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Genetic Structure and Mating System of *Manilkara huberi* (Ducke) A. Chev., a Heavily Logged Amazonian Timber Species

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Abstract

In this work, we report on the population genetic structure of the endangered tree species *Manilkara huberi*, an Amazonian tree species intensely exploited due to the high density and resistance of its wood. We investigated the patterns of spatial distribution, genetic structure, and mating system using 7 microsatellite loci and here discuss the consequences for conservation and management of the species. To examine the population genetic structure, 481 adult trees and 810 seedlings were sampled from an area of 200 ha from a natural population in FLONA Tapajós, PA, Brazil. We found relatively high and consistent inbreeding levels (intrapopulation fixation index [*f*] 0.175 and 0.240) and a significant spatial genetic structure up to a radius of approximately 300 m, most likely due to a limited seed and pollen flow. The multilocus (t_m) population outcrossing rate was high (0.995), suggesting that the species is predominantly allogamous with a pollen flow restricted to 47 m. These results suggest that *M. huberi* is spatially structured, consistent with a model of isolation by distance. Fragmentation may therefore cause the loss of subpopulations, suggesting that management programs for production and conservation should include large areas. The genetic data also revealed that for ex situ conservation, seeds should be collected from more than 175 maternal trees, in order to keep an effective population size of 500. Furthermore, as the species is widely distributed across the Amazon Forest, samples should include several populations in order to represent the highest genetic diversity possible. These results provide a blueprint to guide the production and conservation management policies of this valuable timber species.

The Amazonian Forest covers about 40% of the Brazilian territory, and it is one of the largest world reservoirs of biodiversity. A total of 4000–5000 tree species are estimated to exist in the Amazon region (Ferraz et al. 2004), and valuable species are commercially exploited by selective cutting. In the last 3 decades, after the exhaustion of the native forests of southern Brazil, the Amazon region has become the leading supplier of high-quality timber (Veríssimo et al. 1996).

Timber harvesting by selective exploitation leads to habitat fragmentation and may also reduce drastically the number of reproductive individuals, with serious consequences for the maintenance of genetic diversity and long-term survival of tree populations. Selective harvesting of trees, usually the dominant and reproductive ones, may generate spatial isolation of the remaining individuals, thus increasing inbreeding (Young et al. 1996). Although the uncontrolled exploitation of tropical forests is a common occurrence, very little is known about its effect on the mating system, gene flow, and genetic diversity of the main target species.

A better understanding of the demographic characteristics, population dynamics (Matos and Watkinson 1999), and patterns of genetic diversity (Reis et al. 1997; Ciampi et al. 2000) is a key component of any sustainable production and conservation management plans. However, this information rarely has been considered when devising tropical forest management programs (Sebbenn, Seoane et al. 2000). A genetic assessment of logging impact may help in defining adequate management strategies for each species, which in turn would allow a better combination of use and conservation practices.

Manilkara huberi, commonly known as maçaranduba, is a native tree species of the Amazonian Forest, belonging to the Sapotaceae family. This species grows to a height of about 50 m in height, with an erect and cylindrical trunk measuring up to 2 m in diameter. This is one of the most widespread Amazonian tree species and is classified as a climax species in the successional group. It is a hermaphrodite species with flowering and reproduction occurring in alternate intervals between 3 and 5 years. Due to its very hard and resistant wood, it has been a preferred target of selective harvesting by the timber industry. To the best of our knowledge, no information on the genetics of this species has been available till now.

As part of the Dendrogene Project (Kanashiro et al. 2001), which has the objective of applying scientific knowledge to promote sustainable management of the Brazilian Amazonian forests, efforts have been made to identify seedling dynamics, reproductive ecology, growth, and genetics for some "model" tree species. These species, among which stands *M. huberi*, encompass a variety of ecological characteristics related to population density, reproductive ecology, growth rate, light demands, etc., and are used in simulation studies that consider different logging scenarios (e.g., Vinson 2004; Silva 2005; Cloutier et al. 2007).

Within this context, this study focused on the genetic structure of *M. huberi* by estimating the genetic diversity, spatial genetic structure, mating system, gene flow, and effective population size in a natural forest stand in the Amazon. Based on the genetic data gathered, we discuss the potential impacts of selective timber exploitation on genetic diversity and propose strategies for in situ and ex situ conservation and sustainable management practices of the species.

