

Gene flow in an overexploited population of *Swietenia macrophylla* King (Meliaceae) in the Bolivian Amazon

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Abstract

Pollen and seed movement among and within populations connect individuals and populations, and therefore are among the most important evolutionary processes determining the genetic structure of populations. Seven microsatellite loci were used to investigate the realized pollen dispersal and intra-population spatial genetic structure (SGS) in four permanent plots located in an overexploited big-leaf mahogany (*Swietenia macrophylla* King) population in the Bolivian Amazon. All adult trees found in the plots were mapped, sampled and genotyped. Seedlings were sampled below the canopy of reproductive trees. Private alleles in the sub-population of the adults and the seedlings were observed. The observed heterozygosity was significantly lower and fixation index was significantly higher for the seedlings ($H_o = 0.697$, $F = 0.068$) compared to the adults ($H_o = 0.761$, $F = -0.023$). In one plot, seed immigration was observed (18%). Realized pollen immigration ranged among the plots from zero to 41% and selfing ranged from zero to 5.8%. We observed an average pollen dispersal distance from 75 to 255 m, with the maximum reaching 576 m. We found a significant SGS up to 150 m, showing that near neighbour individuals are relatives. The observed data on pollen- and seed dispersal provides important information for the sustainable management of the endangered mahogany species.

Key words: Logging; mahogany; microsatellite loci; parentage analysis; tropical trees.

Introduction

Among the many merchantable Neotropical timber tree species, *Swietenia macrophylla* King (Meliaceae) or big-leaf mahogany is the most valuable. Mahogany harvesting started in the 16th Century among *Swietenia mahogany* populations of the Caribbean and in *S. macrophylla* populations in Central America (MEJIA et al., 2008). However, large-scale logging only began in

the South American stocks in the mid 20th Century (GROGAN et al., 2002). In Peru, mahogany has been commercially exploited since the 1920s, in Brazil since the 1930s and in Bolivia since the 1950s (MEJIA et al., 2008). Currently, the future of mahogany as a commercial timber species is at risk due to overexploitation and deforestation (VERWER et al., 2008). For example, by 2002, 49% of the mahogany stands in Peru, 65% in Brazil and 79% in Bolivia was commercially extracted predominantly for export to US and European markets (MEJIA et al., 2008). In 2003, because of the high risks to population viability associated with overexploitation and habitat destruction, *S. macrophylla* was listed on the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II.

Genetic conservation strategies and long-term sustainable logging practices depend on the levels of genetic diversity and effective population size of the remaining populations. High logging intensity, associated with a low minimum cutting diameter and short cutting cycles can negatively affect the genetic structure of remaining post-logged populations and, consequently has an impact on sustainable timber production (SEBBENN et al., 2008). As the population genetic structure of a species is partially determined by mating system, pollen dispersal, as well intra-population spatial genetic structure, it is important to study these factors in commercial timber species in order to develop sustainable forest management plans.

A key factor in the sustainable timber production of tropical tree species in natural forests is the maintenance of genetic diversity within the population; logging may directly reduce genetic diversity of the populations (JENNINGS et al., 2001). Immediately after logging, the effects of the genetic bottleneck caused by harvesting of the largest reproductive tree and the mortality associated with logging activity may be detected in the remaining adult trees. Subsequent effects of logging due to genetic drift can be observed by sampling open-pollinated seeds and regeneration.

Many ecological variables may affect the patterns of mating in tree species, such as spatial isolation, flowering phenology, plant density and pollinator activity (CASCANTE et al., 2002; FUCHS et al., 2003). The population density and behavior of pollinator vectors determines mating patterns of tropical tree species and consequently, determines allele recombination, the relatedness and inbreeding of the new generations. Strong evidence suggests that logging can affect mating patterns in tree species (LOURMAS et al., 2007; ANDRÉ et al., 2008; LACERDA et al., 2008a; AGUILAR et al., 2009; CARNEIRO et al., 2011). Logging reduces the density of reproductive

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individuals and the subsequent spatial isolation of trees may affect the reproductive success and mating patterns within and among populations (LACERDA et al., 2008a). The isolation of individuals increases the distance between food resources for pollinators and pollinators may invest more time foraging within one individual, thus increasing the rate of selfing and correlated matings (DICK et al., 2003; FUCHS et al., 2003; LOURMAS et al., 2007; MORAES and SEBBENN, 2011). Selfing increases inbreeding and correlated mating increases the relatedness of individuals of the new generations. Inbreeding is likely to produce inbreeding depression in tropical tree species (GRIBEL and GIBBS, 2002; HUFFORD and HAMRICK, 2003).

In this study, we investigated the genetic diversity, seed and pollen flow and spatial genetic structure of a post-logged population of *S. macrophylla* from the Bolivian Amazon forest. We specifically addressed the two following questions: *i*) How strong was the seed and pollen flow from outside the plots and what is the distribution of distances of pollen dispersal within the plots? *ii*) How strong is the spatial genetic structure in the plots?

Material and Methods

The studied species

Swietenia macrophylla King (Meliaceae) is an economically valuable, large, deciduous and Neotropical tree species (GULLISON et al., 1996). The species has a large geographic distribution, from 20° N in Mexico (Yucatán) to 18° S in Bolivia (LAMB, 1966). Population density of the species is generally low (<1 tree/ha), although densities of tree ≥ 20 cm diameter at breast height (dbh) up to 1.17 trees/ha have been recorded (GROGAN et al., 2008). Mahogany trees are emergent and can reach heights of up to 50 m and dbh >2 m (GULLISON et al., 1996). The species is monoecious and pollinated by small bees, flies, moths and butterflies (STYLES, 1972). Mahogany trees begin fruiting at about 15 years of age (LAMPRECHT, 1990), or at 20 to 30 cm dbh depending on the site (GULLISON et al., 1996). A fruit of *S. macrophylla* can produce more than 40 wind-dispersed seeds (GULLISON et al., 1996). Seedling growth depends on light availability, although survival is possible in the understory of open forest (GULLISON et al., 1996). The average seed dispersal has been estimated between 32 to 36 m (with a maximum of 80 m) and the seed dispersal area in 2 ha (GULLISON et al., 1996).

