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Gene flow and fine-scale spatial genetic structure in *Cabralea canjerana* (Meliaceae), a common tree species from the Brazilian Atlantic forest

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Abstract: The Atlantic forest is the biome most severely affected by deforestation in Brazil. *Cabralea canjerana* spp. *canjerana* is a dioecious tree species with widespread distribution in the Neotropical region. This species is considered a model to ascertain population ecology parameters for endangered plant species from the Atlantic forest. Fine-scale spatial genetic structure and pollen-mediated gene flow are crucial information in landscape genetics and evolutionary ecology. A total of 192 adults and 121 offspring were sampled in seven *C. canjerana* populations in the Southern Minas Gerais State, Brazil, to assess whether pollen-mediated gene flow is able to prevent spatial genetic structure within and among Atlantic forest fragments. Several molecular ecology parameters were estimated using microsatellite loci. High levels of genetic diversity ($H_E = 0.732$) and moderate population structure ($\theta = 0.133$) were recorded. No significant association between kinship and spatial distance amongst individuals within each population ($Sp = 0.000109$) was detected. Current pollen-mediated gene flow occurs mainly within forest fragments, probably due to short-distance flights of the pollinator of *C. canjerana*, and also the forest fragmentation may have restricted flight distance. The high levels of genetic differentiation found amongst the seven sites sampled demonstrated how habitat fragmentation affects the gene flow process in natural areas.

Key Words: Brazil, fine-scale SGS, habitat fragmentation, molecular ecology, pollen dispersal

INTRODUCTION

Forest fragmentation may modify the gene flow pattern amongst and within natural plant populations by restricting pollen and seed dispersal (Ghazoul 2005). Negative consequences, such as a heterogeneous pollen pool, endogamy, increase of genetic drift and consequently decrease of genetic diversity may arise when natural populations reveal a spatial genetic structure (SGS) and limited gene flow pattern as a result of forest fragmentation (Bittencourt & Sebbenn 2008, Dyer & Sork 2001, Dyer *et al.* 2004, Young *et al.* 2001). Pollen-mediated gene flow could be considered the main gene flow component, because pollen dispersal covers a spatial area larger than seed dispersal (Robledo-Arnuncio & Gill 2005, Smouse & Sork 2004). Nevertheless, gene flow via seed dispersal is more important in determining the effective population size and levels of population subdivision, because seeds carry two nuclear alleles, whilst pollen grains carry only one (Hamilton & Miller 2002, Garcia *et al.* 2007). However, it is important to consider all potential gene-flow mechanisms when investigating determinants of spatial genetic structure (Freeland *et al.* 2011).

The kinship and spatial distance between individuals within or amongst populations are useful parameters to quantify SGS levels and gene flow (Hardy *et al.* 2006, Vekemans & Hardy 2004). Landscape genetic models may indicate how habitat fragmentation affects levels of gene-flow, genetic structure, local adaptation and genetic boundaries of a population (Guillot *et al.* 2005a). Consequently, both genetic spatial limits and the current gene flow amongst natural populations could be considered important population parameters providing ecological information about forest fragmentation.

The deforestation of large natural areas, which has been occurring in nearly all Brazilian biomes, is responsible for a vast and irreversible loss of biological diversity (Cardinale *et al.* 2012). Even with high levels of diversity and endemism, more than 90% of the Brazilian Atlantic forest biome has already been lost (Colombo & Joly 2010).

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This process has a direct influence on genetic composition of plant and animal populations (Lowe *et al.* 2005). Several studies have shown how habitat fragmentation affects the genetic diversity of the Brazilian Atlantic forest trees (Auler *et al.* 2002, Conte *et al.* 2008, Franceschinelli *et al.* 2007, Ribeiro *et al.* 2009, Salgueiro *et al.* 2004, Seoane *et al.* 2000, Tarazi *et al.* 2010). However, few studies have analysed the current pollen-mediated gene flow amongst populations and its consequences for their fine-scale spatial genetic structure and inbreeding level.

Therefore, the main goal of our study was to estimate the levels of pollen-mediated gene flow in *C. canjerana* spp. *canjerana* populations located in disturbed and conserved areas, and to assess whether this gene flow is able to prevent the occurrence of a spatial genetic structure within and amongst fragments of montane Atlantic forest of Southern Minas Gerais State, Brazil. Furthermore, the major inquiry that guided this study was related with understanding how the process of habitat fragmentation may affect the pollen dispersal, changing the spatial genetic structure pattern. We hypothesize that forest fragmentation should restrict pollinator movement and gene flow within fragments, enhancing genetic differentiation amongst populations located in separate fragments.

STUDY SPECIES

Cabralea canjerana belongs to a monospecific Meliaceae genus. This species comprises three subspecies, amongst which *C. canjerana* ssp. *canjerana* is a tree that grows up to 25 m tall and occurs in Neotropical forests with wide geographic distribution that ranges from Costa Rica to northern of Argentina (Pennington *et al.* 1981). *Cabralea canjerana* spp. *canjerana* is a dioecious subspecies pollinated through visit of small moths to flowers of both sexes, showing high pollinator dependence on seed production (Franceschinelli *et al.* 2015). In Brazil, *C. canjerana* ssp. *canjerana* is abundant in southern Minas Gerais State, mainly in montane forests (Barreiros & Souza 1986).

STUDY SITES

The sampling sites are located at high altitudes in Atlantic forest fragments (montane forest) in southern Minas Gerais State, in Brazil. This type of forest has many close fragments of varying sizes, which are important biodiversity reservoirs. The Brazilian Forest Code law stipulates that natural areas above 1800 m asl, forests located on steep mountainsides and riverbanks be Permanent Protection Areas (PPA) and completely preserved to prevent erosion. The study area has several close fragments frequently linked through riparian forest or other corridors that may facilitate gene flow amongst them. Specifically, the sampled sites are within an Environmental Protection Area (EPA), called Fernão Dias, which covers 180 073 ha in a well-known region called Serra da Mantiqueira. Samples were taken from six small fragments (area $\leq$ 15 ha), of which two are also considered ecological corridors, and in a large area of continuous forest, amounting to seven sampled sites in an area of *c.* 10 km^2. Each sampled site was considered a population and the distances between them ranged from 0.38 to 4.10 km (Figure 1, Table 1).

The Atlantic forest in this area was continuous until 1910, when human activity increased with intense logging for many years, causing severe habitat fragmentation. The deforestation decreased after 1985, when timber exploitation and the expansion of agriculture became less intense. In 1997, with the implementation of the EPA Fernão Dias, land use and forest exploitation were better controlled (Aguari 2001). Currently, several fragments are well conserved, but others remain subject to selective logging and cattle grazing.

