JUAN MANUEL DIAZ-SOTO

GERMINATION *IN-VITRO*, GENETIC DIVERSITY AND MATING SYSTEM OF *Cedrela fissilis* VELL. IN CENTRAL BRAZIL

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento, para obtenção do título de *Doctor Scientiae*.

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APROVADA: 27 de fevereiro de 2018.

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João Augusto Alves Meira Neto	Lúcio Antonio de Oliveira Campos
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Magali Gonçalves Garcia	Lyderson Facio Viccini

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Luiz Orlando de Oliveira
(Orientador)

To my wife Eydyeliana and daughter Maria Sofia,

to my family, to my country, Colombia,

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BIOGRAPHY

JUAN MANUEL DIAZ SOTO, son of Polidoro Jesus Diaz Echeverria and Ines Idalia Soto, born in Santiago de Cali, Valle del Cauca – Colombia, 8th September 1972.

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He graduated from the Faculty of agricultural sciences, at “Universidad Nacional de Colombia sede Palmira” in the specialization of Recursos Fitogeneticos Neotropicales in February 2006 when he received the grade of Master of Science.

In March 2014, he began to study in the Doctorate Program in Genetic and improvement, in the Federal University of Viçosa, acting in the area of plant genetics.

SUMMARY

RESUMO

DIAZ-SOTO, Juan Manuel, D.Sc., Universidade Federal de Viçosa, fevereiro de 2018. **Germinação in vitro, diversidade genética e sistema reprodutivo de *Cedrela fissilis* Vell, no centro do Brasil**. Orientador: Luiz Orlando de Oliveira.

No norte do estado de Minas Gerais, existe um ecótono formado pela união de três formações vegetais, a Mata Atlântica, o Cerrado e a Caatinga. Esta região está ameaçada pela extração excessiva de madeira e destruição de habitat. Várias espécies de árvores tropicais nativas desta área de ecótono estão em perigo de extinção devido à sobre-exploração e perda de habitat. *Cedrela fissilis* é uma espécie de árvore nativa do Brasil e distribuída em florestas sazonais e matas ciliares no norte de Minas Gerais. Neste estudo, analisou-se a localização genealógica de uma população do Norte de Minas (PAN) em relação com as duas linhagens conhecidos (leste e oeste) de *C.fissilis* e, o estudo da estrutura genética e estimativas do sistema reprodutivo. Para obter tecido germinativo de sementes, adequado para extração de DNA, foram testados três métodos de desinfecção, quatro meios de cultura e um substrato comercial para plantas. Os resultados mostraram que a aplicação de técnicas in vitro com desinfecção aumentou germinação por um fator de 3 a 5 vezes, com respeito a não utilização de técnicas in vitro e a dupla desinfecção aumentou o fator de germinação 1,7 no que diz respeito à desinfecção simples. Encontramos altos valores de diversidade genética que diferenciaram a PAN das linhagens Leste e Oeste; assim, pode ser considerado como uma nova fonte de variabilidade dentro de *C. fissilis*. Os resultados confirmaram *C. fissilis* como espécie de reprodução cruzada com pequena proporção de autofecundação. PAN apresentou acasalamento entre parentes e população estruturada entre progênies como consequência de desvios do acasalamento ao acaso. Concluímos que as restrições no fluxo genético entre árvores reprodutivas levaram aos altos níveis de diferenciação genéticas observadas na população de PAN.

ABSTRACT

DIAZ-SOTO, Juan Manuel, D.Sc., Universidade Federal de Viçosa, February, 2018. **Germination *in vitro*, genetic diversity and mating system of *Cedrela fissilis* Vell, in central Brazil**. Adviser: Luiz Orlando de Oliveira.

In northern Minas Gerais state, there is an ecotone formed by the junction of three vegetation formations, Atlantic dry forest, Cerrado, and Caatinga. This region is threatened by over-logging and habitat destruction. Several tropical tree species native to this ecotone are endangered by extinction, subject to overexploitation and habitat loss. *Cedrela fissilis* is a tropical tree species native to Brazil and distributed in seasonal forests and gallery forests of northern Minas Gerais. In this study, we analyzed the genealogical placement of a population from northern Minas Gerais (PAN) related to the two known lineages (East and West) of *C. fissilis* and carry out genetic structure and mating system analyses of its offspring. To obtain tissue from seedling suitable for DNA extraction we tested three disinfection methods, four culture media and a commercial substrate for plants. The results showed that the application of *in-vitro* techniques jointly with disinfection increased the germination by a factor from 3 to 5 with respect to the non-use of *in-vitro* techniques, and the double disinfection increased the germination by a factor of 1.7 respect of the simple disinfection. We found high values of genetic diversity that differentiate from both the East and West lineages; thus, PAN can be considered as a new source of variability within *C. fissilis*. The results confirmed that *C. fissilis* is as an outcrossed species with some proportion of selfing. PAN presented mating among relatives, and population structure among offspring as a consequence of deviations from random mating. We concluded that restrictions to gene-flow among reproductive trees lead to the high levels of genetic differentiation observed in PAN.

INTRODUCTION

Cedrela P. Browne (Meliaceae) includes 17 species; the genus is distributed from northern Mexico to northwestern Argentina (Styles 1981). The diversification started in the Oligocene and early Miocene and, increased in the late Miocene and early Pliocene (Muellner et al. 2010). Most of the species of the genera are narrow-ranged, however, *C. fissilis and C. odorata* are broadly distributed in Meso America and South America (Pennington and Muellner 2010). *Cedrela fissilis* is found in seasonal forests, gallery forest and within ecotones (Carvalho 1994; Muellner et al. 2010), as the formed by the junction of the Atlantic dry forest, Cerrado, and Caatinga in the northern Minas Gerais. The species develops within the primary forest or as a pioneer on secondary forests (Lorenzi 1998). The ecotones are transitional areas of vegetation between two different plant communities, usually with mixed conditions from each biological community and unique species (Livingston 1903).

The wood of *C. fissilis is* highly appreciated and has a high economic value. The International Union for Conservation of Nature (IUCN 2017) classified the species as endangered mainly for overexploitation of subpopulations and habitat loss. As a result of habitat loss is estimated that some subpopulations are extinct and others had diminished almost 30% in the last 3 generations (Martinelli and Moraes 2013).

The species of *Cedrela* are monoecious and protogynous, mechanisms which help cross-fertilization (Pennington and Muellner 2010).The mating system on *C. fissilis* is characterized by actinomorphic flowers, unisex, of 5 to 10 mm of length reunited in axillary thyrses. The flowers are pale green to red pink. The flowering season is from August to January. The pollination is carried on by insects, as stingless bees and thrips (Patiño 1997; Pennington and Muellner 2010). The fruits are dehiscent woody capsules from 3 to 10 cm in length, enclosing 30 to 300 winged seeds, up to 35 mm long by 15 mm wide, dispersed by the wind (Gandara 2009). The maturity of the fruit is 8-10 months later in the dry season when the tree does not have leaves.

Phylogenetic and phylogeographic studies in *Cedrela* from Brazilian seasonal forest, using sequences of the internal transcriber spacer regions

(ITS), plastid regions (trnS-trnG, psbB-psbT-psbN, trnT-trnL) and microsatellite markers, established that *C. fissilis* is not a monophiletic group. The complex is formed for two distinct lineages separated by the Cerrado, one located on the west side and other located on the east side (Garcia et al. 2011; Mangaravite et al. 2016).

Across Brazil for this specie have been recognized the East lineage and West lineage covering the Chiquitano range and the Atlantic range respectively (Garcia et al. 2011; Mangaravite et al. 2016 Fig 1). These ranges correspond to seasonal forest on either the east or the west of the Brazilian Cerrado. The Chiquitano range covers the Madeira-Tapajós moist forest and Chiquitano dry forest and the Atlantic range covers the Araucaria moist fores, Bahia coastal forest, Bahia Interior forest, Paraná-Paraíba interior forest and Atlantic dry forest. For *C. fissilis* distributed along the ecotone located on northern Minas Gerais there is little information and is unknown their placement related to known lineages.

The gene-flow explains how the individuals within a population are related (Hamilton 2009). The mathematical estimations derived from the relations among individuals can describe how occurred the gene-flow suggesting which were the biological and ecological factors related to the species mating system. However, there is little knowledge about the mating system of *Cedrela* and even less for its species. For the congener, *C. odorata*, were analyzed populations from Costa Rica (James et al. 1998) and for *C. fissilis* were analyzed subpopulations distributed across the Atlantic range (Kageyama et al. 2003) using microsatellites.

The dynamic of plant mating system is narrowly tied to the ecosystem conservation status. Deforestation, for example, can lead to a decrease in the number of reproductively important individuals or treat the pollinator activities; this changes can increase the distance between reproductive trees, modify random mating patterns, increase inbreeding and endogamy, cause diversity depletion, affecting the effective population size and causing loss of alleles of rare or low-frequency (giving rise to population bottleneck), result of genetic drift, reduction in gene flow as well as high depression by inbreeding (Young et al. 1996; Carneiro et al. 2011).

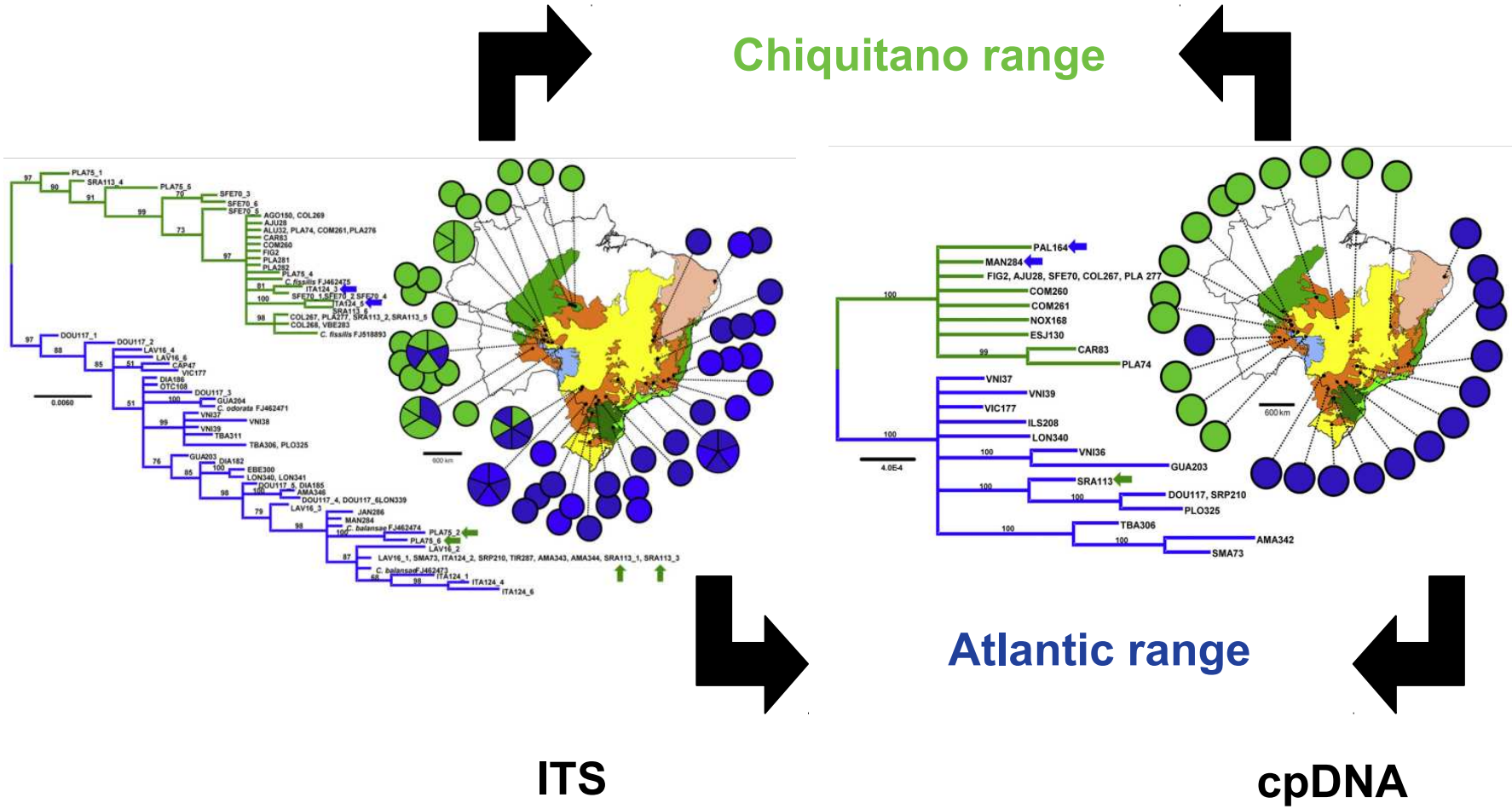

Fig 1. Geographical distribution of the two genealogical lineages split by the Cerrado and placed to the east (Atlantic range blue color) and west (Chiquitano range green color). The phylogenies were built with ITS sequences (left tree) and cpDNA sequences (right tree) (Garcia et al. 2011)

We used the molecular markers to access the genetic diversity of *C. fissilis*. Molecular markers are tools used to uncover the information that is hidden in DNA. These tools are mainly based on Polymerase Chain Reaction (PCR), as SSR (sequence-tagged simple sequence repeats) or Microsatellite molecular markers (Karp and Ingram 2012). Sequence-tagged simple sequence repeats are important tools used in several studies of population genetic diversity, phylogeography, taxonomy and phylogeny, genetic mapping, functional genomics, assisted selection, paternity testing and forensic genetics. These markers are short DNA sequences of 1-6 nucleotides, repeated a number of times and that are scattered throughout the genome of prokaryotic and eukaryotic organisms (Griffiths et al. 2005).

The microsatellite molecular markers shows polymorphism among individuals, population or even species from the same genus due to differences in length and occurrence of different number of repeating units (Morgante and Olivieri 1993). These molecular markers are very useful for genetic diversity and characterization of the population structure due to presence of random and dispersed sequences in the genome, are abundant in plants, multi-allelic, with a high degree of polymorphism, codominant, most selectively neutral and have a high mutation rate (Karp et al. 1997; Matsuoka et al. 2002). The origin of this polymorphism is still under debate but is believed that is a result of the slippage event during DNA replication, in which both the new sequence or standard chain slippages over the other in at least one motif, resulting in an incompatibility of the same (Powell et al. 1996).

In this study, our first aim was understood which was the placement of *C. fissilis* from northern Minas Gerais related to the gene-pool already known, and represented by the East and West lineages. After that, we were interested in how were the genetic relations among trees and among offspring and how was partitioned the genetic variability among offspring families and the implications of our results for the species conservation. For accessing the genetic variability of *C. fissilis* we developed an in-vitro culture procedure to produce a suitable number of seedlings.

REFERENCES

Carvalho P.E. (1994) Especies florestais Brasileiras recomendacoes silviculturais, potencialidades e uso da madeira. Brazil CNPFlorestas-EMBRAPA 639p ISBN 85–85007.

Carneiro F.S., Lacerda A.E.B., Lemes M.R., et al. (2011) Effects of selective logging on the mating system and pollen dispersal of Hymenaea courbaril L. (Leguminosae) in the Eastern Brazilian Amazon as revealed by microsatellite analysis. For Ecol Manage, **262**, 1758–1765.

Gandara FB (2009) Diversidade genética de populações de Cedro (Cedrela fissilis Vell. (Meliaceae) no Centro-Sul do Brasil.

Garcia M.G., Silva R.S., Carniello M.A., et al. (2011) Molecular evidence of cryptic speciation, historical range expansion, and recent intraspecific hybridization in the Neotropical seasonal forest tree Cedrela fissilis (Meliaceae). Mol Phylogenet Evol, **61**, 639–649.

Griffiths A.J.F, Lewontin R.C., Miller J.H., et al. (2005) An Introduction to Genetic Analysis, Eighth edn. W H Freeman & Co, New York.

Hamilton M. (2009) Population genetics., 1er edn. Wiley-Blackwell, Chichester.

IUCN (2017) Americas Regional Workshop (Conservation & Sustainable Management of Trees, Costa Rica, November 1996). 1998. Cedrela fissilis. The IUCN Red List of Threatened Species 1998: e.T33928A9821890. http://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T33928A9821890.en.

James T., Vege S., Aldrich P., & Hamrick J.L. (1998) Mating Systems of Three Tropical Dry Forest Tree Species. Biotropica, **30**, 587–594.

Kageyama P.Y., Sebbenn A.M., Ribas L.A., et al. (2003) Diversidade genética em espécies arbóreas tropicais de diferentes estágios sucessionais por marcadores genéticos. Sci For, **64**, 93–107.

Karp A., Ingram D.S. (2012) Molecular tools for screening biodiversity: plants and animals. Kluwer Academic, Dordrecht.

Karp A., Kresovich S., Bhat K., et al. (1997) Molecular tools in plant genetic resources conservation: a guide to the technologies. International Plant Genetic Resources Institute, Rome, Italy.

Livingston B.E. (1903) The distribution of the upland plant societies of Kent County, Michigan. Bot Gaz, **35**, 36–55.

Lorenzi H. (1998) Arvores Brasileiras Manual de Identifiçao e Cultivo de Plantas Arboreas Nativas do Brasil. Plantarun.

Mangaravite É., Vinson C.C., Rody H.V.S., et al. (2016) Contemporary patterns of genetic diversity of *Cedrela fissilis* offer insight into the shaping of seasonal forests in eastern South America. Am J Bot, **103**, 307–316.

Martinelli G, Moraes M (2013) Livro vermelho da flora do Brasil. Conserv da Flora, Rio 1100.

Matsuoka Y., Mitchell S.E., Kresovich S., et al (2002) Microsatellites in Zea-variability, patterns of mutations, and use for evolutionary studies. TAG Theor Appl Genet, **104**, 436–450.

Morgante M., Olivieri A.M. (1993) PCR-amplified microsatellites as markers in plant genetics. plant J, **3,** 175–182.

Muellner A.N., Pennington T.D., Koecke A., et al. (2010) Biogeography of Cedrela (Meliaceae, Sapindales) in Central and South America. Am J Bot, **97**, 511–518.

Patiño F. (1997) Recursos genéticos de Swietenia y Cedrela en los neotrópicos. Propuestas para acciones coordinadas. FAO, Roma, Italia.

Pennington T.D., Muellner A.N. (2010) A Monograph of Cedrela (Meliaceae). dh Books, Milborne Port.

Powell W., Morgante M., Andre C., et al (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed, **2**, 225–238.

Styles B.T. (1981) Swietenioideae. In: Pennington TD, Styles BT, Taylor DA. (eds). New York, 359–418.

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

CHAPTER I: A rapid and simple method for the *in-vitro* production of seedlings of *Cedrela fissilis*

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ABSTRACT

We tested three disinfection methods, four culture media and a substrate in *Cedrela fissilis* seeds to produce seedlings. The seeds were collected in an ecotone formed by the joined of three vegetation formations: the Cerrado, the Atlantic dry forest and the Caatinga in northern Minas Gerais. The seeds were treated by a combination of different exposition times (2min, 10min, 20min, 30 min) to disinfectant Sodium hypochlorite (25% active chlorine) and Tween20 (4 drops/100ml). Simultaneously were evaluated four liquid culture medium (MS20, DKW, JADS, and WPM) and the substrate Tropstrato HTi® to evaluate the best method to produce seedlings. The first disinfection method (FDM) used 2 min of disinfestation and the substrate Tropstrato HTi® , produced low number of seedlings (28 out of 160) with a percentage of germination of 17.5%. The second disinfection method SDM, evaluated 10min, 20min, 30 min of exposition to disinfectant and the culture medium MS20 and WPM, obtaining 52.7% of germination. The Third method (DDM), evaluated 10min, and 20min of exposition two times to disinfectant and the culture medium MS20, DKW and JADS, this method produced the best percentage of germination 88.9%. The application of *in-vitro* techniques jointly with disinfection with Sodium hypochlorite (25% active chlorine) and Tween20 (4 drops/100ml) increased the germination by a factor from 3 to 5 with respect to the non-use of in-vitro techniques, and the double disinfection method (DDM) increased the germination by a factor of 1.7 respect to the simple disinfection method (SDM).