Study site

This study was carried out in four permanent plots located in the Agroindustrial Forestal La Chonta forestry concession (Figure 1), Bolivia (15°47'S, 62°55'W). Information about the study site was compiled from VERWER et al. (2008). The concession has 100,000 ha and is located 30 km east of Ascención de Guarayos, Santa Cruz State. This semi-deciduous tropical moist forest is a transitional zone between (Chiquitano) dry forest and Amazonian forest. Annual precipitation in the region is about 1,580 mm, with four months receiving <100 mm (May–September) and one month (July) dur-

ing which potential evapotranspiration exceeds rainfall. The area is situated on the south-western border of the Brazilian Shield; it has sandy-loam soils that are rich in nutrients and are characterized by a pH of approximately 7. For trees >10 cm dbh, the plots had an average stem density of 367 stems per hectare, basal area of 19.3 m²/ha, and species richness of 59 tree species per ha. There are about 160 tree species identified at La Chonta, 23 of which are considered marketable (VERWER et al., 2008). The average harvest volumes are about 6 m³/ha. The intended cutting cycle for all species is currently 30 years. The Forest Stewardship Council (FSC) certified the company managing the area since 1998 to 2010. Mahogany was the only species logged in the concession from 1974 to 1995; however, since then the species has not been harvested because the density of reproductive trees was below the required limits (<0.25 tree/ha with >20 cm dbh) (Ministerio de Desarrollo Sostenible y Planificación, 1998). Fires are prevalent in the landscape matrix surrounding La Chonta; most occur during the dry season. In both 1995 and 2004, about 30% of the concession burned.

Sampling

In 2009 all adult trees and all seedlings were sampled in four plots of a mahogany population in La Chonta (Figure 1). The density of reproductive mahogany trees was low in each of the plots (Table 1). Generally, we observed regeneration only close to some reproductive trees. All seedlings were sampled below the canopy of the trees. The seedlings were produced many years after mahogany was logged (last mahogany logging event occurred in 1995) and may represent regeneration from different reproductive events (two to three). As the area where the plots were established burned in 2004 and the height of the sampled seedlings ranged from 0.05 to 1.5 m, the age was probably <4 years. The number of adult trees sampled per plot ranged from 7 to 26. We collected between 13 to 24 seedlings per plot (Table 1). In plot 1, three trees and in plot 3 two trees were dead and not sampled. In plot 3 two trees indicated in a former produced map were not found, and in plot 4, one tree was not found. The dbh of the trees ranged from 9 to 54.5 cm (Table 1). We collected three to four leaves from each individual (adults and seedlings) for DNA extraction and genetic analysis. The leaves were stored in paper bags after collection and further analysis.

Microsatellite analysis

Genomic DNA was extracted from the leaves using a standard CTAB procedure (DOYLE and DOYLE, 1987). Individuals were genotyped at seven highly polymorphic microsatellite loci (sm01, sm22, sm40, sm31, sm46, sm32 and, sm51) previously developed and optimised in multiplexed fluorescence-based systems for *S. macrophylla* (LEMES et al., 2002). PCR was carried out in a final volume of 15 μ l for both single and multiplexed reactions, containing 0.1–0.5 μ M of each forward and reverse primer, 0.6 unit of Taq DNA polymerase, 200 mM of each dNTP, 1X reaction buffer (80 mM Tris-HCl, 20 mM (NH₄)₂SO₄, 0.02% Tween-20), 1.75 mM MgCl₂, 20 ng of template DNA, and ultrapure water.

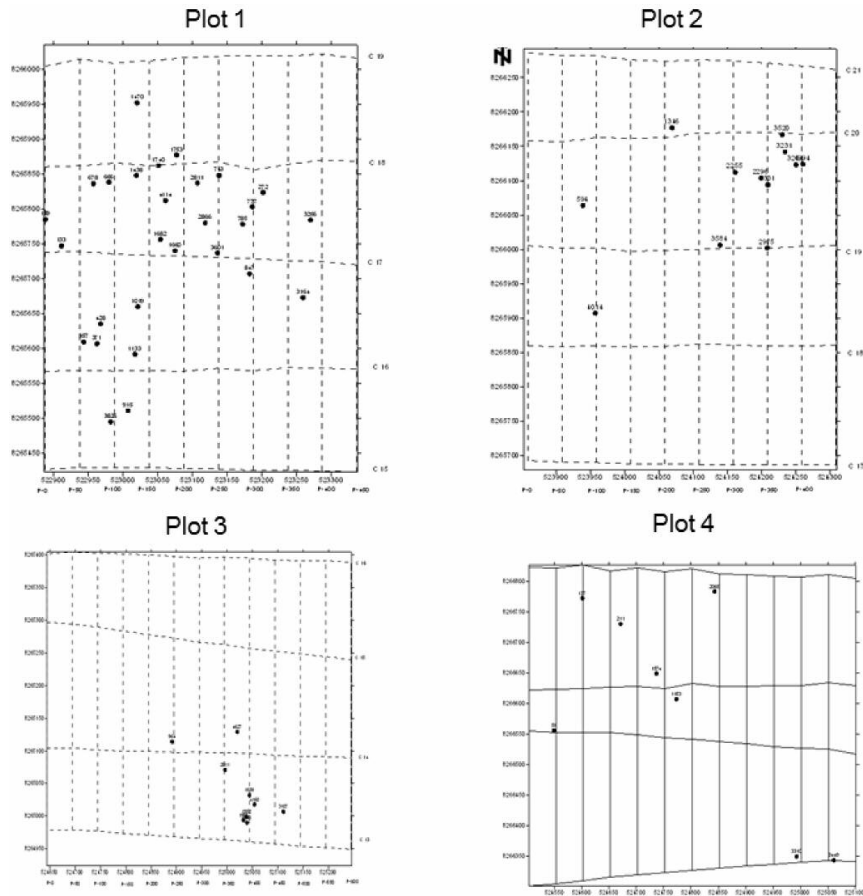


Figure 1. – Spatial distribution of *Swietenia macrophylla* trees in the four plots in La Chonta.