Materials and Methods

Study Site, Sampling, and DNA Extraction

The studied population covers a 200-ha area of primary forest of *terra firme* in the Brazilian Amazon, at Tapajós National Forest (FLONA Tapajós), at kilometer 83 of route BR-163 Santarém-Cuiabá in Belterra county state of Pará (Figure 1). This area is within an Intensive Studies Plot used for ecological and genetic studies in the context of the Dendrogene Project. In total, the population sampled for the genetic study included 294 trees with the diameter at breast height (d.b.h.) starting from 10 cm (50% of the trees selected randomly; Figure 1) and 810 seedlings distributed in 27 open pollinated maternal families. Tissues used for DNA extraction were cambium samples of the adults and leaves from progeny seedlings. Leaf tissue was obtained after germination of seeds collected during the period of fruit dispersion. Genomic DNA extraction from cambium and leaves were carried out by a cetyl trimethyl ammonium bromide–based procedure (Doyle JJ and Doyle JL 1987) adopted from Machado et al. (2002) that uses a Fastprep Cell Disruptor (BIO 101 SAVANT) for tissue homogenization.

Microsatellite Genotyping

Genetic analysis was carried out using microsatellite markers developed and optimized for *M. huberi* (Azevedo et al. 2005). A multiple tailed primer method with fluorescence detection of 6-FAM-, HEX-, and NED-labeled human microsatellite primers (Missiaggia and Grattapaglia 2006) was used. As that system involves 3 primers, for the tailed one, longer than normal (40 bp), new optimizations were necessary for the 12 loci employed. The polymerase chain reaction (PCR) had a final volume of 13 μl, containing 3 ng of genomic DNA, 1× PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 0.27 μM of each primer, 2.0 mM of $MgCl_2$, 0.25 mg/ml of BSA (New England Biolabs, Ipswich, MA), 0.25 mM dNTP and 1.3 U Taq polymerase (Phoneutria, Belo Horizonte, Brazil) and ultrapure water. The amplifications were carried out using a ABI9700 thermocycler (Applied Biosystems, Crescent City, CA) with the following conditions: hot start at 94 °C for 10 min, 35 cycles of 94 °C for 30 s, locus-specific annealing temperature for 45 s (Table 1), and 72 °C extension for 45 s. After 35 cycles, a final stage of extension of 30 min at 72 °C was added. The PCR products were analyzed by electrophoresis in an ABI-Prism 377XL sequencer (Applied Biosystems). The ROX-labeled fluorescent internal size standard used was developed by Brondani and Grattapaglia (2001).

Data Analysis

Genetic Diversity

Seven polymorphic microsatellite loci were characterized for number of alleles per locus (A), allele frequency, and observed (H_o) and expected (H_e) heterozygosity under Hardy–Weinberg expectation (HWE). Tests for HWE and 2-loci linkage disequilibrium were also carried out. Wright fixation indices (f and F, respectively, equivalent to F_{IS} and F_{IT}) were estimated based on all individuals genotyped from both generations. The consistency of the estimates was calculated by a bootstrap resampling using 10 000 permutations. Genetic analyses were carried out using the software Genetic Data Analysis (Lewis and Zaykin 2001).

Spatial Genetic Structure

To analyze the intrapopulation spatial genetic structure, the geographic coordinates (GPS) of the sampled trees were registered. The presence and extension of spatial genetic structure within the population was based on all 7 microsatellite loci and all 294 sampled trees. All the possible pairwise combinations of trees were considered for each one of the predefined distance classes. This estimate was obtained by calculating the Moran's I index (Sokal and Oden 1978) and the Loiselle et al. (1995) coancestry coefficient (θ_{xy}) for each distance class and locus and for the overall locus mean. These analyses were carried out using the Spatial Genetic Software (Degen et al. 2001; Degen 2003) and Spagedi version 1.1 (Hardy and Vekemans 2002), respectively. Moran's I index values were used to test the significance of the deviations of the expected values from the null hypothesis of random distribution using a Bonferroni correction. The 95% confidence interval (CI)

