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Effects of selective logging on the mating system and pollen dispersal of *Hymenaea courbaril* L. (Leguminosae) in the Eastern Brazilian Amazon as revealed by microsatellite analysis

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ABSTRACT

Using nine microsatellite loci, we studied the effects of selective logging on genetic diversity, mating system and pollen dispersal in a population of the tree species *Hymenaea courbaril*, located in a 546 ha plot in the Tapajós National Forest, Pará State, Brazil. We analyzed 250 offspring (nursery reared seedlings) collected after a logging episode from 14 open-pollinated seed trees. These were compared to 367 seedlings from 20 open-pollinated seed trees previously collected from the pre-logging primary forest. The genetic diversity was significantly lower in the post-logging seed cohort. In contrast to the pre-logging population, significant levels of selfing were detected in the post-logging population ($t_m = 0.962$, $P < 0.05$). However, correlated matings were reduced and the effective number of pollen donors almost doubled after harvesting (3.8 against 7.2). Logging also reduced pollen immigration into the plot (from 55% to 38%) and we found no significant correlation between the size of the pollen donors and the number of seeds fathered. Inside the plot, pollen dispersal distance was shorter before logging than after (827 and 952 m, respectively) and the reproductive pollination neighbor area (A_{ep}) was larger (average of 178 ha). The individual and average variance effective population size within families (ranged from 1.80 to 3.21, average of 2.47) was lower than expected in panmictic populations ($N_e = 4$). The results indicate that while logging greatly reduced the levels of genetic diversity after logging, it also increased genetic recombination within the population and constrained crossing among related individuals. The results show that low-density tropical tree species such as *H. courbaril*, when harvested in moderate levels may be resilient to a reduction in the reproductive population and may maintain similar levels of outcrossing and pollen dispersal after logging.

1. Introduction

Timber harvesting in tropical forests using selective logging prescriptions, such as reduced impact logging (RIL), is generally focused on harvesting the largest trees: a practice that can remove the majority of reproductive individuals. Such logging systems may affect mating systems, pollen dispersal patterns and the regeneration of tropical trees species. In studies assessing the impact logging practices have on species, it is especially important to consider if the largest trees are the main contributors to reproduction, as has been reported for tropical species such as *Dicorynia guianensis* (Latouche-Hallé et al., 2004) and *Entandrophragma cylindricum* (Lourmas et al., 2007). The reduction in reproductive tree density tends to increase the distance among reproductive conspecifics (Lacerda et al., 2008a) and change pollinator foraging patterns (Murawski and Hamrick, 1991). Positive associations between population density and outcrossing rates have been documented for numerous tropical trees species (Murawski and Hamrick, 1991; Obayashi et al., 2002; Moraes and Sebbenn, 2011; but see also Kitamura et al., 1994). The reduction in tree density forces pollinator vectors to fly longer distances in order to find resources (nectar and pollen) as compared to natural stands. Such reduction in tree density may contribute to increased selfing (Obayashi et al., 2002; Lemes et al., 2007) and correlated matings, while decreasing the genetic diversity and effective

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population size of post-logging generations (André et al., 2008; Lacerda et al., 2008a; Silva et al., 2008), leading to a significant reduction in the intensity of regeneration (Lobo et al., 2007). Considering that genetic diversity is a key factor in evolution, fitness and survival of tree species populations and individuals, the reduction in genetic diversity may predispose species and populations to disease, reduce productivity and limit breeding (Rajora and Pluhar, 2003).

The expected effects of the genetic bottleneck caused by logging may include a loss of rare (frequency $\leqslant 0.05$) or low-frequency alleles ($0.25 \geqslant$ frequency > 0.05) and changes in the intra-population spatial genetic structure (Lacerda et al., 2008b; Silva et al., 2008). These effects may be especially severe if logging intensity is high, i.e. if more than 50% of the reproductive population is harvested. Genetic bottleneck caused by logging may also produce genetic drift during reproductive events. Short-term effects of genetic drift (e.g. loss of alleles, changes in mating system and pollen and seed dispersal patterns) may be detected one or more generations after logging. However, the cumulative effects of genetic drift caused by successive cutting cycles can only be assessed using genetic simulation models (e.g. Degen et al., 2006; Sebbenn et al., 2008; Wernsdörfer et al., 2010).

Here we investigate the impacts of selective logging practices on the genetic diversity, mating system and pollen dispersal in a population of the tree species *Hymanaea courbaril* (Leguminosae) in the Brazilian Amazon, comparing pre- and post-logging open-pollinated seeds and using microsatellite loci and parentage analysis. In the Brazilian Amazon, RIL regulations permit logging intensities of up to 90% of trees above the minimum cutting diameter (MCD) of 50 cm (diameter at breast height – dbh) in cutting cycles of 25–30 years (IBAMA, 2004). The logging episode that occurred in the study area was less intensive than the required RIL prescriptions, with only trees above 80 cm dbh being logged at an intensity of about 61%. In some of the surrounding stand to the north and east, logging followed the requirements for RIL including higher logging intensities (90%) and lower dbh (>50 cm). This study is unique in that it compares data from a single large-scale plot of primary forest both before and after a logging episode. By comparing the pre- and post-logging population, we demonstrate the potential impacts logging has on tropical tree species and the likely sustainability problems associated with more intensive levels of RIL.