ABBREVIATIONS

DKW: Walnut medium of Driver and Kuniyuki 1984

FDM: first disinfection method

IUCN: International Union for Conservation of Nature

JADS: culture medium of Correia et al. 1995

MS20: culture medium of Murashige and Skoog 1962

SDM: second disinfection method

TDM: third disinfection method

WPM: Woody Plant Medium of McCown and Lloyd 1981

INTRODUCTION

The degradation of Atlantic dry forest, the Cerrado, the Caatinga and other Brazilian vegetation formations has caused that several species of tropical trees, bird, and mammals to become endangered. Native tropical tree species of high economic value are treated of extinction because over-logging (Barbedo et al. 1997), conversion of natural habitats to agricultural land and non-optimal management strategies (Pijut et al. 2012).

Cedrela fissilis Velloso from the Meliaceae family is a native tropical tree from Brazil, known as cedar, pink cedar or red cedar. The International Union for Conservation of nature (IUCN 2017) included this species within a list of threatened species mainly for overexploitation and habitat loss. Native tree species are important because of its value in the recuperation of degraded habitats. However, there is little knowledge about the ecology, morphology and reproductive biology of the species of tropical ecosystems (Barbosa et al. 2003)

The *in-vitro* culture techniques are useful for conservation, genetic breeding, and propagation of tropical trees. However, more research and effort is needed (Pijut et al. 2012). The molecular marker techniques of wide use on conservation and breeding had worked on the early choice of desirable characteristics in several domestic and wild species (Pijut et al. 2011). Nonetheless, the *in-vitro* culture techniques are useful for molecular marker techniques when a suitable source of DNA (deoxyribonucleic acid) is hard to find. For example, for population genetics and mating system studies, the information stored in seeds can be unleashed easily and reliable by means of *in-vitro* propagation and seedling harvesting.

The molecular analyzes of mating system benefit from the *in-vitro* culture by the germination of seeds and production of seedlings. The germination is leaving from the resting state of the embryo and the beginning of metabolic activity with development of the embryo to emerge as a seedling (Rao et al. 2006). This process has several limiting factors as microorganism, nutrients, substrate, water, gases, light and temperature (Floriano 2004). Sometimes when the percentage of germination is low the hormone GA3 (Gibberellic Acid3) is used to increase the number of seedlings (Díaz-Quichimbo et al. 2013).

Mainly the seed samples for this research came from the ecotone formed by the junction of the Cerrado, Caatinga and Atlantic dry forest in northern Minas Gerais. The sampling location corresponds to the gallery forests neighboring the Pandeiros river and Peruaçu river. Our aim was to develop a simple and rapid in-vitro method for production of a suitable number of seedlings of *Cedrela fissilis*.

MATERIAL AND METHODS

1. Study site, sample collection and data analysis

Adult trees sampled were locate within the campus from Viçosa Federal University (VFU) and additionally into an area neighboring the Pandeiros river basin and Peruaçu river basin (Fig 1).

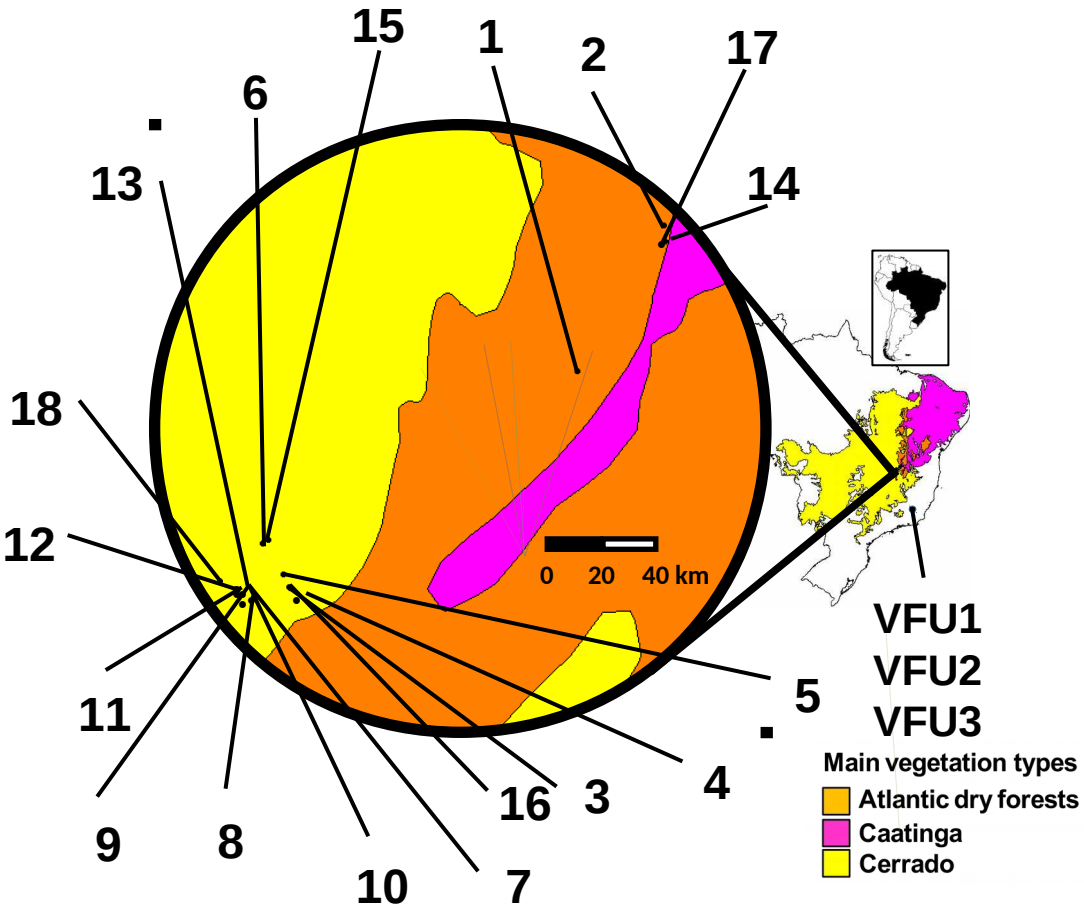

Fig 1. Associated vegetation formations and geographic distribution of adult trees of *Cedrela fissilis* sampled at the Viçosa Federal University and norther Minas Gerais covering Pandeiros and Peruaçu basin's (See Table S1 for codes)

We sampled semi-open fruits with a telescopic pole, and labeled them according to their corresponding origin. They were placed on paper bags, transported to the laboratory, and kept at room conditions until the fruit open and seeds were released, as recommended (Díaz-Quichimbo et al. 2013).

Initially 300 seeds collected from three trees within the Viçosa Federal University without prior disinfection were sowed on plastic cups of 200ml filled with sterilized sand. The plastic cups were leave on greenhouse and watering when needed. The number seedlings were accounted until day 45.

For the next experiments were assayed three disinfection methods and four culture medium to test the better form of producing seedlings to harvest leaf tissue. The First Disinfection Method (FDM) (Fig 2), used seeds without the wing, that were soaked 2 minutes in a 25% solution of sodium hypochlorite, and after rinsed with water for one minute (Oliveira and Barbosa 2014). Disinfected seeds were sowed on plastic cups of 200ml filled with sterilized commercial substrate for plants Tropstrato HTi® (Vida Verde). For this experiment we used 40 seeds from four trees each one. The plastics cups were leave on greenhouse, watering when needed and counting the number of germinate seeds until the 30 day. The evaluated parameter in this experiment and the others was germination, corresponding to the total percentage of germinated seeds until the end of the experiment. We considered germinated seeds those that gave rise to normal seedlings with roots and aerial structures and without any kind of biological contamination.

The Second Disinfection Method (SDM), developed (Fig 3) was adapted from Costa (2002). This method included soaking the seeds in commercial liquid neutral detergent, ethanol 70% and commercial sodium hypochlorite with Tween20 (4 drops/100ml). We assayed three independent times of exposition of seeds to disinfection by sodium hypochlorite with Tween20: 10 minutes, 20 minutes and 30 minutes. After disinfection the seeds were sowed into two culture medium at pH 5.8: MS20 (culture medium of Murashige and Skoog 1962; Table 1) and WPM (Woody Plant Medium of McCown and Lloyd 1981; Table 1) supplemented with Sacarose 2%.

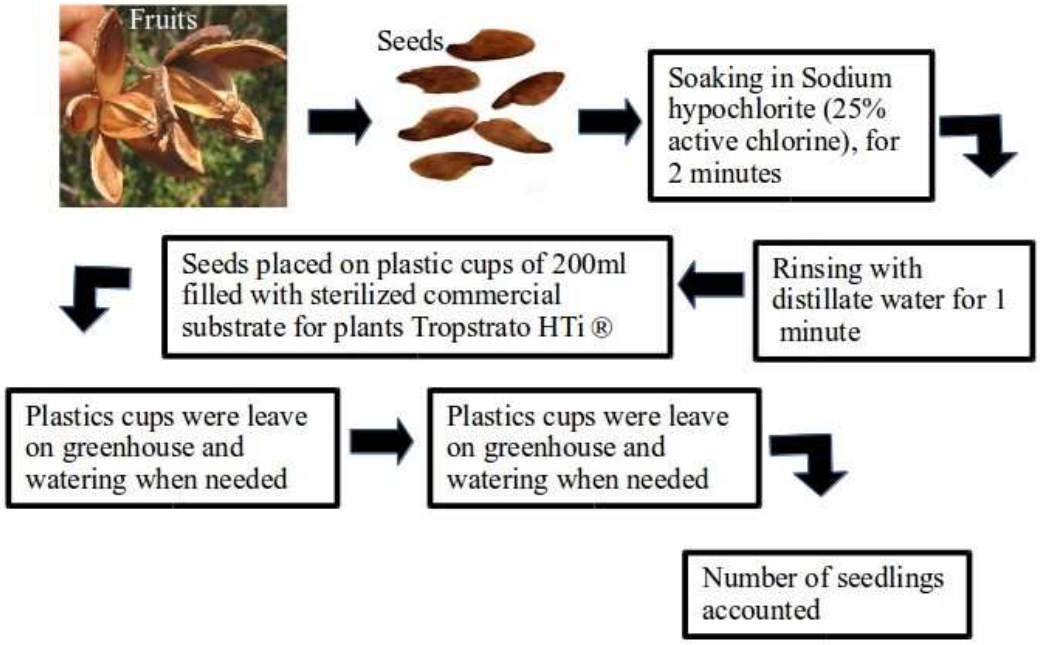

Fig 2. First Disinfection Method (FDM) followed on seeds from *Cedrela fissilis*.

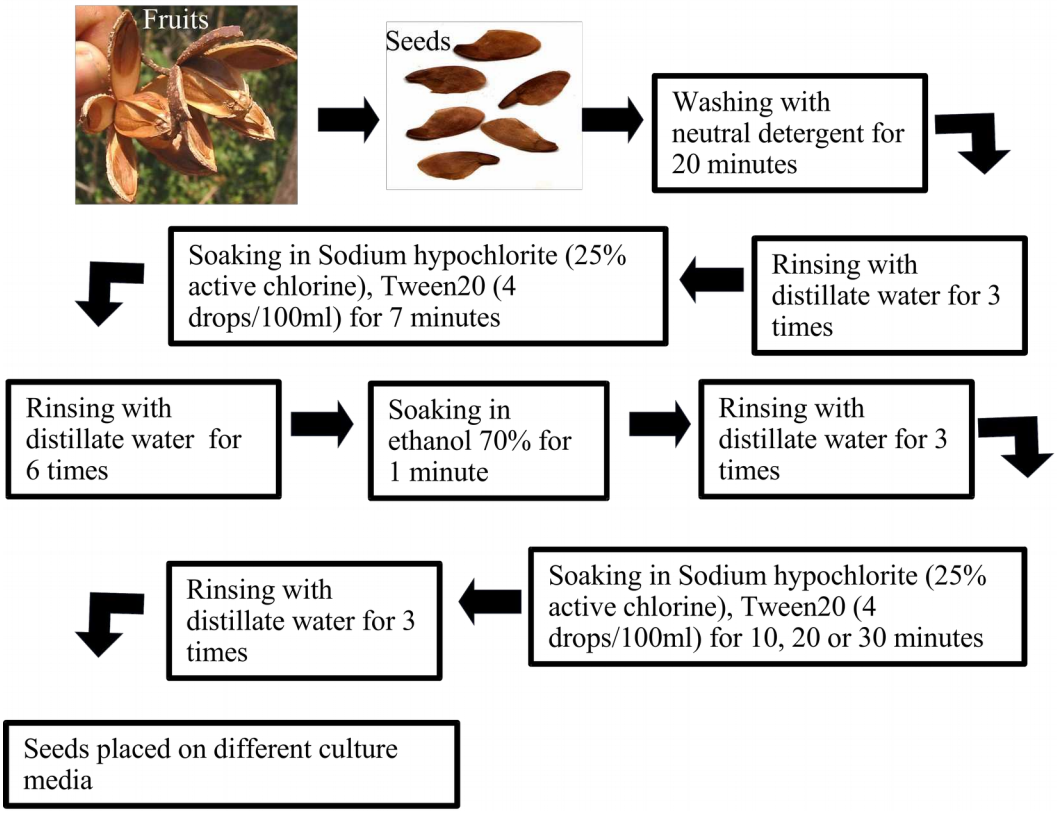

Fig 3. Second Disinfection Method (SDM) followed on seeds from *Cedrela fissilis*.

Table 1 Composition of the four liquid culture media used in this study to germinate seeds of *C. fissilis*

Ingredients	MS20	WPM	JADS	DKW
Sacarose	20g l^{-1}	20g l^{-1}	11.40 M	20g l^{-1}
Major salts				
Ammonium nitrate	1650 mg l^{-1}	400 mg l^{-1}	4.0 mM	1416 mg l^{-1}
Calcium chloride	440 mg l^{-1}	72.5 mg l^{-1}		149 mg l^{-1}
Magnesium sulphate	370 mg l^{-1}	180 mg l^{-1}	3.0 mM	740 mg l^{-1}
Monopotassium phosphate	170 mg l^{-1}	170 mg l^{-1}	3.0 mM	265 mg l^{-1}
Potassium nitrate	1900 mg l^{-1}		8.0 mM	
Potassium sulphate		990 mg l^{-1}		1559 mg l^{-1}
Calcium nitrate		386	5.0 mM	1968 mg l^{-1}
Minor salts				
Boric acid	6.2 mg l^{-1}	6.2 mg l^{-1}	50.1 µM	4.8 mg l^{-1}
Cobalt chloride	0.025 mg l^{-1}		1.0 µM	
Ferrous sulphate	27.8 mg l^{-1}	27.8 mg l^{-1}	200 µM	33.8 mg l^{-1}
Manganese(II) sulphate	22.3 mg l^{-1}	22.3 mg l^{-1}	75.6 µM	33.5 mg l^{-1}
Potassium iodide	0.83 mg l^{-1}			
Sodium molybdate	0.25 mg l^{-1}	0.25 mg l^{-1}	0.62 µM	0.39 mg l^{-1}
Zinc sulpahte	8.6 mg l^{-1}	8.6	14.90 µM	
Zinc nitrate				17.0 mg l^{-1}
Ethylenediaminetetraacetic acid	37.3 mg l^{-1}	37.3 mg l^{-1}	200 µM	45.4 mg l^{-1}
Copper sulphate	0.025 mg l^{-1}	0.25 mg l^{-1}	5.0 µM	0.25 mg l^{-1}
Nickel sulphate				0.005 mg l^{-1}
Vitamins and organics				
Myo-Inositol	100 mg l^{-1}			100 mg l^{-1}
Nicotinic Acid	0.5 mg l^{-1}		4 µM	1.0 mg l^{-1}
Pyridoxine HCl	0.5 mg l^{-1}		2.43 µM	
Thiamine HCl	0.1 mg l^{-1}		14.8 µM	2.0 mg l^{-1}
Glicine	2.0 mg l^{-1}			2.0 mg l^{-1}
Lactalbumin Hydrolysate	1.0 g l^{-1}			
Indole Acetic Acid	1.0 mg l^{-1}			
Kinetin	0.04 mg l^{-1}			
L-arginine			40.20 µM	
L-glutamine			992.3 µM	
L-cysteine			20.60 µM	
Calcium pantotenate			5.0 µM	

For this experiment were sowed 30 seeds for each medium into horizontal laminar flow chamber to avoid contamination. We used acrylic tubes filled with 5 ml of liquid medium. Seeds were laid over a bridge of Germitex paper within each tube to avoid immersion of the seed into liquid medium. After inoculation, the seeds were left from 25 to 30 days at culture room conditions: 25±2 ºC (Oliveira and Barbosa 2014), 16h daylight photo-period, irradiation of 70 µmol m-2 s-1, supplied by white cold fluorescent tubes.

The Third Disinfection Method (TDM) developed (Fig 4), performed twice the procedure with commercial sodium hypochlorite with Tween20 (4 drops/100ml) and a combination of 10 minutes and 20 minutes of disinfection.

After disinfection the seeds were assayed on three liquid medium cultures at pH 5.8: MS20, JADS (Culture medium of Correia et al. 1995; Table 1) and DKW (Walnut medium of Driver and Kuniyuki 1984; Table 1). For this experiment were sowed 21 seeds for each medium into horizontal laminar flow chamber to avoid contamination. In this experiment, we measured the germination and eight morphological characteristics.

The eight morphological characteristics served to evaluate the best culture medium producing leaf tissue suitable for DNA extraction. The following characteristics: The total length (mm), the stem length (mm), and the root length (mm) were measured with a Vernier caliper. The root number and leaf number were measured directly. The leaf fresh weight (g) and the root fresh weight (g) were quantified on a scale. The software ImageJ 1.5.0d (Rasband 1997) measured the leaf area (mm). The coltiledonal leaves were not accounted. Statistical analyses were conducted using JAMOVI 0.8.1.17 (Love et al. 2017). One way analyses of variance (ANOVA) carried out the comparison between results of germination for the disinfection methods and morphological parameters of seedlings yielded.

