Heredity (2016) 116, 339–347

www.nature.com/hdy

ORIGINAL ARTICLE

Small but not isolated: a population genetic survey of the tropical tree *Cariniana estrellensis* (Lecythidaceae) in a highly fragmented habitat

MC Guidugli[1,2], AG Nazareno[3], JM Feres[1,2], EPB Contel[1,2], MA Mestriner[1] and AL Alzate-Marin[1,2]

Here, we explore the mating pattern and genetic structure of a tropical tree species, *Cariniana estrellensis*, in a small population in which progeny arrays ($n=399$), all adults ($n=28$) and all seedlings ($n=39$) were genotyped at nine highly informative microsatellite loci. From progeny arrays we were able to identify the source tree for at least 78% of pollination events. The gene immigration rates, mainly attributable to pollen, were high, varying from 23.5 to 53%. Although gene dispersal over long distance was observed, the effective gene dispersal distances within the small population were relatively short, with mean pollination distances varying from 69.9 to 146.9 m, and seed dispersal distances occurring up to a mean of 119.6 m. Mating system analyses showed that *C. estrellensis* is an allogamous species ($t_m=0.999$), with both biparental inbreeding ($t_m-t_s=-0.016$) and selfing rates ($s=0.001$) that are not significantly different from zero. Even though the population is small, the presence of private alleles in both seedlings and progeny arrays and the elevated rates of gene immigration indicate that the *C. estrellensis* population is not genetically isolated. However, genetic diversity expressed by allelic richness was significantly lower in postfragmentation life stages. Although there was a loss of genetic diversity, indicating susceptibility of *C. estrellensis* to habitat fragmentation, no evidence of inbreeding or spatial genetic structure was observed across generations. Overall, *C. estrellensis* showed some resilience to negative genetic effects of habitat fragmentation, but conservation strategies are needed to preserve the remaining genetic diversity of this population.
Heredity (2016) **116,** 339–347; doi:10.1038/hdy.2015.108; published online 6 January 2016

INTRODUCTION

In forest ecosystems around the world, many plant populations are naturally small, fragmented and sometimes isolated. However, owing to ongoing fragmentation of habitats resulting from anthropogenic processes, especially in tropical areas, an unprecedented number of populations have become smaller and more isolated, representing a potentially serious threat to animal and plant species and their genetic diversity (Saunders *et al.*, 1991; Young *et al.*, 1996). In Brazil, an alarming loss of forests has been reported because of current fragmentation and human-related disturbances (Laurance *et al.*, 2012), resulting in hyperfragmented (Carvalho *et al.*, 2009; Ribeiro *et al.*, 2009) and highly endangered biomes (Ribeiro *et al.*, 2009; Laurance *et al.*, 2011; Gibson *et al.*, 2013; Joly *et al.*, 2014). More than 80% of the remaining Brazilian Atlantic Forest biome is composed of very small fragments (i.e., <50 ha) that are surrounded by open-habitat matrices and isolated by large distances (Ribeiro *et al.*, 2009).

Plant populations that remain small and isolated for many generations after fragmentation suffer an increase in genetic drift and a subsequent loss of genetic diversity and elevated inbreeding, affecting population viability and limiting species' evolutionary potential to respond to environmental change (Young *et al.*, 1996; Lowe *et al.*, 2005). As the effects of habitat fragmentation are dependent on the life history traits of species, such as lifespan and mating system (Jump and Peñuelas, 2006; Leimu *et al.*, 2006; Bacles and Jump, 2011), the consequences of anthropogenic disturbance on the genetics of plant populations remain highly debated (Kramer *et al.*, 2008; Bacles and Jump, 2011). However, a positive relationship between population size and genetic variation has been documented, implying that negative effects of habitat fragmentation on genetic variation of plants are common (Leimu *et al.*, 2006). Moreover, even in highly fragmented landscapes, low levels of pollen or seed dispersal are sufficient to counteract the long-term detrimental effects of inbreeding and loss of genetic diversity resulting from genetic drift, founder effects and genetic erosion (Ellstrand and Elam, 1993; Young *et al.*, 1996; Ellstrand, 2003, 2014). For example, a pollen-flow study involving *Ficus arpazusa* in a highly fragmented landscape in Brazil revealed high levels of genetic diversity and a lack of inbreeding due to pollen dispersal over long distances (Nazareno and Carvalho, 2009).

[1]Universidade de São Paulo, Faculdade de Medicina de Ribeirão Preto, Departamento de Genética—Bloco B, Laboratório de Genética Vegetal, Ribeirão Preto, São Paulo, Brazil; [2]Universidade de São Paulo, Programa de Pós-Graduação em Genética, Departamento de Genética, Ribeirão Preto, São Paulo, Brazil and [3]Universidade de São Paulo, Instituto de Biociências, Departamento de Botânica, Cidade Universitária, São Paulo, São Paulo, Brazil
Correspondence: Dr AG Nazareno. Current address: Universidade de São Paulo, Instituto de Biociências, Departamento de Botânica, Cidade Universitária, Rua do Matão 277, São Paulo, SP CEP 05508-090, Brazil.
E-mail: alison_nazareno@yahoo.com.br
or Dr AL Alzate-Marin. Current address: Universidade de São Paulo, Faculdade de Medicina de Ribeirão Preto, Departamento de Genética—Bloco B, Laboratório de Genética Vegetal, Av. Bandeirantes 3900, Ribeirão Preto, São Paulo CEP 14049-900, Brazil.
E-mail: anaalzate@fmrp.usp.br
Received 4 May 2015; revised 28 September 2015; accepted 30 November 2015; published online 6 January 2016

As population size reduction becomes more common because of habitat fragmentation and degradation, it is vital to measure the contemporary gene flow rates across generations to better understand the effects of habitat changes on the genetic structure of plant species in small and isolated populations. Mating parameters, such as outcrossing rates and the dispersal distances of gene flow, influence patterns of reproduction of tropical plant species in natural populations (Barrett, 2010). These parameters determine how genes are recombined and maintained by a species to perpetuate its natural genetic variability, which is the basis of its continued potential evolution (Brown, 1978; Ritland and Jain, 1981; Sork *et al.*, 2002). Thus, assessing genetic diversity and mating patterns is essential to formulate relevant policies on biodiversity conservation and to design programs for effective management, particularly in fragmented habitats. In this context, there is considerable interest in understanding how mating patterns influence genetic structure of plant species in small and isolated populations.

However, limitations of habitat fragmentation surveys related to experimental design (e.g., sample size, independence of replicates and uncontrolled variation in confounding variables; Nazareno and Jump, 2012) have impaired our ability to understand the ecological mechanisms that underlie the genetic consequences of forest fragmentation. Furthermore, although several studies have focused on assessing the influence of fragmentation on genetic variation among populations, questions remain as to how fragmentation affects intrapopulation genetic diversity. To address the methodological issues that arise from comparisons between undisturbed and disturbed populations (Bacles and Jump, 2011; Nazareno and Jump, 2012), and to explore the genetic consequences of fragmentation, we assessed the temporal dynamics of genetic diversity and mating patterns across generations in one very small population of *Cariniana estrellensis* (Lecythidaceae) in a hyperfragmented landscape of Brazilian Atlantic Forest. We focused primarily on characterizing the mating system and contemporary gene flow for this insect-pollinated and wind-dispersed tropical tree species. Furthermore, we explore the genetic consequences of population fragmentation in *C. estrellensis* by analyzing patterns of genetic diversity in a sample of adults, seedlings and progeny arrays. Our specific objectives were to: (i) document how *C. estrellensis* reproduces; (ii) apply parentage analysis to quantify realized gene dispersal distances, identify immigration of propagules from outside populations and characterize the pollen and seed dispersal kernel for *C. estrellensis*; (iii) assess patterns of fine-scale spatial genetic structure (SGS) across generations; and (iv) infer effects of habitat fragmentation on genetic variability. Our main goal is to generate information about mating patterns and genetic structure dynamics for *C. estrellensis*, a useful starting point for conservation initiatives, management activities and policies for this plant species.

