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Genetic effects of selective logging and pollen gene flow in a low-density population of the dioecious tropical tree *Bagassa guianensis* in the Brazilian Amazon

Marivana Borges Silva [a], Milton Kanashiro [b], Ana Yamaguishi Ciampi [c], Ian Thompson [b], Alexandre Magno Sebbenn [d,*]

[a] *Universidade Federal do Pará, Campus Bragança, Rua Leandro Ribeiro s/n, Bairro Aldeia, CEP68600-000 Bragança-PA, Brazil*
[b] *Embrapa Amazônia Oriental, C.P. 48, 66095-100 Belém-PA, Brazil*
[c] *Embrapa Recursos Genéticos e Biotecnologia, C.P. 02372, 70770-900 Brasília-DF, Brazil*
[d] *Instituto Florestal de São Paulo, CP 1322, São Paulo, SP 01059-970, Brazil*

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Abstract

Forest logging reduces population density and increases the distance between co-specifics and so can cause the loss of alleles, and affect the genetic diversity, spatial genetic structure (SGS), mating system, and pollen flow of the population. These factors were studied in the tropical tree species *Bagassa guianensis* Aubl. occurring in a low-density population in the Brazilian Amazon forest. Genetic diversity was compared among offspring, juveniles and adult trees, before and after selective logging. Non-significant differences were observed between these samples. The harvest of 61% of the adult trees caused the loss of three alleles in the reproductive population. However, these alleles were present in juveniles and offspring and so were not lost from the population. SGS was detected up to 300 m before logging in the overall and adult populations. After logging, significant SGS was not observed. Deviations from random matings were evident throughout biparental inbreeding ($1 - \hat{t}_s = 0.067$, $P < 0.05$), correlated mating ($\hat{r}_{p(m)} = 0.193$, $P < 0.05$), and differentiation in pollen gene pool ($\hat{\Phi}_{ft} = 0.081$, $P < 0.05$). The effective number of pollen donors was estimated as 5–7 trees. The distance of pollen gene dispersal was estimated as 308–961 m, depending on the dispersal model used (normal and exponential) and assumed population density. The estimated neighbourhood pollination area (A_{ep}) ranges from 81 to 812 ha, depending also on the assumed population density. Reproduction by obligatory outcrossing, pollen immigration from trees outside of the plot, the long-pollen dispersal distance, and large A_{ep} suggested that the species can be resilient to the impacts of logging.

Keywords: Tatajuba; Tropical tree species; Dioecious species; Correlated matings; TwoGener analysis

1. Introduction

Sustainable timber management is an important issue in the Brazilian Amazon (Silva et al., 1995). In the Brazilian Amazon forest, selective harvesting of timber has increased in frequency and extent (Asner et al., 2005). Prerequisites for sustainable timber management are information on growth and yield for different management regimes and silvicultural options (Silva et al., 1995). However, population adaptability, productivity and evolutionary potential for sustaining forest health depend on the levels of genetic diversity of the species population (Rajora et al., 2000; Mosseler et al., 2003). Such aspects have received little attention in logging plans of rich Amazon forest. Selective logging of forest trees has a direct impact on the demography and genetics of exploited tree populations. Selective logging removes large individuals and their genes that probably contribute more to reproduction then smaller individuals, reduces the overall population density of reproductive trees and increases the distance between reproductive cospecifics (Jennings et al., 2001; Lowe et al., 2005; Cloutier et al., 2007). Additionally, selective harvesting of the best stem form and fast growing trees (negative phenotypic selection) can led to deterioration in the genetic quality of populations (dysgenic selection). Forest logging may affect also the abundance, diversity and behaviour of animal pollinators that in turn can have an impact on the reproductive biology of the

* Corresponding author. Tel.: +55 19 34351681.
E-mail address: alexandre.sebbenn@pq.cnpq.br (A.M. Sebbenn).

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remaining tree population (Cloutier et al., 2007). Such impacts can cause the loss of specific genes, alterations in the frequency of genes, and can induce changes in the patterns of genetic diversity (such as heterozygosity), mating system, inbreeding, gene flow, and effective size of the population (Jennings et al., 2001; Hawley et al., 2005; Lowe et al., 2005; Cloutier et al., 2007). Some studies comparing unharvested and harvested populations already reported the negative effect of forest harvesting on genetic structure and mating system of tree populations, as loss of alleles (Buchert et al., 1997; Rajora et al., 2000; Hawley et al., 2005; Degen et al., 2006; Lacerda et al., 2008; Sebbenn et al., 2008) and increase in self-fertilization in logged populations (Murawski et al., 1994; Obayashi et al., 2002; Ledig et al., 2005). However, heterozygosities, in general, have been little impacted by selective logging due to their low sensitivity in response to the observed levels of reduction in population size (Rajora et al., 2000; Degen et al., 2006; Lacerda et al., 2008; Sebbenn et al., 2008). Thus, removal of individuals and their genes from stands, reductions in reproductive population size, increase in the distance between cospecífics, and increase in the inbreeding resulting from selective logging may pose significant threats to the long-term viability of harvested tree populations (Hawley et al., 2005; Lowe et al., 2005; Cloutier et al., 2007).

The way trees are spatially structured and their pollination mechanisms are determining aspects of the mating system and the way individuals recombine their genes in successive reproductive events and these have strong effects on the genetic characteristics of the populations (Nason and Hamrick, 1997). Pollen is the dominant vector of gene flow in many temperate tree species (Dyer and Sork, 2001) and probably in many tropical tree species too. Thus, it is important to understand what factors influence the process of gene movement among and within populations in species submitted to forest logging. The low-density population, characteristic of many tropical tree species can favour the heterogeneity in pollen gene pool within populations. Studies in many tropical tree species occurring in low-density populations suggest that trees receive pollen over larger distances (Nason and Hamrick, 1997; Nason et al., 1998; White et al., 2002; Latouche-Hallé et al., 2004; Kenta et al., 2004; Lowe et al., 2005). However, in dioecious tree species the reduction in the population density by fragmentation can be expected to reduce the pollen flow between individuals within fragmented populations (Nason and Hamrick, 1997), and probably also in logged populations. In these species, in low-density logged population, as only one part of the reproductive population produces pollen, some neighbourhoods can be favoured by high pollen diversity and others disfavoured. In forest timber management, this information can help to determine the intensity and distribution of logging in order to preserve reproductive patterns.