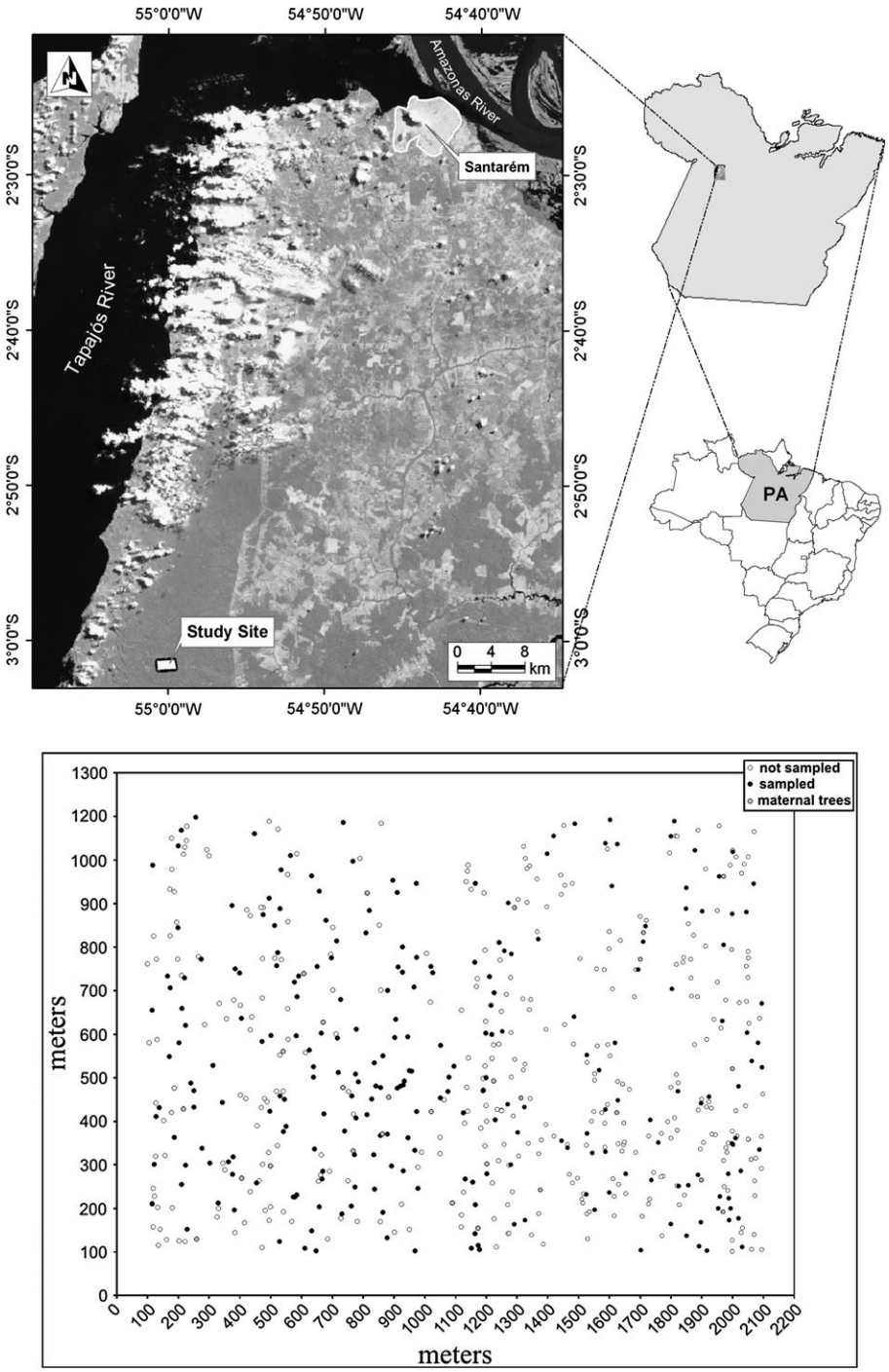

Figure 1. Localization of the study area at the National Forest of Tapajós, Pará, Brazil, from NASA. Distribution of the adult individuals within 200 ha in Brazilian Amazon, at FLONA Tapajós.

of the medium coancestry coefficient for each distance class was built based on the standard deviation of the average of the estimates, obtained by the jackknifed permutation of loci.

Gene Flow

Gene flow through pollen was evaluated within the open pollinated progenies using the software TWO-GENER (Smouse et al. 2001). The principle of this method is to estimate Φ_{ft}, the differentiation of allele frequencies among the pollen pools sampled by several females in the population. The relation between Φ_{ft} and dispersal distance has been derived for given dispersal curves (Austerlitz and Smouse 2001). The parameter is calculated by an analysis of molecular variance (Excoffier et al. 1992). Other estimated

