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Spatial genetic structure in *Milicia excelsa* (Moraceae) indicates extensive gene dispersal in a low-density wind-pollinated tropical tree

J.-P. BIZOUX,*[1] K. DAÏNOU,†[1] N. BOURLAND,† O. J. HARDY,‡ M. HEUERTZ,‡§ G. MAHY* and J.-L. DOUCET†

*Laboratory of Ecology, Gembloux Agricultural University, 2 Passages des Déportés, 5030 Gembloux, Belgium, †Laboratory of Tropical and Subtropical Forestry, Gembloux Agricultural University, 2 Passages des Déportés, 5030 Gembloux, Belgium, ‡Behavioural and Evolutionary Ecology Unit – CP 160/12, Faculté des Sciences, Université Libre de Bruxelles, 50 Av. F. Roosevelt, 1050 Brussels, Belgium, §Department of Forest Systems and Resources, Centre of Forest Research CIFOR-INIA, Carretera de la Coruña km 7.5, 28040 Madrid, Spain

Abstract

In this study, we analysed spatial genetic structure (SGS) patterns and estimated dispersal distances in *Milicia excelsa* (Welw.) C.C. Berg (Moraceae), a threatened wind-pollinated dioecious African tree, with typically low density (~10 adults/km^2). Eight microsatellite markers were used to type 287 individuals in four Cameroonian populations characterized by different habitats and tree densities. Differentiation among populations was very low. Two populations in more open habitat did not display any correlation between genetic relatedness and spatial distance between individuals, whereas significant SGS was detected in two populations situated under continuous forest cover. SGS was weak with a maximum S_p-statistic of 0.006, a value in the lower quartile of SGS estimates for trees in the literature. Using a stepwise approach with Bayesian clustering methods, we demonstrated that SGS resulted from isolation by distance and not colonization by different gene pools. Indirect estimates of gene dispersal distances ranged from σ_g = 1 to 7.1 km, one order of magnitude higher than most estimates found in the literature for tropical tree species. This result can largely be explained by life-history traits of the species. *Milicia excelsa* exhibits a potentially wide-ranging wind-mediated pollen dispersal mechanism as well as very efficient seed dispersal mediated by large frugivorous bats. Estimations of gene flow suggested no major risk of inbreeding because of reduction in population density by exploitation. Different strategy of seed collection may be required for reforestation programmes among populations with different extent of SGS.

Keywords: Central Africa, effective population density, gene dispersal, iroko, *Milicia excelsa*, spatial genetic structure

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Introduction

A quantitative understanding of the genetic dynamics of threatened and/or overexploited plant populations is fundamental for conservation. Seed and pollen dispersal are the two primary factors dictating genetic patterns in plant populations. In tropical tree species, indirect approaches may be required to infer dispersal (Smouse *et al.* 2001; Smouse & Sork 2004; Burczyk & Koralewski 2005). Over time, the interaction of pollen and seed-mediated gene flow with local genetic drift produces patterns of spatial genetic structure (SGS) for neutral molecular markers (Vekemans & Hardy 2004; Hardy *et al.* 2006; Dick *et al.* 2008). Average gene dispersal

Correspondence: K. Daïnou, Fax: +32 (0)81 622342;
E-mail: dainou.k@fsagx.ac.be

[1]These authors have equally contributed to the study.

distances over a few generations can therefore be provided by SGS patterns (Hardy & Vekemans 1999; see below). These data are particularly valuable for management because dispersal is a highly stochastic process, determined by the abundance and behaviour of seed and pollen dispersal vectors, which may vary between years and populations (Nathan *et al.* 2000; Muller-Landau *et al.* 2008). SGS has been detected at several spatial scales in tropical and temperate tree species, and the degree of structure varied significantly because of seed and pollen dispersal vectors (Vekemans & Hardy 2004; Luna *et al.* 2005; Hardy *et al.* 2006; Dick *et al.* 2008).

Demographic parameters, such as tree density and spatial distribution, may also affect SGS in tree populations (Doligez *et al.* 1998; Born *et al.* 2008). Tree density is expected to play a major role in SGS because low densities, exhibited by most tropical tree species, should result in increased SGS because of higher local genetic drift (Vekemans & Hardy 2004; Jump & Penuelas 2006). However, a significant number of studies on tropical tree species (e.g. White *et al.* 2002; Hardy *et al.* 2006; Born *et al.* 2008; Dick *et al.* 2008; Hanson *et al.* 2008) showed that decrease in tree densities could be correlated to an increase in gene dispersal distances, possibly through enhanced pollen flow, reducing SGS. For example, in an African tropical tree, Born *et al.* (2008) found an absence of fine-scale SGS variation among populations with different natural or anthropogenic variation in density and suggested that enhanced gene flow may compensate for lower population density.

Moreover, landscape features, including habitat availability, suitability and distribution; and the effects of human land use may influence dispersal capacities and demographic parameter. Seed and pollen dispersal distance as well as density and spatial distribution may vary according to vegetation type and habitat cover (habitat openness) (Born *et al.* 2008; Hanson *et al.* 2008). These factors can offset the effect of genetic drift and modify SGS (Epperson 2000; Born *et al.* 2008). Therefore, the characterization of SGS in populations from different landscape may be a good strategy to understand factors influencing the range of gene flow in a species.