Bagassa guianensis Aubl. (Moraceae) is a dioecious insect pollinated tropical tree species whish occurs from the Brazilian Amazon to Guyana (Veja, 1976). Presently, this population is intensively exploited by timber companies due the great quality of the wood (Tomazello-Filho et al., 1983). The species is classified as a pioneer species presenting annual flowering in natural populations. The population density is very low (0.2 trees/ha) and trees can reach a diameter (dbh, diameter at breat height) of 190 cm. These characteristics make the species an interesting model to study the effect of forest logging on the levels of genetic diversity. This is the main goal of this study. Thus, we ask the following questions: (i) Are there differences in genetic struture among different ontogenic stages of the population? (ii) Can selective logging reduce the number of alleles and genetic diversity? (iii) How is the spatial genetic distribution of the genotypes in the population before and after logging? (iv) As the species is dioecious, is mating random? (v) How is the pollen gene pool of different seed-trees structured? (vi) What are the distance of pollen flow and the effective neighbourhood pollination area?

2. Materials and methods

2.1. The study site

The study area is a 500 ha experimental plot (Fig. 1) located alongside highway BR-163 (km 83), in the Tapajós National Forest (2° 51′S, 54° 57′W, and 175 m above sea level), 83 km south of Santarém, in the State of Pará, Brazil (Kanashiro et al., 2002). This plot is an Intensive Study Plot (*ISP*) for ecological and genetic investigation before and after Reduced Impact Logging (RIL) established by the Dendrogene project (Embrapa Amazônia Oriental/DFID). The area is located in the Belterra Plateau and has a humid tropical climate (*Ami* under the Köppen system), with an annual rainfall of 1900–2110 mm, a rainy season from December to May, and mean monthly temperatures between 24 and 26 °C throughout the

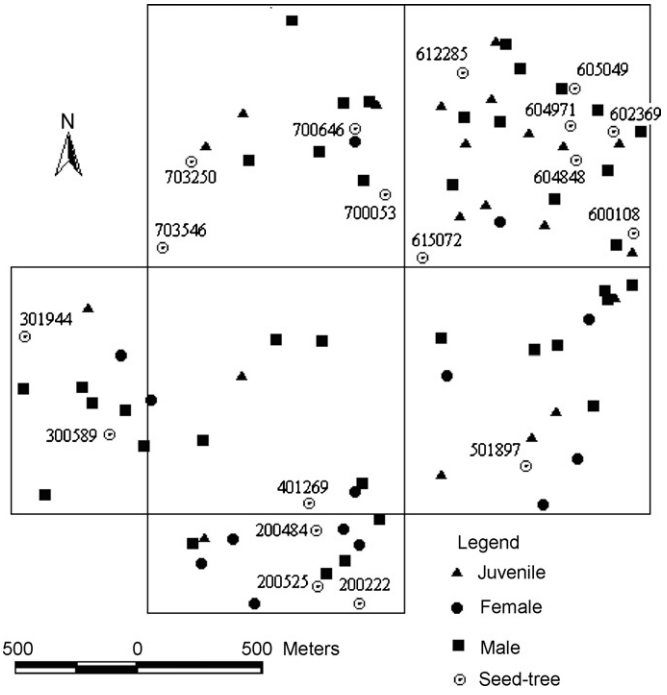

Fig. 1. Spatial distribution of *B. guianensis* trees with dbh > 10 cm before the harvesting in the 500 ha experimental plot in Tapajós National Forest (Flona Tapajós).

year. The area is covered by a luxurious tropical forest classified as *terra firme* Dense Ombrophilous Forest. The canopy reaches up to 60 m or more with several but not uniform strata; the vegetation is usually very dense and several palm trees species and shrubs commonly occupy the understory. The site has many species in common with the rest of the region. The Dendrogene project monitored the study area from 2001 to 2004. In the area, there were 71 *B. guianensis* reproductive trees with dbh $\geq$ 37 cm (37–197 cm), where 33 were females and 38 were males (1:1.19), and 21 juveniles with dbh $\geq$ 18 cm (18–36 cm). The density of reproductive trees before the logging was 0.142 trees/ha, where female-density was 0.066 and male was 0.076. In 2003 (August to December) a timber company logged the area following RIL regulations. During logging, 43 trees (61%) with dbh $\geq$ 62 cm (62–197 cm) were harvested, reducing the reproductive population density to 0.056 trees/ha (37–132 cm), resulting in female density of 0.026 and male of 0.03. The sexual ratio after logging was reduced to 13 female trees and 15 males (1:1.15).

2.2. Sampling

Cambium samples were collected in all individuals of the experimental plot (92 trees with dbh $\geq$ 18 cm). To study the mating system and pollen flow, 490 seeds were collected in 2003 from open-pollination in 18 seed-trees (average of 27 seeds/seed-tree) before logging. The average distance between seed-trees was 1322 m and the distance of the males from these seed-trees ranged from 57 to 2685 m, with an average of 1143 m (standard deviation of 689 m). After collection, the seeds were planted in nurseries at the Embrapa Research Station in Belterra, located approximately 50 km from the study area. The methodology used for DNA sampling following Ciampi et al. (2000) and Silva (2005): cambium samples were extracted and stored in *eppendorf* tubes containing buffer solution (CTAB buffer (1/3) and ethanol (2/3)) and conditioned on ice for subsequent storage (at $-20\,^{\circ}$C); leaves from offspring (seeds) were collected and stored in tubes containing silica-gel for drying. Samples were collected and sent for DNA extraction to the Laboratory for Genetic Research at Embrapa's Genetic Centre (Cenargen) in Brasília.