Table 1. Main features of the microsatellite markers

Loci	Repeat motif	Primer sequence (5′–3′)	Allele range size (bp)	N° ind.	T_m (°C)	*A*	*f*	*F*
Mh04	$(CT)_{12}$	F: GCACTCTCCATGGTTCCAGT; R: AAAGAGTCAATGGCGTGAGC	187–225	1093	51	18	0.170	0.210
Mh06	$(GA)_{14}$	F: ACACGCACAAAACAAACCAA; R: TTCTTGAAGGAGGGTTGCTC	178–210	1065	53	24	0.233	0.237
Mh08	$(CT)_{11}$	F: GTAATGGGAGCCGTTTGAGA; R: CTGGGTAGCATTTGTTGCAT	194–226	1098	53	17	0.103	0.142
Mh17	$(CT)_{13}$	F: CACGATGACCTCTCAGTGGA; R: CCTGTGTATGCGTTCGATTG	240–282	1054	53	24	0.128	0.136
Mh19	$(CT)_{21}$	F: AATTACAACCAAAGCTCCACTT; R: TGAGAGTCTTTTCGCACTTTCA	162–198	1096	53	22	0.405	0.424
Mh20	$(GA)_{13}$	F: GAAGTTTTGACCATTTGGGAAT; R: GACATAACACTAACCCTTCACGA	152–194	1034	53	22	0.217	0.224
Mh22	$(CT)_{15}$	F: CCCATTATAGCCCTCCACCT; R: AGAGAGCACATGCAAGCTCA	186–218	1099	53	17	0.307	0.371
Mean						20.57	0.222	0.249
Upper							0.301	0.330
Lower							0.154	0.178

CI 99%. *N° ind.* Total number of individuals analyzed from both generations; *A*, allele number; *f*, intrapopulation fixation index within generation; *F*, overall fixation index for all individuals.

parameters were as follows: the medium pollen flow distance (δ) and the density of reproductive trees per hectare (*d*). A 95% CI of Φ_{ft} was calculated by performing 10 000 bootstraps resamplings.

Mating System

The preferential system of mating was assessed based on a mixed mating model (Ritland and Jain 1981) and correlated mating model (Ritland 1989), using the software Multilocus MLTR (Ritland 2002). The following estimates were obtained: the multilocus population outcrossing rate (t_m), the average single-locus outcrossing rate (t_s), the biparental inbreeding, that is, mating among related individuals ($t_m - t_s$), the correlation of outcrossed paternity within progeny arrays ($r_{p(m)}$) corresponding to the probability that a randomly chosen pair of progeny individuals from the same family are full sibs (Ritland 1989), the self-pollination correlation ($r_{p(s)}$), the size of the neighborhood, or the number of pollen donors ($1/r_p$). The standard errors were obtained by 1000 bootstraps resamplings across families.

To verify the potential occurrence of a genetic structure within progenies, a coancestry coefficient (θ_{xy}) between plants within progeny was estimated. This coefficient is obtained from the relatedness coefficient (r_{xy}), calculated based on the mating system parameters, according to Ritland (1989)

$$\hat{r}_{xy} = 0.25(1 + \hat{F}_m)[4\hat{s} + (\hat{t}_m^2 + \hat{r}_{p(s)}\hat{t}_m\hat{s})(1 + \hat{r}_{p(m)})], \quad (1)$$

where F_m is the intrapopulation fixation index (F_{IS}) of the parental generation, *s* is the selfing rate ($1 - t_m$), and the other parameters are as defined previously. In diploid species, in the absence of inbreeding, the relatedness coefficient r_{xy} is twice the coancestry coefficient θ_{xy}, that is, $\hat{\theta}_{xy} = \hat{r}_{xy}/2$.

To detect the genetic representativeness of families, the variance effective size ($N_{e(v)}$) was estimated, which, as demonstrated by Cockerham (1969), can be obtained from the coancestry coefficient (θ_{xy}),

$$\hat{N}_{e(v)} = \frac{0.5}{\hat{\theta}_{xy}\left(\frac{n-1}{n}\right) + \frac{1+\hat{F}_p}{2n}}, \quad (2)$$

where F_p is the progeny intrapopulation fixation index (F_{IS}). The maximum theoretical value of $N_{e(v)}$ in an ideal population's simple progeny (infinite size, panmictic, and without selection, mutation, or migration) is approximately 4. From the estimate of the variance effective size ($N_{e(v)}$), the number of maternal trees necessary to keep the effective size ($N_{e(ref)}$) equal to 500 was estimated by $\hat{N}_{e(ref)}/\hat{N}_{e(v)}$, as shown by Sebbenn (2003).