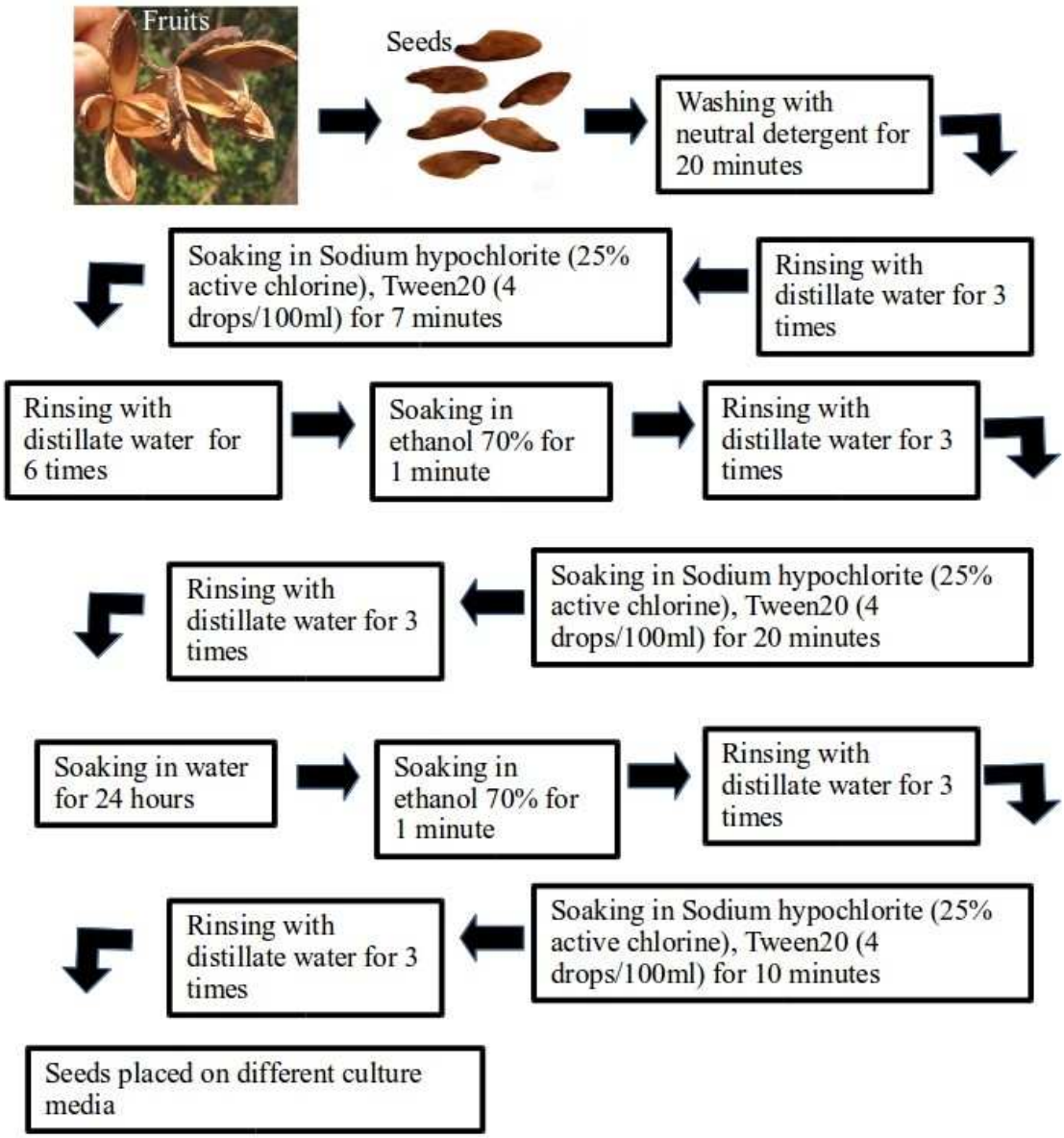

Fig 4. Third Disinfection Method (TDM) followed on seeds from *Cedrela fissilis*.

RESULTS AND DISCUSSION

The first experiment of seed germination, produced until the 30 day nearly 5% of seedlings. The germination began the fifth day after the seeds were sowed and went until the 21th day. The low number of germinated seeds and the presence of rooting seeds presumably by the presence of mold and bacteria, let us assume that for our next experimentation should be included a step of disinfection with the aim to improve the number of seedlings yielded.

The First Disinfection Method (FDM) was the first of three methods that used a solution of sodium hypochlorite like disinfectant agent. The germination with this disinfection method began the third day after the seeds were sowed and went until the 14th day. After this day until the end of the experiment, there was not more germination. The mean germination was 18% (Table 2) and variated among trees ranging from 2.5% to 37.5%. Non germinated seeds presented deformed cotyledons or rotting aspect. There were significant differences (p=0.021) for the percentage of germination. However, there were no significant differences (p=0.392) for the percentage of germination among seedling from used trees. This method was discarded because the environmental and aseptic conditions of the greenhouse were of broad variation, and the percentage of germinated seeds was low.

The results from the Second Disinfection Method (SDM), showed that there were no differences for the number of germinated seeds (mean 53%) (p=0.134; Table 3). There were no differences among the three times of seeds exposition to disinfection (p=0.123; Table 3) and between the culture medium (p=0.817; Table 3).

Table 2 Percentage of germinated of *C. fissilis* on commercial substrate for plants (Tropstrato HTi®) using the first disinfection method (FDM)

Tree	Germination (%)
1	25
2	37.5
3	5
4	2.5
Mean	17.5

40 seeds used from each tree

Table 3 Percentage of germinated seeds of *C. fissilis* on two liquid culture medium using the second disinfection method (SDM)

Culture medium	Disinfection time (min)	Germination (%)
MS20	10	66
	20	50
	30	40
WPM	10	60
	20	50
	30	50
Mean		52.7

30 seeds used for each culture medium
MS20 culture medium of Murashige and Skoog, WPM Woody Plant Medium

The results suggested that the method employed, failed to remove efficiently biological contamination by fungus or bacteria. For the production of seedling, there was not effect upon seeds due to the composition of culture media or the time of disinfection employed. For the third method of disinfection, (TDM), we chose the combined exposition time to disinfection of 10 minutes and 20 minutes to commercial sodium hypochlorite with Tween20 (4 drops/100ml) for seeds. We observed that a prolonged time of exposition of 30 minutes produced some chlorosis and deformations on cotiledonal leaves. This phytotoxicity was too reported for in vitro culture of *C. montana* (Díaz-Quichimbo et al. 2013).

The TDM method showed that there was a significant difference (p<0.001) for the number of germinated seeds (mean 89%; Table 4). However, there was not significant difference because of the effect of the culture medium (p=0.368). Similarly to observed with the DDM, the components of culture media had little effect over the germination. However, this result suggested that the double disinfection caused a dramatic increase in the number of germinated seeds.

The analyses of eight morphological characteristics measured from seedlings using the TDM, were not conclusive to establish which culture medium produced seedlings with a considerable quantity of leaf tissue. The results of the morphological characteristics for the leaf number (p=0.302; Table 5) the total length (mm) (p=0.397; Table 5), the stem length (mm) (p=0.247; Table 5), the root length (mm) (p=0.312; Table 5), the root number (p=0.333; Table 5), the leaf fresh weight (g) (p=0.975; Table 5), the root fresh weight (g) (p=0.159; Table 5) and the leaf area (mm) (p=0.515; Table 5) did not show significant differences among the culture medium MS20, DKW and JADS.

We concluded that for obtaining seedlings of *Cedrela fissilis*, the application of *in-vitro* techniques jointly with disinfection with Sodium hypochlorite (25% active chlorine) and Tween20 (4 drops/100ml) can increase the germination by a factor from 3 to 5 with respect to the non-use of in-vitro techniques. The third disinfection method increased the germination by a factor of 1.7 respect the second disinfection method.

Our experiments *in vitro* had few repetitions and were simple due to difficulty of having and appropriate quantity of seeds that let us establish a more suitable experimental design. Few seed-producing trees were available, due to the hard access to the sampling area, and the difficulty to establish the right time of fructification and that some trees produced seeds of low quality, with low germination percentage.

Table 4 Percentage of germinated seeds of *C. fissilis* on three liquid culture medium using the third disinfection method (DDM)

Culture Media	With germination (%)
DKW	100
JADS	87.7
MS20	89
Mean	88.9

21 seeds used for each culture medium
MS20 culture medium of Murashige and Skoog, JADS culture medium of Correia, DKW Driver-Kuniyuki Walnut medium.

Table 5 Average measurements for eight morphological characteristics of seedlings of *C. fissilis* for the third disinfection method (TDM) across culture medium

	Parameters							
Culture medium	Leaf number	Total length (mm)	Stem length (mm)	Root length (mm)	Root number	Leaf fresh weight (g)	Root fresh weight (g)	Leaf area (mm)
DKW	2.6	71.9	60.7	95.1	1.8	0.21	0.160	339.3
JADS	2.0	55.2	45.5	76.4	2.0	0.19	0.128	269.1
MS20	2.7	54.7	44.4	70.3	1.7	0.18	0.114	325.1
p-value	0.302	0.397	0.247	0.312	0.333	0.975	0.159	0.515

MS20 culture medium of Murashige and Skoog, JADS culture medium of Correia, DKW Driver-Kuniyuki Walnut medium.

SUPPLEMENTARY TABLES

Table S1 Geographic location of samples

Sample	Location	Latitude	Longitude
1	PRB	-15,178	-44,212
2	MSSP	-14,923	-44,060
3	PRB	-15,557	-44,714
4	PRB	-15,557	-44,713
5	PRB	-15,557	-44,713
6	PRB	-15,480	-44,760
7	PRB	-15,563	-44,806
8	PRB	-15,563	-44,806
9	PRB	-15,562	-44,805
10	PRB	-15,563	-44,806
11	PRB	-15,562	-44,806
12	PRB	-15,561	-44,806
13	PRB	-15,561	-44,807
14	MSSP	-14,951	-44,059
15	PRB	-15,473	-44,750
16	PRB	-15,535	-44,724
17	MSSP	-14,950	-44,059
18	PRB	-15,546	-44,835
VFU1	VFU	-20,764	-42,870
VFU2	VFU	-20,762	-42,863
VFU3	VFU	-20.763	-42,868

Families 1 to 18, PRB Peruaçu River Basin, PRB Pandeiros River Basin, MSSP Mata Seca State Park, VFU Viçosa Federal University

REFERENCES

Barbedo C.J., Marcos Filho. J., & Novembre A.D.L.C.A. (1997) Condicionamento osmótico e armazenamento de sementes de cedro-rosa (*Cedrela fissilis* Vell.). Rev Bras Sementes, **19**, 355–361.

Barbosa L.M., Barbosa J.M., Barbosa K.C., et al.. (2003) Recuperação florestal com espécies nativas no Estado de São Paulo: pesquisas apontam mudanças necessárias. Florestar Estatístico, **6**, 28–34.

Correia D., Gonçalves A.N., Couto H.Y.Z., & Ribeiro M.C. (1995) Efeito do meio de cultura líquido e sólido no crescimento e desenvolvimento de gemas de *Eucalyptus grandis* x *Eucalyptus urophylla* na multiplicação in vitro. IPEF, Piracicaba, **48**, 107–116.

Díaz-Quichimbo G, Poma-Angamarca R, Minchala-Patiño J, et al. (2013) In Vitro Clonal Propagation and Germplasm Conservation in the Tropical Timber Tree Spanish White Cedar (*C . Montana* Moritz Ex Turcz .) (Meliaceae). eJ. In Biol. Sci, **7**, 59-69.

Driver J.A., & Kuniyuki A.H. (1984) In vitro propagation of Paradox walnut rootstock [*Juglans hindsii* X *Juglans regia*, tissue culture]. HortScience.

Floriano E.P. (2004) Germinação e dormência de sementes florestais. St Rosa ANORGS

IUCN (2017) Americas Regional Workshop (Conservation & Sustainable Management of Trees, Costa Rica, November 1996). 1998. *Cedrela fissilis*. The IUCN Red List of Threatened Species 1998: e.T33928A9821890. http://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T33928A9821890.en.

Love J, Dropmann D, Selker R (2017) Jamovi Project.

McCown B.H., & Lloyd G. (1981) Woody plant medium (WPM)-a mineral nutrient formulation for microculture of woody plant-species. In: HortScience. Amer Soc Horticultural Science, 453.

Navarro C., Ward S., Hernández M. (2002) The tree *Cedrela odorata* (Meliaceae): a morphologically subdivided species in Costa Rica. Int J Trop Biol Conserv, **50**, 21–29.

Oliveira A.K.M., & Barbosa L.A. (2014) Efeitos da temperatura na germinação de sementes e na formação de plantulas de *Cedrela fissilis*. Floresta, **44**, 441–450..

Pijut P.M., Beasley R.R., Lawson S.S., et al. (2012) In vitro propagation of tropical hardwood tree species–a review (2001–2011). Propag Ornam Plants, **12**, 25–51.

Pijut P.M., Lawson S.S., & Michler C.H. (2011) Biotechnological efforts for preserving and enhancing temperate hardwood tree biodiversity, health, and productivity. Vitr Cell Dev Biol, **47**, 123–147.

Rao N.K., Hanson J., Dulloo M.E., et al. (2006) Manual of seed handling in genebanks. Bioversity International, Rome, Italy

Rasband, W. S. (1997). ImageJ. US National Institutes of Health, Bethesda, MD, USA.

CHAPTER II: Genealogical placement, genetic diversity and mating system of a natural population of *Cedrela fissilis* in central Brazil

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ABSTRACT

Cedrela is monoecious, protogynous and pollination is entomophily. The aims of this study were to investigate the placement of PAN population among East x West lineages of *Cedrela fissilis* and, the genetic structure and the mating system using the information of 283 offspring and 18 mother-trees with 10 fluorescent microsatellite loci. Results suggested the presence of null alleles, but at low frequency (0.060). We found high values of genetic variability for mother-trees (A=11.9; uHe=0.851; Ho=0.799) and offspring (*A*=17.8; *uHe*=0.820; *Ho*=0.736). Bayesian statistics uncovered details of multilocus genetic diversity and population structure with the formation of 3 Bayesian groups of *C. fissilis* among lineages and 6 groups among progenies. A slightly signature of bottleneck was found because there was non mutation drift equilibrium with heterozygote excess for some loci on *IAM* (50%) and *TPM* (20%) and deficit on *SMM* (50%). Estimates of the multilocus out-crossing rate confirm that *C. fissilis* is a outcrossed species (t_m=0.946) with a low selfing rate of 5.4% and crossing between relatives (t_m–t_s=0.066). The estimate of paternity correlation ($r_{p(m)}$) indicated that offspring was composed of mixtures of half-sibs and full-sibs, with the latter occurring at a low-frequency (0.130). The estimated coancestry coefficient within families (Θ_{xy}=0.184) was larger and the effective population size was lower ($Ne_{(v)}$=3.027) than expected in progenies from panmictic population. In terms of conservation, the results show that to keep an effective population size of 150, is necessary to collect seeds from at least 51 seed-trees.

Keywords

Cedrela, Conservation, Genetic diversity, Mating system, Microsatellites

ABBREVIATIONS

A: number of alleles per locus

ALT: Alta Floresta population

Ap: number of private alleles

BLU: Blumenau population

bp: base pairs

CAM: Campinhos population

CAP: Caparao population

DIA: Diamantina population

F: inbreeding coefficient

FIG: Figueropolis population

Fis: Correlation of alleles within an individual relative to the subpopulation in which it occurs

Fit: Correlation of alleles within an individual relative to the entire population

F_{null}: inbreeding coefficient excluding null alleles

FSP: full sibs proportion

Fst: Correlation of randomly chosen alleles within the same subpopulation relative to the entire population. Its measurement is based on allele frequency

Fst_{ENA},: Correlation of randomly chosen alleles within the same subpopulation relative to the entire population excluding null alleles

Gst: measure of population differentiation. Its measurement is based on population frequency

He: expected heterozygosity

Ho: observed heterozygosity

Ho: observed heterozygosity

HSP: half sibs proportion

HWE: Hardy-Weinberg equilibrium

IAM: Infinite alleles model

ITA: Itapora population

IUCN: International Union for Conservation of Nature

Na: number of alleles

NE: number effective of alleles

Nep=1/$r_{(pm)}$: effective number of pollen donors

PAN: Pandeiros population

PCR: Polymerase Chain Reaction

PEU: Parque Estadual Uaimií population

POC: Poconé population

PSB: Parque Serra Do Brigadeiro population

$rp_{(m)}$: paternity multilocus correlation

$rp_{(s)}$: paternity singlelocus correlation

s: selfing rate

SMM: Step-wise mutation model

SSP: selfing sibs proportion

SSRs: simple sequence repeats; microsatellites

t_m-t_s: mating among relatives rate

t_m: multilocus population outcrossing rate

TOC: Tocantins populations

TPM: Two phase model

t_s: singlelocus population outcrossing rate

uHe: unbiased expected heterozygosity

Θxy: The average coefficient of coancestry

INTRODUCTION

Gene flow is the movement of alleles through populations, carried out by individuals or gametes (Hamilton 2009). Gene flow relies heavily on the dispersion capacity of the pollen and seeds, the breeding system (hermaphrodite, monoecious or dioecious), the characteristics of the pollinator agent (insect, bird, mammal, or wind), outcrossing rate, and degree of geographic isolation (Hensen and Oberprieler 2005). Together with selection, gene flow seems to play a crucial role in the formation of gradients in habitats (Frankham et al. 2002). Moreover, habitat fragmentation and isolation may lead to genetic bottlenecks, which is a reduction of genetic interchange and diversity due to few individuals contributing with alleles to the descendant population (Aldrich and Hamrick 1998).

Mathematical models that describe breeding systems allow for the understanding of how gene-flow take place among population and among individuals within populations. Two widely-used models that describes plant mating system are the correlated matings model (Ritland 1989) and the mixed mating model (Ritland and Jain 1981). The first model estimates the proportion of progeny as result of random mating and biparental mating. The second model split the mating process into two components: random mating and self-fertilization.

Cedrela P. Browne (Meliaceae) comprises 17 species; the genus is distributed from northern Mexico to northwestern Argentina (Styles 1981). Several features suggest the dry seasonal forests as the likely origin of *Cedrela*: deciduous leaves, scale-protect gems, dry, capsular fruits, and winged seeds that are wind-dispersed and can survive the dry season (Pennington and Muellner 2010). While most of the congeners are narrow-ranged species, *C. odorata and C. fissilis* detain a broad distribution in Mesoamerica and South America (Pennington and Muellner 2010). They are associated with closed-canopy forests, such as the seasonal forests, the moist forests, and ecotones that are adjacent to seasonal forests (Styles 1981; Pennington and Muellner 2010). *Cedrela fissilis* is a low-density species, with a natural density of about one to tree trees per hectare (Carvalho 1994). Because it is a noble wood, *C. fissilis* experienced intense harvesting throughout much of its range. Currently,

Martinelli and Moraes (2013) established *C. fissilis* as “vulnerable A2cd” facing a high risk of extintion in the wild in the medium-term future, with a reduction of at least 20% reached within the next ten years by habitat loss and overexploitation of subpopulations. Additionally the IUCN Red List (IUCN 2017) categorizes *C. fissilis* as an “Endangered A1acd+2cd” species, facing treats of extinction in the wild in the near future, with reduction of at least 50% over the last 10 years primarily by the reasons already explained.

In eastern South America, *C. fissilis* contains two phylogenetic lineages: the east and west lineages, respectively (Garcia et al. 2011). Microsatellite markers (Mangaravite et al. 2016) largely agree with the phylogenetic analyses (Garcia et al. 2011) and corroborate to the idea that *C. fissilis* split into distinct gene pools. The east lineage occupies the Atlantic range, which occurs at the eastern side of the Cerrado, along the Atlantic coast of southeastern Brazil and includes both the seasonal and moist forests. The west lineage inhabits the Chiquitano range at the western side of the Cerrado, further inland in the Chiquitano dry forests and the Madeira-Tapajós moist forests (in lowland Bolivia and neighboring lands of western Brazil) (Garcia et al. 2011). The present-day savannah-like vegetation of the Cerrado of central Brazil seems to be unsuitable to *Cedrela*. Likely, the Cerrado has been a long-standing geographic barrier that restrained gene exchange between the two lineages of *C. fissilis*; thus, constrained them to evolve under strong genetic differentiation and allopatry (Garcia et al. 2011; Mangaravite et al. 2016).

