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High selfing rate, limited pollen dispersal and inbreeding depression in the emblematic African rain forest tree *Baillonella toxisperma* – Management implications

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ABSTRACT

Mating system and gene flow are major influencing factors of species population dynamics and evolution. These factors are often not characterized in tropical tree species, yet they constitute basic information that must be considered to implement sustainable management practices. In particular, as logging implies a reduction of the density of congeneric mates, the connectivity through pollination between individuals has to be well characterized (selfing versus outcrossing rates, distances between mates). We conducted a genetic-based analysis (using 10 nuclear microsatellites) to determine the mating system and gene flow characteristics of an emblematic timber tree species from lowland rain forests of the Congo Basin, *Baillonella toxisperma* (Sapotaceae). The species, which is frequently exploited for its wood and for a number of non-timber forest products, naturally occurs at low densities (ca. 0.01–0.1 individuals/ha). It is supposedly an entomophilous species whose seeds are probably dispersed by mammals. We have shown that the species presents a mixed-mating system (about 20–40% of selfing depending on analysis method). However, the comparison of inbreeding parameters among cohorts suggests that inbred individuals die between seedling and mature tree stages. The mean pollen dispersal distance was relatively low for such a low-density population species (estimated to be 690 or 777 m depending on analysis method) and, together with a low mean number of pollen donors (N_{EP} = 2.76), it suggests a pattern of nearest-neighbour mating where allo-pollen could be a limiting factor. However, *B. toxisperma* presents a relatively weak genetic structure (*Sp* statistic = 0.0095) indicative of long gene dispersal distance (σ_g = 3–5 km according to the assumed effective population density). Overall, this would indicate that gene flow occurs mainly by extensive seed dispersal in this species. These results suggest that mammals and local populations involved in the dispersal of the species play a key role by lowering biparental inbreeding effects. Sustainable population management might require assisted regeneration using unrelated planting material.

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1. Introduction

Selective logging implies a reduction of 'mate' ('seed tree') densities which in theory can affect plant reproductive success (Aguilar et al., 2008; Eckert et al., 2010). Patterns of mating and pollen dispersal might be particularly concerned (Ratnam et al., 2014) with serious consequences for seed production and viability (Ashman et al., 2004; Knight et al., 2005). A better knowledge of timber species' reproductive biology is required to evaluate the impact of logging on the population dynamics of these species. Given the complex nature of plant reproduction, each species presents singular characteristics. These characteristics are not stable in space and time, as a number of influencing factors interact (e.g. variation in flowering and fruiting success between years, disparate pollinators or pollinators' behaviours in different populations, heterogeneous pressures of pests or herbivores on seeds

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and seedlings, etc.). Accordingly, accurately characterizing plant reproductive characteristics is challenging, especially when we are dealing with trees. Much stronger efforts are needed so that governments, sustainable forest management certifying bodies, and logging companies can rely on scientifically-relevant indicators to ensure sustainable practices.

Plant reproductive success involves flowering and pollination, fruiting and seed dispersal, and germination and seedling establishment (Barrett and Eckert, 2013). Importantly, reproductive success requires efficient natural regeneration through seeds and seedlings, which is a key aspect of sustainable forest management practices (Hall, 2008; Ouédraogo et al., 2011). Pollination patterns are largely influenced by plant mating system (Barrett et al., 1996). A number of factors influence plant mating system, including population density of conspecific individuals, floral synchronism, and post-pollination mechanisms (Goodwillie et al., 2005). Population density can influence the mating system, as the proportion of self-pollination might increase when population density decreases, lowering the outcrossing rates in self-compatible species. However some studies suggest an adjustment of gene flow efficiency to reduced population density (e.g. Côrtes et al., 2013; Duminil et al., 2016), but further data are necessary to confirm this pattern.

Seedling establishment can be affected by the mating system (frequency of selfing). Long-lived plant species such as trees are generally characterized by a high genetic load (high rate of recessive or partially recessive deleterious alleles) (Klekowski, 1988). Such genetic load implies that seedlings resulting from selfing generally exhibit strong inbreeding depression and die at an early stage (Charlesworth and Charlesworth, 1987; Duminil et al., 2009). A disruption of pollen flow between trees can thus have a detrimental effect on their progeny vigour and survival. Such an effect is not usually directly considered by forest management practices and further data on species' mating systems needs to be acquired to allow such considerations to influence management.

The reproductive biology of African tropical timber trees remains largely undocumented (Bawa et al., 1990). Yet, methodological approaches relying on molecular markers are now available to characterize the mating system and patterns of gene flow (Ashley, 2010; Austerlitz et al., 2004; Ouborg et al., 1999). However, the acquisition of new knowledge is hampered by a number of factors, including difficulties in conducting field work in the Tropics and accessing flowers in the high canopy, the limited capacity of developing countries to conduct molecular studies and a lack of awareness at multiple levels of the importance of such factors. Of the twenty most exploited tree species in Central Africa (OFAC data), to the best of our knowledge, the mating system and contemporary gene flow patterns have been characterized for only three species (*Aucoumea klaineana*, Born et al., 2008; *Erythrophleum suaveolens*, Duminil et al., 2016; *Entandrophragma cylindricum*, Lourmas et al., 2007). Historical gene flow patterns were also studied using indirect approaches for at least three other timber species (*Milicia excelsa*, Bizoux et al., 2009; *Distemonanthus benthamianus*, Debout et al., 2010; *Baillonella toxisperma*, Ndiade-Bourobou et al., 2010). However, information on historical gene flow is not sufficient to characterize the current gene dispersal dynamic and the relative contribution of pollen versus seed dispersal to gene flow.

Baillonella toxisperma is an emblematic timber tree species from Central Africa, that is found disseminated in the forest at very low densities (ca. 0.01–0.1 individuals/ha). It is frequently exploited for its wood, and it has been suggested that current management practices put its long-term sustainability at risk (Debroux, 1998), leading to a ban of exploitation in Gabon. Indirect genetic evidences indicate long-distance (historical) seed dispersal (Ndiade-Bourobou et al., 2010) but information on mating system and pollen dispersal are lacking. In the present study we characterize its reproductive biology to inform forest managers and suggest considerations for better orienting current practices towards sustainability. More specifically we document: (i) mating system (level of selfing); (ii) fine-scale spatial genetic structure; and (iii) pollen dispersal characteristics (mean dispersal distance, mean number of effective pollen donors, types of dispersal distribution, effective male population density, pollen immigration rate). These results are interpreted in terms of management practices.