2.3. DNA extraction

The DNA extraction from cambium and leaf followed the method CTAB 2% (Ferreira and Grattapaglia, 1998), adapted to a *Fastprep* machine (BIO 101 SAVANT), to maceration. DNA quantification was conducted by comparison of standard DNA (λ DNA) in 1% agarose gel, using ethidium bromide. The DNA was diluted in 1.0 ng/μl.

2.4. Microsatellite analysis

The microsatellite analysis was conducted using six microsatellite loci of *B. guianensis*. The inheritance analysis of these loci showed a perfect 1:1 Mendelian segregation ratio. The primers were isolated and characterized by Vinson et al. (2005), but used in this study in a different form, as tail primers (Oetting et al., 1995), as these are long primers (about 40 pb) they were again optimized for amplification by PCR. Three kinds of extensions (tail) were alternatively used to synthesize the tail primers. In the same way, three kinds of marking (HEX, NED and 6-FAM) were made in the marked primers. The tail primer, the reverse and the marker with fluorocromo were used for amplification of each of the loci. The reactions have 13 μl of final volume, with genomic DNA (4 ng), PCR reaction buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$) (1×), forward and reverse primers (0.23 μM of each of three), BSA (Bovine Serum Albumin, Biolabs New England) (0.25 mg/ml), dNTP (0.25 mM) and Taq polymerase (Invitrogen, Life Technologies) (1.3 U). A procedure of hot start was used in each PCR reaction, conducted under the following conditions: 30 cycles of 94 °C per 1 min, 52–58 °C per 1 min (depending on the locus used), and 72 °C per 1 min. After 30 cycles, a final phase of alongment of 30 min at 72 °C was added. The amplification of six loci was done in separate PCR reactions, using, while possible, the multiplex system in the moment of allele detection by fluorescence, in automated sequences ABI 377 from PerkinElmer. The data were collected by detection of different fluorescence and analysed using the software GeneScan, which permits automatized allele genotyping, comparing with the values of an internal marker developed for the lab and imported for software Genotyper for filtering, interpretation and final compilation of data.

2.5. Genetic diversity analysis

In order to characterize the temporal genetic structure and to compare the genetic diversity in unharvested and harvested populations, standard genetic diversity indexes were estimated for overall population (juveniles and adults), adults, juveniles, offspring, unharvested adult trees, and harvested trees. The calculated indexes were: overall number of alleles over loci (k), average number of alleles per locus (A), observed heterozygosity (H_o), expected heterozygosity under Hardy-Weinberg equilibrium (H_e), and effective number of alleles per locus ($\hat{A}_e = 1/(1-\hat{H}_e)$). The fixation index ($F$) was calculated as: $\hat{F} = 1 - (\hat{H}_o/\hat{H}_e)$. Statistical significance for the F index was tested using a Bootstrap method (10,000 resembling). All indices cited were calculated using FSTAT program (Goudet, 2002). Also the overall paternity probability of the first [$Pr(Ex_1)$] and second parent [$Pr(Ex_2)$] was estimated for the population, using the program CERVUS 3.0 (Kalinowski et al., 2007).

For the evaluation of the effects of logging on alleles of different frequencies, we classified alleles in three frequency classes: high ($P > 0.25$), low ($0.25 > P \geq 0.01$), and rare ($P < 0.01$). We also highlighted the exclusives alleles and the number of alleles that disappeared from the reproductive and overall population due to the harvest. These methods were applied for overall population (juveniles and adults), adults, offspring, juveniles, unharvested adults, and harvested trees. The gene frequencies were estimated using FSTAT program (Goudet, 2002).

2.6. Spatial genetic structure analysis

To understand the effects of logging on spatial genetic structure (SGS), we performed the analysis for the overall population (juveniles and adults together) before and after logging and reproductive adults before logging. This analysis was not run in adult trees after logging due to the small resulting sample size (28 adult trees). SGS was analysed by the estimate of the average coancestry coefficient (θ_{xy}) between all pairwise individuals into eight distance classes of 300 m. The distance classes were determined to have at least 30 pairs of individuals within each class. The coancestry coefficients were estimated using the estimator of Loiselle et al. (1995). To visualize SGS, θ_{xy} values were averaged over a set of distance classes, and plotted against the distances (classes of 300 m up to 2400 m). In order to test whether there was significant deviation from SGS, the 95% confidence interval was calculated for each observed value and each distance class from 1000 permutation of individuals among locations. The confidence interval was used to construct a coancestry graphic. Coancestry coefficient and confidence interval were calculated using the program SPAGeDi version 1.1b (Hardy and Vekemans, 2002).

2.7. Mating system analysis

Mating system was analysed under the mixed-mating model and correlated mating model using the software Multilocus MLTR version 3.1 (Ritland, 2004). The calculated parameters were multilocus outcrossing rate (t_m); single-locus outcrossing rate (t_s); mating among relatives rate ($t_m - t_s$) and; multilocus paternity correlation ($r_{p(m)}$). Additionally, as *B. guianensis* is a dioecious species, we also estimated the effective biparental inbreeding rate as $1 - t_s$. In this case, the difference between the unity and the multilocus outcrossing rate also occurs due the biparental inbreeding. The analysis was performed on level of population (using Newton-Raphson numerical method). The 95% confidence interval of the parameters was calculated from 500 bootstraps, using families as sample unity. The effective number of pollen donors was calculated as: $\hat{N}_{ep} = 1/\hat{r}_{p(m)}$. To characterize the genetic structure within families the average coancestry coefficient (Θ_{xy}) within families was estimated as (Sousa et al., 2005):