Spatial genetic structure can be characterized by the decay in kinship coefficients between pairs of individuals as a function of the physical distance separating them (kinship-distance curve, reviewed by Vekemans & Hardy 2004). An indirect estimate of gene dispersal distance, σ_g, can be obtained from the regression slope if the SGS results from an isolation-by-distance (IBD) process at drift-dispersal equilibrium and if information on effective population density is available (Rousset 2000; Hardy *et al.* 2006). In addition, the initial curvature of the kinship-distance curve may provide insights into the relative contribution of pollen and seed dispersal to overall gene flow (Heuertz *et al.* 2003).

However, SGS does not necessarily reflect IBD at drift-dispersal equilibrium (Epperson 2000). It can reflect demographic fluctuations or recent colonization (Gapare & Aitken 2005; Troupin *et al.* 2006). The origin of SGS (IBD vs. colonization history) can be established by a stepwise approach as proposed by Born *et al.* (2008). First, genetic homogeneity of each population may be tested using Bayesian clustering methods that detect potential different gene pools. Second, kinship-distance regression may be used to quantify the extent of SGS within populations.

Despite recent advances in our understanding of the genetic dynamics in plant species, current studies are far from depicting the spectrum of diversity in population structure, life-history traits and evolutionary history of tropical trees (Hardy *et al.* 2006; Dick *et al.* 2008). Here, we assess SGS and gene flow in *Milicia excelsa* (Welw.) C.C. Berg (Moraceae), an important African tropical timber tree species (trade name 'iroko'), which exhibits original life-history characteristics, i.e. very low density and wind-pollination. While most SGS studies of tropical tree have been conducted on insect-pollinated species, *M. excelsa* is wind-pollinated (Jøker 2002). Its seeds are mainly dispersed by frugivorous bats, but squirrels, anomalures or parrots can also act as dispersers (Osmaston 1965; Taylor & Kankam 1999). In a large part of the study area in southern Cameroon, *M. excelsa* populations naturally occur at low densities of 2–20 trees/km^2 (dbh ≥ 30 cm, Feteke *et al.* 2004; Form Ecology Consultants 2004), substantially lower than most tropical tree species, previously studied (50–600 trees/km^2, Dick *et al.* 2008). Furthermore, the species is native to different tropical climates, forest types (forest-savannah mosaic, dry forest, moist evergreen and semi-evergreen forest) and landscapes. The abundance and density of *M. excelsa* vary significantly according to geographical location, forest type (Nichols *et al.* 1998) and/or human land use histories. In many countries, *M. excelsa* has been harvested from natural forests for decades, often at unsustainable rates (Ofori & Cobbinah 2007) and is registered in the IUCN Red List as 'Near Threatened'.

In this study, we investigated SGS in *M. excelsa*, a tropical wind-pollinated dioecious tree species with animal dispersed seeds. Our aims were: (i) to examine the variation of SGS extent in four populations distributed in regions differing in density and degree of habitat openness, to understand factors affecting local genetic structure in the species; (ii) to separate cause of SGS (IBD and mixing of gene pool; and (iii) to infer gene dispersal distance in populations that exhibited SGS pattern consistent with IBD.

Materials and methods

Study species

Milicia excelsa (Welw.) C.C. Berg (Moraceae) is a species of large dioecious and deciduous trees native to sub-Saharan Africa. *Milicia excelsa* is commercialized under the trade name 'iroko'. According to White (1966), *M. excelsa* extends from the Ivory Coast and Ghana through Angola, Central and East Africa to Mozambique. *Milicia excelsa* is the only species of *Milicia* occurring in Cameroon (Bosu *et al*. 2006; Ofori & Cobbinah 2007). The species has been described as light demanding (Jøker 2002; Doucet 2003). The inconspicuous male flowers arranged in pendulous catkins indicate that the species is wind-pollinated, and flowering occurs at the end of the dry season when the trees are leafless (Jøker 2002). Females produce fleshy fruit (length: 55.7 ± 11.0 mm, width: 19.2 ± 4.2 mm, weight: 19.6 ± 5.1 g), containing small seeds (78.2 ± 109.1 seeds/fruit) (Nichols *et al*. 1999; K. Daïnou, unpublished). Seeds are primarily dispersed by the large frugivorous bat *Eidolon elvum* Kerr (Osmaston 1965; Taylor & Kankam 1999). Additional seed dispersers are squirrels (*Paraxerus* sp.), an anomalure (*Anomaluris peli*) and parrots (*Poicephalus gulielmi*, *Psittacus erithacus* and *Agapornis swindernianus*; K. Daïnou, personal observation, *Poicephalus robustus*; Taylor & Kankam 1999). Bats can disperse seeds over long distance as they can forage at distance up to 60 km from the roost. In migration periods, bats can travel on average 90 km/day with a maximal distance of 150 km (Richter & Cumming 2008). *Milicia excelsa* individuals can be up to 50-m tall with a diameter not exceeding 200 cm (*c*. 250 years). *Milicia excelsa* is one of the five most intensively logged trees in Cameroon (Amariei 2005). Iroko stands were estimated to have declined in the last decades because of poor regeneration coupled with excessive levels of exploitation (Ofori & Cobbinah 2007).