2. Material and methods

2.1. Species characteristics

Baillonella toxisperma Pierre (1890) belongs to a monospecific genus from the Sapotaceae family, and is commonly known as moabi or African pearwood. It is a large lowland rain forest species that grows up to 60 m high and 5 m in diameter, therefore representing one of the largest tree species from the Guineo-Congolian rain forest. *B. toxisperma* is distributed from South-East Nigeria to North Angola southward and western DRC eastward. It is more abundant in semi-evergreen forest than in evergreen forest (Letouzey, 1968). The species is described as non-pioneer light demanding and does not seem to exhibit regeneration problems in semi-evergreen forest as attested by the distribution of diameter classes (Doucet and Kouadio, 2007). It is a multipurpose species, highly appreciated for hardwood timber and important non-timber forest products (edible fruits, cooking oil extracted from kernels and bark used for medicinal purposes). In 2009, a 25-year ban was imposed on exploitation of the species in Gabon. Given its value to forest residents, the presence of *B. toxisperma* is sometimes considered as an indicator of past human settlements (Plenderleith and Brown, 2004).

Flowers are hermaphrodite and pollen dispersal is probably mediated by insects as in other hermaphrodite species from the Sapotaceae family (Ndiade Bourobou, 2011). In southeast Cameroon, the mean flowering time of *B. toxisperma* individuals is ca. one month, whereas the mean flowering time of the population is ca. two months (F. Feteke, Gembloux Agro-Bio Tech, unpublished results). The production of seeds starts from approximately 40 cm dbh (diameter at breast height), but becomes regular and abundant from about 70 cm dbh (Debroux, 1998). Fruits generally contain two to five seeds. Seed dispersal patterns and mechanisms are still largely undocumented in this species. Dispersion is probably done by large (elephants, gorilla) and small (rodents) mammals, and humans have probably played a significant role in recent millennia as they are consuming its fruits (Debroux, 1998; Ndiade Bourobou, 2011).

2.2. Sampling

Mature trees and offspring (seeds and saplings) were sampled in June 2013 and in February-March 2014 in a population covering about 650 km^2 (decimal latitude longitude at about 0.8°S–13.1°E; Fig. 1) within the FSC-certified 'Precious Woods' logging concession (Ogooué-Lolo province, East Gabon). The sampling zones correspond to different annual allowable cuts that were carried out between 2010 and 2014. The area was harvested before the ban was imposed on moabi, during the late 1980s and the 1990s, so that the species has certainly been exploited, but qualitative or quantitative data are not available. The following cohorts were considered: seeds (N = 61), seedlings (N = 268), saplings (trees < 40 cm dbh; N = 22), mature trees (>40 cm dbh, N = 87). In order to conduct parentage analyses, we attempted to sample mature trees exhaustively in one part of the sampling site (a plot

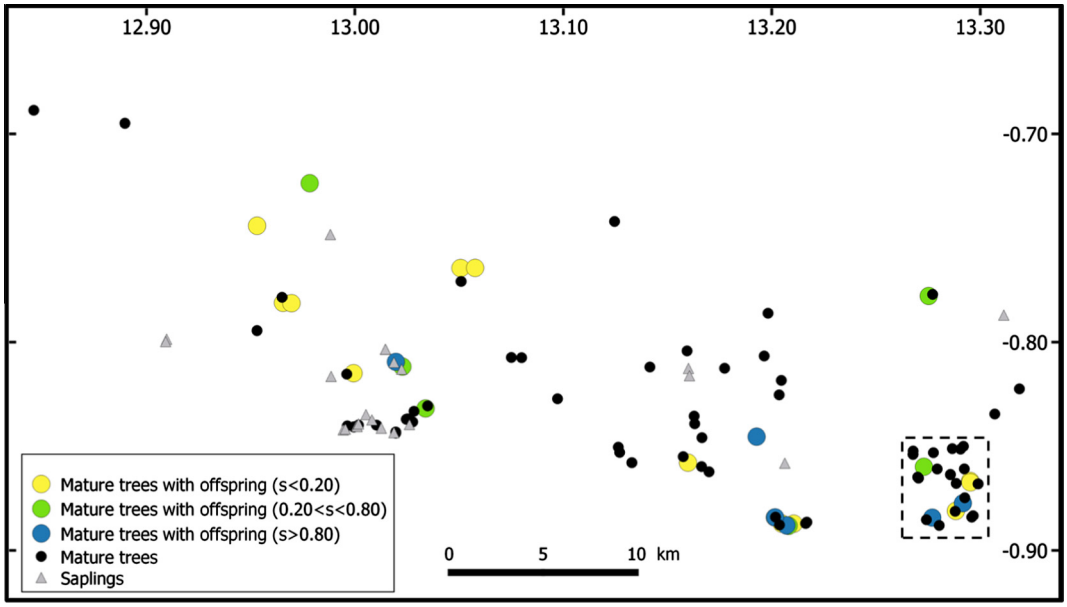

Fig. 1. Sampling of individuals of *B. toxisperma* in East Gabon and assignation of selfing rates for some mature trees (= adults). Yellow, green and blue dots respectively represent seed trees with low (s < 0.20), intermediate (0.20 < s < 0.80) and high rates of selfing (s > 0.80). An exhaustive sampling has been conducted within the hatched zone (South-East of the sampling zone). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of about 12 km^2, containing N = 28 mature trees), and focussed our sampling effort around this zone (Fig. 1). Seedlings and seeds (as many as possible) were sampled below trees when available, comprising 29 families in total (from two to sixteen seeds and/or seedlings per seed tree). The population density (*D*), measured for dbh > 40 cm was 0.0181 individuals per hectare.

