UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE BIOLOGIA GERAL
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA

TESE DE DOUTORADO

Diversidade genética e efeitos da fragmentação de Matas Secas em *Enterolobium contortisiliquum* (Fabaceae): implicações para conservação

ORIENTADA: Patrícia de Abreu Moreira

ORIENTADOR: Dr. Geraldo Wilson Fernandes

CO-ORIENTADOR: Dr. Jorge A. Lobo

BELO HORIZONTE

Agosto – 2012

Patrícia de Abreu Moreira

Diversidade genética e efeitos da fragmentação de Matas Secas em *Enterolobium contortisiliquum* (Fabaceae): implicações para conservação.

Tese apresentada ao Programa de Pós-Graduação em Genética do Departamento de Biologia Geral do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Doutor em Genética.

Orientador: Geraldo W. Fernandes
Co-orientador: Jorge A. Lobo

Belo Horizonte
Agosto – 2012

**“There is no chance, no destiny, no fate,
that can circumvent or hinder or control
the firm resolve of a determined soul.”**

Ella Wheeler Wilcox

AGRADECIMENTOS

Agradeço primeiramente ao meu orientador Prof. Geraldo W. Fernandes por todas as oportunidades oferecidas e pela confiança durante todos esses anos. Agradeço ainda ao co-orientador e amigo Prof. Jorge Lobo por todos os ensinamentos, pela amizade, pela paciência e por todas as críticas e sugestões.

Agradeço à UFMG e a todos os professores pela contribuição na minha formação, em especial ao Prof. Eduardo Tarazona que sempre me apoiou e acreditou em mim e ao Prof. Vasco Azevedo por ter me apoiado sempre. Agradeço ainda a Profª. Bernadete por ter me atendido sempre que precisei, me ajudando e incentivando.

Ao IEF/MG pelas permissões de trabalho no Parque Estadual da Lapa Grande, em especial a Aneliza Melo. Agradeço ainda a CAPES pela concessão da bolsa de doutorado e a FAPEMIG pelo apoio financeiro (APQ-00372-11).

À Unimontes por ter sido a minha casa por tanto tempo, em especial ao Prof. Dario Alves de Oliveira por ter me acolhido, por abrir as portas do Laboratório de Bioprospecção e Recursos Genéticos e por todo apoio financeiro para execução do trabalho. A todos os companheiros de laboratório em especial a Leide "Maria", Ligy e Tânia por terem me ajudado muito e por terem resolvido tantos pepinos.

Ao grupo de trabalho formado "Pesquisa Tamboril" e as "minhas meninas" Nanda, Sa, Naty, Thá e Fa, pela imensa colaboração no campo e no laboratório. Vocês foram fundamentais para a realização desse trabalho!!

A todos os companheiros de Moc city, em especial ao Lemuel, Neila, Dinho, Bia, Nescau, Jegs, Bets, Malu, Raissinha, Comis, Gramps que me ajudaram muito durante esses anos!

Agradeço àqueles que me ajudaram na realização desse trabalho, Magnel Oliveira, Matheus Lopes Souza, Marcílio Fagundes, Murilo M. Brandão, Yule R. F. Nunes, Giovanna Luz, Newton Barbosa.

Um agradecimento especial às queridas amigas, fundamentais durante o doutorado: Aline Medeiros e Marina Beirão... por compartilharem todas as angústias, dúvidas e me ajudarem SEMPRE que precisei!!!

Aos meus pais, a minha irmã Luciana e ao Julinho, a Thaís (Necessaire) e aos meus sobrinhos minha eterna gratidão por todos os momentos felizes, pelo apoio e por toda a preocupação. Amo todos vocês! Agradeço ainda a tia Ângela por ter proporcionado, lá no início, que tudo isso fosse possível... e à família Abreu Costa pelo apoio nas coletas.

À Fundação Moreira-Neves por "comprar" sempre as minhas ideias e pelo apoio financeiro para execução dos meus trabalhos...

Agradeço ao meu marido Fred por ter me levantado sempre que quis desistir, pelo companheirismo e dedicação, pelas discussões dos trabalhos, pela companhia nas coletas, pelas ajudas nas análises, por revisar este trabalho e por permitir que a Fundação Moreira-Neves existisse.

SUMÁRIO

LISTA DE FIGURAS

Capítulo 1

Capítulo 2

Capítulo 3

Capítulo 4

LISTA DE TABELAS

Capítulo 1

Capítulo 2

Capítulo 3

Capítulo 4

RESUMO

O crescente processo de fragmentação de áreas naturais é principal responsável pela perda de biodiversidade. Além da rápida destruição, os habitats que anteriormente ocupavam grandes áreas são fragmentados em virtude das atividades antrópicas. Os resultados dos fatores supracitados são ecossistemas que formam mosaicos de vegetação, mergulhados numa matriz alterada. Devido às mudanças ambientais, populações são reduzidas e subdivididas, o que pode acarretar em alterações de processos ecológicos e genéticos. Dessa forma, estudos ecológicos e sobre a diversidade genética de uma espécie são fundamentais para o delineamento de estratégias de conservação. Nesse contexto, as Matas Secas são formações florestais que se encontram ameaçadas devido à sua distribuição disjunta no ambiente e ações antrópicas, que substituem áreas naturais para o estabelecimento de atividades agropastoris e/ou moradias. A espécie *Enterolobium contortisiliquum*, característica das Matas Secas, está cada vez mais ameaçada em seu habitat natural devido à fragmentação e a retirada indiscriminada de indivíduos. Os frutos dessa espécie são recursos alimentícios para o gado durante a estação seca, porém, as favas são venenosas para esses animais pois provocam abortos e lesões de origem fotossensibilizantes. Assim, muitos pecuaristas optam por eliminar essas árvores das áreas de pastagem. Diante disto, o presente trabalho teve como objetivo avaliar a diversidade genética da espécie *E. contortisiliquum* em áreas disjuntas de Matas Secas e verificar os efeitos da fragmentação do habitat em seu sucesso reprodutivo e nos padrões fenológicos. Uma alta diversidade genética foi observada com marcadores moleculares do tipo ISSR nas populações de *E. contortisiliquum* amostradas nos remanescentes de Matas Secas. A maior parte dessa diversidade genética é atribuída às diferenças entre os indivíduos dentro das populações e uma diferenciação entre as mesmas foi detectada. As populações de *E. contortisiliquum* formaram quatro grupos distintos e o rio São Francisco pode ser uma barreira responsável pela formação da estrutura observada. O efeito da fragmentação no sucesso reprodutivo de *E. contortisiliquum* não foi observado nos seguintes parâmetros analisados: tamanhos dos frutos e sementes, produção de sementes viáveis, produção de sementes abortadas, proporção de sementes predadas, produção de sementes totais e quantidade de sementes germinadas das árvores localizadas em uma matriz conservada se comparadas às árvores localizadas em uma matriz degradada. Contudo, as sementes das árvores localizadas na matriz conservada germinaram mais rápido do que as sementes das árvores

localizadas na matriz fragmentada. Além disso, um efeito da fragmentação foi observado nos padrões fenológicos da espécie. As árvores presentes na matriz conservada apresentam um padrão fenológico distinto das árvores localizadas na matriz fragmentada e das árvores localizadas em uma área urbana. As alterações dos padrões fenológicos reprodutivos podem provocar um isolamento temporal entre os indivíduos de diferentes populações e impossibilitar o fluxo gênico entre os mesmos. Os marcadores moleculares microssatélites desenvolvidos para *E. cyclocarpum* foram transferidos com sucesso para *E. contortisiliquum* e poderão ser utilizados para investigarmos a diversidade genética, o sistema de acasalamento, o fluxo gênico e a estrutura genética espacial de árvores localizadas em áreas com diferentes graus de perturbações, a fim de se verificar os efeitos da fragmentação nesses parâmetros e gerar subsídios para sua conservação. Os resultados encontrados nesse trabalho são importantes para adoção de estratégias de conservação e manejo da espécie *E. contortisiliquum* e para entendermos os efeitos da fragmentação nos padrões ecológicos e de diversidade genética de espécies arbóreas presentes em Matas Secas tropicais.

ABSTRACT

The increasing process of natural areas fragmentation is the major responsible of biodiversity losses. In addition to its rapid destruction, the habitats that previously have occupied large areas are fragmented due to human activities. The results of these factors are vegetation mosaic ecosystems immersed in an altered matrix. Due to environmental changes the populations are reduced and subdivided, which may result in changes of ecological and genetic processes. Thus, ecological and genetic diversity studies of species are fundamental to conservation strategies development. In this context, the seasonally dry tropical forests (SDTF) are in threat because of its disjunct distribution in the environment and anthropic actions that replace natural areas for the human settlements and agricultural activities. *Enterolobium contortisiliquum* species is typical of SDTF and is increasingly threatened in their natural habitat due to fragmentation and indiscriminate removal of individuals. The fruits of this species are resources for cattle feeding during dry season, but its poisonous pods cause abortions and photosensitizers injuries. Thus many farmers choose to remove these trees from the pastures. So, the present study aimed to evaluate the genetic diversity of the *E. contortisiliquum* species and verify the effects of habitat fragmentation on its reproductive success and phenological patterns. High genetic diversity was found using ISSR molecular markers in *E. contortisiliquum* populations in SDTF remnants. Most of this variation was found within populations and differentiation between them was detected. *Enterolobium contortisiliquum* populations formed four distinct groups and São Francisco River may be a barrier responsible for the observed structure. The effect of fragmentation on the reproductive success of *E. contortisiliquum* was not observed on the following parameters: fruit and seed size, undamaged seed production, seed abortion, seed predation, total seed production and number of seed germinated of trees located in a conserved matrix compared to trees located in a degraded matrix. However, seeds of trees located in a conserved matrix germinated faster than seeds of trees located in a degraded matrix. Furthermore, the effect of forest fragmentation was observed on phenological patterns. Trees of conserved matrix exhibited a distinct phenological pattern of trees of fragmented matrix and trees of urban area. Changes in reproductive phenology may cause a temporal isolation between individuals of different populations inhibiting the gene flow among them. The microsatellite markers developed for *E. cyclocarpum* were successfully transferred to *E. contortisiliquum* and may be used to investigate

genetic diversity, the mating system, gene flow and spatial genetic structure of trees in areas with different degrees of disturbance, in order to verify the effects of fragmentation on these parameters and generate support for its conservation. The results of this study are important for adoption of conservation strategies and management of the species *E. contortisiliquum* and to understand the effects of fragmentation in ecological patterns and genetic diversity of tree species present in tropical dry forests.

INTRODUÇÃO GERAL

A fragmentação de habitats devido a atividades antrópicas é considerada a maior responsável pela perda da biodiversidade (Sala et al., 2000; Primack & Rodrigues, 2001; Jordan et al., 2003). O desmatamento, uma das causas mais comuns de destruição do habitat, resulta na supressão de mais da metade das matas primárias ocorrentes nos países tropicais (Townsend et al., 2006). Como resultado, os habitats permanecem distribuídos em mosaicos de vegetação remanescentes, mergulhados numa matriz antropizada.

Nesse processo, populações são reduzidas e subdivididas, o que pode acarretar alterações de serviços ecossistêmicos e padrões genéticos (Ewers & Didham, 2006; Aguilar et al., 2008). Assim, é preciso conhecer e prever as possíveis consequências da alteração de habitats sobre as populações naturais para a elaboração de estratégias de conservação e manejo dos recursos naturais (Turner & Corlett, 1996; Frankham et al., 2002).

A perda de habitat implica na redução de espécies e aumento do isolamento entre os remanescentes, os quais mudam a composição das espécies, a abundância de indivíduos e altera processos ecológicos como as interações animal-planta (Saunders et al., 1991; Debinski & Holt, 2000). Essa alteração é particularmente importante para espécies vegetais que precisam dos animais como vetores para reprodução. Assim, os efeitos da alteração do habitat nos animais têm um impacto negativo na demografia e recrutamento vegetal e coloca em risco os ambientes naturais (Dirzo & Miranda, 1991; Aizen & Feinsinger, 1994).

Dentre as formações florestais tropicais as Florestas Estacionais Deciduais (Matas Secas) representam o ecossistema mais ameaçado das Américas, devido à substituição de suas áreas naturais para o estabelecimento humano e de atividades agrícolas (Mass, 1995; Ewel, 1999). As Matas Secas estão naturalmente distribuídas de forma fragmentada em toda a região Neotropical,

desde o México e Caribe até o Sudeste do Brasil e os Chacos na Argentina (Pennington et al., 2000), mas a maior parte dos remanescentes encontra-se hoje na América do Sul, especificamente no Brasil (Pennington et al., 2006). Algumas áreas foram identificadas como refúgios de Matas Secas na América do Sul e o maior deles corresponde ao nordeste brasileiro e norte do estado de Minas Gerais (Werneck et al., 2011).

Alguns estudos afirmam que a distribuição em manchas das Matas Secas está associada às flutuações climáticas do Quaternário (Prado & Gibbs, 1993, Pennington et al., 2000; Prado, 2000). Essas áreas seriam relíquias de uma floresta contínua no passado, que se expandiu durante o período seco e frio no Pleistoceno (cerca de 21.000 anos desde o presente), no final do último período glacial, formando o "Arco Pleistocênico" (Prado & Gibbs, 1993; Prado, 2000). Posteriormente, com o aumento da temperatura e umidade, a partir do final do último período glacial, estas florestas podem ter retraído (durante o Holoceno) e atingido a distribuição atual. As evidências para construção dessa hipótese do Arco Pleistocênico foram os padrões de endemismo e a distribuição disjunta da flora, como observado em *Myracrodruom urundeuva* e *Enterolobium contortisiliquum* (Prado & Gibbs, 1993; Prado, 2000; Werneck et al., 2011).

Apesar da grande ameaça pouco se conhece sobre as Matas Secas. Essas florestas são formações tropicais caracterizadas por uma acentuada sazonalidade na distribuição das chuvas, onde a precipitação é inferior a 1600 mm por ano, e por vários meses de seca, com um período de pelo menos cinco a seis meses de precipitação inferior a 100 mm (Gentry, 1995; Graham & Dilcher, 1995). Esta fitofisionomia é dominada por árvores com uma deciduidade marcante (com pelo menos 50% de deciduidade no período seco) com um dossel ligeiramente contínuo (Mooney et al., 1995; Sánchez-Azofeifa et al., 2005). Existe uma lacuna de conhecimento em Matas Secas em relação a florestas tropicais úmidas. Apenas 14% dos estudos realizados em florestas tropicais foram realizadas em ambientes secos, enquanto 86% foram realizados em regiões úmidas

(Sánchez-Azofeifa et al., 2005). Além disso, a informação científica em florestas tropicais secas é fragmentada e limitada a poucas áreas, como na América Central, onde ocupavam originalmente 50% dos ambientes florestais (Murphy & Lugo, 1986), principalmente em países como México e Costa Rica (Sánchez-Azofeifa et al., 2005).

As Matas Secas recebem menos investimentos não somente em termos de pesquisas científicas, mas também em termos de recursos para conservação em relação às florestas tropicais úmidas (Pennington et al., 2006). Cerca de 97% das áreas remanescentes estão ameaçadas, principalmente, devido às mudanças climáticas, fragmentação do habitat, fogo, conversão das áreas naturais em áreas agrícolas e de pastagem e aumento da densidade populacional humana (Miles et al., 2006; Quesada et al., 2009). No Brasil a dificuldade em se conservar as Matas Secas envolve o fato de esses remanescentes florestais serem localizados em solos ricos que são utilizados para agricultura ou para implantação de pastagens, como ocorre no estado de Minas Gerais (veja Anaya et al., 2006; Espírito-Santo et al., 2009). Além disso, nas Matas Secas existem árvores com madeira de boa qualidade, o que acelera a fragmentação e dificulta a conservação devido ao corte seletivo. Dessa forma, os altos níveis de perturbação, sobretudo nos últimos dois séculos, decorrentes da retirada indiscriminada de madeira, da pecuária extensiva e do fogo, reduziram seriamente as formações de florestas estacionais deciduais a pequenos fragmentos (Werneck et al., 2000).

As Matas Secas em Minas Gerais estão concentradas na região norte do estado. Estas formações podem ser encontradas tanto nos domínios do Cerrado como da Caatinga, sofrendo influência da fitofisionomia onde estão inseridas (Santos et al., 2007). Essa região está sofrendo intenso processo de fragmentação (Anaya et al., 2006) devido à presença de inúmeras espécies vegetais úteis, utilizadas para fins medicinais, alimentícios, entre outros (Oliveira et al., 2006). Além disso, devido à proximidade do rio São Francisco, as Matas Secas encontram-se sob forte

pressão antrópica, com a implantação de plantios de milho e feijão e pela pecuária (Silva et al., 2004; Anaya et al., 2006, Espírito-Santo et al., 2009). Dessa forma, estudos genéticos e ecológicos de espécies arbóreas de Mata Seca são de suma importância para avaliar a probabilidade de persistência das espécies nos remanescentes (ver Moreira et al., 2008, 2009).

A espécie *Enterolobium contortisiliquum* (Vell.) Morong é uma árvore pertencente a uma das famílias mais dominantes das Matas Secas, a Fabaceae (Gentry, 1995; Mayle, 2004). Essa árvore é característica de Matas Secas (Särkinen et al., 2011) e foi utilizada para definir regiões historicamente pertencentes a essas formações florestais (Prado & Gibbs, 1993; Prado, 2000; Morrone, 2000, 2001, 2009).

Popularmente conhecida como tamboril, orelha de macaco ou orelha de nego, essa espécie arbórea pode atingir de 25 a 30 metros de altura e produz favas de coloração preta com formato característico de orelha humana. A frutificação ocorre entre os meses de junho a julho, mas as favas podem permanecer por mais tempo na árvore (Lorenzi, 1998). Devido às características de suas favas, as mesmas podem representar um Anacronismo Neotropical, em que frutos grandes e indeiscentes eram comidos pela extinta megafauna e suas sementes dispersas pela mesma (Janzen, 1981a, 1981b; Janzen & Martin, 1982, Guimarães Jr et al., 2008). Atualmente roedores promovem a dispersão das sementes (Carvalho, 2003). Acredita-se que suas flores diminutas sejam polinizadas por mariposas noturnas da família Sphingidae e por pequenas abelhas diurnas (P.A. Moreira, observação pessoal), como ocorre na espécie congenérica *E. cyclocarpum* (Janzen, 1982; Rocha & Aguilar, 2001; Frankie et al., 2004; Hamrick & Apsit, 2004).

O tamboril é uma espécie pioneira, de crescimento rápido e, por isso mesmo, tem potencial para ser utilizada na recuperação de áreas degradadas (Durigan et al., 2002; Lorenzi, 1998). Contudo, esta espécie tem sido ameaçada e suas populações naturais diminuídas (Eira et al., 1993) devido à exploração intensiva para utilização em serrarias, móveis e mesmo construção

civil (Alcalay & Amaral, 1982). Além disso, a espécie é retirada do ambiente, principalmente quando próximas às fazendas, por agricultores que utilizam a terra para plantio, e também por pecuaristas para formação de pastagens. Esta prática ocorre devido à intoxicação dos bovinos pelas favas do tamboril a qual pode causar a morte do animal. Os frutos de tamboril são ricos em saponinas e esses compostos metabólitos hemolientes provocam lesões no gado podendo causar a morte do mesmo e, quando ingeridos por vacas prenhas, causam aborto (Grecco et al., 2002; Tokarnia et al., 1999). Bonel-Raposo e colaboradores (2008) pesquisaram a intoxicação e o efeito abortivo das favas do tamboril em cobaias e observaram que a ingestão dos frutos provocou alterações no estômago, nos intestinos delgado e grosso, no fígado e no baço e duas mortes foram constatadas após 12 e 18 horas de ingestão. Além disso, todas as cobaias prenhas que ingeriram as favas abortaram os fetos.

A época de frutificação do tamboril coincide com o período de seca em que ocorre queda da qualidade e quantidade de forrageiras (Bastianetto et al., 2005). A limitação do principal recurso alimentício estimula a ingestão dos frutos de *E. contortisiliquum* pelo gado, aumentando o potencial de intoxicação pelos frutos do tamboril. Costa e colaboradores (2009) alertam que o consumo das favas pelo gado pode ocasionar prejuízos econômicos para o produtor pela diminuição do desempenho produtivo e reprodutivo dos animais bem como pelos gastos com medicamentos e mão-de-obra especializada. Assim, os fazendeiros optam pela alternativa mais barata que é a eliminação das árvores desta espécie nas proximidades das fazendas.

Nesse contexto, estudos ecológicos e genéticos da espécie *E. contortisiliquum* são importantes por fornecerem valiosas informações para a conservação, manejo e manutenção da espécie e do ambiente na qual está inserida, o que é de extrema relevância para aumentarmos o conhecimento sobre esse habitat.

Diante disto, a presente tese teve como objetivos avaliar a diversidade genética da espécie *E. contortisiliquum* em áreas disjuntas de Matas Secas e verificar os efeitos da fragmentação do habitat em seu sucesso reprodutivo e nos padrões fenológicos. Além disso, o trabalho tem como objetivo obter ferramentas moleculares capazes de gerarem informações úteis para sua conservação. Para uma melhor compreensão dos estudos, o presente trabalho foi dividido quatro capítulos, apresentados a seguir.

APRESENTAÇÃO DOS CAPÍTULOS

A presente proposta está organizada em quatro capítulos que correspondem aos artigos gerados a partir dos resultados obtidos.

O capítulo 1 corresponde ao artigo "Genetic diversity and structure of the tree *Enterolobium contortisiliquum* (Vell.) Morong (Fabaceae) associated with patches of a seasonally dry tropical forest" o qual será submetido ao periódico *Genetica*. Esse artigo teve como objetivo avaliar a diversidade e estrutura genética da espécie com marcadores moleculares em áreas disjuntas de Matas Secas no estado de Minas Gerais.

O capítulo 2 refere-se ao artigo "Effects of seasonally dry tropical forest fragmentation on the reproductive success of the Neotropical tree *Enterolobium contortisiliquum* (Fabaceae)" o qual será submetido ao periódico *Plos One*. Esse trabalho teve como objetivo verificar o efeito da fragmentação florestal no tamanho dos frutos e sementes, na produção de sementes, na predação e germinação das sementes.

O capítulo 3 corresponde ao artigo "Effects of different habitat disturbances on phenological patterns of the seasonally dry tropical forest tree *Enterolobium contortisiliquum* (Fabaceae)" o qual será submetido ao periódico *Biological Conservation*. Esse estudo teve como objetivo avaliar os efeitos da fragmentação nos padrões fenológicos de árvores localizadas em áreas com diferentes graus de perturbação.

O capítulo 4, intitulado "Characterization of nine transferred SSR markers in the tropical tree species *Enterolobium contortisiliquum* (Fabaceae)" o qual está aceito no periódico *Genetics and Molecular Research*, trata da transferibilidade de marcadores moleculares microssatélites desenvolvidos para a espécie *Enterolobium cyclocarpum* para a espécie em estudo. Os marcadores microssatélites serão úteis na geração de informações a respeito do sistema de

acasalamento e fluxo gênico entre árvores localizadas em área com uma matriz conservada e uma área com uma matriz alterada.

CAPÍTULO 1

Artigo em preparação a ser submetido ao periódico *Genetica*.

