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

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Running title: Spatial genetic structure of *Milicia excelsa*

Abstract

Spatial genetic structure (SGS) analysis is an effective means to characterize the demographic history and dispersal capacities of tree species, and has shown utility in conservation management programs. In the present study, we analyzed SGS patterns and estimated dispersal distances in *Milicia excelsa* (Welw.) C.C. Berg (Moraceae), a wind-pollinated dioecious African tree. The species is considered threatened in large parts of its range and population densities are typically low (ca. 10 adults/km^2). Eight microsatellite markers were used to type 287 individuals comprising four Cameroonian populations. The populations represented different habitats and tree densities. Differentiation among populations was very low and Bayesian clustering methods inferred a single gene pool. Two populations in more open habitat did not display any correlation between relatedness and spatial distance between individuals, whereas significant SGS was detected in two populations situated under continuous forest cover. Our results showed weak SGS with a maximum *Sp* statistic of 0.006, a value in the lower quartile of SGS estimates for trees in the literature. Indirect estimates of gene dispersal distances ranged from σ_g = 1 km to 7.1 km, corresponding to neighborhood sizes of 126 to 436 individuals. These estimates were 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. *M. excelsa* exhibits a potentially wide-ranging wind-mediated pollen dispersal mechanism as well as very efficient seed dispersal mediated by large frugivorous bats. Implications for conservation are discussed.

Introduction

A quantitative understanding of the genetic dynamics of threatened and/or overexploited plant populations is fundamental in conservation management; and seed and pollen dispersal are the two primary factors dictating genetic patterns. In tropical tree species, direct field measurements of dispersal are often difficult to conduct, and therefore indirect approaches may be required (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). SGS patterns can therefore potentially provide estimates of average gene dispersal distances over a few generations (Hardy & Vekemans 1999, see below). These data are particularly valuable for management because dispersal is a highly stochastic process, determined by the abundance and behavior 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 due to seed and pollen dispersal vectors (Luna *et al.* 2005; Vekemans & Hardy 2004; Hardy *et al.* 2006; Dick *et al.* 2008).

Other factors affecting SGS in tree populations are local tree density and spatial distribution (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, result in increased SGS due to higher local genetic drift (Vekemans & Hardy 2004). Alternatively, a decrease in tree densities could indirectly increase gene dispersal distances through enhanced pollen flow, reducing SGS (Hardy *et al.* 2006; Born *et al.* 2008; Dick *et al.* 2008). 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.

Landscape features, including habitat availability, suitability and distribution; and the effects of human land use also define demographic parameters. Furthermore, local demographic structure may fluctuate due to vegetation type and habitat cover. These factors can offset the effect of drift and modify SGS (Epperson 2000; Born *et al.* 2008). Therefore, the characterization of SGS in populations from different environments with varied land use histories may be a good strategy to understand within species gene flow.

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). The *Sp* statistic, which depends essentially on the slope of the kinship-distance curve, allows quantification and direct comparison of SGS among populations, species and genetic marker types (Hardy 2003; 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 (Hardy et al. 2006; Rousset 2000). In addition, the initial curvature of the kinship-distance curve may provide insights on 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 mirror demographic fluctuations as stated above, or, reflect recent colonization (Gapare & Aitken 2005; Troupin *et al.* 2006). If colonization history involves admixture of differentiated gene pools, a hierarchical approach combining a Bayesian clustering method and kinship-distance regression can be used to establish SGS origins (Born *et al*. 2008).

Despite recent advances in our understanding of the genetic dynamics in tropical trees, current studies are far from depicting the spectrum of diversity in population structure, life-history traits and evolutionary history (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 unique life history characteristics. While most SGS studies have been conducted on insect-pollinated species, *M. excelsa* is wind-pollinated (Jøker 2002). Its seeds are mainly dispersed by frugivorous bat, 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/km² (dbh ≥ 30 cm, Feteke *et al.* 2004; Form Ecology Consultants 2004), substantially lower than most tropical tree species previously studied (50-600 trees/km², Dick *et al.* 2008). Furthermore, the species is native to different tropical climates, varied forest types (forest-savannah mosaic, dry forest, moist evergreen and semi-evergreen forest) and landscapes with different human land use histories. The abundance and density of *M. excelsa* varies significantly according to geographic location and forest type (Nichols *et al.* 1998). 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 the present study, we investigate SGS in *M. excelsa*, a tropical tree species with original biological traits of gene dispersal. Specifically, we examine the extent of SGS variation by studying four populations distributed in regions differing in some landscape features. Our objective was to improve our understanding of the factors affecting local genetic structure in the species. We used a stepwise approach (Born *et al.* 2008) combining a Bayesian clustering method and kinship-distance regressions to identify any influence of