2.3. DNA extraction and genotyping

Total DNA was isolated with a NucleoSpin plant kit (Macherey-Nagel, Düren, Germany). We used 10 nuclear microsatellites developed by Ndiade Bourobou et al. (2009) (Table 1). We first modified the initial protocol by adding linker tails to forward primers and using dyed-labelled tails during the PCR following the protocol of Micheneau et al. (2011). Microsatellites were amplified in two sets of multiplexes: mix I contained loci Bt02, Bt04, Bt05, Bt07, Bt10 and Bt15 and mix II loci Bt03, Bt06, Bt08 and Bt12. Each primer mix was prepared from 10 μM primer solutions, taking 0.15 μL of R primers, 0.10 μL of F primers and 0.15 μL of labelled tails (with FAM, VIC, PET or HEX; Table 1). Polymerase chain reactions (PCRs) were carried out in a TProfessional Thermocycler (Biometra, Göttingen, Germany). PCRs were performed in a total volume of 15 μL containing 1 μL of template DNA (10–100 ng), 7.5 μL of QIAGEN Type-it Multiplex PCR Master Mix, either 2.1 μL of primer mix I or 1.6 μL of primer mix II, and 4.4 μL (with mix I) or 4.9 μL (with mix II) of ddH_2O. The PCR cycling protocol included an initial step of 5 min at 95 °C followed by 21 cycles of 30 s at 95 °C, 1 min 30 s at 57 °C, and 30 s at 72 °C, followed by 9 cycles of 30 s at 95 °C, 1 min 30 s at 53 °C, and 30 s at 72 °C followed by a final incubation at 60 °C for 30 min. Amplified fragments were run on a 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The

Table 1
List of microsatellite loci and genetic diversity in the studied population (mature trees only).

Locus[a]	Ref. locus[b]	Forward primer[c]	Reverse primer	Mix	NA_E[d]	$A_{R(k=190)}$[e]	H_E[f]	H_O[g]	F[h]	*fnull* (SE)[i]
Bt02	mCIRBtH10	Q1-TCCAGAAGTTGTGACCGTTTG	CCCCTATCACTCCTCCATTTG	I	1.37	4.99	0.271	0.272	−0.004	0
Bt03	mCIRBtD051	Q1-TGGAGGATTGTGGCTCTTG	CAGGTTTTGTTCTTTGGCG	II	2.31	14.51	0.566	0.543	0.041	0
Bt04	mCIRBtE051	Q1-AAAACACCAACAGAGCAAG	GACCGCATTAGATTCATTC	I	2.00	4.00	0.499	0.571	−0.145	0
Bt05	mCIRBtE06	Q3-CCCCATGAACAACACAAG	AAACACTCCCACACCTCTC	I	1.73	5.91	0.420	0.202	0.521*	0.227 (0.033)
Bt06	mCIRBtBC01	Q3-TCTCAGTCCATTCTCTCAAC	GCAATCTTATGTACTCGGTG	II	6.32	11.82	0.842	0.76	0.098*	0.005 (0.016)
Bt07	mCIRBtBC06	Q3-ACACACCAAACTTCTATCC	TGCCGTATCCTCTAATG	I	1.73	5.99	0.423	0.433	−0.024	0
Bt08	mCIRBtB05	Q2-ATTTTAACACGGCTTCC	ACCAGGCTCTTGATGAC	II	1.08	2.00	0.073	0.057	0.225	0.050 (0.051)
Bt10	mCIRBtF021	Q2-CCTCTTAAACCTTCAAACAG	CCCTAGATTGGAAACTCAC	I	1.35	2.00	0.260	0.158	0.394*	0.292 (0.113)
Bt12	mCIRBtC03	Q4-CCTCACTTCATTTCACCTG	CACCAACTCAACTCAACTTC	II	1.62	2.9	0.381	0.295	0.227*	0.059 (0.035)
Bt15	mCIRBtE03	Q4-TGGCATCAATCACGACAC	GGTTTTCAGAGGGTTTTGG	I	4.67	7.81	0.786	0.752	0.043*	0.002 (0.012)

[a] Name of the locus as defined in this publication.
[b] Name of the locus as defined in Ndiade Bourobou et al. (2009).
[c] Q1 to Q4 refers to the name of the labelled tails as defined in Micheneau et al. (2011).
[d] NA_E: number of effective alleles.
[e] $A_{R(k190)}$: Standardized allelic richness.
[f] H_E: expected heterozygosity.
[g] H_O: observed heterozygosity.
[h] *F*: deficit in heterozygotes (uncorrected for null alleles.
[i] *fnull*: frequency of null alleles.
* Indicates *F* significantly different than zero, P < 0.05.

lengths of the fragments were determined by comparison with the GeneScan 500 LIZ dye Size Standard (Life Technologies, Carlsbad, CA, USA) using the Peak Scanner v1.0 software (Applied Biosystems).

2.4. Data analyses

As detailed below, data analyses were performed to describe the following: (i) possible inbreeding depression or reduced diversity in offspring (comparison of observed heterozygosity and gene diversity indices between cohorts); (ii) check for evidence of unrelated seedlings under sampled trees (offspring that do not correspond to the expected mother); (iii) characterize the mating system and check the heterogeneity among seed trees (estimation of the selfing rate, correlated paternity and biparental inbreeding in offspring); (iv) estimate historical gene dispersal distances (indirect estimation from the spatial genetic structure of mature trees and saplings); (v) identify contemporary pollen dispersal events (paternity analysis, here successful only for selfing events); (vi) estimate contemporary backward pollen dispersal kernel (indirect approach based on correlated paternity between sibships); and (vii) characterize the contemporary forward pollen dispersal in the exhaustively sampled plot (direct approach based on a neighbourhood model).

2.4.1. Diversity and inbreeding in each cohort

The effective number of alleles (NA_E) (Nielsen et al., 2003), the rarefied allelic richness for a subsample size of k ($k = 36$) gene copies (A_R), the gene diversity corrected for sample size (expected heterozygosity H_E; Nei, 1978), the deficit in heterozygotes estimating the inbreeding coefficient (F) were calculated for each cohort (mature trees, saplings, seedlings, seeds) separately using SPAGeDi 1–5a (Hardy and Vekemans, 2002). We tested if (F) was significantly different from zero after 999 randomization of gene copies among individuals. Differences in genetic diversity parameters between cohorts were tested using an ANOVA procedure in R. The parameters were compared among cohorts accounting for the locus effect.

