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Limited pollen dispersal and biparental inbreeding in *Symphonia globulifera* in French Guiana

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In this paper, we report a study of the mating system and gene flow of *Symphonia globulifera*, a hermaphroditic, mainly bird-pollinated tree species with a large geographic distribution in the tropical Americas and Africa. Using three microsatellites, we analysed 534 seeds of 28 open pollinated families and 164 adults at the experimental site 'Paracou' in French Guiana. We observed, compared to other tropical tree species, relatively high values for the effective number of alleles. Significant spatial genetic structure was detected, with trees at distances up to 150 m more genetically similar than expected at random. We estimated parameters of the mating system and gene flow by using the mixed mating model and the TwoGener approach. The estimated multilocus outcrossing rate, t_m, was 0.920. A significant level of biparental inbreeding and a high proportion of full-sibs were estimated for the 28 seed arrays. We estimated mean pollen dispersal distances between 27 and 53 m according to the dispersal models used. Although the adult population density of *S. globulifera* in Paracou was relatively high, the joint estimation of pollen dispersal and density of reproductive trees gave effective density estimates of 1.6 and 1.3 trees/ha. The parameters of the mating system and gene flow are discussed in the context of spatial genetic and demographic structures, flowering phenology and pollinator composition and behaviour.
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Introduction

For management and conservation of tree populations, it is important to know the distances of effective pollen dispersal and the mating system as a function of tree density (Sork *et al*, 2002). The managers of tropical forests need to know critical limits for remnant tree densities in order to plan selective logging in a way that avoids reproductive isolation (Kanashiro *et al*, 2002), which may result in reduced seed production, lower genetic variation and increased inbreeding depression (Obayashi *et al*, 2002). In areas with forest fragmentation, studies on pollen and seed dispersal distances provide essential information on the level of isolation of remaining forest fragments (Aldrich and Hamrick, 1998; Dick, 2001).

Recently, highly variable microsatellites have been employed to study the gene flow and mating system of tropical trees (Collevatti *et al*, 2001; Dick, 2001; White *et al*, 2002). These studies revealed unexpectedly long pollen flow distances and the impact of pollinator community on pollen dispersal. In most cases, the tree species being studied occurred naturally at low density. Dick *et al* (2003) measured, for the insect-pollinated tropical tree *Dinizia excelsa* with a density of 0.3 trees/ha (dbh $\geq$10 cm), a mean pollen dispersal of 212 m in undisturbed forests and mean pollen dispersal of 1509 m in a fragmented forest with much lower density. There is evidence for a negative relationship between the level of selfing and the density of reproductive trees (Murawski and Hamrick, 1991). The mating system and gene dispersal of tropical trees have been proven to be highly dynamic and sensitive to ecological factors (Franceschinelli and Bawa, 2000). The level of outcrossing and the distribution of effective pollen might change from one flowering event to another and from one population to another simply as a function of demographic structure, phenology and pollinator composition and abundance. Hence, even in undisturbed forests we have a 'natural' variation on these parameters.

We need to know the range of this natural variation before we can analyse the impact of forest exploitation and fragmentation on the mating system and the gene flow. The evolution of tropical trees species seems to have favoured characteristics in the reproductive system, such as specialized animal pollination, that allow them to persist at low densities. Hence, tropical rainforest trees might, to some extent, tolerate an additional local reduction of their density due to forest exploitation. It is further possible that typically abundant species may be more sensitive to reduction in densities than rare species because they are less adapted to this situation.