Hymenaea courbaril L. (Leguminosae) is an ideal tree species to investigate the impacts of selective logging on the species' genetic and ecological processes. It is a hermaphroditic tropical tree species, primarily pollinated by glossophagine bats and its fruits are dispersed by rodents, birds and monkeys (Lacerda et al., 2008a). Additionally, the species has a wide natural distribution (from Southern Brazil to the Central region of Mexico; Lee and Langenheim, 1975) and occurs at very low densities (0.29 tree/ha in Tapajós National Forest for >45 cm dbh). Trees reach more than 45 m in height and 3 m in dbh (Lacerda et al., 2008b). Harvesting levels of *H. courbaril* are usually intense, reflecting the value of the species in the international market due to its desirable timber characteristics. Because *H. courbaril* starts flowering at about 49 cm dbh (similar to RIL prescriptions – MCD of 50 cm) and is harvested in intensities as high as 90% (trees above the MCD), the number of reproductive trees in the Brazilian Amazon has been greatly reduced.

The results herein are based on a post-logging generation of *H. courbaril* for which samples were collected in 2008 (5 years after the stand was logged). These results are compared to a previous phase of the research program conducted in the same stand prior to the selective logging episode of *H. courbaril* trees (Lacerda et al, 2008a,b). The following questions were addressed: (i) Is the reduction of the reproductive population size caused by logging reflected in the genetic diversity and effective population size of a post-logging *H. courbaril* generation? (ii) Is there an increase in selfing due to the reduction in the number of reproductive trees? (iii) Is the rate of mating among relatives reduced after logging due to a disruption of the spatial genetic structure? (iv) Does logging within and outside the study plot influence the pollen immigration rate and patterns and distance of pollen dispersal?

2. Materials and methods

2.1. Study site

The study was carried out in a 546 ha plot (Fig. 1) situated at the Tapajós National Forest, Pará State, in the Brazilian Eastern Amazon (3°02′40″ latitude and 54°58′56″ W longitude; altitude of 175 m). The area is covered by tropical humid forest with trees reaching up to 60 m in height. The vegetation is dense with several palm tree species and shrubs occupying the understory. The rainfall is concentrated during the wet season (January–June), reaching annual averages of 1820 mm in a tropical climate – Am by Köppen classification (IBAMA, 2004). Average annual temperature is 25.5 °C, with a maximum of 30.6 °C and minimum of 21.0 °C. In late 2003 a local logging company harvested *H. courbaril* in the area following the Brazilian RIL regulations (maximum logging intensity of 90%, minimum cutting diameter of 45 cm, and cutting cycles of 30 years); however, due to *H. courbaril*'s timber characteristics, and in order to ensure extracted timber had a high proportion of heartwood, only trees above 80 cm dbh were harvested. In the inventory, 130 reproductive trees of *H. courbaril* (>49 cm dbh) were identified of which 75 were logged (trees $\geqslant$81 cm dbh, Fig. 1). The selection of trees to be harvested was based on the trees with the best trunk form and overall health (neither hollow or rotten). Because of the high stock of timber in the highest diameter classes, the harvesting threshold used by the logging company was set at 80 cm dbh (higher than the 50 cm allowed by law). This MCD threshold was calculated in order to ensure volume limits were respected. The logging reduced the reproductive population density from 0.238 to 0.101 trees/ha. The average distance between reproductive trees before logging was 1164 m (1–2997 m; Lacerda et al., 2008a, median of 1077 m) and after logging it was 1094 m (19–2925 m, median of 1169 m, see results).

2.2. Sampling design, plant collection and DNA extraction

This study is the second stage of the Dendrogene project (Kanashiro et al., 2002). During stage I (pre-logging, 2003) all trees of *H. courbaril* $\geqslant$10 cm dbh were mapped, measured, sampled and genotyped for nine microsatellite loci. Additionally, 367 seeds were collected before logging from 20 *H. courbaril* seed-trees (Lacerda et al., 2008a,b). In the current study (stage II, post-logging), we sampled open-pollinated seeds from *H. courbaril* adults of the remaining population, 5 years after logging. During the sampling period (2008), not all reproductive trees produced fruit and fruiting density was variable among trees. Due to the low fruit production in 2008, we collected 250 seeds from 14 seed-trees (13–20 seeds per seed-tree). Seeds were planted at the Embrapa Research Station in Belterra, Pará state. After 3 months of germination, three to four leaflets were collected from each individual and dried in silica gel for DNA extraction and genetic analysis. Total genomic DNA was extracted using a CTAB protocol (Doyle and Doyle, 1990) optimized by Ferreira and Grattapaglia (1998).

2.3. Microsatellite analysis

For the genetic analyses we initially tested nine nuclear microsatellite markers previously developed for *H. coubaril* (Ciampi et al.,