We also tested for the presence of null alleles using INEST 1.0 (Chybicki and Burczyk, 2009). As null alleles were demonstrated and as they affect population parameter estimates which are based on the proportion of heterozygotes, we also calculated *fnull* (estimate of inbreeding coefficient that control for the presence of null alleles) for each of the cohorts under a population inbreeding model (PIM) using INEST 1.0 (Chybicki and Burczyk, 2009). We tested if the level of inbreeding was significantly different between cohorts (mature trees, saplings, seedlings, seeds) by applying unpaired t-tests on (H_O) per individual (proportion of heterozygous loci) in each cohort, considering only individuals genotyped for at least eight out of ten loci. Under inbreeding depression, if inbred (i.e. less heterozygous) individuals are eliminated through natural selection at early life stages, we expect to observe more heterozygosity in mature trees than in seeds and/or seedlings.

2.4.2. Definition of progeny arrays

Seeds or seedlings have been collected on the ground below mother trees. To assess whether seed dispersal might have occurred, we tested the correspondence between candidate mothers (N = 87) and seeds/seedlings (N = 329) by conducting a maternity analysis using CERVUS 3.0.3 (Marshall et al., 1998). The exclusion power for maternity analysis was checked using the non-exclusion probability estimated for the first parent. CERVUS uses a maximum likelihood approach and assigns maternity according to the highest logarithm of the likelihood (LOD score). Simulations were conducted to estimate the critical values of LOD score required to assign maternity with a given degree of confidence (80% and 95% confidence levels). The following simulation parameters were applied to define the confidence level of maternity analysis assignment: 10 000 simulated mating events; all mature trees as candidate mother plants; individuals typed at a minimum of six loci; 1.0 as the proportion of candidate mothers sampled; genotyping error rate of 0.1.

2.4.3. Mating system

We estimated the selfing rate (s) in the seedling cohort using three different approaches. First, from the observed heterozygosities of the mature trees ($H_{O(A)}$) and the seedling cohort ($H_{O(S)}$) as $s = 2((H_{O(A)} - H_{O(S)})/H_{O(A)})$. This formula assumes that there is no inbreeding in the mature trees cohort, which is the case as outlined by the value of *fnull* obtained for mature trees. This estimator is expected to be relatively robust to the presence of null alleles (similar biases in the numerator and denominator), hence it is here preferred over those based on the comparison between observed and expected heterozygosities.

Second, from progeny arrays (N = 29 families) the following inbreeding parameters were estimated using MLTR 3.2 (Ritland, 2002): the multi-locus outcrossing rate (t_m), the single-locus outcrossing rate (t_s), the correlation of paternity within maternal sibship (r_p, i.e. proportion of pairs of sibs sired by a same father), and the correlation of selfing among families (r_s, i.e. the normalized variance of selfing). All parameters were estimated by the Newton-Raphson algorithm. Mating among relatives (biparental inbreeding) was estimated by the difference ($t_m - t_s$). Standard deviation of these estimators was evaluated through a bootstrap procedure (1000 repetitions).

Third, the selfing rate was also estimated per family through a Bayesian implementation of the mixed mating model using MSF 1.01. (Chybicki and Burczyk, 2013; Chybicki, 2013). MSF accounts for genotyping errors. Only families with at least two offspring were kept for this analysis (N = 25).

2.4.4. Fine-scale spatial genetic structure

Spatial genetic structure (SGS) was assessed using genotypes of mature trees and saplings following the procedure of Vekemans and Hardy (2004) as implemented in SPAGeDi 1–5a (Hardy and Vekemans, 2002). Nason's estimator of pairwise kinship coefficients (F_{ij}) between individuals (Loiselle et al., 1995) and 95% confidence intervals have been estimated at different intervals of geographical distance (log scale). SGS was tested by permuting 10,000 times the position of the individuals. Indirect estimates of neighbourhood size and the corresponding *Sp* statistic (a synthetic measure that quantify the extent of spatial genetic structure; Vekemans and Hardy, 2004) was obtained from the rate of decay of F_{ij} with ln(distance) and the mean pairwise kinship coefficient measured at the first distance class (F_1). Additionally, assuming that the SGS has approached drift-dispersal equilibrium, we have estimated the historical gene dispersal distance σ_g (the square root of half the mean square parent-offspring distance) and the neighbourhood size (Nb) following Vekemans and Hardy (2004). To this end, different values of effective densities (D_E) were tested considering that D_E reaches only half, a quarter or a tenth of the density of sexually mature individuals (Hardy et al., 2006): $D_E = 0.0090$, 0.0045 or 0.00181 ind/ha.

2.4.5. Paternity analysis

We conducted a paternity analysis, with known mothers assigned to the offspring (see Section 2.4.2). We tested the exclusion power for paternity analysis using the non-exclusion probability estimated for the second parent (P = 0.022). The relatively low polymorphism of microsatellite markers in our species, the presence of null alleles and the non-exhaustive sampling of mature trees beyond the 12 km^2 plot did not allow us to conduct a

powerful paternity analysis (only 10% of offspring could be assigned at a 80% confidence level using the algorithm of Cervus, whereas a score of 14% was expected through simulations; results not shown). However, as self-fertilized offspring could still be detected with reasonable power, we relied on this paternity analysis to identify selfed seeds or seedlings.

