

# Outcrossing rate of *Couratari multiflora* (J.Smith) Eyma (Lecythidaceae), a low-density tropical tree species from a central Amazonian rainforest

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## ABSTRACT

A multilocus mixed-mating model was used to evaluate the mating system of a population of *Couratari multiflora*, an emergent tree species found in low densities (1 individual/10 ha) in lowland forests of central Amazonia. We surveyed and observed phenologically 41 trees in an area of 400 ha. From these, only four mother trees were analyzed here because few of them set fruits, which also suffered high predation. No difference was observed between the population multilocus outcrossing rate ( $t_{mp} = 0.953 \pm 0.040$ ) and the average single locus rate ( $t_{sp} = 0.968 \pm 0.132$ ). The four mother trees were highly outcrossed ( $t_m \sim 1$ ). Two out of five loci showed departures from the Hardy-Weinberg Equilibrium (HWE) expectations, and the same results occurred with the mixed-mating model. Besides the low number of trees analyzed, the proportion of loci in HWE suggests random mating in the population. However, the pollen pool was heterogeneous among families, probably due to both the small sample number and the flowering of trees at different times of the flowering season. Reproductive phenology of the population and the results presented here suggest, at least for part of the population, a long-distance pollen movement, around 1,000 m.

**KEYWORDS:** mating system, *Couratari spp.*, genetic conservation, Amazon.

## Taxa de cruzamento de *Couratari multiflora* (J.Smith) Eyma (Lecythidaceae), uma espécie arbórea tropical de baixa densidade da Amazônia central

### RESUMO

Foi utilizado um modelo de cruzamento misto multilocos para analisar o sistema de cruzamento de uma população de *Couratari multiflora*, espécie arbórea emergente encontrada em baixas densidades (1 indivíduo/10 ha) nas florestas de “terra firme” da Amazônia central. Inventariamos e observamos fenologicamente 41 árvores em uma área de 400 ha. Dessas, somente quatro árvores-mãe foram analisadas, pois poucas árvores produziram frutos, os quais também sofreram alta predação. Não foi observada diferença entre a taxa de cruzamento multilocos ( $t_{mp} = 0,953 \pm 0,040$ ) e a taxa média de loco único ( $t_{sp} = 0,968 \pm 0,132$ ). As quatro árvores-mãe apresentaram alta taxa de cruzamento ( $t_m \sim 1$ ). Dois dos cinco locos analisados mostraram desvios das expectativas do Equilíbrio de Hardy-Weinberg (EHW), e os mesmos resultados ocorreram para o modelo misto de cruzamento. Apesar do baixo número de árvores analisadas, a alta proporção de locos em EHW sugere que a população tenha um sistema de cruzamento aleatório. Entretanto, o conjunto polínico foi heterogêneo entre famílias, provavelmente pelo pequeno número e florescimento das árvores analisadas em diferentes fases da estação de florescimento. A fenologia reprodutiva da população e os resultados apresentados neste estudo sugerem que ao menos parte da população cruza via movimento de pólen de longa distância, em torno de 1.000 m.

**PALAVRAS-CHAVE:** taxa de cruzamento, *Couratari spp.*, conservação genética, Amazônia.

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## INTRODUCTION

Many tropical tree species occur in low densities (Hubbell and Foster, 1986; Clark and Clark, 1996; Oliveira and Mori, 1999; Condit *et al.*, 2002), hence there is an important need to understand their genetic structure and mating system in order to ensure their conservation. Studies conducted on low-density species have revealed a mixed to predominant, or complete outcrossing mating system, typically coupled with low genetic differentiation among the populations (Hamrick and Murawski, 1990; Murawski and Hamrick, 1991; Murawski, 1995; Nason and Hamrick, 1997; Lepsch-Cunha *et al.*, 2001). However, the great majority of these studies were developed in relatively small areas and with samples of adult trees, and not with localization of all adult trees continuously inventoried.