METHODS

Samples and molecular procedures

Leaves of adult *C. canjerana* ssp. *canjerana* individuals were randomly collected in the seven study sites. On average, 27 individuals were sampled from each population, totalling 192 adult individuals. On average eight fruits from 15 female trees were also sampled out of these 192 individuals. The females and their offspring were collected from three different populations. We sampled a total of 192 adult individuals and 121 offspring (Table 1). Genomic DNA was extracted using the Slotta *et al.* (2008) protocol. Genotyping was carried out for the six microsatellite loci described by Pereira *et al.* (2011). Capillary electrophoresis was performed with an automated ABI-3100 DNA fragment analyser and genotyping was done in GeneMapper 3.5 software.

Gene diversity and spatial genetic structure

The software Identity1.0 was used to evaluate the power of microsatellite loci based on the exclusion probability and genotype identity values. Allelic richness and unbiased expected heterozygosity (H_E) (Nei 1973) were estimated for each population. The intrapopulation fixation index (f), overall inbreeding coefficient (F) and the measure of genetic structure amongst populations (θ) were estimated according to the parameters of Weir & Cockerham (1984), which are analogous to Wright's F-statistics, F_{IS}, F_{IT} and F_{ST}, respectively. We

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Table 1. Sample sites, geographic coordinates, demographic traits and number of individuals sampled in *Cabralea canjerana* subsp. *canjerana* populations of montane Atlantic forest fragments of Minas Gerais State, Brazil. The degree of isolation is the distance (km) from each site (forest fragment) to the largest sample site (forest fragment 7). Population density refers to the number of adult *C. canjerana* trees per hectare.

Populations	Latitude S	Longitude W	Altitude (m asl)	Plant density (no. ha^{-1})	Isolation degree (km)	Fragment area (ha)	Adult individuals sampled	Number of female/offspring sampled
1	22º 41′ 11″	45º 54′ 28″	1681	26	4.08	0.90	27	5/33
2	22º 40′ 59″	45º 53′ 58″	1667	76	4.10	1.40	35	–
3	22º 41′ 12″	45º 54′ 11″	1630	86	3.89	12.00	30	–
4	22º 41′ 18″	45º 54′ 06″	1603	86	3.51	7.50	33	5/49
5	22º 41′ 35″	45º 53′ 32″	1598	187	2.92	15.00	17	–
6	22º 41′ 43″	45º 53′ 28″	1600	67	2.73	1.95	11	–
7	22º 42′ 19″	45º 52′ 52″	1810	221	–	4000	39	5/39

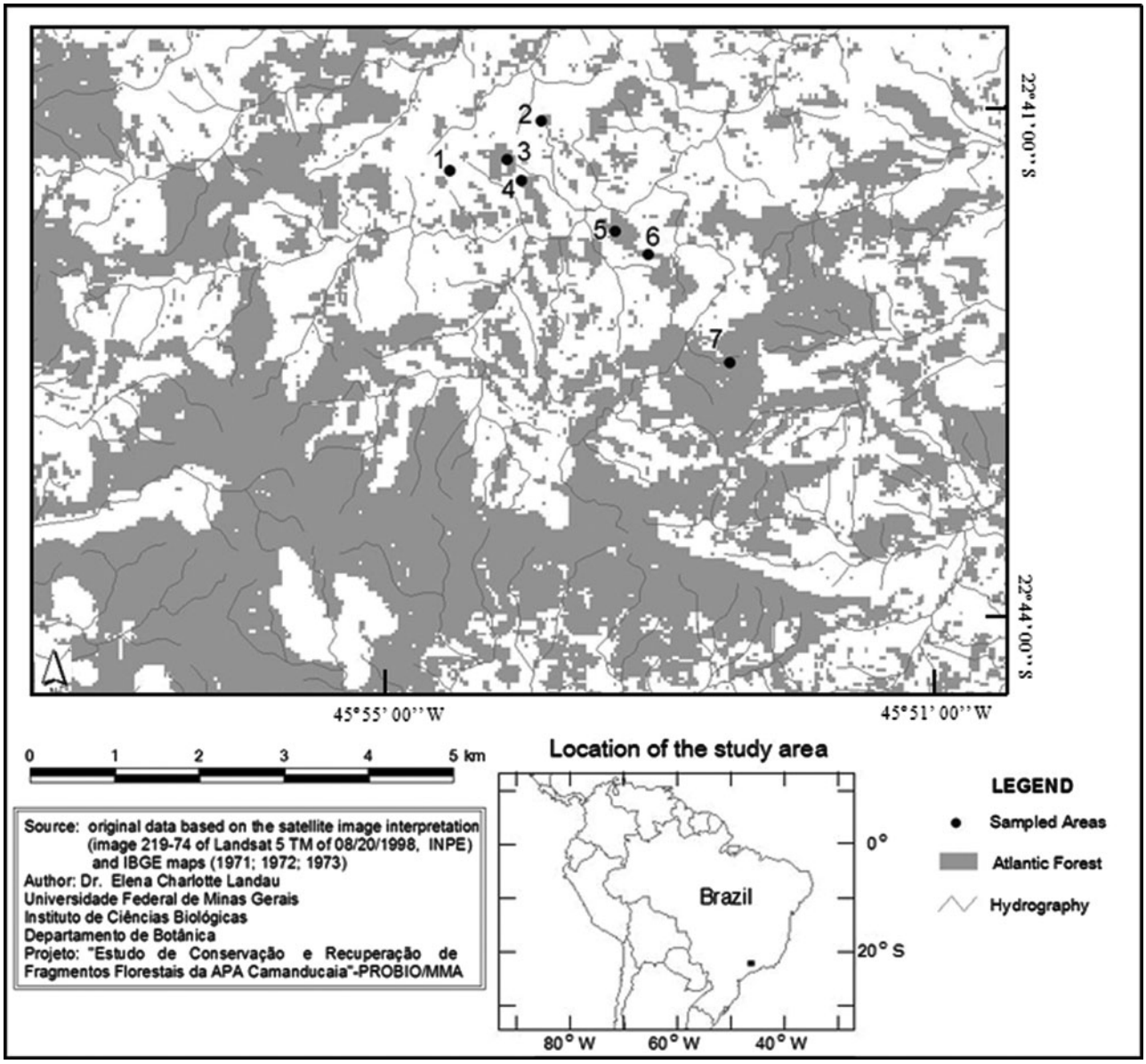

Figure 1. Studied sites in the montane Atlantic Forest of southern Minas Gerais State, Brazil.

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used the software FSTAT 2.9.3.2 for these estimates. Slatkin's R_{ST} genetic structure parameter appropriated for microsatellite loci was estimated using R_{ST}-calc (Goodman 1997). A recent population bottleneck was evaluated for each population using a Luikart & Cornuet (1998) empirical test available in the Bottleneck software (Cornuet & Luikart 1996).