Symphonia globulifera L. f. (Clusiaceae) is an interesting species from this point of view. This hermaphroditic species has an exceptionally large geographic distribution from Mexico south to Rio de Janeiro and it is also present in the tropical West Africa. Dick *et al* (2003) studied the phylogeographic history of *S. globulifera* over this broad geographic range. Their results indicated the

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colonisation of Central and South-America through marine dispersal from Africa during the mid Miocene (ca. 15 million years ago). The density of *S. globulifera* is extremely variable among populations. Counting trees with diameter at breast height (d.b.h.) $\geq$10 cm, densities of 122 N/ha (Quakal swamp forest) and 65 N/ha (Manicole swamp forest) have been reported in Guyana (Andel, 2003). At the other extreme, the population of *S. globulifera* on Barro Colorado Island (Panama) has a density of only 0.5 trees/ha (Center for Tropical Forest Sciences, 2000 forest census). There are also contrasting reports on the community of pollinators of *S. globulifera* at different places. In Costa Rica, Pascarella (1992) observed Lepidoptera as the most important pollinators, in central French Guiana Gill *et al* (1998) identified perching birds as the principal pollinators, whereas Bittrich and Amaral (1996) and Maues (2001) suggested hummingbirds as the pollinators in the Central Amazon. Another advantage of selecting *S. globulifera* as a study subject is the availability of microsatellite markers and additional population genetic data for comparison (Aldrich *et al*, 1998).

In this paper, we report a study of the gene flow and mating system of *S. globulifera* at the experimental site 'Paracou' in French Guiana. This site is characterised by a relatively high density of this species. We will focus on the following questions: Do we also find long distance pollen dispersal for this abundant tropical tree species? Do we observe a spatial genetic autocorrelation in the adult population and, if yes, does it fit to expectations given by the measured gene flow and mating system? Is the mating system of high-density animal-pollinated tree species different from observations of low-density species? The answers to these questions will help to address whether abundant species are more sensitive to logging operations than rare species.

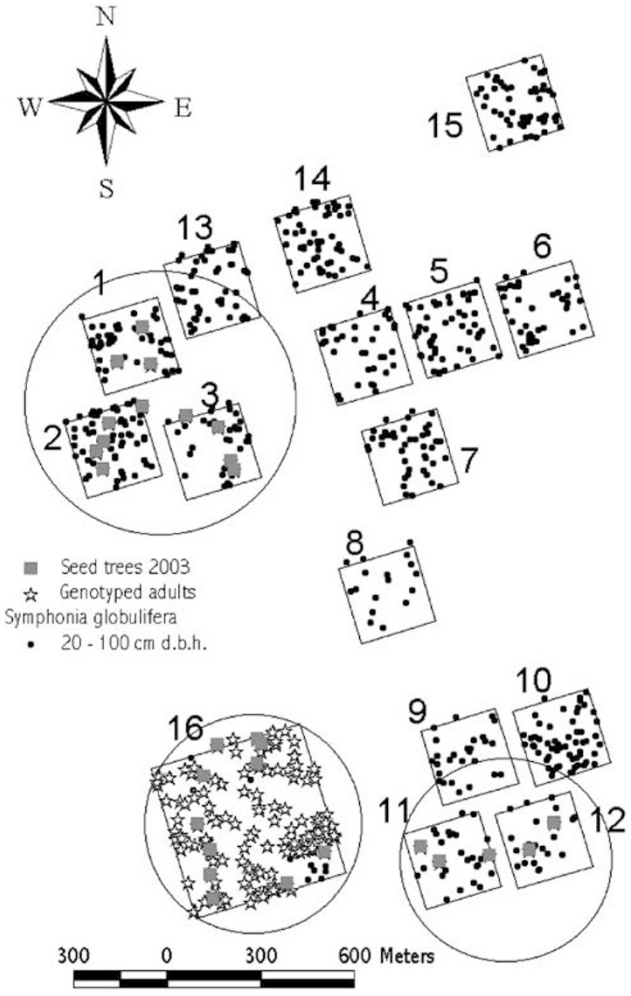

Figure 1 Distribution of *S. globulifera* with d.b.h. $\geq$20 cm in Paracou. Most of the trees sampled for genotyping were located in plot 16 (stars). We sampled 560 seeds from 28 mother trees (squares) in three different parts of Paracou (circles).