Results

Microsatellite Markers

Among the 12 polymorphic loci available from the development study (Azevedo et al. 2005), only one, *Mh12*, did not amplify. Seven were selected for the analysis based on higher information content and robustness of the analysis with the multiple tailed primer method. The mean number of alleles/locus (*A*) in the adults' generation varied from 15 (*Mh22*) to 20 (*Mh17*, *Mh20*), with a mean of 17.7, totaling 124 alleles. In the progeny population, the mean number of alleles/loci varied from 14 (*Mh04*, *Mh22*) to 24 (*Mh06*), with mean of 18.1, totaling 127 alleles (Table 1). High levels of multiallelism were observed at all 7 loci analyzed in both generations. Slightly more number of alleles was observed in the progeny population when compared with the adults, and both generations displayed

private alleles, suggesting that the reproductive population is actually larger than the one genotyped. However, by comparing the frequency of the more frequent alleles between the 2 generations, no significant differences were observed. Discrepancies were thus limited to rarer alleles.

Population Genetic Parameters

All 7 microsatellite loci displayed high levels of polymorphism within both generations. The mean values of expected (H_e) and observed (H_o) heterozygosity and the intrapopulation fixation index (f) were high at almost all loci, and $\hat{H}_e$ was higher than H_o consistently at all loci sampled, with means in adult and seedling cohorts of H_e being 0.86 and 0.82, respectively. The mean fixation index was significantly different from zero in both adult ($f = 0.18$) and seedling ($f = 0.24$) cohorts (Table 2). The mean value of f was not significantly different between the 2 generations, in agreement with the deviation observed in the progeny population. The intrapopulation fixation index (f) for all individuals (both generations) displayed values similar to the ones obtained for each generation separately. A Fisher's exact test revealed deviations from HWE at all loci, with an excess of homozygotes at all loci. Linkage disequilibrium tests detected significance between 2 pairs of loci *Mb17/Mb20* ($P = 0.013$) and *Mb20/Mb22* ($P = 0.001$) for the adult generation.

Table 2. Genetic diversity parameters estimated for both generations with 7 microsatellite marker loci

Locus	*n*	*A*	H_e	H_o	*f*
Adults					
Mb04	294	17	0.840	0.769	0.084
Mb06	288	18	0.864	0.761	0.120
Mb08	294	17	0.863	0.786	0.090
Mb17	276	20	0.890	0.736	0.174
Mb19	294	17	0.884	0.636	0.281
Mb20	280	20	0.894	0.671	0.249
Mb22	294	15	0.759	0.585	0.229
Mean	288.7	17.7	0.856	0.706	0.175
99% CI upper bound					0.222
99% CI lower bound					0.121
Progeny					
Mb04	799	14	0.711	0.564	0.206
Mb06	777	24	0.865	0.627	0.275
Mb08	804	15	0.854	0.762	0.107
Mb17	778	22	0.861	0.766	0.111
Mb19	802	17	0.841	0.460	0.453
Mb20	754	21	0.879	0.699	0.205
Mb22	805	14	0.759	0.504	0.336
Mean	788.4	18.1	0.824	0.626	0.240
99% CI upper bound					0.324
99% CI lower bound					0.156

CI 99%. *A*, allele number; H_e, expected heterozygosity (gene diversity); H_o, observed heterozygosity; *f*, intrapopulation fixation index.

Spatial Genetic Structure

The spatial autocorrelation analysis detected a significant spatial genetic structure up to a radius of approximately 300 m, based on the Moran's *I* index, suggesting that seeds are dispersed at a rather short distance. A continuous decrease in the autocorrelation values was detected with increasing distances, and from 900 m onward, it showed significant negative values, suggesting that nearby trees are genetically related and distant trees are not (Figure 2A). This behavior is consistent with isolation by distance model of gene flow.

A similar result was obtained when using the software Spagedi that calculates the coancestry coefficient based on the method described by Loiselle et al. (1995) (Figure 2B). This analysis also indicated significant positive values for up to 300 m and negative significant values starting from about 1000 m, judging by the 95% CI. The largest coancestry coefficient was estimated in the first class of distance (0–100 m, $\hat{\theta}_{xy} = 0.042$; $P < 0.05$), which is below the one expected for second-degree cousins (0.0312).