In this paper, we report results on the mating system of *Couratari multiflora*, a large tree species found in lowland forests of central Amazonia at low densities (1 tree per each 10 ha). It is probably an uniformly low-density species throughout its geographical range, as the available information on its abundance in other locations shows (Mori and cols., 1987; Mitchel and Mori, 1987). In a previous work on the species, we found high genetic diversity for both seedling and adult trees when compared to other tropical trees (Lepsch-Cunha *et al.*, 1999). In a primary forest, we located all adult s in an area of 400 (ha) continuously, which permitted the monitoring of their phenological behavior at the population level (Lepsch-Cunha and Mori, 1999). The resulting data coupled with the estimated outcrossing rate provided us with an understanding of the mating system of this species. Our objectives here are to estimate the outcrossing rate, to test the assumptions of the mixed-mating system model of Ritland and Jain (1981), and to contribute to the understanding of rare species and its biological conservation implications.

## MATERIAL AND METHODS

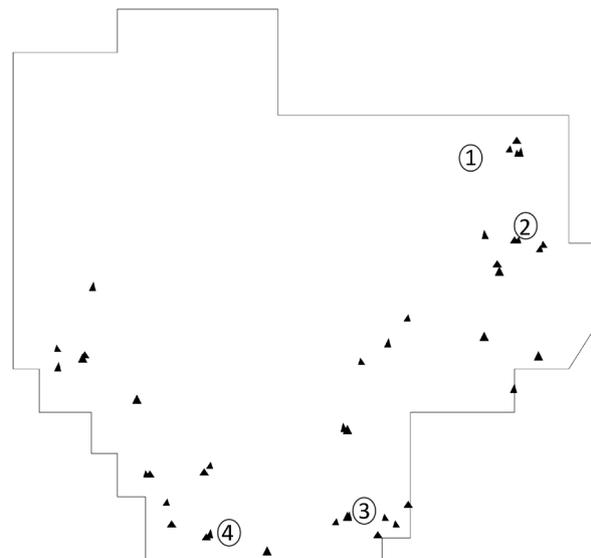
### THE STUDIED SPECIES

*Couratari multiflora* (J.Smith) Eyma (Lecythidaceae) occurs from Venezuela throughout the Guianas and the Brazilian Amazon (Mori and Prance, 1990). This species is represented by emergent trees reaching 40-50 m in height, which possess winged seeds dispersed by the wind (Mori and Lepsch-Cunha, 1995). The flower is zygomorphic and is visited by few, specialized pollinators, such as large euglossine bees (Mori *et al.*, 1980; Mori and Boeke, 1987). The wood is known as "tauari" and is harvested extensively for timber.

### DATA COLLECTION

Field work was conducted in a 10,000-ha reserve, nominated Reserve 1501, inside a continuous primary forest

of the Biological Dynamics of Forest Fragments Project (BDFFP), located about 90 km north of Manaus (Amazon, Brazil, 2° 30' S, 60° W) (Bierregaard and Gascon, 2001). Initially, we searched continuously for all trees considered adults or with DBH  $\geq$  20.0 cm in an area of 400-ha, resulting in 41 trees, and with a density of 0.106 ind./ha (Figure 1). During one reproductive season (Nov 1992 to December 1993), all of them were phenologically observed, resulting in a low production of mature fruits. The population flowered from January to June, with a peak in the mid-wet season (March), and fruits during the dry season, from May to September. Twenty seven trees out 41 flowered, and from these 27, nine were seen bearing just immature fruits, which were highly predated by animals, and nine bore mature ones (Lepsch-Cunha and Mori, 1999). Out of these nine mature-fruited trees, only four seed families (progenies) were sampled, also as consequence of a high predation of fruits by monkeys and macaws. Summing up to this, each of these four trees flowered in different times of flowering season, i.e., in the beginning, peak and end. Even considering the statistical problems imposed by these limitations, which are indicated and commented throughout the results, the phenological population study made before brought strong information that were summed here, considering the urgency to bring biological results about very low density tropical tree species.



**Figure 1** - Spatial distribution of the 41 trees (black triangles) and four studied *C. multiflora* trees (numbers inside the circles) in the Reserve 1501 of the Biological Dynamics of Forest Fragments Project, 90 km north from Manaus, Amazon, Brazil.