Material and methods

Site and sampling

The experimental trial 'Paracou' (5°18′N, 52°53′W) is located in French Guiana near Sinnamary. The forest of Paracou is classified as lowland moist forest on ferralitic soil. The mean annual rainfall in this area is 3.076 mm, with peaks in April–June and December. There are two dry seasons; a long one from August to November and a shorter one in March. The experimental trial consists of 16 plots: 15 are 6.25 ha in size and one covers an area of 25 ha. Since 1984, all trees with a d.b.h. $\geq$10 cm have been measured by CIRAD Forêt. The trees have been mapped and most species have been identified. On the total surface area of 118.7 ha, 1295 trees of *S. globulifera* with a d.b.h. $\geq$10 cm have been identified (10.9 trees/ha). Three silvicultural treatments of different intensities were applied to different plots from 1986 to 1988 but some were left as unlogged controls. After 15 years, the effect of the treatments on the density on *S. globulifera* is quite low. There is even a slightly higher density of *S. globulifera* in the logged plots (12.6 trees/ha) compared to the control plots (10.5 trees/ha). A detailed description of the experimental trial and treatments can be found elsewhere (Schmitt and Bariteau, 1988; Forget *et al*, 1999).

For the genetic study, we collected cambium from 164 trees. This included 147 trees from plot 16 (500×500 m^2) and the 17 sampled seed trees outside plot 16. In February 2003, we collected 560 seeds from 28 mother trees (20 seed each) distributed in three clusters over the experimental site (see Figure 1). The reason of this design was to sample at different spatial scales, because we had no *a priori* information whether the average pollination distance would be in the 30 or 500 m range (Sork *et al*, 2002).

Microsatellites

For adult trees, a disc of 150 mg of cambium stored in a conservation buffer made of 70% ethanol and 0.3 % β-mercaptoethanol was used for DNA extractions. The buffer-preserved discs were flash- frozen in liquid Nitrogen and then ground to a powder using a mortar and pestle. Subsequent DNA extraction followed modifications of the widely used CTAB extraction procedure (Doyle and Doyle, 1987) (2$\times$ CTAB buffer:100 mM Trisma base, 1.4 mM NaCl, 20 mM ethylenediaminetetra-acetic acid (EDTA), 2% Cetyltrimethylammonium bromide (CTAB), 1–4% polyvinylpyrrolidone (PVP)-40, 0.2% ascorbic acid, β-mercaptoethanol 0.3%). Total

genomic DNA was extracted from seed tissues following the same protocol.

Genotypes were scored at three microsatellite loci. One primer pair SgC4 was adopted from Aldrich *et al* (1998). Two additional primer pairs were developed (Sg03 and Sg18) during this study. The clone sequence of Sg03 contained the compound repeat $(CT)^{19}$ and the size range of the nucleotides after polymerase chain reaction (PCR) was 302–354 base pairs (bp). The Sg18 clone contained the repeat $(CT)^{24}$ and the alleles ranged in size from 229 to 333 bp. The primer sequences were as follows: Sg03, forward 5′TCTATCTGCCAAGTGAGACA, Sg03 reverse 5′GAACATTTTTTTGGTGGGAC; Sg18, forward 5′TCTTTTGCCTTTTTAGTTGA, Sg18 reverse TGAGGATTGTTTGCCCAGAA.

The PCR cocktail (20.0 µl total) contained 0.05 µM of each dNTP, 0.012 µM of $MgCl_2$, 1 U of *Taq* polymerase (Invitrogen Corporation), 0.002 µM of each primer and 0.2 ng of DNA. The PCR was performed on an ABI 9700 thermal cycler using the following protocol: 5 min at 94°C; 30 cycles of 45 s at 94°C, 45 s at 55°C and 45 s at 72°C; ending with 5 min at 72°C. The amplification products were visualised by electrophoresis with a LIZ-500 size standard (Applied Biosystems Incorporated, ABI) on an ABI 310 automated sequencer. Allele sizes were scored using the program Genotyper from Applied Biosystems. The observed segregation within families showed codominant inheritance of the microsatellite fragments.