Sampling and study sites

We collected leaf or cambium samples of 287 *M. excelsa* individuals in four different regions in south Cameroon: Belabo, Mindourou, Djoum and Biyeyem with respectively 78, 104, 54 and 51 individuals sampled (Fig. 1). Individuals were mostly sampled in the vicinity of forest tracks accessible by vehicle. According to field observation, the spatial distribution of the species appeared rather well spread within each region except in Biyeyem where individuals seemed to be essentially located nearby disturbed zones (roads, secondary forests). Although the range of this species is continuous in southern Cameroon, we considered individuals from each region as a different population in relation to differences in demographic characteristics and landscape

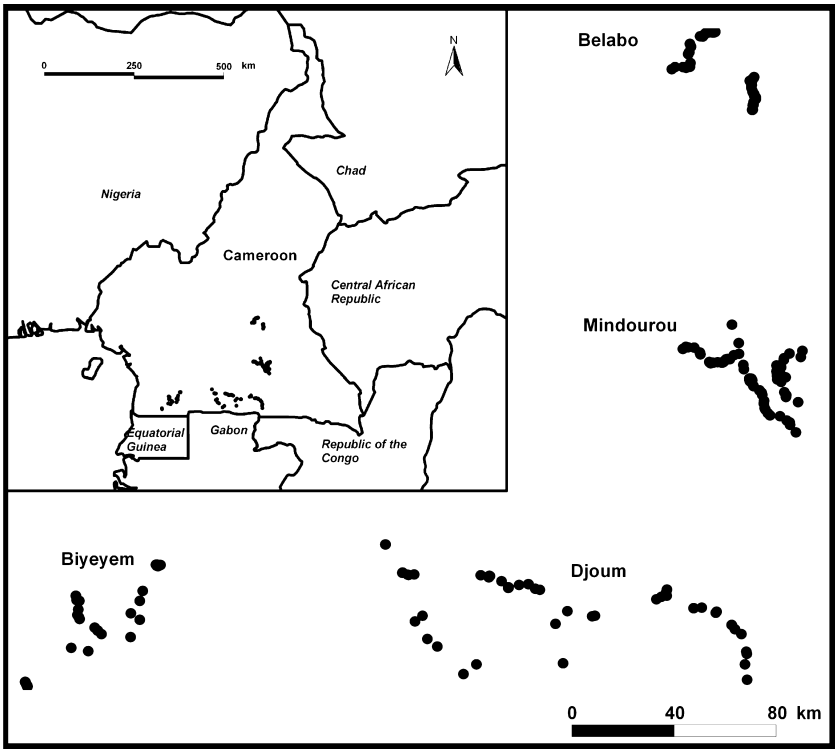

Fig. 1 *Milicia excelsa* sample locations in Cameroon.

features (density, habitat openness). The minimum distance between samples from distinct populations was ~100 km, and the maximum distance between samples within populations was ~60 km, with the exception of Djoum where it reached 150 km. Mindourou and Djoum are located in the East Province of Cameroon, respectively northeast and south of the Dja Wildlife Reserve. The vegetation is dominated by moist semi-evergreen forest rich in lianas (White 1966) and the climate is equatorial with two rainfall peaks and a dry season of 3 months (White 1983; Sonke 1998).The Biyeyem population is located east of the Campo-Ma'an National Park in the transition zone between semi-evergreen and coastal evergreen rainforest (White 1966). The Belabo population is located to the north of the East Province of Cameroon, in an area characterized by forest-savanna mosaic vegetation (transition zone forest, White 1966) with a longer dry season (5–6 months).

From census data and our field knowledge, we know that *M. excelsa* population density varied among populations. Population densities for reproductively mature *M. excelsa* (diameter at breast height >35 cm; K. Daïnou, unpublished data) were estimated to D = 4.9 trees/km^2; at Mindourou and 19.6 trees/km^2; at Djoum (Feteke *et al.* 2004; FORM Ecology Consultants 2004). Our estimation takes into account current observed densities and logging rates of the last decades to obtain reliable density before exploitation. No reliable density estimates were available for Biyeyem and Belabo, but field observations suggested that they were higher than for other populations (K. Daïnou & M. Heuertz, personal observation). Populations also differed in the degree of habitat openness. Mindourou and Djoum populations are typical forest habitats with a high cover canopy. Agriculture is more pronounced in Biyeyem and includes primarily cash crops, such as coffee and cacoa, resulting in more open habitats. *Milicia excelsa* trees in this region were located and sampled in fields or fallows. The most open habitat was found in Belabo area because of forest-savanna habitat and extensive slash and burn agriculture.

Genotyping

DNA was extracted using the DNeasy Plant minikit (QIAGEN, Inc.). Ten specific microsatellite loci characterized by Ouinsavi *et al.* (2006) were tested on ten randomly selected individuals from each population. Eight loci were consistently amplified in polymerase chain reaction (PCR) and were therefore selected for genotyping. Forward primers were labelled with fluorescent dyes(between parenthesis): Mex 51 (6-FAM), Mex 63 (6-FAM), Mex 69 (Hex), Mex 81 (Hex), Mex 95 (6-FAM), Mex 137 (6-FAM), Mex 163a (Ned), Mex 202 (Ned). Loci were segregated into two PCR multiplexes as follows: (i) Mex 51, Mex 81, Mex 137, Mex 163a, Mex 202 and (ii) Mex 63, Mex 69 and Mex 95. Multiplex PCR was performed using the Multiplex PCR Kit (QIAGEN, Inc.) following the manufacturer's protocol in a final reaction volume of 10 μL (5 μL of 2x QIAGEN Multiplex Master Mix, 1 μL of primer mix, 1 μL of Q-solution, 1 μL of H_2O and 2 μL of template DNA). PCR conditions were as follows: 15-min denaturation at 95 °C followed by 30 cycles of 30-s denaturation at 94 °C, 90-s annealing at 59 °C, 60-s extension at 72 °C and 30-min final elongation at 60 °C. Amplifications were conducted using a BIOZYM PTC 200 thermocycler (Biozym Diagnostik GmbH). Genotyping was performed on an ABI PRISM 3100, using a pooled mix of 2 μL PCR product, 13 μL of deionized formamide and 0.6 μL of GS400HD size standard (Applied Biosystems).