The pattern of the fine-scale spatial genetic structure was evaluated using both geographic distances and kinship estimates between adults, based on the algorithm of Loiselle *et al.* (1995). The spatial genetic structure was quantified through $Sp = b/(F_1–1)$, where F_1 is the average kinship coefficient between individuals from the first distance class and b is the regression coefficient proposed by Vekemans & Hardy (2004). SPAGeDi 1.2 software (Hardy & Vekemans 2002) was used in these analyses. The independence between genetic kinship and geographic distance matrix was assessed using the Mantel test with 10 000 permutations (Sokal 1979) in R software. An analysis was performed in a Geneland (Guillot *et al.* 2005b) R package, according to the F spatial model with two groups (K = 2), as previous reported by Melo *et al.* (2014), to identify the number of genetic groups and geographic boundaries between them. A total of 10 000 MCMC interactions and 10 independent runs for each K were used in a Bayesian analysis, considering a burn-in period of 500 steps and sampling frequency of 1 every 100 steps (thinning).

Pollen-mediated gene flow analysis

The global differentiation of the pollen gene pool amongst trees (Φ_{FT}) and pollen dispersal distance (δ) were quantified using three populations (Pop. 1, Pop. 4, Pop. 7), based on a normal and exponential distribution model and a fixed male density (d_e). Both measures were estimated in POLDISP software (Robledo-Arnuncio *et al.* 2007), using 15 female trees and 121 offspring. Austerlitz & Smouse (2001), cautioned that the Φ_{FT} could be overestimated due to the parental inbreeding coefficient (F_p) and proposed a simple correction $\Phi'_{FT} = \Phi_{FT}/(1 + F_p)$. The fixation index ($f$) may be used as a parental inbreeding coefficient (F_p) since *C. canjerana* is a dioecious species. The effective number of pollen donors (N_{ep}) was calculated as $N_{ep} = 1/2\ \Phi'_{FT}$, which was used to estimate the effective pollination neighbourhood area ($A_{ep} = N_{ep}/d_e$), considering the population density (d_e) of each population studied (Austerlitz & Smouse 2001). The variance of the effective population size ($Ne_{(v)}$) could be estimated using the coancestry coefficient (Θ_{xy}), as proposed by Cockerham (1969): $Ne_{(v)} = 0.5/\Theta_{xy}$, where $\Theta_{xy} = 0.125\,(1 + F_p)\,(1 + rp_{(m)})$. The paternity coefficient ($rp_{(m)}$) proposed by Ritland (2002) may be determined as twice of Φ'_{FT} (Hardy *et al.* 2004).

RESULTS

Population genetic diversity

The set of microsatellite loci used to estimate the molecular ecology parameters showed a good performance based on the overall loci estimation of paternity exclusion (0.00000036) and identity probability (0.999). One hundred and thirty-eight alleles were found in the seven *C. canjerana* populations evaluated. The average of Nei genetic diversity was high ($H_E = 0.732$), as well as the average of allelic richness ($A_R = 6.32$). No bottleneck effect was detected, except for population 2, in which an excess of heterozygosity was found according to the Wilcoxon test ($P = 0.015$). We also found a low level of intrapopulational inbreeding coefficient ($f = 0.057$, $CI_{95\%} = -0.103$ to 0.224), and a moderate genetic structure amongst populations ($\theta = 0.133$ $CI_{95\%} = 0.074–0.208$). Approximately 86% of the total genetic diversity was observed within populations (Table 2). The $R_{ST} = 0.105$ ($P < 0.05$) is consistent with evidence of a strong genetic structure amongst populations.

Spatial genetic structure

A non-significant relationship between physical distance amongst adult trees and their kinship coefficient was found ($Sp = 0.000109$) within each population (Table 2). The Mantel test correlogram evaluated the independence between kinship and distance matrices and supported the absence of SGS (Figure 2). The average distance between female individuals was estimated in 127 m, ranging from 10 m (Population 7) to 1040 m (Population 1) amongst the individuals from the same population.

In the landscape genetic analysis (Figure 3), each group of black points represents one population sampled. The Bayesian analyses of allele frequency found that the seven populations are structured in two distinct groups. The first group (Group A), comprising populations 1, 2, 3 and 4, is represented as white in Figure 3a which means these four populations have high a posteriori probability (around 90%) of being correctly assigned to the same group, whilst populations 5, 6 and 7 have low a posteriori probability of belonging to Group A. In Figure 3b, the scenario is the opposite, as populations 5, 6 and 7 (Group B) have high a posteriori probability to be correctly assigned to the same group. Both scenarios are consistent with the real localization of each sampled site.

Pollen-mediated gene-flow analysis

No difference between the normal and exponential dispersal model was found during current pollen-flow

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Table 2. Genetic population parameters of *Cabralea canjerana* subsp. *canjerana* populations located at high altitudes in Atlantic forest fragments in southern Minas Gerais State, in Brazil. A and A_R refer to the average of allele number and average allelic richness, respectively. H_O is the observed heterozygosity and H_E is the Nei gene diversity. ***f*** is the intrapopulation fixation index, θ / R_{ST} are measures of population genetic structure, *b* is the regression of kinship coefficient and Sp is the statistic used to quantify spatial genetic substructure. *b* is the regression of kinship coefficient and Sp is the statistic used to quantify spatial genetic substructure. Except for θ / R_{ST}, these parameters were estimated for each population individually and considered as a single overall population.

Population	A	A_R	H_E	H_O	*f*	θ/R_{ST}	b	Sp
1	8.00	5.56	0.678	0.716	−0.075	–	0.00002	−0.000019
2	8.50	6.37	0.746	0.718	0.100	–	−0.00015	0.00016
3	6.66	6.67	0.760	0.772	0.259	–	0.000002	−0.000002
4	6.33	5.49	0.692	0.732	0.025	–	−0.000021	0.000022
5	9.33	6.65	0.748	0.615	−0.075	–	0.00048	−0.00044
6	8.66	6.24	0.746	0.674	−0.033	–	−0.00098	0.00094
7	10.8	7.27	0.758	0.675	0.158	–	−0.000085	0.0001
Average	8.33	6.32	0.732	0.700	0.057	0.133 / 0.105	−0.000104	0.000109
Overall	15.5	18.6	0.710	0.807	0.103	–	−0.00073	0.00077

estimation. The Φ_{FT} values were statistically different from zero in all three populations analysed, with an average of $\Phi_{FT} = 0.156$ (Table 3). The average of pollen dispersal distance (δ) for each population was 206 m (range = 140–1040 m), 111 m (42–351 m) and 65 m (10–78 m) for populations 1, 4 and 7, respectively (Table 3), using an exponential distribution model and considering 50% of the plants within a population as the male population density. These values revealed that the pollen dispersal patterns are concentrated within populations (average of 127 m) and are inversely related with fragment size and plant density.