Data analysis

Genetic variation and heterozygosity: For each locus, the number of different alleles (A), observed heterozygosity (H_o), expected heterozygosity (H_e), the effective number of alleles $A_e = 1/(1-H_e)$ and the Fixation index $F = 1-(H_o/H_e)$ were calculated as described by Weir (1996). To estimate the significance of F, numerical tests were performed on the basis of Monte–Carlo methods (Manly, 1997). A total of 1000 permutations of homologous alleles among individuals were run to test the significance of the F-values for each locus. Each permutation leads to a new random association of the alleles within the sample of seeds and adults (resampling without replacement). After each permutation, the F-values were recalculated and compared to the observed values. The relative frequency of those cases leading to more extreme F-values than the observed values were used to estimate the probability of significant deviation from the Hardy–Weinberg proportions.

Spatial genetic structure: Moran's Index I_q was computed with the program SGS (Degen *et al*, 2001b) for multilocus genotypes of the adults (Sokal and Oden, 1978a). The index is calculated for a given distance class s_q. For each allele having higher frequency than the threshold of 2% in all samples, I_q was calculated as follows:

$$I_q = \frac{n\sum_{i=1}^{n}\sum_{j\neq i}^{n} w_{ij}(a_i-\bar{a})(a_j-\bar{a})}{W\sum_{i=1}^{n}(a_i-\bar{a})^2} \qquad W = \sum_{i=1}^{n}\sum_{j\neq i}^{n} w_{ij}$$

n is the total number of samples and $w_{ij} = 1$ if the individuals i and j belong both to the spatial interval s_q, otherwise $w_{ij} = 0$. For diploid data, a_i is 1 if the ith individual is homozygous for that allele, 0.5 if heterozygous, and 0 if the individual has no copy of the allele. The value $\bar{a}$ corresponds to the mean value of a_i over all n individuals. Following Streiff *et al* (1998), autocorrelation is calculated over all selected loci summing the numerator and denominator of the first equation over the total number of alleles. The expected values for the case of no autocorrelation are $-1/(n-1)$ (Sokal and Wartenberg, 1983). Higher values indicate positive spatial autocorrelation and smaller values indicate negative spatial autocorrelation.

A permutation procedure using Monte–Carlo simulations was applied to test significant deviation from random spatial distribution of each I_q-value. Each permutation consisted of a random redistribution of the microsatellite genotypes over the spatial coordinates of the sampled trees. For each of the spatial distance classes s_q, observed values were compared with the distribution obtained after 1000 permutations. A 95% confidence interval for the parameters was constructed as the interval from the 25th to the 975th ordered permutation estimates (Streiff *et al*, 1998).

Mating system: By use of the mixed mating model (Ritland and Jain, 1981), we estimated the single- and multilocus outcrossing rates. The outcrossing rates were calculated with the program MLTR version 2.3 (Ritland, 2002) by maximum likelihood, fitting the observed proportions of genotypes descended from a known maternal genotype to the proportions expected under the mixed mating model. The model assumes that: (a) each mating represents a random event of an outcross or a self-fertilization, with probabilities equal to t and $(1-t)$, respectively; (b) no selection and no mutation following fertilization may occur; (3) there is no assortative mating or variability in pollen pool frequencies (Ritland and Jain, 1981). With version 2.3 of MLTR (Ritland, 2002) some departures from these assumptions could be accommodated and be treated as additional facets of the mating system. If mating occurs between relatives (biparental inbreeding), the single-locus selfing rate (s_s) should be higher than the multilocus selfing rate (s_m), and the difference provides a minimum estimate of the apparent selfing due to biparental inbreeding. The reason for this expected difference between s_s and s_m is that the more loci used, the lower the likelihood of confusing selfed and biparentally inbred progeny (Griffin and Eckert, 2003). In addition, we calculated the correlation of outcrossed paternity within progeny arrays (r_p), which is the probability that a randomly chosen pair of seeds from the same family are full-sibs. We performed 500 bootstraps over families to get standard errors for each parameter.