Polymerase chain reaction fragment sizes were qualitatively scored and recorded in base pairs with two decimal place precision using GeneMapper 3.0 (Applied Biosystems). Binning into allele classes was performed with Microsoft Excel. All retained multilocus genotypes were scored for at least six of eight markers. The average of missing data per locus was 2% (Table 1).

Data analyses

Genetic diversity and large-scale structure. The number of alleles per locus, allelic range, the total genetic diversity (H_T), the genetic diversity (H_E) and the inbreeding coefficients (F_{IS}) were estimated using GENEPOP 4.0 (Rousset 2008). The software Microchecker version 2.2.3 (Van Oosterhout *et al.* 2004) was used to detect suspected null alleles per locus and per population under the assumption of random mating. To account for suspected null alleles, genotypes at each specific locus per population were adjusted following Van Oosterhout *et al.* (2004), and F_{IS} was subsequently re-estimated on the transformed data. Deviations from Hardy–Weinberg genotypic expectations at each locus in each population were tested using exact tests in GENEPOP. A sequential Bonferroni procedure was applied to discard significant deviations because of chance (Rice 1989).

Differentiation among populations (F_{ST}) was estimated with SPAGeDI ver. 1.2 (Hardy & Vekemans 2002).

Homogeneity of gene pool. The presence of differentiated gene pools in the overall sample and within each population was explored using the Bayesian clustering algorithm implemented in TESS ver. 2.1 (Chen *et al.* 2007). This method employs a Markov chain Monte Carlo (MCMC) process to estimate allele frequencies and

Table 1 Characteristics of microsatellite loci for *Milicia excelsa*

Locus	% Missing data	N_b of alleles	Size range (bp)	H_T	F_{IS}	F_{IS}*	f_{NA}	F_{IS}/F_{IS}*			
								Mindourou	Djoum	Biyeyem	Belabo
Mex51	0.7	5	159–171	0.316	0.365***	0.194***	0.14	0.057	0.220	0.804*/0.629*	0.723*/0.523*
Mex81	1	8	186–205	0.600	0.138***	0.052***	0.05	0.235*/0.085*	0.142	0.210/0.093	−0.016
Mex163a	1	9	204–219	0.666	0.106^{ns}	0.082^{ns}	0.02	0.108	0.177/0.060	0.116	0.047
Mex202	2.4	5	162–179	0.516	0.085***	−0.048*	0.07	−0.147	0.353*/0.120	−0.090	0.296*/0.053
Mex137	0	8	191–215	0.552	0.020^{ns}	nd	0.00	0.065	−0.020	0.061	−0.037
Mex69	5.2	20	175–215	0.853	0.247***	0.053*	0.09	0.615*/0.026	0.061	0.135/0.113	0.065
Mex63	1.4	8	225–250	0.552	0.242***	0.099***	0.10	0.160	0.372*/0.101	0.262/0.040	0.244*/0.076*
Mex95	4.2	4	184–203	0.386	0.342***	0.136***	0.14	0.240*/0.114	0.465*/0.263	0.374/0.175	0.374*/0.177

N_b, number of alleles; H_T, expected heterozygosity; F_{IS}, inbreeding coefficient; F_{IS}*, inbreeding coefficient following allele frequency adjustment according to Van Oosterhout *et al.* (2004); f_{NA}, estimated frequencies of null alleles per locus.
Overall deviation from Hardy–Weinberg genotypic proportions: *$P < 0.05$; ***$P < 0.001$. Within-population deviation from Hardy–Weinberg genotypic proportions: *significant at a table-wide level of $\alpha = 0.05$ after sequential Bonferroni correction.

assign individuals probabilistically either to distinct gene pools or jointly to two or more gene pools if their genotypes indicate admixture. We used the no-admixture model with an interaction parameter ψ of 0.6 and a degree of trend constant (0) or linear (1). Theses parameters (ψ and degree of trend) affect the relative weight given to spatial position and genotype when assigning an individual to a cluster. Twenty independent analyses were carried out for each number of clusters $1 \leq K \leq 10$, using 15 000 MCMC iterations following a burn-in period of 50 000 steps. Analyses were performed for the whole data set and for each population. The number of clusters, K, that best described the data was identified using the maximum log likelihood of data [ln $P(D|K)$], the minimum variance of [ln $P(D|K)$] and the minimum of deviance information criterion (DIC) (Chen *et al.* 2007). After preliminary computations, we did 50 runs, with a burn-in number of sweeps of 10 000 and 50 000 iterations, for the best number of K. TESS software was preferred to other Bayesian clustering algorithms because it performs better in the case of continuous species distribution and low F_{ST} (Latch *et al.* 2006; Chen *et al.* 2007).