In the past, vegetation shifts took place recurrently over broad geographic scales in South America. Episodes of climate amelioration over time allowed for the recurrent expansion of forests at the expense of dry vegetations, such as the Cerrado of central Brazil; but during drier periods, dry vegetations likely expanded over closed-canopy forests (Whitmore and Prance 1987; Mayle 2004; Pennington et al. 2004). At the present time, gene exchange between the two formerly independent lineages is feasible owing to gallery forests and blocks of seasonal forests that permeate the Cerrado in Central Brazil (Oliveira-Filho and Ratter 1995). Gallery forests and scattered blocks of seasonal forests may allow for genetic connectivity across the Cerrado ─ an otherwise impassable geographic barrier, thus favoring admixturing. Populations of *Cedrela* near ecotone zones between the Cerrado and closed-

canopy forests towards Central Brasil appear to have experienced admixture to a larger degree and may hold biogeographic clues about how the dispersion waves progressed over time towards Central Brasil (Mangaravite et al. 2016).

The northern Minas Gerais State (NMG) comprises a unique mixture of ecosystems; the region congregates plant species from three major vegetation types. At this region, the Caatinga (a scrubland vegetation dominant in the northeastern Brazil) finds its southernmost distribution and meets together with the Cerrado (advancing from the west) and the Atlantic dry forests (advancing from the east). Small tributaries of the larger São Francisco River, the Pandeiros River (Bethonico 2010) and the Peruaçu River (Nunes et al. 2009; Sales et al. 2009) runs through the region and allows for gallery forests and wetlands within an arid ecotone (Lopes et al. 2013).

Differences in the flood regime and soil types along the rivers resulted in distinct floristic composition upstream compared to downstream (Rodrigues et al. 2009). In case such areas detained a more stable climate throughout the late Pleistocene, they may have played an important role as paleo-refuges within Central Brazil. They may congregate greater diversity and ancestral genotypes due to greater long-term persistence and population structure (Carnaval et al. 2009).

The mating system and the reproductive biology of *C. fissilis* remains poorly understood. The trees produce actinomorphic, unisex flowers, of 5 to 10 mm of length reunited in axillary thyrses. The flowers are pale green to red pink. The flowering season is from August to January. The maturity of the fruit is 8-10 months later in the dry season when the trees shed their leaves. The fruits are dehiscent woody capsules from 3 to 10 cm in length, enclosing 30 to 300 winged, wind-dispersed seeds (Pennington and Muellner 2010).

There are some suggestions that the pollination of *C. fissilis* may resemble that of *Cupania guatemalensis*, in which *Trigona* stingless bees play an important role (Bawa 1977); or may be similar to that of *C. odorata,* which exhibits reported monoecious reproduction with pollination carried out by nocturnal butterflies (Bawa et al. 1985). In *C. fissilis*, monoecy and protogyny are traits that may favor cross-fertilization (Pennington and Muellner 2010). Cross-fertilization, entomophily, and long-life cycles are traits that may suggest

high levels of genetic diversity within the population and low differentiation among populations (Hensen and Oberprieler 2005).

In *C. fissilis*, however, the low density in which the trees naturally occurs together with the short-distance pollen dispersers travel may result in a scenario in which the genetic diversity is scattered in small assemblages within the population. When visiting a given flower, a pollinator may deposit several pollen grains transported from the nearest donor tree; thus, the fruit will yield a progeny of full-sibs (Muona et al. 1991). A tendency to progenies with low genetic diversity can also take place in case the pollen comes from few male donors or when mating takes place between closely-related neighbors (Surles et al. 1990).

In this study, we explored the potential role of the unique mixture of ecosystems in NMG may have played in shaping the evolutionary history of *C. fissilis*, an arboreal species associated with seasonal forests. Using microsatellite data from representative populations of the Atlantic and the Chiquitano ranges, we carried out an array of analyses to inquire the genealogical placement of a natural population of *C. fissilis* sampled from NMG. With microsatellite data obtained from mother-trees and their offspring, we investigated levels of genetic diversity and structure and mating system within the sampled population. The three questions addressed by this research were as follows: (1) Which gene pool (East x West lineage) would a population of *C. fissilis* from NMG fit in? Or will it exhibit genetic admixture? (2) What levels of genetic diversity and genetic structure does *C. fissilis* from NMG exhibit? Is there evidence for a genetic bottleneck? (3) What do the mating system estimators tell us about *C. fissilis* from NMG? We also present the implications of our results for the genetic conservation of *C. fissilis* and for the debate about the origins of seasonal forest distributions in eastern South America.

MATERIAL AND METHODS

1. Study site and sample collection

Adult trees were located within three neighboring sampling sites: Pandeiros River Basin, Peruaçu River Basin, and Mata Seca State Park (Appendix 1). The sampling sites are located in Northern Minas Gerais State, towards Central Brazil. Wherever we found fruit-bearing trees, we recorded the location using a Global Positioning System (GPS) receiver. We used the Terrestrial Ecoregions of the World database from the World Wildlife Fund (Olson et al. 2001) to define the associated vegetation formations that surrounded the sampling sites.

For the fruit season 2016-2017, a total of 18 bearing-fruit trees were located. Hereafter, those 18 trees are referred to as the population “Pandeiros” (PAN); each specimen is referred to as a ‘mother-tree’. For DNA analyses, we sampled each mother-tree of PAN for leaf tissue; leaf samples were dried immediately using silica gel and kept at room temperature until subsequent use. From distinct branches around each mother-tree, we sampled semi-open fruits, and labeled them according to their mother-tree. They were placed on paper bags, transported to the laboratory, and kept at room conditions until the fruit open and seeds were released, as recommended (Díaz-Quichimbo et al. 2013).

The seeds were sowed in Murashige-Skoog (MS20) liquid media (Saccharose 20g/L, Mio-Inositol 100mg/L, MS-salts 4.33g/L, MS-Vitamins 10ml/L, pH5.8) (Murashige and Skoog 1962) and let growing for 25-30 days. When the plantlets developed at least two leaflets, leaf tissue was taken and stored on silica gel at room temperature until subsequent use. For each mother-tree, we obtained a set of plantlets, hereafter referred to as an “offspring”.

For DNA extraction, we also sampled leaf tissues from two populations of *Cedrela fissilis*: Uaimií State Park (PEU) and Serra do Brigadeiro State Park (PSB). Both populations were located within the limits of the Atlantic range of *C. fissilis*. From PEU and PSB, we sampled 17 and 10 specimens, respectively.

2. DNA extraction and microsatellite marker analyses

DNA extractions were carried out according to the protocol described previously (Cota-Sánchez et al. 2006), with modifications (Riahi et al. 2009). We genotyped each sample using 11 nuclear microsatellite loci (Table 1). Eight markers (Ced2, Ced18, Ced41, Ced44, Ced54, Ced65, Ced95, and Ced131) were obtained for *C. odorata* (Hernández et al. 2008) and two markers (CF26, CF66) were for *C. fissilis* (Gandara 2009). The primer pair CF66 amplified two loci ─ CF66A and CF66B, respectively (Gandara et al. 2014). Distinct ranges of allele sizes (113–175 bp for CF66A; 199–253 bp for CF66B) allow for clear differentiation between the genotypes of each of the two loci (Mangaravite et al. 2016).

Following a previously developed strategy (Mangaravite et al. 2016), the polymerase chain reaction (PCR) was performed using two multiplex (M1: Ced54-Ced41-Ced95; M2: Ced2-Ced65-Ced131), a duplex (D1: CF26-Ced18) and a simplex (S1: CF66 and S2: Ced44) systems. We used a final volume of 12 µL containing 15 ng of DNA, 1X buffer (10 mM Tris-HCl, pH 8.4, 50 mM KCl, 1% Triton X-100) 0,2–0.3 mM of each primer (forward and reverse), 2.75mM MgCl2, 0.25 mg/ml BSA (Bovine Serum Albumin Invitrogen), 0.2 mM dNTPs and 1 U Taq polymerase (Phytoneutria 7 Biotechnology). We used the following PCR program: 96°C for 2 minutes, 30 cycles of 94° C for 1 minute, annealing temperature 55 °C for 1 minute and 72 °C for 1 minute; and 72 °C for 20 minutes. The forward primers were labeled with the fluorescence 6-FAM, HEX (MWG- Biotech) and NED (Applied Biosystems). Fragments were separated on a 96 capillary sequencer ABI PRISM 3730xl DNA Analyzer (Applied Biosystems) and their sizes were measured using GS500LIZ size standard (Thermo Fisher Scientific). The fragments were scored using GENEMAPPER 4.0 (Applied Biosystems).

Table 1 Primers used for SSR amplification with locus identification.

L	Primer sequence (5'→3')	*Ar*	*Ft*	T_m (°C)	*AR (pb)*	*A*
Ced2	F: TTTGCTTTGAGAAACCTTGT* R: AACTTTCGAATTGGTTAAGG	$(GA)_{20}$	6-FAM	55	133-171	17
Ced18	F: CAAAGACCAAGATTTGATGC* R: ACTATGGGTGGCACAACTAC	$(GA)_{23}$	HEX	55	119-149	16
Ced41	F: TCATTCTTGGATCCTGCTAT* R: GTGGGAAAGATTGTGAAGAA	$(TC)_{18}$	HEX	55	114-150	19
Ced44	F: ACTCCATTAACTGCCATGAA* R: ATTTTCATTCCCTTTTAGCC	$(TG)_{14}(AG)_{17}$	6-FAM	55	154-232	34
Ced54	F: GATCTCACCCACTTGAAAAA* R: GCTCATATTTGAGAGGCATT	$(GA)_{15}(AG)_6G(GA)_5$	6-FAM	55	176-200	7
Ced65	F: GAGTGAGAAGAAGAATCGTGATAGC* R: GAGGTTCGATCAGGTCTTGG	$(GA)_7(CA)_{14}$	HEX	55	149-177	10
Ced95§	F: ATTTTCATTCCCTTTTAGCC* R: TTATCATCTCCCTCACTCCA	$(CT)_{17}(AC)_{13}$	NED	55		
Ced131	F: CTCGTAATAATCCCATTCCA* R: GGAGATATTTTTGGGGTTTT	$(CT)_{16}$	NED	55	82-104	12
CF26	F: CCAAATTCCAGAGGAGAG* R: GTTCTGCTTCATCGAAGG	$(AG/TC)_{13}$	6-FAM	55	135-189	21
CF66A	F: CAGCAGTTCTGAAACAGTAA* R: ATTCAGCAACTTGAGAGC	$(AG/TC)_{13}$	6-FAM	55	119-179	27
CF66B	F: CAGCAGTTCTGAAACAGTAA* R: ATTCAGCAACTTGAGAGC	$(AG/TC)_{13}$	6-FAM	55	183-243	26

L locus, Ar array, *Ft* florescence type, T_m melting temperature, *AR* allelic range, *A* average number of alleles per locus. * fluorescence labeled primers, §: marker removed for subsequent analysis for excess of missing data.

3. Genealogical placement of PAN

Microsatellite raw data from a previous study (Mangaravite et al. 2016) provided information from five populations of the west lineage (ALT, FIG, TOC, POC, and ITA; from the Chiquitano range) and four populations of the east lineage (CAP, BLU, CAM, and DIA; from the Atlantic range) of *C. fissilis*. Moreover, the representation of the east lineage was enlarged with microsatellite data from two additional populations (PEU and PSB). Given that the raw microsatellite data from the previous study (Mangaravite et al. 2016) and data from this study (populations PAN, PEU, and PSB) were obtained using the identical strategies, they were compatible and could be combined to form a single data set for analyzes. Therefore, this dataset consisted of specimens from 12 populations: PAN, five populations west lineage, and six populations of the east lineage.

Two distinct approaches examined the likely placement of PAN within the two lineages of *C. fissilis*. Firstly, we carried out a principal coordinate analysis (PCoA) using the chord distance (Cavalli-Sforza and Edwards 1967). The software POPULATIONS (Langella 1999) produced the pairwise distance matrix; the PCoA was carried out in GENALEX 6.5 (Peakall and Smouse 2012) (Goudet 2005). Custom-made scripts in the RGL software (Team 2017) allowed for the visualization of the first three principal coordinates.

Secondly, the Bayesian model-based approach using the “Clustering of individuals” module, as implemented in BAPS 6.0 (Corander et al. 2008), inferred hidden genetic structure within the dataset. In BAPS the number of clusters is treated as an unknown parameter (Corander et al. 2003). We input the following models independently for the ‘Population mixture analysis’: K=2, and K=3, with 5 replicates for each model. Then, BAPs performed the ‘Population admixture analysis’ for each of the three set of K. This time, we selected the ‘admixture based on mixture clustering’; BAPs was not provided with the information about the population of origin of the specimen. For the admixture analysis, the minimum size of the population and the number of reference individuals was 5, with 100 overall iterations, and 10 iterations for reference individuals.

4. Genetic diversity

Initially, MICROCHECKER (Van Oosterhout et al. 2004) estimated the frequency of scoring errors and presence of null alleles. Following previous recommendations (Chapuis and Estoup 2007), we made corrections applying the method ENA (Excluding Null Alleles) to avoid the positive bias on *Fst* induced by presence of null alleles (Weir 1996). Subsequently, FREENA (Chapuis and Estoup 2007) made estimations to produce global and pairwise on both *Fst* and Fst_{ENA} with a total of 10.000 replicates to calculate the bootstrap 95% confidence interval (95%CI).

We defined the following three arrays: 1. mother-trees. 2. offspring. 3. families. The software GENALEX 6.5 (Peakall and Smouse 2012) inferred the average number of alleles per locus (*A*), number effective of alleles (*NE*), number of private alleles (*Ap*), unbiased expected heterozygosity (u*He*), observed heterozygosity (*Ho*), coefficient of inbreeding (*F*), fixation indexes (*Fis* and *Fit*) (Hartl and Clark 1997) , and the estimators of genetic differentiation (*Gst* and *Gis*) (Nei 1973). Statistical analyses were conducted using JAMOVI 0.8.1.17 (Love et al. 2017). The Mann-Whitney U test and Kruskal-Wallis analyses of variance carried out comparison between medians from genetic parameters.

The software POPGENE 4.7.0 (Rousset 2008) estimated the Fisher's exact test to assess deviations from the Hardy-Weinberg equilibrium (*HWE*). For locus across groups, we applied the multiple sample *score U test* for heterozyigote deficiency and heterozygote excess (Raymond and Rousset 1995). Additionally, we performed the exact test for genotypic disequilibrium between all pairs of loci for offspring, with the following settings 10.000 MCMC (Markov Chain Monte Carlo), 100 batches, and 5.000 iterations per batch (Rousset 2008).

The effect of null alleles over inbreeding was uncovered by obtaining the average inbreeding coefficient with the null alleles corrections (F_{null}) for offspring using INEST 2.2 (Chybicki and Burczyk 2009). The estimations used a Bayesian approach, with 500.000 MCMC iterations, the thinning parameter was set to 5.000 and burning to 50.000 cycles. We analyzed the full model (nfb), that includes estimations for the presence of null alleles (*n*), inbreeding (*f*) and

genotyping failures (*b*). We measured the robustness of the model by comparing the analysis using a random mating model (*F*=0), determining which model fit the data better by choosing the model with the lowest *DIC* (Deviance Information Criterion) value (Chybicki and Burczyk 2009).

5. Genetic structure

This time, we carried out a PCoA for progenies, with the chord distance (Cavalli-Sforza and Edwards 1967). The software Populations (Langella 1999) produced the pairwise distance matrix; the PCoA was carried out in GENALEX 6.5 (Peakall and Smouse 2012). Custom-made scripts in the R software (Team 2017) allowed for the visualization of the first three principal coordinates. The package NbClust (Charrad et al. 2014) used a total of 23 methods to search the eigenvectors for determining the best number of clusters. Then, NbClust used the majority rule to suggest the number of cluster that fit best the PCoA dataset. Next, a Bayesian model-based approach used the "Clustering on groups of individuals", as implemented in BAPS 6.0 (Corander et al. 2008). The best number of cluster NbClust suggested was taken as the upper bound of the number models (*K*) we tested in BAPs. Then, we input the models independently for the 'Population mixture analysis', with 5 replicates for each model. Subsequently, we selected "admixture based on mixture clustering". BAPs was not provided with the information about the mother-tree that gave rise to the progenies.

For the admixture analysis, the minimum size of the population and the number of reference individuals was 5, with the number of iterations was 100, and the number of iterations for reference individuals was 10. Additionally, we estimated the gene flow among inferred Bayesian groups from "admixture analysis" using the option "Plot Gene-Flow" as implemented in BAPS 6.0 (Corander et al. 2008; Tang et al. 2009).

6. Mating system

The mating system of PAN was inferred using data from the progenies of the 18 mother-trees. Analyzes used both mixed mating (Ritland and Jain 1981) and correlated mating models (Ritland 1989) models and the maximum expectation method (EM) as implemented in MLTR 3.4 (Ritland 2002).

The standard error of the parameters was calculated from 1.000 bootstraps; families were used as the re-sample unit. The following statistics were estimated: the multilocus population outcrossing rate (t_m), the singlelocus population outcrossing rate (t_s), the mating among relatives rate (t_m-t_s), the paternity multilocus correlation ($r_{p(m)}$), the paternity singlelocus correlation ($r_{p(s)}$), effective number of pollen donors ($N_{ep}=1/r_{(pm)}$), selfing sibs proportion (*SSP*), full sibs proportion (*FSP*), and half sibs proportion (*HSP*). The average coefficient of coancestry (Θ_{xy}) evaluated the genetic structure of the progeny within individuals between families (Sebbenn 2002; Carneiro et al. 2011).

The variance effective population size ($Ne_{(V)}$) and the number of seed-trees for seed collection ($m_{(150)}$) required to achieve a reference effective population size of 150 trees were calculated in accordance with a previous suggestion (Carneiro et al. 2011).

7. Detection of bottleneck

Detection of bottleneck was investigated using the software BOTTLENECK 1.2.02 (Piry et al. 1999), with the infinite alleles model (*IAM*), two phase model (*TPM*), and step-wise mutation model (*SMM*). The *IAM* explain the origin of new alleles that does not exist previously (KIMURA and CROW 1964). The *SMM* assumes that mutations are originated by one step forward or backwards, therefore allowing for reverse mutations to existing states (Chakraborty and Nei 1977; Piry et al. 1999).

The *IAM* and *SMM* are too extreme models to fit correctly all locus datasets (Cornuet and Luikart 1996) . Hence, *TPM* (Di Rienzo et al. 1994) came as an intermediate model that employs both models and the user can choose the proportion of each model *IAM* or *SMM* to be used during analyses.

Detection of bottleneck required two tests: The sign test and the two-tailed Wilcoxon test. The sign test compares the number of loci that can present a heterozygote excess to the number of such loci expected by chance alone. The parameter for TPM was 5% of SMM and 95% of IAM with variance among multiple steps of 12 and 10.000 replications (Piry et al. 1999).

The two-tailed Wilcoxon test perform a statistical analysis of heterozygote excess (Luikart et al. 1998). This test is based on the assumption of allele neutrality and mutation-drift equilibrium. This means that near the mutation drift equilibrium the expected heterozygous (H_{eq}) equals the measured *HWE* heterozygosity (uH_e) in non-bottleneck population and when the population has undergone a recent bottleneck $uH_e > H_{eq}$ (Nei 1975; Cornuet and Luikart 1996).