TwoGener: Following Smouse *et al* (2001) we made a twogener analysis on the progeny arrays of Paracou. The principle of this method is to estimate Φ_{FT}, the differentiation of allelic frequencies among the pollen pools sampled by several mother trees in the population. The relation between Φ_{FT} and dispersal distance has been shown for given dispersal curves (Austerlitz and Smouse, 2001), allowing the development of several estimates of pollen dispersal (Austerlitz and Smouse, 2002; Austerlitz *et al*, 2004). A general estimate is based on the global Φ_{FT} measured on all the seed trees of the

Table 1 Sample size (*N*), number of alleles (*A*), effective number of alleles (A_e), observed and expected frequencies of heterozygotes (H_o, H_e), Fixation index (*F*) and probability for departure from Hardy–Weinberg Heterozygosity (*P*) for adults and seeds in Paracou

Locus	*Adults* (N = 164)						*Seeds* (N = 534)					
	A	A_e	H_o	H_e	F	P	A	A_e	H_o	H_e	F	P
Sg03	26	9.38	0.59	0.89	0.33	0.000	20	8.46	0.70	0.88	0.20	0.000
SgC4	30	14.52	0.93	0.93	0.00	0.500	26	14.02	0.90	0.92	0.02	0.026
Sg18	13	5.03	0.66	0.80	0.17	0.000	17	7.25	0.74	0.86	0.13	0.000
Mean	*24*	*8.02*	*0.73*	*0.87*	*0.16*	*0.000*	*21*	*9.16*	*0.78*	*0.89*	*0.12*	*0.000*

population. This provides an estimate of the pollen dispersal distance (delta) assuming a given dispersal curve and a density of reproducing adults (*d*) in the landscape. We tested the normal and exponential dispersal functions and used, according to former phenological observations, a proportion of the known adult density as entry into the estimation. Also, the pairwise Φ_{FT} between all mother trees in the population were calculated to design pairwise estimates that allow one to jointly infer the parameters pollen dispersal distance (delta) and a density of reproducing adults (*d*). As noted by Austerlitz and Smouse (2002), the pairwise analysis yields more accurate estimates of Φ_{FT} but requires larger sample sizes. We computed the 99% confidence interval of Φ_{FT} by bootstrapping among loci with 1000 replicates (Weir, 1996).

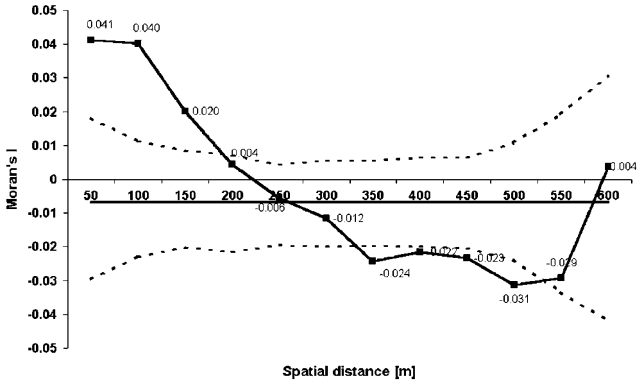

Figure 2 Mean Moran's Index among trees in different spatial distance classes (line with squares), 95% confidence interval as drawn from 1000 permutations (dotted line) and expected Moran's Index for absence of spatial genetic structure (black central line).

Results

Genetic variation and heterozygosity

The genetic variation measured by the mean number of alleles was, despite a lower sample size, higher for the adults compared to the seeds (adults: $A = 24$; seeds: $A = 21$, Table 1). However, the alleles were more evenly distributed in the seeds, as shown by the higher mean values of the effective number of alleles (A_e). Both ontogenetic stages had a highly significant excess of homozygotes (mean adults: $F = 0.16$; mean seeds: $F = 0.12$). For both samples, there was a high variation of the *F*-values among loci with a strong excess of homozygotes for loci Sg03 and Sg18 and nearly Hardy–Weinberg proportions for locus SgC4.