MATERIALS AND METHODS

Study species and study site

C. estrellensis (Raddi) Kuntze (Lecythidaceae), commonly known as jequitibá-branco, is an emergent neotropical tree that prefers wet and deep soils. This climax forest tree can reach up to 45 m in height, with a diameter at breast height up to 120 cm. *C. estrellensis* is found mainly in Brazil and also occurs in Bolivia, Paraguay and Peru (Leite, 2007). Different densities of the species have been reported, ranging from 5 to 27 trees ha^{-1} (Toledo Filho *et al.*, 2000; Nunes and Petrere, 2012). *C. estrellensis* flowers—creamy white and clustered in axillary racemes—are well distributed over the crown but not produced in large numbers, and physical barriers protect pollen and stigma from insects. During pollination, insects must enter the urceolus to touch the stigma. According to Prance and Mori (1979), *Trigona* and *Melipona* are the primary stingless bees involved in pollination of *C. legalis*, a species closely related to *C. estrellensis*. As allogamy predominates in the Lecythidaceae family (Mori *et al.*, 2010), we expected to find some evidence of self-incompatibility in *C. estrellensis*. Fruits are small, cylindrical-oblong, woody, brown fibrous pyxis containing up to 35 alate seeds with membranous wings. *Cariniana* trees generally flower toward the end of the dry season, and the fruit mature at the beginning of the subsequent dry season (Prance and Mori, 1979). During the dry season, the wind-dispersed, winged seeds are released when the operculum is spontaneously dropped. Seed predation by black howler monkeys has been reported for the species (Oliveira-filho and Galetti, 1996). *C. estrellensis* wood is of high commercial value and the species is important for degraded area recovery in the tropics (Leite, 2007).

This study was conducted during the dry season of 2007 in a semideciduous, 8.0 ha forest fragment (21°17′47″S, 47°40′29″W; Figure 1a) located in Ribeirão Preto (São Paulo State, Southeastern Brazil). This internationally known agricultural region is one of the most anthropogenically devastated forest landscapes in Brazil. The removal of natural vegetation in this area began more than 100 years ago to make room for coffee production. The remaining natural vegetation is highly fragmented and exposed to continued processes of degradation due to urban settlement and sugarcane production. A rapid

Figure 1 Satellite images showing (**a**) the location of the studied fragment of Atlantic Forest (dashed dark line) within the highly fragmented landscape in Ribeirão Preto, São Paulo State, Southeastern Brazil; the spatial distribution of adults (**b**) and seedlings (**c**) of *C. estrellensis*. The image was accessed via Google in January 2015.

floristic survey conducted in 99 forest remnants (sizes varying from 1.5 to 247.0 ha) in the region identified *C. estrellensis* in 27 remnants (3.0–75.0 ha), although no information exists about their densities (Kotchetkoff-Henriques, 2003). The majority ($n=23$) of these fragments are isolated by long distances and are very small (<50 ha). Based on floristic surveys in the region, the nearest *C. estrellensis* individual to the studied population is located 1 km away.

Sample collection, DNA extraction and SSR amplification

Samples of *C. estrellensis* were taken from leaf material of individuals from three ontogenetic stages (adults, seedlings and progeny arrays). In the studied population (density of 3.5 trees ha^{-1}), all adult trees (28 plants reaching a height of 20 m with a trunk diameter ranging from 1 to 5 m; Figure 1b) and seedlings (39 regenerated juvenile trees with heights ranging from 0.15 to 1.5 m; Figure 1c) were sampled, tagged with a numbered aluminum plate and mapped using GPS with ± 6 m accuracy (Garmin, Atchison, KS, USA). As *C. estrellensis* is a long-lived tropical tree species, and based on the size of the adult trees in the studied population, we assume that current adult individuals predate recent fragmentation.

From all adult trees that blossomed in 2007 ($n=7$), we obtained a total of 399 progeny arrays (65 progeny from five seed trees; 50 progeny from one seed tree and 24 progeny from another seed tree). Although the estimates of inbreeding and genetic diversity can be biased when seeds are collected directly from mother trees, we decided to use this sampling method because the botanical identification of seeds germinated on the forest floor is extremely difficult. Seeds were raised in a native tree nursery at the Ribeirão Preto Campus, University of São Paulo, and information was recorded for each seed tree. Leaves from all 466 individuals (28 adult trees, 39 seedlings and 399 progeny arrays) were stored at -20 °C until DNA extraction.

Genomic DNA was extracted from leaves of each sample using a modified CTAB protocol as described in Alzate-Marin *et al.* (2009). Amplification protocols for nine microsatellite loci can be found in Guidugli *et al.* (2009). Amplicons (10 μl) were denatured and separated with 10% denaturing polyacrylamide gels (39:1 acrylamide to bisacrylamide) and stained with silver nitrate. Gels were run with 1 × TBE buffer (90 mM Tris, 92 mM boric acid and 2.5 mM ethylenediaminetetraacetic acid) using a vertical electrophoresis apparatus at constant voltage (550 V) and electric current (23 mA) for 4 h. Allele sizes were estimated by comparison with a 10 base-pair DNA ladder standard (Invitrogen, Carlsbad, CA, USA). To minimize possible genotyping error in size-calling alleles, we ran amplicons of progeny arrays together with their maternal seed tree. After calling alleles for each locus, we verified the sizes by ranking alleles, from lowest to highest, in a denaturing polyacrylamide gel, which was run using the same method described above.

Genetic diversity parameters

As genetic structure, parentage analysis and mating system presuppose that alleles at different loci segregate independently, we undertook Mendelian inheritance analyses for each locus, based on the mother tree and her progeny as proposed by Gillet and Hattemer (1989). Considering that gametic disequilibrium creates pseudoreplication for analyses in which the loci are assumed to be independent samples of the genome, we used the FSTAT 2.9.3.2 program (Goudet, 2002) to test all loci for linkage disequilibrium, applying the Bonferroni correction for multiple comparisons (Rice, 1989). We used the MICROCHECKER 2.2.3 program (Van Oosterhout *et al.*, 2004) to detect null alleles and genotyping artifacts in the data. If null alleles were detected, we estimated their frequency following the method of maximum-likelihood estimator as described by Kalinowski and Taper (2006).

Allele frequencies and descriptive population genetic parameters (number of alleles, number of private alleles, observed heterozygosity, unbiased expected heterozygosity (Nei, 1978) and tests for Hardy–Weinberg equilibrium) for adults, seedlings and progeny arrays of *C. estrellensis* were obtained using GenAlEx (Peakall and Smouse, 2006). Allelic richness (A_R) independent of the sample size was also obtained for adults, seedlings and progeny arrays. We followed the rarefaction procedure method proposed by Kalinowski (2005), which is used to standardize the allelic richness to the smallest sample size across generations. The 95% confidence interval (CI) of the standard error of this parameter was calculated using a jackknife procedure across all loci.

Determination of mating system

The progeny arrays ($n=399$) from seven *C. estrellensis* seed trees were analyzed to study mating system. Genotypes obtained were analyzed under mixed-mating and correlated mating models using the multilocus mating system program MLTR version 3.2 (Ritland, 2008). Our analysis took into account that all loci may contain null alleles even if there are none. The mating system parameters estimated were multilocus outcrossing rate (t_m), single-locus outcrossing rate (t_s), selfing rate ($1-t_m$), biparental inbreeding (or mating among relatives, t_m-t_s) and multilocus paternity correlation ($r_{p(m)}$). The average number of pollen donors per seed tree, N_{ep}, was calculated as $1/\hat{r}_{p(m)}$ (Ritland, 1989). We also calculated the inbreeding coefficient of seed trees and progeny arrays. Mating system parameters were estimated for individual families ($n=7$ families) and at the population level ($n=399$). Analyses at the population level were carried out using the probabilities of expectation maximization method, and analyses at the individual level used the method of moments according to Ritland (2008). The standard error for each parameter was calculated from 1000 bootstrap replicates with resampling among families. We also quantified the coefficient of pollen pool structure Φ_{ft} from two-generation analyses (TWOGENER; Smouse *et al.*, 2001). This statistic is analogous to Wright's F_{ST} and ranges from 0 to 1. The standard error for Φ_{ft} was calculated using a jackknife procedure over loci. All calculations were carried out using the R language for TWOGENER analysis as described by Hirao (available at http://hosho.ees.hokudai.ac.jp/~hirao/TWOGENER/TwoGener.html).