Amplifications were performed using a thermal cycler from Sensoquest using the following program: 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 sec, 54–60 °C (depending on annealing temperature of the primers) for 30 sec, and 68 °C for 2 min, and a final elongation step at 68 °C for 15 min. PCR products were combined with 8 μ l diluted size standard ET-400 ROX (GE Healthcare). Separation and detection of the fragments were carried out on a MegaBACE 1000 96 capillary automated sequencer (GE Healthcare) using Fragment Profiler software version 1.2 (GE Healthcare).

Analysis of genetic diversity, inbreeding and differentiation among plots

The genetic diversity was analysed for the all adults and all juveniles merged from all plots. We computed the total number of alleles across all loci (k), average number of alleles per locus (A), allelic richness (R), expected heterozygosity (H_e) and observed heterozygosity (H_o). The allelic richness (R) was estimated by rarefaction (EL MOUSSADIK and PETIT, 1996). We estimated the fixation index (F) as an indicator of the levels of inbreeding, and tested the statistical significance by using 1,000 Monte Carlo permutations (alleles among

individuals re-sampled). These analyses were carried out using the FSTAT program (GOUDET, 1995).

Parentage analysis

Parentage analysis was carried out within each plot using a maximum likelihood categorical parentage analysis (MEAGHER and THOMPSON, 1987), implemented in the CERVUS 3.0 program (MARSHALL et al., 1998; KALINOWSKI et al., 2007). To verify the exclusion power for parentage analysis, we used a non-exclusion probability estimated for the parent pair (P_{Pair}) with CERVUS 3.0. The cryptic gene flow was estimated by $C_{gf} = 1 - P_{Pair}^2$ (DOW and ASHLEY, 1996). The parent pair of each seedling from each plot was determined using the Δ statistic (MARSHALL et al., 1998). The cryptic values of Δ to determine if a seed or pollen comes from outside plots were calculated using simulations in CERVUS 3.0, considering 50,000 repetitions (simulated seedlings), 0.01 as the proportion of error in the loci, all reproductive trees as putative maternal and paternal parents, and a confidence level of 80% (MARSHALL et al., 1998). We also considered the possibility of selfing; if a pair of parental candidates of a seedling was the same individual. The selfing rate (s) was calculated as the

Table 1. – Sample size (n) for adult trees and seedlings, diameter at breast height (dbh) and spatial distances among adult trees of *Swietenia macrophylla* in four plots in the Bolivian Amazon forest. P_{pair} is the theoretical combined non-exclusion probability of the parent pair; C_{gf} is the cryptic gene flow; Q_i is the combined non-exclusion probability of genetic identity.

Plot	n trees	Reproductive trees	Reproductive density/ha	Average dbh (Min-Max) cm	Average distance (Min-Max) m	Median distance (m)	n seedlings	\hat{P}_{pair}	\hat{C}_{gf} (%)	\hat{Q}_i
Plot 1	26	22	0.88	31.7 (13-54.5)	196±86 (19-459)	188	24	0.000275	0.71	0.00000030
Plot 2	11	9	0.36	39.0 (12-46)	142±99 (10-377)	114	13	0.000275	0.30	0.00000034
Plot 3	10	9	0.36	25.9 (9-42)	116±87 (8-395)	71	24	0.001094	1.09	0.00000246
Plot 4	7	7	0.28	37.0 (20-51)	267±146 (55-576)	212	17	0.001357	0.95	0.00000266

proportion of selfed seedlings (n_{selfed}), divided by the total number of sampled seedlings (n) within each plot. If we identified two parents inside the plot, the parent located closest to the seedling was considered the seed parent (NAKANISHI et al., 2008; SEBBENN et al., 2011). This assumption is supported by the observation that seeds of *S. macrophylla* are relatively heavy and tend to fall close to the mother tree (LAMB, 1966). In mahogany, bees, flies, moths and butterflies disperse pollen (STYLES, 1972) and these insects have the potential for long pollen dispersal distance, thus favouring a system of shorter seed dispersal than pollen dispersal in the species. The rate of pollen immigration from outside was estimated by the proportion of seedlings for which only one parent was assigned inside of the plot, divided by the total number of sampled seedlings.

As all trees in each plot were mapped, the average, median, minimum and maximum distances of pollen dispersal was calculated for each seedling as the distance between the mother tree and the assigned father tree. We compared the distribution of pollen distances with the distribution of the distances among all reproductive trees using the Kolmogorov-Smirnov test (SOKAL and ROHLF, 1995). We used a Pearson product-moment correlation to verify if trees with a large diameter at breast height (dbh) mothered or fathered more sampled seedlings. The effective neighbourhood pollination area (A_{ep}) was estimated for each plot from the axial pollen-dispersal variance (σ^2), assuming a circular area around a central mother tree, $A_{ep} = 2\pi\sigma^2$ (LEVIN, 1988).