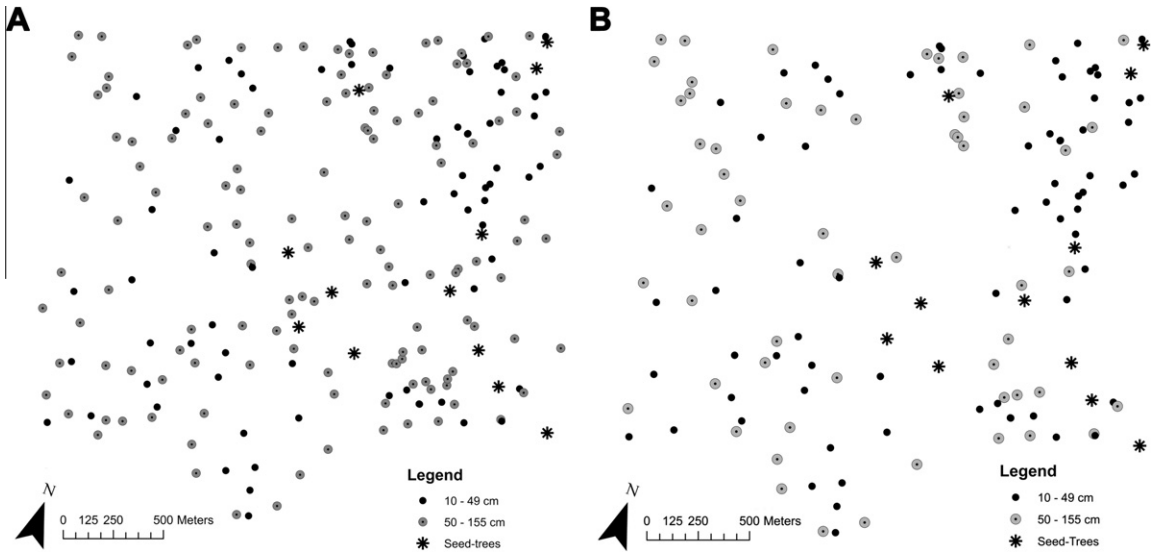

Fig. 1. Spatial distribution of *Hymenaea courbaril* trees with dbh ⩾10 cm before (a) and after selective logging (b) in a 546 ha plot in the Tapajós National Forest.

2000). PCR amplification was performed with one primer labeled with a fluorescent dye, either FAM, NED, and HEX. Primers were multiplexed into three different combinations in which primers of similar sizes were marked with different dyes. PCRs were carried out in a total volume of 10 μl containing 1× PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 200 μM dNTPs, BSA (2.5 mg ml^{-1}), 1.25 μM of each forward and reverse primers, 1 U Taq DNA polymerase and 5.0 ng of genomic DNA using a Veriti (ABI Inc.) thermal cycler. The amplification procedures were slightly different from Lacerda et al. (2008b) and consisted of the following steps: (1) 95 °C for 15 min, followed by 30 cycles of (2) denaturing at 94 °C for 30 s, (3) annealing for 90 s at primer temperature, (4) extension at 72 °C for 1 min, and (5) a final extension at 60 °C for 30 min. Multiplexed PCRs were analyzed in a 3130 XL ABI platform (ABI Inc.) and the amplified fragment sizes estimated using the *GeneMapper*® Software (ABI Inc).

2.4. Genetic diversity and fixation index

In order to compare genetic diversity among adults (dbh ⩾ 49 cm, $n = 55$) and offspring collected after logging ($n = 250$), the total number of alleles (k), average number of alleles (A), effective number of alleles ($A_e = 1/\sum_{i=1}^{k} p_i^2$, where p_i is the frequency of the allele i), observed (H_o) and expected heterozygosity (H_e) were estimated. Private alleles or alleles that occur only in adults or offspring were also identified. The fixation index (F) was used to estimate inbreeding among juveniles and adult trees and to test its significance (i.e. if F is significantly different from zero); we used 1000 Monte Carlo permutations (alleles among individuals) and a Bonferroni correction (95%, $\alpha = 0.05$). These analyses were carried out using FSTAT program (Goudet, 1995). To compare the average values of A, A_e, H_o, and H_e between adult-trees and seedlings, the 95% confidence interval of the standard error (95% CI ± 1.96 × SE) of these parameters was calculated using a jackknife procedure for all loci. In the offspring, the intra-individual fixation index was calculated using reference allele frequencies calculated for adult trees, using SPAGeDI version 1.3 (Hardy and Vekemans, 2002). The significance of F values in offspring was also tested using a 1000 Monte Carlo permutations of alleles among individuals and applying a Bonferroni correction (95%, = 0.05), implemented by SPAGeDI 1.3.

2.5. Mating system analysis

Mixed and correlated mating models (Ritland and Jain, 1981; Ritland, 1989) implemented by the MLTR 3.1 program (Ritland, 2002) were used to estimate the following mating system parameters at family and population levels: multilocus outcrossing rate (t_m), single-locus outcrossing rate (t_s), mating among relatives ($t_m - t_s$), correlation of selfing (r_s) and multilocus paternity correlation ($r_{p(m)}$); a 95% confidence interval was estimated by 1000 bootstraps. Based on the multilocus paternity correlation, we estimated the effective number of pollen donors ($\hat{N}_{ep} = 1/r_{p(m)}$), the average coefficient of coancestry (Θ) and effective population size (N_e) within families. The average coancestry coefficient was estimated using the method developed by Sebbenn (2006), which accommodates mating among relatives, individual variation in outcrossing rate, selfing and correlated matings. This method allows for the calculation of the average coancestry at the population level through the following expression:

$$\Theta = 0.125(1+F_p)\{4s[(1-r_s)(s+t_s)+r_s]+[t_s^2(1-\hat{r}_s)+t_s r_s](1+r_p)\}+0.25(t_m-t_s)(1-r_s)[2(1+F_p+2\Theta_p)(s+t_s)+t_s(1+F_p+6\Theta_p)(1-r_p)]+0.125[(t_m-t_s)^2(1-r_s)+(t_m-t_s)r_s][(1+F_p+6\Theta_p)(1-r_p)+(1+F_p+2\Theta_p)2r_p].$$