Fine-scale spatial genetic structure. We assessed SGS by spatial autocorrelation analyses within populations following Vekemans & Hardy (2004) using SPAGeDI ver. 1.2 (Hardy & Vekemans 2002). Kinship coefficients (F_{ij}) were estimated between individuals i and j using J. Nason's estimator (Loiselle *et al.* 1995). F_{ij} was regressed on the natural logarithm of the spatial distance separating individuals, ln(d_{ij}), which provided regression slopes b_{Ld}. To test for SGS, spatial positions of individuals were permuted 10 000 times to obtain the frequency distribution of b_{Ld} under the null hypothesis that F_{ij} and ln(d_{ij}) were uncorrelated. The extent of SGS was quantified using the S_p-statistic (Vekemans & Hardy 2004), calculated as $-b_{Ld40}/(1 - F_1)$, where F_1 represented the mean F_{ij} for the first distance interval (0–2 km, an approximation of the mean kinship between neighbours) and the b_{Ld40} regression slope of F_{ij} on ln(d_{ij}) for $d_{ij} \leq 40$ km. This distance corresponded to the maximum inter-individual distance that could be obtained in all populations. The S_p-statistic, which depends essentially on the slope of the kinship-distance curve, allows quantification and direct comparison of SGS among populations (Hardy 2003; Vekemans & Hardy 2004). To visualize SGS, kinship coefficients were also averaged over a set of distance intervals (d), giving $F(d)$, and plotted against the logarithm of geographical distance. Five distance classes were chosen to achieve the most uniform scale over populations: 0–2, 2–6, 6–18, 18–80 and >80 km.

Gene dispersal estimates. If SGS in a two-dimensional space results from IBD, gene dispersal estimates can be obtained from the b_{Ld} regression slope and the kinship coefficient between neighbouring individuals (F_1) by the relationship: $N_b \equiv 4\pi D_e \sigma_g^2 = -(1 - F_N)/b_{Ld}$, where D_e is the effective population density, σ_g^2 is half the mean squared gene dispersal distance (0.71 times the quadratic average gene dispersal distance) and N_b may be interpreted as neighbourhood size (Rousset 1997; Vekemans & Hardy 2004). Regression linearity is expected, if it is performed on distances ranging from σ_g to $\sigma_g/(2\mu)^{1/2}$, where μ is the mutation rate (Rousset 2000). An assumed mutation rate of 10^{-3}–10^{-4} per generation for microsatellites translates into an upper distance limit of $\sim 20\sigma_g$. We used an iterative approach to estimate N_b and σ_g knowing D_e, as implemented in SPAGeDI (Hardy & Vekemans 2002). D_e was approximated as the census density D times the effective vs. census population

Table 2 Estimates of population genetics and SGS parameters for each population

Population	N	H_E	F_{IS}	F_{IS}*	F_1	b_{Ld}	b_{Ld40}	S_p (40 km) (SE)	k
Mindourou	104	0.553	0.184***	0.060***	0.022	−0.0063**	−0.0062**	0.0063 (0.0016)	>0
Djoum	54	0.531	0.198***	0.093***	0.035	−0.0101**	-0.0037^{ns}	0.0039 (0.0051)	<0
Biyeyem	51	0.545	0.192***	0.096**	0.013	-0.0014^{ns}	-0.0020^{ns}	0.0020 (0.0019)	nd
Belabo	78	0.561	0.151***	0.060***	0.014	-0.0002^{ns}	-0.0002^{ns}	0.0002 (0.0010)	nd

N, number of individuals sampled; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; F_{IS}*, inbreeding coefficient accounting for null alleles; F_1, kinship coefficients between individuals separated by <2 km; b_{Ld} (b_{Ld40}), slope of the regression of kinship coefficients on the logarithm of spatial distance (between 0 and 40 km); S_p (40 km), intensity of SGS calculated for pairwise distances between individuals up to 40 km in each population; k, initial curvature of the kinship-distance curve (see text); nd, not determined; ns, not significant. *P < 0.05; **P < 0.01; ***P < 0.001.

size ratio (N_e/N, $D_e = D^*N_e/N$) (Vekemans & Hardy 2004). The analysis may only be realized for populations with reliable census density D (Djoum and Mindourou). Demographic studies have demonstrated that N_e/N ratios in adult populations typically range from 0.1 to 0.5 (Frankham 1995). As *M. excelsa* is dioecious this ratio may be further reduced if sex ratio is unbalanced (Nunney 1993). In the Mindourou population, the sex ratio was rather one male for one female (K. Daïnou, unpublished) but no information was available for other populations. In addition, all individuals do not flower each year in a population (K. Daïnou, unpublished). To take into account these characteristics, D_e was estimated under different scenario with $D/2$, $D/4$ and $D/10$.

The relative contributions of pollen and seed dispersal can be explained by the shape of the kinship-distance curve, as Heuertz *et al.* (2003) showed in a simulation study using bivariate isotropic normal dispersal functions of pollen and seeds. The second derivative, k, of a third degree polynomial regression of F_{ij} on the logarithm of short distance indicates the initial kinship-distance-plot curvature (Vekemans & Hardy 2004). A concave shape ($k > 0$) at short distance indicates leptokurtic gene flow, which occurs when the short-distance component of dispersal, often seed dissemination, is spatially restricted. A convex shape ($k < 0$) at short distance indicates no such restriction.

Results

Genetic diversity and large-scale structure

The number of alleles per locus ranged from four to 20, resulting in values of total genetic diversity ranging from $H_T = 0.316$ to 0.853 (Table 1). Inbreeding coefficients (F_{IS}) were significantly positive for six loci of eight loci and null alleles were suspected in all populations and at eight loci, with the exception of Mex137 (Table 1). Allele frequencies were subsequently adjusted for null alleles following Van Oosterhout *et al.* (2004), and F_{IS} remained significantly positive for five loci (Table 1). At the population level, genetic diversity (H_E) ranged from 0.531 to 0.561 (Table 2). A significant heterozygote deficit was detected even after adjusting for null alleles, with the inbreeding coefficient ranging from $F_{IS} = 0.060$ to 0.096 (Table 2). Differentiation among populations was very low ($F_{ST} = 0.01$).