colonization history and IBD. We consequently examined gene dispersal distance in populations that exhibited a 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. *M. excelsa* is the only species of *Milicia* occurring in Cameroon (Ofori & Cobbinah 2007; Bosu et al. 2006). 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, 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*; Daïnou, pers. obs., *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 90km/day with a maximal distance of 150 km (Richter and Cumming, 2008). *M. excelsa* individuals can be up to 50 m tall with a diameter not exceeding 200 cm. *M. excelsa* is one of the five most heavily logged trees in Cameroon (Amariei 2005). Iroko stands were estimated to have declined in the

last decades due to 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 features. The minimum distance between samples from distinct populations was approximately 100 km, and the maximum distance between samples within populations was approximately 60 km, with the exception of Djoum where it reached 150 km. Mindourou and Djoum are located in the East Province of Cameroon, respectively north-east 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 three 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 a region characterized by forest-savanna mosaic vegetation (transition zone forest, White 1966) with a longer dry season (5-6 months).

M. excelsa population density and degree of habitat openness varied among regions. Such variation could influence SGS and gene dispersal. Population densities for reproductively mature *M. excelsa* (diameter at breast height >35 cm, Daïnou unpublished data) were $D = 4.9$

trees/km² at Mindourou and 19.6 trees/km² at Djoum (Feteke *et al.* 2004; FORM Ecology Consultants 2004). No reliable density estimates were available for Biyeyem and Belabo, but field observations suggested they were higher than for other populations (Daïnou & Heuertz, pers. obs.). 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 cocoa, resulting in more open habitats. *M. excelsa* trees in this region were located and sampled in fields or fallows. Finally, the comparatively most open habitat was found in Belabo area due to 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 individuals with representatives form each population. Eight loci were consistently amplified in PCR and were therefore selected for genotyping. Forward primers were labeled with fluorescent dies (between brackets): 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 (5µlL 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 in 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).

PCR 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 6 of 8 markers. The average missing data per locus was 2% (Table 1).

Data analyses

Genetic diversity and large-scale structure

The number of alleles per locus, allelic range, genetic diversity (H_E) and 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 due to chance (Rice 1989).

Differentiation among populations (F_{ST}) was estimated with SPAGeDi ver. 1.2 (Hardy & Vekemans 2002). 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). The method employs a Markov chain Monte Carlo (MCMC) process to estimate allele frequencies and assign individuals probabilistically to either 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 affect the relative weight given to spatial position

and genotype when assigning an individual to a cluster. 20 independent analyses were carried out for each number of clusters $1 \leq K \leq 10$, using 15000 MCMC iterations following a burn-in period of 50000 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 [LnP(D|K)], the minimum variance of [LnP(D|K)] and the minimum of DIC (Chen *et al.* 2007). After preliminary computations, we did 50 runs, with a burn-in number of sweeps of 10000 and 50000 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 analysis within populations following Vekemans and 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 10000 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 spatial genetic structure was quantified using the *Sp* 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 neighbors) 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. 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 best uniform scale over populations: 0-2 km, 2-6 km, 6-18km, 18- 80 km and > 80 km.

Gene dispersal estimates

If SGS in a two-dimensional space results from isolation by distance, gene dispersal estimates can be obtained from the b_{Ld} regression slope and the kinship coefficient between neighboring individuals (F_1) by the relationship: $Nb \equiv 4\pi D_e \sigma_g^2 = -(1 - F_N)/b_{Ld}$, 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 *Nb* may be interpreted as neighborhood 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} to 10^{-4} per generation for microsatellites translates to an upper distance limit of about $20\sigma_g$. We used an iterative approach to estimate *Nb* 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 size ratio (N_e/N, $D_e = D * N_e/N$) (Vekemans & Hardy 2004). Demographic studies have demonstrated that N_e/N ratios in adult populations typically range from 0.1 to 0.5 (Frankham 1995). Because *M. excelsa* is dioecious, this ratio may be further reduced if sex ratio is unbalanced (Nunney 1993). Therefore, *D*/2, *D*/4 and *D*/10 were used as alternative estimates of D_e.