Enzyme extracts were made from leaves of an average of 25 seedlings per mother-tree (Table 3), using the complete extraction buffer of *Liriodendron* sp. (Alfenas *et al.*, 1991). The horizontal electrophoresis run was performed with penetrose gel at a concentration of 13% in the Universidade de São Paulo (USP/ESALQ) laboratory in Piracicaba city, State of São Paulo, Brazil. Histidine pH 7.0 with 4 % sucrose (Brown *et al.*, 1975) and morpholine-citrate pH 6.1 (Clayton and Tretiak, 1972) buffers were used, allowing us to interpret 5 enzyme systems: malate dehydrogenase (MDH - EC 1.1.1.37), phosphoglucoisomerase (PGI - EC 5.3.1.9), phosphoglucomutase (PGM - EC 5.4.2.2), peroxidase (PRX - EC 1.11.1.7), and shikimic dehydrogenase (SKDH - EC 1.1.1.25). Enzyme stain recipes were used as described by Murphy *et al.* (1990), Soltis and Soltis (1990) and Alfenas *et al.* (1991), and three replicates were made for each tree assayed.

## DATA ANALYSIS

We used MLTR software of Ritland (Version 1996), based on the mixed mating model (Clegg, 1980; Ritland and Jain, 1981; Brown *et al.* 1984). From progeny array data, we estimated: 1. the multilocus outcrossing rate for the population ( $t_{mp}$ ) and families ( $t_m$ ), by the Newton-Raphson method, 2. the single locus outcrossing rate,  $t_s$ , and its average for all loci,  $t_{sp}$ , which was calculated as the arithmetic mean of all  $t_s$  values, 3. the ovule (o) and pollen (p) allele frequencies, which were obtained by the expectation maximization method, 4. the variances through the bootstrap technique (1000 bootstraps used), and 5. the mother genotypes. For the calculation of the multilocus outcrossing rate of the individual trees ( $t_m$ ) and its variances, we held the population allele frequencies constant. Departure from the model's assumptions was tested for each locus through Chi-square goodness-of-fit tests made by the MLT program of Ritland (1990). Chi-square or exact-Fisher tests (Swofford and Selander, 1989) were used to test whether genotype numbers in the progeny arrays departed significantly from Hardy-Weinberg Equilibrium proportions, as expected if mating was not at random. We tested the differences between the ovule and pollen allele frequencies' contributions to the seed gene pool through the Chi-square statistic,  $c^2 = NF_{ST}(k-1)$ , with  $(k-1)$  degrees of freedom, in which  $N$  is the number of gametes in the pollen and ovule group, and  $k$  is the number of alleles. In this case, the Wright's  $F_{ST}$  (1965) is being used as a measure of genetic diversity between pollen and ovule pools, and, as in the next case, these pools are being treated as separate subpopulations. We tested the assumption of the homogeneity of the pollen pool in the population that contributed to seedling genotypes of different mother trees

through Chi-square tests, as suggested by Murawski and Hamrick (1991). Treating each family as a subpopulation and using their  $F_{ST}$ 's, we examined among family diversities in the pollen allele frequencies that contributed to the gene pool of seeds. One  $F_{ST}$  value was calculated for each locus based on  $F_{ST} = \sigma^2/[p_i(1-p_i)]$ , where  $\sigma^2$  is the variance in pollen allele frequencies among seed tree families, and  $p_i$  is the frequency of the  $i^{th}$  pollen allele averaged over all mother trees. The tests of significance for the  $F_{ST}$  values were made using Chi-square statistics suggested by Workman and Niswander (1970), namely  $c^2 = 2SF_{ST}(k-1)$  with  $[(k-1)(s-1)]$  degrees of freedom, where  $k$  = the allele number,  $s$  = the number of subpopulations (or families), and  $S$  = the total number of seedlings.