Categorical parentage analysis

Estimates of gene flow from pollen and seeds were carried out by parentage assignment (paternity or maternity) using the maximum-likelihood-based method of Marshall *et al.* (1998), implemented in the program CERVUS version 3.0.3 (Kalinowski *et al.*, 2007). Seedlings ($n=39$) and progeny arrays ($n=399$) from seven *C. estrellensis* seed trees were analyzed to study pollen dispersal, with all adult trees ($n=28$) as paternal candidates. To study seed dispersal, we analyzed the genotypes of all seedlings and adult trees, and all adult trees were considered as putative maternal and/or paternal candidates.

We used a two-phased approach: first, we performed 10 000 simulated genotypes to obtain the critical value of Delta (Δ_{crit}); second, paternity assignment was performed to obtain the value of Delta (Δ), which is the difference between the highest and the second highest logarithm of odds scores. Parentage analyses were performed using all loci (with a genotyping error rate of 0.01 per locus estimated in Microchecker) and a confidence level of 95%. Only the potential parent with $\Delta > \Delta_{crit}$ was considered to be the true parent of the analyzed progeny arrays or seedlings. Additionally, we used the set of assigned individuals to assess pollen and seed dispersal patterns in terms of distance. Pollen and seed dispersal (Euclidean) distances were calculated based on the position of the parent tree and the putative pollen or seed parent within the population. Pollen dispersal distance was calculated for progeny arrays and seedlings based on the position of the seed-tree and putative pollen parent within the small population. For seedlings, we adopted one of the following procedures: (a) when a seedling had only one putative parent within the population, we assumed that the assigned parent was the mother, and this parent was used to infer the distance of seed dispersal; (b) when a seedling had two parents within the population, the candidate nearest to the seedling was assumed as the maternal parent and the further candidate was the paternal parent. Although the later procedure can introduce bias in estimating distance of seed dispersal, it has been used when identifying the maternal parent is impossible, as is the case with monoecious and hermaphroditic species (Dow and Ashley, 1996).

A Kolmogorov–Smirnov test was applied to determine whether the success of pollination is dependent on the geographical distance between adult trees. For this, we compared the distributions of frequencies of pollen dispersal with the frequency distribution of the distances among all adult trees in the population. Additionally, pollen and seed immigration rates (m_p and m_s, respectively) were calculated as the percentage of genotypes not assigned to a candidate parent within the population (Smouse and Sork, 2004). We also estimated cryptic pollen flow, a value that represents the fraction of genotypes assigned to a putative father within the population when the true father is

outside the population (Dow and Ashley, 1996). Assuming a circular area around each seed tree, the effective pollination neighborhood area ($A_{ep} = 2\pi\sigma^2$; Levin, 1988) was calculated from the variance of pollen flow distance (σ^2).

Pollen and seed dispersal kernels

We used a maximum-likelihood procedure to determine an individual pollen dispersal distance probability density function based on genotypes of seedlings ($n = 39$) and progeny arrays ($n = 399$). We estimated the probability of pollen and seeds traveling from their original locations to a given position (i.e., pollen and seed dispersal kernels) for *C. estrellensis* using a spatially explicit NEIGHBORHOOD model as proposed by Burczyk *et al.* (2002). Using a maximum-likelihood fractional parent assignment, the NEIGHBORHOOD model was calculated in the software NM+ version 1.1 (Chybicki and Burczyk, 2010). The neighborhood parameter was set to 'infinite' to include all sampled adults in our small population as the neighborhood size (Chybicki and Burczyk, 2010). Initial setting values used in the NEIGHBORHOOD model were those estimated from mating system (*s*, selfing rate) and categorical parentage analysis (d_p, mean pollination distance; d_s, mean seed dispersal; *m*, apparent pollen immigration; m_s, seed immigration rate). Pollen and seed dispersal were modeled using the three most useful models (i.e., Exponential-power, Weibull and 2Dt) related to dispersal kernels in plants (Austerlitz *et al.*, 2004; Chybicki and Burczyk, 2010; Ottewell *et al.*, 2012; Côrtes *et al.*, 2013). The shape of dispersal kernel tails (*b*), their scale (*a*), the average distance of pollen (d_p) and seed dispersal (d_s), and immigration rates (m_p for pollen and m_s for seeds) were estimated for all dispersal kernel models. If null alleles were present, the rate of genotype mistyping errors corresponding to the frequency of null alleles at each locus was taken into account in the analysis.

Fine-scale genetic structure

Wright's inbreeding index, F_{IS}, was calculated and significance tested using the software SPAGEDI v.1.2 (Hardy and Vekemans, 2002). To explore whether *C. estrellensis* has the ability to survive in a relictual population following habitat fragmentation, we compared the inbreeding index and allelic richness between adult trees (prefragmentation), seedlings and progeny arrays (postfragmentation). We used allelic richness in the comparisons as it is highly influenced by population reduction because of rapid elimination of rare alleles (Cornuet and Luikart, 1996) and because it may more accurately reflect current levels of genetic diversity within the fragmented populations (Jump and Peñuelas, 2006).

We assessed fine-scale SGS for adults and seedlings using SPAGEDI v.1.2 (Hardy and Vekemans, 2002). We calculated Nason's estimator of kinship coefficient (F_{ij}), as described in Loiselle *et al.* (1995), because it displays robust statistical properties (Vekemans and Hardy, 2004). At least seven distance classes were identified to reach a minimum of 70 and 100 pairs of individuals per distance class for adult trees and seedlings, respectively. Spatial locations were permuted 20 000 times to obtain error estimates for the null hypothesis ($F_{ij} = 0$). Error estimates for the observed kinship values among adult trees and seedlings were calculated by jackknifing loci 20 000 times. To visualize SGS, we plotted the kinship coefficient against geographical distance. The strength of SGS was assessed using the Sp statistic as described by Vekemans and Hardy (2004), calculated as $-b_{log}/(1-F_1)$, where b_{log} is the mean slope of regression of kinship coefficients on log 10 distance and F_1 is the mean kinship coefficient of the first distance class. We tested SGS by assessing the significance of the regression slope using SPAGeDi v.1.2 (Hardy and Vekemans, 2002).

RESULTS

Genetic diversity parameters

The microsatellite set for *C. estrellensis* had a Mendelian inheritance (Supplementary Table 1) and we found no significant linkage disequilibrium after Bonferroni correction ($P < 0.001$) between loci for all life stages. MICROCHECKER 2.2.3 revealed that locus *CES02* may contain null alleles at moderate frequencies ranging from 0.031 (adult trees) to 0.132 (seedlings). However, we chose to include it in this study and accounted for null alleles in our mating system and parentage analyses.

Table 1 Population genetic estimates for one small *C. estrellensis* population, in São Paulo State, Southeast Brazil

	n	K	A_R	A_P	H_E	H_O	F_{IS}
Adults	28	62	6.5 (6.33–6.78)	5	0.618	0.653	−0.02
Seedlings	39	55	5.7 (5.35–5.86)	2	0.574	0.555	0.07
Progeny	399	56	4.7 (4.47–4.88)	2	0.554	0.578	−0.04

Abbreviations: A_P, number of private alleles; A_R, allelic richness based on rarefaction from a sample of 28 for each sample size; F_{IS}, inbreeding index; H_E and H_O, expected and observed heterozygosity, respectively; *K*, total number of alleles; *n*, sample size.