The indirect estimates of gene flow (N_{em}), based on Wright's F statistics (θ = 0.133), suggested the occurrence of 1.63 migrant individuals amongst populations per generation. In addition, the effective number of pollen donors (N_{ep}) was approximately three individuals for each population. Based on the inbreeding coefficient in parental generation (F_p) and co-ancestry coefficient within the progeny (Θ_{xy}), the variance of effective population size ($N_{e(v)}$) was 3.30 for Population 1, 3.03 for Population 4 and 2.63 for Population 7. Furthermore, we estimated the effective pollination neighbourhood area (A_{ep}), for populations 1, 4 and 7 as 1244 m^2, 406 m^2 and 146 m^2 (Table 3), respectively.

DISCUSSION

Genetic diversity

The high levels of genetic diversity ($H_E = 0.732$) found in *C. canjerana* populations could be explained by its sexual system, because in dioecious species outcrossing amongst individuals is required. The high levels of intra-population heterozygosity, commonly encountered in microsatellite DNA loci, can lead to very low genetic differentiation measures, as pointed out by Hedrick (1999). However, the estimated θ and R_{ST} values revealed that a high proportion of genetic diversity is concentrated within rather than amongst populations. This genetic structure pattern is common amongst outcrossing plant species (Loveless & Hamrick 1984). The similarity between θ and R_{ST} estimates suggests that genetic drift is the main force determining the genetic structure of the population and that the dispersive effect of mutation is still of minor importance (Holsinger & Weir 2009). The forest fragmentation scenario may have enhanced the levels of genetic differentiation amongst populations through genetic drift and genetic isolation.

The location of population 7 may explain the high levels of genetic diversity found for this population. The forest fragment where this population occurs is one of the largest in the region (about 4000 ha), and is located above 1800 m asl in a steep landscape. According to the Brazilian environmental laws, exploration activities are prohibited in areas located above 1800 m asl. Therefore, human activities are more difficult in the area of population 7, maintaining a high plant density and genetic diversity. Franceschinelli *et al.* (2007), studying young plants of *Myrciaria floribunda* in the same area, found higher levels of genetic diversity within populations in higher altitudes as well. Our results show the importance of this type of law in protecting the genetic diversity of Atlantic forest trees. There are significant correlations between altitude, population density and genetic diversity in *C. canjerana* populations from Fernão Dias EPA (Melo *et al.* 2015).

Short-distance pollen-mediated gene flow

The pollen dispersal distance found was on average 127 m for the *C. canjerana* population evaluated. Considering this dispersal distance and that a female plant is generally pollinated by pollens that come from

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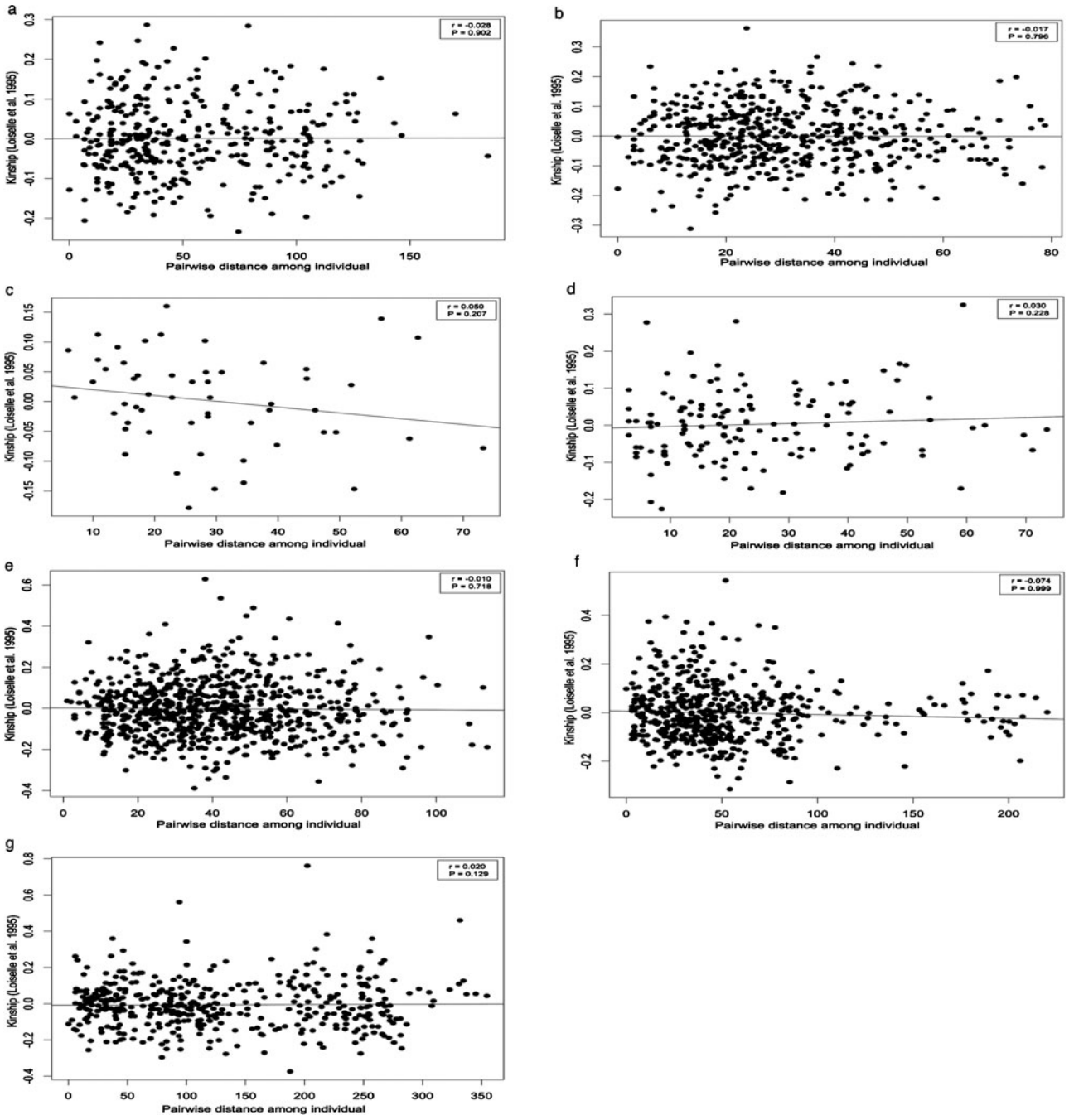

Figure 2. Mantel test correlogram showing the pairwise relationship between Loiselle *et al.* (1995) kinship (F_{ij}) and the geographic distance (metres) between individuals for the seven *Cabralea canjerana* subsp. *canjerana* populations evaluated at high altitudes in Atlantic forest fragments in southern Minas Gerais State, in Brazil.

three different male trees, most male plants have a high chance of pollinating female plants of the same population. Thus, pollen dispersal is restricted between a few neighbouring plants, mainly in forest fragments with low population density of dioecious plants. However, pollination may eventually happen amongst plants of different populations considering that pollen dispersal distance ranged from 10 to 1040 m.