2.4.6. Spatial structure of pollen pools and backward dispersal kernel

We used mapped mother-offspring genotypic data (N = 28 progeny arrays, with a mean of 7.14 offspring per array) to infer contemporary pollen dispersal characteristics with KINDIST and TWOGENER as implemented in POLDISP 1.0c (Robledo-Arnuncio et al., 2007). As recommended in POLDISP user's manual the input dataset was prepared as follows: offspring resulting from selfing have not been considered, no mother-offspring genotyping mismatch (mismatches were coded as missing data in offspring), no missing data for the mothers, a minimum of two offspring per family. The correlation of paternity within and among maternal families was first estimated with KINDIST. The mean number of effective pollen donors (N_{EP}) that participate in pollination was estimated from the within-sibship correlated paternity (r_P) as $N_{EP} = 1/r_P$. The slope of the relationship between among-sibship correlated paternity and distance was tested with a Mantel procedure using the zt software (Van de Peer, 2002). The slope was negative and significant, a necessary condition to test the fit of the different dispersal distributions available in KINDIST (Appendix A). We used 10,000 m as reference threshold distances to define unrelated pollen pools. The best dispersal distribution was chosen by comparing their least-square residuals. The mean pollen dispersal distance was obtained for the same dispersal distribution. We then used TWOGENER to estimate the effective male population density (D_{Em}) using as input the pollen dispersal distribution parameters estimated with KINDIST. The ratio (D_{Em}/D) provides an indication of the proportion of reproductive trees that have contributed to reproduction within the population for one year (assuming similar male and female reproductive successes per individual).

2.4.7. Pollen dispersal characterization using the neighbourhood model

We made inferences on plant gene dispersal and mating patterns by modelling parentage probabilities of offspring using the neighbourhood model as implemented in NM+ 1.1 (Chybicki and Burczyk, 2010). NM+ estimates through a maximum likelihood approach, the proportion of offspring resulting from selfing (s), pollen and seed immigration from outside a defined study zone (m_p and m_s respectively) and parameters of pollen and seed dispersal kernels (shape parameters, b_p and b_s respectively). We used two different data sets: (i) the whole data set where sampling of mature trees can be considered as non-exhaustive (28 progeny arrays and 87 mature trees); (ii) the 12 km^2 plot where mature trees have been exhaustively sampled (eight progeny arrays and 28 mature trees).

3. Results

3.1. Diversity and inbreeding

We observed similar levels of NA_E, A_R and H_E between cohorts (Table 2). F tended to decrease from seeds to mature trees and was significantly different from zero in all cohorts. Two loci (Bt05, Bt10) displayed high frequencies of null alleles (>0.200; Table 1). Accounting for them, the corrected estimator of the inbreeding coefficient *fnull* was close to zero in all cohorts.

The unpaired t-test on observed heterozygosity between cohorts was significant (P < 0.05) only between mature trees and seedlings, and between mature trees and seeds (Table 2). This suggests inbreeding depression and that inbred individuals are eliminated through natural selection between the seedling and mature trees stages.

Table 2
Genetic diversity and inbreeding statistics of the different cohorts.

Cohort	N[1]	NA_E[2]	A_R[3]	H_E[4]	H_O[5]	F[6]	$F_{(null)}$[7] (SE)
Mature trees	87	2.38	4.44	0.472	0.410[a]	0.134*	0 (0)
Saplings	22	2.75	4.34	0.430	0.411[ab]	0.207*	0 (0)
Seedlings	268	2.29	4.13	0.459	0.354[b]	0.228*	0.003 (0.004)
Seeds	61	2.46	4.10	0.484	0.359[b]	0.272*	0.106 (0.174)

[1] Sample size.
[2] Effective number of alleles.
[3] Allelic richness (k = 36).
[4] Expected heterozygosity (gene diversity corrected for sample size).
[5] Observed heterozygosity.
[6] Fixation index estimating the inbreeding coefficient without accounting for null alleles.
[7] Estimation of the inbreeding coefficient accounting for null alleles (standard error estimated by jackknife). Letters: H_O values sharing a common letter do not differ significantly (P > 0.05) according to the unpaired t test.
* Indicates $F > 0$ at P < 0.01.

3.2. Definition of progeny arrays

The combined non-exclusion probability (first parent) was 0.143. Fifty-four out of 61 seeds (about 88%) and 217 out of 268 seedlings (about 81%) were correctly assigned to the expected mother. Only the 271 correctly assigned offspring have been used in following analyses.

3.3. Mating system

Outcrossing rate ($t = 1 - s$) estimated from $s = 2((H_{O(ST)} - H_{O(S)})/H_{O(ST)})$, was 0.775. According to MLTR, multilocus (t_m) and single-locus (t_S) outcrossing rate estimations reached respectively 0.730 (SE of 0.069) and 0.596 (SE of 0.067). There is a signal of biparental inbreeding as ($t_m - t_s$) estimates is 0.134 (SE of 0.043). Correlated paternity (r_p) was 0.384 (SE of 0.086), indicating that sibs were often sired by the same father. Correlation of selfing (r_s) reached 0.426 (SE of 0.117), indicating heterogeneous selfing rate among seed trees, as confirmed by per family estimates: 13 families having $t > 0.80$, six families having $t < 0.20$ and six families presenting intermediate t values (Appendix B). We found no relationship between levels of outcrossing and number of surrounding mature trees (Fig. 1).

According to paternity analyses the outcrossing rate was about 0.760 as 63 out of 271 correctly-assigned seeds/seedlings were demonstrated to correspond to selfing events (see 'Assignments of offspring to mother' section below).

3.4. Fine-scale spatial genetic structure and inference of historical gene dispersal distances

Pairwise kinship coefficients F_{ij} decayed fairly linearly (except for some scatter) with the logarithm of the geographical distance (Fig. 2), as expected under isolation by distance, and the Mantel test was significant (P < 0.05). The resulting *Sp* statistic was 0.0095 (SE of 0.0037).