DNA analysis of nine microsatellite markers in the total sample of 466 genotypes revealed 68 alleles (Supplementary Table 2). The number of alleles per locus ranged from 2 (*Ces14*) to 14 (*Ces04*), with averages ranging from 6.11 (seedlings) to 6.89 (adults) (Supplementary Table 3). The allelic richness differed significantly between life stages according to the 95% CI (Table 1). Of the nine private alleles observed, five (55.5%) were found in *C. estrellensis* adults (Table 1). Some alleles were also found exclusively in seedlings or progeny arrays (Table 1). The number of alleles and the observed and expected heterozygosity are presented in Table 1. Allele frequencies for all life stages are provided as Supplementary Information (Supplementary Table 2).

Across generations of *C. estrellensis*, a test for Hardy–Weinberg equilibrium found that of 27 locus generations, 2, 1 and 4 (7.4%, 3.7% and 14.8%) showed significant deviation at $P < 0.05$, 0.01 and 0.001, respectively. However, these deviations were observed in only seedlings and progeny arrays. In relation to the dynamics of inbreeding between generations, we observed values of F_{IS} ranging from negative to positive. The mean F_{IS} between loci was not significantly different from zero for all generations (Table 1).

Mating system

C. estrellensis was highly outbred with a multilocus outcrossing rate not significantly different from unity (Table 2). The difference between the multilocus and single-locus outcrossing rate was negative but not significantly different from 0 (Table 2). As the multilocus outcrossing rates were not significantly different from unity, the selfing rate is considered nil. Furthermore, inbreeding was not detected in either adult trees ($F = -0.057$; 95% CI = −0.121 to 0.017) or progeny arrays ($F = -0.043$; 95% CI = −0.183 to 0.061). Family-level estimates of the multilocus outcrossing rates were high but not significantly different from unity (Supplementary Table 4). These results indicate that *C. estrellensis* is an effectively outcrossing species and it seems to be self-incompatible. A moderate degree of correlated paternity within seeds ($r_p = 0.121$) was observed. This paternal identity rate within progeny arrays translated into an estimated average of 8.2 effective pollen donors per seed tree (Table 2). The coefficient of pollen pool structure showed low but significant global differentiation between pollen pools fertilizing the seed trees ($\Phi_{ft} = 0.065$, $P < 0.05$). Considering no correlated paternity among seed-tree pairs, this translates into an estimate of 1/2 $\Phi_{ft} = 7.7$ effective pollen donors per seed tree (see Equation (27) in Austerlitz and Smouse, 2001).

Paternity analysis

We assigned a paternal parent within the fragment to 305 (76%) of 399 progeny arrays with at least 80% confidence. With 95% confidence, we assigned paternity for 237 of the 305 sampled progeny arrays (78%) to *C. estrellensis* adult trees located within the study population. In addition, 26 of the 28 adult trees contributed paternity

to progeny arrays from the analyzed seed trees. Male reproductive success was highly skewed towards a few individuals. For instance, the majority of pollen donor trees contributed single progeny arrays, whereas seven of the 26 pollen donors contributed more than 63.9% of progeny arrays. The percentage of progeny arrays that do not have a parent tree assigned within the population (i.e., pollen immigration) was high ($m_p = 23.5\%$; Table 2). With a probability of cryptic gene flow of 13% (Table 2), the total (actual) pollen immigration for progeny arrays (i.e., pollen immigration rate plus cryptic gene flow) was 36.5%. Given the high exclusion probability ($EP = 0.993$; Supplementary Table S3) and sampling of all adult trees within the *C. estrellensis* population, the high pollen immigration rate indicates that the small forest fragment studied herein is not genetically isolated.

Our results demonstrate pollen movement beyond the boundaries of the small fragment over distances possibly >1 km. However, effective pollination distances within the small population were relatively short, with 50% of pollination events occurring at distances up to 72 m (Figure 2). The mean, minimum and maximum pollen dispersal distances to the nearest *C. estrellensis* pollen donor were 69.9 ± 7.9 (s.d.), 4 and 355 m, respectively (Table 2). Nevertheless, the frequency distribution of observed pollination distances for progeny arrays and the frequency distribution of intertree distances of all adult trees relative to the sampled seed trees were significantly different ($D = 0.391$, $P < 0.01$; Supplementary Figure 1). Overall, the number of pollen donors for each seed tree ranged from 3.8 to 24.6, with an average of 12.9 paternal parents contributing to progeny arrays (Table 1). Effective pollination neighborhood (A_{ep}) for *C. estrellensis* ranged from 1.0 to 4.4 ha between seed trees, with an average of 2.3 ha (Table 2), equivalent to a circle with a radius of 86 m around a seed tree.

Table 2 Population-level estimates of mating system and paternity analysis for a small *Cariniana estrellensis* (Raddi) Kuntze population in Ribeirão Preto, Sao Paulo State, Southeast Brazil

Mating system	
Number of seed trees/number of progeny arrays	7/399
Multilocus outcrossing rate (t_m)	0.999 (0.937–1.061)
Single-locus outcrossing rate (t_s)	1.015 (0.990–1.039)
Mating among relatives ($t_m - t_s$)	−0.016 (−0.078 to 0.047)
Selfing rate ($1 - t_m$)	0.001
Multilocus paternity correlation (r_p)	0.121 (0.102–0.141)
Mean number of pollen donors	8.2
Paternity analysis	
Pollination distances (m)	
Mean (s.d.)	69.9 (7.9)
Minimum	4.0
Maximum	355.0
Pollination neighborhood area (A_{ep}) (ha)	
Mean (s.d.)	2.3 (0.7)
Minimum	1.0
Maximum	4.4
Mean pollen immigration	23.5%
Cryptic gene flow	13.1%
Mean number of pollen donors (N_{ep})	12.9

Numbers within parentheses indicate 95% confidence interval obtained by 1000 bootstraps. Confidence intervals that fall within 1 (for t_m and t_s estimates) or 0 (for $t_m - t_s$ estimate) are not significant.

Parentage analysis

We assigned a paternal and maternal parent from within the fragment to 18 (46%) of the 39 seedlings with 95% confidence. For the remaining 21 seedlings, a putative mother tree was assigned within the population. Overall, the assignment of paternity and maternity to seedlings indicates that seed immigration was more restricted than pollen immigration (Table 3); 62% of the seedlings (53% of apparent pollen immigration along with 9% cryptic gene flow) were fathered by trees located outside of the population. Nevertheless, the actual seed immigration rate may be close to 10% based on the estimate of cryptic seed flow (9.3%).

Compared with the paternity results, pollen immigration and mean pollen dispersal distance for seedlings was greater than that observed for progeny arrays (Table 2). In addition, the effective pollination distance was also greater than the effective seed migration distance, with 50% of pollination events occurring at distances of up to 85 m. The seed dispersal distance varied from 13 to 345 m, with an average of 119.64 ± 8.2 m (s.d.) (Table 3). Approximately 51% of seedlings were dispersed up to 100 m, although 13% of them were dispersed as much as 180 m from the putative mother tree (Supplementary Figure 2).

Pollen and seed dispersal kernel

The best-fitting dispersal distributions were fat-tailed exponential-power curves ($b < 1$; Tables 4 and 5; see Austerlitz *et al.*, 2004) for both progeny arrays and seedlings. The exponential-power dispersal kernel had the highest model likelihood for both progeny arrays and seedlings (Tables 4 and 5), indicating a higher rate of pollen immigration ($m_{p(progeny)} = 0.26$ vs 0.23, $m_{p(seedlings)} = 0.69$ vs 0.53)

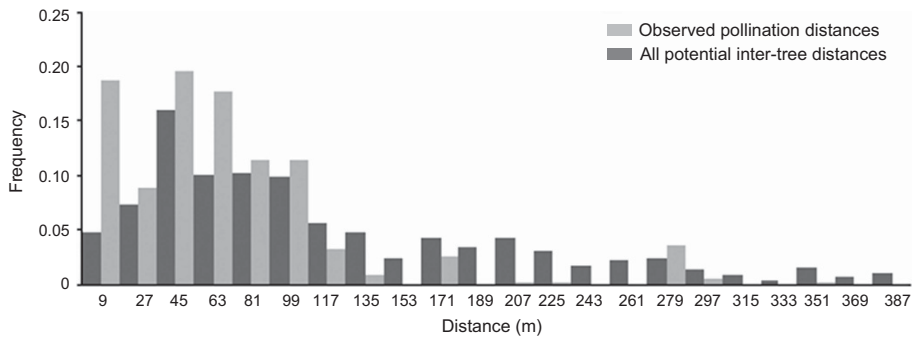

Figure 2 Histogram of observed pollination distances for progeny estimated from paternity analyses (gray bars) and intertree distances of all *C. estrellensis* trees relative to the sampled seed trees (dark gray bars).

and seed immigration ($m_s = 0.16$ vs 0.00) than expected from the categorical parentage analysis.