$$\hat{\Theta}_{xy} = 0.125(1 + \hat{F}_p)(1 - \hat{r}_{p(m)}),$$

where F_p is the inbreeding coefficient in the parental generation. Additionally, the average variance effective size ($\hat{N}_{e(v)}$) was estimated using Cockerham (1969) estimator and assuming that an infinite number of seeds were collected in each seed-tree:

$$\hat{N}_{e(v)} = \frac{0.5}{\hat{\Theta}_{xy}}.$$

The number of seed-trees (m) necessary to collect seeds, aiming to retain the reference effective population size ($N_{(e)reference}$) of 150 was calculated following Sebbenn (2003):

$$\hat{m} = \frac{N_{(e)reference}}{\hat{N}_{e(v)}}.$$

2.8. TwoGener analysis

Pollen dispersal distance (δ) was investigated using the TwoGener approach (Austerlitz and Smouse, 2001a; Smouse et al., 2001), using genotypes of progeny arrays, seed-trees genotypes and respective spatial position, and genotypes of male reproductive adult trees (due the fact that the species is dioecious). The TwoGener analyses were conducted using the Poldisp 1.0 program (Robledo-Arnucio et al., 2007). The program estimates the differentiation in pollen gene pool within the set of crossed pollen (Φ_{ft}) among different seed-trees, using analysis of molecular variance (Amova). The 95% confidence level of the global Φ_{ft} among all seed-trees was estimated by permutation males gametes among seed-donors (1000 times). Following the suggestion of Smouse et al. (2001), the parameter Φ_{ft} was used to estimate the effective number of pollen donors: $\hat{N}_{ep} = 1/2\hat{\Phi}_{ft}$. To estimate δ, we assumed two dispersal models: a bivariate normal model and an exponential model (Austerlitz and Smouse, 2001a). Pollen dispersal distance can be calculated from global Φ_{ft} and pairwise Φ_{ft} between all seed-trees, jointly with the effective density reproductive population (d_e). Our joint estimated produced unrealistic values for both normal bivariate and exponential models (δ = 47 m for normal bivariate and 52 m for exponential dispersal function), considering that the minimum distance between a male tree and a sampled seed-tree was 57 m. Thus, we have not presented these estimates. Additionally, for simplicity, we used only the pairwise estimate, as the global estimate showed values not statistically different to those of the pairwise estimate. How the relationship between the effective number of reproductive trees and the number sense of reproductive trees (N_e/N) in a population can range from about one to 1/10, due to the variation in fertility and nonsynchronous flowering (Frankhan, 1995), following the method used by Cloutier et al. (2007), we estimated δ from Φ_{ft} for two contrasting population densities. The observed density of reproductive male trees in the experimental plot (0.076 trees/ha) was used as an upper limit estimate of density of reproductive trees (d_{max}) and 1/10 (0.0076 trees/ha), as the lower limit of density of reproductive trees ($d_{min} = d_{max}/10$). Effective neighbourhood pollination area (A_{ep}) was calculated as: $\hat{A}_{ep} = 4\pi\hat{\sigma}^2 = \hat{N}_{ep}/d$ (Austerlitz and Smouse, 2001a).

3. Results

3.1. Genetic diversity and fixation index

The overall number of alleles (k) over six study loci ranged from 31 in juveniles to 44 in offspring (Table 1), indicating low levels of polymorphism for microsatellite loci. The overall paternity exclusion probabilities over six SSR loci of the first

Table 1
Genetic parameters for *B. guianensis* in different sample populations

Sample	n	k	$\hat{A}$	$\hat{A}_e$	$\hat{H}_e$	$\hat{H}_o$	$\hat{F}$
Overall population	92	37	6.17 (0.40)	2.70 (0.22)	0.630 (0.031)	0.659 (0.042)	−0.047 (0.038)
Adults	71	36	6.00 (0.31)	2.71 (0.32)	0.632 (0.036)	0.663 (0.066)	−0.050 (0.096)
Offspring	490	44	7.33 (0.49)	2.58 (0.26)	0.612 (0.038)	0.599 (0.049)	0.022 (0.045)
Juveniles	21	31	5.17 (0.84)	2.66 (0.16)	0.624 (0.025)	0.646 (0.024)	−0.036 (0.050)
Unharvested adults	28	33	5.50 (0.40)	2.65 (0.22)	0.622 (0.031)	0.705 (0.050)	−0.136 (0.051)
Harvested	43	32	5.33 (0.50)	2.71 (0.22)	0.631 (0.030)	0.636 (0.043)	−0.008 (0.037)

Sample size (n); overall number of alleles (k); average number of alleles per locus (A); effective number of alleles per locus (A_e); observed heterozygosity (H_o); expected heterozygosity (H_e); fixation index (F).

and second parent were 0.7643 and 0.9345, respectively. Offspring have seven exclusive alleles that were not present in juveniles and adult trees, suggesting pollen and/or seed immigration from outside the plot. Similar to k pattern, juveniles have also the smallest average number of alleles per locus (A) and offspring the highest. However, offspring have a smaller effective number of alleles (A_e) than adults, indicating that, although offspring have more alleles, many of these alleles have low frequency. Adults showed highest A_e, expected (H_e) and observed heterozygosity (H_o), followed by juveniles, and then offspring, but only A_e was significantly different between adults and offspring ($P < 0.05$). The fixation index (F) was low and not significantly different from zero ($P > 0.05$) for all samples, indicating that the gene and genotypic frequencies of the population fit to Hardy-Weinberg expectations.

According to the overall number of alleles over loci, harvesting of 61% of the adult population caused the loss of three alleles (8.3%). All the lost alleles have low frequency (0.008–0.037) and one other common allele changed its frequency to low frequency (Table 2). However, the alleles lost from the adult reproductive population were not lost from the overall population. The three alleles were found in the juvenile and offspring stage, where they have low frequency (0.029–0.071). Forest logging also reduces A, A_e, and H_e, although increases H_o (Table 1). However, these differences were not significant ($P > 0.05$). In contrast, the fixation index was significantly lower in adults after logging than adults before logging.