Spatial genetic structure analysis

To investigate whether the adult trees displayed an aggregate distribution inside the plots, we estimated the dispersal index of CLARK and EVANS (1954) using SGS software (DEGEN et al., 2001). This analysis was carried out for the all adults merged from all plots. The following equation was used: $\hat{R}_{CE} = \hat{R}_o / \hat{R}_E$, where R_o is the average distance of the closest neighbour and R_E is the expected average distance of the closest neighbour. If $R_{CE} = 1$, $R_{CE} < 1$ or $R_{CE} > 1$, then the distribution of the individuals is considered random, aggregate, or uniform, respectively. The spatial genetic structure analysis was run only for the adults because we do not have the exact spatial position (x and y coordinates) of each seedling. To study the intra-population spatial genetic structure (SGS) in the adults of all plots, we used the Spagedi 1.3 program (HARDY and VEKEMANS, 2002) and coancestry coefficient (LOISELLE et al., 1995). The genetic SGS was analysed for the all adults merged from all plots.

Results

Genetic diversity and fixation index

For all sampled 142 individuals (adults+seedlings), the number of alleles per locus (k) ranged from 7 to 21, with an average of 13.9 and a total of 97 alleles. The observed heterozygosity varied from 0.600 to 0.730, with an average of 0.701, and the expected heterozygosity ranged from 0.601 to 0.836, average of 0.724. We observed fixation indices between -0.082 to 0.150, with an average of 0.027.

In the adults, the number of alleles per locus (k) ranged from 6 to 16, with an average of 10.9 and a total of 76 alleles (Table 2). In the seedlings, the number of alleles per locus (k) ranged from 7 to 17, with an average of 12.3 and a total of 86 alleles (Table 2). We observed 11 private alleles for the adults and 21 for the seedlings. The observed heterozygosity (H_o) was significantly lower for the seedling than adults. The fixation index (F) was significant different from zero for the average among loci in seedlings and significant different between adults and seedlings.

Seed and pollen immigration

The theoretical combined non-exclusion probability of parent pair was low for adults of each sampled plot (Table 1). Consequently, the estimated cryptic gene flow, or the probability of assigning a parent pair for a seedling when the true parent pair was outside of the plot was very low (<1.1%). The combined non-exclusion probability of genetic identity was also low (maximum 0.00000572), indicating that the loci battery used is suitable for parentage analysis because all adults of each plot have a unique multilocus genotype.

A probable mother was assigned for all seedlings in plots 1, 2 and 3, indicating no seed immigration (Table 3). In contrast, in the plot 4, no mother and father were found inside of the plot for three seedlings, suggesting 18% seed immigration. Pollen donors were assigned to 37% of the seedlings in the plot 1, all seedlings of the plot 2, 50% seedlings of the plot 3, and 41% seedlings of the plot 4. Realized selfing ranged among plots from zero to 5.8%. Excluding selfing, the assigned seedlings were likely fathered by 55% (12 trees) of the reproductive trees in the plot 1, 89% (8 trees) in the plot 2, 56% (5 trees) in the plot 3 and, 43% (3 trees) in the plot 4.

Male and female fertility

The estimate of the Spearman correlation coefficient between the dbh of mothers and the number of seedlings

Table 2. – Genetic diversity and fixation index of adult trees and seedlings of *Swietenia macrophylla* in the Bolivian Amazon forest.

Locus	Adults (N=54)					Seedlings (N=78)				
	A	R	H_o	H_e	F	A	R	H_o	H_e	F
sm01	6	6.0	0.773	0.722	-0.071	7	7.0	0.709	0.723	0.019
sm22	11	11.0	0.866	0.773	-0.120	15	13.9	0.836	0.798	-0.047
sm40	6	6.0	0.600	0.656	0.086	7	6.8	0.658	0.683	0.037
sm31	12	11.9	0.637	0.661	0.037	13	12.2	0.683	0.662	-0.032
sm46	14	13.9	0.758	0.765	0.009	17	16.1	0.684	0.751	0.089
sm32	11	11.0	0.830	0.752	-0.104	12	11.6	0.671	0.751	0.107
sm51	16	16.0	0.862	0.877	0.017	15	14.7	0.640	0.868	0.263*
Mean	10.9	10.8	0.761	0.744	-0.023	12.3	11.7	0.697	0.748	0.068*
CI _{95%} lower	10.0	9.97	0.743	0.722	-0.039	11.5	11.02	0.674	0.728	0.029
CI _{95%} upper	11.7	11.64	0.788	0.758	-0.004	13.2	12.57	0.707	0.762	0.081
Total	76	--	--	--	--	86	--	--	--	--

A is the number of alleles; R is the allelic richness for 65 multilocus genotypes; H_o is the observed heterozygosity; H_e is the expected heterozygosity; F is the fixation index.

* P < 0.05.

CI_{95%}: 95% standard error calculated by the jackknife method. The parameters H_e and F are significantly different between adults and seedlings according to the 95% confidence interval calculated by the jackknife method.

Table 3. – Realized seed and pollen immigration, pollen dispersal distance and selfing (s) in four experimental plots of *Swietenia macrophylla* in Bolivian Amazon forest. n is the sample size.

Plot	n seedlings	Immigration		Pollen dispersal distance			
		Seed	Pollen	Within plot	\hat{s}	Mean±SD [Median] (m)	Min-Max (m)
Plot 1	24	0 (0%)	9 (37%)	15 (63%)	1 (4.2%)	128±92 [117]	16-316
Plot 2	13	0 (0%)	0 (0%)	13 (100%)	0 (0%)	75±26 [78]	48-128
Plot 3	24	0 (0%)	12 (50%)	12 (50%)	1 (4.2%)	176±79 [189]	8-244
		3 (12.5%)	7 (41%)	10 (59%)		255±131	83-576
Plot 4	17	(18%)			1 (5.8%)	[183]	

Inbreeding from selfing (F_s): Individual 54 from plot 1 $F_s = -0.018$, Individual 127 plot 3, $F_s = 0.599$, Individual 102 plot 4 $F_s = 0.071$, where F_s is the individual fixation index estimated using Spagedi program (HARDY and VEKEMANS, 2002).

mothered by each tree shows a significant and positive correlation ($r=0.43$, $df=20$, $P<0.05$), showing that larger trees mothered more seedlings. However, no association was detected between the dbh of pollen donors and the number of seedlings fathered ($r=0.14$, $df=22$, $P>0.05$).