Fine-scale genetic structure

We observed limited SGS between adults and seedlings overall ($Sp_{adults} = 0.016$ and $Sp_{seedlings} = 0.006$), with a weak kinship coefficient between adjacent *C. estrellensis* individuals in the first distance class ($F_{1\ adults} = 0.0351$, 226.4 m; and $F_{1\ seedlings} = 0.0226$, 125.9 m; Figure 3). In addition, slopes (b_{log}) of the correlograms for adult and seedling *C. estrellensis* generations were not significantly different from the null hypothesis of no SGS ($b_{log\ adults} = -0.0156$ with associated determination coefficient (R^2) equal to 0.012; $b_{log\ seedlings} = -0.0061$ with $R^2 = 0.012$).

DISCUSSION

Mating system

To date, there have been no published descriptions of *C. estrellensis* mating system. The high multilocus outcrossing rate detected in this study indicates that it is an effectively outcrossing tree species displaying mechanisms of self-incompatibility. In other species belonging to the same family (Lecythidaceae), such as *Bertholletia excelsa* (O'Malley *et al.*, 1988), *Cariniana legalis* (Tambarussi, 2013) and *Eschweilera ovata* (Gusson *et al.*, 2006), the estimated rates of reproduction indicate a mixed system with a predominance of outcrossing and a probable absence of mechanisms for self-incompatibility in these species. Outcrossing rates for hermaphroditic species such as *C. estrellensis* depend on factors such as the presence and intensity of a self-incompatibility system, the degree of protandry and protogeny, pollinator foraging behavior between and within reproductive trees and flowering tree density in the population (Murawski and Hamrick, 1991). Nevertheless, according to Bawa *et al.* (1985), most hermaphroditic tropical species have strong barriers to selfing; for example, the floral structure of *C. estrellensis* likely prevents selfing in this species. According to Mori *et al.* (2010), representatives of the Lecythidaceae family with actinomorphic flowers (such as *C. estrellensis*) tend to have their stigmas (i.e., female receptive

Table 3 Parentage estimates for *Cariniana estrellensis* (Raddi) Kuntze seedlings in a small population in Southeast Brazil

Number of plants with both parents identified	18
Number of plants with one identified parent (maternal parent)	21
Pollination distances (m)	
Mean (s.d.)	146.9 (9.8)
Minimum	36.0
Maximum	310.0
Seed dispersal distances (m)	
Mean (s.d.)	119.6 (8.2)
Minimum	13.0
Maximum	345.0
Immigration rates	
Mean pollen immigration	53%
Mean seed immigration	0%
Cryptic gene flow	9.3%

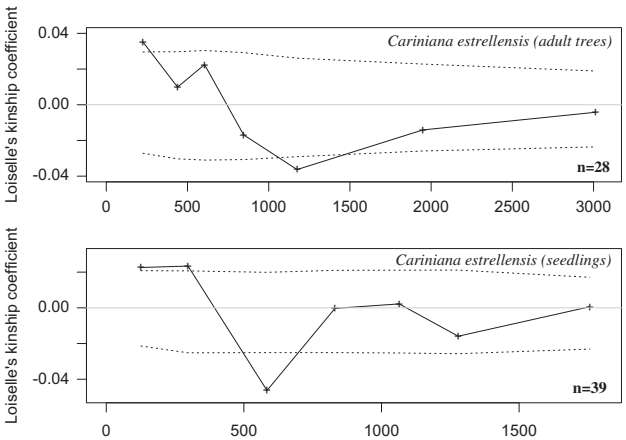

Figure 3 Average Loiselle's kinship coefficient plotted against geographical distance between individuals (solid lines) for adults and seedlings of a smaller *C. estrellensis* population, São Paulo State, Southeast Brazil. When the kinship coefficient lies above its 95% CIs (dotted lines), individuals are significantly more similar than would be expected through random sampling.

Table 4 Model LogL, m_p, d_p, *a* and *b* from *Cariniana estrellensis* (Raddi) Kuntze progeny arrays ($n = 399$)

Model	*LogL*	m_p	d_p	a	b
Exponential-power	−4552.89	0.259 (0.02)	227.36 (36.41)	134.83	0.81
Weibull	−4555.64	0.259 (0.02)	199.28 (25.55)	224.58	1.58
2Dt	−4557.81	0.259 (0.02)	231.32 (34.76)	212.84	2.37

Abbreviations: *a*, dispersal kernel scale; *b*, shape parameters; d_p, mean distance of pollen dispersal; LogL, log likelihoods; m_p, pollen immigration rate.
Values shown within parentheses are the standard deviations.

Table 5 Model LogL, m_p, m_s, d_p and d_s, a_p and a_s, and b_p and b_s from *Cariniana estrellensis* (Raddi) Kuntze seedlings ($n = 39$)

Model	*LogL*	m_p	m_s	d_p	d_s	a_p	a_s	b_p	b_s
Exponential-power	−675.27	0.695 (0.10)	0.160 (0.09)	1061.03 (281.21)	126.56 (31.06)	530.51	63.28	0.93	0.64
Weibull	−677.71	0.698 (0.10)	0.167 (0.08)	433.78 (670.41)	123.61 (18.85)	489.47	139.48	2.01	1.97
2Dt	−675.36	0.693 (0.10)	0.165 (0.09)	682.47 (910.52)	141.83 (36.09)	593.63	123.37	2.19	2.05

Abbreviations: a_p, dispersal kernel scale for pollen; a_s, dispersal kernel scale for seed; b_p, shape parameter for pollen; b_s, shape parameter for seed; d_p, mean distance of pollen dispersal; d_s, seed dispersal; LogL, log likelihoods; m_p, pollen immigration rate; m_s, seed immigration rate.
Values shown within parentheses are the standard deviations.

region) oriented in a specific position that ensures that the pollen is deposited on the receptive field as the pollinator enters the flower but not as it is leaving. Thus, pollen is not deposited on the stigma of the same flower from which it was collected. Specific studies of the genetic control of self-incompatibility in *C. estrellensis* are necessary to better understand this mechanism.

The results suggest that the reproductive system of *C. estrellensis* is resilient to a reduction in population size, as the estimates of biparental inbreeding indicate no mating between relatives. A lack of biparental inbreeding—or any inbreeding (Table 2)—in the studied population can be explained by the absence of SGS in the adult generation and by the occurrence of pollen flow over long distances. This is in contrast to several other fragmented tree species that have shown elevated levels of mating between related individuals (Ismail *et al.*, 2012; Tambarussi, 2013), genetic structuring (Dubreuil *et al.*, 2010; Ismail *et al.*, 2012; Saro *et al.*, 2014) and inbreeding (Fuchs *et al.*, 2003; Jump and Peñuelas, 2006; Kettle *et al.*, 2007; Dick *et al.*, 2008; Vranckx *et al.*, 2011; Zhang *et al.*, 2012; Tambarussi, 2013; Finger *et al.*, 2014).

The elevated outcrossing rates, accompanied by moderate values for the estimated paternity correlations observed in this study, strongly corroborate the high number of effective pollen donors involved in seed production in *C. estrellensis*. The low density of reproductive adult trees in the studied population (3.5 trees ha^{-1}) may have contributed to pollination vectors of *C. estrellensis* visiting a larger number of individuals. This behavior ensures the maintenance of high rates of outcrossing and moderate correlated paternity rate in this population.