An overall analysis of the 287 individuals using TESS yielded the best clustering of the data for $K = 2$, ([ln $P(D \mid K = 2)$] = −5153, DIC = 10 273) with an assignment of all individuals to one genetic cluster (estimated mixing proportions for $K = 2$: 0.98, 0.02), suggesting that the sample belongs to a single genetic unit. One genetic unit was also inferred within each population.

Fine-scale spatial genetic structure

The regression slope b_{Ld} of pairwise kinship coefficients on the logarithm of spatial distance was significantly negative in two populations: $b_{Ld} = -0.0063$ ($P = 0.005$) for Mindourou; and $b_{Ld} = -0.0101$ ($P = 0.003$) for Djoum (Table 2, Fig. 2). The intensity of SGS assessed at <40-km scale was $S_p = 0.0063$ (0.0016, SE) for Mindourou and $S_p = 0.0039$ (0.0051, SE) for Djoum (Table 2). In the Biyeyem and Belabo populations, slopes (b_{Ld}) were not significantly different from zero (Table 2, Fig. 2).

Gene dispersal estimates

Gene dispersal estimates ranged from $\sigma_g = 3.7$ to 7.1 km in Mindourou and from $\sigma_g = 1$ to 2.6 km in Djoum. These results corresponded to neighbourhood sizes of $N_b = 310$–436 trees in Mindourou and 126–303 trees in Djoum (Table 3). The initial curvature of the kinship-distance curve was concave ($k > 0$ for distances smaller than 2 km) for Mindourou, suggesting a limitation in short-range dispersal. In Djoum, such a limitation was not observed ($k < 0$, Table 2).

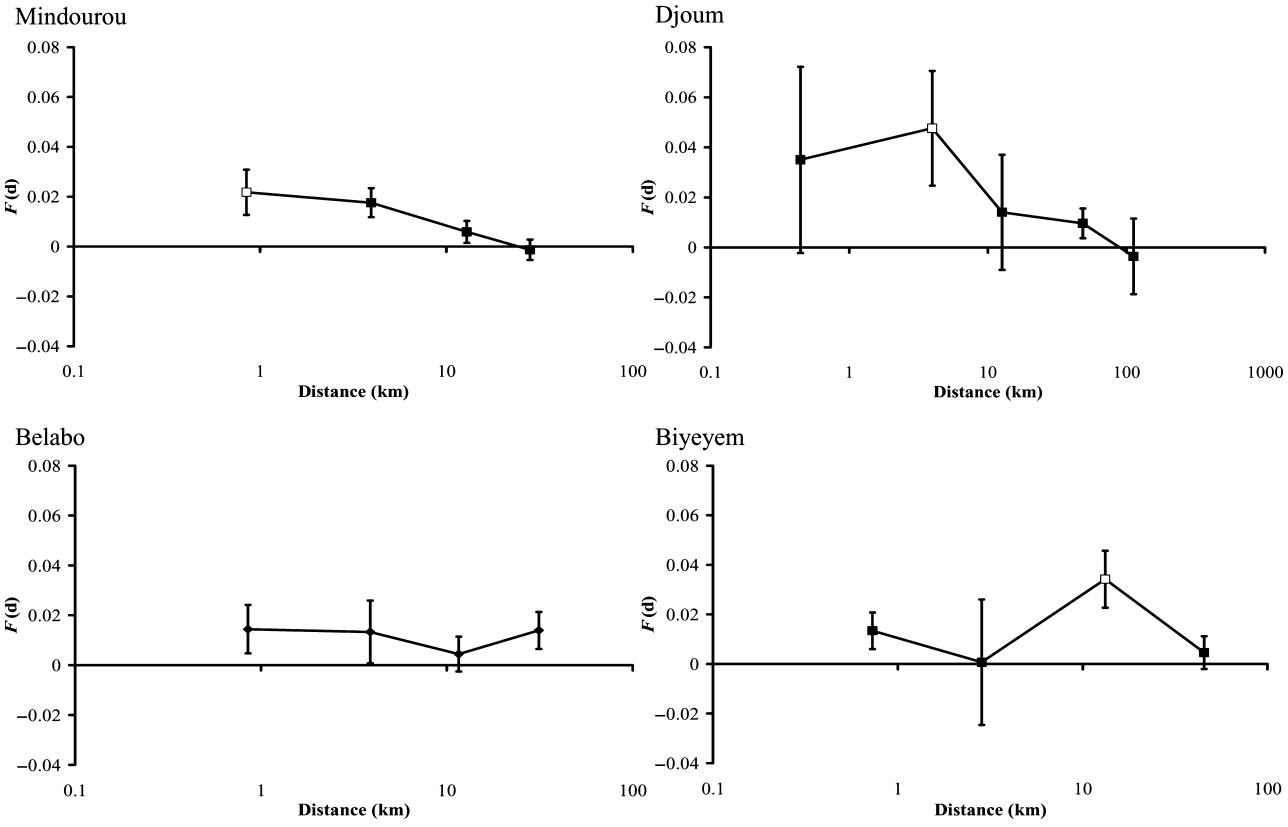

Fig. 2 Average kinship-distance curves, $F(d)$, of each study population, Mindourou, Djoum, Belabo and Biyeyem. Unfilled symbols represent significant ($P < 0.05$) average kinship coefficient values and bars represent standard errors estimated by jackknife.