RESULTS

1. Genealogical placement of PAN

Principal coordinate analysis (PCoA) used the Cavalli-Sforza and Edwards distance measure; the results showed that the populations split according to their range of origin (Fig. 1). There were two major groupings: one of the groups brought together the six populations of the East lineage of *C. fissilis* that had been sampled at the Atlantic range (Fig. 1; depicted in blue); the second group contained the five populations of the west lineage, from the Chiquitano range (Fig. 1; depicted in green). Away from the west lineage, PAN was a population that occupied a placement in the border of the east lineage (Fig. 1; depicted in purple). Together, the three principal coordinates explained about 48% of the total variation.

Admixture inferences shed further light into the genealogical placement of PAN within the East lineage; moreover the BAPS analyses revealed hidden aspects of the genetic structure of PAN. When the *K*=2 model was used (Fig. 2; top), the BAPS analysis indicated that a small Bayesian group (12 mother-trees of PAN) was split away from a much larger group (6 mother-trees of PAN together with 98 specimens of the six populations of the east lineage and 77 specimens of the five populations of the west lineage. When the *K*=3 model was used (Fig. 2; bottom), the BAPS analysis showed that the previous split of PAN into two distinct Bayesian groups was statistically stable. Furthermore, the *K*=3 model indicated that the first group of the *K*=2 model gave origin to two new groups, containing each either the east or the west lineages of *C. fissilis*, with few exceptions. In the *K*=3 model, few specimens of the east lineage came together within the group formed mostly with the specimens of the west lineage, and vice-versa.

Once we have established the genealogical placement of PAN within the east lineage of *C. fissilis*, we shifted tools. Instead of tools for analyses at large geographic scales, we used statistical tools that allowed us to understand local forces that can shape genetic diversity and differentiation within a single population.

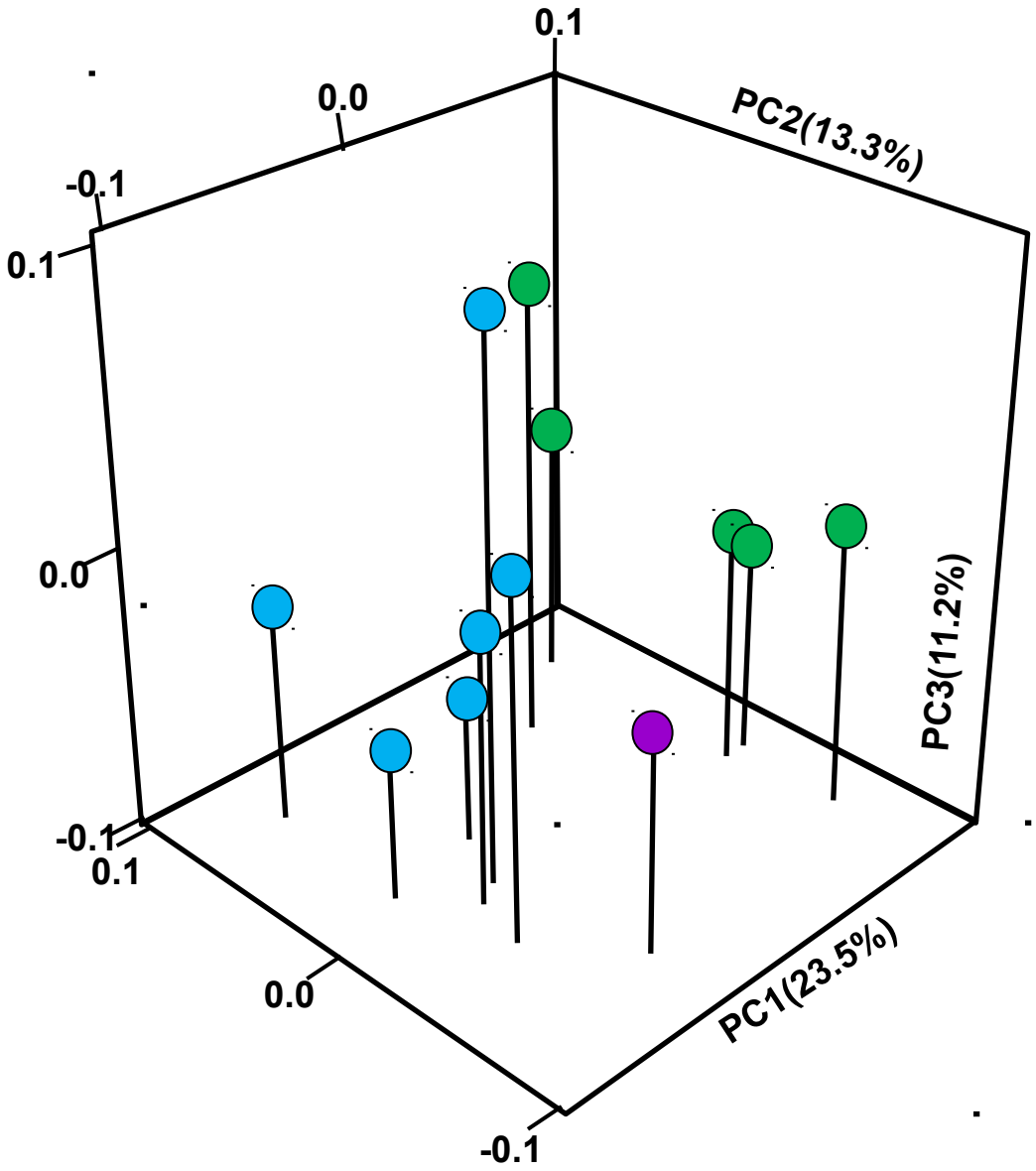

Fig. 1 Plot of the first three principal coordinates for 12 populations of *Cedrela fissilis*, based on the Cavalli-Sforza and Edwards distance measure. Color code according to origin: blue, six populations of the Atlantic range; green, five populations of the Chiquitano range; purple, population PAN. Eigenvalues for each of the principal coordinate are shown in parenthesis

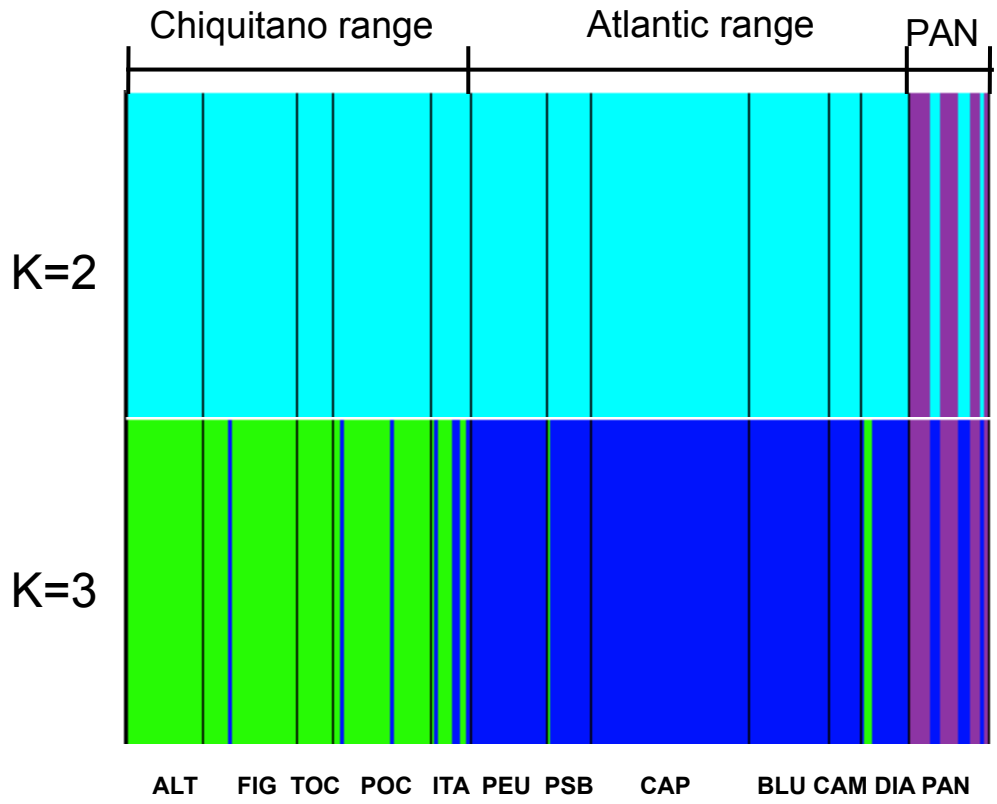

Fig. 2 Plots from admixture inferences (for *K*=2 and *K*=3, obtained from BAPS) for each of 12 populations of *Cedrela fissilis* (six populations of the Atlantic range, five populations of the Chiquitano range, and the population PAN). Along de x-axis, each vertical bar represents a specimen. Along the y-axis, membership coefficient of a sample represents the fraction of the sample's genome that has ancestry in a given Bayesian group (color-coded as indicated)

2. Genetic diversity

The frequencies of null alleles were below the threshold value of 0.19; thus, dataset from all ten loci of the microsatellite markers were considered suitable for the subsequent analyses.

Ten nuclear microsatellite loci allowed us to identify a total of 189 alleles (Table 1) in PAN: 119 for the set of 18 mother-trees and 178 for the set of 283 offspring. Therefore, there was an influx of 59 new alleles through pollen gene flow originated from non-sampled donor trees. All ten loci were polymorphic, both in mother-trees and offspring. The mean number of alleles per locus (*A*) across markers within mother-trees, ranged from four (Ced54) to 18 (CF66B) (Table 2). Within the offspring, it ranged from seven (Ced54) to 32 (Ced44) (Table 3). Across families, values of *A* ranged from 4.2 (*Fam*1) to 8.0 (*Fam*17) (Table 4).

For the set of 18 mother-trees, the overall mean of the observed heterozygosity (*Ho*=0.80) was lower than the observed unbiased heterozygosity (*uHe*=0.85); the overall mean number of private alleles (*Ap*=1.1) was very low (Table 2). Compared to mother-trees, the offspring showed lower mean values of *Ho* (0.74) and *uHe* (0.82) and a much higher value of *Ap* (7.1; Table 3). There was a significant difference ($p<0.01$) between values of *Ap* of mother-trees compared to offspring.

For the mother-trees, the coefficient of inbreeding (*F*) ranged from -0.048 (Ced2) to 0.232 (CF66A) (Table 2). For the offspring, values of *F* ranged from 0.001 (Ced44) to 0.228 (Ced131) (Table 3). The overall mean values of *F* for the mother-trees (*F*=0.06) and the offspring (0.10) were significantly different from zero ($p<0.05$).

For the offspring, six out of 10 loci showed a significant deviation from the Hardy-Weinberg equilibrium (*HWE*). There were five loci (Ced2, Ced41, Ced44, Ced54, and CF66A) that exhibited heterozygote excess, while one loci (CF66B) showed heterozygote deficit (Table 3). The Holm-Bonferroni correction method (Holm 1979) indicated ($p<0.05$) that there was linkage disequilibrium (*LD*) between pairs of loci in five families: 4 (Ced41-CF26, Ced41-Ced2, Ced41-Ced131), 6 (Ced2-Ced44), 14 (Ced2-Ced44), 15 (Ced54-CF66B, Ced54-Ced44), and 17 (Ced2-Ced44).

Table 2 Within-population genetic diversity of mother-trees across 10 loci.

L	*A*	*NE*	*Ap*	*uHe*	*Ho*	*F*
Ced2	11	8.00	1	0.90	0.94	-0.048
Ced18	10	7.71	0	0.90	0.93	-0.040
Ced41	16	10.29	1	0.93	0.89	0.043
Ced44	15	10.32	2	0.93	0.88	0.052
Ced54	4	1.80	0	0.46	0.44	0.026
Ced65	8	3.75	0	0.76	0.71	0.065
Ced131	10	6.49	0	0.87	0.82	0.051
CF26	13	8.1	0	0.90	0.83	0.076
CF66A	14	11.57	3	0.94	0.72	0.232
CF66B	18	13.13	4	0.95	0.81	0.147
Mean	11.9	8.12	1.1	0.85	0.80	0.06

L locus, *A* average number of alleles per locus, *NE* number of effective alleles, A_p number of private alleles, *uHe* unbiased expected heterozygosity, *Ho* observed heterozygosity, *F* fixation index.

Table 3 Within-population genetic diversity of offspring across 10 loci.

L	*A*	*NE*	*Ap*	*uHe*	*Ho*	*F*	*Score U test*	*Null**
Ced2	16	9.39	6	0.90	0.85	0.053	Excess	0.065
Ced18	16	8.53	5	0.88	0.76	0.144	HWE	0.080
Ced41	18	10.61	3	0.91	0.91	0.002	Excess	0.009
Ced44	32	13.24	19	0.93	0.93	0.001	Excess	0.021
Ced54	7	1.60	3	0.37	0.37	0.008	Excess	0.003
Ced65	10	3.69	3	0.72	0.62	0.143	HWE	0.063
Ced131	12	6.24	2	0.84	0.65	0.228	HWE	0.151
CF26	21	8.11	8	0.88	0.77	0.121	HWE	0.071
CF66A	24	6.75	13	0.85	0.79	0.076	Excess	0.039
CF66B	22	11.83	9	0.92	0.72	0.210	Deficit	0.098
Mean	17.8	8.0	7.1	0.82	0.74	0.10		0.06

L locus, A average number of alleles per locus, *NE* number of effective alleles, A_p number of private alleles, *uHe* unbiased expected heterozygosity, *Ho* observed heterozygosity , *F* fixation index, *HWE* Hardy Weinberg Equilibrium, Excess: heterozygote excess, Deficit: heterozygote deficit. *: Observed loci with positive presence of null alleles; the number shows null allele frequency using the EM algorithm (Dempster et al. 1977).

Within families, the overall mean *Ho* (0.732) was higher than *uHe* (0.689; Table 4) and the values significantly different (*Ho*>*uHe*; $p<0.001$). As a consequence; the fixation index was negative ($F=-0.061$) and significantly different from zero ($p<0.001$), which suggested deviations from random mating (Table 5). Among families, there were significant differences ($p<0.05$) for *A, uHe* and the number of effective alleles (*NE*; Table. 4).

The overall mean of the inbreeding coefficient across families with null allele corrections ($F_{null}=0.020$) was obtained using INEST. Overall mean F_{null} was different from zero ($p<0.001$). The inbreeding component of the model (*f*) played an important role in explaining the departures of *HWE* or *LD* in a total of five out of 18 families (4, 6, 11 17, and 18). A single family (17) exhibited F_{null} that did not overlap zero (Table 4).

3. Differentiation among the 18 families

There was a significant (p<0.01) genetic divergence (*Fst*=0.174; Fst_{ENA}=0.169, and *Gst*=0.163) among the 18 families of the population PAN (Table 5). The presence of null alleles biased the estimation of the fixation index, with Fst_{ENA} being significantly different (p<0.01) from *Fst.*

The hierarchical analysis of molecular variance (*AMOVA*) using *Fst* as the molecular distance indicated that there was not significant divergence (*Fst*=0.004; p=0.057) between mother-trees and their offspring (Table 6). Heterozygote deficit (*Fis*=0.100) reached 10% of the variability among individuals. When *Rst* was as molecular distance, *AMOVA* showed that the genetic divergence was strong (*Rst*=0.317; p<0.001). Thus, *SMM* explains about 32% of the differences between mother-trees and their offspring (Table 6).

Among families, *AMOVA* was significant for both *Fst* (0.167; p<0.001) and *Rst* (0.047; p<0.001), which corresponded to about 16% and 5% of total variance, respectively (Table 6). Differences among individuals within families (*Fis*=-0.066; p=1.00, *Ris*=0.652; p<0.001) were small and non-significant for the first and large and significant for the second (Table 6).

Principal coordinate analysis revealed hidden genetic structures among the 18 families (Fig. 3). According to the majority rule, seven methods suggested that the best number of clusters was six (Fig. 3; depicted as A to F). The number of families within a given cluster ranged from one to eight (Table 4).

During the mixture analysis in BAPS, we used six models to probe how clustering would take place amongst the 283 offspring. The models are based on parameter *K,* that estimated the maximum number of genetically diverge groups. The models ranged from *K*=2 up to *K*=6, as six was the best number of clusters according to the preceding NbClust analysis. The results from three models (*K*=2, *K*=4, and *K*=6) are shown (Fig. 4), with the 283 offspring arranged according to their family of origin (1 to 18). In the *K*=2 model, the offspring were split into either of two Bayesian groups. Across the subsequent analyses (*K*=4 and *K*=6), the groups recovered in *K*=2 model underwent further subdivisions.

Table 4 Within-population genetic diversity for families across 10 loci.

F	*N*	*A*	*NE*	*Ap*	*uHe*	*Ho*	*Fx*	*Nu*	*HWE*	F_{null}	*DIC* *nb* *nfb*	*95% CI*	*NbC*
1	10	4.2	2.87	0.1	0.652	0.620	0.054	Yes°°	0.685ns	0.046	**427.00**-430.43	0.00-0.13	F
2	21	4.9	3.33	0.6	0.628	0.653	-0.038	Yes°°	**<0.000**	0.015	**619.22**-623.19	0.00-0.06	F
3	9	5.2	3.44	0.4	0.705	0.711	-0.013	Yes°	0.229ns	0.021	**460.75**-462.01	0.00-0.08	E
4	20	5.8	3.35	0.0	0.695	0.735	-0.064	Yes°	**0.000**	0.007	966.03-**963.39**	0.00-0.03	E
5	13	6.4	3.78	0.1	0.731	0.746	-0.030	Yes°	**0.006**	0.021	**720.77**-723.81	0.00-0.08	E
6	14	5.3	2.60	0.2	0.633	0.664	-0.043	Yes°°	0.418ns	0.024	605.71-**604.23**	0.00-0.10	C
7	11	6.3	3.29	0.2	0.657	0.767	-0.145	Yes°°	0.998ns	0.088	**573.43**-575.91	0.00-0.03	A
8	21	6.7	3.73	0.2	0.733	0.802	-0.094	Yes°	0.161ns	0.007	**1141.16**-1144.75	0.00-0.03	A
9	18	6.6	3.27	0.2	0.650	0.733	-0.112	No	0.549ns	0.006	**884.08**-887.08	0.00-0.02	A
10	21	7.3	3.68	0.1	0.681	0.789	-0.149	No	0.162ns	0.006	**875.49**-1106.24	0.00-0.02	A
11	18	6.4	3.16	0.1	0.642	0.698	-0.096	No	0.452ns	0.008	875.49-**873.18**	0.00-0.03	A
12	21	6.7	3.68	0.2	0.701	0.728	-0.040	Yes°°	**0.007**	0.009	**1100.73**-1108.90	0.00-0.03	A
13	14	6.0	3.39	0.1	0.675	0.734	-0.078	Yes°	0.539ns	0.008	**706.02**-713.37	0.00-0.03	A
14	11	4.8	3.33	0.2	0.679	0.791	-0.150	No	0.134ns	0.008	**503.948**-510.02	0.00-0.03	D
15	21	6.1	3.69	0.7	0.705	0.783	-0.108	Yes°	**0.013**	0.005	**1066.78**-1070.11	0.00-0.02	B
16	12	4.7	3.05	0.0	0.669	0.692	-0.014	Yes**	**0.008**	0.015	**536.68**-538.75	0.00-0.05	B
17	15	8.0	5.38	0.2	0.774	0.729	0.052	Yes*	**0.004**	0.051	1356.40-**1353.71**	0.01-0.09	D
18	13	6.9	4.43	0.1	0.782	0.797	-0.022	Yes*	0.141ns	0.019	810.34-**809.19**	0.00-0.06	A
Mean	15.72	6.02	3.52	0.21	0.689	0.732	-0.061			0.020			

F family, *N* number of individuals, *A* average number of alleles per locus, *NE* number of effective alleles, A_p number of private alleles, *uHe* unbiased expected heterozygosity, *Ho* observed heterozygosity, *Fx* fixation index, *Nu*: Yes: Presence of null alleles in any of 10 locus; ° null alleles with low frequency. °° null alleles with medium frequency. No: Absence of null alleles or null alleles with the lowest frequency in any locus using the software FREENA (Chapuis and Estoup 2007), *HWE* deviation from Hardy-Weinberg Equilibrium; ns: not significant; significant p-values shown in bold ($p \leq 0.05$), F_{null} average inbreeding coefficient with null alleles corrections, DIC deviance information criterion; DIC values for nb and nfb models are shown, best model was chosen based on DIC and are indicated in bold, 95% CI confidence interval, NbC: groups identified from A to F with package NbClust.