Pollen dispersal distance and patterns

Pollen dispersal distance was estimated excluding selfing (Table 3). The estimated average distance of pollen dispersal ranged among plots from 75 to 255 m. The maximum distance of pollen dispersal was 576 m (plot 4). In plot 1, 2 and 4, the mean and median pollen dispersal distance (Table 3) were lower than the mean and median distance among all sampled trees in the plots (Table 1), indicating a pattern of near neighbour pollen dispersal. In contrast, in plot 3, the mean and median pollen dispersal distance was higher than the mean and median distance among all reproductive trees

in the plot. If we group the pollen dispersal distance of the four plots, 91% of the pollen was dispersed in a radius up to 250 m. No significant difference was

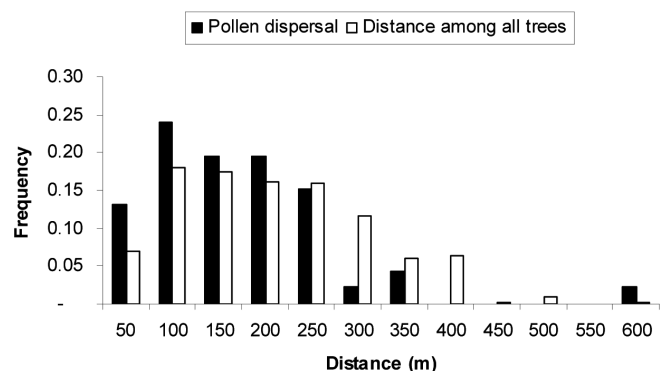


Figure 2. – Frequency of effective pollen dispersal and the distance between reproductive trees and seed trees in a *Swietenia macrophylla* population.

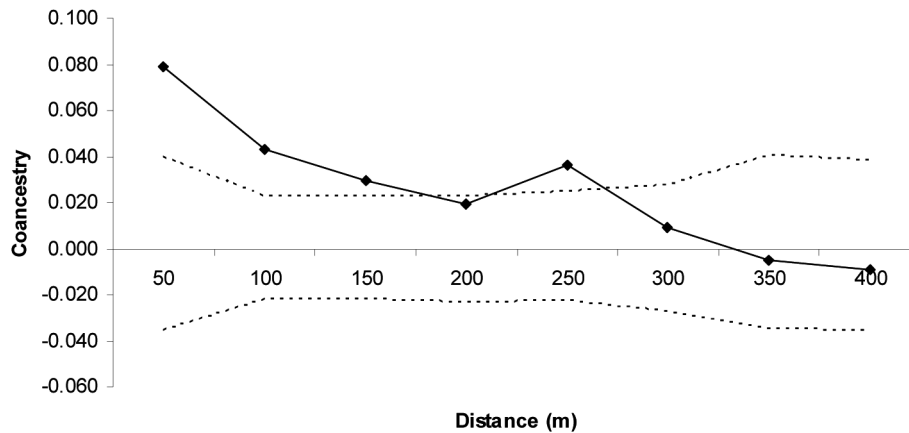


Figure 3. – Intra-population spatial genetic structure among reproductive trees in *Swietenia macrophylla*.

observed between the frequency curve of pollen dispersal and the frequency curve of the distances between all reproductive trees ($D=0.174$, $P=0.158$; Figure 2), indicating that the spatial distance among trees alone could explain the observed mating patterns. The reproductive pollination neighbour area (A_{ep}) ranged from 0.42 to 10.8 ha.

Aggregation and spatial genetic structure

The dispersal index ($R_{CE}=0.42$, $P<0.01$) estimation in the adults indicated that the distribution of the individuals within the plots was aggregated (Figure 1). The observed average distance of the closest neighbour was 59 m. We found a significant SGS at least until 150 m (Figure 3), suggesting that near neighbour individuals are relatives.

Discussion

Genetic diversity and inbreeding

We observed a higher heterozygosity (H_o) in the adults than in the seedling and we found significant departure of Hardy-Weinberg heterozygosity only in seedling stage. ANDRÉ et al. (2008) reported a similar result for a pre- and post-logging population of mahogany in the Brazilian Amazon. The authors detected a significant reduction in the observed heterozygosity and a significant increase in the fixation index in post-logging seedlings ($H_o=0.646$; $F=0.129$) as compared to the pre-logging adult cohort ($H_o=0.722$; $F=0.062$). This phenomenon has also been observed in other tropical tree species occurring in fragmented populations, as for example *Myracrodruon urundeuva* (GAINO et al., 2010) and *Copaifera langsdorffii* (SEBBENN et al., 2011). The lower heterozygosity and higher inbreeding in seedlings than adults can be caused by selfing and mating among relatives, but later on selective mortality may increase the heterozygosity and decrease the inbreeding from the seedlings stage to the adult stage (HUFFORD and HAMRICK, 2003). Furthermore, the reduction in the heterozygosity of seedlings in relation to adults depends somewhat on the foraging patterns of the pollinators vectors. In some cases, the pollinators may compensate for the

decreased reproductive adult tree density by foraging over longer distances, as have been observed in fragmented populations for *Swietenia humilis* (WHITE et al., 2002) and *Dinizia excelsa* (DICK et al., 2003) and logged population for *Hymenaea courbaril* (CARNEIRO et al., 2011).