Gene flow

In the small *C. estrellensis* population, we found that gene dispersal is enhanced, in contrast to the prediction that a reduction in population size following habitat fragmentation disrupts gene dispersal. However, gene flow for *C. estrellensis* was somewhat lower compared with the values reported for wind-dispersed tropical trees and species with different dispersal patterns (Nakanishi *et al.*, 2009; Berens *et al.*, 2013) even in small populations (Ottewell *et al.*, 2012; Tambarissu, 2013).

Realized gene dispersal distances in this small population were higher for pollen than seeds. This finding indicates that gene flow for *C. estrellensis* is maintained primarily by pollen dispersal, which is consistent with other studies (Ndiade-Bourobou *et al.*, 2010; Berens *et al.*, 2013). As a matter of course, plants like *C. estrellensis*, which are effectively outcrossing and insect-pollinated, should show pollen dispersal over long distances, as has been observed for other tropical tree species (Nason *et al.*, 1998; Gaiotto *et al.*, 2003; Nazareno and Carvalho, 2009). Indeed, even in a highly fragmented landscape, we observed a moderate to high frequency (23.5–53%) of gene immigration, indicating that pollen movement beyond the boundaries of the small fragment can reach distances >1 km (i.e., the distance between the small studied population and the nearest pollen source). Gene flow by pollen dispersal beyond the edges of seemingly isolated forest fragments has been reported for several plant species, including species that are animal-pollinated (Nason and Hamrick, 1997; Dow and Ashley, 1998; Dick *et al.*, 2003; Sato *et al.*, 2006; Nazareno and Carvalho, 2009; Buschbom *et al.*, 2011; Ottewell *et al.*, 2012; Côrtes *et al.*, 2013; Tambarussi, 2013; Saro *et al.*, 2014).

The fractional paternity approach revealed an exponential-power distribution with a fat-tailed dispersal curve ($b<1$) for seedlings and progeny arrays. This finding is in line with the trend towards fat-tailed distributions of gene dispersal in plants (Dick *et al.*, 2003; Austerlitz *et al.*, 2004; Oddou-Muratorio *et al.*, 2005; Klein *et al.*, 2006). The fat-tailed dispersal distribution observed for *C. estrellensis* implies that long-distance dispersal is more frequent in this species than in plant species exhibiting thin-tailed distributions. However, immigration rates, most notably for seeds, and mean gene dispersal distances, principally for pollen, were inconsistent between fractional paternity analysis and categorical paternity assignments (Tables 2–5). A discrepancy between paternity approaches has been reported in other mating studies for tree species in small populations (Ottewell *et al.*, 2012; Saro *et al.*, 2014), and this difference likely arises owing to the nature of each analytical approach (Burczyk *et al.*, 2002; Kalinowski *et al.*, 2007).

Although we have not measured functional traits of *C. estrellensis*, such as the seed terminal velocity and the height of seed release above the vegetation cover, or quantified wind speed and vertical turbulence around the edges of the studied population, it is known that these traits can affect the immigration rates for wind-dispersed plant species (Heydel *et al.*, 2014). In this context, we hypothesize that the influence of these factors—besides the small number of seedlings in the studied fragment and the nature of the method used—could limit the amount of seed immigration into the *C. estrellensis* population, potentially leading to an underestimation of realized seed dispersal in this species using parentage analysis. Furthermore, the pattern of seed dispersal for *C. estrellensis* may be influenced by the degree of seed predation by primates, such as *Alouatta caraya,* that eat the seeds at the base of the wing (Oliveira-Filho and Galetti, 1996).

Overall, our findings indicate that this small population of *C. estrellensis* is not genetically isolated. Therefore, in the fragmented landscape, there are isolated trees or relictual populations that may contribute to the genetic pool of this specific population. Further studies linking the functional traits of the species and meteorological variables to the estimates of effective gene dispersal, in both fragmented and continuous populations, can strengthen our understanding of the mating pattern of this tropical species.

Diversity and fine-scale genetic structure

Significant reduction in allelic richness but no inbreeding was detected in both postfragmentation generations of *C. estrellensis*, pointing to a loss of genetic diversity between life stages in this small population. Nonetheless, these results do not fit well with the theory that inbreeding is observed immediately following fragmentation, but genetic diversity is lost slowly over generations (Lowe *et al.*, 2005; Bacles and Jump, 2011); for long-lived plant species, this process may take decades.

Although a significant reduction in allelic richness following fragmentation was observed for *C. estrellensis*, the extensive gene flow detected in the small population could be attributed to a lack of inbreeding and SGS between the life stages of this species. As has been documented for other outcrossing species in small populations (Balloux, 2004; Saro *et al.*, 2014), we observed an excess of heterozygotes in all life stages of *C. estrellensis*. Owing to the relatively large range of pollen dispersal, one could expect an effective mixing of genes by pollen flow or even seed dispersal within the studied population, hampering SGS. Moreover, because the number of *C. estrellensis* individuals within the population was too small to assess SGS with statistical rigor, our SGS results should be viewed with caution.

Another explanation for these findings is that the current adult and seedlings generations have developed in different landscape fragmentation contexts. Thus, it is expected that the structure of genetic variation in the adult generation is composed of overlapping successive generations from different gene pools with different histories of

establishment, reflecting a historic situation where habitat fragmentation was less extensive. The seedling life stage, in turn, resulted from the germination of seeds in the current forest fragment. One must consider that this generation was subjected to a selection process and this process excluded inbred or less vigorous individuals. The same could be hypothesized for the progeny arrays, which were germinated in unnatural conditions. Indeed, we observed a low to moderate germination rate (at least 50%, data not shown) for progeny arrays. Given these considerations, the establishment and permanence of seedlings within this population of *C. estrellensis* can be considered a key factor in the replacement of genetic diversity for the species. Nonetheless, within the studied fragment, the small population of *C. estrellensis* showed a senescent demographic structure (data not shown) with a greater proportion of adults of older generations. We also observed a lack of regenerating individuals taller than 2 m, which may be a consequence of habitat fragmentation affecting establishment and/or *C. estrellensis* continuation within the area. This finding, along with the loss of genetic diversity across life stages of *C. estrellensis*, suggests that even recent habitat fragmentation is adversely affecting *C. estrellensis* and could compromise the long-term survival of this small population.

Based on our results, it is necessary to take urgent action to increase gene flow for *C. estrellensis* coupled with management strategies to promote more favorable conditions for the establishment and retention of new generations of seedlings in the habitat. In addition, to develop an efficient *ex situ* conservation strategy for *C. estrellensis*—considering also that rare and unique alleles were found in all life stages—seeds should be collected from all reproductive individuals in this small population because they were not genetically related. In parallel, to ensure that even rare and unique alleles present in non-reproductive trees can also be preserved, the use of cutting techniques such as those developed recently for *C. estrellensis* (Hernandes *et al.*, 2013) is also feasible. Furthermore, collecting information about the number and size of populations throughout the species distribution area and carrying out long-term ecological and genetic studies should be a priority as this information is necessary in designing effective conservation strategies.

DATA ARCHIVING

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.4b18b.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the São Paulo Research Foundation (FAPESP, Grant 2011/08883-3 to ALAM), Conselho Nacional de Pesquisa (CNPq, Grant 470975/20113 to ALAM), Fundação de Apoio ao Ensino, Pesquisa e Assistência do Hospital das Clínicas de Ribeirão Preto (FAEPA to MAM) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Programa de Excelência Acadêmica (CAPES-PROEX). MCG and JMF were supported by FAPESP DS fellowships (2007/04787-4 and 2009/14200-6, respectively). ALAM was supported by a Research Assistantship from CNPq (PDS 150277/2009-1, PV 300140/2011-8). We also thank MLM Freitas (Instituto Florestal de São Paulo/IF-SP), R Ferreira-Ramos (USP/RP), AJ Silva (USP/RP), MAC Reis (Verde Tambaú-SP) for their sampling support, and ER Nimmo for editing the manuscript.

