AIDE A L'APPLICATION DES NORMES FSC SUR LA REGENERATION ET LA DIVERSITE GENETIQUE DES ESSENCES DU BASSIN DU CONGO

Contrats C102, 103, 104, 105

Rapport final d'activités

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1. Contexte du projet

Les normes d'aménagement et de certification promeuvent la durabilité des pratiques d'exploitation forestière. Or certaines de ces normes sont parfois très complexes et difficilement applicables par les exploitants forestiers sans l'apport de la recherche appliquée. Tel est le cas du critère FSC relatif à la régénération et au maintien de la diversité génétique des espèces (critère 6.3).

Le potentiel de régénération naturelle des espèces exploitées du Bassin du Congo demeure souvent méconnu, ce qui constitue un handicap majeur à la mise en œuvre d'une production soutenue. Ce potentiel inclut la capacité (i) pour les adultes reproducteurs d'échanger efficacement du pollen, (ii) pour les graines produites de se disperser efficacement et, (iii) pour les graines dispersées de germer et de croître dans un environnement optimal. En conséquence, le projet "*Aide à l'application des normes FSC sur la régénération et la diversité génétique des essences du Bassin du Congo*" avait pour objectif de participer au développement de normes de gestion durable des populations d'essences forestières d'importance économique majeure dans la sous-région. Plus particulièrement, il avait pour ambition de proposer des recommandations sur des densités minimales d'arbres semenciers à maintenir afin de garantir une diversité génétique et un potentiel de régénération suffisants.

Le projet s'est focalisé sur certaines espèces prioritaires (moabi, doussié, sapelli, assaméla, iroko, tali et sipo) pour les raisons suivantes : (1) plusieurs de ces espèces à tendance héliophile affichent un déficit de régénération naturelle, (2) certaines sont menacées par l'exploitation d'une forte fraction de la population semencière, (3) une de ces espèces est sous statut CITES (assaméla), (4) certains taxons fournissent d'importants produits forestiers non ligneux pour les populations locales (moabi, sapelli). Enfin, le choix a été guidé par la disponibilité de marqueurs moléculaires permettant leur étude endéans la durée du projet.

Deux volets principaux ont chapeauté les activités :

> (i) *L'estimation des distances de dispersion du pollen et des graines par des outils de biologie moléculaire.* Des analyses de paternité, de parenté ou de caractérisation des flux géniques entre individus, tenant compte de la localisation des parents, devaient permettre d'estimer les distances de dispersion en fonction de la densité de population.
>
> (ii) *L'effet de l'isolement spatial sur le succès reproducteur.* Il devait être évalué sur base des données génétiques, et par des approches d'écologique classique. Les données génétiques devaient permettre d'estimer les niveaux de consanguinité et les taux d'autofécondation des populations étudiées en relation avec leur isolement spatial. Parallèlement, des observations devaient être faites sur le terrain en combinant trois aspects : l'abondance de la production de graines ; l'abondance et la qualité de la dispersion des graines par les animaux disperseurs selon l'habitat ; et le taux de germination des graines.

En parallèle, le projet devait permettre *le renforcement des capacités locales via la formation* d'étudiants aux techniques de biologie moléculaire et d'écologie de la régénération des arbres tropicaux.

Les sites d'étude principaux ont été situés dans les Unités Forestières d'Aménagement (UFA) gérées par la société Pallisco, au Cameroun. Le projet financé par le PPECF se place dans la continuité des travaux menés par les partenaires dans d'autres sites et sur base de financements complémentaires (projets Dynaffor-FFEM, P3FAC-FFEM, AFRITIMB-FNRS, Herbaxylaredd-BELSPO, Beyond Timber-CBFF). Les résultats présentés dans ce rapport sont le fruit d'une valorisation conjointe.

Le projet a démarré fin janvier 2016 et s'est terminé en mai 2017. Ainsi que stipulé dans « l'Annexe II TDR du projet », l'obtention des résultats dans les délais annoncés était fondamentalement tributaire de deux facteurs : la production de graines des espèces étudiées sur la période du projet, et le polymorphisme (variabilité) des marqueurs génétiques sur les populations étudiées.