Spatial genetic structure

The shape of the correlogram showed a general pattern of decreasing spatial autocorrelation among trees with increasing spatial distances (Figure 2). The mean value of the Moran's *I* among adult trees at distances of up to 150 m was significantly higher, and the value among trees at distances of 300–500 m was significantly lower than expected for a random spatial genetic structure. The values varied between +0.041 and –0.031 indicating only a weak spatial genetic structure.

Mating system

The multilocus outcrossing rate (t_m) had a value of 0.92 (0.017). There was a significant difference between the multilocus and single-locus estimates $t_m - t_s = 0.156$ (0.022). This implies a relatively high proportion of biparental inbreeding in Paracou. Another striking result was the significant high value for the correlation of outcrossed paternity $r_p = 0.47$ (0.059). Hence, the number of effective pollen donors in a family was very limited, despite a relatively high density of trees in Paracou.

Table 2 Density of reproductive trees and mean pollen dispersal distance (delta) estimated for the normal and exponential dispersal model. The error is a quadratic criterion for the fit between expected and observed values for pairwise Φ_{ij} estimates (Austerlitz and Smouse, 2002)

Dispersal function	*Density constraint*	*Density of reproductive trees (N/ha)*	*Delta (m)*	*Error*
Normal	Fixed	4.0	27.4	1.70
Normal	Estimated	1.6	42.9	1.69
Exponential	Fixed	4.0	30.9	1.69
Exponential	Estimated	1.3	53.1	1.68

Gene flow

The distance between sampled seed trees varied between 45 and 2010 m with a mean of 955 m. The differentiation of allelic frequencies among the pollen pools was $\Phi_{FT} = 0.205$ with the 99% confidence interval from 0.147 to 0.288. The estimates of the mean pollen dispersal (delta) for the normal model were 27.4 m with fixed tree density and 42.9 m for the joint estimation of tree density and delta (Table 2). The values for the exponential model

were, respectively, 30.9 and 53.1 m. The joint estimation of delta and density of reproductive trees reduced the estimated tree density down to 1.6 trees/ha (normal model) and 1.3 trees/ha (exponential model). The differences for the error, calculated as a quadratic criterion by fitting the observed and expected pairwise Φ_{ij} values (Austerlitz and Smouse, 2002), were quite small among the models. The smallest error was obtained for the joint estimation in the exponential model.

Discussion

Level of diversity

With mean values of $A_e = 8.02$ and 9.16, we observed relatively high values for the effective number of alleles. The seeds were sampled in three different parts of the Paracou sites, whereas the adults are mostly from one area (plot 16). This might explain the higher variation in the seed array. Our values were slightly higher than the mean value observed by Aldrich *et al* (1998) for *S. globulifera* in Costa Rica ($A_e = 7.02$). Most other neotropical tree species had lower genetic diversity. In French Guiana, Latouche-Halle *et al* (2003) observed a mean value of $A_e = 3.23$ for the tree species *Dicorynia guianensis*, and in the same region Dutech *et al* (2002) found values between 1.69 and 2.08 for *Voucapoua americana*. Dick *et al* (2003) measured a mean value of $A_e = 3.50$ for *Dinizia excelsa* in Manaus (Brazil). Comparison among species should be carried out with caution because of the limited number of analysed loci and possible differences in mutation rate between di- and trinucleotide microsatellite loci. Nevertheless, results with different kind of gene markers (RAPDs, AFLPs and microsatellites) showed very similar ranking of tropical tree species diversity (Degen *et al*, 2003). The high level of diversity is probably linked to the large geographic distribution of *S. globulifera*, fitting the pattern seen for many tropical tree species using allozymes (Loveless, 1992).