Realized pollen dispersal

This study represents the first published genetic data available on seed and pollen flow for *S. macrophylla*. We sampled seedlings below the canopy and within a radius of 10 m of large trees. In other parts of the plots no regeneration of mahogany was observed. Our parentage analysis suggested that for the majority of seedlings sampled, near neighbour tree of the each seedling's was the mother trees. Seed immigration from outside was observed only for one plot, indicating a strong tendency toward short-distance seed dispersal. Our results agree with the previous ecological observation that seed are dispersed generally close to the mother tree due to the large size and weight of the seeds (GULLISON et al., 1996). The significant SGS in adults until 150 m also support the hypothesis of short distance seed dispersal. In contrast, substantial realized pollen immigration was detected in three of the four studied plots, indicating that seed dispersal is more restricted than pollen dispersal. As noted above, pollen of *S. macrophylla* is dispersed by bees, moths, flies, and butterflies (STYLES, 1972) and seeds are dispersed by wind (LAMB, 1966). However, seedling establishment is dependent on light availability, although survival is possible in the understory in open forest (GULLISON et al., 1996). Both wind and insects as dispersal vectors have the potential for long distance dispersal, but as the high vegetation density of the studied tropical forest (VERWER et al., 2008) may restrict more the distance of seed dispersal than the movement of insects transporting pollen of the species.

A low level of realized selfing was observed in the plots (0 to 5.8%). Similar values have been found by LEMES et al. (2007) in open-pollinated seeds in a logged *S. macrophylla* population in Brazil.

Our estimated pollen dispersal distances in three of the four plots are obviously an underestimation because a large proportion of pollen immigrated into the plot. The size of the plots also limited the maximum distance that can be detected. For example, the maximum distance that a mating event could be detected was 576 m, which represents the maximum distance between two reproductive trees in the plot 4 (Table 1). Average pollen dispersal was also lower in areas of high mahogany density. In the plot 2, pollen dispersal distance reached a maximum of 128 m. This maximum distance is lower than the average distance among reproductive trees in this plot (142 m), clearly indicating a pattern of near-neighbour pollen dispersal. In the other plots, pollen dispersal was greater, reaching the maximum possible distance in the plot 4, at 576 m. However, with the exception of the plot 3, the median pollen dispersal distance was shorter than the median distance among reproductive trees. This difference was most pronounced in the plots 1 and 2. This result suggests also a pattern of near-neighbour pollen dispersal. However, when we grouped the information of pollen dispersal distance for all plots, the distance among all reproductive trees of all plots explains the pattern of pollen dispersal. This result can be explained by the fact that the trees in the plots are grouped (Figure 1) and mating occurred generally between near-neighbour trees. The pollen dispersal function is probably bimodal seeing also the significant pollen flow from outside the plots.

Intra-population spatial genetic structure

Selective logging is expected to reduce SGS of reproductive trees by reducing the population density and increasing the distance among conspecifics. However, we found that the spatial aggregation of the adults in the plots was associated to genetic aggregation. A significant SGS until 150 m was found, showing that nearby individuals are relatives. The probable cause of the observed SGS in the adults is the limited seed dispersal in the population, as already commended. In contrast to our results, SILVA et al. (2008) reported no SGS among post-logging adult trees of the very low density dioecious Neotropical tree *Bagassa guianensis*, in the Brazilian Amazon, and LACERDA et al. (2008b) reported a decrease in SGS after logging in *Hymenaea courbaril*.

Implications for sustainable forest management

An important aspect of sustainable forest logging in natural tropical forest is the maintenance of the genetic diversity within populations. The main conclusion of this study is that although the population was overexploited, 14 year after logging, it is recovering. The reproductive trees have no inbreeding, the observed selfing was low and the significant inbreeding in seedlings may be reduced by inbreeding depression, when this generation reaches reproductive stage. We even observed many private alleles in both adults and seedlings and substantial levels gene flow from outside of the plots, by both seeds and pollen. Moreover, the studied population of mahogany was logged before minimum requirements were established in Bolivia, which resulted in an overexploitation of the species. However, new rules are cur-

rently in place in Bolivia. Studies about the effects of logging on demography of mahogany has been suggested that the species may be sustainably logged in cutting cycles >100 years (GULLISON et al., 1996). Similar result was observed in the Neotropical tree *Hymenaea courbaril* in Brazil (LACERDA et al., 2012). This new silvicultural practice may reduce the impacts of harvesting on the genetic diversity, mating system and demography of species populations because a large number of reproductive individuals will be remain in the populations after logging. Moreover, *S. macrophylla* individuals reach their reproductive maturity between 70 to 80 cm dbh (GULLISON et al., 1996). A reduction in the logging intensity associated with some levels of disturbance of the forest may favour genetic recombination through mating, seed production and natural regeneration. Obviously, this must be empirically investigated. New studies on the impact of logging on genetic diversity, mating system, intra-population spatial genetic structure, pollen and seed dispersal patterns and demography of mahogany populations are necessary in order to develop sustainable harvesting practices for the species. Studies examining the long term effects of logging must also be investigated using modelling and simulation, such as EcoGene (DEGEN et al., 1996).