3.2. Spatial genetic structure

The fine-scale of spatial genetic structure analysis in overall and reproductive populations before logging detected significant ($P < 0.05$) structuring at distances up to 300 m (Fig. 2). The coancestry coefficient in the first distance class was about that expected between cousins (0.0625). However, after logging, overall population did not show SGS, suggesting that logging ruptures the previous spatial structure.

3.3. Mating system

Multilocus and single-locus outcrossing rates were high (>0.9), but significantly different from unity ($P < 0.05$, Table 3). Differences among multilocus and single-locus outcrossing rates ($\hat{t}_m - \hat{t}_s = 0.052$) and single-locus and unity ($1 - \hat{t}_s = 0.067$) were significant different from zero ($P < 0.05$), suggesting mating among relatives. The estimate of paternity correlation was significantly different from zero ($\hat{r}_{p(m)} = 0.193$, $P < 0.05$), indicating that part of the offspring are full-sibs and that there is a restricted effective number of pollen donors mating with each seed-tree ($\hat{N}_{ep} = 5.18$). The coancestry coefficient within families was higher than expected in half-sib families ($\Theta_{xy} = 0.125$), resulting in the variance effective size lower than expected in panmitic populations ($N_{e(v)} = 4$). From the variance effective size, it was determined that to retain the effective size of 150 in seed sample collections, it is necessary to collect seeds from at least 45 seed-trees.

3.4. TwoGener analysis

The differentiation among pollen gene pools ($\hat{\Phi}_{ft} = 0.081$) was significant different from zero ($P < 0.05$), indicating that seed-trees received different pollen pools (Table 3). The number of pollen donors, estimated from Φ_{ft} ($\hat{N}_{ep} = 6.17$) was higher than estimated from $r_{p(m)}$, but not statistically different ($P > 0.05$), confirming that a low number of pollen donors effective sired the seed-trees. The pollen dispersal distance estimated from pairwise Φ_{ft} ranges from 308 to 844 m for the bivariate normal and from 347 to 961 m for an exponential model, suggesting long-pollen dispersal distance. The average neighbourhood pollination area was 81 ha for maximum

Table 2
Number of alleles per classes of frequency, exclusivity and loss of alleles in different ontogenic stages, reproductive and pre- and post-logging sub-populations of *B. guianensis*

Allele frequency class	Population	Adults	Offspring	Juveniles	Unharvested adults	Harvested
Total number of alleles	37	36	44	31	33	32
Common $P > 0.25$	9 (24%)	10 (28%)	10 (23%)	8 (26%)	9 (27%)	9 (28%)
Low $0.25 \geq P > 0.01$	25 (68%)	20 (56%)	20 (45%)	23 (74%)	24 (73%)	23 (72%)
Rare $P \leq 0.01$	3 (8%)	6 (17%)	14 (32%)	0 (0%)	0 (0%)	0 (0%)