## RESULTS

### GENETIC INTERPRETATIONS

From the five enzyme systems assayed, we used five loci to estimate outcrossing rates, and seven loci for the other parameters (see explanation thereafter). From seven loci, all were polymorphic. The criteria used for a locus to be considered polymorphic was an allele frequency of 1% or more. Malate dehydrogenase (MDH) presented three banding regions, the two external ones, Mdh-1 and Mdh-3, were considered as individual loci and exhibited a dimeric pattern, with three alleles in both loci. Phosphoglucoisomerase (PGI) exhibited two banding regions, but only the slower was interpretable, dimeric and with four alleles. Phosphoglucomutase (PGM) exhibited two banding regions, considered as two monomeric loci, Pgm-1 and Pgm-2, with five alleles. Peroxidase (PRX) presented three banding regions, the two external ones distinctly as monomeric loci, but the center zone, although not entirely interpretable, appeared to be represented by more than one locus, only Prx-3 showed good staining in most seedlings, with three alleles. Shikimic dehydrogenase (SKDH) exhibited a duplicated monomeric pattern, as described by Weeden and Gottlieb (1980), with lighter duplication bands (ghost bands), which were rejected in the interpretation, the darker banding zone was interpreted as a monomeric locus with 3 alleles. All enzymes, except for peroxidase (PRX), were indifferent or showed better resolution utilizing the complete extraction buffer of *Liriodendrum* (with MTT and mercaptoethanol). PRX showed interpretable staining only without mercaptoethanol, being negatively affected by concentrations larger than 3% of hydrogen peroxide. Using PGM and SKDH without mercaptoethanol and MTT impaired the staining.

**Table 1** - Maximum likelihood estimates of the gene frequencies in the pollen and the ovule pools in progeny arrays of four *C. multiflora* trees in Central Amazonia, and corresponding  $c^2$  heterogeneity tests,  $F_{ST}$  is being used as a measure of genetic diversity between these two gametic pool (see text for details).

Locus	S <sup>a</sup>	Allele	Pollen pool (SD) <sup>c</sup>	Ovule pool (SD)	$F_{ST}$ <sup>d</sup>	$c^2$
Mdh-1	103	1	0.640(0.047)	0.625(0.047)	0.0003	0.0745
		2	0.355(0.047)	0.375(0.047)		
		3	0.005(0.007) <sup>b</sup>	<b>0.000</b>		
Mdh-3	103	1	0.897(0.029)	1	0.0400	8.57*
		2	0.052(0.021)	<b>0.000</b>		
		3	0.052(0.021)	<b>0.000</b>		
Pgi-1	103	1	0.451(0.049)	0.500(0.049)	0.0078	1.66
		2	0.364(0.047)	0.250(0.042)		
		3	0.185(0.038)	0.250(0.042)		
Pgm-1	95	1	0.605(0.050)	0.625(0.049)	0.0061	1.30
		2	0.338(0.048)	0.250(0.044)		
		3	0.057(0.023)	0.125(0.033)		
Pgm-2	102	1	0.781(0.041)	0.875(0.032)	0.0393	8.40*
		2	0.042(0.019)	0.125(0.032)		
		3	0.177(0.037)	<b>0.000</b>		
Prx-2	99	1	0.718(0.045)	0.750(0.043)	0.0011	0.24ns
		2	0.276(0.044)	0.250(0.043)		
		3	0.005(0.007) <sup>b</sup>	<b>0.000</b>		
Skdh-1	103	1	0.247(0.042)	0.625(0.047)	0.0895	19.14**
		2	0.343(0.046)	0.250(0.042)		
		3	0.410(0.048)	0.125(0.032)		

<sup>a</sup> S = number of seedlings.

<sup>b</sup> immigrant allele absent in the adult population.

<sup>c</sup> SD = standard deviation.