Le budget total du projet était de 220.056 €. Le PPECF a apporté une contribution représentant 59% de ce montant, le reste étant le cofinancement des partenaires de mise en œuvre du projet : Gembloux Agro-Bio Tech / Université de Liège, Nature+, Université Libre de Bruxelles et Bioversity International.

2. Fondements méthodologiques des outils génétiques

Les outils génétiques (marqueurs « microsatellites » dans le cas présent) permettent de déduire des liens de parentés entre individus génotypés. Dès lors, le génotypage de familles de mères connues (graines récoltées ou plantules germées en pépinière) permet potentiellement d'identifier le père de chaque descendant et donc d'estimer la distance de dispersion du pollen par analyse de paternité (identification du père). Le génotypage de juvéniles établis (plantules et jeunes tiges installées en forêt) permet d'estimer la distance de dispersion des graines, mais aussi la distance de dispersion du pollen, par analyse de parenté (identification des parents des juvéniles). Les analyses de paternité et de parenté nécessitent de génotyper tous les adultes susceptibles d'être les parents des descendants analysés, ce qui implique un échantillonnage exhaustif des adultes sur une aire suffisamment étendue. Toutefois, d'autres méthodes d'analyse génétique permettent d'estimer la dispersion du pollen et/ou des graines sans recourir à un échantillonnage exhaustif. En conséquence, en fonction des jeux de données accessibles, différentes analyses sont possibles :

- *En présence d'un échantillon d'adultes et de descendants issus de relevés exhaustifs* dans une aire bien déterminée (les parcelles de 400 ou 900 ha, voir point 3.1), des analyses de parenté et de paternité sont réalisables ;
- *En présence d'un échantillon d'adultes et de lots de familles collectés aléatoirement dans une zone donnée* (échantillon non exhaustif), l'analyse de paternité n'est pas possible, mais il existe des méthodes dites « indirectes » (comparaison des pools polliniques) pour estimer la dispersion du pollen ;
- *Avec un jeu de données comprenant essentiellement des adultes* (collecte exhaustive ou aléatoire), on peut estimer les distances de dispersion génique, combinant indistinctement la dispersion du pollen et des graines. Cette méthode est basée sur le principe de l'isolement par la distance.

  L'isolement par la distance (*isolation by distance* ou IBD) est basé sur le principe que les individus géographiquement proches ont tendance à être plus apparentés que des individus plus éloignés : le coefficient de parenté entre paire d'individus (*kinship coefficient*) diminue donc avec la distance spatiale entre ces individus. Ce signal est d'autant plus fort que les distances

moyennes de dispersion génique d'un arbre (pollen et graines) sont faibles. Ainsi, pour une espèce d'arbre dont les vecteurs de dispersion assurent de grandes distances de dispersion des graines et du pollen, on s'attendra à une faible relation entre le coefficient de parenté et la distance spatiale. La relation entre les deux paramètres (coefficient de parenté et distance spatiale, entre paires d'individus) peut être testée par une simple régression. La pente de cette régression mesure l'intensité de la relation et permet d'estimer la distance de dispersion des gènes, nommée σ_g (Rousset 1997[1] ; Vekemans et Hardy 2004[2]).

Outre l'estimation des distances de dispersion des graines et du pollen, les outils génétiques permettent de mesurer les taux d'autofécondation (proportion de graines ou plantules dont le père est aussi la mère) et le degré de consanguinité des différentes cohortes (graines, plantules, adultes). Une conséquence potentiellement néfaste de l'autofécondation, ou d'autres formes de croisements consanguins (entre arbres frères ou entre parents et descendants), est que la viabilité ou la vigueur des individus consanguins est souvent réduite par rapport aux individus non consanguins (c'est-à-dire issus de croisements entre individus non apparentés), un phénomène appelé "dépression de consanguinité". L'existence d'une dépression de consanguinité peut être constatée indirectement par la décroissance du degré de consanguinité moyen du stade graine au stade adulte. Si ce phénomène apparait, et que des croisements consanguins sont fréquents (par autofécondation ou par une faible dispersion des graines et du pollen), on peut craindre une perte de qualité moyenne des génotypes.