Gene Flow and Mating System

The global estimate of Φ_{ft} was 0.145 (95% CI). Given that the average distance between the sampled females ($\bar{D}$) was 739 m, this translated into an estimate of a pollen dispersal distance δ of 47 m ($\pm$0.73 m), assuming a normal pollen dispersal function. The estimate of the density of reproductive trees for hectare (d) was 1.9. Multilocus outcrossing rates ranged from 0.96 to 1.2 across the 27 families, with the average across all seedlings ($n = 810$) being 0.995 ($\pm$0.003), indicating that *M. huberi*, like most tropical trees studied to date, is predominantly outcrossed but capable of self-fertilization (Nason and Hamrick 1997). Single-locus estimates varied from 0.90 to 1.2 across families, with the average for all seedlings of 0.718 ($\pm$0.012), which is lower and significantly different from the multilocus outcrossing rate. The difference between the 2 estimates ($\hat{t}_m - \hat{t}_s = 0.277 \pm 0.012$) was highly significant, suggesting that biparental inbreeding, that is, mating among relatives, is a frequent occurrence. The multilocus correlation of outcrossed paternity ($r_{p(m)}$) suggests that 18.9% of the progeny are full sibs (0.189 ± 0.022) (Table 3). The single-locus correlation of outcrossed paternity ($\hat{r}_{p(s)} = 0.197$ or 19.7%) was higher than the multilocus but not significantly different. Coancestry estimations within the progenies showed values ($\hat{\theta}_{xy} = 0.175$) that were between those expected in half sibs (0.125) and full sibs (0.25). With the estimation of the variance effective size $\hat{N}_{e(v)} = 2.85$ (Equation 2), the minimum number of mother trees to be considered in a seed collection program for ex situ conservation was estimated. For long-term maintenance of genetic diversity, seeds should be collected from at least 175 trees.

Subpopulation Analysis

As the studied population covered a relatively large area of 200 ha, a tentative subpopulation analysis of all adult individuals was carried out by distributing all adults in 10

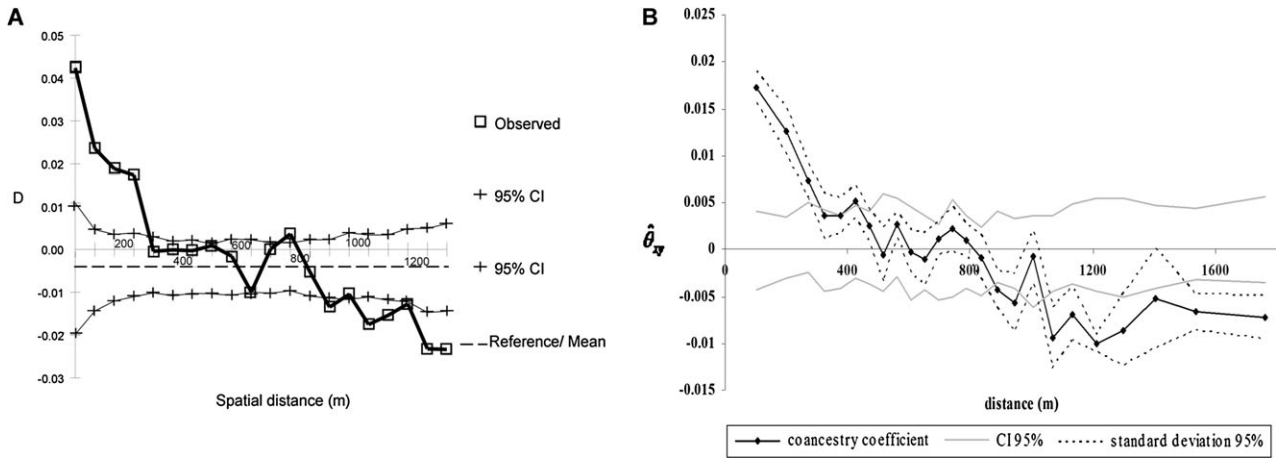

Figure 2. Correlograms of the spatial genetic structure of *Manilkara huberi*. (**A**) Index I Moran correlogram; (**B**) Coancestry coefficient histogram, Loiselle et al. (1995).

d.b.h. classes. Little significant variation for the estimated parameters was observed across classes. The mean values of f and F were positive and significant, indicating excess of homozygotes within each d.b.h. class, which simply reflects what had been detected previously for the whole population. No significant difference among the d.b.h. classes, as estimated by $\theta - P$ (F_{ST}), was detected. The 21- to 30-cm and 71- to 80-cm classes displayed the higher values of f (0.235 and 0.228, respectively) and the 101- to 140-cm class had the lowest value (0.086). When subdivided in 2 subpopulations, 1) noncommercial trees (d.b.h. $<$ 45 cm) and 2) commercial trees (d.b.h. $>$ 45 cm), the genetic divergence between them was low and also not significant (data not shown).