Table 3 Gene dispersal distance (σ_g) and neighbourhood size (N_b) estimates with respective 95% confidence intervals for the Mindourou and Djoum populations using three estimates of effective densities ($D_e = D/2$, $D/4$ and $D/10$)

Population	D_e	Trees/km^2	σ_g (km)	N_b
Mindourou	$D/2$	2.48	**3.72** (2.12–∞)	432 (140–∞)
Mindourou	$D/4$	1.24	5.29 (2.77–∞)	436 (120–∞)
Mindourou	$D/10$	0.49	**7.10** (4.16–∞)	310 (107–∞)
Djoum	$D/2$	9.8	**1.01** (0.57–∞)	126 (40–∞)
Djoum	$D/4$	4.9	2.22 (0.82–∞)	303 (42–∞)
Djoum	$D/10$	1.96	**2.64** (1.04–∞)	171 (27–∞)

Dispersal distances in bold represent average values for the iterative estimation method cycle (nonconvergence of the method).

Discussion

Extent and causes of SGS variation among populations

This study showed that SGS varied among populations of *Milicia excelsa*, with two populations on four exhibiting SGS and two populations exhibiting random spatial genetic arrangement of individuals.

The use of Bayesian clustering method allows the rejection of the hypothesis of admixed gene pool, and therefore demonstrates that observed SGS patterns in two populations result from IBD even in a species with high dispersal abilities and colonization dynamics. S_p-values (<0.006) in *M. excelsa* were lower than most values reported for gravity or rodent-dispersed tropical tree species, but of the same order of magnitude as those in bat- or bird-dispersed species (Hardy *et al.* 2006; Dick *et al.* 2008). Compared with S_p for wind-pollinated temperate tree species (Vekemans & Hardy 2004; Dick *et al.* 2008, no data on tropical are available), our values are quite similar.

Both tree density and habitat openness may affect strength of SGS in tree populations. From census data and from our field observations, we know that density of *M. excelsa* shows an increase from Mindourou and Djoum forest habitat, to field and fallow habitats of Biyeyem, and, to the open habitat of Belabo (forest-savanna mosaic). This might be because *M. excelsa* prefers increased light conditions and/or is better adapted to climates with a longer dry season such as that of Belabo (Nichols *et al.* 1998; Doucet 2003). SGS was significant in only the low-density populations under continuous

forest cover. Stronger SGS is expected in low- compared with high-density populations because of the increase in local genetic drift at lower densities (Williams 1994; Gehring & Delph 1999; Vekemans & Hardy 2004). Alternatively, the absence of SGS in populations from high-density may well be a direct consequence of more open habitat in these populations. In contrast to the situation described for *M. excelsa*, open habitats have often been associated with low tropical tree densities, for instance, in comparisons between intact and fragmented forests (Young & Merriam 1994; Nason & Hamrick 1997; Sork *et al.* 2002; Jump & Penuelas 2006). However, in most studies, open habitats, even if they present lower tree density, led to an increase in gene dispersal distances, especially by pollen and irrespectively of the pollen dispersal vector (wind-pollination: El-Kassaby & Jaquish 1996; insect-pollination: White *et al.* 2002; Dick *et al.* 2003; Hanson *et al.* 2008; but see Jump & Penuelas 2006; Sork *et al.* 2002).

Human impact may also affect SGS. Current SGS patterns reflect gene flow during the last five to ten generations (e.g. Heuertz *et al.* 2003), which in *M. excelsa* may represent a few thousand of years. *Milicia excelsa* is one of the trees preserved for shading plantations of coffee and cacao and therefore it is unlikely that population sizes have declined because of agriculture. Logging *M. excelsa* is a fairly recent practice, initiated during the last decades that cannot yet impact SGS of adult tree.

Gene flow in a wind-pollinated tropical tree species

Our approach to assess gene flow from the decay of the kinship-distance curve provided indirect estimates of the extent of gene dispersal mediated by pollen and seed movements over the past few generations. Indirect methods are very useful especially for tropical tree species where direct field measurements of dispersal are often difficult to conduct (Smouse *et al.* 2001; Smouse & Sork 2004). Such estimates are usually not very precise and do not distinguish per se the impact of seed vs. pollen dispersal, but simulation studies and comparisons between direct and indirect estimates in different organisms indicate that they are fairly reliable (Austerlitz *et al.* 2004; Vekemans & Hardy 2004; Hardy *et al.* 2006; Leblois *et al.* 2006). Estimates of gene dispersal (σ_g) ranged from 1 to 7.1 km in the two rainforest populations, depending on the assumptions of effective density (D_e). This result was one order of magnitude greater than σ_g estimates in insect-pollinated tropical trees (~100–500 m, Hardy *et al.* 2006; Born *et al.* 2008). Neighbourhood sizes ranged from 126 to 436 individuals, corresponding to areas of 13–633 km^2. Despite our density rectification, it is possible that as a result of intense logging, density estimates in *M. excelsa* may underestimate historical densities. If so, we overestimated dispersal distances, although it is difficult to determine to what extent. In Mindourou, for instance, iroko harvesting should be fairly recent (since 1990; R. Feteke, personal communication). Gene dispersal probably even exceeds these estimations in the more open habitats, where our study indicated no kinship-distance correlation over distances of ~40 km.