Heterozygosity

For both the seeds ($F = 0.12$) and the adults ($F = 0.16$), we observed a significant excess of homozygotes compared to the expected Hardy–Weinberg proportions. This excess of homozygotes could be explained by the presence of null alleles, selfing and/or biparental inbreeding. Using information on the selfing rate (s), mean pollen dispersal (delta) and spatial genetic structure of adults, Fenster *et al* (2003) provided a formula to estimate an expected Fixation index (F_{exp}) due to selfing and biparental inbreeding: $F_{exp} = (2(1-s)F_x + s)/(2-s)$. Here F_x is the biparental inbreeding coefficient (inbreeding coefficient of truly outcrossed progenies) calculated as kinship coefficient among mates within the radius of mean pollen dispersal. As has been shown by Hardy and Vekemans (1999), the kinship coefficient can be computed as approximately half of the Moran's I (see Figure 2). Using the above formula, we calculated for our population an expected value of $F_{exp} = 0.065$. Hence inbreeding would explain a part of the observed excess of homozygotes. There seems to be no strong selection against inbred progeny because the adult sample also had an excess of homozygotes. In other studies, a decrease of *F*-values has been reported with increasing age of the analysed ontogenetic stages (Morgante *et al*, 1993).

Another factor that might have contributed to the high values of *F* in Paracou is the Wahlund effect. In French Guiana, two different ecotypes of *S. globulifera* have been observed. These ecotypes differ in leaf and seed size and have different environmental preferences (Loubry, 1994). About 90% of the observed trees in Paracou belong to a first type with relatively small leaves and seeds, which can be found in dry and swamp areas of the site. The remaining 10% of the trees belong to a second type with large leaves and seeds, which occurs preferentially in the swampy areas of the stand. If these types are not in reproductive contact and have different allele frequencies at the studied loci, this might be responsible for a part of the excess of homozygotes in Paracou and could explain the variation of *F*-values among loci. We measured the mean leaf size, of 60 adults in dry and swampy areas of plot 16 to test the presence of the Wahlund effect. These trees were included in the genetic study. After ranking the trees by leaf size, we classified them in two groups, one with big and one with small leaves. We did not find significant differences in allele frequencies among the two groups. Moreover, both groups still had a significant excess of homozygotes. Hence, we could not prove the impact of a Wahlund effect. Furthermore, Dick *et al* (2003), in context of their phylogeographic study, did not find any nucleotide differences at the sequenced internal transcribed spacer (ITS) between the two morphological types from Paracou. This also indicated no or only very little genetic difference between the two morphological types.

Outcrossing rates

The estimated multilocus outcrossing rate t_m was 0.920. Our results fit well to the selfing rates between 0.098 and 0.261 observed for saplings by Aldrich and Hamrich (1998) in Costa Rica and confirm that *S. globulifera* is a predominantly outcrossing species. We observed a significant level of biparental inbreeding in Paracou ($t_m - t_s = 0.156$), which may be explained by limited pollen dispersal within the range of family structures. If there is a family structure, this can be evaluated by the analysis of spatial genetic structure of the adults. We found a significant positive spatial autocorrelation of genotypes for the adults up to 150 m (Figure 2). The high biparental inbreeding can be explained, if we compare the estimated range of mean pollen dispersal of 27–53 m with the scale of the observed spatial structure. It is quite clear that in Paracou most of the pollen flow is within a range of significant spatial structure. Another striking result was the high proportion of full-sibs ($r_p = 0.47$). This fits together with the high level of biparental inbreeding. In Paracou, the trees are pollinated by a rather limited number of trees close to the mother tree. Stacy *et al* (1996) also observed for tropical tree species at Panama a strong correlation of paternity of crossed seeds, since pollinators visit neighbouring trees when trees are closely spaced. The population in Paracou has a high density of adult trees. Hence, we expected to have more different pollen donors represented in the offspring in Paracou. This contradictory result might be explained by unsynchronised flowering phenology in Paracou and by the composition and behaviour of the pollinators. As

shown for the South American shrub species *Helicteres brevispira*, the behaviour of pollinating hummingbirds can change according to the density of flowering trees from promoting outcrossing by traplining to territorial pollination promoting selfing (Franceschinelli and Bawa, 2000). Hence, the trees in Paracou might be pollinated by rather territorial pollinators.