3. Dispositifs installés et matériel collecté

Pour les analyses génétiques de paternité et de parenté, des parcelles exhaustivement inventoriées et échantillonnées (fragments de feuille ou de cambium) sont nécessaires. Outre les *arbres adultes*[3] (tiges de diamètre ≥ 10 cm), l'analyse de parenté permettant d'estimer les distances de dispersion du pollen et des graines, nécessite des échantillons de *juvéniles établis* (les jeunes plants naturellement installés et de diamètre < 10 cm), l'ensemble devant être géoréférencé. Quant à l'analyse de paternité, comme expliqué au chapitre 2, elle impose la collecte de *familles de graines* (lots séparés de semences issus d'arbres mères connus).

Dans la mesure où le projet visait à tester la relation entre les caractéristiques génétiques et le succès reproducteur, les autres aspects (pluie de graines, disperseurs, taux de germination) devaient également être menés sur les populations étudiées pour les aspects génétiques.

3.1. Délimitation et inventaire des populations à étudier

Dans le cadre du projet, des dispositifs existants (en RDC, au Cameroun et au Gabon) ont été valorisés et des dispositifs complémentaires ont été implantés dans la concession de Pallisco ainsi que dans la Réserve de Faune du Dja.

[1] Rousset, F. (1997). Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, 145, 1219–1228.

[2] Vekemans, X. & Hardy O. J. (2004). New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, 13, 921–935.

[3] Pour des raisons de terminologie, nous désignerons par adulte les tiges de diamètre ≥ 10 cm, bien que le diamètre de maturité sexuelle soit généralement supérieur et variable d'une espèce à l'autre.

Compte tenu de la variation spatiale de l'abondance des espèces cibles du projet entre les UFA de Pallisco, aucune zone particulière n'offrait les effectifs nécessaires à l'étude de toutes ces espèces. Au moins deux parcelles étaient donc nécessaires. Elles permettaient aussi de mieux évaluer l'effet de la densité de population sur le potentiel de régénération.

Il a été observé qu'une parcelle existante de 400 ha (Pallisco 1), destinée au suivi de la dynamique forestière et implantée dans les UFA 10.030-10.031 (Assiette Annuelle de Coupe (AAC) 11 ; **Figure 1**), recelait un nombre d'échantillons satisfaisant pour les espèces suivantes : le doussié, le sapelli et le tali.

Suite à une analyse des données d'inventaire d'exploitation, il a été décidé d'implanter une seconde parcelle de 900 ha (Pallisco 2) dans l'AAC 9 de l'UFA 10.042 de la même société forestière. La **Figure 2** montre la répartition spatiale des collectes effectuées dans cette parcelle.