Genetic diversity and structure of the tree *Enterolobium contortisiliquum* (Vell.) Morong (Fabaceae) associated with patches of a seasonally dry tropical forest

Running title: Genetic diversity and structure of *Enterolobium contortisiliquum*

Abstract

Fragmentation of tropical forests is one of the major threats to biodiversity conservation and viable natural populations. In this way, Brazilian seasonally dry tropical forests (SDTF) are endangered because of human occupation, conversion of lands to agriculture and high deforestation rates in these fertile soils. *Enterolobium contortisiliquum* tree species has been removed from SDTF natural areas owing to the advance of cattle and agriculture in Brazilian SDTFs. To aim in the conservation efforts of the species we used molecular markers to study the genetic diversity and structure in populations of *E. contortisiliquum* in the Brazilian SDTF patches. A total of 263 individuals in 13 populations were analyzed with 103 ISSR fragments. Despite being found scattered in SDTF patches, high genetic diversity was found in *E. contortisiliquum* populations (mean Shannon's index of diversity = 0.384; mean gene diversity = 0.280). However, genetic divergence between populations was detected (Φ_{ST} =0.155). UPGMA dendrogram, principal coordinate analysis and Bayesian analyses showed that in *E. contortisiliquum* populations were clustered in four groups. No significant correlation was found between geographical distance and genetic distance (r = 0.119, p = 0.197). Nevertheless, the São

Francisco River appears to influence in the genetic structure observed in *E. contortisiliquum* populations. Jointly to the threat posed by the disjunct distributions of SDTFs, the current fragmentation caused by human activities is reducing the natural habitats of *E. contortisiliquum* tree and represent a threat to this species survivor.

Introduction

The current fragmentation of tropical forests is one of the major threats to biodiversity conservation and viable natural populations (Saunders et al.1999). This is especially worrisome when seasonally dry tropical forests (SDTF) are considered because SDTF are the most endangered tropical ecosystem (Janzen 1988, Miles et al. 2006, Steininger et al. 2001).

SDTFs occurs in regions characterized by pronounced seasonality in rainfall distribution, where precipitation is less than 1600mm/yr, and by several months of drought, with a period at least five or six months receiving less than 100 mm (Gentry 1995, Graham and Dilcher 1995). SDTFs are dominated by deciduous trees with a more or less continuous canopy (Mooney et al. 1995, Sánchez-Azofeifa et al. 2005). The most dominant families are Fabaceae and Bignoniaceae (Gentry 1995, Mayle 2004).

The tropical dry forests exhibit a disjunct distribution scattered throughout the Neotropics (Pennington et al. 2006). The discontinuous distribution of SDTF species, as *Anadenanthera columbrina* (Vell.) Brenan and *Enterolobium contortisiliquum* (Vell.) Morong, indicate that in the past these forests represented a large and contiguous formation during cooler and drier periods of the Pleistocene. Today, the remnants of tropical dry forests are present in patterns called the “Pleistocenic Arc” (Prado and Gibbs 1993, Prado 2000). The SDTF distribution shifts

may have occurred as a consequence of Quaternary climatic changes (Werneck 2011) and STDFs were restricted into actual fragmented patches.

The largest areas of SDTFs are found in South America, specifically in Brazil (Pennington et al. 2004), where they are represented in 3.21% of its territory (Sevilha et al. 2004). As in other localities, Brazilian SDTFs are associated with fertile soils with a moderate to high pH and high nutrient content (Espírito-Santo et al. 2009, Pennington et al. 2006). Brazilian SDTFs are endangered because of human occupation, conversion of lands to agriculture and high deforestation rates in these fertile soils. In this way, SDTF can be considered the most threatened ecosystem in Brazil (Espírito-Santo et al. 2009).

Enterolobium contortisiliquum (Vell.) Morong is a Neotropical tree species belonging to the most SDTF dominant family Fabaceae (Gentry 1995, Mayle 2004). This species is considered as a SDTF specialist (Särkinen et al. 2011) and it was used as a "track" to define the Chacoan subregion of the Neotropical region in a historical biogeographic scheme of Latin America and the Caribbean regions (Morrone, 2000, 2001, 2009). This Chacoan subregion corresponds to Pleistocenic Arc (Prado and Gibbs 1993, Prado 2000). The species flowering occurs from a short period, September and October, when tree is leafless, while their fruits ripen between June and July (P.A. Moreira, *personal observations*). The mature fruits and seeds of *E. contortisiliquum* resemble other *Enterolobium* species. As *E. cyclocarpum*, its fruits are smooth, shiny, indehiscent and deep brown (Rocha and Aguilar 2001a) and seeds are hard, ovoid and brown (Janzen 1982).

This tree species has been removed from natural areas owing to the advance of cattle and agriculture in Brazilian SDTFs because of its poisonous fruits. The ingestion of *E. contortisiliquum* pods is harmful to cattle, causing photosensitivity reactions and abortion (Bonel-Raposo et al. 2008, Costa et al. 2009). As a result many farmers have cut trees of this species near their ranches because the fruiting period of *E. contortisiliquum* occurs during the dry season,

coincident with low forage availability. The resulting selective logging has leaded the populations of *E. contortisiliquum* to become vulnerable in the disjunct SDTF matrix. The understanding of the effects of fragmentation and selective logging of this tree species is urgently needed for its conservation.

To aim in the conservation efforts of the species we used molecular markers to study the genetic diversity and structure in populations of *E. contortisiliquum* in the Brazilian SDTF patches. By using inter simple sequence repeats (ISSRs) we addressed the following questions: (1) what is the level of genetic diversity in *E. contortisiliquum* in the Brazilian SDTF patches? and (2) how the genetic variation is structured among patches?

Materials and methods

Plant materials sampling

Enterolobium contortisiliquum is a common species in the Brazilian SDTFs (Oliveira-Filho 2006). From 15 to 29 adult trees were sampled in 13 Brazilian SDTF patches in this study, totaling 263 *E. contortisiliquum* individuals (Figure 1, Table 1). Each SDTF remnants was treated as a population: Unaí (UNA), Paracatu (PAR), João Pinheiro (JOP), Felício dos Santos (FES), Gouveira (GOU), Buenópolis (BUE), Manga (MAN), Itacarambi (ITA), Verdelândia (VER), Jequitaí (JEQ), Várzea da Palma (VAR), Beltrão (BEL) and Montes Claros (MOC). Expanded leaves were collected from all individual trees and stored on silica gel until DNA extractions were carried out.

DNA extraction and ISSR amplification

Genomic DNA extraction followed the standard CTAB procedure (Doyle and Doyle 1987). DNA integrity was performed by electrophoresis on 1.0% agarose gels with 1 X TBE buffer, stained with ethidium bromide and photographed under UV light. Then, DNA obtained was diluted in ultrapure water up to final concentration of 5.0 ng/µL prior to PCR amplifications.

An initial screening of 20 primers was realized for ISSR analysis. ISSR amplifications were performed in a 15 µL volume containing 20.0 ng of template DNA, PCR buffer (50 mm KCl, 10 mm, Tris-HCl pH 8.4, 0.1% Triton X-100, Phoneutria), 0.50 mM $MgCl_2$, 0.25 µg of BSA, 250 µM of each dNTP, 10.0 µM of primer and 1 unit of Taq DNA polymerase (Phoneutria). Amplifications were performed using Veriti thermal controller (Applied Biosystems) under the following conditions: 94°C for 4 min (one cycle); 94°C for 1 min, 46 or 47°C for 1 min (according to each primer), 72°C for 1 min (37 cycles); and 72°C for 7 min (one cycle). ISSR amplified products were separated electrophoretically at a constant voltage of 100 V for 4 hours in a 1.5% agarose gels with 1X TBE buffer, stained with ethidium bromide and photographed under UV light (Gel Logic 212 Pro, Carestream). A 100-bp DNA ladder (Fermentas) was used as a standard molecular weight to estimate the molecular size of the fragments.

Data analysis

Fragments amplified by ISSR were scored as present (1) or absent (0) by manual verification to create a binary matrix. We assumed that amplified products of similar size using the same primer were homologous. To avoid problems associated with the analysis of dominant markers, only data from intensely stained, clear and unambiguous bands were used for genetic analyses. Genetic diversity was measured by percentage of polymorphic loci (*P*), the observed number of alleles (*Na*), effective number of alleles (*Ne*) and Shannon's index (*I*) (Lewontin 1972). These genetic analyses were performed with the software PopGene v.1.32 (Yeh et al. 1999). The measures of genetic diversity included a Bayesian analysis described by Holsinger et al. (2002), implemented in Hickory v1.0.4 (Holsinger and Lewis 2005). We estimated *hs*, which is analogous to Nei's (1978) unbiased expected heterozygosity (H_E).

The Analysis of Molecular Variance (AMOVA) was performed using Arlequin v. 3.01 (Excoffier et al. 2005) to estimate variance components and to partition the variation of each species within and among populations. Statistical significance of the proportion of variance associated with the fixation index Φ_{ST} was determined through permutation tests against a null distribution generated by the data. The pairwise Θ^B (a Bayesian analysis analogous to F_{ST}) was obtained with the software Hickory v1.0.4 (Holsinger and Lewis 2005) for all populations pairs and used to construct a UPGMA (unweighted pair-group method with arithmetic averages) dendrogram using NTSYS v.2.2 (Rohlf 2000) and to carry out a Principal Components Analysis (PCA) with the software GenAlEx (Peakall and Smouse 2006). To verify the isolation by distance a Mantel test was performed using PC-ORD v.6 (McCune and Mefford 2011).

To determine the number of genetic cluster (K), a Bayesian analysis of population structure was carried out with the software STRUCTURE 2.2. (Pritchard et al. 2000). A burn-in period of 100,000 generations and 100,000 steps of Markov Chain Monte Carlo simulations were

used to estimate lnPr (X/K), F_{ST} and Q (individual ancestry) for different values of K. Admixture model (individuals may have mixed ancestry), a reasonably model for dealing with many of the complexities of real populations, and correlated allele frequencies model (frequencies in the different populations are likely to be similar probably due to migration or shared ancestry), which performs better with inbreeding were considered for all analyses (see Pritchard et al. 2007). For each K value, 10 runs were carried out to verify the consistency of the results.

A Bayesian analysis of genetic structure was performed in Hickory v1.0.4 (Holsinger and Lewis 2005). The following coefficients of population differentiation were estimated: f (within-population inbreeding coefficient analogous to F_{IS}), Θ^B (which is analogous to F_{ST}) and G_{ST}-B (which is analogous to Nei's G_{ST}). Three different models were tested: a full model (this model estimates f and Θ^B), $f = 0$ model (this model maintain $f = 0$ and estimates Θ^B) and $\Theta^B = 0$ model (this model maintain $\Theta^B = 0$ and estimates f). For each one of the models five runs were carried out to verify the consistency of the results. A burn-in period of 5,000 generations, a run of 5,000 iterations and a thinning factor of 5 were used.

Results

From 20 ISSR primers tested 10 were used and resulted in 103 clearly identifiable fragments (Table 2). Each primer amplified from six to 18 fragments and all 103 scored bands were polymorphic at the species level and at population level the polymorphism ranged from 61.54 to 95.19% (Table 3). The average effective number of alleles per locus at the population level was 1.436 and 1.589 at the species level (Table 3). The gene diversity (hs) and Shannon's index (I) were 0.370 and 0.519, respectively, for the species. Populations exhibited high genetic

variability with mean *hs* and *I* equal to 0.280 and 0.384, respectively. VER population showed the lowest genetic diversity (*hs* = 0.241, *I* = 0.338) and UNA population the highest diversity (*hs* = 0.339, *I* = 0.492).

The AMOVA results revealed that most of the variation was found within populations (84.46%, Φ_{ST} =0.155, p < 0.0001) and only 15.54% of the variation occurred among populations (Table 4). The UPGMA dendrogram shows *E. contortisiliquum* populations into four main clusters. The group I corresponds to UNA, JOP, PAR, MAN and ITA populations, the group II corresponds to VER, JEQ, BEL and GOU, the group III corresponds to VAR and BUE populations and the last group (IV) corresponds to FES and MOC populations (Figure 2). The pairwise Θ^B ranged from 0.054 (between UNA and JOP) to 0.392 (between VAR and FES). The highest geographic distance is among FES and UNA which showed Θ^B = 0.231 and the nearest distance is between VAR and JEQ which showed Θ^B = 0.271 (Table 5). The Mantel test showed no significant correlation between geographical distance and genetic distance based on ISSR data (r = 0.119, p = 0.197).

In spite that the PCA analysis showed an intermediate position for MAN and GOU populations, PCA results were consistent with UPGMA dendrogram. The first two axes explained most of the total variability (axis 1 = 39.95% and axis 2 = 26.30%) (Figure 3). The UPGMA and PCA analyses showed congruence with the STRUCTURE analysis, which indicated a high hierarchical structure when *K* = 4 (Figure 4).

The Deviance Information Criterion (DIC) generated by each model of Hickory was used to chosen the best model. Lower DIC values indicate a better fit of the model to the data. However, a difference of larger than five or six units among different models is necessary to prefer one model over another (Holsinger and Lewis 2005). Thus, after comparison of DIC

values, the full model was chosen (Table 5). Differences in DIC values between the full model and $f = 0$ model provide evidence for inbreeding at species level. Likewise, comparison of DIC values between the full model and $\Theta^B = 0$ model suggest genetic differentiation among populations at species level (Table 6).

Discussion

The ISSR markers revealed similar levels of genetic diversity of *Enterolobium contortisiliquum* populations to other outcrossing species analyzed with another dominant molecular marker, RAPD which estimates are comparable with ISSR (Nybom 2004). The high levels of genetic diversity found in *E. contortisiliquum* populations (*hs* = 0.280, *I* = 0.384) are similar to other Leguminosae species as *Derris trifoliata* and *Ormosia hosiei* (Wu et al. 2012, Zhang et al. 2012, respectively) and is higher than two others tree species from Brazilian Cerrado, *Dimorphandra mollis* and *Dimorphandra wilsonii* (Souza and Lovato 2010). This result may be related to *E. contortisiliquum* breeding behavior, its long lifespan and SDTF history. Although the *E. contortisiliquum* mating system is still unknown, its congener *E. cyclocarpum* is predominantly outcrossed (Rocha and Aguilar 2001b). We postulate that *E. contortisiliquum* could be an outcrossing species, which could explain the high levels of genetic diversity found. Furthermore, *E. contortisiliquum* is found in SDTFs enclaves which occur scattered throughout the Brazilian Cerrado and Caatinga biomes (Werneck 2011). This current disjunct distribution of SDTFs supposedly represents remnants a more continuous distribution of dry forests in the past during the Late Pleistocene which formed the 'Pleistocenic Arc' (Prado and Gibbs 1993, Prado 2000). During the Pleistocenic Arc, in a much larger single formation of SDTFs, the gene flow in

E. contortisiliquum could be held among more trees and promote an increase of genetic diversity. Thus, the contraction of Pleistocenic Arc with increases in temperature and precipitation could be caused losses of *E. contortisiliquum* trees. Nevertheless, woody plants as *E. contortisiliquum* may have persisted in remnant populations in SDTFs enclaves for long time and our results probably reflect the historical genetic diversity of adult trees. In a recent meta-analysis it was confirmed that adults exhibited greater genetic variation than offspring in a fragmented landscape because of persistence in habitats (Vranckx 2011).

The fixation index of *E. contortisiliquum* (Φ_{ST} =0.155) was more correspondent with outcrossing, long-lived perennial species which have seeds dispersed through ingestion by animals (Nybom 2004). In addition, the pairwise Θ^B and the full model showed a genetic divergence among *E. contortisiliquum* populations which is not correlated with geographic distances. These results were interpreted as evidence of historical seed dispersal before SDTFs fragmentation. The *E. contortisiliquum* pods can be regarded as Neotropical Anachronism, whose large and indehiscent fruits were eaten by the extinct megafauna and seeds were dispersed by them (Janzen 1981a, b, Janzen and Martin 1982, Guimarães Jr et al. 2008). Thus, the extinct megafauna could have promoted gene flow through seed dispersal in the past and because of this there is no isolation by distance among *E. contortisiliquum* populations. However, the São Francisco River appears to influence the genetic structure of the species.

The UPGMA dendrogram shows that the group I was on the left bank of the São Francisco River and the three others (group II, group III and group IV) were on the right bank of the river (Figure 1). It is possible that São Francisco River acts as a geographic barrier to animals movement and consequently avoids *E. contortisiliquum* gene flow. Rodents, as agoutis, are probably the present-day *E. contortisiliquum* seed disperser. These animals could be feeding on

pods since these fruits were plentiful because of Pleistocene megafaunal extinction (Janzen and Martin 1982) and the rodents may maintain the genetic structure generated by megafauna. ISSR molecular markers have been already used to identify barriers to gene flow in tropical plants. The Espinhaço Range was detected as a geographic barrier among populations of *Vellozia gigantea* (Lousada et al. 2011) and the Cerrado biome was postulated to act as a geographic barrier to gene flow between populations of *Psychotria ipecacuanha* (Rossi et al. 2009). Rossi et al. (2009) suggest that populations of *P. ipecacuanha* investigated were relicts from a larger population before fragmentation of the Pleistocenic Arc.

The Bayesian analysis and PCA results were consistent with four clusters although MAN and GOU position in PCA were not clear. It occurs because MAN shares gene pool with group I and group II as well as GOU shares gene pool with group IV (Figure 4). This genetic differentiation among trees on opposite river banks may be related to ecological factors such as pollination and seed dispersal of *E. contortisiliquum*. Despite that the *E. contortisiliquum* pollinators are unknown, flowers are apparently pollinated by moths, as in the congener *E. cyclocarpum* (Janzen 1982, Frankie et al. 2004, Hamrick and Apsit 2004) and diurnal insects as small bees (P.A. Moreira, *personal observations*). It is possible that small pollinators cannot cross large rivers as the São Francisco. Furthermore, endozoochorous seeds can be dispersed by animals which achieve long distances but cannot cross large rivers because of its poor swimming ability (Fragoso et al. 2003).

The full model considered in Bayesian analysis of genetic structure was congruent with differentiation among populations. Moreover, full model provides evidence for inbreeding at the species level (Table 6). In spite the lack of progeny analysis and the unknowledge of mating system of *E. contortisiliquum*, biparental inbreeding was detected in its congener *E. cyclocarpum*. However, the level of biparental inbreeding was very low (Rocha and Aguilar, 2001b).

Despite the current fragmentary distribution of SDTFs be due to climatic changes during the Quaternary (Prado and Gibbs 1993; Pennington et al. 2000) the present-day deforestation caused by human activities is reducing natural habitats of *E. contortisiliquum* tree and represent a threat to this species survivor. From 13 populations analyzed in this study only MAN and MOC are located in protected areas, respectively Mata Seca State Park and Lapa Grande State Park. The most genetic diversity analyzed is outside of conservation units. These data indicate that, it is necessary creation of more protected areas to ensure the survival of *E. contortisiliquum* populations. The monitoring of these populations will also contribute to the preservation of the very much threatened Brazilian SDTF remnants.

Acknowledgments

We thank Universidade Estadual de Montes Claros for logistical support during our laboratory work, F.S. Neves for his help with field work and N.P.U. Barbosa for producing the map used here. We gratefully acknowledge a postgraduate scholarship to P.A. Moreira from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). We thank the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) (APQ-04105-10, APQ-00372-11) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (3352/2010-8) for financial support. This study is a partial fulfillment of the requirements for the doctoral degree in genetics for P.A. Moreira at Universidade Federal de Minas Gerais.

References

Bonel-Raposo J, Riet-Correa F, Guim TN, Schuch ID, Grecco FN et al. (2008) Intoxicação aguda e abortos em cobaias pelas favas de *Enterolobium contortisiliquum* (Leg. Mimosoideae). Pesquisa Veterinária Brasileira 28:593-596

Costa RLD, Marini A, Tanaka D, Berndt A, Andrade FME (2009) Um caso de intoxicação de bovinos por *Enterolobium contortisiliquum* (Timboril) no Brasil. Archivos de Zootecnia 58:313-316

Doyle JJ, Doyle JL (1987) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Espírito-Santo MM, Sevilha AC, Anaya F, Barbosa R, Fernandes GW et al. (2009) Sustainability of tropical dry forests: two case studies in southeastern and central Brazil. For Ecol Manag 258:922-930

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Fragoso JMV, Silvius KM, Correa LA (2003) Long-distance seed dispersal by tapirs increases seed survival and aggregates tropical trees: long-distance dispersal. Ecology 84:1998–2006

Frankie GW, Haber WA, Vinson SB, Bawa KS, Ronchi PS et al. (2004) Flowering phenology and pollination systems diversity in the seasonal dry forest. In: Frankie GW, Mata A, Vinson SB, editors. Biodiversity conservation in Costa Rica: learning the lessons in a seasonal dry forest. University of California Press, Berkeley. pp 17-29

Gentry AH (1995) Diversity and floristic composition of neotropical dry forests. In: Bullock SH, Mooney HA, Medina E (eds.) Seasonally Dry Tropical Forests. Cambridge University Press, Cambridge, pp 146-194

Guimarães Jr PR, Galetti M, Jordano P (2008) Seed dispersal Anachronisms: rethinking the fruits extinct megafauna ate. PLoS ONE 3:e1745.

Graham A, Dilcher D (1995) The Cenozoic record of tropical dry forest in northern Latin America and the southern United States. In: Bullock SH, Mooney HA, Medina E (eds.) Seasonally Dry Tropical Forests. Cambridge University Press, Cambridge, pp 124-145

Hamrick JL, Apsit VJ (2004) Breeding structure of neotropical dry forest tree species in fragmented landscapes. In: Frankie GW, Mata A, Vinson SB, editors. Biodiversity conservation in Costa Rica: learning the lessons in a seasonal dry forest. University of California Press, Berkeley. pp 30–37

Holsinger KE, Lewis PO (2005) Hickory: a package for analysis of population genetic data v.1.0.4. Department of Ecology and Evolutionary Biology, University of Connecticut.

Holsinger KE, Lewis PO, Dey DK (2002) A Bayesian approach to inferring population structure from dominant markers. Mol Ecol 11:1157-1164

Janzen DH (1981a) *Enterolobium cyclocarpum* seed passage rate and survival in horses, Costa Rican Pleistocene seed dispersal agents. Ecol 62:593-601

Janzen DH (1981b) Guanacaste tree seed-swallowing by Costa Rican range horses. Ecol 62:587-592

Janzen DH (1982) Variation in average seed size and fruit seediness in a fruit crop of a Guanacaste tree (Leguminosae: *Enterolobium cyclocarpum*). Am J Bot 69: 1169–1178

Janzen DH (1988) Management of habitat fragments in a tropical dry forest: growth. Ann Mo Bot Gard 75:105–116

Janzen DH, Martin PS (1982) Neotropical anachronisms: the fruits the Gomphotheres ate. Science 215:19-27

Lewontin RC (1972) The apportionment of human diversity. Evol Biol 6:381-398

Lousada JM, Borba EL, Ribeiro KT, Ribeiro LC, Lovato MA (2011) Genetic structure and variability of the endemic and vulnerable *Vellozia gigantea* (Velloziaceae) associated with

the landscape in the Espinhaço Range, in southeastern Brazil: implications for conservation. Genetica 139:431-440

Mayle F (2004) Assessmen t of the Neotropical dry forest refugia hypothesis in the light of palaeoecological data and vegetation model simulations. J Quat Sci 19:713-720

McCune B, Mefford MJ (2011) PC-ORD: multivariate analysis of ecological data, ver. 6. MjM Software, Gleneden Beach, Oregon

Miles L, Newton AC, Fries RS, Ravilious C, May I, Blyth S, Kapos V, Gordon JE (2006) A global overview of the conservation status of tropical dry forests. J Biogeogr 33:491–505

Mooney HA, Bullock SH, Medina E (1995) Introduction. In: Bullock SH, Mooney HA, Medina E (eds.) Seasonally Dry Tropical Forests. Cambridge University Press, Cambridge, pp 1.

Morrone JJ (2000) What is the Chacoan subregion? Neotropica 46:51-68

Morrone JJ (2001) Biogeografia de América Latina y el Caribe. Manuales & Tesis, Sociedad Entomológica Aragonesa, Zaragoza.