The TwoGener approach assumes homogeneous tree densities and independent pollen dispersal events following an isotropic distribution. Using Clark and Evan's index (R), it could be shown that *S. globulifera* trees have a random spatial distribution in Paracou (Degen *et al*, 2001a). If pollinator behaviour causes strong preferential mating among particular adults, the mean dispersal distance (delta) should be underestimated. Similarly, variation in flowering intensities or phenology among adults causes delta to be underestimated when the density is fixed. Nevertheless, these effects should be minimised by the joint estimation.

Effective pollen dispersal

By use of the TwoGener approach we estimated, for the population in Paracou, mean pollen dispersal distances (delta, Table 2) between 27 and 53 m. The values differed according to the dispersal model used (normal *vs* exponential model) and the estimation method (only delta estimation *vs* joint estimation of delta and density). The joint estimation calculated an effective density of 1.6 reproductive trees/ha for the normal model and 1.3 reproductive trees/ha for the exponential model. This would imply that effectively about 13% of all trees $\geq$10 cm d.b.h. contributed to reproduction. Using the TwoGener approach Sork *et al* (2002) measured a mean pollen dispersal of 64.8 m for the wind pollinated *Quercus lobata* with a density of 1.19 trees/ha. With the same approach, Dick *et al* (2003) measured a mean pollen dispersal of 1509 m in a fragmented landscape and 212 m in undisturbed forests for the insect-pollinated tropical tree *Dinizia excelsa* with a density of 0.3 trees/ha. In comparison to these results, the pollen dispersal of *S. globulifera* is much shorter. This raises the possibility that birds, as the suspected main pollinators of *S. globulifera*, might be less efficient than bees or the wind. In general pollen dispersal seems to be negatively correlated with the tree density: the high tree density in Paracou led to short pollen dispersal. Such a correlation has been also found by Stacy *et al* (1996) and might result if pollinator behaviour fitted postulated optimal foraging strategy according to a cost-benefit function (Waddington and Holden, 1979).

In contrast to former studies with RAPDs (Degen *et al*, 2001a) we found a weak but significant positive spatial genetic autocorrelation up to 150 m (maximum of Moran's $I = 0.041$) and a significant negative autocorrelation from 300 to 500 m (minimum of Moran's $I = -0.031$). This means close individuals are genetically more similar and pairs of individuals in a distance between 300 and 500 m are more different than expected for a random distribution. This is a pattern expected for clinal variation due to limited gene flow (Sokal and Oden, 1978b). This range of Moran's I is small compared to other tree species studied at the same site. In comparison, Latouche-Halle *et al* (2003) observed for the rather aggregated tree species *Dicorynia guianensis* maximum values for Moran's I at microsatellite loci between 0.1 and 0.25 in the first distance class up to 50 m. Hence, of the two species, *S. globulifera* has a weaker but larger spatial genetic structure. This can be explained by long distance seed dispersal (Hardy and Vekemans, 1999) and an overlapping of seed shadows. Seed dispersal is bat mediated in *S. globulifera*. This species probably has a more limited pollen dispersal than seed dispersal, in contrast to other species, due to its pollinator behaviour.

This paper is the first of a planned series on our results on *S. globulifera*. A second paper will describe the interannual variation of the mating system and gene flow in a Brazilian population with low density of *S. globulifera*. This population was subject to logging operations at the end of 2003. We will continue to study the mating system and gene flow of the species in the same stand for the next 2 years. This time series will enable us to compare the variation of mating system and gene flow under natural and disturbed conditions.

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