Table 5 Among-populations genetic diversity of offspring.

	Fis	*Fst*	*Fst$_{ENA}$*§	*Fit*	*Gis*	*Gst*
Offspring	-0.076	**0.174**	**0.169**	**0.112**	-0.065	**0.163**
95%CI		0.167-0.181	0.163-0.174			0.157-0.169

Fst genetic differentiation among populations including null alleles, *Fst$_{ENA}$* genetic differentiation among populations excluding null alleles; NS non-significant, significant p-values shown in bold ($p \leq 0.01$). § estimation of null allele frequency using software FREENA (Chapuis and Estoup 2007).

The composition of the groups of NbClust showed no apparent correspondence with the composition of the groups recovered in BAPS. Nevertheless, the composition of Bayesian groups exhibited some degree of association with the geographic distribution of the families (Fig. 5). For instance, eight families (7 to 12, and 18; depicted in purple) were each sampled from mother-trees located in close proximity within a single sampling site at the Pandeiros River Basin. Three families (2, 14, and 17; depicted in green) were collected each from neighboring mother-trees at the Mata Seca State Park. Another set of three families (3, 4, and 5; depicted in pink) were from a separate sampling site at the Pandeiros River Basin. However, there were instances in which two neighboring mother-trees gave rise each to a family that belonged to a discrete Bayesian groups. For example, the mother-trees that gave rise to families 6 and 15 were from a single site but the families belonged to distinct groups. The pink group (3, 4, 5) excluded family 16. Indeed, the geographic distribution of the families across the sampling area seems to have contributed to decrease gene flow amongst the 6 groups, as the Bayesian analysis of gene flow had demonstrated (Fig. 6). Mother-trees within each of the six clusters received the contribution from genetically-related pollen donors, therefore decreasing the rate of admixture among distinct genetic groups.

Table 6 Analysis of molecular variance (AMOVA) for three hierarchical analyses of *Cedrela fissilis* populations using the distance matrix for the number of alleles (*Fst*) and the distance matrix for the sum of square size differences (*Rst*).

Source of variation	*d.f*	*Sum of squares*	*Variance components*	*Fixation index*	*Percentage variation*	*P-Value**
Three hierarchical levels for mother threes and offspring: number of alleles						
Among adult and offspring	1	5.52	0.02	*Fst*=0.004	0	0.057
Among individuals	299	1353.53	0.41	***Fis*=0.100**	10	0.001
Within individuals	301	1114.74	3.70	***Fit*=0.103**	90	0.001
Totals	601	2473.79	4.13		100	
Three hierarchical levels for mother threes and offspring: sum of square size difference						
Among adult and offspring	1	25753.25	367.08	***Rst*=0.317**	32	0.001
Among individuals	299	270265.89	113.90	***Ris*=0.144**	10	0.001
Within individuals	301	203508.20	676.11	***Rit*=0.416**	58	0.001
Totals	601	499527.34	1157.09		100	

Source of variation	*d.f*	*Sum of squares*	*Variance components*	*Fixation index*	*Percentage variation*	*P-Value*
Three hierarchical levels for offspring families: number of alleles						
Among offspring families	17	423.87	0.69	***Fst*=0.167**	16	0.001
Among individuals	265	853.91	0.00	*Fis*=-0.066	0	1.000
Within individuals	283	1041.26	3.68	***Fit*=0.112**	84	0.001
Totals	565	2319.02	4.37		100	
Three hierarchical levels for offspring families: sum of square size difference						
Among offspring families	17	71893.75	114.68	***Rst*=0.047**	5	0.001
Among individuals	265	169236.17	0.000	***Ris*=-0.652**	62	0.001
Within individuals	283	189936.60	671.15	***Rit*=0.668**	33	0.001
Totals	565	431066.62	785.83		100	

* *P-values* are the probabilities of having a more extreme variance component than the observed values by chance alone. Probabilities were calculated by 1000 random permutations, significant p-values shown in bold ($p \leq 0.001$).

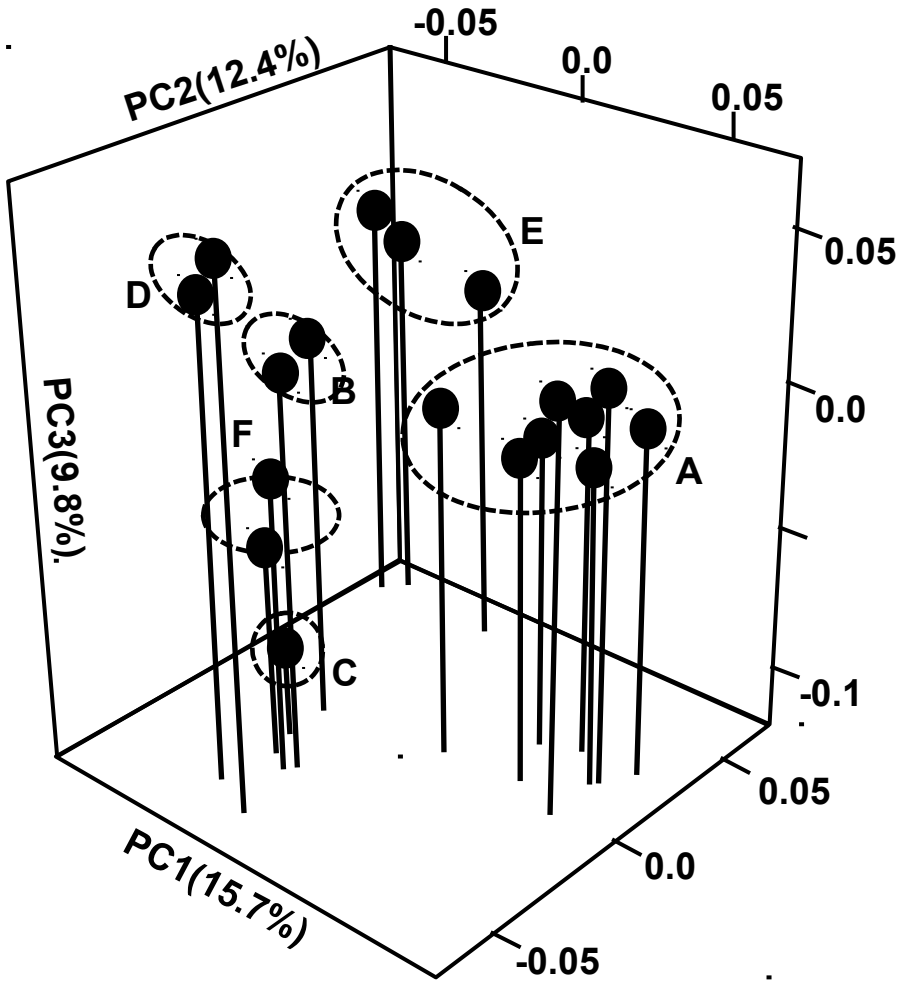

Fig. 3 Plot of the first three principal coordinates for 18 families of the PAN population of *Cedrela fissilis* based on the Cavalli-Sforza and Edwards distance measure. NbClust defined the number of groups (A to F). Eigenvalues for each of the principal coordinate are shown in parenthesis

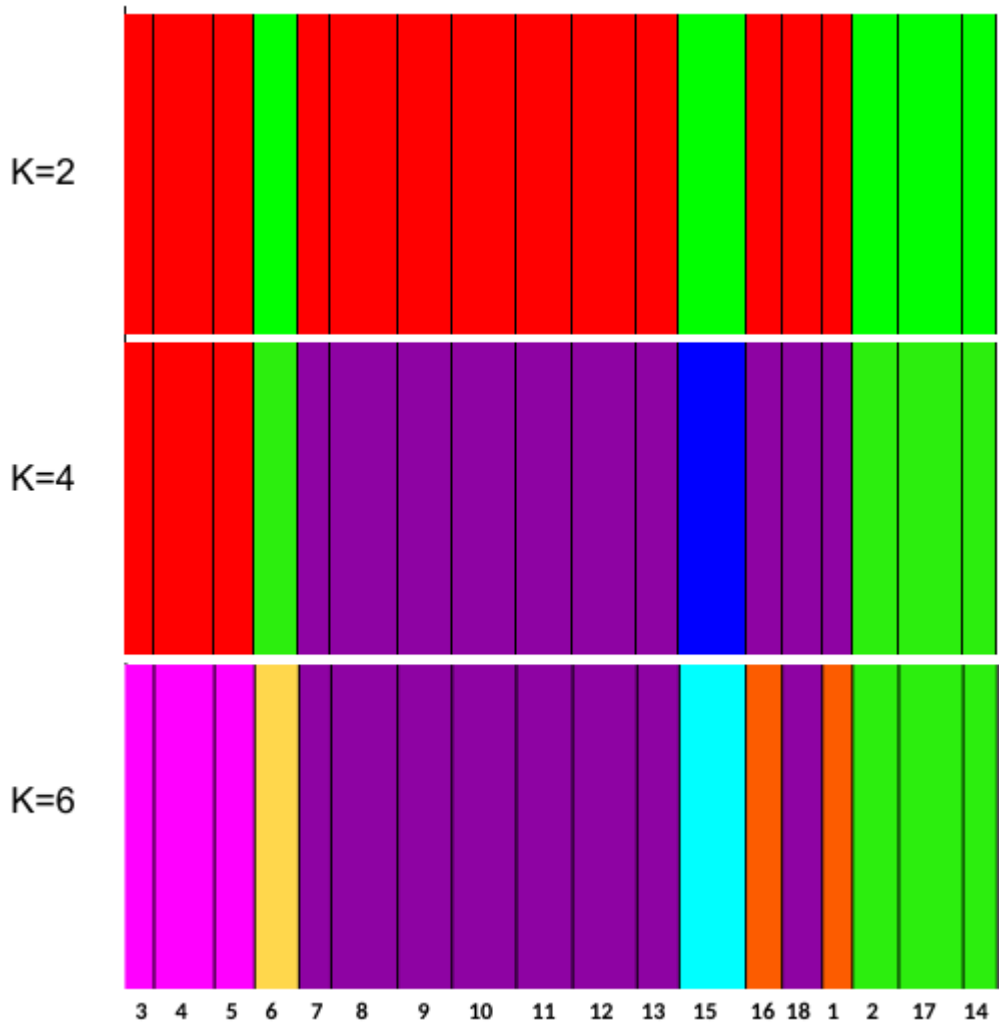

Fig. 4 Plots from mixture inferences (*K*=2, *K*=4, and *K*=6, obtained from BAPS) for each of 18 families of the PAN population of *Cedrela fissilis*. Along de x-axis, each vertical bar represents a specimen. Along the y-axis, membership coefficient of a sample represents the fraction of the sample's genome that has ancestry in a given Bayesian group (color-coded as indicated)

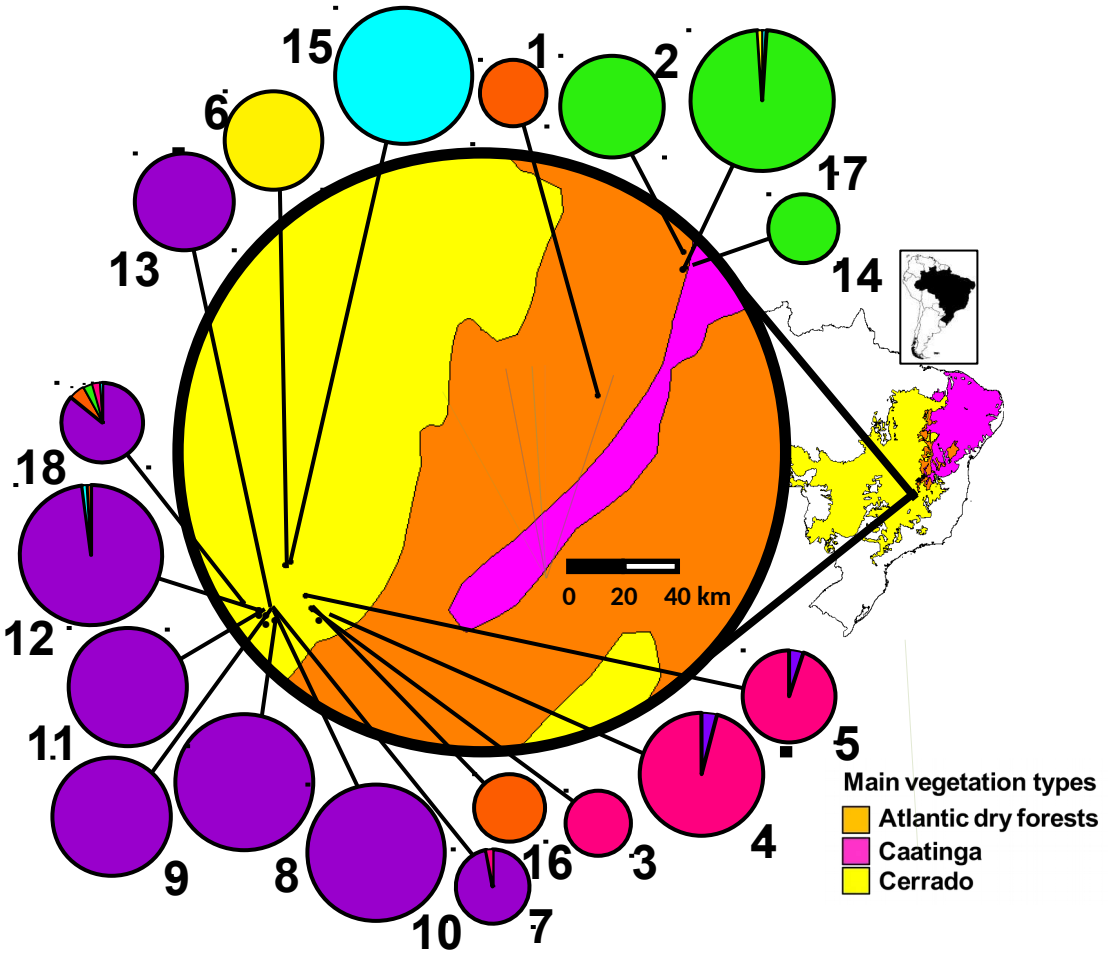

Fig. 5 Associated vegetation formations and geographic distribution of the 18 mother-trees of the PAN population of *Cedrela fissilis* and their derived families. Each pie diagram represents the mean of the membership coefficients (taken from K=6, from BAPS) for the progenies within each of the 18 families. The pie size is proportional to the sample size of the family. NbClust defined the number of groups (N=6; color-coded as indicated)

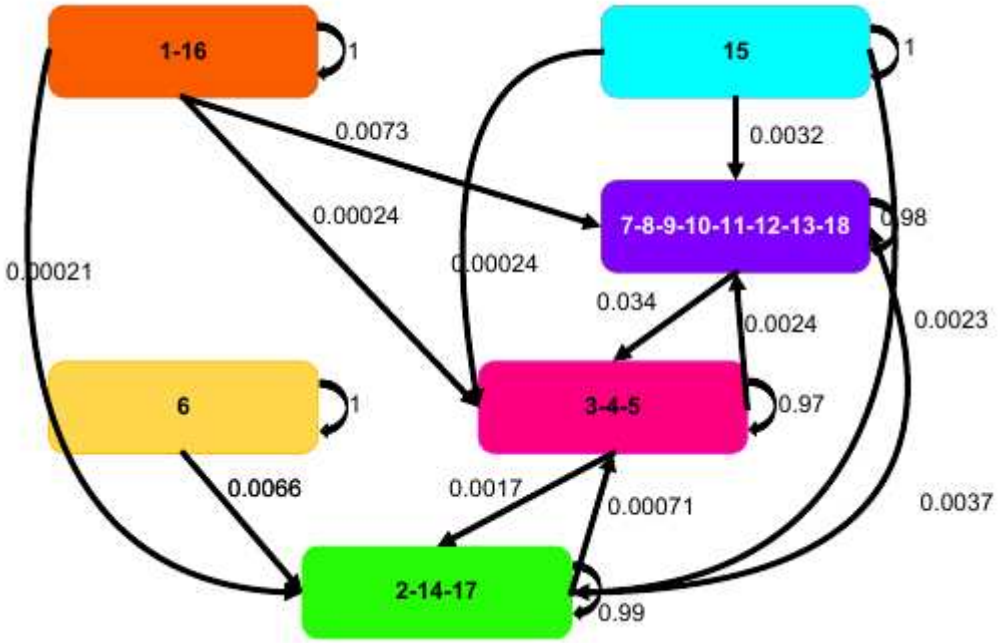

Fig. 6 Gene flow network, with *K*=6 obtained from BAPS, among 18 families of the PAN population of *Cedrela fissilis*. A self-looping arrow denotes the own genetic composition of a cluster composed by a family or families. The direct arrow emerging from each cluster denotes the contributions made by means of gene flow

4. Mating system

The overall mean for the multilocus outcrossing rate (t_m) was 0.95. Across families, it ranged from 0.466 (family 6) to 0.998 (family 15) (Table 7). The averaged proportion of full-sibs (*FSP*=13%), the overall mean half-sibs proportion (*HSP*=82%), and the self-sib proportion (SSP=5%) were shown (Table 7). Mating among genetically related individuals (t_m-t_s) showed that 7% of all progenies resulted from crossing among related trees (Table 7).

The value for the averaged paternity multilocus correlation ($r_{p(m)}$=0.17) indicated that the probability of sharing the same pollen donor was slightly low for a given family. However, $r_{p(m)}$ varied across families. There were instances in which most of the offspring of a given family were sired from the same pollen donor, such as the offspring of families 2 (0.59), 4 (0.35), 14 (0.24), 15 (0.26), and 16 (0.28). Thus, within each of those five families, the offspring combined half-sibs with self-sibs or self-half-sibs mostly (Table 7). The overall value of the crossing between pollinated related trees ($r_{p(s)}$-$r_{p(m)}$=-0.04) was low and not significant (p=0.236); thus, as a general trend, a given mother trees received pollen from unrelated donors. The overall mean for coancestry coefficient within families (Θ_{xy}=0.184; p<0.001) was significantly. Among families, values of Θ_{xy} ranged from 0.154 (family 5) to 0.292 (family 6; Table 7); therefore, families 5 and 6 detained highly distinct composition. Whereas the progeny of family 5 consisted mostly of half-sibs (*HSP*=0.83), the progeny of family 6 was mostly self-sibs (*SSP*=0.53; Table 8). The variance effective size ($Ne_{(v)}$) was 3.03 and the number of seed trees per seed collection ($m_{(150)}$) was 50.7.(Table 7).