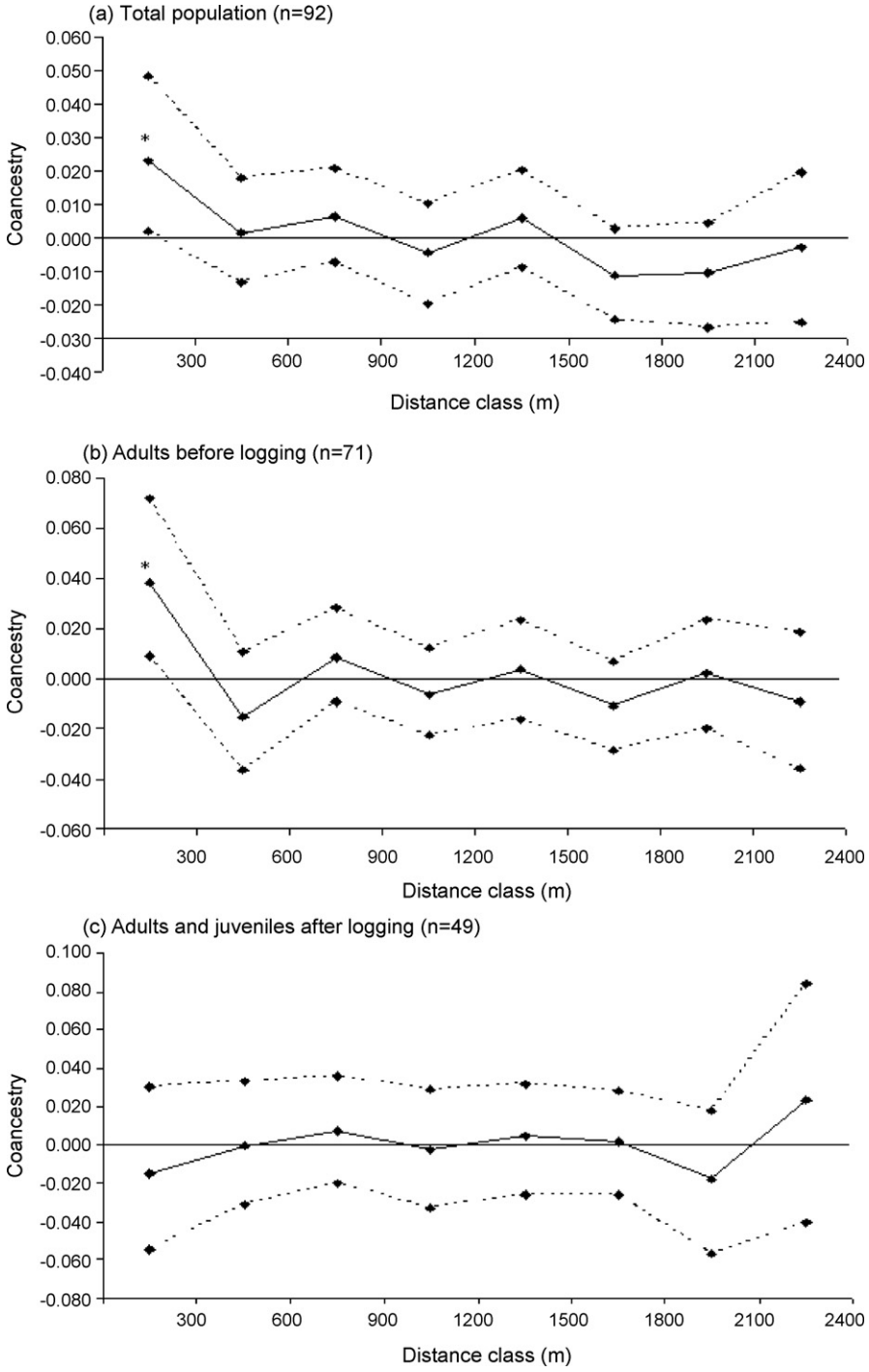

Fig. 2. Coefficient of coancestry (θ_{xy}) between pairwise individual of *B. guianensis* for eight distance classes: (a) overall population before logging; (b) reproductive population before logging; (c) overall population after logging, (dashed lines indicate the limits of error with a confidence interval at 95% of probability; continuous line is the coancestry coefficient; $^{*}P < 0.05$).

density and 812 ha for minimum reproductive population density.

4. Discussion

4.1. Genetic diversity

The results of the six used microsatellite loci showed low levels of genetic diversity for the population. The cause of the low genetic diversity in this species is probably associated to the low number of alleles detected in the six SSR loci (ranged from 5 to 10 alleles per locus), the low-density of the populations (<1 tree/ha) and the small size of the species (the species is endemic, occurring only in a part of Amazon). Population of small number of individuals of endemic species are expect to present low levels of genetic diversity than species with ocurr in high density populations and present widespread distribution (Hamrick and Godt, 1990).

Table 3
Estimate of mating system and TwoGener parameters in *B. guianensis*

Parameter	Estimate (95% confidence interval)
Mating system	
Multilocus crossing rate: t_m	0.985 (0.979–0.991)
Single-locus crossing rate: t_s	0.933 (0.915–0.951)
Mating among relatives rate: $\hat{t}_m - \hat{t}_s$	0.052 (0.035–0.069)
Effective mating among relatives rate: $1 - \hat{t}_s$	0.067 (0.049–0.085)
Multilocus paternity correlation: $r_{p(m)}$	0.193 (0.182–0.204)
Effective number of pollen donors: $\hat{N}_{ep} = 1/\hat{r}_{p(m)}$	5.18 (4.90–5.49)
Coancestry coefficient within families: $\hat{\Theta}_{xy} = 0.125(1 + \hat{F}_p)(1 + \hat{r}_{p(m)})$	0.149 (0.148–0.151)
Variance effective size: $\hat{N}_{e(v)} = 0.5/\hat{\Theta}_{xy}$	3.35 (3.32–3.38)
Number of seed-trees for retain the $N_{e(reference)} = 150$: $\hat{m} = 150/\hat{N}_{e(v)}$	45 (44–46)
TwoGener analysis	
Differentiation in pollen gene pool: Φ_{ft}	0.081 (0.024–0.123)
Effective number of pollen donors: $\hat{N}_{ep} = 1/2\hat{\Phi}_{fp}$	6.17 (4.07–20.83)
Effective neighbourhood pollination area $\hat{A}_{ep} = 4\pi\hat{\sigma} = \hat{N}_{ep}/d_{max}$	81 (53–274)
Effective neighbourhood pollination area $\hat{A}_{ep} = 4\pi\hat{\sigma} = \hat{N}_{ep}/d_{min}$	812 (535–2741)

d_{max} = 0.076 trees/ha; d_{min} = 0.0076 trees/ha.

4.2. Effects of forest logging

Our results show that the RIL affects the gene pool of the reproductive population. The harvesting of 61% of the reproductive adult population caused the loss of three low frequency alleles (8.3%) and changed gene frequency of an allele from the common class to low frequency (Table 2). However, the alleles lost from the adult reproductive population were present in juvenile and offspring and these alleles not will be lost from the population. Juveniles can put back to the population these alleles when they reach the reproductive stage, if these individuals do not die due to the logging or other causes subsequently. Still, gene flow from pollen and seeds from the surrounding forests can also put back these alleles. However, the surrounding forests were also submitted to RIL and there is the possibility that trees with these alleles have been cut. In addition, during logging, 15–25% of the remaining trees can die due to damage caused by the harvesting process (Degen et al., 2006; Sebbenn et al., 2008) and these and other rare alleles can be lost or have their frequency reduced. Another aspect is the relationship between the time for juveniles to become reproductive and the time before the second cutting cycle is applied. *B. guianensis* has an average annual increment of about 0.35 cm/ha year^{-1} (S.D. = 0.31 cm/ha year^{-1}; Silva, 2005). The lost alleles were found in juvenile plants with minimum dbh of 32 cm. Trees in the species generally start to produce pollen and seeds when they reach about 42 cm. Thus, these alleles will be transferred from juveniles for the next generation in about 30 years [(42 − 32)/0.35] after logging (year ~2034). The cutting cycle in Brazil is 30 years and the area will be exploited in 2034 around the time these trees start flowering. Again rare alleles can be lost in the logging. On the other hand, *B. guianensis* is a pioneer species and needs light to reach the canopy. Selective logging opens the canopy of the forest, favouring the regeneration of the species. An increase in the population can retain these and other rare alleles. Another point is that seed may remain viable in the soil for a number of years and respond to the gaps opened by logging. These seeds will represent the genetic characteristics of the populations pre-logging.