The procedures to estimate gene dispersal parameters σ_g (historical gene dispersal distance) and *Nb* (neighbourhood size) resulted in convergence only for $De = D/2$ and $D/4$ (but no convergence was obtained using the jackknifing over loci option, thus no standard error could be estimated). Assuming an effective popula-

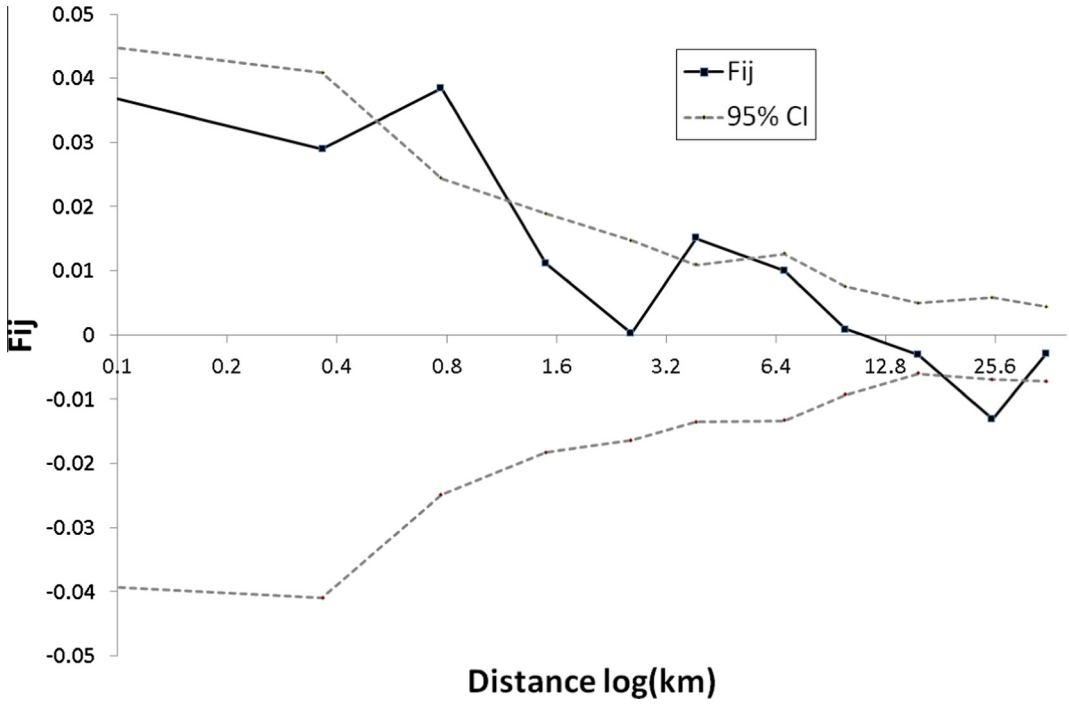

Fig. 2. Average kinship coefficients Fij between pairs of individuals plotted against the logarithm of geographical distance in the whole population.

tion density *De* = 0.0090 ind/ha we found σ_g = 3.16 km and neighbourhood size *Nb* = 114, and assuming *De* = 0.0045 ind/ha we found σ_g = 5.35 km, and *Nb* = 163.

3.5. Spatial structure of pollen pools and backward dispersal kernel

According to Poldisp, the mean within-sibship correlated paternity (r_p) was 0.362 which is similar to the value estimated by MLTR (r_p = 0.384) and corresponds to a mean number of pollen donors (N_{EP}) of 2.76 (no confidence interval available). Correlated paternity between sibships significantly decayed with the logarithm of the distance separating seed trees (Pval < 0.001). From this pattern of decay, KINDIST inferred that the exponential dispersal distribution appeared as the most likely backward pollen dispersal kernel with a mean dispersal distance of 777 m (no convergence was reached for the exponential power distribution). TWOGENER provided an estimate of effective density of pollen producers (D_{Em}) of 0.0064. Accordingly D_{Em}/D = 0.35, as if 35% of individuals had contributed (equally) to pollination events (or a higher proportion of individuals contributed unequally to pollination).

3.6. Pollen dispersal according to the Neighbourhood model

Based on the whole dataset, fixing for an exponential form of the kernel (b_p = 1) following Poldisp analyses (see above), we obtained the following estimates and standard errors (in brackets): selfing = 40% (±4%), mean distance of forward pollen dispersal kernel (d_p) of 311 m (±66 m), and mean within-neighbourhood pollen dispersal distance of 690 m.

Based on the dataset from the exhaustively sampled 12 km^2 area, still with a fixed exponential kernel form (b_p = 1), we obtained: selfing = 46% (±7%), d_p = 211 m (±82 m), and a percentage of pollen immigration (m_p) coming from outside the exhaustive zone of 16% (±6%).

4. Discussion

As it is highly appreciated for the quality of its wood and for non-timber forest products, the hermaphroditic lowland rain forest tree species *Baillonella toxisperma* has a high economic and social importance. The species typically occurs at a low-density (about one mature tree per 10–100 ha). As a consequence, logging practices can have detrimental effects on its regeneration dynamics if gene flow is limited. Here, although we confirm extensive gene dispersal, probably mediated by seed dispersal, we found unusual patterns of reproduction for a low-density species, characterized by limited nearest-neighbour pollen dispersal, a substantial selfing rate and evidence of inbreeding depression, patterns that need to be taken into account to develop sustainable management strategies.

4.1. High selfing rate resulting in unfit progeny

Rates of selfing (estimated at between 20 and 40% depending on the type of analyses) were unexpectedly high compared to other tropical trees (Dick et al., 2008; Ward et al., 2005). Such rates may be more common in low-density species as the result of a relaxed incompatibility system, a necessary consequence of low availability of conspecific mates (Dick et al., 2008; Murawski and Hamrick, 1991). We found that selfing varied significantly among mature trees (selfing rates ranged from 0.02 to 0.94, leading to a high correlation of selfing r_s = 0.43; Appendix B), but without any obvious link with spatial isolation (Fig. 1). However, the presence of conspecific mature trees close to seed trees is not a sufficient condition for outcrossing; the trees also need synchronous flowering. We observed that moabi trees flower irregularly, and that all trees do not flower every year (data not shown). It will be necessary to combine genetic analyses and phenological observations (intra-specific heterogeneity of the flowering period) to better interpret our results. Whatever the origin of this pattern, our results indicate that *B. toxisperma*

can self-fertilize. Moreover, our results further imply that seeds resulting from selfing probably do not result in viable offspring as suggested by the decrease of the inbreeding between seed and mature trees cohorts (Table 2) and as confirmed by the unpaired *t* test on observed heterozygosity (Table 2). The inbreeding depression seems to be expressed predominantly at the seedling stage (seedlings resulting from selfing do not reach the sapling stage). A deficit in outcross pollen is thus detrimental in *B. toxisperma* as is generally the case in tree species (Duminil et al., 2009).