Morrone JJ (2009) Evolutionary biogeography: an integrative approach with case studies. Columbia University Press, New York.

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

Nybom H (2004) Comparison off different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol 13:143–1155

Oliveira-Filho AT (2006) Catálogo das árvores nativas de Minas Gerais: mapeamento e inventário da flora nativa e dos reflorestamentos de Minas Gerais. Editora UFLA, Lavras.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Pennington RT, Lavin M, Prado DE, Pendry CA, Pell SK, Butterworth CA (2004) Historical climate change and speciation: neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification. Phil Trans R Soc Lond B 359:515-538

Pennington RT, Lewis GP, Ratter JA (2006) An overview of the plant diversity, biogeography and conservation of neotropical savannas and seasonally dry forests. In: Pennington RT, Lewis GP, Ratter JA (eds.) Neotropical savannas and seasonally dry forests: plant diversity, biogeography and conservation. CRC Press Taylor & Francis Group, Boca Raton, London, New York, pp 1-29

Pennington RT, Prado DE, Pendry CA (2000) Neotropical seasonally dry forest and quaternary vegetation changes. J Biogeogr 27:261–273

Prado DE (2000) Seasonally dry forests of tropical South America: from forgotten ecosystems to a new phytogeographic unit. Edinburgh J Bot 57:437-461

Prado DE, Gibbs PE (1993) Patterns of species distributions in the dry seasonal forests of South America. Ann Mo Bot Gard 80:902-927

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Pritchard JK, Wena X, Falush D (2007). Documentation for Structure software, ver 2.2. http://pritch.bsd.uchicago.edu/software. Accessed 15 February 2012

Rocha OJ, Aguilar G (2001a) Reproductive biology of the dry forest tree *Enterolobium cyclocarpum* (Guanacaste) in Costa Rica: a comparison between trees left in pastures and trees in continuous forest. Am J Bot 88:1607-1614

Rocha OJ, Aguilar G (2001b) Variation in the breeding behavior of the dry forest tree *Enterolobium cyclocarpum* (Guanacaste) in Costa Rica. Am J Bot 88:1600-1606

Rohlf FJ (2000) NTSYS-pc. Numerical taxonomy and multivariate analysis system, ver. 2.2. Exeter Software, Setauket

Rossi AAB, Oliveira LO, Venturini BA, Silva RS (2009) Genetic diversity and geographic differentiation of disjunct Atlantic and Amazonian populations of *Psychotria ipecacuanha* (Rubiaceae). Genetica 136:57–67

Sánchez-Azofeira GA, Kalácska M, Quesada M, Calvo-Alvarado JC, Nassar JM, Rodrigues JP (2005) Need for integrated research for a sustainable future in tropical dry forests. Conserv Biol 19:285-286

Särkinen T, Iganci JRV, Linares-Palomino R, Simon MF, Prado DE (2011) Forgotten forests - issues and prospects in biome mapping using seasonally dry tropical forests as a case study. BMC Ecol 11:1-15

Saunders DA, Hobbs RJ, Margules CR (1991) Biological consequences of ecosystem fragmentation: a review. Conserv Biol 5:18-32

Sevilha AC, Scariot A, Noronha SE (2004) Estado atual da representatividade de unidades de conservação em florestas estacionais deciduais no Brasil. In: Sociedade Brasileira de Botânica (eds.) Biomas florestais. Annals of the 55th Congresso Nacional de Botânica, Sociedade Brasileira de Botânica, São Paulo. pp 1-63

Steininger MK, Tucker CJ, Ersts P, Killeen J, Villegas Z, Hecht SB (2001) Clearance and fragmentation of tropical deciduous forest in the Tierras Bajas, Santa Cruz, Bolivia. Conserv Biol 15:856–66.

Souza HAV, Lovato MB (2010) Genetic diversity and structure of the critically endangered tree *Dimorphandra wilsonii* and of the widespread in the Brazilian Cerrado *Dimorphandra mollis*: implications for conservation. Biochem Syst Ecol 38:49-56

Vranckx G, Jacquemyn H, Muys B, Honnay O (2011) Meta-analysis of susceptibility of woody plants to loss of genetic diversity through habitat fragmentation. Conserv Biol 26:228-237

Werneck FP (2011) The diversification of eastern South American open vegetation biomes: historical biogeography and perspectives. Quat Sci Rev 30:1630-1648

Wu B, Geng SL, Shu B (2012) Genetic variation and the conservation of isolated populations of *Derris trifoliata* (Leguminosae), a mangrove-associated vine, in southern China. Biochem Syst Ecol 40:118-125

Yeh FC, Yang RC, Boyle T (1999) POPGENE Microsoft window-based freeware for population genetic analysis: release 1.32. University of Alberta, Edmonton. http://www.ualberta.ca/~fyeh/popgene_download.html. Accessed 14 February 2012

Zhang R, Zhou Z, Du K (2012) Genetic diversity of natural populations of endangered *Ormosia hosiei*, endemic to China. Biochem Syst Ecol 40:13-18

FIGURES

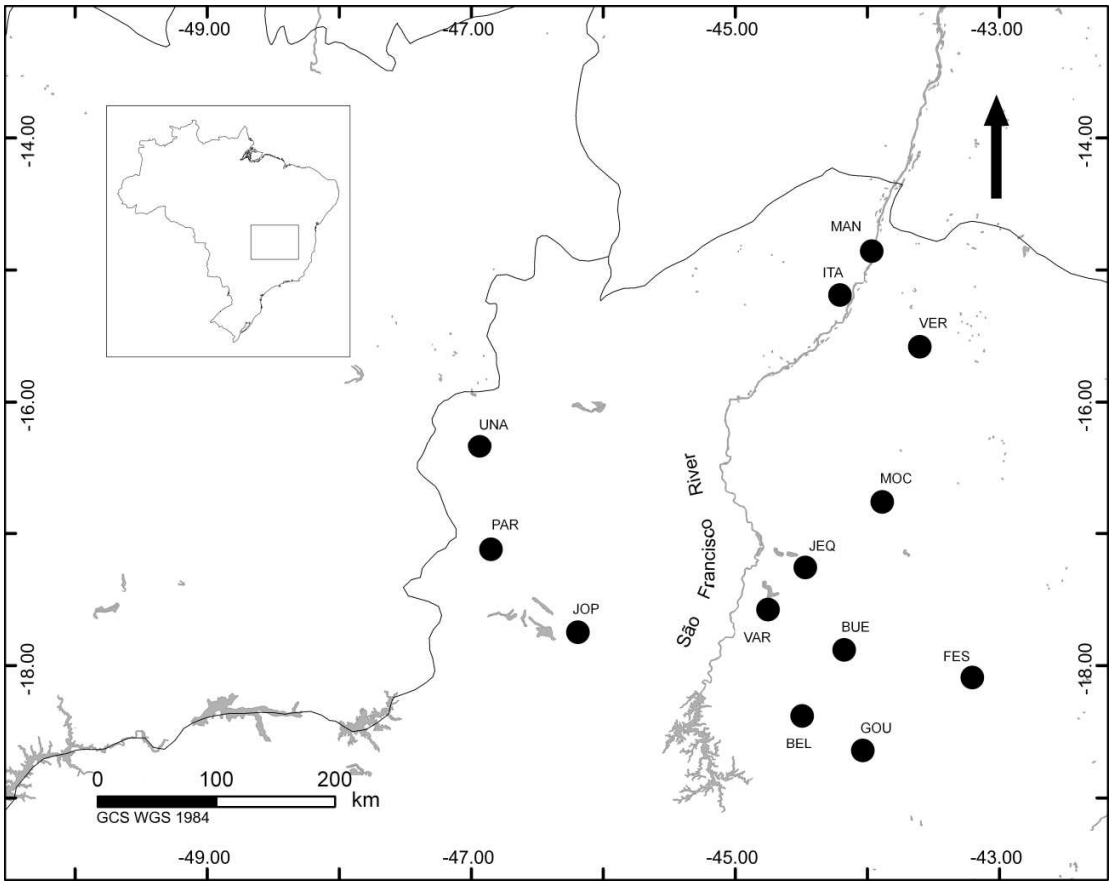


Fig. 1 Geographic distribution of the *Enterolobium contortisiliquum* populations in Brazilian seasonally dry tropical forests.

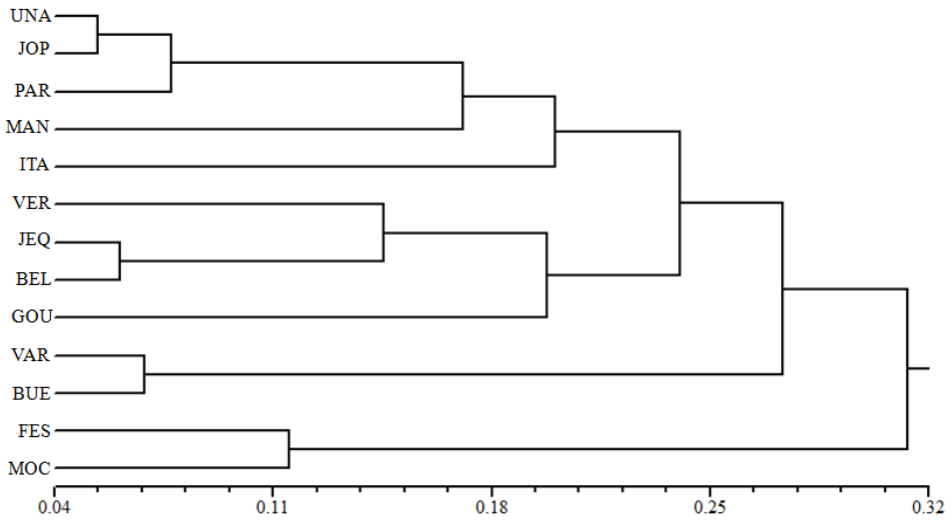


Fig. 2 UPGMA dendrogram showing the relationship based on pairwise Θ^B values among *Enterolobium contortisiliquum* populations in Brazilian seasonally dry tropical forests.

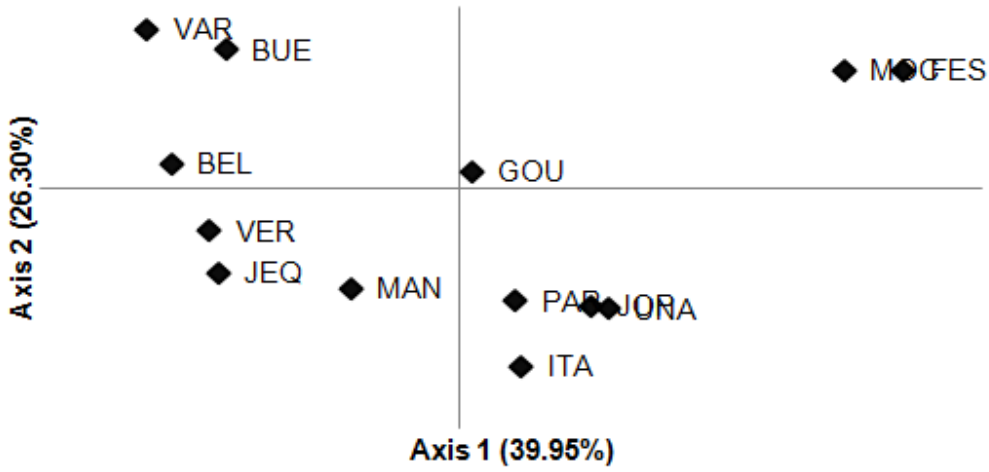


Fig. 3 Principal components analysis based on the *Enterolobium contortisiliquum* populations distance matrix. Axes 1 and 2 explained 39.95 and 26.30% of the variation, respectively.

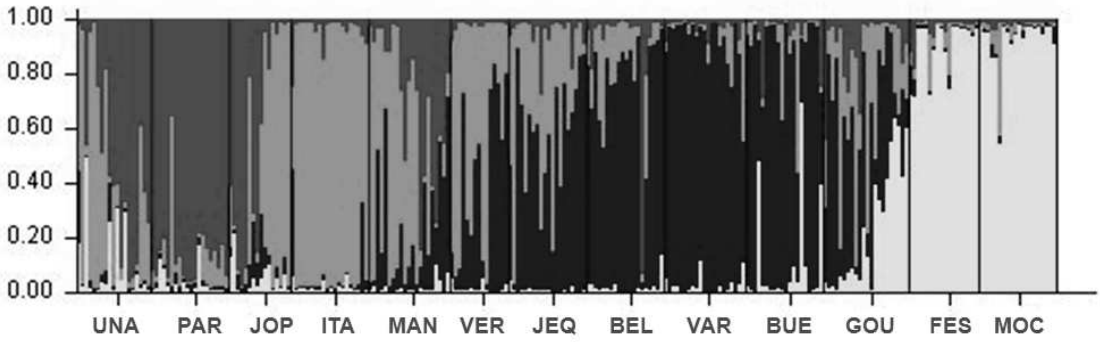


Fig. 4 Bayesian analysis of *Enterolobium contortisiliquum* individuals from Brazilian seasonally dry tropical forests remnants. Populations are separated by vertical bars.

TABLES

Table 1 Geographical location and sampling details of *Enterolobium contortisiliquum* populations studied.

Populations	Population code	Coordinates	*N*
Unaí	UNA	16°19' S 46°55' W	20
Paracatu	PAR	17°06' S 46°50 W	20
João Pinheiro	JOP	17°44' S 46°11' W	16
Felício dos Santos	FES	18°04' S 43°12'W	20
Gouveia	GOU	18°37'S 44°01' W	22
Buenópolis	BUE	17°52'S 44°10'W	20
Manga	MAN	14°50'S 43°57'W	21
Itacarambi	ITA	15°10'S 44°12'W	20
Verdelândia	VER	15°34'S 43°35'W	15
Jequitaí	JEQ	17°15'S 44°27'W	20
Várzea da Palma	VAR	17°34'S 44°44'W	29
Beltrão	BEL	18°22'S 44°29'W	20
Montes Claros	MOC	16°41'S 43°56'W	20
Total			263

N = number of trees sampled. For population locations see Figure 1.

Table 2 ISSR primers used for PCR amplifications of *Enterolobium contortisiliquum*.

Primer name	Sequence (5' – 3')[a]	Ta (°C)[b]	*N*[c]
John	$(AG)_7YC$	47	10
Manny	$(CAC)_4RC$	47	7
Mao	$(CTC)_4RC$	47	6
Terry	$(GTG)_3GGTGRC$	46	14
UBC810	$(GA)_8T$	47	9
UBC827	$(AC)_8G$	47	10
UBC840	$(GA)_8YT$	47	18
UBC879	$(CTTCA)_3$	47	6
UBC880	GGAGAGGAGAGGAGA	47	11
UBC899	$(CA)_6RG$	47	12
Total			103

[a] Y = C or T; R = A or G
[b] Annealing temperature of primers
[c] *N* = number of fragments for each primer. All fragments were polymorphic when considering all populations.

Table 3 Genetic diversity parameters of 13 *Enterolobium contortisiliquum* populations detected by ISSR analysis.

Population	*Na*	*Ne*	*hs*	*I*	*P (%)*
UNA	1.952	1.571	0.339	0.492	95.19
PAR	1.875	1.490	0.313	0.439	87.50
JOP	1.883	1.527	0.316	0.454	79.81
FES	1.711	1.463	0.254	0.390	71.15
GOU	1.701	1.392	0.277	0.349	70.19
BUE	1.721	1.342	0.255	0.315	72.12
MAN	1.779	1.414	0.273	0.369	77.88
ITA	1.740	1.472	0.272	0.392	74.04
VER	1.659	1.397	0.241	0.338	61.54
JEQ	1.798	1.431	0.259	0.385	79.81
VAR	1.711	1.308	0.269	0.302	71.15
BEL	1.769	1.406	0.279	0.370	76.92
MOC	1.762	1.458	0.298	0.401	71.15
Mean	1.774	1.436	0.280	0.384	76.03
Species	2.000	1.589	0.370	0.519	98.78

Na = number of alleles per locus, *Ne* = effective number of alleles per locus, *hs* = expected Bayesian heterozygosity (without assuming Hardy–Weinberg equilibrium), *I* = Shannon's information index, *P* = Percentage of polymorphic bands.

Table 4 Analysis of molecular variance (AMOVA) among and within *Enterolobium contortisiliquum* populations.

Source of variation	d.f.*	Sum of squares	Variance components	% Total variance	Φ_{ST}	*P*-value
Among populations	12	139.54	0.512	15.54	0.155	< 0.0001
Within populations	250	612.90	2.785	84.46		< 0.0001

*- degree of freedom.

Table 5 Matrix of population pairwise Θ^B (bellow diagonal) and geographic distance in km (above diagonal) among 13 *Enterolobium contortisiliquum* populations.

	UNA	PAR	JOP	ITA	MAN	VER	JEQ	BEL	VAR	BUE	GOU	FES	MOC
UNA	-	98	173	323	361	370	282	348	271	341	397	442	323
PAR	0.073	-	102	356	398	386	253	287	225	295	340	402	312
JOP	0.054	0.082	-	353	393	370	191	194	154	214	251	320	268
ITA	0.194	0.224	0.133	-	49	79	234	358	272	297	380	337	170
MAN	0.178	0.191	0.143	0.251	-	95	275	400	317	338	422	370	206
VER	0.238	0.286	0.175	0.244	0.199	-	210	328	253	264	346	281	129
JEQ	0.202	0.239	0.171	0.253	0.193	0.139	-	125	48	77	160	163	80
BEL	0.271	0.259	0.215	0.326	0.228	0.152	0.061	-	93	67	56	141	196
VAR	0.333	0.327	0.282	0.356	0.275	0.264	0.271	0.213	-	69	139	174	130
BUE	0.279	0.298	0.243	0.333	0.246	0.163	0.229	0.185	0.069	-	88	106	132
GOU	0.243	0.277	0.202	0.325	0.260	0.256	0.179	0.158	0.351	0.273	-	109	216
FES	0.231	0.286	0.234	0.341	0.337	0.365	0.373	0.367	0.392	0.321	0.283	-	172
MOC	0.237	0.248	0.238	0.341	0.332	0.341	0.347	0.339	0.368	0.310	0.261	0.115	-

Table 6 Model comparison of Bayesian analysis at species level.

Model	f	Θ^B	G_{ST}-B	DIC
Full	0.884	0.256	0.241	5083.02
$f = 0$		0.190	0.178	5145.92
$\Theta^B = 0$	0.912			9685.24

CAPÍTULO 2

Artigo em preparação a ser submetido ao periódico *Plos One*.

Effects of seasonally dry tropical forest fragmentation on the reproductive success of the Neotropical tree *Enterolobium contortisiliquum* (Fabaceae)

Running title: Fragmentation effects on the reproductive success of *Enterolobium contortisiliquum*

Abstract

Fragmentation is the major threat for biodiversity conservation and viable natural populations since the deforestation has modified the natural habitats into smaller and separated remnants. Thus, forest disturbance alters ecological processes such as animal-plant interactions. In this way, many researchers investigated the effects of forest fragmentation on plant reproduction. In this study, we determine the effects of tropical dry forest fragmentation by evaluating fruit and seed size, seed production, seed abortion, seed predation and seed germination on a typical seasonally tropical dry forest tree, *Enterolobium contortisiliquum*, by comparing trees present in continuous forest with trees present in fragmented matrix. Fragmented habitat condition did not affected length or width of fruits only the thickness was larger in fruits from trees of continuous habitat. None of the biometric measures differed for the seeds between trees from different habitat condition. Although total seed production was slightly higher in the fragmented habitat than in the continuous habitat, the difference was not statistically significant. The number of undamaged seeds, aborted seeds and pre-dispersal predated seeds were independent of tree condition. We did not observe any effect of forest fragmentation on germination rates. However, seeds of trees

sampled in the continuous habitat germinated faster than seeds of trees sampled in fragmented habitat. For many years researchers have evaluated the effects of forest fragmentation on the reproduction of trees. Nevertheless, trees have different responses to forest disruption. Ours results highlight the absence of clear patterns of fragmentation effects on plant reproductive success. In *E. contortisiliquum* there is no clear evidence for negative effects of forest fragmentation on its reproduction.

1. Introduction

Fragmentation of native forests has modified the natural habitats into smaller and separated remnants. Indeed, forest destruction is currently the major threat for biodiversity conservation and viable natural populations [1]. The conservation of biodiversity in the progressively more fragmented habitats requires an understanding of the effects of forest destruction on community dynamics [2]. Habitat loss implies in the reduction of species and increases the isolation among remnants, which may change the composition and abundance of species, thus altering ecological processes such as animal-plant interactions [1,3]. This is particular important for most of woody plants which needs animal vectors to reproduce sexually. Animals act in many stages of life cycle of plants and the effect of habitat alteration in them has a profound effect on plant demography and recruitment [4,5].

Due to the intimate interactions with pollinators and seed dispersers the sexual reproduction success of many plant species has been negatively affected by habitat fragmentation [4]. In addition, tropical trees are predicted to be vulnerable to the effects of habitat fragmentation due to their low densities, self-incompatibility systems and high rates of outcrossing [6]. In some cases, habitat disruption negatively affects pollinator activity, flower production, pollen

deposition, fruit and seed set and seed germination [4,6-9]. In some species, forest fragmentation increases the number of aborted seeds [10] and may affect seed predation [4,6,11] as well as seed production [12,13]. Trees in smaller isolated remnants may suffer a reduction of seed production and seed germination when compared with trees in continuous forest [6,15]. Besides, genetic estimates indicates that the progeny of trees in fragments or isolated trees tends to present lower genetic diversity, as a result of a decrease in the number of pollen donors [6,16,17]. Also, due to the scarcity of resources and increasing distance between them, pollinators can invest more time foraging within the same plant increasing the selfing rate or reducing the number of donors [6,16].

Over the last decade many researchers investigated the effects of forest fragmentation on plant reproduction. However, plants have different responses to forest disruption and results do not show clear patterns on the effects of fragmentation on some aspects of plant reproductive success [4,7, 18-24]. It has been proposed that tropical trees species could be more adaptable and resilient to habitat fragmentation than previously considered because of their longevity, high intra-population genetic diversity and high rates of pollen movement [22,23]. In 29 species out of 123 studied there was no affected by habitat disturbance in any aspect of plant reproduction success evaluated [24]. No effects of fragmentation were observed in pollen loads, number of seeds per fruit, aborted seeds, undamaged seed and progeny genetic diversity in trees of *Samanea saman* from different habitat condition [6]. In addition, genetic diversity of progenies and production of fruits and seeds were similar in isolated trees and trees from continuous populations in *Pachira quinata* [16]. The gene flow was not affected by fragmentation in *Swietenia humilis*, *Spondias mombin* and *Acer saccharum* [22,25,26,respectively].

Most of the studies on the effects of forest fragmentation have involved perennial species and only a few were performed on trees from SDTF [but see 4,6-8,11,13,17]. In spite of the large

distribution and importance of seasonally dry tropical forests (SDTF) in the tropics, these ecosystems are largely endangered [27-30]. The soils of STDF are often fertile [27] and conversion of these forests to pasture and agriculture has increased recently [31,32]. Thus, the understanding of the fragmentation effects on SDTF are needed to avoid the increased of deforestation.

In this study, we determine the effects of tropical dry forest fragmentation by evaluating fruit and seed size, seed production, seed abortion, seed predation and seed germination on a typical seasonally tropical dry forest tree, *Enterolobium contortisiliquum*, by comparing trees present in continuous forest with trees present in fragmented matrix.