The pollinator flight behaviour amongst plants and flowers of a plant is an important factor that defines distance over which pollen is dispersed (Dick *et al.* 2003). The concentration of pollen-mediated gene flow within populations may be the consequence of the limited flight distance of *C. canjerana* pollinators. This subspecies is commonly pollinated by small nocturnal moths (Franceschinelli *et al.* 2015), which usually have

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Table 3. Current pollen mediated gene-flow parameters of three *Cabralea canjerana* subsp. *canjerana* populations located at high altitudes in Atlantic forest fragments in southern Minas Gerais State, in Brazil. N/d_e is the number of adult individuals sampled divided by population density. Φ_{FT} = structure of the pollen cloud that fertilized different female trees; $\Phi'_{FT} = \Phi_{FT}/(1 + F_p)$ (Austerlitz & Smouse 2001); δ = pollen dispersal distance between different female trees; N_{ep} = effective population size of pollen donor; Θ_{xy} = coancestry coefficient; $N_{e(v)}$ = variance of the effective population size and A_{ep} = effective size of the neighbourhood area (m^2).

Populations	N/d*e*	Φ_{FT}	Φ'_{FT}	δ (range)	N_{ep}	Θ_{xy}	$N_{e(v)}$	A_{ep}
1	27/26	0.143	0.154	206 (140–1040)	3.23	0.151	3.30	1244
4	33/86	0.147	0.143	111 (42–351)	3.48	0.164	3.03	406
7	39/221	0.180	0.155	65 (10–78)	3.21	0.189	2.63	146
Average	–	0.156	0.151	127 (10–1040)	3.31	–	2.99	598

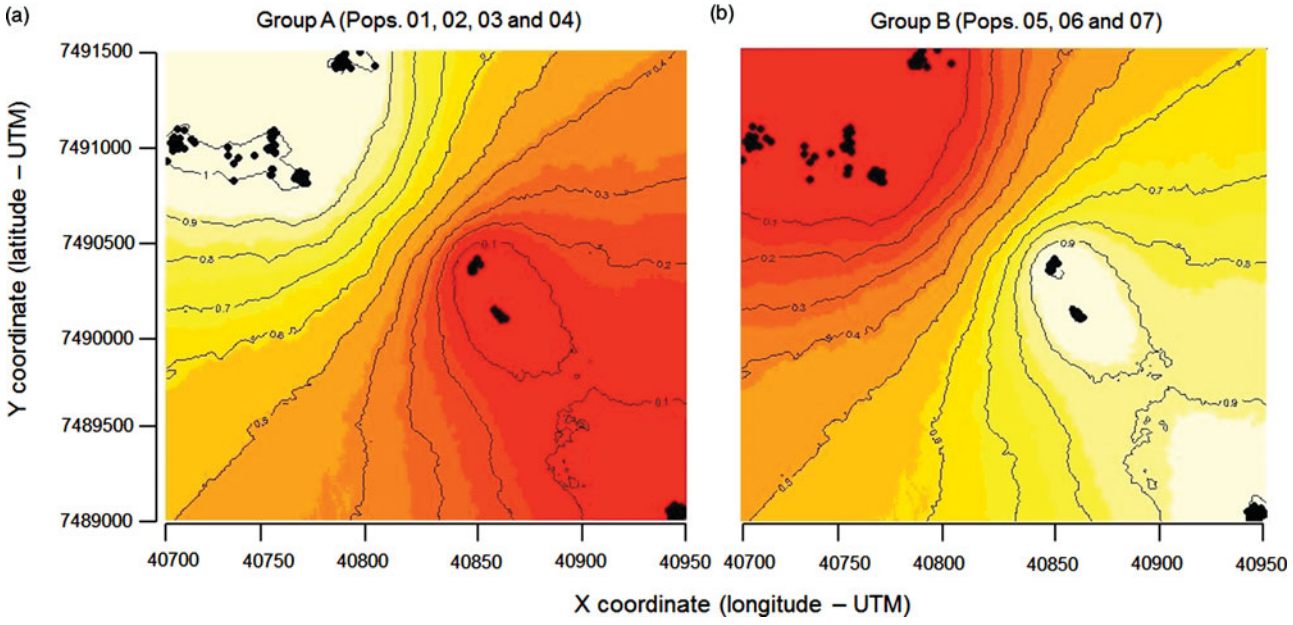

Figure 3. Geneland Bayesian allele frequency analysis and graphic representation showing the genetic structure of the population and the genetic boundaries of *Cabralea canjerana* subsp. *canjerana* populations in the landscape of the sites studied. Each group of black points represents one population. Populations 1 to 4 are represented in white, meaning that these four populations have a high posteriori probability (around 90%) of being correctly assigned to the same group. Populations 5, 6 and 7, represented in red, have a low posteriori probability (lower than 10%) of belonging to Group A (a), whilst in Group B (b), an opposite scenario is observed.

a short flight distance (Vilarinho *et al.* 2011), limiting the pollen flow within populations. This short flight behaviour causes pollination to occur mainly between closer trees, which also explains the small effective pollination in the surrounding area ($A_{ep} = 598\ m^2$) and effective population size ($N_{e(v)} = 3.31$).

Short-distance pollen dispersal has also been recorded for other tropical tree species pollinated by insects, such as *Copaifera langsdorffii* in a remnant of semi-deciduous Atlantic forest (Sebbenn *et al.* 2011), *Himatanthus drasticus* in fragments of dry forest in Brazil (Baldaulf *et al.* 2014), and *Eurycorymbus cavaleriei* in a highly fragmented forest of South-East Asia (Wang *et al.* 2011). Short-distance pollen dispersal was also reported in continuous forests for other insect-pollinated species, such as *Carapa guianensis* (Cloutier *et al.* 2007) and *Dinizia excelsa* in the Amazon rain forest (Dick *et al.* 2003), as well as *Calophyllum longifolium, Spondias mombin* and *Turpinia occidentalis* in Barro Colorado Island, Panama (Stacy *et al.* 1996). These studies suggested that the pollen dispersal distance depends mainly on the degree of plant density and aggregation amongst plants within a population.