At the family level the average coancestry was estimated using the same equation but assuming $r_s = 0$. This method assumes that all positive differences between t_m and t_s occur due to mating among relatives. Furthermore, the equation considers that, as inbreeding in a generation is equal to the coancestry in parental population ($\Theta_p = F_{t_m - t_s}$), the average coancestry coefficient between mated trees (Θ_p) in the parental population can be estimated from the difference between the inbreeding coefficient in the offspring and the expected inbreeding due to selfing: $\hat{F}_{t_m-t_s} = \hat{F}_o - \hat{F}_s$; F_o is estimated as a fixation index and negative values are assumed as zero (the inbreeding coefficient range from zero to 1.0); $\hat{F}_s = 0.5\hat{s}(1+\hat{F}_p)$, where F_p is the coefficient of inbreeding in the parental population (Sebbenn, 2006). The variance effective population size (N_e) within families was estimated using the expression: $\hat{N}_e = 0.5/\{\hat{\Theta}[(n-1)/n]+[(1+\hat{F}_o)/2n]\}$ (Cockerham, 1969), where n is the sample size. The number of seed-trees to be sampled aiming to retain the reference effective population size ($N_{e(\text{reference})}$) was

estimated by: $\hat{m} = N_{e(\text{reference})}/\hat{N}_e$ (Sebbenn, 2006). This estimate was based on two suppositions: (i) the seed-trees are not related and do not mate with each other; (ii) seed-trees do not receive an overlapping pollen pool. As reference, we chose an effective population size of 150 as it is likely sufficient to retain the genetic diversity in a population in the short-term, or 10 generations (Nunney and Campbell, 1993).

2.6. Paternity analysis

To verify the exclusion power for paternity analysis, we used a non-exclusion probability estimated for the second parent [$\Pr(Ex_2)$] and the CERVUS 3.0 program (Marshall et al., 1998). We used a maximum likelihood of a categorical paternity analysis (Meagher and Thompson, 1986) for the estimation of the selfing rate and pollen dispersal distance and patterns; such calculations are also available in the CERVUS 3.0 program. The paternity of each seed from every seed-tree was assigned using the Δ statistic (the difference between the LOD score of the most likely paternal parent and the second most likely pollen donor (Marshall et al., 1998). The critical values of Δ were calculated using simulations in CERVUS 3.0 considering 50,000 repetitions (simulated offspring), 0.01 as the proportion of errors in the loci, all reproductive trees as putative paternal parents (n = 55), 50% as the proportion of sampled pollen donors (due to the likelihood of many pollen donors existing outside of the study area) and finally we adopted a confidence level of 80% as relaxed in the paternity calculation (Marshall et al., 1998). We also considered the possible occurrence of selfing. For the Δ statistics, if a candidate father of a seed has a calculated Δ higher than a critical Δ value, it was considered the true father of the seed. If a father candidate has the same genotype as the mother, the seed was considered as selfed. Thus, the selfing rate (s) was calculated as the number of selfed seeds (n_{selfed}) divided by the total number of sampled seeds (n). The cryptic gene flow was estimated by $C_{\text{gf}} = 1 - (1 - P_{\text{nexcl}})^2$ (Dow and Ashley, 1996). The rate of pollen immigration ($n_{\text{immigrant}}$) into the plot was estimated as the number of seeds for which a father was not assigned inside of the plot divided by the total number of seeds sampled (n = 250). As each tree in the plot was mapped, the distance of pollen dispersal was calculated for each seed as the distance between the seed-trees and the putative pollen donor. To estimate the spatial distance between all pairwise trees, we used the SPAGEDI program (Hardy and Vekemans, 2002). To determine if pollen dispersal distance inside the plot was a function of the spatial distance between all trees, we compared the frequency curve of the effective pollen dispersal determined within the plot with the frequency curve of the distances between all reproductive trees in relation to the 14 seed-trees, using the Kolmogorov–Smirnov test (Sokal and Rohlf, 1995). To verify if trees with a larger dbh were more likely to pollinate seeds than smaller trees, the Spearman's rank correlation coefficient between the dbh of pollen donors and the number of seeds fathered by each tree was estimated using MS Excel.

Using paternity analysis, we also estimated the effective number of pollen donors, using the unbiased Nielsen et al. (2003) method:

$\hat{N}_{\text{ep}} = (n-1)^2/\sum_{i=1}^{k_o}\hat{p}_i^2(n+1)(n-2) + (3-n)$, where p_i^2 is the observed square frequency of offspring fathered by a tree i ($p_i = x_i/n$), x_i is the number of offspring fathered by tree i, and n is the sample size. From N_{ep}, we estimated the paternity correlation ($\hat{r}_p = 1/\hat{N}_{\text{ep}}$) or the probability that a sample of two seeds from the same family were fathered by the same tree. From selfing rate and paternity correlation, we also calculated the coancestry and the variance effective population size within families, using the same expression described above for the mating system analysis.

3. Results

3.1. Genetic diversity

We found a total of 120 alleles over nine SSR loci in 305 plants distributed among adults and offspring. The number of alleles among loci ranged from 2 to 24, with an average of 13.3. The adults exhibited more alleles (k = 103) than offspring (k = 84) although the number of sampled offspring was about five times higher than adults (Table 1). The analysis also showed more exclusive alleles for offspring than for adults (14 and 9, respectively), suggesting pollen immigration from outside the plot. The average number of alleles per locus and the observed and expected heterozygosities were significantly lower in offspring ($\hat{A}$ = 9.3, $\hat{H}_o$ = 0.536, $\hat{H}_e$ = 0.562) than adults ($\hat{A}$ = 11.4, $\hat{H}_o$ = 0.592, $\hat{H}_e$ = 0.666) (Table 1). The fixation index was positive and significantly different from zero in all samples (Table 1), suggesting inbreeding, especially in offspring ($\hat{F}_{\text{offs}} = 0.254$).

3.2. Mating system

We found a high multilocus outcrossing rate (0.907 –0.992) (Table 2) for the 14 open-pollinated arrays of *H. courbaril*. The values were statistically significant different from the 1.0 for 12 of the 14 seed-trees and for the average population, suggesting

Table 1
Genetic diversity and fixation index of *Hymenaea courbaril* adults and offspring after logging: k is the number of alleles; H_o and H_e are the observed and expected heterozygosity; F_{ad} and F_{of} are the fixation index for adults and offspring; $\hat{P}_{\text{Exc2}}$ is the theoretical combined non-exclusion probability of the second parent.