2. Material and methods

2.1 Study species and area

Enterolobium contortisiliquum (Vell.) Morong is a Neotropical leguminous tree frequently found in SDTF of Brazil [33]. Flowering occurs from a short period, September and October, while their fruits ripen between June and July (P.A. Moreira, *personal observations*). The mature fruits and seeds of *E. contortisiliquum* resemble other *Enterolobium* species. Fruits are smooth, shiny, indehiscent and deep brown as in *E. cyclocarpum* [13], while seeds are hard, ovoid and brown [35]. Development of fruits occurs during almost one year and their dispersal is during dry season before its flowering. In this context, mature fruits correspond to pollination of the last year. Seeds of *E. contortisiliquum* are pre-dispersal predated by larvae of the bruchid *Merobruchus bicoloripes* (Pic, 1930) [35,36]. The Bruchidae beetles are known for their association with the Fabaceae seeds; ca. 85% of the host records for bruchids are on Fabaceae

[37]. The oviposition of bruchid females are on or near the fruits. When the eggs hatch, the larvae enter through the pericarp and go into a seed, where they undergo different larval stages. The bruchids complete their life cycle consuming one or more seeds and emerge from the fruit as adults [38].

Fruits of this tree species are poisonous for cattle. Because of this, the trees have been gradually removed from natural and agricultural areas, as a result of the advance of cattle ranching and agricultural fields in central Brazil. The ingestion of *E. contortisiliquum* pods is harmful to cattle, causing photosensitivity reactions and abortion [39,40]. As a result many farmers have cut trees of this species near their ranches because the fruiting period of *E. contortisiliquum* occurs during the dry season, coincident with low forage availability. Therefore is required understanding the effects of the fragmentation and selective logging in this tree species to generate useful information for its conservation.

The study was conducted in northern Minas Gerais state (southeastern Brazil) within and surrounding the Lapa Grande State Parks (LGSP) (ca 16°42'S, 43°56'W), a state reserve of 7.000 ha which is characterized by marked dry winters from May to September and rainy summers from November to March. The predominant climate is tropical semiarid (Aw in Köppen's classification) with average rainfall ranging from 700 to 1.200 mm and average temperature among 21 and 25°C [41]. The vegetation of LGSP is composed by cerrado and SDTF. In northern Minas Gerais SDTF has been replaced by agriculture, silviculture and extensive cattle ranching [31,42], which has resulted in a fragmented matrix with isolated trees. We studied 20 reproductive trees in continuous habitat (LGSP) and 20 reproductive trees in fragmented habitat (Figure 1). These last trees were surrounding by an altered matrix of cattle ranching, agricultural fields and farms.

2.2 Fruit and seed size

The study was conducted during the fruiting period in 2011. On each mature tree, we collected between 26-30 fruits. These fruits were produced by natural pollination in the previous year. We determined the fruit biometry measuring length (in longitudinal direction), width (in transverse direction) and thickness using a digital caliper (mm). After that, fruits were opened and all seeds were extracted in the laboratory. Seed biometry was obtained as previously described for fruits. Due to its irregular shape aborted seeds were excluded from biometric measures.

To test the forest fragmentation effects on fruits and seeds biometry we used generalized linear mixed model (GLMM) with Gaussian error distribution and logit-link function. We used the lmer function in software R for this analysis. Our model included habitat condition (continuous or fragmented) as a fixed effect and tree as a random effect nested within condition. The response variables were (1) length, (2) width; and (3) thickness of fruits and seeds.

2.3 Seed production

For each fruit, we counted the number of undamaged seeds, the number of aborted seeds, the number of pre-dispersal predated seeds by bruchid beetles and the number of total seeds produced as the sum of all previous categories. Intact seeds without injuries were considered potentially viable (undamaged seeds). The seeds were considered aborted when exhibited irregular shape with a brown and dry endosperm [8]. Bruchid beetle damage was identified by the characteristic role left on seed when adult beetle emerge [43].

To determine the effect of forest fragmentation on seed production we used generalized linear mixed model (GLMM) with Binomial error distribution and logit-link function. We used the glmer function in software R for this analysis [44]. Our model also included habitat condition (continuous or fragmented) as a fixed effect and tree as a random effect nested within condition. The response variables were (1) proportion of undamaged seeds; (2) proportion of aborted seeds; (3) proportion of pre-dispersal predated seeds by bruchid beetles; and (4) total of seeds production.

2.4 Seed germination

To verify if forest fragmentation affects seed germination we collected a sample of 10 seeds per maternal tree from each habitat condition. Seeds used in this experiment were considered as undamaged seeds in previous analysis of seed production. A total of 400 seeds were subjected to scarification by carefully sanding the seed cover to break seed dormancy. After that, seeds were placed in Petri dishes covered with a sheet of filter paper and moistened with distilled water. Then, seeds were incubated in a germination chamber (B.O.D. type) under 12 hours photoperiod with controlled temperature of 25°C on dark and 30°C on light. All Petri dishes were observed at 24 h intervals for 30 days and seeds were considered germinated once the radicle protrusion was observed [45].

The effect of forest fragmentation on seed germination was evaluated by comparing germination time and number of seeds germinated in 30 days between treatments. The germination speed was evaluated with regression analysis using Weibull parametric survival distribution. We used the survival function in software R for this analysis. The number of

germinated seeds was test with generalized linear model (GLM) with Poisson error distribution in software R [44].

3. Results

3.2 Fruit and seed size

A total of 1.196 fruits and 19.529 seeds were obtained for morphometric analysis (the sum of undamaged and pre-dispersal predated seeds). Fragmented habitat condition did not affected length (X^2 =0.10, df = 1, p = 0.75) or width (X^2 =2.50, df = 1, p = 0.11) of fruits. However, the thickness was larger in fruits from trees of continuous habitat (X^2 =5.40, df = 1, p = 0.02). None of the biometric measures differed for the seeds in trees from different habitat condition (X^2 =0.07, df = 1, p=0.79 for length, X^2 =0.81, df = 1, p=0.37 for width, and X^2 =0.31, df = 1, p=0.58 for thickness) (Table 01).

3.3 Seed production

Although total seed production was slightly higher in the fragmented habitat than in the continuous habitat, the difference was not statistically significant (X^2 =0.14, df = 1, p=0.71). The number of undamaged seeds, aborted seeds and pre-dispersal predated seeds were independent of tree condition, fragmented or continuous (X^2 =0.03, df = 1, p=0.87, X^2 =1.19, df = 1, p=0.27, X^2 =0.51, df = 1, p=0.48, respectively) (Table 2, Figure 02). Trees of both conditions produced an average of 18 seeds per fruit, of which 80-82% were undamaged or potentially viable seeds, 8-9% were aborted seeds and 9-11% was pre-dispersal predated seeds (Figure 02).

3.4 Seed germination

We did not observe any effect of forest fragmentation on germination rates of *E. contortisiliquum* (p = 0.78). However, there was a significant difference in the time of germination. Seeds of trees sampled in the continuous habitat germinated faster than seeds of trees sampled in fragmented habitat (p = 0.003) (Figure 03).

4. Discussion

Forest fragmentation had no effect on the biometrics of the fruits and seeds of *Enterolobium contortisiliquum*. Only fruit thickness differed between tree individuals inhabiting continuous and fragmented dry forests. However, seeds more thick were not formed. Our results indicate that in spite of being in disturbed sites with an altered matrix, trees in fragmented habitats did not suffer enough stress to reallocate fruit/seed development resources to other purposes. In this way, fruits and seeds from both habitat conditions were much similar in regard to their size.

Our results also indicated that the reproductive success of *E. contortisiliquum* was similar among trees in continuous and fragmented conditions. It was expected that trees in disturbed habitat produce fewer seeds since the fragmentation may affect the efficiency and activity of pollinators [13]. However, no difference was observed on total seed production. A possible explanation for this result may be related to pollinators which have deposited a quantity of pollen sufficient to maintain the same level of production of seeds in trees of continuous and fragmented habitat. Despite a lower density of trees in disturbed area, trees may not suffer from pollen

limitation. Other studies also failed to find a relationship between seed production and forest fragmentation. Other tropical dry forest trees, *Samanea saman* [6], *Ceiba aesculifolia* [8] and *Pachira quinata* [16] showed similar total seed production in disturbed and undisturbed habitats.

Fragmentation theory predicts that habitat loss reduces the number of mating partners, increases inbreeding, limits pollen availability and reduces the quality and/or quantity of seeds [46,47]. A reduction in the number of pollen sources and genetic diversity in the offspring was observed in trees of *Swietenia humilis* in fragmented patches on the Pacific Coast in the state of Jalisco, Mexico [17] and seeds of isolated *S. saman* trees were more inbred and seedling growth less vigorous [6]. However, no effect of forest fragmentation on gene flow was detected in *S. humilis* in the Punta Ratón region in Honduras [22] or in *Spondias mombin* [25] or in *Acer saccharum* [26]. Although we did not study pollen gene flow or the relatedness of the progeny produced by trees, our study suggests that pollination is not hindered in fragmented habitat because the undamaged and aborted seeds were similar in both habitat conditions. It is possible that pollinators are travelling among patches to feed and this behavior could be favoring more compatible crosses between unrelated trees. Despite *E. contortisiliquum* pollinator is unknown, flowers are apparently pollinated by moths, as congeners *E. cyclocarpum* [48,49]. Some moths are capable to fly long distances and can visit many trees in a foraging route [50]. Moths can carry large pollen loads [51] and cover great distances between consecutively visited plants [52]. In addition, a reduction of the number of potentially viable seeds and an increase of the number of aborted seeds is expected as a consequence of a negative effect of fragmentation on mating system. Nevertheless, habitat condition did not affect the production of potentially viable seeds or seed abortion in *E. contortisiliquum* as found in *C. aesculifolia* [8] and *S. saman* [6]. Habitat condition also had no effects on undamaged seed production in *P. quinata* [16] and the number of

potentially viable seeds was greater in trees in disturbed habitat in *Cercidium praecox* and *Acacia aroma*, which also shows no effect fragmentation on the number of seed abortion [10].

Seed predation of *E. contortisiliquum* was not affected by forest fragmentation, with similar predation rate of damaged seeds in continuous and fragmented habitats. Despite fruit and seed predation be a force that affects plant reproductive success [53] the effects of fragmentation on this interaction has not been well studied [11] and no pattern of the effects of habitat disturbance on fruit and seed predation was also detected. Fragmentation affected pre-dispersal predated seeds of *S. saman* [6], *C. praecox* [10] and *C. aesculifolia* [11] with greater seed predation in trees of continuous forest than isolated trees. However, no fragmentation effect on pre-dispersal seed predation was detected in this study with *E. contortisiliquum* as in the tropical tree species *A. aroma* [10] and the temperate tree species *Nothofagus glauca*, which has seeds predated by the *Perzelia* sp. microlepidopteran larva [54]. Adult insects associated with *E. contortisiliquum* fruits and nymphs found in attacked seeds which metamorphosed into adults confirmed bruchid *Merobruchus bicoloripes* as seed predator. It is possible that fragmentation did not depress *M. bicoloripes* population on fragmented habitat or the matrix between studied areas has enough resources to allow bruchid movement and consequently maintain the same pattern of attack seeds.

Forest disturbance may affect seed germination [6,15,25,55] and our results suggest that in *E. contortisiliquum* time to germinate was negatively influenced by forest fragmentation. The germination time was significantly greater for seeds of trees in continuous habitat than for seeds of trees in fragmented habitat. Although time to germination was affected by forest disturbance seeds obtained from trees of both habitat conditions showed the same germination rate. It is expected that seeds produced in forest patches suffer more inbreeding [56] and, consequently, a reduction in germination rates [15]. Even though we did not study gene flow of individuals we

believe that inbreeding depression is not responsible for difference on seed germination time because viable and aborted seed were the same for trees from both conditions. As assumed above, it is possible that there is no pollen limitation and pollinators could be favoring compatible crosses between unrelated trees. Hence, a possible explanation for the germination result is the environment of fragmented habitat. Trees in altered area occur in more disturbed soil with lower water availability and fluxes of radiation and air altered [1]. Therefore, this environmental condition and maternal factors other than those from genetic paternal effects could be responsible for the findings [57].

Agreeing with our results, progeny from trees in continuous populations of *E. cyclocarpum* took less time to germinate [13] and no effect of habitat disturbance was observed on seed germination in *N. glauca* [54] or in *Astrocaryum mexicanum* [58] or in seedling emergence in *Macadamia integrifolia* [59]. However, trees in fragmented populations suffered a reduction in germination rate in *E. cyclocarpum* [13], *S. mombin* [25] and *S. saman* [6], although trees of the last species in different habitat condition did not show difference between days to seed germination [6].

For many years researchers have evaluated the effects of forest fragmentation on trees reproduction. However, trees have different responses to forest disruption. Negative effect has been detected on the reproductive success of some species [7-11,15,16,54,60-62]. On the other hand, no effect of deforestation was observed in some reproductive success parameters [6,8,10,54,60]. Even a positive effect of forest fragmentation was detected in some tree species [8,25,60,63]. Ours results highlight the absence of clear patterns of fragmentation effects on plant reproductive success. In *E. contortisiliquum* there is no clear evidence for negative effects of forest fragmentation on its reproduction.

We thank Universidade Estadual de Montes Claros for logistical support during our laboratory work, SAS Sousa and FA Oliveira for their kind help with laboratory work, M.L. Oliveira and M. L. Souza for statistical assistance and N.P.U. Barbosa for producing the map used here. We thank CS Ribeiro-Costa from Universidade Federal do Paraná for insect identification. We are very grateful to the Instituto Estadual de Florestas (IEF) for logistical support during field work. We gratefully acknowledge a postgraduate scholarship to P.A. Moreira from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). We thank the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) (APQ-04105-10, APQ-00372-11) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (3352/2010-8) for financial support. This study is a partial fulfillment of the requirements for the doctoral degree in genetics for P.A. Moreira at Universidade Federal de Minas Gerais.

References

1. Saunders DA, Hobbs RJ, Margules CR (1991) Biological consequences of ecosystem fragmentation: a review. Conserv Biol 5: 18-32.
2. Burkey TV (1993) Edge effects in seed and egg predation at two neotropical rainforest sites. Biol Conserv 66: 139-143.
3. Debinski DM, Holt RD (2000) A survey overview of habitat fragmentation experiments. Conserv Biol 14: 342-355.
4. Aizen MA, Feinsinger P (1994) Forest fragmentation, pollination, and plant reproduction in a Chaco Dry Forest, Argentina. Ecol 75: 330-351.
5. Dirzo R, Miranda A (1991) Altered patterns of herbivory and diversity in the forest understory: a case study of the possible consequences of contemporary defaunation. In: Price PW, Lewinsohn TW, Fernandes GW, Benson WW, editors. Plant-animal

interaction: evolutionary ecology in tropical and temperate regions. New York: Wiley. pp. 273-287.

6. Cascante A, Quesada M, Lobo JA, Fuchs EA (2002) Effects of dry forest fragmentation on the reproductive success and genetic structure of the tree *Samanea saman.* Conserv Biol 16: 137-147.

7. Aizen MA, Feinsinger P (1994) Habitat fragmentation, native insect pollinators, and feral honey bees in Argentine "Chaco Serrano". Ecol Apl 7: 378-392.

8. Herrerías-Diego Y, Quesada M, Stoner KE, Lobo JA (2006) Effects of forest fragmentation on phenological patterns and reproductive success of the tropical dry forest tree *Ceiba aesculifolia.* Conserv Biol 20: 1111-1120.

9. Quesada M, Stoner KE, Rosas-Guerrero V, Palacios-Guevara C, Lobo JA (2003) Effects of habitat disruption on the activity of nectarivorous bats (Chiroptera: Phyllostomidae) in a dry tropical forest: implications for the reproductive success of the neotropical tree *Ceiba grandiflora*. Oecologia 135: 400-406.

10. Chacoff N, Morales JM, Vaquera MP (2004) Efectos de la fragmentación sobre la aborción y depredación de semillas em el Chaco Serrano. Biotropica 36: 109-117.

11. Herrerías-Diego Y, Quesada M, Stoner KE, Lobo JA, Hernández-Flores Y et al. (2008) Effect of forest fragmentation on fruit and seed predation of the tropical dry Forest tree *Ceiba aesculifolia*. Biol Conserv 141: 241-248.

12. Aguilar R, Galetto L (2004) Effects of forest fragmentation on male and female reproductive success in *Cestrum parqui* (Solanaceae). Oecologia 138: 513-520.

13. Rocha OJ, Aguilar G (2001) Reproductive biology of the dry forest tree *Enterolobium cyclocarpum* (Guanacaste) in Costa Rica: a comparison between trees left in pastures and trees in continuous forest. Am J Bot 88: 1607-1614.

14. Harper JL (1977) Population biology of plants. London: Academic Press. 892 p.

15. Bruna E (1999) Seed germination in rainforest fragments. Nat 402: 139.

16. Fuchs EJ, Lobo JA, Quesada M (2003) Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropical dry forest tree *Pachira quinata*. Conserv Biol 17: 149-157.

17. Rosas F, Quesada M, Lobo JA, Sork V (2011) Effects of habitat fragmentation on pollen flow and genetic diversity of the endangered tropical tree *Swietenia humilis* (Meliaceae). Biol Conserv 144: 3082-3088.

18. Donaldson J, Nänni I, Zachariades C, Kemper J (2002) Effects of habitat fragmentation on pollinator diversity and plant reproductive success in Renosterveld Shrublands of South Africa. Conserv Biol 16: 1267-1276.

19. Hobb RJ, Yates CJ (2003) Impacts of ecosystem fragmentation on plant populations: generalizing the idiosyncratic. Aust J Bot 51: 471–488.

20. Aguilar R, Ashworth L, Galetto L, Aizen MA (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecol Lett 9: 968–980.

21. Steffan-Dewenter I, Westphal C (2008) The interplay of pollinator diversity, pollination services and landscape change. J Appl Ecol 45: 737– 741.

22. White GM, Boshier DH, Powell W (2002) Increased pollen flow counteracts fragmentation in a tropical dry forest: an example from *Swietenia humilis* Zuccarini. Proc Natl Acad Sci 99: 2038-2042.

23. Hamrick JL (2004) Response of forest trees to global environmental changes. For Ecol Manag 197: 323-335.

24. Ghazoul (2005) Pollen and seed dispersal among dispersed plants. Biol Rev 80: 413-443.

25. Nason JD, Hamrick JL (1997) Reproductive and genetic consequences of forest fragmentation: two case studies of neotropical canopy trees. J Hered 88: 264-276.

26. Foré SA, Hickey RJ, Vankat JL, Guttman SI, Schaefer RL (1992) Genetic structure after forest fragmentation: a landscape ecology perspective on *Acer saccharum*. Can J Bot 70: 1659-1668.

27. Murphy PG, Lugo AE (1986) Ecology of tropical dry forests. Annu Rev Ecol Syst 17: 67–88.

28. Janzen DH (1988) Management of habitat fragments in a tropical dry forest: growth. Ann Mo Bot Gard 75:105–116.

29. Sánchez-Azofeifa GA, Kalacska M, Quesada M, Calvo-Alvarado JC, Nassar JM et al. (2005) Need for integrated research for a sustainable future in tropical dry forests. Conserv Biol 19: 1–2.

30. Sánchez-Azofeifa GA, Quesada M, Cuevas-Reyes P, Castillo A, Sánchez-Montoya G (2009) Land cover and conservation in the area of influence of the Chamela-Cuixmala Biosphere Reserve, Mexico. For Ecol Manag 258: 907–912.

31. Espírito-Santo MM, Sevilha AC, Anaya F, Barbosa R, Fernandes GW et al. (2009) Sustainability of tropical dry forests: two case studies in southeastern and central Brazil. For Ecol Manag 258: 922-930.

32. Mass JM (1995) Conservation of tropical dry forest to pasture and agriculture. In: Bullock SH, Mooney HA, Medina E, editors. Seasonally dry tropical forests. New York: Cambridge University Press. pp. 399-422.

33. Oliveira-Filho AT (2006) Catálogo das árvores nativas de Minas Gerais: mapeamento e inventário da flora nativa e dos reflorestamentos de Minas Gerais. Lavras: Editora UFLA. 423 p.

34. Janzen DH (1982) Variation in average seed size and fruit seediness in a fruit crop of a Guanacaste tree (Leguminosae: *Enterolobium cyclocarpum*). Am J Bot 69: 1169–1178.

35. Link D, Costa EC (1995) Danos causados por insetos em sementes de timbaúva, *Enterolobium contortisiliquum* (Vell.) Morong. Ciência Florestal 5: 113-122.

36. Morandini MN, Viana ML (2009) Depredación pre-dispersiva de semillas en tres poblaciones del árbol *Enterolobium contortisiliquum* (Fabaceae). Int J Trop Biol 57: 781-788.

37. Johnson CD (1985) Potential useful tropical Legumes and their relationships with Bruchid beetles. In: Misra KC, editor. Ecology resource management in tropics: resented papers, silver jubilee Symposium of International Society for Tropical Ecology. Varanasi: Bhargava Book Depot, pp. 206-223.

38. Janzen DH (1969) Seed eaters versus seed size, number, toxicity and dispersal. Evol 23:1-27.

39. Bonel-Raposo J, Riet-Correa F, Guim TN, Schuch ID, Grecco FN et al. (2008) Intoxicação aguda e abortos em cobaias pelas favas de *Enterolobium contortisiliquum* (Leg. Mimosoideae). Pesquisa Veterinária Brasileira 28: 593-596.

40. Costa RLD, Marini A, Tanaka D, Berndt A, Andrade FME (2009) Um caso de intoxicação de bovinos por *Enterolobium contortisiliquum* (Timboril) no Brasil. Archivos de Zootecnia 58: 313-316.

41. Antunes, FZ (1994) Caracterização climática – Caatinga do estado de Minas Gerais. Informe Agropecuário 17: 15-19.

42. Rodrigues L (2000) Formação econômica do norte de Minas e o período recente. In: Oliveira MFM, Rodrigues L, Machado JMA, Botelho TR, editors. Formação social e econômica do norte de Minas Gerais. Montes Claros: Editora Unimontes. pp. 105-170.

43. Janzen DH (1977) The interaction of seed predators and seed chemistry. In: Labeyrie V, editor. Comportement des insects et milieu trophique. Paris: Colloques Internationaux du C.N.R.S. pp. 415-428.

44. R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

45. Gomes V, Madeira JA, Fernandes GW, Lemos-Filho JP (2001) Seed dormancy and germination of sympatric species of *Chamaecrista* (Leguminosae) in a rupestrian field. Int J Ecol Environ Sci 27: 191-197.

46. Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Annu Rev Ecol Syst 18: 237-268.

47. Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. Annu Rev Ecol Syst 24: 217-242.

48. Frankie GW, Haber WA, Vinson SB, Bawa KS, Ronchi PS et al. (2004) Flowering phenology and pollination systems diversity in the seasonal dry forest. In: Frankie GW, Mata A, Vinson SB, editors. Biodiversity conservation in Costa Rica: learning the lessons in a seasonal dry forest. Berkeley: University of California Press. pp. 17-29.

49. Hamrick JL, Apsit VJ (2004) Breeding structure of neotropical dry forest tree species in fragmented landscapes. In: Frankie GW, Mata A, Vinson SB, editors. Biodiversity conservation in Costa Rica: learning the lessons in a seasonal dry forest. Berkeley: University of California Press. pp. 30–37

50. Haber WA, Frankie GW (1989) A tropical hawkmoth community: Costa Rican dry forest Sphingidae. Biotropica 21: 155–172.

51. Willmont AP, Burquez A (1996) The pollination of *Merremia palmeri* (Convolvulaceae): can hawk moths be trusted? Am J Bot 83: 1050-1056.

52. Linhart YB, Mendenhall JA (1977) Pollen dispersal by hawkmoths in a Lindenia rivalis Benth population in Belize. Biotropica 9: 143-143.