The shape of the kinship-distance curve can explain the relative contributions of pollen and seed dispersal, 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 (for details, see 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 total gene diversities ranging from H_T = 0.316 to 0.853 (Table 1). Inbreeding coefficients (F_{IS}) were significantly positive for six loci and null alleles were suspected in all populations and at all 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 better clustering of the data for K=2, ([LnP(D|K=2)] = -5153, Dic =10273) with an assignment of all individuals to one genetic cluster (estimated mixing proportions for K = 2: 0.98, 0.02), suggesting that the sample comprised 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 a < 40 km scale was Sp = 0.0063 (0.0016, SE) for Mindourou; and Sp = 0.0039 (0.0051, SE) for

Djoum (Table 2). In the Biyeyem and Belabo populations, slopes were not significantly different from zero (Table 2, Fig. 2).

Gene dispersal estimates

Gene dispersal estimates ranged from σ_g = 3.7 to 7.1 km in Mindourou; and σ_g = 1 to 2.6 km in Djoum. These results corresponded to neighborhood sizes of *Nb* = 310 to 436 trees in Mindourou; and 126 to 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).

Discussion

Our results point to extensive gene flow in *M. excelsa* a wind pollinated low density tropical tree. However extend of SGS varied among studied populations.

This variation may be expected from the complex combination of factors that determine SGS, including seed and pollen dispersal (gene flow), demographic structure and population history.

SGS variation among populations

One of the most striking results of the present study was the SGS variation among populations, with two populations exhibiting SGS consistent with IBD and two populations exhibiting random spatial genetic arrangement of individuals. *Sp* 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 (Dick *et al.* 2008).

315 Conspecific tree density in *M. excelsa* showed an increase from Mindourou and
316 Djoum forest populations, to field and fallow habitats of Biyeyem, and, to the Belabo
317 population exhibiting an open habitat (forest-savanna mosaic). SGS was significant in only
318 the low-density populations under continuous forest cover. Stronger SGS in low compared to
319 high-density populations is expected because of the increase in local drift at lower densities
320 (Williams 1994; Gehring & Delph 1999; Vekemans & Hardy 2004).

321 In our study, conspecific tree density increased as habitat openness increased. This
322 might be because *M. excelsa* prefers increased light conditions and/or is better adapted to
323 climates with a longer dry season such as that of Belabo (Nichols *et al.* 1998; Doucet 2003).
324 Contrastingly, the literature indicates that open habitats were often associated with low tree
325 densities, for instance, in comparisons of intact to anthropically fragmented forests (Young &
326 Merriam 1994; Nason & Hamrick 1997; Jump & Peñuelas 2006; Sork *et al.* 2002). Higher
327 SGS due to drift is expected in fragmented forests. In most cases, however, this expectation
328 was not supported (e.g., Young & Merriam 1994) because open habitats led to an increase in
329 dispersal distances, especially by pollen and irrespectively of the pollen dispersal vector
330 (wind-pollination: El-Kassaby & Jaquish 1996; insect-pollination: White *et al.* 2002; Dick *et*
331 *al.* 2003; Hanson *et al.* 2008; but see Jump & Peñuelas 2006; Sork *et al.* 2002). In our study,
332 direct estimates of pollen dispersal would be necessary to test for increased pollen
333 dissemination in open/disturbed habitats. However, the absence of SGS in populations from
334 open habitats may well be a direct consequence of higher tree density causing better genetic
335 mixing.

336 Human impact may also affect SGS. Current SGS patterns reflect gene flow during the
337 last five to ten generations (e.g. Heuertz *et al.* 2003), which in *M. excelsa* represents hundreds
338 of years. *M. excelsa* is one of the trees preserved for shading in coffee and cacao plantations,
339 therefore it is unlikely population sizes have declined due to agriculture. Logging *M. excelsa*

is a fairly recent practice, initiated during the last decades (see also below). Therefore, the effect of recent human landscape alterations on SGS is probably low.

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. Such estimates are usually not very precise and do not distinguish per se the impact of seed versus pollen dispersal but simulation studies and comparisons between direct and indirect estimates in different organisms indicate that they are fairly reliable (Hardy *et al.* 2006, Leblois *et al.* 2006 and Vekemans & Hardy 2004). Estimates of gene dispersal (σ_g) ranged from 1 km 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 (about 100 – 500m, Hardy *et al.* 2006; Born *et al.* 2008). Neighborhood sizes ranged from 126 to 436 individuals, corresponding to areas of 13 to 633 km^2. It is possible that due to logging, current density estimates in *M. excelsa* underestimated 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 pers. com.). Gene dispersal probably even exceeds the previous estimates in the more open habitats, where our study indicated no kinship-distance correlation over distances of about 40km.