4.2. Extensive seed dispersal but pollen dispersal limited to nearest neighbour

Overall our results suggest that gene flow can be extensive in *B. toxisperma* but is mainly mediated by seeds. The spatial genetic structure of the studied population was weak ($Sp = 0.0095$) and close to the mean value found in tropical trees dispersed by efficient seed dispersers such as birds, bats or monkeys (mean $Sp = 0.009$, $n = 6$, Dick et al., 2008). Accordingly, the resulting estimates of historical gene dispersal distance were relatively large (σ_g between 3 and 6 km), and on the same order as a previous estimate made in a population from northwest Gabon ($\sigma_g = 6.6$–9.9 km; Ndiade-Bourobou et al., 2010). However, contemporary pollen-mediated gene dispersal was much more limited with estimates of mean realized dispersal distance ranging between 600 and 800 m (NM+ and KINDIST). This discrepancy between estimates of dispersal suggests either that pollen-mediated gene flow is less important than it was in the past (under the assumption that pollen is the main contributor to gene flow, as reported in a majority of plant species; Bittencourt and Sebbenn, 2007; Gaino et al., 2010; Petit et al., 2005), or that seed-mediated gene flow is much higher in this species than pollen-mediated gene flow. As the species is likely pollinated by insects, we do not see any obvious explanation for a reduction of pollen-mediated gene flow in recent time. Trees may have been harvested in the area 20–30 years ago, but we do not have data to estimate past population density. If the density of mature trees has been reduced (probable), we might expect that pollen-mediated gene flow would be higher at lower population density as demonstrated in other species (e.g. Duminil et al., 2016), not the contrary. Using maternally inherited markers, Ndiade-Bourobou et al. (2010) inferred mean historical seed dispersal distances of 4–6.3 km. Hence, there is more support for the alternative hypothesis that seed dispersal is the main vector of gene flow in this species.

There is a controversy between experts on seed dispersal vectors in *B. toxisperma*. Although elephants are often invoked as the main disperser, the regeneration of the species is lower in study sites where elephants are relatively abundant than in places where elephants are relatively rare (Doucet et al., 2009). Accordingly it is still unclear if elephants really disperse or mostly consume seeds, grinding them while eating the fruits and consuming the seedlings or destroying them by trampling below seed trees. Primates (gorillas in particular) that eat the fruits can also disperse seeds by transporting the fruits (Forget et al., 2007; Gautier-Hion et al., 1985), though probably not over long distances. Rodents also eat the seeds and they could contribute to their dispersal by storing them in locations hidden from the sight of conspecifics and forgetting some of their hoards (Debroux, 1998). Finally, human might also have played a significant role. However, their role should be limited to the last centuries or millennia, a tiny proportion of *B. toxisperma* evolutionary existence.

Contrary to our results, Ndiade-Bourobou et al. (2010) suggested long distance pollen dispersal (ca. 10 km), based on a difference between their estimates of gene dispersal distance and seed dispersal distance. However, this very indirect approach offers extremely low precision and, considering our current results, their pollen dispersal estimate does not appear reliable. Here, with a density of ca. 1.8 mature trees per km^2, the distance between nearest-neighbours under a regular distribution would be 745 m, which is very close to the mean pollen dispersal distance inferred from the backward kernel (777 m, POLDIST approach) or the within neighbourhood mean dispersal distance (690 m, NM+ approach). Such limited pollen dispersal explains the signal of biparental inbreeding detected (i.e. significant difference between t_m and t_s) because mature trees separated by less than a kilometre tend to be related (mean kinship coefficient around 0.04; Fig. 2). Nearest-neighbour mating also explains the high rate of correlated paternity of outcrossed progenies (mean r_p 0.36–0.38) with a corresponding low mean effective number of pollen donors per mother tree ($N_{EP} = 2.76$).

According to the neighbourhood model, the mean distance of the forward pollen dispersal kernel would even be smaller (d_p close to 200–300 m) than the mean effective dispersal distance (backward dispersal). This forward kernel better represents the pollen dispersal distance expected in a high density population. To better understand how this discrepancy between forward and backward dispersal kernels can be interpreted, it is useful to recall what they represent. The forward dispersal kernel is the spatial distribution of propagule arrival points around its source. In the neighbourhood model, it is not constrained by the assumption that propagules established (i.e. that a pollen grain fertilized an ovule), despite the fact that the kernel is fitted on data corresponding to established propagules (pollen genotype inferred from seed and mother genotypes). By contrast, the backward dispersal kernel represents the spatial distribution of sources of propagules around their arrival point, under the assumption that these propagules established (ovule fertilization in the case of pollen dispersal). Hence, the backward dispersal kernel, which may be seen as a realized kernel, depends both on the forward dispersal kernel and the distribution of the sources and recipients. In the limit of a high density of sources and recipients (i.e. high mature trees density), forward and backward dispersal kernels should converge, but they can diverge substantially under low density (because only propagules having dispersed over a sufficient distance might reach an adequate arrival point for establishment), or under an aggregated spatial distribution (in which case the mean dispersal under backward kernel can become shorter than for the forward kernel). Here, as the mean distance of the forward pollen dispersal kernel d_p is much smaller than the mean realized pollen dispersal distance, it seems that only pollen grains from the tail of the forward kernel are able to reach *B. toxisperma* flowers from another individual, leading to a pattern of nearest-neighbour mating. This suggests that allo-pollen might be a limiting resource for ovule fertilization, explaining both the high rate of selfing despite its detrimental effect through inbreeding depression, and the low effective number of fathers (Nep = 2.76), limited to closest neighbours.