53. Schupp EW (1988) Factors affecting post-dispersal seed survival in a tropical forest. Oecologia 76: 525–530.

54. Burgos A, Grez A, Bustamante RO (2008) Seed production, pre-dispersal seed predation and germination of *Nothofagus glauca* (Nothofagaceae) in a temperate fragmented forest in Chile. For Ecol Manag 255: 1226–1233.

55. Menges ES (1991) Seed germination percentage increases with population size in a fragmented prairie species. Conserv Biol 5: 158-164.

56. Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. Trends Ecol Evol 11:413–418.

57. Stephenson AG (1992) The regulation of maternal investment in plants. In: Marshall C, Grace J, editors. Fruit and seed production. New York: Cambridge University Press. pp. 151-171.

58. Aguirre A, Dirzo R (2008) Effects of fragmentation on pollinator abundance and fruit set of an abundant understory palm in a Mexican tropical forest. Biol Conserv 141: 375 - 384.

59. Neal JM, Hardner CM, Gross CL (2010) Population demography and fecundity do not decline with habitat fragmentation in the rainforest tree *Macadamia integrifolia* (Proteaceae). Biol Conserv 143: 2591 -2600.

60. Quesada M, Stoner KE, Lobo JA, Herrerías-Diego Y, Palacios-Guevara C et al. (2004) Effects of forest fragmentation on pollinator activity and consequences for plant reproductive success and mating patterns in bat-pollinated Bombacaceous trees. Biotropica 36: 131-138.

61. Cunningham SA (2000) Effects of habitat fragmentation on the reproductive ecology of four plant species in Mallee Woodland. Conserv Biol 14: 758-768.

62. Cunningham SA (2000) Depressed pollination in habitat fragments causes low fruit set. Proc R Soc Lond 267: 114 9-1152.

63. Dick CW (2001) Genetic rescue of remnant tropical trees by an alien pollinator. Proc R Soc Lond 268:2391–2396.

FIGURES

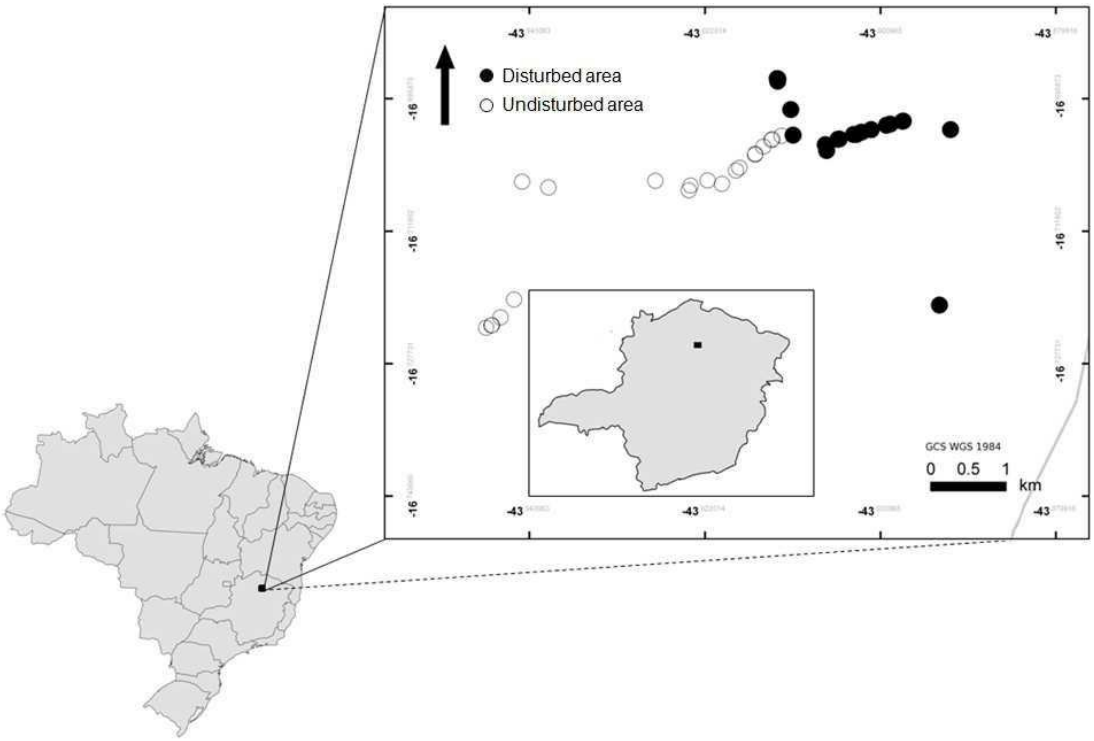


Figure 1- Spatial distribution of *Enterolobium contortisiliquum* trees in the study area. Empty circles represent trees sampled in a undisturbed area inside the Lapa Grande State Park and black circles are trees sampled in a disturbed area where they are surrounded by agriculture fields and pastures.

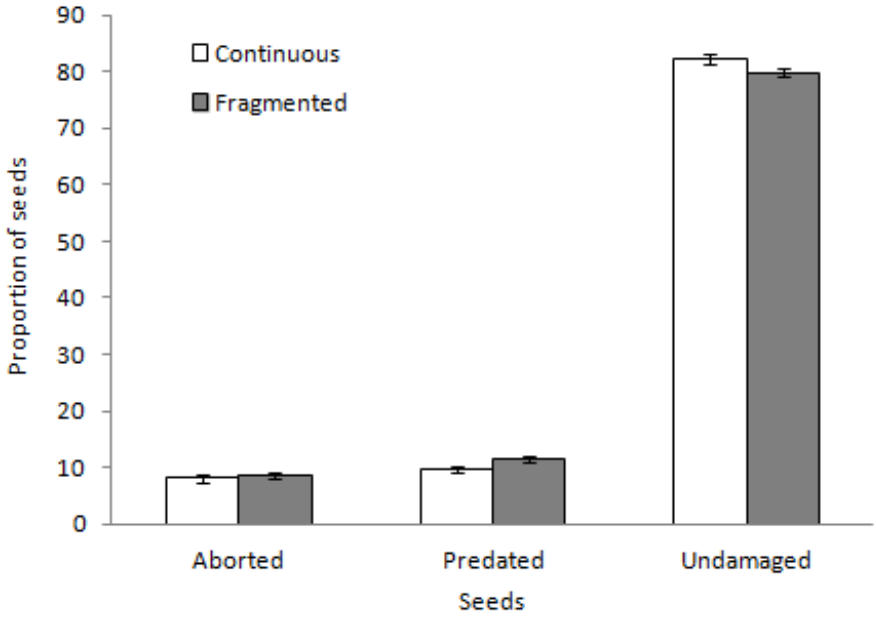


Figure 2- Proportion of aborted seeds, predated seeds and undamaged seeds of *Enterolobium contortisiliquum* in undisturbed and disturbed area.

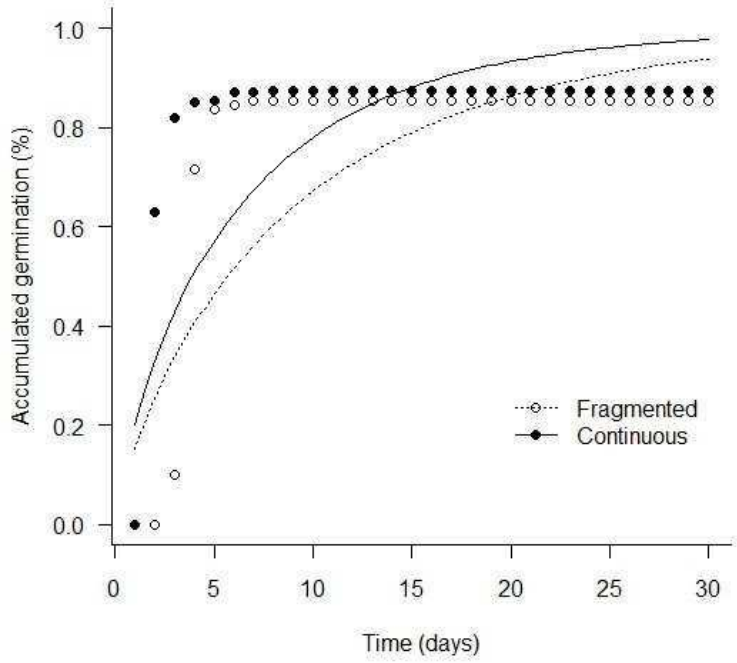


Figure 3- Accumulated percentage of the seeds germination of *Enterolobium contortisiliquum* trees in undisturbed and disturbed area.

TABLES

Table 01 – Morphometric measures (average ± SE) of fruits and seeds of *Enterolobium contortisiliquum* trees in continuous and fragmented habitats. Different letters indicate significant differences between the parameters.

	Fruits		Seeds	
Habitat	Continuous	Fragmented	Continuous	Fragmented
Length (mm)	67.03±0.43	67.74±0.45	12.38±0.15	12.55±0.25
Width (mm)	37.81±0.25	40.49±0.39	7.45±0.13	7.20±0.11
Thickness (mm)	13.27±0.15 [a]	12.13±0.14[b]	5.13±0.01	5.36±0.42

Table 02 – Average number of undamaged, aborted, predated and total of seeds production per fruit of *Enterolobium contortisiliquum* trees in continuous and fragmented habitats.

Habitat	Undamaged seeds	Aborted seeds	Predated seeds	Total of seeds
Continuous	14.54	1.26	1.75	17.55
Fragmented	14.36	1.50	2.01	17.87
Total	14.45	1.38	1.88	17.71

CAPÍTULO 3

Artigo em preparação a ser submetido ao periódico *Biological Conservation*.

Effects of different habitat disturbances on phenological patterns of the seasonally dry tropical forest tree *Enterolobium contortisiliquum* (Fabaceae)

Running title: Fragmentation effects on the phenological patterns of *Enterolobium contortisiliquum*

Abstract

Seasonally tropical dry forest (SDTF) is one of the most threatened ecosystems in the world. Brazilian SDTFs are endangered because of human occupation, conversion of lands to agriculture and deforestation. The replacement of forests to pasture and cropland increases temperature and decreases precipitation. In this context, changing in regional climate caused by deforestation may alter phenological patterns of trees. We sought to determine the effects of habitat disturbance on phenological patterns of the SDTF tree *Enterolobium contortisiliquum* reviewing reproductive phenophases and vegetative phenophases in areas with different habitat disturbances. The patterns of flowering observed were similar to other SDTF. Despite this, a difference was detected among areas. Flower buds occurred first in urban area and anthesis was recorded first in disturbed and urban areas. Besides, urban area exhibited lowest activity index of flower buds and flowering and lowest percentage of intensity of both phenophases. Immature fruits were first observed in undisturbed and disturbed areas with more production in disturbed area. Leaf flushing was most pronounced in urban area. The intensity of leaf emergence was too small in disturbed area and the intensity of flushing was different among populations. The lowest activity

of green leaves was detected in undisturbed area and the highest leaf abscission in disturbed area. The NMDS ordination showed clear differences among trees which were clustered according to its phenological data in three groups formed according to its habitat condition. Our results suggest that habitat disturbance may trigger differences in phenological responses of *E. contortisiliquum* trees.

1. Introduction

Seasonally dry tropical forests (SDTF) are tropical forests formations that occur in regions characterized by pronounced seasonality in rainfall distribution, where precipitation is less than 1600 mm/yr, and by several months of drought, with a period at least five or six months receiving less than 100 mm (Gentry, 1995; Graham and Dilcher, 1995). SDTFs are dominated by deciduous trees with a more or less continuous canopy (Mooney et al., 1995; Sánchez-Azofeifa et al., 2005). The most dominant families are Fabaceae and Bignoniaceae (Gentry, 1995; Mayle, 2004).

This ecosystem exhibit a disjunct distribution scattered throughout the Neotropics (Pennington et al., 2006) and is one of the most threatened ecosystems in the world (Janzen, 1988; Miles et al., 2006; Steininger et al., 2001). Despite this, SDTF is the least protected forest and its natural areas have been replaced by pasture and agriculture (Mass, 1995; Espírito-Santo et al., 2009). The largest areas of SDTF are found in South America, specifically in Brazil (Pennington et al., 2004), where it represents 3.21% of its territory (Sevilha et al., 2004). As in other localities, the Brazilian SDTFs are associated with fertile soils with a moderate to high pH and high nutrient content (Espírito-Santo et al., 2009; Pennington et al., 2006). As a result, Brazilian SDTFs are endangered because of human occupation, conversion of lands to agriculture

and deforestation. In this way, SDTF can be considered the most threatened ecosystem in Brazil (Espírito-Santo et al., 2009).

Deforestation modifies natural habitats into smaller and isolated remnants and decreases density of tree population. The spatial isolation and reduction of populations may negatively affect the reproductive success of tropical tree species. In some cases, habitat disruption negatively affects pollinator activity, flower production, pollen deposition, fruit and seed set and seed germination (Aizen and Feinsinger, 1994a, 1994b; Cascante et al., 2002; Herrerías-Diego et al., 2006; Quesada et al., 2003). In addition, due to the scarcity of resources and increasing distance between them, pollinators can invest more time foraging within the same plant increasing the selfing rate or reducing the number of donors (Cascante et al., 2002; Fuchs et al., 2003).

Habitat fragmentation may have important consequences to local climate. The replacement of forests to pasture and cropland increases temperature and decreases precipitation (Sampaio et al., 2007). In this context, changes in local climate caused by deforestation may alter phenological patterns of trees which are influenced by them (Primack et al., 2009; Jochner et al., 2011). Temporal isolation caused by asynchronous flowering is predicted to have negative consequences for success reproduction and genetic structure of plants in fragmented habitat (Murawski and Hamrick, 1992; Nason and Hamrick, 1997). The gene flow via pollen is determined by flowering phenophase which means that the number of pollen donors and the density of flowering individuals are both very important (Murawski and Hamrick, 1992).

Due to the relevance and threat to SDTF, understanding how tree species of this ecosystem will respond to climate change and deforestation is necessary for conservation strategies (Giraldo and Holbrook, 2011). Thus, phenological studies of SDTF tree species may help us to comprehending plant responses to climate condition of this region (Fournier, 1974)

where seasonality is outstanding and phenological patterns are particularly important to understanding dry forests function (Justiniano and Fredericksen, 2000).

We sought to determine the effects of habitat disturbance on reproductive and vegetative phenology of the SDTF tree *Enterolobium contortisiliquum* (Vell.) Morong reviewing the following reproductive phenophases: (1) flower buds, (2) flowering opening, (3) fruiting, and (4) ripe fruit; and the following vegetative phenophases: (5) leaf flushing, (6) mature leaves, and (7) leaf fall. We expect that deforestation may affect the percentage of intensity (Fournier, 1974) and the activity index of theses phenophases (Bencke and Morellato, 2002).

2. Materials and methods

2.1. Study species

Enterolobium contortisiliquum (Vell.) Morong is a Neotropical tree species belonging to the most SDTF dominant family Fabaceae (Gentry, 1995; Mayle, 2004) which is consider as specialist of SDTF (Särkinen et al., 2011). The ripe fruits and seeds of *E. contortisiliquum* resemble other *Enterolobium* species. As *E. cyclocarpum*, its fruits are smooth, shiny, indehiscent and deep brown (Rocha and Aguilar, 2001) while seeds are hard, ovoid and brown (Janzen, 1982) and pre-dispersal predated by larvae of the bruchid *Merobruchus bicoloripes* (Pic, 1930) (Link and Costa, 1995; Morandini and Viana, 2009). The *E. contortisiliquum* pods can be regarded as Neotropical Anachronism, whose large and indehiscent fruits were probably eaten by extinct megafauna and seeds were dispersed by them (Janzen, 1981a, 1981b; Janzen and Martin, 1982). It is believed that rodents are the present-day *E. contortisiliquum* seed dispersers. The tree produces several inflorescences with white flowers and despite *E. contortisiliquum* pollinators are

unknown, it is believed that this species is pollinated by nocturnal insects as moths, similar to its congeners *E. cyclocarpum* (Frankie et al., 2004; Hamrick and Apsit, 2004; Janzen, 1982) and diurnal insects as small bees (P.A. Moreira, *personal observations*).

2.2. Study area and sampling

The study was conducted in northern Minas Gerais state (southeastern Brazil) in Montes Claros city and within and surrounding the Lapa Grande State Park (LGSP) (ca 16°42'S, 43°56'W). The LGSP encompasses 7.000 ha and is characterized by marked dry winters from May to September and rainy summers from November to March. The predominant climate is tropical semiarid (Aw in Köppen's classification) with average rainfall ranging from 700 to 1.200 mm and average temperature among 21 and 25°C (Antunes 1994). The original vegetation of LGSP is composed by cerrado and SDTF. In northern Minas Gerais SDTF has been replaced by agriculture, silviculture and extensive cattle ranching (Espírito-Santo et al., 2009; Rodrigues, 2000), which has resulted in a fragmented matrix with isolated trees.

To test the effects of different habitat disturbances in *E. contortisiliquum* phenology, we compared trees in three habitat conditions: undisturbed, disturbed and urban. Trees in undisturbed area are located inside the park in a conserved matrix while trees in disturbed area are located outside of the park surrounding by an altered matrix of cattle ranching, agricultural fields and farms. Trees in the urban area were within urban habitat consisting of residential and commercial buildings which is more complex than matrices of other landscapes, as agricultural fields (Culley et al., 2007). We studied 15 adult trees of *E. contortisiliquum* in disturbed and undisturbed habitats. In urban habitat 13 trees were studied because two trees were cut off during the study (Fig. 1).

2.3. Phenological patterns and data analysis

The phenology of each tree was recorded monthly from January to December in 2011. However, the phenological data will be registered until December of 2012. The phenophases were surveyed through careful observation of the canopy using binoculars when necessary. The following reproductive phenophases were registered: (1) flower buds (pre-anthesis), (2) flowering opening (anthesis), (3) fruiting (immature fruits), and (4) ripe fruit (brown fruits). The following vegetative phenophases were also recorded: (5) leaf flushing (recently emerged leaves with bright green color), (6) mature leaves (developed leaves with dark green color), and (7) leaf fall (senescent dry leaves with brownish color).

To quantify the phenological events the percentage of phenophase intensity (Fournier, 1974) and activity index (Bencke and Morellato, 2002) were estimated. The percentage of phenophase intensity was used in a semi-quantitative scale of five categories (0 to 4) with a 25% interval between them. The sum of intensity obtained for all individuals of each habitat was divided by the maximum possible value (number of total individuals studied multiplied by four) in each month. The value obtained corresponds to a proportion and then this value is multiplied by 100 to turn it into a percentage value. The activity index indicates the percentage of individuals expressing an event at the same moment of sampling, with the presence or absence of some particular phenophase being recorded. At the population level the activity index is quantitative and refers to the percentage of individuals in the population that is displaying a particular phenological event. Through this method the synchrony between individuals in a population can be estimated (Bencke and Morellato, 2002).

To verify if trees in distinct habitats are differently affected by meteorological parameters the phenological data were correlated with total precipitation and average temperature, in the same period (month) (Fig. 2). Spearman's non-parametric correlation tests were performed (Zar, 1996). In addition non-metric multidimensional scaling (NMDS) was used to check for differences among trees according to its phenological data between habitats using the Jaccard index computed from presence/absence of the phenophases. To test if the structure of trees associated with phenological patterns differs among habitat condition an analysis of similarity was performed (ANOSIM, Clarke, 1993). These analyses were conducted using the PAST software (Hammer et al., 2001).

3. Results

3.1. Reproductive phenology

Flower buds phenophase was concentrated in late dry season. In all three areas flower buds started in September, but urban area showed a major peak of flower bud in this month and undisturbed and disturbed areas showing a major peak in October, one month later. Furthermore, the end of flower buds occurred on November in disturbed and urban areas and one month later (December) in undisturbed habitat (Fig. 3). This last habitat exhibited the lowest percentage of this phenophase (Fig. 4). Despite undisturbed and disturbed areas provided the same activity index of flower buds, the percentage of intensity was lower in undisturbed area than disturbed (Fig. 4). Flowering occurred during the transition from the dry to wet season in all sites. The anthesis started in September in urban and disturbed areas and in undisturbed area was in October. In this month, a major peak of this activity index was observed in all habitats (Fig. 3).

Once more, the urban area exhibited the lower activity index and percentage of intensity of flowering. Despite undisturbed area showed lower activity index of flowering than disturbed area, the percentage of intensity was similar in the same time (Fig. 4). *Enterolobium contortisiliquum* bloom just before first rains, as soon as its congeners *E. cyclocarpum* (Frankie et al., 1974).

Fruiting phenology started in the wet season and finished in the middle of dry season in all habitats. The activity of index and percentage of intensity of fruiting varied among areas (Fig. 3 and 4). Fruiting in undisturbed and disturbed areas started in February and in March in urban area (Fig. 3). Disturbed area exhibited the higher activity index of this phenophase (Fig. 3) but the percentage of intensity was low, as soon as undisturbed and urban areas (Fig. 4). The major peak of activity index was in the same month of major peak of percentage index for all sites, but the month was different between then occurring in April in disturbed area, in May in undisturbed area and in June in urban area (Fig. 4). Despite this, the activity index and percentage of intensity of ripe fruits were similar between undisturbed and disturbed areas, where major peak of activity index and percentage of this phenophase was in July. In urban area the major peak of activity index and percentage of ripe fruits was in August. However, ripe fruits occurred during all months every area (Fig. 3 and 4).

3.2. Vegetative phenology

At the end of wet season a slightly leaf flushing was recorded for all areas but major peak of activity index and percentage of intensity of flushing was registered in September in all populations just before first rains (Fig. 3, 5) with undisturbed area showing the lowest percentage of intensity (Fig. 6). A major peak of activity index of leaf fall was registered from April to

August in disturbed area with the peak of percentage of intensity in July. In undisturbed area the peak of activity index was recorded in June and the peak of percentage of intensity in April. The peak of activity of leaf shedding in urban area was from May to July with the major peak of percentage of intensity occurring in March, May and July. The maximum activity index of mature leaves (dark green leaves) was observed during all year in undisturbed and disturbed areas, except in August when leaf loss of the last months decreased mature leaves (Fig. 5). A high activity index of mature leaves was registered in urban area also, with a slight exception occurring during March, July and August (Fig. 5). In all areas mature leaves were registered until mid-August, just before the occurrence of leaf flushing. During the end of the rains, a decrease of mature leaves intensity was verified and the increased of leaves was recorded before the first rains (Fig. 6).

3.3. Phenophases versus climate variables

The influence of climatic variables was different between areas. Trees of *E. contortisiliquum* in undisturbed area were positively affected by precipitation and temperature only in the intensity of mature leaves phenophase. There were no interactions between climatic variables and reproductive phenology in undisturbed area (Table 1). However, the activity and intensity of ripe fruits were negatively influenced by precipitation and temperature in disturbed area. As observed in undisturbed area the intensity of mature leaves was positively affected by climatic variables in the disturbed area. Besides that, the activity of leaf fall was negatively influenced by precipitation and temperature and the intensity of this phenophase was negatively affected only by precipitation (Table 1).

In urban area as well as disturbed area the intensity of ripe fruits were negatively influenced by climatic variables (Table 1). The activity of leaf senescence was negatively affected only by temperature while the intensity of mature leaves was positively influenced by both climatic variables. The intensity of this last phenophase was the only phenological characteristic correlated with precipitation and temperature in all different areas (Table 1).

The NMDS ordination and ANOSIM results identified differences among *E. contortisiliquum* trees which were clustered according to its phenological data ($p = 0.0003$). Three phenological groups are formed with trees corresponding to its habitat condition (Table 2; Fig. 7).