M. excelsa is wind-pollinated (Osmaton 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 due to 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 conspecific densities in rainforest populations and given the dioecious mating system in *M. excelsa*, wind-pollination might be superior to insect-pollination in providing reproductive assurance and 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 & Sebenn 2007; de-Lucas *et al.* 2008; but see Bacles *et al.* 2006). Wind-pollination has independently evolved multiple times in angiosperms in response to pollinator limitation (Culley *et al.* 2002). In rainforest species, wind pollination has been proposed for shade-tolerant trees with inconspicuous flowers, including many understory species (Bawa 1990; Bullock 1994), but has also been documented in trees higher in the canopy (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). *E. elvum* can travel average daily distances of 29 km (Richter & Cumming 2008), and therefore may explain, in part, high gene dispersal distances in *M. excelsa*. 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 due to 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. Direct estimates of seed and pollen dispersal are needed to test this hypothesis.

Management implications

Despite clear differences in spatial genetic structure, 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. Born *et al.* 2008; Hanson *et al.* 2008; White *et al.* 1999; Dutech *et al.* 2002).

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 to 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 due to 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 geographic areas. Indirect estimates of gene dispersal indicated that seeds and/or pollen must disperse over several kilometers 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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662 Figure legends:

663 **Fig. 1** *M. excelsa* sample locations in Cameroon.

664 **Fig. 2** Average kinship-distance curves, $F(d)$, of each study population, Mindourou, Djoum,

665 Bellabo and Biyeyem. Unfilled symbols represent significant ($P < 0.05$) average kinship

666 coefficient values and bars represent standard errors estimated by jackknife.

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Table 1 Characteristics of microsatellite loci for *M. excelsa*: Number of alleles; size range; H_T, expected heterozygosity; F_{IS}, inbreeding coefficient; F_{IS}*, inbreeding coefficient following allele frequency adjustment according to van Oosterhout *et al.* (2004). Overall deviation from Hardy-Weinberg genotypic proportions: ***P<0.001; *P<0.05. Within-population deviation from Hardy-Weinberg genotypic proportions: *, significant at a table-wide level of α=0.05 after sequential Bonferroni correction.

Locus	% missing data	Nb of alleles	Size range (bp)	H_T	F_{IS}	F_{IS}*	F_{IS} / F_{IS}*			
							Mindourou	Djoum	Biyeyem	Belabo
Mex51	0.7	5	159-171	0.316	0.365***	0.194***	0.057	0.220	0.804*/0.629*	0.723*/0.523*
Mex81	1	8	186-205	0.600	0.138***	0.052***	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}	0.108	0.177/0.060	0.116	0.047
Mex202	2.4	5	162-179	0.516	0.085***	-0.048*	-0.147	0.353*/0.120	-0.090	0.296*/0.053
Mex137	0	8	191-215	0.552	0.020^{ns}	nd	0.065	-0.020	0.061	-0.037
Mex69	5.2	20	175-215	0.853	0.247***	0.053*	0.615*/0.026	0.061	0.135/0.113	0.065
Mex63	1.4	8	225-250	0.552	0.242***	0.099***	0.160	0.372*/0.101	0.262/0.040	0.244*/0.076*
Mex95	4.2	4	184-203	0.386	0.342***	0.136***	0.240*/0.114	0.465*/0.263	0.374/0.175	0.374*/0.177

Table 2 Estimates of population genetics and SGS parameters for each population. 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 less than 2 km; b_{Ld} ($b_{Ld\ 40}$), slope of the regression of kinship coefficients on the logarithm of spatial distance (between 0 and 40 km); *Sp* (40km), 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. Significance values: ns, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Population	N	H_E	F_{IS}	F_{IS}*	F_1	b_{Ld}	$b_{Ld\ 40}$	*Sp* (40km) (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

Table 3 Gene dispersal distance (σ_g) and neighborhood size (*Nb*) 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). Dispersal distances in bold represent average values for the iterative estimation method cycle (non-convergence of the method).