Table. 7 Estimates of mating system of 283 offspring from 18 mother-trees of *Cedrela fissilis*

Parameters / Families																			
Mating system analyses	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*Mean*
Multilocus outcrossing rate: t_m	0.817	0.993	0.980	0.997	0.923	0.466	0.986	0.951	0.996	0.998	0.996	0.998	0.992	0.986	0.998	0.989	0.979	0.984	0.946
	(0.007)	(0.003)	(0.009)	(0.000)	(0.009)	(0.017)	(0.007)	(0.000)	(0.001)	(0.003)	(0.002)	(0.003)	(0.005)	(0.007)	(0.003)	(0.006)	(0.008)	(0.007)	
Single locus outcrossing rate: t_s	0.767	0.806	0.889	0.912	0.932	0.627	0.924	0.940	0.892	0.932	0.855	0.930	0.901	0.930	0.919	0.849	0.919	0.920	0.880
	(0.078)	(0.040)	(0.008)	(0.014)	(0.015)	(0.072)	(0.013)	(0.011)	(0.014)	(0.015)	(0.016)	(0.014)	(0.008)	(0.013)	(0.015)	(0.019)	(0.012)	(0.013)	
Mating among relatives rate: t_m-t_s	0.050	0.187	0.091	0.085	-0.009	-0.161	0.062	0.011	0.104	0.066	0.141	0.068	0.091	0.056	0.079	0.140	0.060	0.064	0.066
	(0.071)	(0.042)	(0.013)	(0.014)	(0.017)	(0.056)	(0.009)	(0.011)	(0.014)	(0.014)	(0.017)	(0.013)	(0.008)	(0.010)	(0.014)	(0.024)	(0.005)	(0.008)	
Paternity multilocus correlation: $r_{p(m)}$	0.209	0.591	0.105	0.349	0.101	0.110	0.069	0.124	0.095	0.111	0.067	0.129	0.054	0.243	0.262	0.280	0.051	0.113	0.170
	(0.052)	(0.072)	(0.006)	(0.021)	(0.013)	(0.005)	(0.008)	(0.010)	(0.012)	(0.015)	(0.014)	(0.013)	(0.015)	(0.025)	(0.023)	(0.029)	(0.014)	(0.010)	
Paternity singlelocus correlation: $r_{p(s)}$	0.121	0.278	0.101	0.175	0.105	0.109	0.082	0.115	0.096	0.130	0.110	0.129	0.098	0.185	0.181	0.145	0.091	0.110	0.13
	(0.011)	(0.101)	(0.004)	(0.040)	(0.005)	(0.002)	(0.012)	(0.012	(0.010)	(0.019)	(0.010)	(0.013)	(0.007)	(0.034)	(0.021	(0.028)	(0.008)	(0.006)	
Crossing between pollinated related trees: $r_{p(s)}$-$r_{p(m)}$	-0.088	-0.313	-0.004	-0.174	0.004	-0.001	0.013	-0.009	0.001	0.020	0.043	0.000	0.044	-0.058	-0.081	-0.135	0.040	-0.003	-0.04
	(0.041)	(0.077)	(0.003)	(0.042)	(0.010)	(0.006)	(0.004)	(0.004)	(0.006)	(0.006)	(0.008)	(0.009)	(0.010)	(0.014)	(0.024)	(0.014)	(0.007)	(0.009)	
Effective number of pollen donors: $N_{ep}=1/r_{(pm)}$	4.79	1.69	9.52	2.87	9.90	9.09	14.49	8.07	10.53	9.01	14.93	7.75	18.52	4.12	3.82	3.57	19.61	8.85	8.95
Genetic diverse of crossed pollen among mother trees: $\Phi ft=r_{p(s)}/2$	0.06	0.14	0.05	0.09	0.05	0.06	0.04	0.06	0.05	0.07	0.06	0.07	0.05	0.09	0.09	0.07	0.05	0.06	0.07
Self-sib proportion: $SSP=(1- t_m)$	0.18	0.01	0.02	0.003	0.08	0.53	0.01	0.05	0.004	0.002	0.004	0.002	0.01	0.01	0.002	0.01	0.02	0.02	0.05
Full-sibs proportion: $FSP=r_{p(s)}t_m$	0.10	0.28	0.10	0.17	0.10	0.05	0.08	0.11	0.10	0.13	0.11	0.13	0.10	0.18	0.18	0.14	0.09	0.11	0.13
Half-sibs proportion: $HSP=(1-r_{p(s)})\ t_m$	0.72	0.72	0.88	0.82	0.83	0.42	0.91	0.84	0.90	0.87	0.89	0.87	0.90	0.80	0.82	0.85	0.89	0.88	0.82
Coancestry coefficient within families: Θ_{xy}	0.205	0.227	0.175	0.192	0.154	0.292	0.161	0.156	0.175	0.164	0.184	0.166	0.168	0.176	0.182	0.200	0.162	0.166	0.184
Variance effective size: $Ne_{(v)}$	2.77	2.33	3.28	2.77	3.55	1.88	3.47	3.38	3.05	3.23	2.90	3.18	3.2	3.17	2.90	2.77	3.34	3.30	3.03
Number of seed-trees for seed collection: $m_{(150)}$	54.23	64.34	45.79	54.21	42.23	79.84	43.29	44.37	49.23	46.50	51.77	47.18	46.37	47.30	51.66	54.21	44.90	45.47	50.72

standard errors in parenthesis

5. Bottleneck

The offspring is not at mutation-drift equilibrium. There were sets of loci with significant excess of heterozygotes in each of the three models we tested: there were four loci (*Ced02, Ced18, Ced41*, and *CF66B*) in the *IAM* model, two loci (*Ced02* and *Ced41*) in the *TPM* model, and five loci (*Ced44, Ced54, Ced65, CF26* and *CF66A*) in the *SMM* model (Table 8).

When the *TPM* model was used, the Sign test showed that heterozygote excess or deficiency was not significant (p=0.16). However, there were significant excess (or deficiency) of heterozygotes in models *IAM* (p=0.049) and *SMM* (p=0.015). To elucidate the matter further, the Wilconxon test revealed a significant (p=0.002) heterozygote excess in model IAM and significant (p=0.002) heterozygote deficiency in model *SMM.*

Table 8 Genetic analysis of population bottlenecking from *Cedrela fissilis* offspring across 10 loci

			IAM				*TPM*				*SMM*			
L	*A*	*uHe*	*Heq*	*S.D.*	*DH/S.D.**	*Prob*	*Heq*	*S.D.*	*DH/S.D.*	*Prob*	*Heq*	*S.D.*	*DH/S.D.*	*Prob*
Ced2	16	0.895	0.754	0.097	1.457	**0.002**	0.822	0.055	1.335	**0.022**	0.893	0.02	0.100	0.485
Ced18	16	0.884	0.754	0.095	1.37	**0.007**	0.821	0.055	1.145	0.059	0.893	0.02	-0.455	0.280
Ced41	18	0.907	0.779	0.085	1.498	**0.001**	0.843	0.047	1.367	**0.018**	0.906	0.017	0.067	0.475
Ced44	32	0.926	0.88	0.041	1.112	0.055	0.923	0.018	0.152	0.546	0.947	0.021	-0.996	**0.031**
Ced54	7	0.374	0.519	0.172	-0.842	0.210	0.580	0.146	-1.410	0.108	0.746	0.06	-6.196	**0.000**
Ced65	10	0.721	0.628	0.143	0.717	0.267	0.699	0.102	0.309	0.458	0.824	0.038	-2.447	**0.028**
Ced131	12	0.841	0.682	0.122	1.304	0.019	0.755	0.081	1.069	0.085	0.856	0.028	-0.539	0.251
CF26	21	0.878	0.811	0.072	0.928	0.125	0.871	0.036	0.206	0.517	0.92	0.019	-2.223	**0.015**
CF66A	24	0.853	0.836	0.06	0.289	0.482	0.89	0.029	-1.273	0.104	0.93	0.016	-4.750	**0.002**
CF66B	22	0.917	0.819	0.069	1.428	**0.003**	0.878	0.033	1.187	0.057	0.923	0.016	-0.380	0.275

L locus, *A* number of alleles observed, *uHe* observed heterozygosity, *Heq* expected heterozygosity, *S.D* standard deviation, * Positive *DH/S.D* values indicates a heterozygosity excess, negative values identify a deficiency. (*DH=uHe-Heq*), ** significant p-values shown in bold ($p \leq 0.05$).

DISCUSSION

1. A third gene pool located in Central Brazil

This study investigated PAN, a population of *C. fissilis* that is located within ecotonal areas in NMG, towards Central Brazil. Our initial question addressed a previous hypothesis, by which populations of *C. fissilis* from Central Brazil would detain gene pools entirely derived from parental sources located in seasonal forests at either the eastern or western side of the Cerrado, with possible admixture taking place where the two lineages reconnected (Garcia et al. 2011; Mangaravite et al. 2016). Unexpectedly, about ⅔ of the mother-trees from PAN clustered together into a third Bayesian group, a finding that did not fit within the genetic variation expected for either the East lineage or the West lineage of *C. fissilis*. This third lineage was not apparent from previous large-scale surveys carried out using microsatellite data (Mangaravite et al. 2016). The genetic variation of the remaining ⅓ of the mother-trees of PAN matched the pattern expected for the East lineage. Thus, the current gene pool of PAN detained some degree of admixture; it combined genetic components of both the East lineage (from the Atlantic range) and the third lineage.

The likely origin of the third lineage remains enigmatic. Shifts in vegetations at local and regional scales were probable triggered when cycles of cooling/warming and dry/wet climates operated in the Neotropics during the last 2 million years of the Pleistocene (Whitmore and Prance 1987), or even earlier (Mayle 2004; Pennington et al. 2004). In Central Brazil, blocks of seasonal forests can be found locked within areas that hold a suitable combination of wetter conditions and favorable soil types, but are surrounded by the drier vegetation of the Cerrado of Central Brazil (Oliveira-Filho and Ratter 1995; Ratter et al. 2006) and the Caatinga of Northeastern Brazil (Oliveira-Filho et al. 2006). The extent of which those small, scattered blocks maintained genetic connectivity with the larger, distant blocks of seasonal forests continues unexplored. It is plausible that the changes in ecological conditions that lead to contractions and expansions of forests in Eastern South America (Martins 2011) also included NMG. Those changes may have fragmented the otherwise

interconnected populations of the East lineage of *C. fissilis*. If geographically-isolated populations endured genetic isolation within NMG, novel genetic variation had the chance to accumulate over time through genetic drift and could explain why PAN exhibited some degree of differentiation from the remaining populations of the Atlantic range. In case this process was a recurrent one, we expect it also triggered genetic differentiation among additional populations of *C. fissilis* of Central Brazil, with levels of differentiation varying depending upon the time the population remained in genetic isolation. It is plausible to assume this process extended further and included other plant species that are co-distributed with *C. fissilis*; they may have developed similar patterns of genetic differentiation when their populations remained trapped within geographic isolated blocks of seasonal forests within the Cerrado or Caatinga.

2. Genetic diversity scattered in small sub-populations

The set of ten microsatellite markers uncover an appreciable amount of genetic diversity and a number of alleles within the 18 families of PAN. The dataset allowed for the realization of parentage estimations with confidence. Initially, we suspected that the presence of null alleles could explain the significant deviations from random mating we observed within each family. Deviations from the Hardy-Weinberg equilibrium and linkage disequilibrium have been suggested as a result of the presence of null alleles (Goicoechea et al. 2015; Islam et al. 2015). In our study, however, the frequencies of null alleles remained below the threshold value of 0.19 (Chapuis et al. 2008); thus, the presence of null alleles was assumed to detain little or no effect on shaping diversity and mating system estimations in PAN.

At first, we anticipated that PAN would behave as a single, homogeneous population with high levels of gene flow. At a first glance, there were no observable barriers to gene flow within the ecotonal area in NMG that could subdivide PAN into distinct sub-populations. Unexpectedly, the split of the 18 families of PAN into six Bayesian groups suggested that gene-flow was restricted within PAN. Gene flow was low even among mother-trees that were

located within a very short distance from each other. For example, the mother-trees that gave rise to families 6 and 15 were only 1.4km apart; while the mother-trees of families 3, 4, 5, and 16 were located up to 2.8 km apart from each other within a single site (Fig. 5). We concluded that restrictions to gene-flow among reproductive trees lead to the high levels of genetic differentiation we observed in PAN. Habitat fragmentation and ecological barriers, together with restrictions to pollen or seed availability may contribute to populations to become structured (Arnold 2006).

In *Cedrela*, the inflorescences display thyrses with a proportion of female to male flowers of about 1♀:2♂ (Gouvêa et al. 2008). Several genera within Swietenioideae, including *Cedrela*, share a inflorescence arrangement in which the central flower of a cyme or a three-flowered cymule is female, while the lateral flowers are male (Styles 1972). On certain occasions, however, cymules may display only male flowers; more rarely, the flowers may become all functional females. Thus, the number of male flowers may exceed the number of functional female flowers (Gouvêa et al. 2008). Nutrition and other environmental factors may influence the tendency towards either maleness or femaleness; moreover, the proportion of female to male flowers can also vary during a given flowering season (Styles 1972). In PAN, we hypothesize that differences in the availability of flowers, different sex proportions, protogyny, and phenological differences contributed to gene flow to become restricted among neighboring mother-trees and increased the differences among sub-populations.

Two statistics (*Ap*, and *F*) showed significant differences between mother-trees and offspring, thus suggesting that PAN is under inbreeding, because a higher number of private alleles for offspring means less genetic flow among families and therefore more gene-flow within relatives. Although there were non-sampled donor trees as sources of new alleles, the origin of some families were highly dependent upon the mating among relatives. We found significant differences between the fixation indexes *Fst* and Fst_{ENA}. Such bias (about 3%) likely took place when true heterozygotes were falsely considered as (null) homozygotes during data acquisition prior to analyses with FREENA. The strong differentiation among families confirmed that barriers at the local scale interrupted pollen flow among sub-populations. Within the landscape, for

example, the sub-population of eight mother-trees that gave rise to families that came together as the group A (Fig. 3) ─ or came together as the purple Bayesian group (Fig. 4) ─ were neighbors within a grassland area of about 5.6 ha; this sub-population was about 9 km from any other mother-tree sampled for this study. Moreover, the sub-population likely comprised descendents from a recent ancestor; thus, the probability of correlated mating became high within the site and may explain the origin of group A ─ and the purple Bayesian group.

The gene-flow diagram (Fig 6) suggests that the assemblages detained differential reproductive and phenological conditions. Thus, we suppose, based on observations from Gouvêa et al. (2008), that groups orange, cyan and yellow represent families originated from mother-trees with a higher proportion of male flowers while the other groups of families groups (purple, pink and green) had femaleness or less male flowers than expected. This condition caused that only these groups purple, pink and green had effective gene-flow from other sources.

3. High levels of kinship within families

With an average value of t_m close to 1.0 for most families, *Cedrela fissilis* of PAN behaved predominantly as an outcrossing species. However, at least some of the seeds within each family resulted from selfing or bi-parental inbreeding. With exceptionally low values of t_m, families 1 (t_m=0.82) and 6 (t_m=0.47) displayed a mixed mating system ($0.2<t_m\leq0.8$; (Goodwillie et al. 2005), with levels of selfing and mating between related parents higher than the average we uncovered in PAN. Family 1 comes from a mother-tree that was apparently an isolated tree located within a landscape with no observable pollen donors nearby. Family 6 comes from a mother-tree that had three mature trees at close proximity (less than 50 meters), but those trees did not provide pollen as they did not set flowers during the study season. Family 1 and 6 fit well within the suggestion that outcrossing rates and distances among reproductive individuals are negatively associated, with the tendency of geographically isolated trees to become reproductive isolated trees (Rymer et al. 2015).

Correlated mating within families ($r_{p(m)}$=0.17; Table 7) indicated that the parents held some degree of relatedness. The low value of the effective number of pollen donors (*Nep*=8.95; Table 7) indicated that the majority of the offspring within a given family resulted from related crosses, with only 2 to 10 pollen donors per family. The coancestry coefficient within families (Θ_{xy}=0.184; Table 7) was closer to the value expected for half-sibs families than full-sibs families (Θ_{xy}=0.125 and Θ_{xy}=0.250, respectively; (Sebbenn 2006) . In PAN, therefore, Θ_{xy} was about 47% higher than the value expected for a panmictic population (Sebbenn 2006). Mating among relatives, such as that we uncovered in PAN, can arise when pollinators visit persistently related neighbors. Nocturnal bees, butterflies, and thrips (*Thysanoptera*) are able to carry out pollination in several species of Meliaceae (Patiño 1997). In the tropical tree species *Bagassa guianensis*, wind-born trips arrive in great numbers as they visit receptive flowers and distribute pollen among trees (Silva et al. 2008); as trips are short flyers, pollination by trips may take place among nearby ─ possible related ─ trees in PAN.

Given that the value of the half-sibs proportion (HSP=0.82) was higher than the full-sibs proportion (FSP=0.13) and that sharing of pollen donors was an uncommon feature ($r_{p(m)}$=0,17), we concluded that PAN is under a non-random mating pattern. Flowering asynchrony, small population size, and the foraging behavior of pollinators systematically visiting near neighbor trees (Sebbenn 2006) can be factors that account for the correlated mating we observed in PAN. Moreover, in a low-density species, such as *Cedrela fissilis* (Carvalho 1994), pollen availability is naturally less diverse. In contrast, high-density species may rely on multiple pollen sources and pollen donors are shared with high frequency (Murawski and Hamrick 1991). Supporting evidence for a genetic bottleneck in PAN was the absence of mutation drift equilibrium with heterozygote excess for *IAM* and *TPM*, heterozygote deficit for *SMM*, and linkage disequilibrium (Frankham et al. 2002), was present in PAN.

4. Conservation perspectives

The variance effective size ($Ne_{(v)}$=3.0) was lower than expected under the random mating expectations ($Ne_{(v)}$=4.0). In PAN, therefore, seed collection for conservation genetics, progeny tests, and reforestation must be taken from about 51 mother-trees ($m_{(150)}$=50.7). In case PAN was a panmictic population, seeds collection would require only 38 mother trees ($m_{(150)}$=37.4; (Sebbenn 2006).

Cedrela fissilis is a threatened species because its wood is widely used for its high economical value, diminishing natural populations and increasing forest fragmentation and consequently affecting the natural dynamics of gene-flow. The effective number of pollen donors (N_{ep}=8.95) was considerable, but there were mother-trees with exceptionally low number of pollen donors and little pollen diversity, these situations produces selfing and correlated mating. Isolated trees on grassland or forest patches will sire offspring with low genetic divergence reducing the species aptitude. This risk is increased by the maleness or femaleness observed in this genera.

While the largest proportion of seedling was not sired by random mating the coancestry coefficient showed that the offspring are far from what should be expected under equilibrium for a panmictic population. The Bayesian analysis showed an effect of population substructure as a result of parent differential contributions to the offspring. We believe that similar to others population accessed in Brazil and Central America, the natural population of *C. fissilis* from northern Minas Gerais even conserve appropriate levels of diversity. However, the signature of a slight bottleneck arises, suggesting that some events are affecting the genetic structure of the remaining natural population.

Although the visited trees produce a lot of seeds we did not see seedlings occurring naturally in the field. Because, the high genetic differentiation observed among families, we suggest the formation of a seed bank to rescue at least the actual genetic variability and establish ex situ propagation to avoid the effects of logging and environmental degradation, to accomplish it the results indicate that to retain an effective population size of 150 trees, is necessary to collect seeds from at least 50.72 individuals. It would be useful to identify potential seed and pollen donors to increase the genetic

pool of *C. fissilis* and diminish the effect of an anthropic landscape that threat the biological communities from the Brazilian savanna (Cerrado) and Brazilian Atlantic forest.