Discussion

All the 7 loci used for the genetic analysis in this work detected high levels of multiallelism, confirming the high expected genetic information content of these markers for studies of *M. huberi* populations (Azevedo et al. 2005). The detection of alleles in the progeny and not in the adults can be attributed as much to pollens from outside the sampled area as to pollens from individuals of the area that were not sampled. Alleles detected in the adults and not in the progeny implicate differential reproductive success or sampling effect of progeny individuals.

Table 3. Mating system parameters for *Manilkara huberi*

Parameter	Estimate
t_m	0.995 (0.003)
t_s	0.718 (0.012)
$t_m - t_s$	0.277 (0.012)
$r_{p(m)}$	0.169 (0.010)
$r_{p(s)}$	0.197 (0.022)
$1/r_{p(m)}$	5.9

Standard errors through 1000 bootstraps over families in parentheses. t_m, multilocus outcrossing rate; t_s, single-locus outcrossing rate; $t_m - t_s$, biparental inbreeding; $r_{p(m)}$, paternity correlation; $r_{p(s)}$, self-pollination correlation; $1/r_{p(m)}$, number of pollen donors per mother tree.

The mean heterozygosity estimates ($\hat{H}_e = 0.843$ and $\hat{H}_o = 0.648$) indicate high genetic diversity and a relatively high level of inbreeding in the studied population, evidenced by the high value of f (0.231) in both generations and in all d.b.h. classes of the sampled adults, favoring the hypothesis that frequent consanguineous matings are occurring in this population and not the overestimation due to the presence of null alleles, thus deviating from panmixia. Similar results were obtained for *Swietenia macrophylla* by Novick et al. (2003), *Caryocar brasiliense* by Collevatti et al. (2001), *Dinizia excelsa* by Dick et al. (2003), and *Symphonia globulifera*, *Vouacapoua americana*, and *Sextonia rubra* by Hardy et al. (2006). Different results for the intrapopulation fixation index have been found for other tropical species such as *Carapa guianensis* (Dayanandan et al. 1999) and *Swietenia humilis* (White and Powell 1997), where no deviation from HW proportions was seen.

The establishment of large-scale sampling programs to assess genetic diversity and allow an accurate view of the genetic landscape is usually the limiting factor when the goal is to apply autocorrelation techniques (Diniz-Filho and Telles 2001). Some researchers have pointed out that spatial autocorrelation analyses should not be carried out with fewer than 20 or 30 samples (Sokal and Jaquez 1991). In the case of *M. huberi*, the spatial patterns are clear as the spatial autocorrelation seems to be powerful enough to detect population spatial structure. The application of the spatial autocorrelation analysis to define units for conservation is useful when it is possible to recover the main patterns of genetic variation across the geographic landscape (Diniz-Filho and Telles 2001). This is expected to preserve most of the existing genetic variation.

Restricted dispersion of seeds has been observed for other tropical species for which dispersers are small animals,

as in the case of *C. guianensis* (182 m) and *V. americana* (176 m), and even for species for which dispersers are bats, birds, and arboreal mammals, such as *S. globulifera* (141 m) (Hardy et al. 2006). For a tropical forest tree species *Cordia alliodora*, Boshier et al. (1995) showed that near neighbors were highly genetically related, in comparison with distant trees. Hamrick (1993) showed that a spatial genetic structure was present in intermediate-diameter classes for *Platypodium elegans*, *Alseis blackiana*, and *Swartzia simplex* in Panama.

In spite of the high outcrossing rates (99%) detected in the population, the test of HWE showed deviation from random mating, which might have been caused by mating among relatives. Due to the floral structure of the species, selfing within the same flower is prevented because the pollen shed and stigma maturation occur at different times (Dendrogene 2004). Results indicating a low selfing level were expected because hermaphrodite species commonly possess autoincompatibility systems to avoid it. Murawski (1995) showed that at least 34% of the tropical tree species show some autoincompatibility. In spite of this low selfing rate, the outcrossing rate among relatives is high (27.7%), an aspect that contributes to inbreeding. Due to the restricted seed flow and population structure, mating among relatives may happen once the species shows high density in the primary forest and synchronous flowering, which generates a dense offer of flowers at a short distance.

The correlation of outcrossed paternity within progeny arrays ($r_{p(m)}$) in *M. huberi* was estimated as 18.9%, indicating that this phenomenon contributes considerably to the inbreeding seen in the species. The presence of correlated paternity in the progeny derived from open pollination indicates nonrandom pollen sampling and is likely due to the availability of few pollen sources (Sampson 1998). The estimated number of pollen donors per tree was 5.9, which is lower than the estimate for other tropical tree species such as *Solanum lycocarpum* (10.2–10.49), by Martins et al. (2006). Due to its influence on the creation, maintenance, and erosion of the genetic structure of the adult population, it is important to understand which factors influence pollen movement. If pollen flow is restricted, a few local, reproductively active trees may be overrepresented in succeeding generations (Dyer and Sork 2001).