	Adults (n = 55)					Offspring from after logging (n = 250)			
Locus	k	$\hat{H}_o$	$\hat{H}_e$	$\hat{F}_{\text{ad}}$	$\hat{P}_{\text{Exc2}}$	k	$\hat{H}_o$	$\hat{H}_e$	$\hat{F}_{\text{of}}$
HC06	9	0.634	0.791	0.198	0.381	7	0.414	0.452	0.487*
HC12	3	0.415	0.457	0.092	0.689	4	0.437	0.417	0.571*
HC42	17	0.755	0.833	0.094	0.314	11	0.720	0.745	0.050*
HC14	8	0.289	0.266	−0.085	0.699	2	0.236	0.237	0.147*
HC40	20	0.963	0.927	−0.039	0.163	17	0.905	0.915	0.047
HC34	9	0.744	0.758	0.018	0.418	11	0.772	0.694	0.194*
HC33	2	0.111	0.282	0.606*	0.846	2	0.078	0.091	0.123*
HC17	18	0.673	0.782	0.140	0.230	11	0.701	0.729	0.203*
HC25	17	0.744	0.900	0.173	0.187	19	0.564	0.776	0.361*
Mean	11.4	0.592	0.666	0.111*	–	9.3	0.536	0.562	0.254*
$CI_{95\%}$–Inf	10.9	0.570	0.645	0.095	–	8.8	0.514	0.539	0.123
$CI_{95\%}$–Sup	12.0	0.614	0.687	0.127	–	9.8	0.558	0.584	0.386
Total	103	–	–	–	0.00014	84	–	–	–

$\hat{F}_{\text{prog}}$: intra-individual fixation index calculated with reference allele frequencies obtained for the adult trees using the SPAGeDI program.
* $P < 0.05$.

Table 2
Mating system and within family genetic parameters for *Hymenaea courbaril* seed-trees and population: t_m is the multilocus outcrossing rate; $t_m - t_s$ is the biparental outcrossing rate; $r_{p(m)}$ is the multilocus paternity correlation; N_{ep} is the effective number of pollen donors; Θ is the coefficient of coancestry within families; $N_{e(v)}$ is the size within progenies; m is the number of seed-trees necessary for seed collection; average ±95% confidence interval of standard error; ( ) 95% confidence interval of standard error.

Seed-tree	t_m	$t_m - t_s$	$r_{p(m)}$	N_{ep}	Θ	$N_{e(v)}$	m
400131	0.907±0.057	0.000±0.045	0.053±0.306	18.9	0.172	2.64	57
400440	0.992±0.003	0.076±0.018	0.097±0.197	10.3	0.159	2.83	54
401048	0.949±0.041	0.027±0.029	0.087±0.169	11.5	0.165	2.73	55
401169	0.943±0.045	0.051±0.040	0.225±0.112	4.4	0.184	2.49	61
500182	0.978±0.014	0.066±0.017	0.068±0.330	14.7	0.157	2.85	53
501383	0.965±0.035	0.056±0.036	0.058±0.237	17.2	0.158	2.83	54
501785	0.990±0.002	0.111±0.026	0.069±0.271	14.5	0.157	2.85	53
502348	0.937±0.047	0.048±0.032	0.214±0.217	4.7	0.184	2.49	61
600959	0.963±0.038	0.045±0.038	0.058±0.229	17.2	0.158	2.83	54
602696	0.950±0.040	0.031±0.032	0.035±0.358	28.6	0.158	2.83	54
606001	0.950±0.041	0.070±0.032	0.119±0.210	8.4	0.170	2.67	57
700119	0.949±0.040	0.066±0.027	0.036±0.367	27.8	0.160	2.81	54
700321	0.923±0.056	0.058±0.048	0.231±0.264	4.3	0.189	2.44	62
702357	0.983±0.001	0.099±0.020	0.203±0.290	4.9	0.176	2.59	58
Population	0.962 (0.947–0.979)	0.066 (0.037–0.084)	0.139 (0.077–0.164)	7.2 (6.1–13.0)	0.170 (0.156–0.179)	2.66 (2.55–2.87)	56 (52–59)

Selfing correlation: $r_s = 0.066$ (ranging from 0.036 to 0.066).

that some selfing occurred ($t_m = 0.962$, $P < 0.05$). The difference between multilocus and single-locus outcrossing rate was significant (different from zero) in 11 of 14 seed-trees and for the average population ($t_m - t_s = 0.066$), indicating biparental inbreeding in the offspring (Table 2). Paternity correlation among seed-trees ranged from 0.035 to 0.225, with an average of 0.139 (Table 2), indicating 4.3–28.6 pollen donors mated with each seed-tree (average of 7.2) (Table 2). The coancestry coefficient within families was high (0.170, Table 2) and consequently the variance effective population size ($N_{e(v)} = 2.66$) was lower than expected in panmictic populations ($N_{e(v)} = 4$). The number of seed-trees (m) for seed collection ranged among seed-trees from 53 to 62, with an average for the whole population of 56.

3.3. Pollen flow and dispersal patterns

As the combined non-exclusion probability (Table 1) was very low [$\Pr(Ex_2) = 0.00014$], the theoretical cryptic gene flow was equally low (<1%, [$0.0077 = 1-(1-0.00014)^{55}$)]), showing that our estimates of pollen flow are unbiased (i.e. the theoretical chance of two seeds being wrongly assigned (250 seed × 0.0077)). From our 250-seed sample, a possible father tree from inside the plot was detected for 155 seeds. Therefore, the other 95 seeds were likely fathered by trees from outside the plot, suggesting a pollen immigration of 38% (Table 3). Furthermore, the 155 seeds were apparently fathered by 65% of the reproductive trees (36 of the 55 reproductive trees). The estimate of the Spearman's correlation coefficient between the dbh of pollen donors (ranging from 49 to 102 cm) and the number of seeds fathered by each tree showed no statistical significance ($R^2 = 0.11$, $P > 0.05$), suggesting no association between the size of the pollen donors and the number of seeds fathered. From the total number of seeds (250), 11 (4%) were fertilized by the same seed-tree, suggesting selfing.