Nevertheless, it has been found that isolated trees and populations occurring in highly fragmented landscapes, including *Symphonia globulifera* (Aldrich & Hamrick 1998), *Swietenia macrophylla* (White *et al.* 2002), *Swietenia humilis* (Rosas *et al.* 2011) and *Dinizia excelsa* (Dick *et al.* 2003), may exhibit longer pollen dispersal distance. Here, we found similar results. Populations located in smaller and disturbed fragments showed higher-distance pollen flow than population 7, for example, which is located in a very large and pristine fragment. Probably, the pollinator moths in population 7 only visit neighbouring plants and do not have to fly much

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further than 65 m to find the necessary resource for their survival. Still, the moths have to travel further to feed in disturbed fragments, such as was shown for populations 1 and 4 (Table 3).

A low Φ'_{FT} value indicates less population differentiation and is consistent with more gene flow within forest fragment. The Φ'_{FT} values estimated for *C. canjerana* (Table 3) were higher than for other tropical tree species. For example, Bittencourt & Sebbenn (2008) found $\Phi'_{FT} = 0.084$ for *Araucaria angustifolia*, a dominant tree species in *Araucaria* forests of southern Brazil. Cloutier *et al.* (2007), studying two Amazon rain-forest species, found $\Phi'_{FT} = 0.053$ and $\Phi'_{FT} = 0.064$ for *Carapa guianensis* and *Sextonia rubra*, respectively. However Dick *et al.* (2003), evaluating populations of an Amazon forest emergent tree, *Dinizia excelsa*, found $\Phi'_{FT} = 0.104$, a similar value to that found for *C. canjerana*. However, in contrast to the reports for *D. excelsa*, Franceschinelli *et al.* (2015a) found lower fruit and seed production for *C. canjerana* individuals located in smaller fragments than for those located within a continuous forest. A lower rate of pollinator visits in small fragments than in a continuous forest has also been reported (Franceschinelli *et al.* 2015b). Therefore, pollen may be moving further in smaller fragments due to the lower plant density, but pollinators are either less abundant or are moving less frequently amongst plants in smaller fragments, causing a pollination deficit in *C. canjerana* trees of smaller areas.

No pattern of fine-scale spatial genetic structure

Strong SGS was detected among populations although no pattern of fine-scale spatial genetic structure was found. Melo *et al.* (2014) have already reported a strong SGS amongst these same populations and concluded that isolation by distance and fragmentation could explain this pattern of genetic structure. In our study, the Bayesian analysis of allele frequencies clearly showed that the seven populations are genetically structured in two distinct groups, according to landscape, i.e. the populations from the same group are located in the same mountainside. In addition, populations 1 to 4 are located very close each other, whereas the area of population 6 is an ecological corridor that connects population 5 to the large continuous forest where population 7 is located (Figure 3). Therefore, the isolation amongst close fragments located in the same mountainside or connected through corridors can be weaker.

Although *C. canjerana* pollinators disperse pollen amongst neighbouring plants, no genetic substructure was found within populations. The absence of an intrapopulational spatial genetic structure (Sp = 0.000109) was not expected because some birds eat the seed aril of *C. canjerana* in the study fragments and drop the seeds very close to the mother plants (Carmo 2005). However, Pizo & Oliveira (1998) highlighted that birds performed an efficient seed-dispersal mechanism for *C. canjerana* subsp. *canjerana* and in some situations it can fly long distances and efficiently disperse the seeds carried in their bodies, even in a fragmented landscape. Nonetheless, some studies verified that habitat fragmentation might restrict the number of seed dispersers and could change the ecological behaviour of seed dispersers and their relative contribution to plant gene flow (Cordeiro & Howe 2003, Ricketts 2001). Then, forest fragmentation may also restrict the access of seed dispersers of *C. canjerana* within populations, contributing to an increase in population differentiation in each generation.

A strong SGS and low level of gene flow was found for populations of other Atlantic forest trees, such as *Ocotea catharinensis* (Tarazi *et al.* 2010), *Copaifera langsdorffii* (Sebbenn *et al.* 2011) and *Esenbeckia leiocarpa* (Forti *et al.* 2014). Forest fragmentation was considered the main factor restricting the gene flow and promoting SGS amongst these populations. Furthermore, Wang *et al.* (2011) evaluated the fine-scale SGS in populations of *Castanopsis sclerophylla* before and after forest fragmentation in the south-eastern section of Qiandao Lake in China. Contrary to our results, significantly greater fine-scale SGS was found in post-fragmentation subpopulations in the most fragmented habitat. *Castanopsis sclerophylla* is wind-pollinated and seeds are dispersed by gravity and secondarily by rodents. According to the authors, fragmentation may be restricting the secondary dispersal of these seeds. In the present study, fragmentation may be reducing seed dispersal amongst fragments. However, this reduction is not strong enough to cause fine-scale genetic structure within populations.

Therefore, we can conclude that current pollen-mediated gene flow occurs mainly within forest fragment due to short-distance flights of the pollinator of *C. canjerana* and also due to the forest fragmentation that may have reduced this distance. In addition, plant density decreased with fragmentation, reducing the number of pollinator visits amongst plants. We did not detect any fine-scale SGS in the *C. canjerana* populations studied, probably because seed-dispersal is an efficient mechanism that promotes gene flow within populations. The high levels of genetic differentiation found amongst these seven sampled sites reinforced how habitat fragmentation affects the gene flow process in natural areas, although a natural corridor and low distance amongst fragments may reduce genetic differentiation.

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LITERATURE CITED

AGUARI. 2001. *Ações sociais para a preservação de fragmentos florestais na bacia do Rio Camanducaia*. *PROBIO/MMA,* Camanducaia. 40 pp.

ALDRICH, P. R. & HAMRICK, J. L. 1998. Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. *Science* 281:103–105.

AULER, N. M. F., REIS, M. S., GUERRA, M. P. & NODARI, R. O. 2002. The genetics and conservation of *Araucaria angustifolia*: I. Genetic structure and diversity of natural populations by means of non-adaptive variation in the State of Santa Catarina, Brazil. *Genetics and Molecular Biology* 25:329–338.

AUSTERLITZ, F. & SMOUSE, P. 2001. Two-generation analysis of pollen flow across a landscape. II. Relation between Φ_{FT}, *pollen dispersal and inter female distance. Genetics* 157:851–857.

BALDAULF, C., CIAMPI-GUILLARDI, M., AGUIRRA, T. J., CORRÊA, C. E., SANTOS, F. A. M., SOUZA, A. P. & SEBBENN, A. M. 2014. Genetic diversity, spatial genetic structure and realised seed and pollen dispersal of *Himatanthus drasticus* (Apocynaceae) in the Brazilian savanna. *Conservation Genetics* 15:1073–1083.