Alzate-Marin AL, Guidugli MC, Soriani HH, Martinez CAH, Mestriner MA (2009). A DNA minipreparation procedure suitable for PCR/SSR and RAPD analyses in tropical forest tree species. *Braz Arch Biol Technol* **52**: 1217–1224.
Austerlitz F, Dick CW, Dutech C, Klein EK, Oddou-Muratorio S, Smouse PE *et al.* (2004). Using genetic markers to estimate the pollen dispersal curve. *Mol Ecol* **13**: 937–954.
Austerlitz F, Smouse PE (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.
Bacles CFE, Jump AS (2011). Taking a tree's perspective on forest fragmentation genetics. *Trends Plant Sci* **16**: 13–18.
Balloux F (2004). Heterozygote excess in small populations and the heterozygote-excess effective population size. *Evolution* **58**: 1891–1900.
Barrett SCH (2010). Understanding plant reproductive diversity. *Philos Trans R Soc Lond Ser B* **365**: 99–109.
Bawa KS, Perry DR, Beach JH (1985). Reproductive biology of tropical lowland rain forest trees. I. Sexual systems and incompatibility mechanisms. *Am J Bot* **72**: 331–345.
Berens DG, Griebeler EM, Braun C, Chituyi BB, Nathan R *et al.* (2013). Changes of effective gene dispersal distances by pollen and seeds across successive life stages in a tropical tree. *Oikos* **122**: 1616–1625.
Brown AHD (1978). Isozymes, plant population genetic structure and genetic conservation. *TAG* **52**: 145–157.
Burczyk J, Adams WT, Moran GF, Griffin AR (2002). Complex patterns of mating revealed in a *Eucalyptus regnans* seed orchard using allozyme markers and the neighbourhood model. *Mol Ecol* **11**: 2379–2391.
Buschbom J, Yanbaev Y, Degen B (2011). Efficient long-distance gene flow into an isolated relict oak stand. *J Hered* **102**: 464–472.
Carvalho FMV, Júnior PDM, Ferreira LG (2009). The Cerrado into-pieces: habitat fragmentation as a function of landscape use in the savannas of central Brazil. *Biol Conserv* **142**: 1392–1403.
Chybicki IJ, Burczyk J (2010). NM+: software implementing parentage-based models for estimating gene dispersal and mating patterns in plants. *Mol Ecol Resour* **10**: 1071–1075.
Cornuet JM, Luikart G (1996). Description and power analysis of two tests for inferring recent population bottlenecks from allele frequency data. *Genetics* **144**: 2001–2014.
Côrtes MC, Uriarte M, Lemes MR, Gribel R, Kress WJ, Smouse PE *et al.* (2013). Low plant density enhances gene dispersal in the Amazonian understory herb *Heliconia acuminata*. *Mol Ecol* **22**: 5716–5729.
Dick CW, Etchelecu G, Austerlitz F (2003). Pollen dispersal of Neotropical trees (*Dinizia excelsa*: Fabaceae) by native insects and african honeybees in pristine and fragmented Amazonian rainforest. *Mol Ecol* **12**: 753–764.
Dick CW, Hardy OJ, Jones FA, Petit RJ (2008). Spatial scales of pollen and seed-Mediated gene flow in tropical rain forest trees. *Trop Plant Biol* **1**: 20–33.
Dow B, Ashley M (1996). Microsatellite analysis of seed dispersal and parentage of saplings in bur oak *Quercus macrocarpa*. *Mol Ecol* **5**: 615–627.
Dow B, Ashley M (1998). High levels of gene flow in bur oak revealed by paternity analysis using microsatellites. *J Hered* **89**: 62–70.
Dubreuil M, Riba M, Gonzalez-Martinez SC, Vendramin GG, Sebastiani F, Mayol M (2010). Genetic effects of chronic habitat fragementation revisited: Strong genetic structure in a temperate tree, *Taxus baccata* (taxaceae), with great dispersal capability. *Am J Bot* **97**: 303–310.
Ellstrand NC (2003). Current knowledge of gene flow in plants. *Philos Trans R Soc Lond Ser B* **358**: 1163–1170.
Ellstrand NC (2014). Is gene flow the most important evolutionary force in plants? *Am J Bot* **101**: 737–753.
Ellstrand NC, Elam DR (1993). Population genetic consequences of small population size: implications for plant conservation. *Annu Rev Ecol Syst* **24**: 217–224.
Finger A, Kaiser-Bunbury CN, Kettle CJ, Valentin T, Ghazoul J (2014). Genetic connectivity of the moth pollinated tree *Glionnetia sericea* in a highly fragmented habitat. *PLoS One* **9**: e111111.
Fuchs EJ, Lobo JA, Quesada M (2003). Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropical dry forest tree *Pachira quinata*. *Conserv Biol* **17**: 149–157.
Gaiotto FA, Grattapaglia D, Vencovksy R (2003). Genetic struture, mating system, and long-distance gene flow in heart of palm (*Euterpe edulis* Mart.). *J Hered* **94**: 399–406.
Gibson L, Lynam AJ, Bradshaw CJA, He F, Bickford DP, Woodruff DS *et al.* (2013). Near-complete extinction of native small mammal fauna 25 years after forest fragmentation. *Science* **341**: 1508–1510.
Gillet E, Hattemer HH (1989). Genetic analysis of isoenzyme phenotypes using single tree progenies. *Heredity* **63**: 135–141.
Goudet J (2002). *FSTAT: a program to estimate and test gene diversities and fixation indices. Version 2.9.3.2*. Available at: http:www2.unil.ch/popgen/softwares/fstat.htm (last accessed 18 December 2015).
Guidugli MC, Campos T, Sousa ACB, Feres JM, Sebbenn AM, Mestriner MA *et al.* (2009). Development and characterization of 15 microsatellite loci for *Cariniana estrellensis* and transferability to *Cariniana legalis*, two endangered tropical tree species. *Conserv Gen* **10**: 1001–1004.
Gusson E, Sebbenn AM, Kageyama PY (2006). Mating system in *Eschweilera ovata* (Cambess.) Miers populations. *Rev Árvore* **30**: 491–503.
Hardy OJ, Vekemans X (2002). SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Mol Ecol Notes* **2**: 618–620.
Hernandez W, Xavier A, Paiva HN, Wendling I (2013). Vegetative propagation of jequitibá-rosa (*Cariniana estrellensis* (Raddi) Kuntze) by cuttings. *Revista Árvore* **37**: 955–967.

Heydel F, Cunze S, Bernhardt-Romermann M, Tackenberg O (2014). Long-distance seed dispersal by wind: disentangling the effects of species traits, vegetation types, vertical turbulence and wind speed. *Ecol Res* **29**: 641–651.

Ismail SA, Ghazoul J, Ravikanth G, Shaanker RU, Kushalappa G, Kettle CJ (2012). Does long-distance pollen dispersal preclude inbreeding in tropical trees? Fragmentation genetics of *Dysoxylum malabaricum* in an agro-forest landscape. *Mol Ecol* **21**: 5484–5496.

Joly CA, Metzger JP, Tabarelli M (2014). Experiences from the Brazilian Atlantic Forest: ecological findings and conservation initiatives. *New Phytol* **204**: 459–473.

Jump AS, Peñuelas J (2006). Genetic effects of chronic habitat fragmentation in a wind-pollinated tree. *Proc Natl Acad Sci USA* **103**: 8096–8100.

Kalinowski ST (2005). HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Mol Ecol Notes* **5**: 187–189.

Kalinowski ST, Taper ML (2006). Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. *Conserv Genet* **7**: 991–995.

Kalinowski ST, Taper ML, Marshall TC (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol* **16**: 1099–1106.