Including selfing in the calculation, pollen dispersal distance (δ) ranged from 0 to 2204 m, with an average of 898 m (Table 3, Fig. 2), and median of 865 m. Without selfing, the pollen dispersal distance ranged from 114 to 2204 m, with an average of 952 m (Table 3), and median of 869 m. Within the plot, about 40% of the pollen was dispersed over 1000 m, showing that long-distance pollination by bats is very effective for this *H. courbaril* population. Actual pollen dispersal is likely even greater if we consider the high proportion of migrant pollen coming from outside of the population

Table 3
Pollen dispersal and genetic structure within families assessed by paternity analysis from a *Hymenaea courbaril* population: s is the selfing rate; N_{ep} is the number of pollen donors; N_{ep} is the unbiased number of pollen donors (Nielsen et al., 2003); r_p is the paternity correlation; F_m is the fixation index in seed-trees; Θ is the coancestry within families; N_e is the effective population size within families; δ is the distance of pollen dispersal (average ±SD, SD is the standard deviation); A_{ep} is the circular effective pollination area.

Seed-tree	n	Migrant number (% pollen immigration)	Pollen movement within plot							
			s	N_{ep}	r_p	Θ	N_e	With selfing (mean (DP) (m)	Without selfing (mean (DP) (m)	A_{ep} (ha)
400131	20	10 (0.50)	2 (0.10)	4.6	0.219	0.201	2.17	504 ± 368	631 ± 288	52
400440	20	10 (0.50)	2 (0.10)	12.0	0.083	0.193	2.23	497 ± 274	497 ± 274	47
401048	20	9 (0.45)	1 (0.05)	20.2	0.050	0.156	2.67	749 ± 500	824 ± 458	132
401169	17	6 (0.35)	0 (0.00)	6.3	0.158	0.152	2.73	914 ± 646	914 ± 646	262
500182	18	7 (0.39)	1 (0.06)	24.3	0.041	0.165	2.56	1418 ± 744	1519 ± 656	271
501383	17	4 (0.24)	0 (0.00)	6.6	0.152	0.144	2.92	864 ± 521	864 ± 521	171
501785	17	4 (0.24)	0 (0.00)	15.6	0.064	0.169	2.57	790 ± 625	790 ± 625	245
502348	16	4 (0.25)	1 (0.06)	8.9	0.112	0.170	2.53	1106 ± 460	1207 ± 315	62
600959	20	6 (0.30)	0 (0.00)	10.2	0.098	0.172	2.56	968 ± 631	968 ± 631	251
602696	20	9 (0.45)	1 (0.05)	8.7	0.114	0.191	2.28	968 ± 726	1065 ± 687	296
606001	20	9 (0.45)	2 (0.10)	15.0	0.067	0.255	1.80	632 ± 431	773 ± 332	69
700119	19	3 (0.16)	0 (0.00)	13.7	0.073	0.134	3.21	994 ± 515	994 ± 515	167
700321	13	6 (0.46)	1 (0.08)	13.5	0.074	0.208	2.00	932 ± 823	1087±782	384
702357	13	8 (0.62)	0 (0.00)	5.3	0.190	0.149	2.28	758 ± 368	758±368	85
Mean	–	–	–	11.8 ± 5.7	0.107 ± 0.054	0.176 ± 0.032	2.47 ± 0.37	864 ± 242	921 ± 254	178 ± 108
Total	250	95 (0.38)	11 (0.04)	–	–	–	–	898 ± 601	952 ± 575	

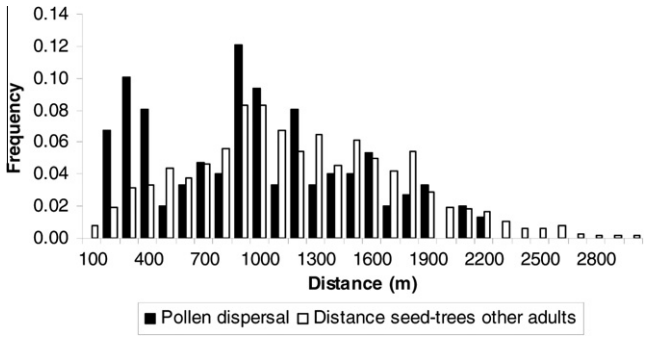

Fig. 2. Frequency of effective pollen dispersal and the distance among reproductive tree and the seed-trees in a *Hymenaea courbaril* population after logging.

that cannot be assessed for the estimate of the pollen flow. The Kolmogorov–Smirnov test was significantly different from the expected value ($D = 0.163, P = 0.0036$; Fig. 2), indicating that the distance among trees did not explain the observed mating pattern. The reproductive pollination neighbor area (A_{ep}) was large, ranging from 47 a 384 ha, with average of 178 ha (not considering selfing).

3.4. Coancestry and effective population size within families

The unbiased effective number of pollen donors (N_{ep}) estimated from paternity analysis ranged widely among seed-trees (4.6–24.3, average of 11.8). Thus, the estimated paternity correlation also ranged widely among seed-trees (0.041–0.219, average 0.107). The coancestry coefficient within families (0.176) was higher than expected in half-sib families (0.125). Consequently, the individual and average variance effective population size within families (ranged from 1.80 to 3.21, average of 2.47) was lower than expected in panmictic populations ($N_e = 4$).