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References

- AGUILAR, R., M. QUESADA, L. ASHWORTH, Y. HERRERIAS-DIEGO and J. LOBO (2008): Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. *Molecular Ecology* **17**: 5177–5188.
- ANDRÉ, T., M. R. LEMES, J. GROGAN and R. GRIBEL (2008): Post-logging loss of genetic diversity in a mahogany (*Swietenia macrophylla* King, Meliaceae) population in Brazilian Amazonia. *Forest Ecology and Management* **255**: 430–345.
- CARNEIRO, F. S., A. E. B. LACERDA, M. R. LEMES, R. GRIBEL, M. KANASHIRO, L. H. O. WADT and A. M. SEBBENN (2011): Effects of selective logging on the mating system and pollen dispersal of *Hymenaea courbaril* L. (Leguminosae) in the Eastern Brazilian Amazon as revealed by microsatellite analysis. *Forest Ecology and Management* **262**: 1758–1765.
- CASCANTE, A., M. QUESADA, J. J. LOBO and E. A. FUCHS (2002): Effects of dry tropical forest fragmentation on the reproductive success and genetic structure of the tree *Samanea saman*. *Conservation Biology* **16**: 137–147.

- CLARK, D. A. and F. C. EVANS (1954): Distance to nearest neighbour as a measure of spatial relationship in populations. *Ecology* **35**: 445–456.
- DEGEN, B., R. PETIT and A. KREMER (2001): SGS – Spatial Genetic Software: a computer program for analysis of spatial genetic and phenotypic structure of individuals and populations. *Journal of Heredity* **92**: 447–448.
- DEGEN, B., H.-R. GREGORIUS and F. SCHOLTZ (1996): ECOGENE: a model for simulation studies on the spatial and temporal dynamics of genetic structures of tree populations. *Silvae Genetica* **45**: 323–329.
- DICK, C. W., G. ETCHALECU and F. AUSTERLITZ (2003): Pollen dispersal of Neotropical trees (*Dinizia excelsa*: Fabaceae) by native insects and Africa honeybees in pristine and fragmented Amazonian rainforest. *Molecular Ecology* **12**: 753–764.
- DOW, B. D. and M. V. ASHLEY (1996): Microsatellite analysis of seed dispersal and parentage of sampling in bur oak, *Quercus macrocarpa*. *Molecular Ecology* **5**: 615–627.
- DOYLE, J. J. and J. L. DOYLE (1990): Isolation of plant DNA from fresh tissue. *Focus* **12**: 13–15.
- EL MOUSSADIK, A. and R. J. PETIT (1996): High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. *Theoretical and Applied Genetics* **92**: 832–839.
- FUCHS, E. J., J. A. LOBO and M. QUESADA (2003): Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropical dry forest tree *Pachira quinata*. *Conservation Biology* **17**: 149–157.
- GAINO, A. P. S. C., A. M. SILVA, M. A. MORAES, P. F. ALVES, M. L. T. M. MORAES, M. L. M. FREITAS and A. M. SEBBENN (2010): Understanding the effects of isolation on seed and pollen flow, spatial genetic structure and effective population size of the dioecious tropical tree *Myracrodruon urundeuva*. *Conservation Genetics* **11**: 1631–1643.
- GOUDET, J. (1995): Fstat. (Version 2.9.3.2.): a computer program to calculate *F*-statistics. *Journal of Heredity* **86**: 485–486.
- GRIBEL, R. and P. E. GIBBS (2002): High outbreeding as a consequence of selfed ovule mortality and single vector bat pollination in the Amazonian tree *Pseudobombax munguba* (Bombacaceae). *International Journal of Plant of Science* **163**: 1035–1043.
- GROGAN, J., O. BARRETO and A. VERRÍSSIMO (2002): Mahogany in the Brazil Amazon: Ecology and Perspectives on Management. IMAZON, Belém, Pará, Brazil. 60 pp.
- GROGAN, J., S. B. JENNINGS, R. M. LANDIS, M. SCHULZE, M., A. M. V. BAIMA, J. C. A. LOPES, J. M. NORGHAUER, L. R. OLIVEIRA, F. PANTOJA, D. PINTO, J. N. M. SILVA, E. VIDAL and B. L. ZIMMERMAN (2008): What loggers leave behind: Impacts on big-leaf mahogany (*Swietenia macrophylla*) commercial populations and potential for post-logging recovery in the Brazilian Amazon. *Forest Ecology and Management* **255**: 269–281.
- GULLISON, R. E., S. N. PANFIL, J. J. STROUSE and S. P. HUBBELL (1996): Ecology and management of mahogany (*Swietenia macrophylla* King.) in the Chimanes Forest, Beni, Bolivia. *Botanical Journal of the Linnaean Society* **122**: 9–34.
- HARDY, O. and X. VEKEMANS (2002): SPAGeDI: a versatile computer program to analyze spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* **2**: 618–620.
- HUFFORD, K. M. and J. L. HAMRICK (2003): Variability selection at three early life stages of the tropical tree *Platypodium elegans* (Fabaceae, Papilionoidae). *Evolution* **57**: 518–526.
- JENNINGS, S. B., N. D. BROWN, D. H. BOSHIER, T. C. WHITMORE and J. C. A. LOPES (2001): Ecology provides a pragmatic solution to the maintenance of genetic diversity in sustainably managed tropical rain forests. *Forest Ecology and Management* **154**: 1–10.
- KALINOWSKI, S. T., M. L. TAPER and T. C. MARSHALL (2007): Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* **16**: 1099–1106.
- LACERDA, E. B. L., E. R. NIMMO and A. M. SEBBENN (2012): Modeling the long-term impacts of logging on genetic diversity and demography of *Hymenaea courbaril*. *Forest Science* (in press).
- LACERDA, E. B. L., M. KANASHIRO and A. M. SEBBENN (2008a): Long-pollen movement and deviation of random mating in a low-density continuous population of *Hymenaea courbaril* in the Brazilian Amazon. *Biotropica* **40**: 462–470.
- LACERDA, E. B. L., M. KANASHIRO and A. M. SEBBENN (2008b): Effects of Reduced Impact Logging on genetic diversity and spatial genetic structure of a *Hymenaea courbaril* population in the Brazilian Amazon Forest. *Forest Ecology and Management* **255**: 1034–1043.
- LAMB, F. B. (1966): Mahogany of Tropical America: its Ecology and Management. University of Michigan Press, Ann Arbor, MI, USA.
- LAMPRECHT, H. (1990): *Silvicultura nos tropicos*. Eschborn: GTZ, 343 pp.
- LEMES, M. R., R. P. V. BRONDANI and D. GRATTAPAGLIA (2002): Multiplexed systems of microsatellite markers for genetic analysis in mahogany, *Swietenia macrophylla* King (Meliaceae), a threatened Neotropical timber species. *Journal of Heredity* **93**: 287–290.
- LEMES, M. R., D. GRATTAPAGLIA, J. GROGAN, J. PROCTOR and R. GRIBEL (2007): Flexible mating system in a logged population of *Swietenia macrophylla* King (Meliaceae): implications for the management of a threatened neotropical tree species. *Plant Ecology* **192**: 169–179.
- LEVIN, D. A. (1988): The paternity pool plants. *American Naturalist* **132**: 309–317.
- LOISELLE, B. A., V. L. SORK, J. NASON and C. GRAHAM (1995): Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany* **82**: 1420–1425.
- LOURMAS, M., F. KJELLBERG, H. DESSARD, H. I. JOLY and M.-H. CHEVALLIER (2007): Reduced density due to logging and its consequences on mating system and pollen flow in the African mahogany *Entandrophragma cylindricum*. *Heredity* **99**: 151–160.
- MARSHALL, T. C., J. SLATE, L. E. B. KRUK and J. M. PEMBERTON (1998): Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**: 639–655.
- MEAGHER, T. R. and E. THOMPSON (1987): Analysis of parentage for naturally established seedlings of *Chamaelirium luteum* (Liliaceae). *Ecology* **68**: 803–812.
- MEJÍA, E., X. BUITRÓN, M. PEÑA-CLAROS and J. GROGAN (2008): Big-leaf mahogany (*Swietenia macrophylla*) in Peru, Bolivia and Brazil. NDF Workshop Case Studies. WG1-Trees, Case Study 4 *Swietenia macrophylla*. Mexico. 36 pp.