Our results suggest that RIL changes the SGS of the *B. guianensis* population. After logging, SGS was broken (Fig. 2). This is a positive aspect of harvesting. SGS can produce biparental inbreeding, resulting in inbreeding depression. In fact, significant biparental inbreeding (6.7%) was observed and biparental inbreeding producing biparental inbreeding depression in tropical tree species was reported by Stacy (2001). Thus, with reduction in the SGS by logging, we can expect a reduction in the biparental inbreeding after logging.

Forest logging tended to reduce the average number and effective number of alleles per locus and the expected heterozygosity (Table 1), although these differences were not significant ($P > 0.05$). In fact, these parameters have been found to have low sensitivity for effects of forest logging (Buchert et al., 1997; Rajora et al., 2000; Degen et al., 2006; Lacerda et al., 2008; Sebbenn et al., 2008). Heterozygosity and effective number of alleles can be depleted by genetic drift very slowly, at the rate of $1/2N_e$ per generation (Savolainen and Kärkkäinen, 1992). For example, theoretically the 28 remaining post-logging individuals will experience only 2% of reduction in H_e. Changes in genetic diversity following a decrease in population size take a number of generations to become apparent (Lowe et al., 2005).

4.3. Mating system

Although the species is dioecious, the matings were not random. Differences between single-locus outcrossing rate and unity indicates significant mating among relatives (6.7%) and the paternity correlation ($\hat{r}_{p(m)} = 0.193$) indicates that matings are correlated. The observed biparental inbreeding can be explained by SGS in the reproductive population (Fig. 2b), as already commented, and by the fact that part of the pollen came from short distance dispersal (Table 4). Correlated mating

Table 4
Density of reproductive trees (d) and mean pollen dispersal distance (δ) estimated for the normal and exponential dispersal model (TwoGener)

Dispersal function	Parameter	Density	$\hat{\delta}$ (m)
Normal	δ_{min}	d_{max} = 0.076 trees/ha	308 (273–343)
	δ_{max}	d_{min} = 0.0076 trees/ha	844 (786–902)
Exponential	δ_{min}	d_{max} = 0.076 trees/ha	347 (310–384)
	δ_{max}	d_{min} = 0.0076 trees/ha	961 (899–1023)

arises from flowers fertilizing from a pollen pool unrepresentative of the population, due to a pollen sample from a small number of male trees or few events of pollination (Sampson, 1998). Asynchronous flowering phenologies, low reproductive population size, and behaviour of pollinators visiting neighbourhood trees are the most probable causes of correlated mating in *B. guianensis*. The analysis of flower phenology of the reproductive event which produced the analysed seed was very synchronous between males and female trees, although some trees did not produce pollen and seeds (Silva, 2005). Considering that seeds were collected from different fruits in seed-trees, the result indicates that the pollinator is visiting many flowers of the same trees or many pollinators are visiting the same trees. In *B. guianensis*, pollination can occur by very small insects, known as thrips (from 3 to 5 mm), from the *Thysanoptera* order (Silva, 2005). However, until the moment, wind cannot be discarded as responsible for part of the pollination process. Thrips are considered low active fliers and apparently use the wind currents to fly long-distances, characterizing an interaction between wind x thrips for effective pollination of the species (Silva, 2005). In fact, in a monitored flowering female individual in the area, simultaneous visits of hundreds of thrips, forming true ''thrips clouds'' borne by the wind, were registered during the receptivity of the flowers. This behaviour could explain the correlated mating, because hundred of thrips that simultaneously came from one tree to another can efficiently fertilize in the same time many flowers, including different flowers of the same plant. In addition, in *B. guianensis*, the female flowers in near branches are receptive synchronously, within a female flowering phenology showing a strongly defined period of flowering lasting on average two weeks. On the other hand, male trees flowering phenology lasts on average two months, which permits that a male tree can fecund flowers of many female trees in the population. In summary, the correlated mating must be caused primarily by the behaviour of the pollinator of the species.

4.4. Inbreeding and heterosis

Although a significant rate of biparental inbreeding (6.9%) was observed no significant inbreeding was detected in offspring. No differences in fixation index were also detected among offspring and adult trees, but the pattern of the fixation index indicates selection in favour of heterozygous between offspring and adult stage ($F_{adults} < F_{juveniles} < F_{offspring}$, Table 1). Excess or deficiency in frequency of heterozygots in relation to that expected from the Hardy-Weinberg model can also occur due to non-panmixia. In the absence of evolutive advantage of heterozygous over the homozygous, the not panmitic excess of heterozygous in a population can arise by fixation of hybrids for agamospermy or apomixy (vegetative reproduction) or preferential mating among different individuals, known as dissociative mating (Richards, 1986). As dioecism necessarily involves the union of gametes from individuals of different sexes (males and females), the possibility of mating is limited in low-density population of tree species, resulting in situations of non-panmixia, justifying the heterozygous excess.

4.5. Pollen heterogeneity

In dioecious species, pollen gene pool heterogeneity among seed-trees can be affected by inbreeding and SGS in parental population (Austerlitz and Smouse, 2001b). We observed absence of inbreeding and weak SGS in the *B. guianensis* parental population (Fig. 2). Thus, the estimated pollen gene pool heterogeneity ($\hat{\Phi}_{ft} = 0.081$) seems to be robust. The levels of Φ_{ft} in *B. guianensis* were lower than detected in other animal or insect pollinated tropical tree species. For example, in *Symphonia globulifera*, Φ_{ft} was calculated as 0.205 in a high-density population (>10 trees/ha) in French Guiana (Degen et al., 2004), and was 0.124 and 0.159 in two consecutive years in a low-density population (0.046 trees/ha) occurring in the same experimental plot to this study (Carneiro et al., 2007). The present value was also smaller than detected in a *Hymenaea courbaril* ($\hat{\Phi}_{ft} = 0.131$: Lacerda et al., 2007), and a *Dinizia excelsa* population occurring in a continuous Amazon forest ($\hat{\Phi}_{ft} = 0.104$, Dick et al., 2003). However, this Φ_{ft} was higher than detected in *D. excelsa* trees occurring in a very low-density population (0.10 trees/ha) in fragmented landscape ($\hat{\Phi}_{ft} = 0.002$, Dick et al., 2003), in *Sextonia rubra* ($\hat{\Phi}_{ft} = 0.061$, Veron et al., 2005), and in a high density *Carapa guianensis* population occurring in the present plot before and after logging ($\hat{\Phi}_{ft} = 0.044$ and 0.054, respectively; Cloutier et al., 2007). Thus, the estimated Φ_{ft} suggest that different *B. guianensis* seed-trees can share a differentiated gene pool. The explanations for this result are the same presented for correlated matings.