BARREIROS, H. D. S. & SOUZA, D. S. E. 1986. Notas geográficas e taxonômicas sobre *Cabralea canjerana* (Vell.) Mart. no Brasil (Meliaceae). *Revista Brasileira de Biologia* 46:17–26.

BITTENCOURT, J. V. M. & SEBBENN, A. M. 2008. Pollen movement within a continuous forest of wind-pollinated *Araucaria angustifolia*, inferred from paternity and TwoGener analysis. *Conservation Genetics* 9:855–868.

CARDINALE, B. J., DUFFY, J. E., GONZALEZ, A. HOOPER, D. U., PERRINGS, C., VENAIL, P., NARWANI, A., MACE, G. M., TILMAN, D., WARDLE, D. A., KINZIG, A. P., DAILY, G. C., LOREAU, M., GRACE, J. B., LARIGAUDERIE, A., SRIVASTAVA, D. S. & NAEEM, S. 2012. Biodiversity loss and its impact on humanity. *Nature Review* 486:59–67.

CARMO, R. M. 2005. *Biologia reprodutiva de Cabralea canjerana subsp. canjerana em fragmentos de Mata Atlântica do sul do estado Minas Gerais*. Ph.D thesis, Universidade Federal de Minas Gerais, Brasil.

CLOUTIER, D., HARDY, O. J., CARON, H., CIAMPI, A. Y., DEGEN, B., KANASHIRO, M. & SCHOEN, D. J. 2007. Low inbreeding and high pollen dispersal distances in populations of two Amazonian Forest tree species. *Biotropica* 39:406–415.

COCKERHAM, C. C. 1969. Variance of gene frequencies. *Evolution* 23:72–84.

COLOMBO, A. F. & JOLY, C. A. 2010. Brazilian Atlantic Forest lato sensu: the most ancient Brazilian forest, and a biodiversity hotspot, is highly threatened by climate change. *Brazilian Journal of Biology* 70:697–708.

CONTE, R., REIS, M. S., MANTOVANI, A. & VENCOVSKY, R. 2008. Genetic structure and mating system of *Euterpe edulis* Mart. populations: a comparative analysis using microsatellite and allozyme markers. *Journal of Heredity* 99:476–482.

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.

CORDEIRO, N. J. & HOWE, H. F. 2003. Forest fragmentation severs mutualism between seed dispersers and an endemic African tree. *Proceedings of the National Academy of Sciences USA* 100:14052–14054.

DICK, C. W., ETCHELECU, G. & AUSTERLITZ, F. 2003. Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Molecular Ecology* 12:753–764.

DYER, R. J. & SORK, L. J. 2001. Pollen pool heterogeneity in shortleaf pine, *Pinus schinata* Mill. *Molecular Ecology* 10:859–866.

DYER, R. J., WESTFALL, R. D., SORK, V. L. & SMOUSE, P. E. 2004. Two-generation analysis of pollen flow across a landscape V: a stepwise approach for extracting factors contributing to pollen structure. *Heredity* 92:204–211.

FRANCESCHINELLI, E. V., VASCONCELOS, G. M. P., LANDAU, E. C., ONO, K. Y. & SANTOS, F. A. M. 2007. The genetic diversity of *Myrciaria floribunda* (Myrtaceae) in Atlantic forest fragments of different size. *Journal of Tropical Ecology* 23:361–367.

FRANCESCHINELLI, E. V., CARMO, R. M., SILVA-NETO, C. M. & MESQUITA-NETO, J. N. 2015a. Functional dioecy and moth pollination in *Cabralea canjerana* subsp. *canjerana* (Meliaceae). *Darwiniana* 3:96–107.

FRANCESCHINELLI, E. V., CARMO, R. M., SILVA-NETO, C. M., GONÇALVES, B. B. & BERGAMINI, L. 2015b. Reproductive success of *Cabralea canjerana* (Meliaceae) in Atlantic forest fragments, Brazil. *Revista de Biologia Tropical* 63:515–524.

FREELAND, J. R., BISS, P. & SILVERTOWN, J. 2011. Contrasting patterns of pollen and speed flow influence the spatial genetic structure of sweet vernal grass (*Anthoxanthum odoratum*) populations. *Journal of Heredity* 103:28–35.

FORTI, G., TAMBARUSSI, E. V., KAGEYAMA, P. Y., MORENO, M. A., FERRAZ, E. M., IBAÑES, B., VENCOVSKY, R., MORI, G. M. & SEBBENN, A. M. 2014. Low genetic diversity and intrapopulation spatial genetic structure of the Atlantic Forest tree, *Esenbeckia leiocarpa* Engl. *(Rutaceae)*. *Annals of Forest Research* 57:165–174.

GARCIA, C., JORDANO, P. & GODOY, J. A. 2007. Contemporary pollen and seed dispersal in a *Prunus mahaleb* population: patterns in distance and direction. *Molecular Ecology* 16:1947–1955.

GHAZOUL, J. 2005. Pollen and seed dispersal among dispersed plants. *Biological Review* 80:413–443.

GOODMAN, S. J. 1997. Rst-Calc: a collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and a determining their significance. *Molecular Ecology* 6:881–885.

GUILLOT, G., ESTOUP, A., MORTIER, F. & COSSON, J. F. 2005a. A spatial statistical model for landscape genetics. *Genetics* 170:1261–1280.

GUILLOT, G., MORTIER, F. & ESTOUP, A. 2005b. GENELAND: a computer package for landscape genetics. *Molecular Ecology Notes* 5:712–715.

HAMILTON, M. B. & MILLER, J. R. 2002. Comparing relative rates of pollen and seed gene flow in the island model using nuclear and organelle measure of population structure. *Genetics* 162:1897–1909.

HARDY, O. J. & VEKEMANS, X. 2002. SPAGeDi: a versatile computer program to analyses spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* 2:618–620.

HARDY, O. J., GONZALEZ-MARTINEZ, S. C., COLAS, B., FREVILLE, H., MIGNOT, A. & OLIVERI, I. 2004. Fine-scale genetic structure and gene dispersal in *Centaurea corymbosa* (Asteraceae). II. Correlated paternity within and among sibships. *Genetics* 168:1601–1614.

HARDY, O. J., MAGGIA, L., BANDOU, E., BREYNE, P., CARON, H., CHEVALLIER, M. H., DOLIGNEZ, A., DUTECH, C., KREMER, A., HALLÉ, C. L., TROISPOUX, V., VERON, V. & DEGEN, B. 2006. Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. *Molecular Ecology* 15:559–571.

HEDRICK, P. W. 1999. Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution* 53:313–318.