GLOSSARY OF TERMS

Amova: the Analysis of Molecular Variance is a statistical method to study the variation within species and partition of the total genetic variation into components within and among populations or groups at different levels of hierarchical subdivision. Its estimation can be accomplished by the number of alleles (Fst) or by the sum of square size difference (Rst).

Coefficient of inbreeding (F): a measure of the level of inbreeding in a population that determines the probability that an individual possesses two alleles at a locus that are identical by decent.

Effective number of pollen donors (Nep=1/$r_{(pm)}$): estimation of the number of individuals who contributed with pollen to originate a set of genetically related individuals (family).

Family: set of individuals that share at least one parent. Families can share two parents and are of full sibs or share one parent and are of half sibs.

Fixation index (Fst, Fst_{ENA}, F_{null}, Rst): reduction in homozygosity caused through population subdivision.

Full sibs proportion (FSP): proportion of sibs that share two parents.

Gene flow: exchange of genetic information between populations through migration of seeds or pollen.

Half sibs proportion (HSP): proportion of sibs that share at least one parent. The auto-pollination also can give rise to self-half-sibs.

Heterozygosity: a measure of genetic variation that accounts for either the observed (Ho), or expected (He) proportion of individuals in a population that are heterozygotes.

Heterozygote: diploid individual that has different alleles in one or several genetic loci

Homozygosity: a measure of the proportion of individuals in a population that are homozygous.

Homozygote: diploid individual that has identical alleles in one or several genetic loci

Hardy-Weinberg equilibrium (HWE): principle that establish that allele and genotype frequencies will reach the equilibrium after one generation and remain

constant in large populations in absence of migration, mutation, selection or non-random mating.

Infinite alleles model (IAM): a mutation model that states that any new alleles are produced by independent events, each mutational event creates a new allele unlike any other allele currently in the population.

Mating among relatives rate (t_m-t_s): estimation of out-crossing between individuals genetically related.

Multi-locus population out-crossing rate (t_m): estimation of out-crossing accounted from multiple locus.

Null alleles: an allele that is not detectable either due to an artifact that avoid to produce a functional product or a mutation in a primer site that prevent amplification.

Number effective of alleles (NE): the number of alleles of equal frequency that would result in the observed heterozygosity or homozygosity.

Number of alleles per locus (A): the estimation of accounted alleles for a set of individuals or population.

Number of private alleles (Ap): alleles that are found only in one population.

Parent: each adult that contribute with genetic material to offspring.

Paternity multi-locus correlation ($rp_{(m)}$): estimation of relatedness between male parents accounted from multiple locus.

Paternity single-locus correlation ($rp_{(s)}$): estimation of relatedness between male parents accounted from the average of a single locus.

Population: Set of individuals that share a common gene-pool and breed among them.

Selfing sibs proportion (SSP): proportion of sibs that have only one parent and are originated by selfing.

Single-locus population out-crossing rate (t_s): estimation of out-crossing accounted from the average of a single locus.

Step-wise mutation model (SMM): a mutation model where the allelic states produced by mutation depend on the initial state of and allele. Alleles with more differences in mutational state undergo more mutational events.

The average coefficient of coancestry (Θxy): the probability of sampling at random two homologous alleles in the same locus and that those alleles are identical per descendant.

Two phase model (TPM): an intermediate mutation model between SMM and IAM in which the new mutation can be originated by change in the allelic state by a one-step mutation or a multi-step mutation.

Unbiased expected heterozygosity (uHe): a measure of genetic variation that accounts for either the observed, or expected proportion of individuals in a population that are heterozygotes corrected for size population.

SUPPLEMENTARY TABLES

Table S1 Geographic location of samples

Sample	Location	Latitude	Longitude
1	PRB	-15,178	-44,212
2	MSSP	-14,923	-44,060
3	PRB	-15,557	-44,714
4	PRB	-15,557	-44,713
5	PRB	-15,557	-44,713
6	PRB	-15,480	-44,760
7	PRB	-15,563	-44,806
8	PRB	-15,563	-44,806
9	PRB	-15,562	-44,805
10	PRB	-15,563	-44,806
11	PRB	-15,562	-44,806
12	PRB	-15,561	-44,806
13	PRB	-15,561	-44,807
14	MSSP	-14,951	-44,059
15	PRB	-15,473	-44,750
16	PRB	-15,535	-44,724
17	MSSP	-14,950	-44,059
18	PRB	-15,546	-44,835
AR	PEU	-20,245	-43,588
AR	PSB	-20,688	-42,455
VFU1	VFU	-20,764	-42,870
VFU2	VFU	-20,762	-42,863
VFU3	VFU	-20.763	-42,868

Families 1 to 18, AR Atlantic range, PRB Peruaçu River Basin, PRB Pandeiros River Basin, MSSP Mata Seca State Park, PEU Uaimií State Park, PSB Serra do Brigadeiro State Park, VFU Viçosa Federal University

REFERENCES

Aldrich P.R., Hamrick J.L., (1998) Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. Science, **281**, 103–105.

Alves R.M., Artero A.S., Sebbenn A.M., & Figueira A. (2003) Mating system in a natural population of Theobroma grandiflorum (Willd. ex Spreng.) Schum., by microsatellite markers. Genet Mol Biol, **26**, 373–379.

Arnold M.L. (2006) Evolution through genetic exchange. Oxford University Press

Bawa K.S. (1977) The reproductive biology of *Cupania guatemalensis* Radlk. (Sapindaceae). Evolution (N Y) **31**, 52–63.

Bawa K.S., Perry D.R., & Beach J.H. (1985) Reproductive biology of tropical lowland rain forest trees. I. Sexual systems and incompatibility mechanisms. Am J Bot, 331–345.

Bethonico M.B. de M. (2010) Rio Pandeiros: território e história de uma área de proteção ambiental no norte de Minas Gerais. Acta Geográfica, **3**, 23–38.

Carnaval A.C., Hickerson M.J., Haddad C.F.B., et al. (2009) Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. Science, **323**, 785–789.

Carneiro F.S., Lacerda A.E.B., Lemes M.R., et al. (2011) Effects of selective logging on the mating system and pollen dispersal of *Hymenaea courbaril* L. (Leguminosae) in the Eastern Brazilian Amazon as revealed by microsatellite analysis. For Ecol Manage, **262**, 1758–1765.

Carvalho P.E. (1994) Especies florestais Brasileiras recomendacoes silviculturais, potencialidades e uso da madeira. Brazil CNPFlorestas-EMBRAPA 639p ISBN 85–85007

Cavalli-Sforza L.L., & Edwards A.W.F. (1967) Phylogenetic analysis: models and estimation procedures. Evolution, **21**, 550–570

Chakraborty R., & Nei M. (1977) Bottleneck effects on average heterozygosity and genetic distance with the stepwise mutation model. Evolution, **31**, 347–356.

Chapuis M.P., & Estoup A. (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol, **24**, 621–631.

Chapuis M.P., Lecoq M., Michalakis Y., et al. (2008) Do outbreaks affect genetic population structure? A worldwide survey in Locusta migratoria, a pest plagued by microsatellite null alleles. Mol Ecol, **17**, 3640–3653.

Charrad M., Ghazzali N., Boiteau V., et al. (2014) Package “NbClust.” J Stat Softw, **61**, 1–36.

Chybicki I.J., & Burczyk J. (2009) Simultaneous estimation of null alleles and inbreeding coefficients. J Hered 100:106–113.

Clegg M.T. (1980) Measuring Plant Mating Systems. Biosci, **30**, 814–818.

Corander J., Marttinen P., Sirén J., & Tang J. (2008) Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. BMC Bioinformatics, **9,** 539.

Corander J.., Waldmann P., & Sillanpää M.J. (2003) Bayesian analysis of genetic differentiation between populations. Genetics, **163**, 367–374.

Cornuet J.M., & Luikart G. (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics, **144**, 2001–2014.

Cota-Sánchez J.H., Remarchuk K., & Ubayasena K. (2006) Ready-to-use DNA extracted with a CTAB method adapted for herbarium specimens and mucilaginous plant tissue. Plant Mol Biol Report, **24**, 161–167.

Di Rienzo A., Peterson A.C., Garza J.C., et al. (1994) Mutational processes of simple-sequence repeat loci in human populations. Proc Natl Acad Sci, **91,** 3166–3170.

Díaz-Quichimbo G., Poma-Angamarca R., Minchala-Patiño J., et al. (2013) In Vitro Clonal Propagation and Germplasm Conservation in the Tropical Timber Tree Spanish White Cedar (C . Montana Moritz Ex Turcz .) (Meliaceae). eJ. In Biol. Sci, **7**, 59-69.

Frankham R., Ballou J.D., & Briscoe D.A. (2002) Introduction to Conservation Genetics, First edn. Cambridge University, Cambridge

Gandara F.B. (2009) Diversidade genética de populações de Cedro (*Cedrela fissilis* Vell. (Meliaceae) no Centro-Sul do Brasil.

Gandara F.B., Tambarussi E.V., Sebbenn A.M., et al. (2014) Development and characterization of microsatellite loci for *Cedrela fissilis* Vell (Meliaceae), an endangered tropical tree species. SILVAE Genet. **63** 240–243.

Garcia M.G., Silva R.S., Carniello M.A., et al. (2011) Molecular evidence of cryptic speciation, historical range expansion, and recent intraspecific hybridization in the Neotropical seasonal forest tree *Cedrela fissilis* (Meliaceae). Mol Phylogenet Evol, **61** 639–649.

Goicoechea P.G, Herrán A., Durand J., et al. (2015) A linkage disequilibrium perspective on the genetic mosaic of speciation in two hybridizing Mediterranean white oaks. Heredity (Edinb), **114**, 373.

Goodwillie C., Kalisz S., Eckert C.G. (2005) The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. Annu Rev Ecol Evol Syst, **36**, 47–79.

Goudet J. (2005) Hierfstat, a package for R to compute and test hierarchical F-statistics. Mol Ecol Notes, **5**, 184–186.

Gouvêa C.F., Dornelas M.C., & Rodriguez A.P.M. (2008) Floral development in the tribe Cedreleae (Meliaceae, sub-family Swietenioideae): *Cedrela* and *Toona*. Ann Bot, **101**, 39–48.

Hamilton M. (2009) Population genetics., 1er edn. Wiley-Blackwell, Chichester

Hartl DL, Clark AG (1997) Principles of population genetics, 3er edn. Sinauer associates, Sunderland. MA

Hensen I., & Oberprieler C. (2005) Effects of population size on genetic diversity and seed production in the rare *Dictamnus albus*(Rutaceae) in central Germany. Conserv Genet, **6**, 63–73.

Hernández G., Buonamici A., Walker K., et al. (2008) Isolation and characterization of microsatellite markers for *Cedrela odorata* L. (Meliaceae), a high value neotropical tree. Conserv Genet, **9**, 457–459.

Holm S. (1979) A simple sequentially rejective multiple test procedure. Scand J Stat, 65–70.

Islam M.S., Lian C., Kameyama N., & Hogetsu T. (2015) Analysis of the mating system, reproductive characteristics, and spatial genetic structure in a natural mangrove tree (*Bruguiera gymnorrhiza*) population at its northern biogeographic limit in the southern Japanese archipelago. J For Res, **20**, 293–300.

IUCN (2017) Americas Regional Workshop (Conservation & Sustainable Management of Trees, Costa Rica, November 1996). 1998. *Cedrela fissilis*. The IUCN Red List of Threatened Species 1998: e.T33928A9821890. http://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T33928A9821890.en.

Kimura M., & Crow J.F. (1964) the Number of Alleles That Can Be Maintained in a Finite Population. Genetics, **49**, 725–738.

Langella O. (1999) Populations, 1.2. 28. http://www cnrs-gif fr/pge

Lopes L.E., Neto S.D., Leite L.O., et al. (2013) Birds from Rio Pandeiros, southeastern Brazil: a wetland in an arid ecotone. Rev Bras Ornitol J Ornithol, **18**, 16.

Love J., Dropmann D., & Selker R. (2017) Jamovi Project

Luikart G., Sherwin W.B., Steele B.M., & Allendorf F.W. (1998) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. Mol Ecol, **7**, 963–974.

Mangaravite É., Vinson C.C., Rody H.V.S., et al. (2016) Contemporary patterns of genetic diversity of *Cedrela fissilis* offer insight into the shaping of seasonal forests in eastern South America. Am J Bot, **103**, 307–316.

Martinelli, G., & Moraes, M. A. (2013). Livro vermelho da flora do Brasil.

Martins F.D.M. (2011) Historical biogeography of the Brazilian Atlantic Forest and the Carnaval-Moritz model of Pleistocene refugia what do phylogeographical studies tell us. Biol J Linn Soc, **104**, 499–509.

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

Muona O., Moran G.F., & Bell J.C. (1991) Hierarchical patterns of correlated mating in *Acacia melanoxylon*. Genetics, **127**, 619–626

Murashige T., & Skoog F. (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant, **15**, 473–497

Murawski D.A., & Hamrick J.L. (1991) The effect of the density of flowering individuals on the mating systems of nine tropical tree species. Heredity, **67**, 167–174.

Nei M. (1975) Molecular Population Genetics and evolution. North Holland, Amsterdan

Nei M. (1973) Analysis of gene diversity in subdivided populations. Proc Nat Acad Sci, **70,** 3321–3323.

Nunes Y.R.F., Azevedo I.F.P., Neves W.V., et al. (2009) Pandeiros: o Pantanal Mineiro. MGBiota, **2**, 4–17.

Oliveira-Filho A.T., Jarenkow J.A., & Rodal M. (2006) Floristic relationships of seasonally dry forests of eastern South America based on tree species distribution patterns. Neotrop Savannas Seas Dry For Plant Divers Biogeogr Conserv, 159–192.

Oliveira-Filho A.T., & Ratter J.A. (1995) A study of the origin of central Brazilian forests by the analysis of plant species distribution patterns. J Bot, **52**, 141.

Olson D.M., Dinerstein E., Wikramanayake E.D., et al. (2001) Terrestrial Ecoregions of the World: A New Map of Life on Earth: A new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. Bioscience, **51**, 933–938.

Patiño F. (1997) Recursos genéticos de *Swietenia* y *Cedrela* en los neotrópicos. Propuestas para acciones coordinadas. FAO, Roma, Italia.

Peakall R., & Smouse P. (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic soft- ware for teaching and research – an update. **1**, 6–8.

Pennington R.T., Lavin M., Prado D.E., et al. (2004) Historical climate change and speciation: neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification. Philos Trans R Soc London Ser B Biol Sci, **359**, 515–538.

Pennington T.D., Muellner A.N. (2010) A Monograph of *Cedrela* (Meliaceae). dh Books, Milborne Port

Piry S., Luikart G., Cornuet J.M. (1999) Computer note. BOTTLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. J Hered, **90**, 502–503.

Ratter J.A., Bridgewater S., & Ribeiro J.F. (2006) Biodiversity patterns of the woody vegetation of the Brazilian Cerrados. Syst Assoc Spec, **69,** 31.

Raymond M., & Rousset F. (1995) An Exact Test for Population Differentiation. Evolution, **49**, 1280.

Riahi M., Zarre S., Maassoumi A.A., et al. (2009) An inexpensive and rapid method for extracting papilionoid genomic DNA from herbarium specimens. Genet Mol Res, **9**, 1334–1342.

Ritland K. (2002) Extensions of models for the estimation of mating systems using n independent loci. Heredity (Edinb), **88**, 221–228.

Ritland K. (1989) Correlated matings in the partial selfer *Mimulus guttatus*. Evolution, 848–859.

Ritland K., & Jain S. (1981) A model for the estimation of outcrossing rate and gene frequencies using n independent loci. Heredity (Edinb), **47**, 35–52.

Rodrigues P.M.S., de Azevedo I.F.P., das Dores M., et al. (2009) Riqueza florística da vegetação ciliar do rio Pandeiros, norte de Minas Gerais. MG Biota, **2,** 18–37.

Rousset F. (2008) GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Resour, **8**, 103–106.

Rymer P.D., Sandiford M., Harris S.A., et al. (2015) Remnant *Pachira quinata* pasture trees have greater opportunities to self and suffer reduced reproductive success due to inbreeding depression. Heredity (Edinb), **115**, 115–124.

Sales H., Souza S., Luz G., et al. (2009) Flora arbórea de uma floresta estacional decidual na APA Estadual do Rio Pandeiros, Januária/MG. MG Biota, **2**, 31–41.

Sebbenn A.M. (2006) Sistemas de reprodução em espécies tropicais e suas implicações para a seleção de árvores matrizes para reflorestamentos ambientais. In: Higa AR, Duque SL (eds) Pomares de sementes de espécies florestais nativas. FUPEF, Curitiva-Parana, 93–138

Sebbenn A.M. (2002) Número de árvores matrizes e conceitos genéticos na coleta de sementes para reflorestamentos com espécies nativas. Rev do Inst Florest, **14**, 115–132.

Silva M.B., Kanashiro M., Ciampi A.Y., et al. (2008) Genetic effects of selective logging and pollen gene flow in a low-density population of the dioecious tropical tree *Bagassa guianensis* in the Brazilian Amazon. For Ecol Manage, **255,** 1548–1558.

Styles B.B.T. (1972) The flower biology of the Meliaceae and its bearing on tree breeding. Silvae Genet, **5**, 175–182.

Styles B.T. (1981) Swietenioideae. In: Pennington TD, Styles BT, Taylor DA. (eds). New York, 359–418.

Surles S.E., Arnold J., Schnabel A., et al. (1990) Genetic relatedness in open-pollinated families of two leguminous tree species, *Robinia pseudoacacia* L. and *Gleditsia triacanthos* L. Theor Appl Genet, **80**, 49–56.

Tang J., Hanage W.P., Fraser C., & Corander J. (2009) Identifying currents in the gene pool for bacterial populations using an integrative approach. PLoS Comput Biol, **5**, 8:e1000455

Team R.C. (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2016

Van Oosterhout C., Hutchinson W.F., Wills D.P.M., et al. (2004) Micro-Checker: Software for Identifying and Correcting Genotyping Errors in Microsatellite Data. Mol Ecol Notes, **4**, 535–538.

Weir B.S. (1996) Genetic data analysis II: Methods for discrete population genetic data. Sinauer Assoc Sunderland, MA 376.

Whitmore T.C., & Prance G.T. (1987) Biogeography and Quaternary history in tropical America. Clarendon Press

CONCLUSIONS

- The application of *in-vitro* techniques jointly with disinfection with Sodium hypochlorite (25% active chlorine) and Tween20 (4 drops/100ml) can increase the germination by a factor from 3 to 5 with respect to the non-use of in-vitro techniques. The double disinfection method increase the germination by a factor of 1.7 respect the simple disinfection method.
- PAN population belongs to a third gene pool, distinct from the West (Chiquitano range) and East (Atlantic range) lineages. However, PAN showed some levels of admixture with east lineage (Atlantic range).
- The offspring showed differentiation among families and signatures of restricted genetic flow, suggesting habitat fragmentation and ecological barriers.
- In PAN the mating system estimations showed that *Cedrela fissilis* is an outcrossed species with 5% of selfing and 7% of mating among relatives due to the parents held some degree of relatedness.
- PAN was not under random mating equilibrium and the coancestry coefficient was higher than expected for a panmictic population. Therefore, for conservation purposes should be sampled 51 mother-trees , which is 26% more that expected on panmictic populations.
- The breeding trees found in the ecotone Caatinga-Cerrado Atlantic forest are not enough to conserve the reproductive potential of PAN because is necessary collect a bigger sample to represent efficiently the gene-pool