Milicia excelsa is wind-pollinated (Osmaston 1965; Jøker 2002), a rare feature in tropical trees, where animal-pollination is most commonly observed (Bawa 1990; Dick *et al.* 2003). Wind-pollination is an inefficient pollination strategy in rainforests because of low species densities and because pollen grains are easily washed to the ground by heavy rains (Dick *et al.* 2008 and references therein). Paradoxically, our results suggested that under conditions of extremely low population density in rainforest populations and given the dioecious mating system in *M. excelsa*, wind-pollination may, in part, explain the large gene dispersal distances estimated in our study. Wind can carry pollen over long distances and pollen dissemination, on average, ranges farther than seed dispersal (Sato *et al.* 2006; Bittencourt & Sebbenn 2007; de-Lucas *et al.* 2008; but see Bacles *et al.* 2006). Wind-pollination may have independently evolved multiple times in angiosperms in response to pollinator limitation (Culley *et al.* 2002). In rainforest species, wind-pollination has been not only proposed for shade-tolerant trees with inconspicuous flowers, including many understory species (Bawa 1990; Bullock 1994) but has also been documented for canopy trees (Atluri *et al.* 2004). Even though *M. excelsa* occupies its specific niche in rainforests, it may be better adapted to semi-deciduous forests and their associated savannahs (Tondeur 1939; Nichols *et al.* 1998), where wind is an efficient pollen dispersal agent.

Tree species with fleshy fruits typically exhibit efficient animal-mediated seed dispersal, suggested from low among-population structure at maternally inherited markers (e.g. Raspé *et al.* 2000; Petit *et al.* 2003). The main seed disperser of *M. excelsa* in the dry semi-deciduous forest of the Afram Headwaters Forest Reserve in Ghana is the bat *Eidolon elvum* (Taylor & Kankam 1999). *Eidolon elvum* travels average daily distances of 29 km (Richter & Cumming 2008). High gene dispersal distances in *M. excelsa* may be explained, in part, by this fact. Congruent with putative bat-dispersal, the Djoum rainforest population from our study displayed a convex kinship-distance curve at short distances ($k < 0$), indicating the absence of any limitations to short-range gene flow. Conversely, in the Mindourou rainforest population, short-range gene flow was apparently limited ($k > 0$). This result might reflect more limited seed dispersal as a result of variation in disperser assemblages (Cordeiro & Howe 2003), which may affect

dispersal distances and SGS patterns. Preliminary observations suggested that squirrels and parrots removed most seeds in this population (K. Daïnou, unpublished field observations). An alternative explanation for the difference in curvature between Djoum and Mindourou is that population density in Mindourou is substantially lower, increasing effective pollen dispersal distances because there are few nearby trees. Hence, in Mindourou population, pollen might disperse over larger distances than seeds, while in Djoum population, pollen and seeds would disperse over similar distances.

Management implications

xDespite clear differences in SGS, overall values of genetic diversity and inbreeding coefficients were relatively homogeneous across all populations. Furthermore, genetic diversity was similar to other tropical tree species (e.g. White *et al.* 1999; Dutech *et al.* 2002; Born *et al.* 2008; Hanson *et al.* 2008). In dioecious taxa, the mating system is 100%, outcrossed and inbreeding can therefore not be attributed to selfing. In populations with SGS, Mindourou and Djoum, the moderate levels of inbreeding observed might be explained by mating among relatives (biparental inbreeding). Alternatively, undetected null alleles are another possible explanation (White *et al.* 1999).

The observation of SGS in different populations of this threatened tropical timber tree species has direct implications for conservation and forest management. Information on SGS levels is important for seed collections to develop reforestation strategies. In comparison with panmictic populations, seed collection in populations exhibiting SGS requires greater distances among trees (here at least 10–20 km) and large sample sizes to avoid collecting seed of related trees that represent only a subset of the genetic diversity (Bittencourt & Sebbenn 2008).

A potential genetic risk for heavily exploited tree species, and particularly dioecious species, is that low pollen source diversity in a given tree becomes a limiting factor for reproductive output and/or the genetic diversity of seeds, which may further cause substantial inbreeding (Robledo-Arnuncio *et al.* 2004). Our indirect estimates of gene dispersal distance were extensive and suggested no major risk of inbreeding because of low population density. However, the risk that pollen may be a limiting factor cannot be assessed with our data, and the likelihood that pollen dispersal is more limited than seed dispersal should not be overlooked.

Conclusion

Patterns of genetic variation in *Milicia excelsa* in four areas of south Cameroon reveal surprisingly low levels of SGS for a species that occurs at very low densities in at least two geographical areas. Indirect estimates of gene dispersal indicated that seeds and/or pollen must disperse over several kilometres to explain this pattern. To distinguish the relative roles of seed and pollen dispersal and elucidate the contributions of dispersal agents and distances, further insights should be obtained (i) by observing seed removal in additional populations, (ii) by investigating SGS at chloroplast markers that might reveal the extent of seed dispersal, and (iii) by genotyping progeny arrays that should provide contemporary estimates of pollen dispersal distances (e.g. using TwoGener by Smouse *et al.* 2001; or KinDist by Robledo-Arnuncio *et al.* 2006).

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The various laboratories involved in this work have collaborated for many years to study the ecology and population genetics of timber trees in the tropical forests of Central Africa. The aim of these research projects is to contribute to the development of best management practices to ensure the sustainability of local forest resources.