4. Discussion

Phenological studies have compared populations in areas under different environmental conditions (Borchet, 1980; Frankie et al., 1974; Lobo et al., 2003) and some of them assessed the effect of warming on vegetation phenology (Mimet et al., 2009; White et al., 2002; Zhang et al., 2004b). However, most of them studied the impact of urban heat island (UHI) comparing trees in two different sites: towns and rural areas. In this study we observed the effects of habitat disturbances on phenological patterns of *Enterolobium contortisiliquum* in SDTF areas with distinct degrees of fragmentation: an undisturbed STDF area, a disturbed SDTF area due to cattle ranching, agricultural fields and farms and an urban area. The heterogeneity and water availability are crucial factors in plant phenology of dry environments (Borchert, 1994; Devineau, 1999; Reich and Borchert, 1994; Seghieri et al,. 1995; Seghieri and Simier, 2002; Opler et al., 1976). Nevertheless, few of these studies have compared the same species in different habitat conditions.

The patterns of flowering observed in *E. contortisiliquum* were similar to other SDTF species (Justiniano and Fredericksen, 2000; Morellato et al., 1989; Morellato and Leitão-Filho, 1990; Nunes et al., 2012; Pezzini, 2008) with flower buds occurring from September to October and anthesis during September to November. Despite flowering phenophases of *E. contortisiliquum* being similar to others species, a difference was detected among areas. The asynchrony and difference on percentage of intensity of reproductive phenology between trees may affect reproductive success of plant species reducing pollinator activity (Aizen and Feinsinger, 1994b; Quesada et al., 2003) and pollen deposition (Cunningham, 2000; Cascante et al., 2002; Quesada et al., 2003) and the variation on vegetative phenology may affect assemblage of herbivores associated with plants leading to fluctuations in population size (Peters et al., 2001).

According to Bullock (1995) synchronous flowering within populations is more frequent in dry areas, because seasonality is much more pronounced. We observed a synchrony of flowering phenophases among trees within population. However, there is a difference when we compare areas. Flower buds occurred first in urban area and anthesis was recorded first in disturbed and urban areas. Besides, urban area exhibited lowest activity index of flower buds and flowering and lowest percentage of intensity of both phenophases. The percentage of intensity of anthesis is almost twenty times smaller in urban area than in undisturbed and disturbed areas. Urbanization creates a dome of warm air called UHI which influence on climate (Oke, 1973) and induces early flowering (Mimet et al., 2009; White et al., 2002; Zhang et al., 2004a, 2004b). The urbanization also affect primary productivity (Imhoff et al., 2000), biodiversity and biogeochemical cycles (Bonan, 2002). In this way, deforestation could negatively affect plants creating spatial patchiness which may reduce the gene flow between areas because of the difference in flowering phenology.

Fruits were observed throughout the year, but the intensity of them was low. Immature fruits could be observed in the late wet season and fruit ripening occurred during the dry season, when the intensity of ripe fruits was higher. Immature fruits were first observed in undisturbed and disturbed areas with more production in disturbed area. It is important to note that fruiting activity began before flower buds. Fruiting began in the middle of wet season while flower buds occurred in the end of dry season which mean that immature fruits observed just before buds correspond to pollination of the last year. This pattern is known as "dry season fruiters" which correspond to flowers of the previous dry season in tropical dry forest (Frankie et al., 1974). This was observed before for some tropical dry species, as *E. cyclocarpum*, in a tropical dry forest of Costa Rica (Frankie et al., 1974) and *Ceiba aesculifolia* in Mexico and Costa Rica (Lobo et al., 2003).

Despite variations in reproductive phenology among areas climate variables influenced only fruiting. A negatively correlation was verified in disturbed and urban areas between ripe fruits and precipitation and temperature. Once more, fruit ripening occurred in dry season when there are lowest temperatures and no precipitation. Ripe fruits were observed in trees when deciduousness was highest.

Precipitation and temperature positively affected occurrence of presence of mature leaves in all areas. However, leaf fall was negatively affected by temperature in disturbed and urban area and negatively affected by precipitation only in disturbed area.

Enterolobium contortisiliquum trees abscised their leaves at the beginning of dry season achieving the peak of leaf shedding in the end of this season. The onset of leaf flushing was at the beginning of wet season just before the first rains. These patterns of vegetative phenology are similar to other SDTF species (Nunes et al., 2012; Pezzini, 2008). As a feature of tropical dry species, *E. contortisiliquum* responds to environmental signal associated with the production of

leaves as the increased water and light availability (Borchert, 1994; Lieberman and Lieberman, 1984). In contrast, increased of water stress and leaf ageing is responsible for leaf fall (Elliott et al., 2006).

In spite the similarity of *E. contortisiliquum* vegetative phenology with other SDTF species, some differences were observed among populations. Leaf flushing was verified in late wet season and this phenophase was most pronounced in urban area. In the same time, intensity of leaf emergence was too small in disturbed area. Despite the peak of flushing occurred in September for all areas, the intensity of this phenophase was different. The lowest activity of green leaves was detected in undisturbed area and the highest leaf abscission in disturbed area.

Our results suggest that habitat disturbance may trigger differences in phenological responses. Trees located in areas with the same habitat condition were clustered. As noted in some studies environmental factors have influenced phenological events in tropical plants (Borchert, 1994; Opler et al., 1976; Reich and Borchert, 1984). Thus we believed that habitat disturbances have changed microclimate, such as soil moisture and irradiation, which affect differently phenological patterns in all areas. Besides, biotic factors, such as competition for pollinators or pollinator attraction (Appanah, 1985; Augspurger, 1981; Gentry, 1974; Janzen, 1967; Murray et al., 1987; Robertson, 1895; Sakai et al., 1999; Stiles, 1975), which have been proposed as responsible for phenological patterns in tropical species, may trigger differences found between areas. Although pollinators have not been investigated in this study, habitat disruption negatively affects pollinator activity (Quesada et al., 2003) which could be associated with phenological results. In addition, our results showed that only phylogenetic membership is not enough to constrain phenological patterns in *E. contortisiliquum* in these areas. Some studies revealed that even in different latitudes phenological patterns of species and genera of the same family are similar (Lobo et al., 2003). However, we showed that even in near areas phenological

patterns were different and this result may be caused by habitat fragmentation. Finally, we concluded that deforestation is negatively affecting phenological patterns of *E. contortisiliquum* in Brazilian SDTF areas.

Acknowledgements

We thank Universidade Estadual de Montes Claros for logistical support during our work and LG Sousa, SAS Sousa and FA Oliveira for their help with field work. We also thank N.P.U. Barbosa for producing the map used here. We are very grateful to the Instituto Estadual de Florestas (IEF) for logistical support during field work. We gratefully acknowledge a postgraduate scholarship to P.A. Moreira from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). We thank the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) (APQ-04105-10, APQ - 00372-11) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (3352/2010-8) for financial support. This study is a partial fulfillment of the requirements for the doctoral degree in genetics for P.A. Moreira at Universidade Federal de Minas Gerais.

References

Aizen, M.A., Feinsinger, P., 1994a. Forest fragmentation, pollination, and plant reproduction in a Chaco Dry Forest, Argentina. Ecol. 75, 330-351.

Aizen, M.A., Feinsinger, P., 1994b. Habitat fragmentation, native insect pollinators, and feral honey bees in Argentine “Chaco Serrano”. Ecol. Apl. 7, 378-392.

Antunes, F.Z., 1994. Caracterização climática do Estado de Minas Gerais: climatologia agrícola. Informe Agropecuário. 17, 9-13.

Appanah, S., 1985. General flowering in the climax rain forests of south-east Asia. J. Trop. Ecol. 1, 225–240.

Augspurger, C.K., 1981. Reproductive synchrony of a tropical shrub: experimental studies on effects of pollinator and seed predators on *Hybanthus prunifolius* (Violaceae). Ecol. 6, 775–788.

Bencke, C.S.C., Morellato, P.C., 2002. Comparação de dois métodos de avaliação da fenologia de plantas, sua interpretação e representação. Rev. Bras. Bot. 25, 269-275.

Bonan, G., 2002. Ecological Climatology-Concepts and Applications, Cambridge University Press, New York.

Borchert, R., 1980. Phenology and ecophysiology of tropical trees: *Erythrina poeppigiana* O. F. Cook, Ecol. 61, 1065-1074.

Borchert, R., 1994. Soil and stem water storage determine phenology and distribution of tropical dry forest trees. Ecol. 75, 1437–1449.

Bullock, S.H.,1995. Plant reproduction in neotropical dry forests, in: Bullock, S.H., Mooney, H.A., Medina, E. (Eds.), Seasonally dry tropical forests, Cambridge University Press, Cambridge, pp. 277-303.

Cascante, A., Quesada, M., Lobo, J.A., Fuchs, E.A., 2002. Effects of dry forest fragmentation on the reproductive success and genetic structure of the tree *Samanea saman*. Conserv. Biol. 16, 137-147.

Clarke, K.R., 1993. Non-parametric multivariate analysis of changes in community structure. Aust. J. Ecol. 18, 117-143.

Culley, T.M., Sbita, S.J., Wick, A., 2007. Population genetic effects of urban habitat fragmentation in the perennial herb *Viola pubescens* (Violaceae) using ISSR markers. Ann. Bot. 100, 91-100.

Cunningham, S.A., 2000. Depressed pollination in habitat fragments causes low fruit set. Proc. R. Soc. Lond. 267, 114 9-1152.

Devineau, J.L., 1999. Seasonal rhythms and phenological plasticity of savanna woody species in a fallow farming system (south-west Burkina Faso). J. Trop. Ecol. 15, 497-513.

Elliott, S., Baker, J.P., Borchert, R., 2006. Leaf flushing during the dry season: the paradox of Asian monsoon forests. Glob. Ecol. Biogeogr. 15, 248-257.

Espírito-Santo, M.M., Sevilha, A.C., Anaya, F.C., Barbosa, R., Fernandes, G.W., Sánchez-Azofeifa, G., Scariot, A., Noronha, S.E., Sampaio, C.A., 2009. Sustainability of tropical dry forests: two case studies in southeastern and central Brazil. For. Ecol. Manage. 258, 922-930.

Fournier, L.A.O., 1974. Un método cuantitativo para la medición de características fenológicas en árboles. Turrialba. 24, 422-423.

Frankie, G.W., Baker, H.G., Opler, P.A., 1974. Comparative phenological studies of trees in tropical wet and dry forests in the lowlands of Costa Rica. J. Ecol. 62, 881-919.

Frankie, G.W., Haber, W.A., Vinson, S.B., Bawa, K.S., Ronchi, P.S., Zamora, N., 2004. Flowering phenology and pollination systems diversity in the seasonal dry forest, in: Frankie, G.W., Mata, A., Vinson, S.B. (Eds.), Biodiversity conservation in Costa Rica: learning the lessons in a seasonal dry forest. University of California Press, Berkeley, pp. 17-29.

Fuchs, E.J., Lobo, J.A., Quesada, M., 2003. Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropical dry forest tree *Pachira quinata*. Conserv. Biol. 17, 149-157.

Gentry, A.H., 1974. Flowering phenology and diversity in tropical Bignoniaceae. Biotropica. 6, 64–68.

Gentry, A.H., 1995. Diversity of floristic composition of Neotropical dry forests, in: Mooney, H.A., Bullock, S.H., Medina E. (Eds), Seasonally Tropical Forests. Cambridge University Press, Cambridge, pp. 146-194.

Giraldo, J.P., Holbrook, N.M., 2011. Physiological mechanisms underlying the seasonality of leaf senescence and renewal in seasonally dry tropical forests trees, in:

Dirzo, R., Young, H., Mooney H., Ceballos, G. (Eds.), Seasonally Dry Tropical Forests: Ecology and Conservation. Island Press, Washington DC, pp. 129-140.

Graham, A., Dilcher, D., 1995. The Cenozoic record of tropical dry forest in northern Latin America and the southern United States, in: Mooney, H.A., Bullock, S.H., Medina E. (Eds), Seasonally Tropical Forests. Cambridge University Press, Cambridge, pp. 124-145.

Hamrick, J.L., Apsit, V.J., 2004. Breeding structure of neotropical dry forest tree species in fragmented landscapes, in: Frankie, G.W., Mata, A., Vinson, S.B. (Eds.), Biodiversity conservation in Costa Rica: learning the lessons in a seasonal dry forest. University of California Press, Berkeley, pp 30–37.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontol. Electronica. 4, 1-9.

Herrerías-Diego, Y., Quesada, M., Stoner, K.E., Lobo, J.A. 2006. Effects of forest fragmentation on phenological patterns and reproductive success of the tropical dry forest tree *Ceiba aesculifolia*. Conserv. Biol. 20, 1111-1120.

Imhoff, M.L., Tucker,C.J., Lawrence,W.T., Stutzer. D.C., 2000. The use of multisource satel lite and geospatial data to study the effect of urbanization on primary productivity in the United States, IEEE Trans. Geosci. Remote Sens. 36, 2549 – 2556.

Janzen, D.H., 1967. Synchronization of sexual reproduction of trees within the dry season in Central America. Evol. 21, 620–637.

Janzen, D.H., 1981a. *Enterolobium cyclocarpum* seed passage rate and survival in horses, Costa Rican Pleistocene seed dispersal agents. Ecol. 62, 593-601.

Janzen, D.H., 1981b. Guanacaste tree seed-swallowing by Costa Rican range horses. Ecol. 62, 587-592.

Janzen, D.H., 1982. Variation in average seed size and fruit seediness in a fruit crop of a Guanacaste tree (Leguminosae: *Enterolobium cyclocarpum*). Am. J. Bot. 69, 1169–1178.

Janzen, D.H., 1988. Management of habitat fragments in a tropical dry forest: growth. Ann. Mo. Bot. Gard. 75,105–116.

Janzen, D.H., Martin, P.S., 1982. Neotropical anachronisms: the fruits the Gomphotheres ate. Sci. 215, 19-27.

Jochner, S.C., Beck, I., Behrendt, H., Traidl-Hoffmann, C., Menzel, A., 2001. Effects of extreme spring temperatures on urban phenology and pollen production: a case study in Munich and Ingolstadt. Clim. Res. 49, 101-112.

Justiniano, M.J., Fredericksen, T.S., 2000. Phenology of tree species in Bolivian dry forests. Biotropica. 32, 276-281.

Lieberman, D., Lieberman, M., 1984. The causes and consequences of synchronous flushing in a dry tropical forest. Biotropica. 16, 193-201.

Link, D., Costa, E.C., 1995. Danos causados por insetos em sementes de timbaúva, *Enterolobium contortisiliquum* (Vell.) Morong. Ciência Florestal, 5, 113-122.

Lobo, J.A., Quesada, M., Stoner, K.E., Fuchs, E.J., Herrerias-Diego, Y., Rojas, J., Saborio, G., 2003. Fators affecting phenological patterns of Bombacaceae trees in seasonal forests in Costa Rica and México. Am. J. Bot. 90, 1054–1063.

Mass, J.M., 1995. Conversion of tropical dry forest to pasture and agriculture, in: Mooney, H.A., Bullock, S.H., Medina E. (Eds), Seasonally Tropical Forests. Cambridge University Press, Cambridge, pp. 399-422.

Mayle, F.E., 2004. Assessment of the Neotropical dry forest refugia hypothesis in the light of palaeoecological data and vegetation model simulations. J. Quat. Sci. 19, 713-720.

Miles, L., Newton, A.C., Fries, R.S., Ravilious, C., May, I., Blyth, S., Kapos, V., Gordon, J.E., 2006. A global overview of the conservation status of tropical dry forests. J. Biogeogr. 33, 491–505.

Mimet, A., Pellissier, V., Quénol, H., Aguejdad, R., Dubreuil V., Rozé, F., 2009. Urbanisation induces early flowering: evidence from *Platanus acerifolia* and *Prunus cerasus*. Int. J. Biometeorol. 53, 287-298.

Mooney, H.A., Bullock, S.H., Medina E., 1995. Seasonally Tropical Forests. Cambridge University Press, Cambridge.

Morandini, M.N., Viana, M.L., 2009. Depredación pre-dispersiva de semillas en tres poblaciones del árbol *Enterolobium contortisiliquum* (Fabaceae). Int. J. Trop. Biol. 57, 781-788.

Morellato, L.P.C., Leitão-Filho, H.F., 1990. Estratégias fenológicas de espécies arbóreas em floresta mesófila na Serra do Japi, Jundiaí, São Paulo. Rev. Bras. Biol. 50, 163-173.

Morellato, L.P.C., Rodrigues, R.R., Leitão-Filho, H.F., Joly, C.A., 1989. Estudo comparativo da fenologia de espécies arbóreas de floresta de altitude e floresta mesófila semidecídua na Serra do Japí, Jundiaí, São Paulo. Rev. Bras. Bot. 12, 85-98.

Murawski, D.A., Hamrick, J.L., 1992. The mating system of *Cavanillesia platanifolia* under extremes of flowering tree density: A test of predictions. Biotropica. 24, 99–101.

Murray, K.G., Feinsinger, P., Busby, W.H., Linhart,Y.B., Beach, J.H., Kinsman, S., 1987. Evaluation of character displacement among plants in two tropical pollination guilds. Ecol, 68, 1283–1293.

Nason, J.D., Hamrick, J.L., 1997. Reproductive and genetic consequences of forest fragmentation: two case studies of neotropical canopy trees. J. Hered. 88, 264-276.

Nunes, Y.R.F., Luz, G.R., Braga, L.L., 2012. Phenology of tree species populations in tropical dry forests of southeastern Brazil, in: Zhang, X. (Ed.). Phenology and climate change. InTech, Rijeka, Croatia, pp. 125-142.

Oke, T.R., 1973. City size and the urban heat island. Atmos. Environ. 7, 769–779.

Opler, P.A., Frankie, G.W., Baker, H.G., 1976. Rainfall as a factor in the release, timing, and synchronization of anthesis by tropical trees and shrubs. J. Biogeogr. 3, 231–236.

Pennington, R.T., Lavin, M., Prado, D.E., Pendry, C.A., Pell, S.K., Butterworth, C.A., 2004. Historical climate change and speciation: neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification. Philos. Trans. R. Soc. Lond. B Biol. Sci. 359, 515-538.

Pennington, R.T., Lewis, G.P., Ratter, J.A., 2006. An overview of the plant diversity, biogeography and conservation of neotropical savannas and seasonally dry forests, in: Pennington, R.T., Lewis, G.P., Ratter, J.A. (Eds.), Neotropical savannas and seasonally dry forests: plant diversity, biogeography and conservation. CRC Press Taylor & Francis Group, Boca Raton, New York, pp. 1-29.

Peters, P.J., Read, J., Sanson, G.D., 2001. Variation in the guild composition of herbivorous insect assemblages among co-occuring plant species, Austral Ecol 26: 385-399.

Pezzini, F.F., 2008. Fenologia e características reprodutivas em comunidades arbóreas de três estágios sucessionais em Floresta Estacional Decidual do norte de Minas Gerais. Dissertação de Mestrado, Universidade Federal de Minas Gerais, Belo Horizonte, 130p.

Primack, R.B., Ibáñez, I., Higuchi, H., Lee, S.D., Miller-Rushing, A.J., Wilson, A.M., Silander Jr, J.A., 2009. Spatial and interspecific variability in phenological responses to warming temperatures. Biol. Conserv. 142, 2569-2577.

Quesada, M., Stoner, K.E., Rosas-Guerrero, V., Palacios-Guevara, C., Lobo, J.A., 2003. Effects of habitat disruption on the activity of nectarivorous bats (Chiroptera: Phyllostomidae) in a dry tropical forest: implications for the reproductive success of the neotropical tree *Ceiba grandiflora*. Oecologia. 135, 400-406.

Reich, P.B., Borchert, R., 1984. Water stress and tree phenology in a tropical dry forest in the lowlands of Costa Rica. J. Ecol. 72, 61–74.

Robertson, C., 1895. The philosophy of flower seasons, and the phenological relations of the entomophilous flora and the anthophilous insect fauna. Am. Nat. 29, 97–117.

Rocha, O.J., Aguilar, G., 2001. Reproductive biology of the dry forest tree *Enterolobium cyclocarpum* (Guanacaste) in Costa Rica: a comparison between trees left in pastures and trees in continuous forest. Am. J. Bot. 88, 1607-1614.

Rodrigues, L., 2000. Formação econômica do norte de Minas e o período recente, in: Oliveira, M.F.M., Rodrigues, L., Machado, J.M.A., Botelho, T.R. (Eds.), Formação social e econômica do norte de Minas Gerais. Editora Unimontes, Montes Claros, pp. 105-170.

Sakai, S., Momose, K., Yumoto, T., Nagamitsu, T., Nagamasu, H., Hamid, A.A., Nakashizuka, T., 1999. Plant reproductive phenology over four years including an episode of general flowering in a lowland dipterocarp forest, Sarawak, Malaysia. Am. J. Bot. 86, 1414–1436.

Sampaio, G., Nobre, C., Costa, M.H., Satyamurty, P., Soares-Filho, B.B., Cardoso, M., 2007. Regional climate change over easter Amazonia caused by pasture and soybean cropland expansion. Geophys. Res. Lett. 34, 1-7.

Sánchez-Azofeira, G,A., Kalácska, M., Quesada, M., Calvo-Alvarado, J.C., Nassar, J.M., Rodrigues, J.P., 2005. Need for integrated research for a sustainable future in tropical dry forests. Conserv. Biol. 19, 285-286.

Särkinen, T., Iganci, J.R.V., Linares-Palomino, R., Simon, M.F., Prado, D.E., 2011. Forgotten forests – issues and prospects in biome mapping using Seasonally Dry Tropical Forests as a case study. BMC Ecol. 11, 1-15.

Seghieri, J., Floret, C., Pontanier, R., 1995. Plant phenology in relation to water availability: herbaceous and woody species in the savannas of northern Cameroon. J. Trop. Ecol. 11, 237–254.

Seghieri, J., Simier, M., 2002. Variation in phenology of a residual invasive shrub species in Sahelian fallow savannas, south-west Niger. J. Trop. Ecol. 18, 897–912.

Sevilha, A.C., Scariot, A., Noronha, S.E., 2004. Estado atual da representatividade de unidades de conservação em florestas estacionais deciduais no Brasil, in: Sociedade Brasileira de Botânica (Eds.), Biomas Florestais-Annals of the 55th Congresso Nacional de Botânica, Sociedade Brasileira de Botânica, São Paulo, pp. 1-63.

Steininger, M.K., Tucker, C.J., Ersts, P., Killeen, J., Villegas, Z., Hecht, S.B., 2001. Clearance and fragmentation of tropical deciduous forest in the Tierras Bajas, Santa Cruz, Bolivia. Conserv. Biol. 15, 856–66.

Stiles, F.G., 1975. Ecology, flowering phenology, and hummingbird pollination of some Costa Rican *Heliconia* species. Ecol. 56, 285–301.

White, M.A., Nemani, R.R., Thor, P.E., Running, S.W., 2002. Satellite evidence of phenological differences between urbanized and rural areas of the eastern United States deciduous broadleaf forest. Ecosystems. 5, 260-277.

Zar, J.H., 1996. Biostatistical analysis, Prentice-Hall, New Jersey.

Zhang, X., Friedl, M.A., Schaaf, C.B., Strahler, A.H., 2004a. Climate controls on vegetation phenological patterns in northern mid- and high latitudes inferred from MODIS data. Glob. Chang. Biol. 10, 1133–1145.

Zhang, X., Friedl, M.A., Schaaf, C.B., Strahler, A.H., Schneider A., 2004b. The footprint of urban climates on vegetation phenology. Geophys. Res. Lett. 31, 1-4.