<sup>d</sup> genetic diversity between ovule and pollen pool

\* P < 0.05, \*\* P < 0.01

## MIXED MATING MODEL ASSUMPTIONS

Estimates of allele frequencies attributed to the pollen and ovules in the gene pool of seedlings showed significant difference at  $P < 0.01$  for Skdh-1, followed by Mdh-3 and Pgm-2 at  $P < 0.05$ . These differences could be attributed to: 1. relative differences in male and female contribution among trees, 2. immigrant pollen to the sampled population, 3. selection between the pollination time and the seedling sample, or 4. the lack of a representative sample of mother trees (Ritland and Jain, 1981). The allelic frequencies of the Mdh-3 and Pgm-2 loci showed the presence of pollen alleles that are not present in the ovule pool (in bold at Table 1). However, these pollen alleles were present in the adult population (400 ha) with a representative frequency as shown by Lepsch-Cunha *et al.* (1999). Therefore, the significant differences are not explained by the presence of immigrant pollen to the sampled population, but probably by the presence of these pollen alleles in the pollen donor population, which were absent in the pollen pool of mother trees. Immigrant alleles to the

total population (seedlings and adults) were encountered in the seedling population at other loci (Mdh-1 and Prx-2), but not in Skdh-1, Mdh-3 and Pgm-2. The significant differences probably are due to the low number of mother trees sampled. The seedling population presented a majority of loci (72%) in Hardy-Weinberg Equilibrium (HWE) expectations, with the exception of the Pgi-1 and the Skdh-1 loci (Table 3), indicating that the observed frequencies for some loci did not diverge significantly from the Hardy-Weinberg's model expected frequencies. These results are not expected because both the differences in male and female contribution among trees and the selection between the pollination time and the seedling sample may occur. The expectation in these cases would be greater departures from the HWE. The pollen pool in the population resulted in significant differences among families ( $P < 0.01$ ) for all loci, but Pgm-2 (Table 2). Therefore, the mother trees are receiving a differentiated pollen pool from the population.

**Table 2** - Pollen allele frequencies and  $c^2$  tests for verification of departures from pollen pool homogeneity received by each mother trees. The  $F_{ST}$  statistics is being used as a measure of genetic diversity, considering families (progeny arrays) as subpopulations.

Locus	Allele	Family 1	Family 2	Family 3	Familia 4	Media n	$F_{ST}^b$
Mdh-1	1	0.790	0.750	0.330	0.310	0.545	
	2	0.180	0.250	0.670	0.690	0.448	
	3	0.030	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	0.008	0.210***
Mdh-3	1	0.970	0.870	0.840	0.930	0.903	
	2	<b>0.000</b>	<b>0.000</b>	0.160	0.070	0.058	
	3	0.030	0.130	<b>0.000</b>	<b>0.000</b>	0.040	0.054**
Pgi-1	1	0.390	0.200	0.880	0.080	0.388	
	2	0.370	0.550	0.120	0.800	0.460	
	3	0.240	0.250	<b>0.000</b>	0.110	0.150	0.271***
Pgm-1	1	0.830	0.660	0.340	0.680	0.628	
	2	0.140	0.250	0.660	0.210	0.315	
	3	0.030	0.090	<b>0.000</b>	0.110	0.058	0.150***
Pgm-2	1	0.780	0.810	0.880	0.790	0.815	
	2	0.010	0.030	<b>0.000</b>	0.070	0.028	
	3	0.210	0.160	0.120	0.140	0.158	0.011
Prx-1	1	0.960	0.590	0.640	0.750	0.735	
	2	0.040	0.410	0.032	0.250	0.255	
	3	<b>0.000</b>	<b>0.000</b>	0.040	<b>0.000</b>	0.010	0.099***
Skdh-1	1	0.110	0.330	0.090	0.330	0.215	
	2	0.590	0.060	0.290	0.670	0.403	
	3	0.300	0.610	0.620	0.000	0.383	0.213***
						$F_{STmed}^a$	0.144

<sup>a</sup>  $F_{STmed} = F_{ST}$  averaged;

<sup>b</sup> diversity among families

\*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ;

