTGCTCTTGCT	(CT)11; (AT)8; (GT)18	207	53	MK075841

Table A6. Allele frequency for each locus on the managed and unmanaged stands

Ere02		**Ere03**		**Ere04**		**Ere07**		**Ere08**		**Ere14**	
Allele	Managed	Allele	Managed	Allele	Managed	Allele	Managed	Allele	Managed	Allele	Managed
77	0,003	147	0,000	395	1,000	146	0,581	221	0,593	176	0,294
88	0,503	173	0,003			164	0,419	227	0,407	184	0,683
96	0,144	176	0,215							186	0,023
98	0,350	179	0,101								
		185	0,125								
		191	0,557								
Allele	Unmanaged	Allele	Managed	Allele	Managed	Allele	Unmanaged	Allele	Unmanaged	Allele	Unmanaged
77	0,000	147	0,008	395	1,000	146	0,590	0,719	221	176	0,349
88	0,500	173	0,000			164	0,410	0,281	227	184	0,651
96	0,254	176	0,100							186	0,000
98	0,246	179	0,150								
		185	0,092								
		191	0,650								

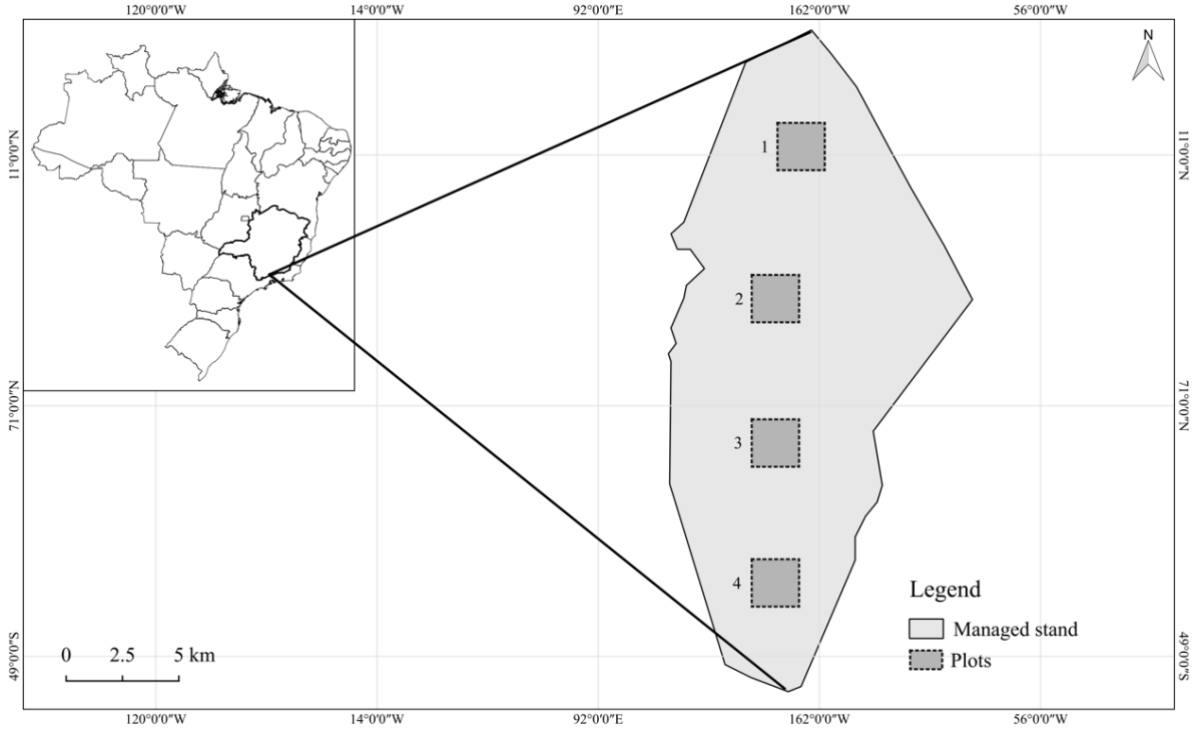

Figure A1. Sampling location at Itamonte, southern Minas Gerais state. Dark squares with dots represent plot distribution and light shaded area represents the managed stand.

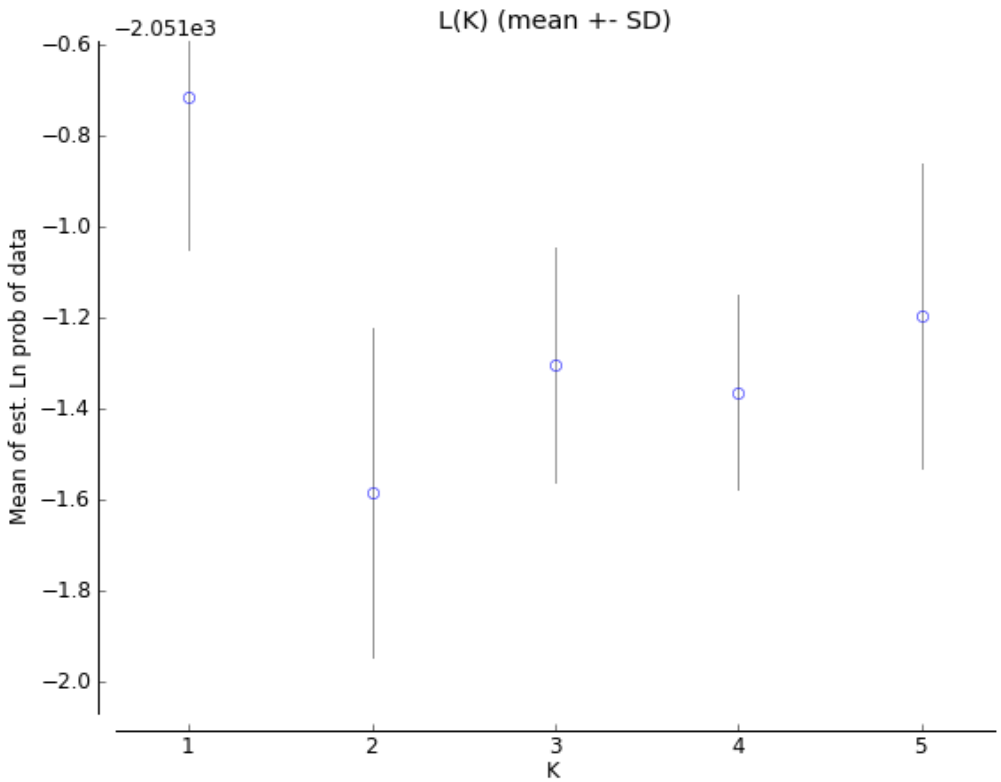

Figure A2. Values of k and their respective average probabilities, *LnP* (D), obtained by means of the Bayesian approach, showing k = 1 as the probable value of groupings of the total number of individuals.