4. Discussion

4.1. Genetic diversity

Our results showed that logging had a negative impact on the genetic diversity of the *H. courbaril* offspring. The total number of alleles in the offspring decreased from 159 before logging (Lacerda et al., 2008b) to 84 after logging (this study): a reduction of 47%. One explanation could be that logging 61% of the trees >80 cm dbh reduced the size of the *H. courbaril* reproductive population (bottleneck effect) to such an extent that it may have directly influenced the number of alleles in the ovule and pollen pool and, consequently, the number of alleles passed on to the offspring. A similar after-logging decline in the genetic diversity was also observed for mahogany (*Swietenia macrophylla*), the most valuable timber species from the Neotropics, in a study carried out in the Brazilian Amazon (André et al., 2008).

The genetic diversity (A, H_o and H_e) in offspring after logging was also reduced in relation to the remaining adult population. Reproductive adults have exclusive alleles not found in offspring, suggesting genetic drift during reproductive events since not all alleles observed in the adults were passed on to the offspring. The occurrence of genetic drift could be related to a variation in flower phenology. *H. courbaril* in general flowers annually in the Tapajós National Forest in a synchronized fashion, therefore flowering does not explain the observed genetic drift. However, the species does present a variation in the number of trees that flower per year (Lacerda, 2007) which might be correlated with droughts or natural species variation. We assume that the only phenologically related process affecting genetic diversity in the offspring is the number of trees flowering per year. The other explanation is a reduction in the number of reproductive trees caused by logging; in the study area, the reproductive population was reduced by 42%. Therefore, intense logging caused an important reduction in genetic diversity in the offspring which may have been enhanced by the species' natural variation in the number of trees flowering in the sampling year.

4.2. Mating system

In contrast to the pre-logging analysis (Lacerda et al., 2008b), selfing was detected after logging (Tables 2 and 3). These results show a strong coherence between estimates of outcrossing rate determined under the mixed-mating model (Ritland and Jain, 1981) and categorical paternity analysis. The detection of selfing in the *H. courbaril* population is surprising, considering other studies suggest that the species is self-incompatible, based on controlled hand pollination (Bawa, 1974) and genetic markers (Dunphy et al., 2004; Lacerda et al., 2008b). However, the observed self-incompatibility in *H. courbaril* could be considered "latent", meaning that under specific circumstances some levels of selfing can occur, for example when trees are reproductively isolated from other conspecifics. Based on the observations of Gibbs et al. (1999) for the pollen tube development of *Hymenaea stigonocarpa* Mart. ex Hayne, it is likely that *H. courbaril* also lacks any pre-zygotic self-incompatibility mechanism. The occurrence of selfing in *Hymenaea* may be influenced by the relative abundance of cross-fertilized ovules. Under conditions of low levels of cross fertilization, such as in spatially or temporally isolated reproductive trees, it is possible that a small proportion of selfed ovules escape selective abortion, developing into the ovary (selfed embryos) together with other embryos resulting from crossing, until fruit maturation. This flexible post-zygotic control of selfing in *Hymenaea* may explain the 4% selfing observed here for *H. courbaril*. This "self-incompatibility system" in isolated trees has been reported for other tropical tree legumes, such as *Dipteryx panamensis* (Hanson et al., 2008), and for the malvaceous *Paquira quinata* (Fuchs et al., 2003).

Considering a 95% confidence interval, mating among relatives in the logged population ($t_m - t_s = 0.066$) was lower, but not significantly different from that detected before logging ($t_m - t_s = 0.096$, Lacerda et al., 2008a). In the studied area, logging reduced the detected genetic structuring from a distance of 800 to 200 m, but did not totally eliminate it (Lacerda et al., 2008b). The post-logging disruption of the SGS in the plot may explain the lower rate of mating among relatives in the post-logging population.

The results suggest a slightly significant level of inbreeding in our sample after logging, whereas the fixation index for adults was similar to that observed before logging ($\hat{F}_{adul} = 0.149$, Lacerda et al., 2008b). However, a significant SGS can result in positive and high fixation index due to the Wahlund effect (Bittencourt and Sebbenn, 2007). Lacerda et al. (2008a) attributed their high values of fixation index to this effect and not to inbreeding *per se*. The same argument can be used to explain the high fixation indexes found for adults of *H. courbaril* in the post-logging population. In contrast, the high fixation index observed in offspring after logging may be the result of true inbreeding, caused mainly by self-fertilization.

Our results showed that post-logging offspring were not true half-sibs (Table 3). The proportion of full-sibs after logging was significantly lower than that detected before logging (average of 0.264, Lacerda et al., 2008a), rejecting the hypothesis that logging increases correlated mating. The effective number of pollen donors (N_{ep}) was significantly lower before (average of 3.8) than after logging (Table 3). This suggests that logging may improve pollen dispersal, likely due to a reduction in the vegetal density which increases the porosity of the forest, allows for greater ease of

pollinator movement, thus contributing to an increase in the number of effective pollen donors.

4.3. Relatedness and effective population size within families

Logging favored genetic recombination among the remaining trees and reduced the relatedness within families. The average coancestry within families was significantly higher than expected in half-sib families (0.125) and also significantly lower than before logging (0.187, Lacerda et al., 2008a). The reduction in the relatedness within families after logging was caused by a decrease in correlated mating. In contrast, the estimated variance effective population size after ($N_{e(v)}$ = 2.66, Table 3) and before logging ($N_{e(v)}$ = 2.68, Lacerda et al., 2008a) were very similar and not statistically significantly different. For conservation purposes, this result indicates that seed collection (seed sourcing), whether the stand was logged or not, must sample seeds from at least 56 seed trees that are not related, that do not mate among themselves and do not receive an overlapping pollen pool, if the final objective is to keep the effective population size of at least 150 individuals.