## ESTIMATES OF OUTCROSSING RATES

We found departures from the mixed-mating model in two (Pgi-1, Skdh-1) of seven loci (Table 3, the estimates of outcrossing rates used just five loci, see below). These departures have had little effect on the estimates when using multilocus methods to obtain outcrossing rates as pointed out by Ritland (1983) and Brown *et al.* (1984). We rerunning the data without these significant loci, and neither the outcrossing rate nor other parameters were noticeably altered. In consideration of the small number of families in this study, we first ran the single locus program to verify the convergence in the likelihood maximization estimates for each locus in the different families. The only locus without a convergence in the likelihood maximization estimates was Mdh-1. Furthermore, from seven loci initially analyzed, two (Mdh-1 and Pgm-2) showed family outcrossing rates without convergence of  $t$ -estimates ( $t \geq 2$ ). Therefore, these two loci were eliminated from our calculations of the average single locus outcrossing rate ( $t_{sp}$ ) and also the population multilocus rate ( $t_{mp}$ ). Both averaged single locus ( $t_{sp}$ ) and multilocus ( $t_{mp}$ ) estimates of the

outcrossing rate were close to one. As suggested by Brown *et al.* (1984), values of  $t > 1$  (or 100 percent) are due to a failure of the model to deal with multiple heterozygous mother genotypes, rather than disassortative mating per se, or due to the invalidity of the mixed-mating model. Murawski and Hamrick (1992b) also pointed out that these  $t$  values  $> 1$  are statistically valid estimates. We found single locus outcrossing estimates ( $t_s$ ) ranged from 0.48 to 1.00, being very different from locus to locus (Table 3). This range has been also shown in many studies (Shaw and Allard, 1981; Ritland and El-Kassaby, 1985). For isozyme markers, Brown *et al.* (1984) discussed the possible factors contributing to this problem, and concluded that this heterogeneity of estimates over loci may not necessarily argue that the single loci estimation, or the mixed-mating model are unsatisfactory. All individual-family multilocus outcrossing estimates also resulted in values of  $t_m = 1.00$  (Table 3). The expected variance among the families of outcrossing estimates calculated by the program was zero. It is noteworthy that when the run was conducted with less than 4 loci in any combination this variance and the non-convergence of  $t$  estimates began to appear.

**Table 3** - The single locus ( $t_s$ ) and the multilocus outcrossing rates of the individual trees ( $t_m$ ) of *C. multiflora*. Probabilities of goodness of fit tests for Hardy-Weinberg Equilibrium expectations ( $PX^2$ ) and  $c^2$  fit tests for multilocus mixed mating model are given, both for individual loci.

Locus	$PX^2$ (HWE)	$c^2$ (mixed mating model)	$t_s$ (SE)	Individual mother tree	No. of seedlings	$t_m$ (SE)
Mdh-3	1.000	0.000	0.480 (0.195)	1	30	1.135 (0.076)
Pgj-1	0.000 **	45.25 **	1.313 (0.099)	2	30	1.116 (0.075)
Pgm-1	1.000	2.222	0.970 (0.108)	3	27	1.019 (0.076)
Prx-2	0.878	9.551	1.478 (0.148)	4	16	1.032 (0.103)
Skdh-1	0.004 **	39.77 **	0.598 (0.086)			
Mdh-1	0.679	0.044	not used			
Pgm-2	0.528	7.848	not used			
$t_s$ (SE)			0.968 (0.132)	$t_m$ (SE)		0.953 (0.040)

## DISCUSSION

The *C. multiflora* mating system analysis showed multilocus outcrossing rates of individual mother trees and of the population equal to one. Two out of five loci showed significant deviations from the mixed mating model and also from the Hardy-Weinberg Equilibrium (HWE) expectations, indicating random mating in this population. The contribution of male and female gametes to the seed pool was homogenous, with the exception of one locus ( $P < 0.05$ ). Nevertheless, the pollen pool was differentiated among families, with mother trees receiving a differentiated pollen pool from the population.

Clegg (1980) suggested that spatial heterogeneity in pollen allele frequencies could be due to the family structure in the population. However, the proportion of loci in HWE, the homogeneity of pollen and ovule allele frequency distributions within families, and the population phenological study observations made in the same year as the seed collection was undertaken (Lepsch-Cunha and Mori, 1999), gave rise to a different explanation. This pollen pool heterogeneity appears to be a consequence of (i) the seed sampling period of each analyzed mother-tree within a long flowering season (6 months, at the beginning, peak, and end of the flowering season), (ii) the smaller number of flowered trees and their greater spatial clustering at the end of the flowering season, represented by two of four families under study, (iii) the small number of families analyzed, and (iv) the variation in the flowering time of each mother-tree.