Kettle CJ, Hollingsworth PM, Jaffré T, Moran B, Ennos RA (2007). Identifying the early genetic consequences of habitat degradation in a highly threatened tropical conifer, *Araucaria nemorosa* Laubenfels. *Mol Ecol* **16**: 3581–3591.

Klein E, Lavigne C, Gouyon P (2006). Mixing of propagules from discrete sources at long distance: comparing a dispersal tail to an exponential. *BMC Ecol* **6**: 3.

Kotchetkoff-Henriques O (2003). Characterization of the Natural Vegetation in Ribeirão Preto, SP: Bases for Conservation. DSc thesis, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto. Available at: http://www.bv.fapesp.br/pt/dissertacoes-teses/6724/caracterizacao-vegetacao-natural-ribeirao-preto/ (last accessed 16 September 2015).

Kramer AT, Ison JL, Ashley MV, Howe HF (2008). The paradox of forest fragmentation genetics. *Conserv Biol* **22**: 878–885.

Laurance WF, Camargo JLC, Luizão RCC, Laurance SG, Pimm SL, Bruna EM *et al.* (2011). The fate of Amazonian forest fragments: a 32-year investigation. *Biol Conserv* **144**: 56–67.

Laurance WF, Useche DC, Rendeiro J, Kalka M, Bradshaw CJA, Sloan SP *et al.* (2012). Averting biodiversity collapse in tropical forest protected areas. *Nature* **489**: 290–294.

Leimu R, Mutikainen P, Koricheva J, Fischer M (2006). How general are positive relationships between plant population size, fitness and genetic variation? *J Ecol* **94**: 942–952.

Leite EJ (2007). State-of-knowledge on *Cariniana estrellensis* (Raddi) Kuntze (Lecythidaceae) for genetic conservation in Brazil. *Res J Bot* **2**: 138–160.

Levin DA (1988). The paternity pool plants. *Am Nat* **132**: 309–317.

Loiselle BA, Sork VL, Nason J, Graham C (1995). Spatial genetic structure of a tropical understory shurb, *Psychotria officinalis* (Rubiaceae). *Am J Bot* **82**: 1420–1425.

Lowe AJ, Boshier D, Ward D, Bacles CFE, Navarro C (2005). Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for Neotropical trees. *Heredity* **95**: 256–273.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol* **7**: 639–655.

Mori SA, Smith NP, Cornejo X, Prance GT (2010). *The Lecythidaceae Pages*. The New York Botanical Garden: New York, NY, USA. Available at: http://sweetgum.nybg.org/lp/index.php (last accessed in 25 April 2015).

Murawski DA, Hamrick JL (1991). The effect of the density of flowering individuals on the mating systems of nine tropical tree species. *Heredity* **67**: 167–174.

Nakanish A, Tomaru N, Yoshimaru H, Manabe T, Yamamoto S (2009). Effects of seed- and pollen-mediated gene dispersal on genetic structure among *Quercus salicina* saplings. *Heredity* **102**: 182–189.

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

Nason JD, Herre EA, Hamrick JL (1998). The breeding structure of a tropical keystone plant resource. *Nature* **391**: 685–687.

Nazareno AG, Carvalho D (2009). What the reasons for no inbreeding and high genetic diversity of the neotropical fig tree *Ficus arpazusa*? *Conserv Genet* **10**: 1789–1793.

Nazareno AG, Jump AS (2012). Species-genetic diversity correlations in habitat fragmentation can be biased by small sample sizes. *Mol Ecol* **21**: 2847–2849.

Ndiade-Bourobou D, Hardy OJ, Favreau B, Moussavou H, Nzengue E *et al.* (2010). Long-distance seed and pollen dispersal inferred from spatial genetic structure in the very low-density rainforest tree, *Baillonella toxisperma* Pierre, in central Africa. *Mol Ecol* **19**: 4949–4962.

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

Nunes YRF, Petrere M (2012). Structure and dynamics of a *Cariniana estrellensis* (Lecythidaceae) population in a fragment of Atlantic Forest in Minas Gerais, Brazil. *Rodrigués*ia **63**: 257–267.

O'Malley DM, Buckley DP, Prance GT, Bawa KS (1988). Genetics of Brazil nut (*Bertholletia excelsa* Humb. & Bonpl. Lecythidaceae), 2. Mating system. *Theor Appl Genet* **76**: 929–932.

Oddou-Muratorio S, Klein E, Austerlitz F (2005). Pollen flow in the wildservice tree, *Sorbus torminalis* (L.) Crantz. II. Pollen dispersal and heterogeneity in mating success inferred from parent–offspring analysis. *Mol Ecol* **14**: 4441–4452.

Oliveira-Filho AT, Galetti M (1996). Seed predation of *Cariniana estrellensis* (Lecythidaceae) by black howler monkeys, *Alouatta caraya*. *Primates* **37**: 87–90.

Ottewell K, Grey E, Castillo F, Karubian J (2012). The pollen dispersal kernel and mating system of an insect-pollinated tropical palm, *Oenocarpus bataua*. *Heredity* **109**: 332–339.

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

Prance GT, Mori S (1979). A Lecythidaceae-Part I: The Actinomorphic-Flowered New World Lecythidaceae (Asteranthos, Gustavia, Grias, Allantoma, & Cariniana). *Flora Neotrop* **21**: 1–272.

Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM (2009). The Brazilian Atlantic Forest: how much is left, and how is the remaining forest distributed? Implications for conservation. *Biol Conserv* **142**: 1141–1153.

Rice WR (1989). Analyzing tables of statistical tests. *Evolution* **43**: 223–225.

Ritland K (1989). Correlated matings in the partial selfer, *Mimulus guttatus*. *Evolution* **43**: 848–859.

Ritland K (2008). *Multilocus Mating System Program—MLTR version 3.2*. University of British Columbia: Vancouver, BC, Canada. Available at: http://genetics.forestry.ubc.ca/ritland/programs (last accessed 11 June 2012).

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

Saro I, Robledo-Arnuncio JJ, Gonzáles-Pérez MA, Sosa PA (2014). Patterns of pollen dispersal in a small population of the Canarian endemic palm (*Phoenix canariensis*). *Heredity* **113**: 215–223.

Sato T, Isagi Y, Sakio H, Osumi K, Goto S (2006). Effect of gene flow on spatial genetic structure in the riparian canopy tree *Cercidiphyllum japonicum* revealed by microsatellite analysis. *Heredity* **96**: 79–84.

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

Smouse PE, Dyer RJ, Westfall RD, Sork VL (2001). Two-generation analysis of pollen flow across a landscape. I. Male gamete heterogeneity among females. *Evolution* **55**: 260–271.

Smouse PE, Sork VL (2004). Measuring pollen flow in forest trees: an exposition of alternative approaches. *For Ecol Manage* **197**: 21–38.

Sork VL, Davis FW, Smouse PE, Apsit VJ, Dyer RJ *et al.* (2002). Pollen movement in declining populations of Califonia Valley oak, *Quercus lobata*: where have all the fathers gone? *Mol Ecol* **11**: 1657–1668.

Tambarussi EV (2013). Contemporary gene flow, mating system, and spatial genetic structure in a Jequitibá-rosa (*Cariniana legalis* Mart. Kuntze) fragmented population by microsatellite markers. Thesis—Esalq, Universidade de São Paulo.

Toledo Filho DV, Bertoni JE, Batista EA, Parente PR (2000). Fitossociologia de um fragmento Florestal à margem do Rio do Peixe, Município de Lindóia (SP). *Rev Inst Flor* **12**: 37–45.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004). Microchecker: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* **4**: 535–538.

Vekemans X, Hardy O (2004). New insights from fine-scale spatial genetic structure analyses in plant populations. *Mol Ecol* **13**: 921–935.

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

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

Zhang Z, Shi M-M, Shen D-W, Chen X-Y (2012). Habitat loss other than fragmentation per se decreased nuclear and chloroplast genetic diversity in a monoecious tree. *PLoS One* **7**: e39146.

Supplementary Information accompanies this paper on Heredity website (http://www.nature.com/hdy)