4.6. Distance of pollen flow

Pollination observation indicated that *B. guianensis* is primary pollinated by Thrips (Silva, 2005). The results indicated that thrips were very efficient in terms of pollen dispersal distance. The average effective pollen dispersal distance ranges from 308 to 961 m, depending on the model and assumed effective reproductive density. These distances are lower than the average distance between males and the sampled seed-trees (1357 m, ranging from 57 to 2849 m), suggesting a predominance of mating between nearby trees. The pollen dispersal distance depends on distance among reproductive cospecífics, the phenology of flowering, and the behaviour of the pollinator. The present population has very low density and

the distances between cospecifics are generally high. Male flowering phenology was perfectly synchronous with female flowering, but not all males produced flowers all years. This can affect the distance among cospecific reproductive male and female trees each year. The observed pollinator (thrips) seems to use wind to fly between trees. Thus, we can explain this pattern arguing that, possibly, thrips forage in the nearer reproductive trees.

The observed long distance of pollen dispersal agrees to that observed in other low-density tropical trees species, where results have been suggesting that average of pollen dispersal distance is generally higher than 200 m (Nason and Hamrick, 1997; Nason et al., 1998; Kenta et al., 2004; Konuma et al., 2000; White et al., 2002; Dick et al., 2003; Isagi et al., 2004; Latouche-Hallé et al., 2004; Carneiro et al., 2007; Cloutier et al., 2007; Lacerda et al., 2007).

4.7. Effective pollination neighbourhood area

The effective number of pollen donors (N_{ep}) and the population density permit estimation of the effective neighbourhood pollination area (A_{ep}). Both estimatives of paternity correlation and differentiation in pollen gene pool indicates that a restricted number of pollen donors (5.18–6.17) produced the seeds of the sampled seed-trees. Restricted values of N_{ep} estimates from r_p and Φ_{ft} have been reported in many tropical trees species occurring in both high- and low-density populations, suggesting that this can be a pattern for these species. Values of N_{ep} have ranged from two to 12 in the tropical tree species: *Carapa procera* ($\hat{N}_{ep} = 12$; Doliguez and Joly, 1997), *Cariniana legalis* ($\hat{N}_{ep} = 3 - 5$; Sebbenn et al., 2000), *Myracrodruon urundeuva* ($\hat{N}_{ep} = 2$; Moraes et al., 2004), *Symphonia globulifera* ($\hat{N}_{ep} = 3 - 4$; Degen et al., 2004; Carneiro et al., 2007), and *H. courbaril* ($\hat{N}_{ep} = 3 - 4$, Lacerda et al., 2007). This number associated with the population density of reproductive trees will determine the A_{ep}. Our estimates of A_{ep} range from 81 to 812 ha, according to assumed population density. The largest A_{ep} can correspond to a circle of radius of 1601 m around a central seed-tree and is about 1.6 times the area of the plot. Thus, although a low number of pollen donors apparently mated with each seed-tree, the very low-density population promotes a large affective neighbourhood pollination area. These areas are one of the biggest detected in tropical tree species. *Turpinia occidentalis* has a A_{ep} of only 4 ha (Stacy et al., 1996), *Spondia monbin* of 19.6 ha (Stacy et al., 1996), *Cordia aliodora* of 24.9 ha (Chese et al., 1996), *Pithecellobiun elegans* of 63.6 ha, (Nason et al., 1997), *Neobalanocarpus heimii* of 86 ha (Konuma et al., 2000), and *Calophyllum longifolium* of 1241 ha (Stacy et al., 1996). The species with higher A_{ep} is the very low-density *Ficus dugandii* population with 63,170 ha (Nason et al., 1998). The large A_{ep} for low-density population probably make this species resilient to reduction in population density and increase of distance among cospecific trees by forest fragmentation and logging. In fact, the harvesting of 61% of reproductive trees led to the distance between male and female-trees ranging from 57 to 2547 m, with an average of 1175 m. The average distance was lower than the distance among cospecifics and we can expect that these trees will not be reproductive isolates.

4.8. Genetic conservation

The detected non-random mating has important implication for genetic conservation and seed-collection programs of *B. guianensis*. To retain the effective population size of 150, seed collection for *ex situ* conservation or reforestation needs to include at least 45 seed-trees. According to detected SGS, if the seed harvesting will be undertaken in natural populations of the species, seed-trees need to be distant at least 300 m, to avoid collecting seeds from female relatives. In collection in logged populations, this is apparently not necessary, considering that logging broke the previous spatial genetic structure.

Finally, our results suggested that logging caused the loss of some rare alleles, but these alleles were probably not lost from the overall population due to their presence in juveniles and offspring. The distance of pollen dispersal and the gene flow from surrounding populations seems to be high, according to the measured distance of pollen dispersal and the high number of exclusive alleles detected in offspring. Thus, pollen flow from surrounding populations can possibly compensate the lost alleles in present population. Gap regeneration typical of a pioneer species, coupled with longevity of seed in the soil will also help reduce the risks of lost alleles.

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