The presence of a strong genetic structure at short distances, characterized by a steeper decay of kinship with ln(distance) at short than at medium or large distances, is generally explained by seed dispersal limitation: part of the seeds that are produced by a seed tree are not dispersed far away (Heuertz et al., 2003). The absence of such SGS pattern at short distance in *B. toxisperma* (Fig. 2) is consistent with efficient seed dispersal. However, a number of (non-dispersed) seedlings were collected below seed trees. It could thus be surprising that *B. toxisperma* does not show a genetic structure at short distances. This suggests that seedlings that grow near their mother tree do not reach the mature trees stage. This can

be a direct consequence of elephants' behaviours; attracted by the seeds, they may also eat seedlings and damage them by trampling, or more generally a consequence of the Janzen-Connell effect (Connell, 1971; Janzen, 1970). This can also be explained by the light requirements of the seedlings. *B. toxisperma* is classified among the non-pioneer light demanding species (Meunier et al., 2015) and seedlings only grow in canopy gap (Doucet et al., 2016). The genetic structure of *B. toxisperma* populations where elephants are not present should be investigated to better understand this pattern.

4.3. Management implications

Our results have implications for the management of forest genetic resources of *B. toxisperma*. The healthy population dynamic of the species seems to rely predominantly on seed-mediated gene flow. Being particularly sensitive to inbreeding depression and having relatively reduced pollen-mediated gene dispersal, the long distance dispersal of seeds is the only factor that can limit mating between related individuals. Thus, our results suggest that in the absence of its main dispersers, the species would be highly threatened if no assisted silvicultural strategies are developed. This is generally the case for tree species dependent on megafaunal dispersal, but is particularly true for *B. toxisperma* given its restricted pollen dispersal and its susceptibility to inbreeding. As mentioned by Beaune et al. (2013), this suggests that specific silvicultural strategies for *B. toxisperma* population management need to be developed to ensure its regeneration, such as the planting nursery-grown seedlings in logging gaps (Doucet et al., 2009). To this end, one needs to establish a tracking system for planting material and to avoid planting seedlings near their parent trees.

5. Conclusions

B. toxisperma has a mixed mating system, with potential for high levels of selfing. It is highly susceptible to inbreeding depression, and seeds or seedlings resulting from selfing are eliminated at early life stages. Moreover, *B. toxisperma* exhibits relatively short distance pollen dispersal (less than one kilometre), which is surprising for a species occurring at low density (ca. 0.01–0.1 individuals/ha). Offspring from mating between related individuals will also probably suffer from inbreeding depression and be counter-selected. Our results support earlier findings that unlike pollen dispersal, realized (historical) seed dispersal in *B. toxisperma* is efficient and typically reaches more than one kilometre. Overall this suggests that *B. toxisperma*'s regeneration dynamic largely depends on seed dispersal. This study is one of the accumulating examples that point out the dramatic effects associated with the empty forest syndrome, which might be tempered here by the role played by humans that collect and consume the fruits. In the absence of efficient seed dispersal, genetic resources of the species will need to be carefully managed with the establishment of assisted regeneration protocols that include, in particular fine-scale tracking of reproductive material collection, growth in nursery and replanting. Reproductive material needs to be planted at a reasonable distance from their parent trees.

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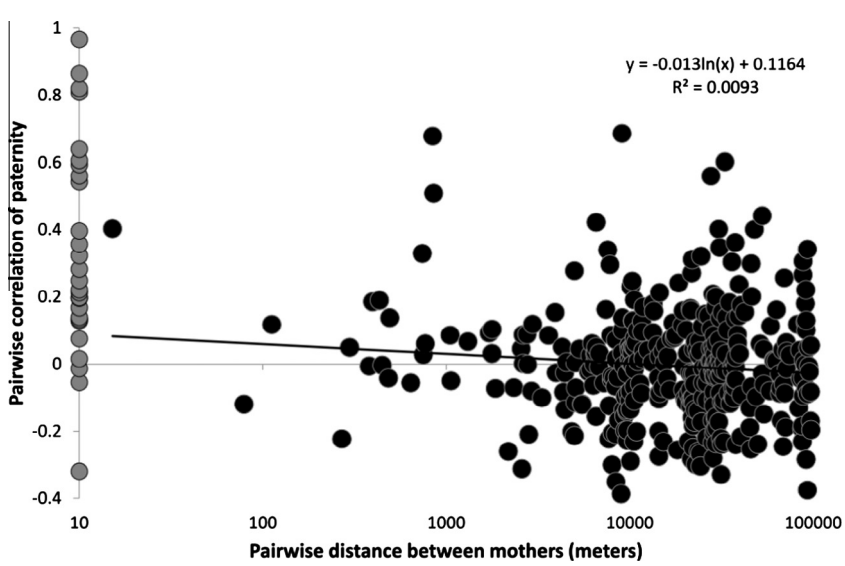
Appendix A

Pairwise correlation of paternity as a function of pairwise log (distance) between mothers. Black dots correspond to among-sibship correlated paternity estimates. Grey dots correspond to within-sibship correlated paternity estimates (N = 28).



Appendix B

Estimation of selfing and correlated paternity per family (MSF software).

Mother id	Nu. of offspring	Rates of selfing		r_p [b]
		Mean	HPD (95%) [a]	
66	12	0.022	0.043–0.488	0.356
83	5	0.660	0.074–0.962	0.196
90	8	0.865	0–0.106	0.607
94	6	0.676	0.136–1	0.129
116	8	0.037	0.54–1	0.249
6	11	0.024	0.184–1	0.966
7	14	0.066	0–0.193	0.139
8	15	0.828	0–0.12	0.820
14	4	0.058	0–0.244	1.030
20	13	0.940	0.619–1	0.594
23	2	0.244	0–0.299	-0.319
39	10	0.409	0.784–1	-0.055
46	3	0.075	0–0.889	0.221
57	14	0.067	0.148–0.733	0.396
58	10	0.897	0–0.347	0.865
59	3	0.804	0–0.319	0.560
60	2	0.490	0.626–1	0.640
61	6	0.053	0.278–1	0.283
65	13	0.081	0–1	0.198
73	10	0.822	0–0.27	0.168
86	2	0.173	0–0.282	-0.013
91	14	0.540	0.582–1	0.016
117	14	0.029	0–0.879	0.075
120	13	0.060	0.203–0.841	0.323
122	14	0.175	0–0.686	0.212

[a] Highest posterior density interval.
[b] Within-sibship correlated paternity (r_p).

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