FIGURES

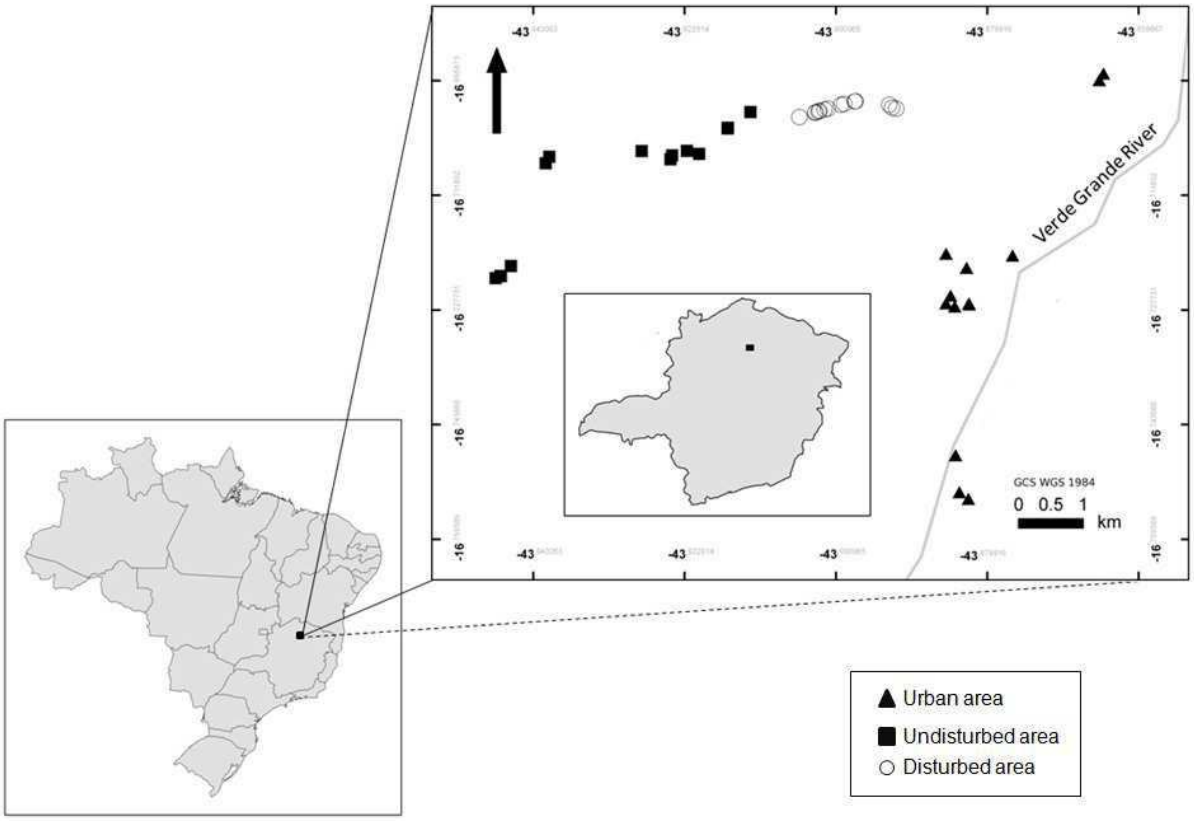


Fig. 1. Spatial distribution of *Enterolobium contortisiliquum* trees in undisturbed area of the Lapa Grande State Park (■), in a disturbed area, where they are surrounded by agriculture fields and pastures (○) and urban area (▲).

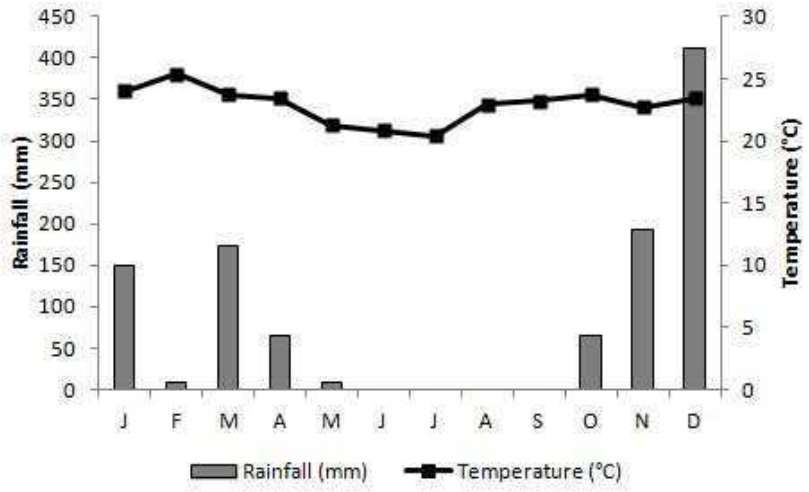


Fig. 2. Climate description of the study SDTF areas: total precipitation (bars) and average monthly temperatures.

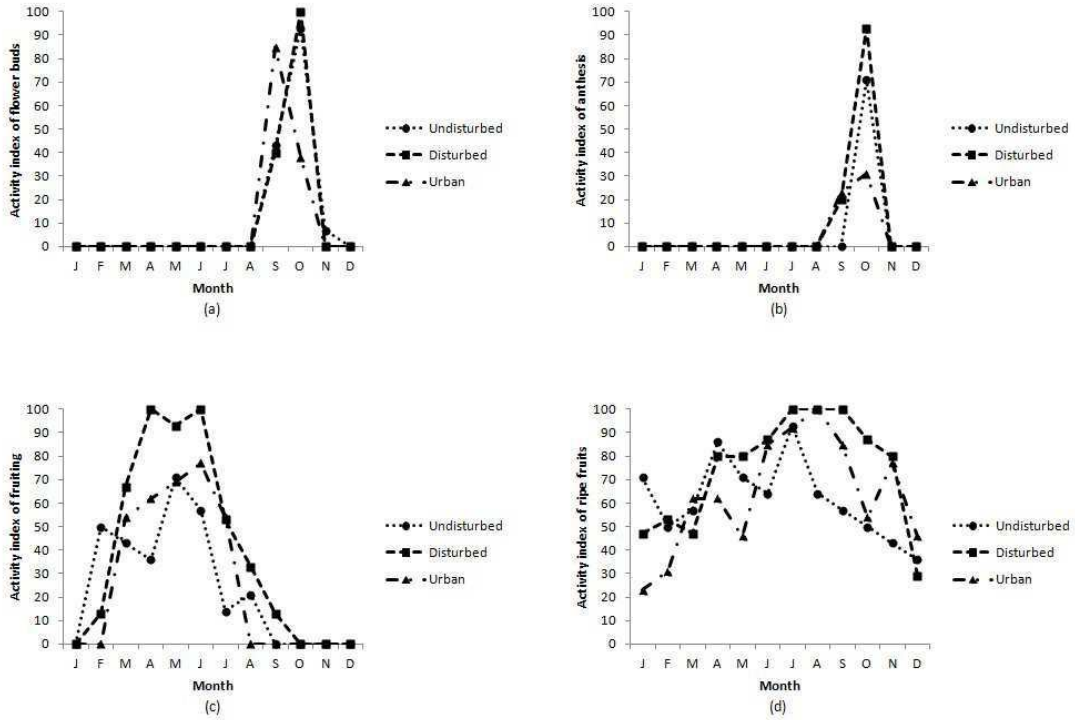


Fig. 3. Activity index of *Enterolobium contortisiliquum* reproductive phenology in three different areas from January to December.

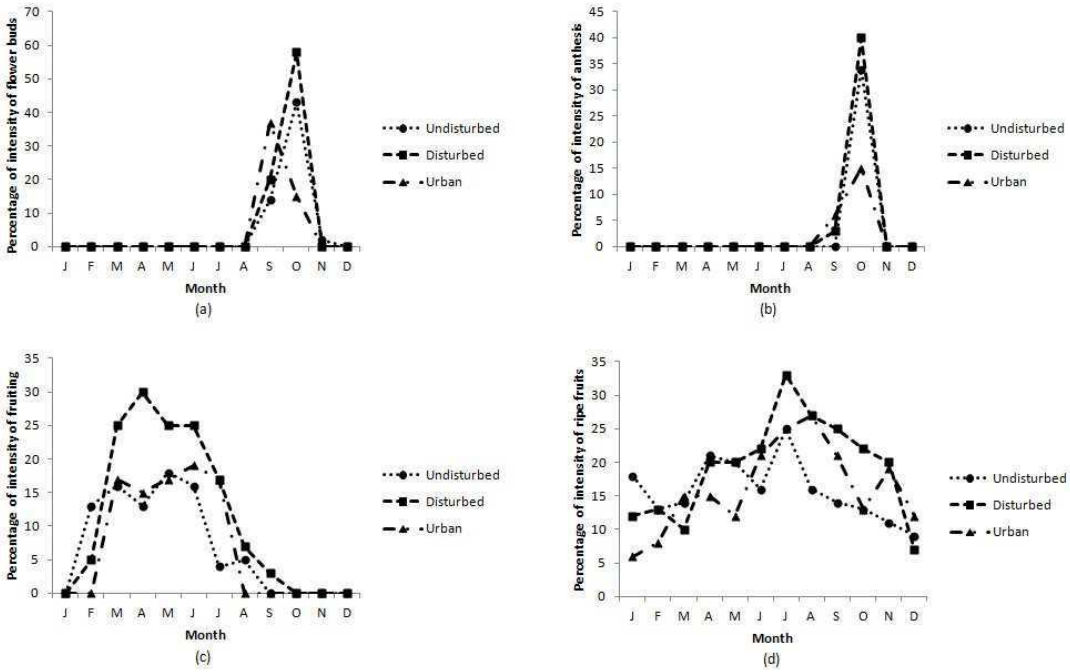


Fig. 4. Percentage of intensity of *Enterolobium contortisiliquum* reproductive phenology in three different areas from January to December.

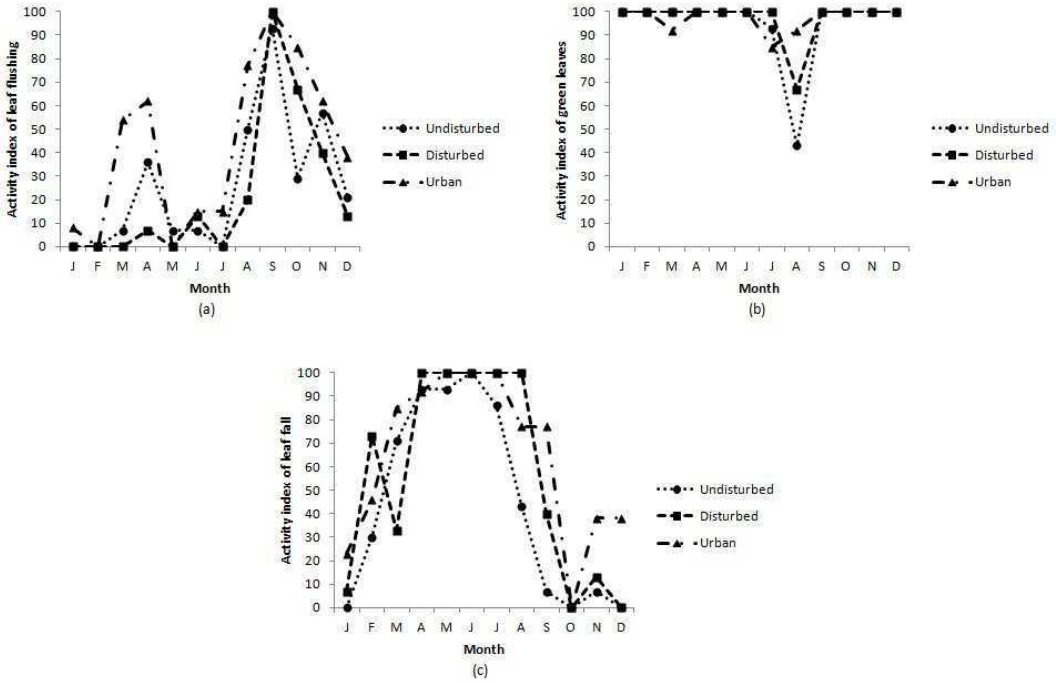


Fig. 5. Activity index of *Enterolobium contortisiliquum* vegetative phenology in three different areas from January to December.

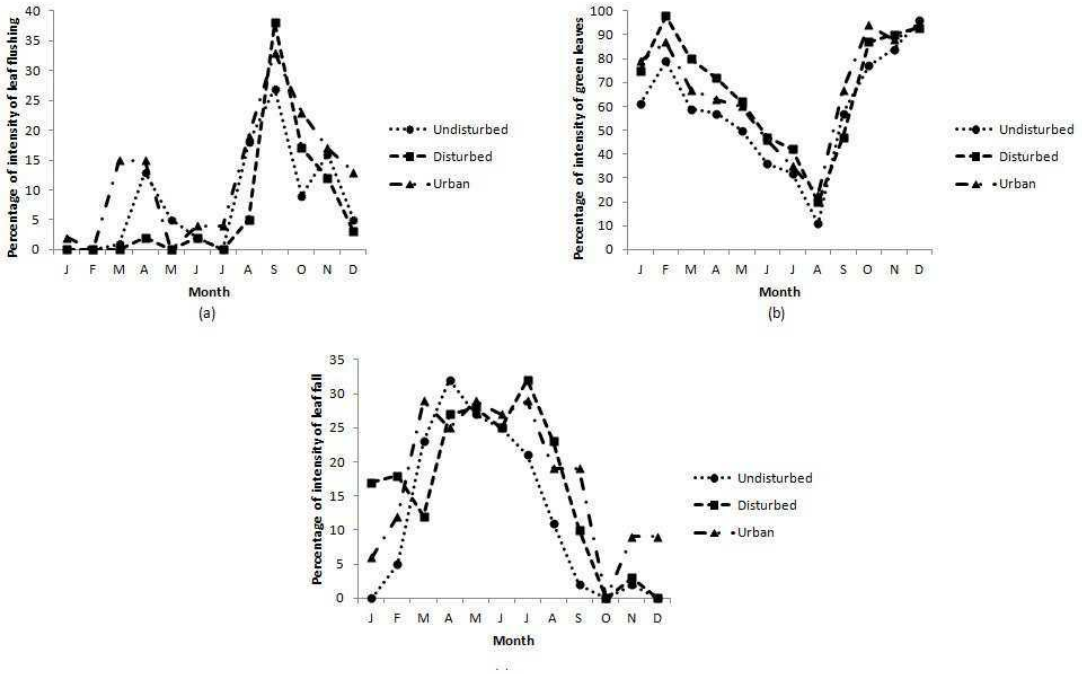


Fig. 6. Percentage of intensity of *Enterolobium contortisiliquum* vegetative phenology in three different areas from January to December.

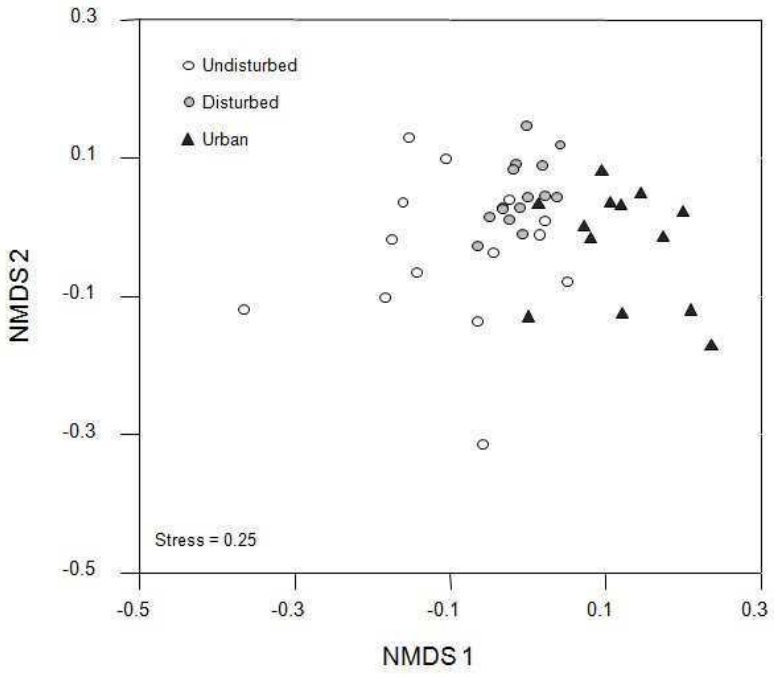


Fig. 7. Non-metric multidimensional scaling (NMDS) ordination of *Enterolobium contortisiliquum* trees according to its phenological patterns.

TABLES

Table 1

Spearman correlation (r) between climate variables and *Enterolobium contortisiliquum* phenophases activity and intensity in different SDTF areas.

Area	Climate variable[a]	Phenophase	Activity		Intensity	
			r	p	r	p
Undisturbed	P	Green leaves			0.81	0.001
Undisturbed	T	Green leaves			0.61	0.033
Disturbed	P	Leaf fall	-0.75	0.005	-0.62	0.032
Disturbed	T	Leaf fall	-0.58	0.046		
Disturbed	P	Green leaves			0.76	0.003
Disturbed	T	Green leaves			0.65	0.021
Disturbed	P	Ripe fruits	-0.81	0.001	-0.81	0.001
Disturbed	T	Ripe fruits	-0.60	0.040	-0.62	0.031
Urban	T	Leaf fall	-0.67	0.017		
Urban	P	Green leaves			0.75	0.004
Urban	T	Green leaves			0.62	0.032
Urban	P	Ripe fruits	-0.58	0.046	-0.58	0.046
Urban	T	Ripe fruits	-0.69	0.012	-0.69	0.012

[a] P = precipitation, T = temperature.

Table 2

Non-parametric analyses of similarity (ANOSIM) testing for differences in the ordination similarities for trees in three different areas grouped by phenophases activity.

Parameter	Areas	R[a]
Phenophases activity	Undisturbed x Disturbed	0.268
	Undisturbed x Urban	0.354
	Disturbed x Urban	0.413

[a] All values of R are significant (p = 0.0003, adjusted nominal 5% level with Bonferroni correction)

CAPÍTULO 4

Artigo aceito para publicação no periódico *Genetics and Molecular Research*.

Characterization of nine transferred SSR markers in the tropical tree species *Enterolobium contortisiliquum* (Fabaceae)

Running title: Transferability of SSR in the Neotropical tree *Enterolobium contortisiliquum*

Abstract

Transfer of molecular markers is widely used in conservation genetics studies. We investigated the transferability of simple sequence repeats (SSR) markers developed for *Enterolobium cyclocarpum* to *E. contortisiliquum*, a tropical tree widely distributed in dry forests. A set of 9 evaluated SSR markers were amplified in *E. contortisiliquum* and the degree of polymorphism was assessed in 8 trees sampled from each of 5 populations from central Brazil. All loci were polymorphic and the mean number of alleles for all loci was 6. In addition, all pairs of SSR markers were in linkage equilibrium. For most loci, the observed heterozygosity was higher than expected under Hardy–Weinberg equilibrium, with fixation indices not significantly different from zero. The combined probability of paternity exclusion was high and the probability of identity was very low. We conclude that these SSR markers developed for *E. cyclocarpum* are applicable for genetic studies of *E. contortisiliquum*.

Key words: Simple sequence repeats; Markers transferability; Tropical seasonally dry forest; *Enterolobium contortisiliquum*.

INTRODUCTION

Molecular markers are an efficient tool for detecting polymorphism at the DNA level. Many molecular markers are available, but studies with microsatellite or simple sequence repeats (SSRs) have increased during the last decade. SSR molecular markers have many advantages because they are highly polymorphic, robust, codominant, and easily detected by polymerase chain reaction (PCR). In addition, SSR markers are widespread in genomes (Goldstein and Schlotterer, 1999). Despite these positive qualities, however, the development of

SSR markers are time-consuming and expensive. Therefore, cross-species amplification is an effective alternative for the application of this molecular tool to the study of ecological issues in high-diversity tropical ecosystems because it eliminates the labor and cost involved in developing SSR markers for new species (Cristofani-Yali et al., 2011). Because of the rapid fragmentation of natural habitats and the long time needed to develop SSR markers, the transferability of these molecular markers among related species is an efficient tool for protecting and managing populations. SSR molecular markers can provide important information that is used by conservation genetics to evaluate and monitor species (Frankham et al., 2002).

Heterologous amplification of SSR markers has been successfully applied to the genetic analysis of tropical tree species (Zucchi et al., 2002). The transfer of polymorphic markers in plants is mainly successful within genera. Many studies have demonstrated the use of SSR markers developed from one species to another of the same genus. This transfer occurs because of sequence conservation in the primer sites flanking the microsatellite loci and the stability of those sequences during evolution (Dayanandan et al., 1997; Ciampi et al., 2008; Feres et al., 2009).

Enterolobium contortisiliquum (Vell.) Morong (Leguminosae) is a poisonous tropical tree species that has been removed from natural areas owing to the advance of cattle production and agriculture in central Brazil. The ingestion of *E. contortisiliquum* pods is harmful to cattle, causing abortion and severe injuries in the skin, mainly in photosensitive places without pigmentation (Bonel-Raposo et al., 2008; Costa et al., 2009). Therefore, many farmers have cut trees of this species near their ranches because the fruiting period of *E. contortisiliquum* occurs during the dry season, coincident with low forage availability. Molecular markers are needed to understand the effects of fragmentation and selective logging in this tree species and to generate useful information for its conservation.

To save time in *E. contortisiliquum* conservation, we aimed to evaluate in *E. contortisiliquum* the transferability and characterization of SSR molecular markers previously developed for *Enterolobium cyclocarpum*. These SSR markers may be a valuable tool for the investigation of the genetic diversity, mating system, gene flow, and spatial genetic structure of this species.

MATERIAL and METHODS

Five populations of *E. contortisiliquum* in different tropical dry forests were sampled, 4 in Minas Gerais State (Unaí, Felícios dos Santos, Montes Claros and Manga, respectively, UNA, FES, MOC, and MAN) and one in Bahia State (Vitória da Conquista, VIC). The shortest distance between these populations was 170 km (between FES and MOC), and the longest was 674 km (between UNA and VIC; Figure 1). In each population, expanded leaves from 8 reproductive trees of *E. contortisiliquum* were collected and stored in silica gel until DNA extraction. Genomic DNA extraction was carried out using a standard cetyltrimethylammonium bromide procedure

(Doyle and Doyle, 1990). The concentration of DNA extracted was quantified visually in 1.0% agarose gel through comparison with standard DNA concentrations. The DNA was diluted in TE buffer (10 mM Tris-HCl [pH 8.0], and 1 mM ethylenediaminetetraacetic acid) to a final concentration of 3 ng/µL before PCR amplifications.

Nine microsatellite loci previously developed for the Guanacaste tree *E. cyclocarpum* (Peters et al., 2008) were used for PCR amplification in *E. contortisiliquum*. Microsatellite amplifications were performed in a 10 µL volume containing 10.0 µM of each primer, 1 U *Taq* DNA polymerase, 250 µM of each deoxyribonucleotide triphosphate, 1X reaction buffer (10 mM Tris–HCl [pH 8.3], 50 mM KCl, and 1.5 mM $MgCl_2$), 0.25 µg bovine serum albumin, and 9.0 ng template DNA. Amplifications were performed using a Veriti® Thermal Cycler (Applied Biosystems) under the following conditions: 94°C for 5 min (one cycle); 94°C for 1 min, 54–58°C for 1 min (according to the primer), and 72°C for 1 min (35 cycles); and 72°C for 30 min (one cycle). The PCR products were genotyped on a 3500 Genetic Analyzer (Applied Biosystems) and were sized through comparison to a GeneScan™ 600 LIZ® Size Standard (Applied Biosystems). Fluorescent PCR products were automatically sized using GeneMapper® (Applied Biosystems).