The phenological monitoring of 41 surveyed trees showed a tendency for early and late trees to flower for longer periods of time (~ 45-60 days) than peak-flowering trees (~ 15 days) (Lepsch-Cunha and Mori, 1999). This flowering-time

variation in the population promoted a greater phenological flowering overlap than had been supposed by the long, annual flowering season. These features conferred some similarity in the number of possible pollen donors during the whole flowering season, with the exception of the late trees. In this period, the number of flowered trees decreased and they were in general closer or more aggregated, differently from the early and peak trees. Two of four mother-trees (#3 and #4) analyzed here flowered during the late flowering season, the tree #4 being one of the last in the population to flower. These patterns can be seen in the differences among progeny arrays: the mother-tree #1 did not possess 2 of 21 pollen alleles, whereas trees #2, #3 and #4 lacked 3, 4 and 5 of them, respectively (Table 2). Therefore, deviations from some assumptions of the mixed mating model appear to be a consequence of outcrossing among few, and close trees at the end of the flowering season, which does not corroborate family structuring. Furthermore, although the reproductive phenology showed that there was a relatively similar number of trees flowering at any time during the long flowering season, the reproductive-tree assemblage varied throughout the whole season. All individual-family multilocus outcrossing estimates also resulted in values of  $t_m = 1.00$  (Table 3). This reveals that regardless of the flowering period of seed collection (early, peak and late flowering season), these four mother trees outcrossed completely, or selfed seeds were aborted (selection before the sampling collection).

Lepsch-Cunha and Mori (1999) calculated that the majority of synchronic flowering trees of *C. multiflora* studied here were 600-1000 m distant of each other during the flowering season. The apparently aggregated spatial pattern of some trees (Figure 1) did not correspond to that of synchronic flowering trees, the latter being much more widely spaced.

The small values of third quartiles (or deviations from median in smaller distances) reflected a low probability of mating between neighbors. These results, coupled with the complete outcrossing rates in different families throughout the long flowering season, suggest that most mating probabilities in these four mother-trees occurred between distant trees, except at the end of the flowering season. This result is shared with many other tropical tree species studied to date, whose distances as long as 3 km or more among trees are not an obstacle to pollen flow and effective outcrossing (Gandara 1996, Nason and Hamrick 1997, Aldrich and Hamrick 1998, White *et al.* 1998, Dick 2001, Ghazoul and McLeish 2001).

Indeed, the majority of tropical low-density tree species have shown predominant to complete outcrossing rates (i.e.  $T_m \geq 0.85$ ) (O'Malley and Bawa, 1987; Murawski and Hamrick, 1991; Gandara, 1996, Stacy *et al.*, 1996; Sebbenn *et al.*, 2000, see also Tables in Murawski, 1995; Nason and Hamrick, 1997; Lepsch-Cunha *et al.*, 2001). Exceptions are restricted to some families, i.e. Bombacaceae (Murawski *et al.*, 1990, 1992a; Murawski and Hamrick, 1992b; Gribel *et al.*, 1999), and to populations that suffered decreasing numbers of individuals or area, as tree thinning and forest fragmentation, which have shown mixed mating systems ( $\leq 0.8$ ) (review in Lepsch-Cunha *et al.*, 2001).

## CONCLUSION

The *Couratari multiflora* trees analyzed here outcrossed at rates equal to 100% and flowered during a reproductive season when flowered trees in the population were distant from each other among 600 to 1000 m. These results support the assumption that this species is pollinated by pollinators travelling distances as far as 1,000 m. The constancy of complete outcrossing rates in trees collected in different periods of a relative long flowering season and in different flowering densities suggests that this species has a high probability to have some mechanism of self-incompatibility. If not, the selection favoring outcrossed seeds is very possible. Understanding the relationship between genetic and ecological/demographic parameters will help to make appropriate guidelines for management and conservation practices to be developed.

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