It is also important to keep in mind that the number of potential pollen donors after logging depends on the minimum cutting dbh and the logging intensity of trees above the minimum cutting diameter. In the present study, only trees with more than 80 cm in dbh were logged. After logging, a relatively large number of reproductive trees (55 trees) remained in the forest. However, *H. courbaril* usually starts flowering only when its crown reaches the upper stratum in the canopy, or when a large proportion of its crown is exposed to sunlight. At this point the tree reaches a size of about 49 cm in dbh. In Brazil, logging rules permit logging of up to 90% of trees with a dbh >50 cm. If the maximum intensity of 90% had been applied to this plot, only a small number of reproductive trees would remain in the forest and only a small proportion of these trees would participate in the mating process. This likely would have contributed to an increase in correlated matings, pollen pool homogeneity and coancestry within families. On the other hand, such a scenario would decrease the variance of effective population size within families. Because the logging episode was somewhat anomalous, it is necessary to carry out studies to further evaluate logging impacts on the mating patterns on this economically important species. However, it is clear that more intense logging parameters as permitted by RIL would have negative impacts on the remaining stand in relation to mating system and effective population size.

4.4. Pollen immigration and patterns of pollen dispersal

Our results showed a lower pollen immigration rate after logging (Table 3), compared to the rate estimated before logging (m_{pollen} = 0.55, Lacerda et al., 2008b). The reduction in pollen immigration was likely caused by logging within the plot but was also due to logging that took place in part of the surrounding stand where logging intensities were much higher. Outside the plot, the logging company harvested 90% of the trees >50 cm of dbh. Hence, logging decreased the density of reproductive trees more intensively outside of the Tapajos plot than inside the plot. Pollen immigration rate in the stand after logging was also lower than detected for other tropical tree species sampled in the same plot: 43% in *Symphonia globulifera* (Carneiro et al., 2009) before logging; 74% pre-logging and 85% post-logging in *Dypterix odorata*; and 49% pre- and 43% post-logging in *Jacaranda copaia* (Vinson, 2009). Logging both within the study area and in the neighboring primary forest, clearly had an impact on the availability of pollen and its movement across the landscape. In the long-term this reduction could lead to a loss of genetic diversity and increase of correlated matings.

The pattern of pollen dispersal of *H. courbaril* in the plot did not change substantially after logging. Before logging, 62% of the pollen was dispersed up to 1000 m (Lacerda et al., 2008a) and after logging 60% of the detected pollen dispersal showed the same pattern. Pre-logging, the mean pollen dispersal distances was 827 m, ranging from 46 to 1943 m (Lacerda et al., 2008a), and after logging it ranged from 114 to 2204 m, with an average of 952 m (Table 3). Before logging took place there were no significant differences between the curve of pollen dispersal frequencies and the frequency curve of distances among reproductive trees and the sampled seed-trees, suggesting that the patterns of mating in *H. courbaril* could be explained by the distances among reproductive trees. Post-logging, however, both curves were significantly different and the distance among reproductive trees cannot explain the observed mating patterns. Still, the estimated average pollination neighbor area for *H. courbaril* (Table 3) corresponds to about 1/3 of the plot (546 ha), indicating a large pollination neighbor area. This area is expected to contain about 18 reproductive trees and was much larger than that detected before logging (average of 116 ha, Lacerda et al., 2008b). These results clearly suggest that forest disturbance by logging affected the foraging behavior of the pollinators, the generalist glossophagine and phyllostomine bats. In the primary forest, these bat species probably foraged in small, well defined areas, exploiting several food resources like nectar, pollen, fruits, and insects. In the disturbed forest it is likely that the *H. courbaril* pollinators forage over broader areas than in the primary forest because food resources were sparse and the forest structure more porous. It is important to note that the logging operation exploited more than 45 timber species in the plot, thus causing considerable disturbance in the forest structure.

4.5. Conclusion

This study provides results which demonstrate the impacts of selective logging practices on the *H. courbaril* species. Logging had a significant negative impact on genetic diversity in the post-logging generation and caused significant changes in mating system, pollen dispersal and pollen vector behavior. The results demonstrate a decrease in the genetic diversity of seeds; however, logging did not have an impact on the effective population size within families. After logging a high rate of pollen immigration into the plot remained, but mating was not random and some levels selfing were detected. Finally, logging reduced the density of reproductive trees and increased the distance of pollen dispersal and pollination neighbor area. While it is clear that reserves of genetic diversity exist due to pollen immigration, it is important to consider the implications of reduced genetic diversity in the long-term. The logging intensity used in this forest plot was much less intense than that normally applied in RIL; therefore, the negative impacts are a clear indication of more significant impacts that will occur with greater logging intensities. In the Amazon, logging has been linked to land conversion and forest fragmentation which could further amplify the impacts described herein; with increased distance between fragments and smaller populations, individuals of low-density species such as *H. courbaril* might become genetically isolated as pollen flow might not be possible and local populations might become extinct.

Future studies on this population should focus on monitoring the regeneration of the population after logging to gain further information about realized seed and pollen immigration in the Tapajós National Forest. Long term studies such as this one on the effects of forest logging on genetic diversity, demography, mating system and pollen dispersal should continue with other forest tree species as well in order to elucidate and advocate for sustainable logging strategies, genetic conservation, and long term management planning and conservation of the Amazonian tropical

forest. The long term study of logged areas, a rare event in the Amazonian region, is a key factor in bringing about sustainable forest management programs in the region.

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