- MORAES, M. L. T. and A. M. SEBBENN (2011): Pollen dispersal between isolated trees in the Brazilian savannah: A case study of the Neotropical tree *Hymenaea stigonocarpa*. *Biotropica* **43**: 199–199.
- NAKANISHI, A., N. TOMARU, H. YOSHIMARU, T. MANABE and S. YAMAMOTO (2008): Effects of seeds- and pollen-mediated gene dispersal on genetic structure among *Quercus salicina* samplings. *Heredity* **102**: 182–189.
- SEBBENN, A. M., B. DEGEN, V. C. R. AZEVEDO, M. B. SILVA, A. E. B. LACERDA, A. Y. CIAMPI, M. KANASHIRO, F. S. CARNEIRO, I. TOMPSON and M. D. LOVELESS (2008): Modelling the long term impact of selective logging on genetic diversity and demographic structure of four tropical tree species in the Amazon forest. *Forest Ecology and Management* **254**: 335–349.
- SILVA, M. B., M. KANASHIRO, A. Y. CIAMPI, I. TOMPSON and A. M. SEBBENN (2008): Genetic effects of selective logging and pollen gene flow in a low-density population of the dioecious tropical tree *Bagassa guianensis* in the Brazilian Amazon. *Forest Ecology and Management* **255**: 1548–1558.
- SOKAL, R. R. and F. J. ROHLF (1995): *Biometry: principles and practices of statistics in biological research*. Third Edition. W.H. Freeman and Company, New York.
- STYLES, B. T. (1972): The flowering biology of the Meliaceae and its bearing on tree breeding. *Silvae Genetica* **21**: 172–182.
- VERWER, C., M. PEÑA-CLAROS, D. VAN DER STAAK, K. OHLSON-KIEHN and F. STERCK (2008): Silviculture enhances the recovery of overexploited mahogany *Swietenia macrophylla*. *Journal Applied Ecology* **45**: 1770–1779.
- WHITE, G. M., D. BOSHIER and W. POWELL (2002): Increased pollen flow counteracts fragmentation in tropical dry Forest: an example from *Swietenia humilis* Zuccarini. *Proceeding Natiolan Academy of Science USA* **99**: 2038–2042.

Book-Review

Holzmerkmale. By C. RICHTER. DRW Verlag Weinbrenner, Leinfelden-Echterdingen, 2010, 3rd issue, 224 pages with 782 coloured figures. ISBN 978-3-97181-781-6. (39.90 €).

In 2002 the author started a series of articles in the *Holz-Zentralblatt*. Five years later the articles were published as the book “*Holzmerkmale*” [Wood characters] (82 pages). Three years later the third widened issue is available.

The book is divided into four chapters: It started with the description of the wood structure and the development of wood characters. In the second chapter wood characters which are determined naturally by biotic or abiotic effects, and types of breaks are listed in tables. The heart of the book is the third chapter which follows the structure of the previous tables. Each character is described according its importance by a unique scheme: description of the Character, measurement, reason, pre-

vention, effects on utilisation, and technological adaptation. Typical occurrences in nature are added in figures. These informative photos are of high quality. The descriptions are, compared to the former issues, explicitly widened. The effects of wood characters on sorting rules are listed in the fourth chapter. Detailed sources and a key word list complete the book.

The book is written for all those who are handling wood and those who are interested in correlation of the surface and the constitution inside and those who want to identify the reasons of specific wood characters. If in such a short time three issues of a book are published, it needs no further word on the quality of the book. The price of the book is more then suitable. The book can be recommended to wood technologists, foresters, tree workers and dendrologists.

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