Population	D_e	Trees/km²	σ_g (km)	*Nb*
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-∞)

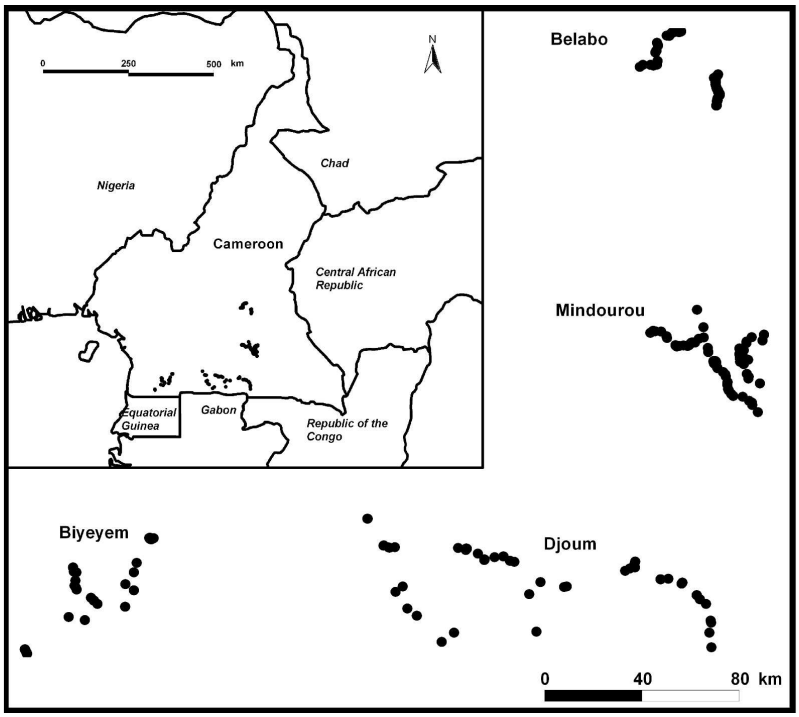

Fig. 1: *M. excelsa* sample locations in Cameroon.
168x119mm (600 x 600 DPI)

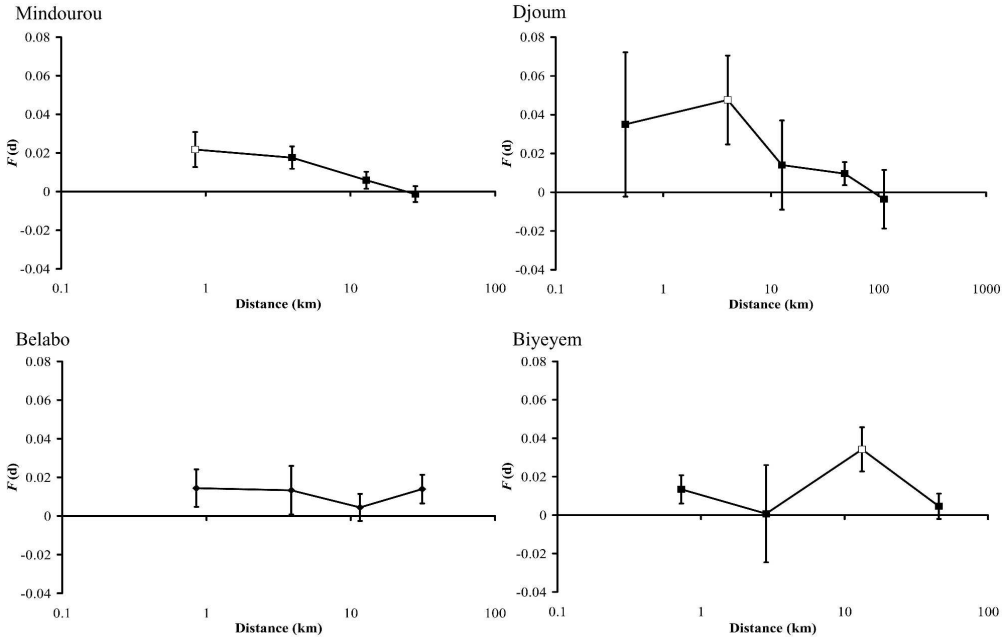

Fig. 2: Average kinship-distance curves, $F(d)$, of each study population, Mindourou, Djoum, Bellabo and Biyeyem. Unfilled symbols represent significant ($P < 0.05$) average kinship coefficient values and bars represent standard errors estimated by jackknife
168x119mm (600 x 600 DPI)