SSR loci were characterized based on 40 adult individuals for the number of alleles per locus (A) and observed (H_O) and expected (H_E) heterozygosity under Hardy-Weinberg equilibrium (Nei, 1978). The inbreeding coefficient (f), for each locus and over all loci was also estimated (Nei, 1978). All pairs of loci were tested for linkage equilibrium. Analyses and randomization based tests with Bonferroni correction were performed with FSTAT 2.9.3.2 (Goudet et al., 1996; Goudet, 2002). The probability of genetic identity (I) (Chakravaratt and Li, 1983) and the probability of paternity exclusion (Q) (Weir, 1996) were estimated for each locus,

and the combined probability of genetic identity, $IC = \Pi Ii$, and combined probability of paternity exclusion, $QC = 1 - [\Pi(1-Qi)]$, were estimated for the loci overall.

RESULTS

The set of SSR primers of *E. cyclocarpum* exhibited amplification in *E. contortisiliquum* under various annealing temperatures (Table 1). All loci were polymorphic, and the average allele number over all loci was 6, with the number of alleles ranging from three (Ency-21 and Ency-22) to 11 (Ency-24; Table 2). All microsatellite loci pairs were in linkage equilibrium ($P > 0.001389$, adjusted nominal 5% level with Bonferroni correction).

For most loci, the observed heterozygosity was higher than that expected under Hardy–Weinberg equilibrium, with fixation indices not statistically different from zero (see Table 2). Nevertheless, the combined probability of paternity exclusion was high (QC =0.9929) and the probability of identity was very low ($\sim 10^{-36}$; see Table 2), showing that the battery of loci is appropriate for population genetic analyses. The cross-transferability of Guanacaste tree SSR markers to *E. contortisiliquum* observed in this study renders this set of primers useful for investigating the structure and genetic diversity of *E. contortisiliquum* populations.

DISCUSSION

SSR molecular markers derived from *E. cyclocarpum* were successfully transferred to *E. contortisiliquum* in our study, confirming that the regions flanking these microsatellites are conserved enough to permit locus amplification. The transfer of SSR markers is unequally distributed across taxa, but a high success of SSR transferability is found within plant genera

(reaching rates close to 60% in eudicots) (Barbará et al., 2007). The SSR transferability for Leguminosae species, including *Enterolobium* species, has been successfully reported. Six pairs of primers developed for the tropical rain forest tree *Pithecellobium elegans* could amplify SSR fragments in *Enterolobium schomburgkii*, and three of these could do so in *E. cyclocarpum* (Dayanandan et al., 1997).

Our results show that the polymorphism level founded in *E. contortisiliquum* is satisfactory. In fact, the high percentage transfer capability of Guanacaste tree SSR markers to *E. contortisiliquum* observed in this study makes these molecular markers valuable tools for the investigation of the genetic diversity, mating system, gene flow, and spatial genetic structure of *E. contortisiliquum* populations.

ACKNOWLEDGMENTS

We thank Universidade Estadual de Montes Claros for logistical support during our laboratory work. We also thank F.S. Neves for his help with field work and E.V. Menezes for help with laboratory work. We are grateful to J. Lobo for his comments on the early versions of this manuscript. We gratefully acknowledge a postgraduate scholarship to P.A. Moreira from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), a scholarship of Fundação de Amparo à Pesquisa do Estado de Minas Gerais (PIBIC/FAPEMIG) to F.A. Oliveira and a scholarship of Universidade Estadual de Montes Claros (BIC/UNI-UNIMONTES) to S.A.S. Soares. We thank the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) (APQ -00372-11) for support. This study is a partial fulfillment of the requirements for the doctoral degree in genetics for P.A. Moreira at Universidade Federal de Minas Gerais.

References

Barbará T, Palma-Silva C, Paggi GM, Bered F, Fay MF, Lexer C (2007) Cross-species transfer of nuclear microstellite markers: potential and limitations. *Mol. Ecol.* 16:3759–3767.

Bonel-Raposo J, Riet-Correa F, Guim TM, Schuch ID, Grecco FB, Fernandes CG (2008) Intoxicação aguda e abortos em cobaias pelas favas de *Enterolobium contortisiliquum* (Leg. Mimosoideae). *Pesqui. Veter. Bras.* 28:593-596.

Braga AC, Reis AMM, Leoi LT, Pereira RW, Collevatti RG (2007) Development and characterization of microsatellite markers for the tropical tree species *Tabebuia aurea* (Bignoniaceae). *Mol. Ecol. Notes* 7:53-56.

Chakravaratt I, Li CC (1983) The effect of linkage on paternity calculations. In: Walkera RH (ed). Inclusion Probabilities in Parentage Testing. American Association of Blood Banks: Arlington, pp 411–420.

Ciamp AY, Azevedo VCR, Gaiotto FA, Ramos ACS, Lovato MB (2008) Isolation and characterization of microsatellite loci for *Hymenaea courbaril* and transferability *to Hymenaea stigonocarpa*, two tropical timber species. *Mo.l Ecol. Resour.* 8:1074-1077.

Costa RLD, Marini A, Tanaka D, Berndt A, Andrade FME (2009) Intoxication of bovines by *Enterolobium contortisiliquum* (Timboril) in Brazil. *Arch. Zootec.* 58:313-316.

Cristofani-Yali M, Novelli VN, Bastianel M, Machado MA (2011) Transferibility and level of heterozygosity of microsatellite markers in *Citrus* species. *Plant. Mol. Biol. Rep.* 29:418-423.

Dayanandan S, Bawa KS, Kesseli R (1997) Conservation of microsatellites among tropical trees (Leguminosae). *Am. J. Bot.* 84:1658-1663.

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 12:13-15.

Feres JM, Martinez MLL, Martinez CA, Mestriner MA, Alzate-Marin AL (2009) Transferability and characterization of nine microsatellite for the tropical tree species *Tabebuia roseo-alba*. *Mol. Ecol. Resour.* 9:434-437.

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to Consevation Genetics. Cambridge University Press, Cambridge, UK.

Goldstein DB, Schlotterer C (1999) Microsatellites: evolution and applications. Oxford University Press, Oxford.

Goudet J (2002) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). Available at [http://www.unil.ch/izea/softwares/ fstat.html]. Accessed July 03, 2011.

Goudet J, Raymond M, de-Meeus T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics* 144:1933-1940.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individual. *Genetics* 89: 83–590.

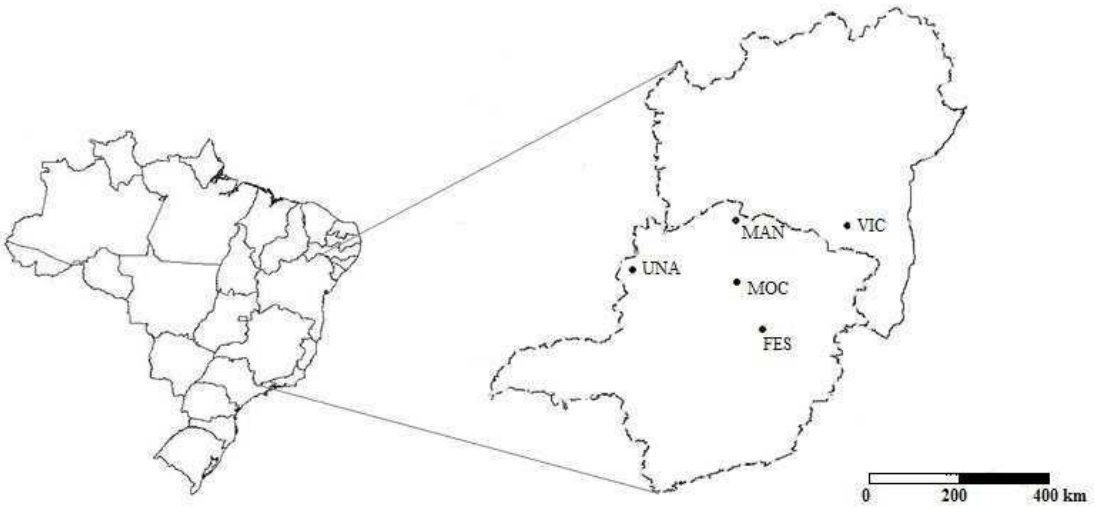
Peters MB, Hagen C, Trapnell DW, Hamrick JL, Rocha O, Smouse PE, Glenn TC (2008) Isolation and characterization of microsatellite loci in the Guanacaste tree, *Enterolobium cyclocarpum*. *Mol. Ecol. Resour.* 8:129-131.

Zucchi MI, Brondani RPV, Pinheiro JB, Brondani C, Vencovsky R (2002) Transferability of microsatellite markers from *Eucalyptus* spp. to *Eugenia dysenterica* (Myrtaceae family). *Mol. Ecol. Notes* 2:512-513.

Weir BS (1996). Genetic Data Analysis II – Methods for discrete population genetic data. Sinauer Associates: Sunderland.

FIGURE

Figure 1. Localization of the populations of *Enterolobium contortisiliquum* of central Brazil.



TABLES

Table 1. Description of SSR primers developed for *Enterolobium cyclocarpum* and transferred to *E. contortisiliquum* with repeat motif of each loci, the allele size range founded in *E. contortisiliquum* for each loci and the annealing temperature of each primer used for *E. contortisiliquum*.

Locus	Repeat motif	Allele size range (bp)	Ta (°C)
Ency-04	(AC)9...(ACAT)4...(AAAC)5	164-202	56°C
Ency-08	(AAAG)4	192-206	58°C
Ency-09	(AG)16	158-172	56°C
Ency-13	(ATC)10	204-220	58°C
Ency-17	(GTTT)6	266-282	56°C
Ency-21	(AC)8...(ACAT)12	192-198	56°C
Ency-22	(CT)14...(ACTC)4	192-198	56°C
Ency-24	(CT)15	106-136	54°C
Ency-33	(ACAT)8	124-150	54°C

Table 2 Characterization of the genetic diversity of nine primers developed for *Enterolobium cyclocarpum* and transferred to *E. contortisiliquum*.

Locus	*A*	H_E	H_O	*f*	*Q*	*I*
Ency-04	9	0.657	0.750	-0.144	0.4211	0.0000
Ency-08	6	0.644	0.833	-0.300	0.4306	0.0000
Ency-09	4	0.579	0.278	0.524*	0.3105	0.0004
Ency-13	4	0.692	0.516	0.257	0.4340	0.0000
Ency-17	5	0.630	0.150	0.764*	0.3779	0.0000
Ency-21	3	0.628	1.000	-0.610	0.3638	0.0001
Ency-22	3	0.601	0.472	0.216	0.3302	0.0002
Ency-24	11	0.848	0.917	-0.082	0.6826	0.0000
Ency-33	9	0.536	0.639	-0.195	0.3463	0.0002
Over all loci	6	0.646	0.617	0.045	*QC*=0.9929	*IC*=1.004X10^{-36}

A, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; *f*, inbreeding coefficient, *Q*, probability of paternity exclusion; *QC*, combined probability of paternity exclusion; *I*, probability of genetic identity; *IC*, combined probability of genetic identity. Values followed by * are statistically differ from zero, for P = 0.005, Bonferroni adjusted p value for a nominal level of 5%.

CONSIDERAÇÕES FINAIS

As análises de diversidade genética de populações de *Enterolobium contortisiliquum* realizadas com marcadores moleculares do tipo ISSR revelam uma alta diversidade genética. A maior parte da variabilidade é atribuída às diferenças entre os indivíduos dentro das populações. Os resultados mostraram a formação de quatro grupos distintos, provavelmente determinados por uma barreira geográfica, o Rio São Francisco.

Além de uma estrutura genética das populações foi possível verificar uma maior velocidade de germinação das sementes das árvores localizadas em uma matriz conservada, embora uma diferença na quantidade de sementes germinadas em relação às árvores localizadas em uma matriz fragmentada não tenha sido detectada. Além disso, os efeitos da alteração do habitat não foram observados nos tamanhos dos frutos e sementes, bem como na produção de sementes viáveis e inviáveis (abortadas), na proporção de sementes predadas pelo bruquídeo *Merobruchus bicoloripes* e na produção de sementes totais.

Ainda que os efeitos da fragmentação do habitat não tenham sido observados nos atributos acima citados, modificações nos padrões fenológicos foram observadas quando comparamos árvores localizadas em uma matriz conservada, com árvores em uma matriz degradada e/ou em uma área urbana. Essas diferenças são importantes pois podem provocar um isolamento temporal entre os indivíduos de diferentes populações, impossibilitando o fluxo gênico entre os mesmos.

Os marcadores moleculares microssatélites desenvolvidos para *E. cyclocarpum* foram transferidos com sucesso para *E. contortisiliquum*. Essa valiosa ferramenta será útil para investigarmos a diversidade genética de adultos e progênie, o sistema de acasalamento, o fluxo gênico e a estrutura genética espacial de árvores localizadas em áreas com diferentes graus de

perturbações, verificar os efeitos da fragmentação nesses parâmetros e gerar subsídios para a conservação da espécie.

Os resultados observados nesse trabalho são importantes por ampliar o conhecimento sobre a espécie *E. contortisiliquum* e sobre as Matas Secas brasileiras. Além disso, os resultados encontrados são fundamentais para adoção de estratégias de conservação e manejo da espécie em estudo, bem como do ambiente na qual ela está inserida.

REFERÊNCIA BIBLIOGRÁFICAS

Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J. (2008) Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Molecular Ecology, 17, 5177 – 5188.

Alcalay N, Amaral DMI. (1982). Quebra de dormência em sementes de timbaúva – *Enterolobium contortisiliquum* (Vell.) Morong. Silvicultura em São Paulo, 16, 1149-1152.

Anaya F, Barbosa R, Sampaio C. (2006) Sociedade e biodiversidade na Mata Seca Mineira. Unimontes Científica, 8, 35-41.

Bastianetto E, Cunha AP, Bello ACPP, Melo MM. (2005). Intoxicação de bezerros búfalos por *Lantana* spp. em Minas Gerais: relato de caso. Revista Brasileira de Reprodução Animal, 29, 570-590.

Bonel-Raposo J, Riet-Correa F, Guim TN, Schuch ID, Grecco FB, Fernández CG.(2008). Intoxicação aguda e abortos em cobaias pelas favas de *Enterolobium contortisiliquum* (Leg. Minosoidae). Pesquisa Veterinária Brasileira, 28, 593-596.

Carvalho PER. (2003). Espécies arbóreas brasileiras, Brasília: Embrapa informação Tecnológica, Colombo/PR: Embrapa Florestas.

Costa RLD, Marini A, Tanaka D, Berndt A, Andrade FME. (2009). Um caso de intoxicação de bovinos por *Enterolobium contortisiliquum* (Tamboril) no Brasil, Archivos de Zootecnia, 58, 313-316.

Debinski DM, Holt RD. (2000). A survey overview of habitat fragmentation experiments. Conservation Biology, 14, 342-355.

Durigan G, Nishikawa DLL, Rocha E, Silveira ER, Pulitano FM, Regalado LB, Carvalhaes MA, Paranaguá PA, Ramilu VEL. (2002). Caracterização de dois estados da vegetação de uma área de cerrado no município de Brotas, SP, Brasil. Acta Botânica Brasílica,16, 251-262.

Eira MTS, Freitas RWA, Mello CMC. (1993). Superação da dormência de sementes de *Enterolobium contortisiliquum* (Vell.) Morong.-Leguminosae. Revista Brasileira de Sementes, 15, 177-181.

Espírito-Santo MM, Sevilha AC, Anaya F, Barbosa R, Fernandes GW et al. (2009) Sustainability of tropical dry forests: two case studies in southeastern and central Brazil. Forest Ecology and Management, 258, 922-930

Ewel JJ. (1999). Natural systems as models for design of sustainable systems of land use. Agroforestry Systems, 45, 1-21.

Ewers RM, Didham RK. (2006). Confounding factors in the detection of species responses to habitat fragmentation. Biological Reviews, 81, 117–142.

Frankham R, Ballou JD, Briscoe DA. (2002). Conservation Genetics. Cambridge University Press: Cambridge UK.

Frankie GW, Haber WA, Vinson SB, Bawa KS, Ronchi PS et al. (2004). Flowering phenology and pollination systems diversity in the seasonal dry forest. In: Frankie GW, Mata A, Vinson SB, editors. Biodiversity conservation in Costa Rica: learning the lessons in a seasonal dry forest. University of California Press, Berkeley. pp 17-29.

Gentry AH. (1995). Diversity and floristic composition of neotropical dry forests. In: Bullock SH, Mooney HA, Medina E (eds.) Seasonally Dry Tropical Forests. Cambridge University Press, Cambridge, pp 146-194.

Graham A, Dilcher D. (1995). The Cenozoic record of tropical dry forest in northern Latin America and the southern United States. In: Bullock SH, Mooney HA, Medina E (eds.) Seasonally Dry Tropical Forests. Cambridge University Press, Cambridge, pp 124-145.

Grecco FB, Dantas AFM, Riet-Correa F, Leite CGD, Raposo JB. (2002). Cattle intoxication from *Enterolobium contortisiliquum* pods. Veterinary and Human Toxicology 44, 160-162.

Hamrick JL, Apsit VJ. (2004). Breeding structure of neotropical dry forest tree species in fragmented landscapes. In: Frankie GW, Mata A, Vinson SB, editors. Biodiversity conservation in Costa Rica: learning the lessons in a seasonal dry forest. University of California Press, Berkeley. pp 30–37.

Janzen DH. (1981a). *Enterolobium cyclocarpum* seed passage rate and survival in horses, Costa Rican Pleistocene seed dispersal agents. Ecology, 62, 593-601.

Janzen DH. (1981b). Guanacaste tree seed-swallowing by Costa Rican range horses. Ecology, 62, 587-592.

Janzen DH. (1982). Variation in average seed size and fruit seediness in a fruit crop of a Guanacaste tree (Leguminosae: *Enterolobium cyclocarpum*). American Journal of Botany, 69, 1169–1178.

Janzen DH, Martin PS. (1982). Neotropical anachronisms: the fruits the Gomphotheres ate. Science, 215, 19-27.

Jordan L, Baldi A, Orci KM, Racz I, Varga Z. (2003). Characterizing the importance of habitat patches and corridors in maintaining the landscape connectivity of a *Pholidoptera transsylvanica* (Orthoptera) metapopulation. Landscape Ecology, 18, 83-92.

Lorenzi H. (1998). Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil. Ed. Plantarum: Nova Odessa.

Mass JM. (1995). Conservation of tropical dry forest to pasture and agriculture. In: Bullock SH, Mooney HA, Medina E, editors. Seasonally dry tropical forests. New York: Cambridge University Press. pp. 399-422.

Mayle F. (2004). Assessmen t of the Neotropical dry forest refugia hypothesis in the light of palaeoecological data and vegetation model simulations. Journal of Quaternary Science, 19, 713-720.

Miles L, Newton AC, Fries RS, Ravilious C, May I, Blyth S, Kapos V, Gordon JE. (2006). A global overview of the conservation status of tropical dry forests. Journal of Biogeography, 33, 491–505.

Moreira PA, Braga AC, Collevatti RG, Ferreira MFM, Silva GM, Melo-Júnior, AF. (2008). A genética da conservação das Matas Secas, MG Biota, 1, 111-121.

Moreira PA, Fernandes GW, Collevatti RG. (2009). Fragmentation and spatial genetic structure in *Tabebuia ochracea* (Bignoniaceae) a seasonally dry Neotropical tree. Forest Ecology and Management, 258, 2690–2695.

Mooney HA, Bullock SH, Medina E. (1995). Introduction. In: Bullock SH, Mooney HA, Medina E (eds.) Seasonally Dry Tropical Forests. Cambridge University Press, Cambridge, pp 1.

Morrone JJ. (2000). What is the Chacoan subregion? Neotropica, 46, 51-68.

Morrone JJ. (2001). Biogeografia de América Latina y el Caribe. Manuales & Tesis, Sociedad Entomológica Aragonesa, Zaragoza.

Morrone JJ. (2009). Evolutionary biogeography: an integrative approach with case studies. Columbia University Press, New York.

Murphy PG, Lugo AE. (1986). Ecology of tropical dry forests. Annual Review Ecology and Systatics, 17, 67–88.

Oliveira DA, Moreira PA, Melo-Júnior A.F, Pimenta MAS. (2006). Potencial da biodiversidade vegetal da região Norte do Estado de Minas Gerais. Unimontes Científica, 8, 23-33.

Pennington RT, Lewis GP, Ratter JA. (2006). An overview of the plant diversity, biogeography and conservation of neotropical savannas and seasonally dry forests. In: Pennington RT, Lewis GP, Ratter JA (eds.) Neotropical savannas and seasonally dry forests: plant diversity, biogeography and conservation. CRC Press Taylor & Francis Group, Boca Raton, London, New York, pp 1-29.

Pennington RT, Prado DE, Pendry CA. (2000). Neotropical seasonally dry forest and quaternary vegetation changes. Journal of Biogeography, 27, 261–273.

Prado DE. (2000). Seasonally dry forests of tropical South America: from forgotten ecosystems to a new phytogeographic unit. Edinburgh Journal of Botany 57, 437-461.

Prado DE, Gibbs PE. (1993). Patterns of species distributions in the dry seasonal forests of South America. Annals of the Missouri Botanical Garden, 80, 902-927.

Primack RB, Rodrigues E. (2001). Biologia da Conservação. Midiograf: Londrina, PR.

Quesada M, Sánchez-Azofeira GA, Alvarez-Anõrve M, Stoner KE et al. (2009). Succession and management of tropical dry forests in the Americas: Review and new perspectives. Forest Ecology and Management, 258, 1014–1024.

Rocha OJ, Aguilar G. (2001). Reproductive biology of the dry forest tree *Enterolobium cyclocarpum (*Guanacaste) in Costa Rica: a comparison between trees left in pastures and trees in continuous forest. American Journal of Botany, 88,1607-1614.

Santos RM, Vieira FA, Fagundes M, Nunes YRF, Gusmão E. (2007). Riqueza e similaridade florística de oito remanescentes florestais no norte de Minas Gerais, Brasil. Revista Árvore, 31, 135-144.

Sala OE, Chapin FS, Armesto JJ, et al., 2000. Global biodiversity scenarios for the year 2100. Science, 287, 1770–1774.

Sánchez-Azofeira GA, Kalácska M, Quesada M, Calvo-Alvarado JC, Nassar JM, Rodrigues JP. (2005). Need for integrated research for a sustainable future in tropical dry forests. Conservation Biology, 19, 285-286.

Särkinen T, Iganci JRV, Linares-Palomino R, Simon MF, Prado DE. (2011). Forgotten forests - issues and prospects in biome mapping using seasonally dry tropical forests as a case study. BioMed Central Ecology, 11, 1-15.

Saunders DA, Hobbs RJ, Margules CR. (1991). Biological consequences of ecosystem fragmentation: a review. Conservation Biology, 5, 18-32.

Silva JMC, Tabarelli M, Fonseca MT, Lins LV. (2004). Biodiversidade da Caatinga: áreas e ações prioritárias para a conservação. Ministério do Meio Ambiente, Brasília.

Tokarnia CH, Döbereiner J, Dutra IS, Chagas BR, França TN, Brust LAG. (1999). Experimentos em bovinos com favas de *Enterolobium contortisiliquum* e *Enterolobium timbouva* para verificar propriedades fotossensibilizantes e/ou abortivas. Pesquisa Veterinária Brasileira, 19, 39-45.

Townsend C, Begon M, Harper JL. (2006). Fundamentos em ecologia, 2 ed, Porto Alegre: Artmed.

Turner IM, Corlett RT. (1996). The conservation value of small, isolated fragments of lowland tropical rain forest. Trends in Ecology and Evolution, 11, 330-333.

Werneck FP. (2011). The diversification of eastern South American open vegetation biomes: historical biogeography and perspectives. Quaternary Science Reviews, 30, 1630-1648.

Werneck MS, Franceschinelli EV, Tameirão-Neto E. (2000). Mudanças na florística e estrutura de uma floresta decídua durante um período de quatro anos (1994-1998), na região do Triângulo Mineiro, MG. Revista Brasileira de Botânica, 23, 401-413.