HOLSINGER, K. E. & WEIR, B. S. 2009. Genetics in geographically structured populations: defining, estimating and interpreting FST. *Nature Review Genetics* 10: 639–650.

LOISELLE, B. A., SORK, V. L., NASON, J. & GRAHAM, C. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany* 82:1420–1425.

LOVELESS, M. D. & HAMRICK, J. L. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecological System* 15:65–95.

LOWE, A. J., BOSHIER, D., WARD, M., BACLES, C. F. E. & NAVARRO, C. 2005. Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. *Heredity* 95:255–273.

LUIKART, G. & CORNUET, J. M. 1998. Empirical evaluation of a testing for identifying recently bottleneck populations from allele frequency data. *Conservation Biology* 12:228–237.

MELO, A. T. O., COELHO, A. S. G., PEREIRA, M. F., BLANCO, A. J. V. & FRANCESCHINELLI, E. V. 2014. High genetic diversity and strong spatial genetic structure in *Cabralea canjerana* (Vell.) Mart. (Meliaceae): implications to Brazilian Atlantic Forest tree conservation. *Natureza & Conservação* 12:129–133.

MELO, A. T. O., COELHO, A. S. G., PEREIRA, M. F., BLANCO, A. J. V. & FRANCESCHINELLI, E. V. 2015. Genética da conservação de Cabralea canjerana (Vell.) Mart. (Meliaceae) em fragmentos florestais de Mata Atlântica na APA Fernão Dias. *Revista Árvore* 39:365–374.

NEI, M. 1973. Analysis of gene diversity in subdivided population. *Proceedings of the National Academy of Sciences USA* 70:3321–3323.

PENNINGTON, T. D., STYLES, B. D. & TAYLOR, D. A. H. 1981. Meliaceae. *Flora Neotropica* 28:235–244.

PEREIRA, M. F., BANDEIRA, L. F., BLANCO, A. J. V., COELHO, A. S. G, CIAMPI, A. Y. & FRANCESCHINELLI, E. V. 2011. Isolation and characterization of microsatellite locis in *Cabralea canjerana* (Meliaceae). *American Journal of Botany* 98:1–3.

PIZO, M. A. & OLIVEIRA, P. S. 1998. Interaction between ants and seeds of a nonmyrmecochorous neotropical tree, *Cabralea canjerana* (Meliaceae), in the Atlantic forest of southeast Brazil. *American Journal of Botany* 85:669–674.

RIBEIRO, M. C., METZGER, J. P., MARTENSEN, A. C., PONZONI, F. J. & HIROTA, M. M. 2009. The Brazilian Atlantic Forest: how much is left, and how is the remaining forest distributed? Implications for conservation. *Biological Conservation* 142:1141–1153.

RICKETTS, T. H. 2001. The matrix matters: effective isolation in fragmented landscapes. *American Naturalist* 158:87–99.

RITLAND, K. 2002. Extensions of models for the estimation of mating systems using n independent loci. *Heredity* 88:221–228.

ROBLEDO-ARNUNCIO, J. J. & GILL, L. 2005. Patterns of pollen dispersal in a small population of *Pinus sylvestris* L. revealed by total exclusion paternity analysis. *Heredity* 94:13–22.

ROBLEDO-ARNUNCIO, J. J., AUSTERLITZ, F. & SMOUSE, P. E. 2007. POLDISP: a software package for indirect estimation of contemporary pollen dispersal. *Molecular Ecology Notes* 7:763–766.

ROSAS, F. QUESADA, M., LOBO, J. A. & SORK, V. L. 2011. Effects of habitat fragmentation on pollen flow and genetic diversity of the endangered tropical tree *Swietenia humilis* (Meliaceae). *Biological Conservation* 144:3082–3088.

SALGUEIRO, F., FELIX, D., CALDAS, J. F., PINHEIRO, M. M. & MARGIS, R. 2004. Even population differentiation for maternal and biparental gene markers in *Eugenia uniflora*, a widely distributed species from the Brazilian coastal Atlantic rain forest. *Diversity and Distributions* 10:201–210.

SEBBENN, A. M., CARVALHO, A. C. M., FREITAS, M. L. M., MORAES, S. M. B., GAINO, A. P. S. C., DA, SILVA, J. M., JOLIVET, C. & MORAES, M. L. T. 2011. Low levels of realized seed and pollen gene flow and strong spatial genetic structure in a small, isolated and fragmented population of the tropical tree *Copaifera langsdorffii* Desf. *Heredity* 106:134–145.

SEOANE, C. E. S., KAGEYAMA, P. Y. & SEBBEN, A. M. 2000. Forest fragmentation effects in population genetic structure of *Esenbeckia leiocarpa* Engl. (Guarantã). *Scientia Forestalis* 57:123–139.

SLOTTA, T. A. B., BRADY, L. & CHAO, S. 2008. High throughput tissue preparation for large-scale genotyping experiments. *Molecular Ecology Resource* 8:83–87.

SMOUSE, P. E. & SORK, V. L. 2004. Measuring pollen flow in forest trees: an exposition of alternative approaches. *Forest Ecology and Management* 197:21–38.

SOKAL, R. R. 1979. Testing statistical significance of geographic variation patterns. *Systematic Zoology* 28:227–232.

STACY, E. A., HAMRICK, J. L., NASON, J. D., HUBBELL, S. P., FOSTER, R. B. & CONDIT, R. 1996. Pollen dispersal in low-density populations of three Neotropical tree species. *American Naturalist* 148:275–298.

TARAZI, R., MANTOVANI, A. & REIS, M. S. 2010. Fine-scale spatial genetic structure and allozymic diversity in natural populations of *Ocotea catharinensis* Mez (Lauraceae). *Conservation Genetics* 11:965–976.

VEKEMANS, X. & HARDY, O. J. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology* 13:921–935.

VILARINHO, E. C., FERNANDES, O. A., HUNT, T. E. & CAIXETA, D. F. 2011. Movement of *Spodoptera frugiperda* adults (Lepidoptera: Noctuidae) in maize in Brazil. *Florida Entomologist* 94:480–488.

YOUNG, A., BOYLE, T. & BROWN, T. 2001. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution* 11:413–418.

WANG, R., COMPTON, S. G. & CHEN, X. Y. 2011. Fragmentation can increase spatial genetic structure without decreasing pollen-mediated gene flow in a wind-pollinated tree. *Molecular Ecology* 20:4421–4432.

WEIR, B. S. & COCKERHAM, C. C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370.

WHITE, G. M., BOSHIER, D. H. & POWELL, W. 2002. Increased pollen flow counteracts fragmentation in tropical dry forest: an example from *Swietenia humilis* Zuccarini. *Proceedings of the National Academy of Sciences USA* 99:2038–2042.

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