The mean distance of pollen flow obtained was extremely low ($\hat{\delta}$ = 47 m). The restricted pollen flow can be related to the reproductive biology of the species. *Manilkara huberi* flowers suprannually, though this is intense and synchronous. The intense localized offer of flowers allows the pollinators to forage restricted areas as it is unnecessary to search for food over long distances. Pollinators of *M. huberi* are insects such as flies and bees of medium size, which can travel up to 2000 m while foraging, a distance much greater than the pollen flow detected. In a similar fashion, low pollen flow was detected in an undisturbed population of *D. excelsa* (212 m), a tropical tree species, by Dick et al. (2003).

The estimate of genetic divergence of the crossed pollen revealed that the pollen that fertilized the different maternal trees was genetically heterogeneous (Φ_{ft} = 0.14). In other words, 14% of the variation in the allele frequencies of the crossed pollen in the population was among the maternal trees. This estimate of the divergence among the crossed pollen obtained for *M. huberi* is much higher than the estimate obtained for the species pollinated by animals *Albizia julibrissin* (Φ_{ft} = 0.062; Irwin et al. 2003). High estimates of Φ_{ft} indicate patchy pollen pools and likely low pollen flow.

Implications for Conservation and Sustainable Use

Microsatellite markers have been important tools for generating detailed pictures of genetic diversity and population structures. These data, in turn, aid in the development of strategies for sustainable forest conservation and management practices. Lemes et al. (2003) suggested in situ conservation strategies for *S. macrophylla* that preserve large areas to minimize the loss of diversity due to genetic drift. Bottin et al. (2005), in their study of *Santalum austrocaledonicum*, defined 2 evolutionary significant units (ESUs) in their study populations, based on the estimates of F_{ST} values. They also recommended large reserves to conserve tree species diversity.

The relatively high and consistent inbreeding levels and high and significant spatial structuring, due to low seed as well as low pollen flow, suggest that isolation by distance is a major factor shaping the population structure of *M. huberi*. The pattern found in this study is consistent with other large-scale studies of tropical trees, such as *Euterpe edulis* in the Atlantic Forest (Cardoso et al. 2000), *C. brasiliense* in the Cerrado of Brazil (Collevatti et al. 2001), and *S. macrophylla* in the Amazon region (Lemes et al. 2003; Novick et al. 2003). The few old and tall adults of a forest often contribute the most gametes to succeeding generations, and some of them at least should be the targets of highest priority when establishing in situ genetic reserves.

Common forest management practices often remove about 90% of the major reproduces with d.b.h. greater than 45 cm, greatly lowering the reproductive potential of the population. The decreased density will likely reduce mating among relatives but may increase self-fertilization because of the greater distance between remaining individuals. However, selfing appears to be rare in *M. huberi* and density may remain high even after exploitation. Depending on the severity of the selective logging, genetic bottlenecks may result, but Dick et al. (2001) reported an increase in outcrossed seed production in disturbed habitats for *D. excelsa*. In the specific case of *M. huberi*, a careful logging system actually may help in eliminating part of the observed inbreeding. We are currently following this approach in a parallel study and expect to acquire data within a few years. Simulation studies considering different logging scenarios have also been carried out. We expect that these results will contribute to designing a sounder management strategy for this species and the whole tropical forest.

Deviations from random mating, high correlated paternity, and the likely restricted pollen flow found for *M. huberi* all have strong implications for in situ conservation

as well as seed collection strategies for prebreeding programs or recovery of degraded areas. In a species such as *M. huberi*, genetic conservation must be integrated into forest management. The establishment of in situ conservation programs by defining parks and reserves may represent the major component of a conservation strategy, especially if those areas are not already fragmented. Management programs must include large areas in order to conserve the highest genetic diversity possible because *M. huberi* is highly spatially structured and fragmentation may lead to the loss of subpopulations. This strategy may minimize the loss of diversity due to genetic drift and maximize the conservation efficiency of the regional genotypic diversity. For ex situ conservation, seeds should be collected from at least 175 maternal trees. As the species is highly distributed throughout the Amazon forest, samples must include the largest number of populations in order to conserve as much genetic diversity as possible, thus maintaining a large effective population size of several hundred individuals.

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