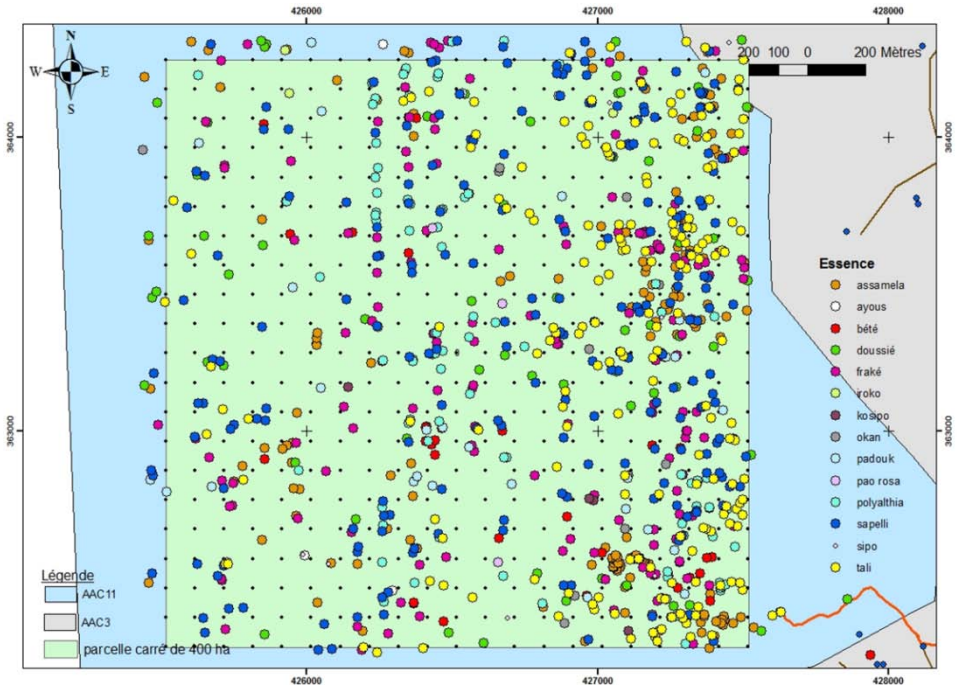


Figure 1. Répartition de diverses essences prioritaires dans la parcelle « Pallisco1 » de 400 ha de l'AAC 11 des UFA 10.030-10.031. D'autres essences suivies pour la dynamique de population sont également mentionnées dans la légende.

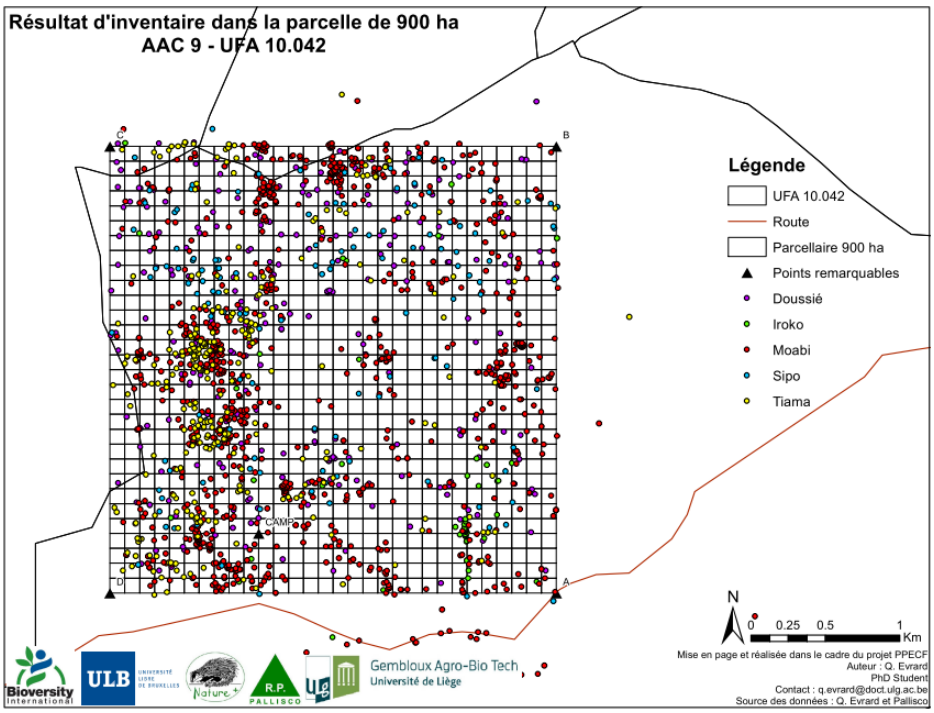


Figure 2. Répartition des espèces-cibles dans la seconde parcelle « Pallisco 2 » de 900 ha.

Deux faits ont toutefois conduit à procéder à un échantillonnage aléatoire en dehors des parcelles pour deux espèces cibles (iroko et assaméla) :

1. L'échantillonnage obtenu dans les deux parcelles était trop faible du fait d'une densité de population faible de l'espèce sur l'ensemble des UFA de Pallisco : c'est le cas de l'iroko ;
2. La population présente dans les parcelles n'a pas fructifié durant l'année 2016 : c'est le cas de l'assaméla.

Pour ces deux essences, l'échantillonnage des parcelles a donc été complété par des collectes aléatoires en d'autres lieux des concessions de Pallisco.

Enfin, une troisième parcelle de 400 ha a été implantée dans la Réserve de Faune du Dja afin d'étudier une zone a priori plus riche en faune, laquelle intervient dans les mécanismes de dispersion. Les résultats liés à l'inventaire de cette parcelle seront valorisés ultérieurement. La **Figure 3** montre la répartition spatiale des collectes effectuées dans cette parcelle.

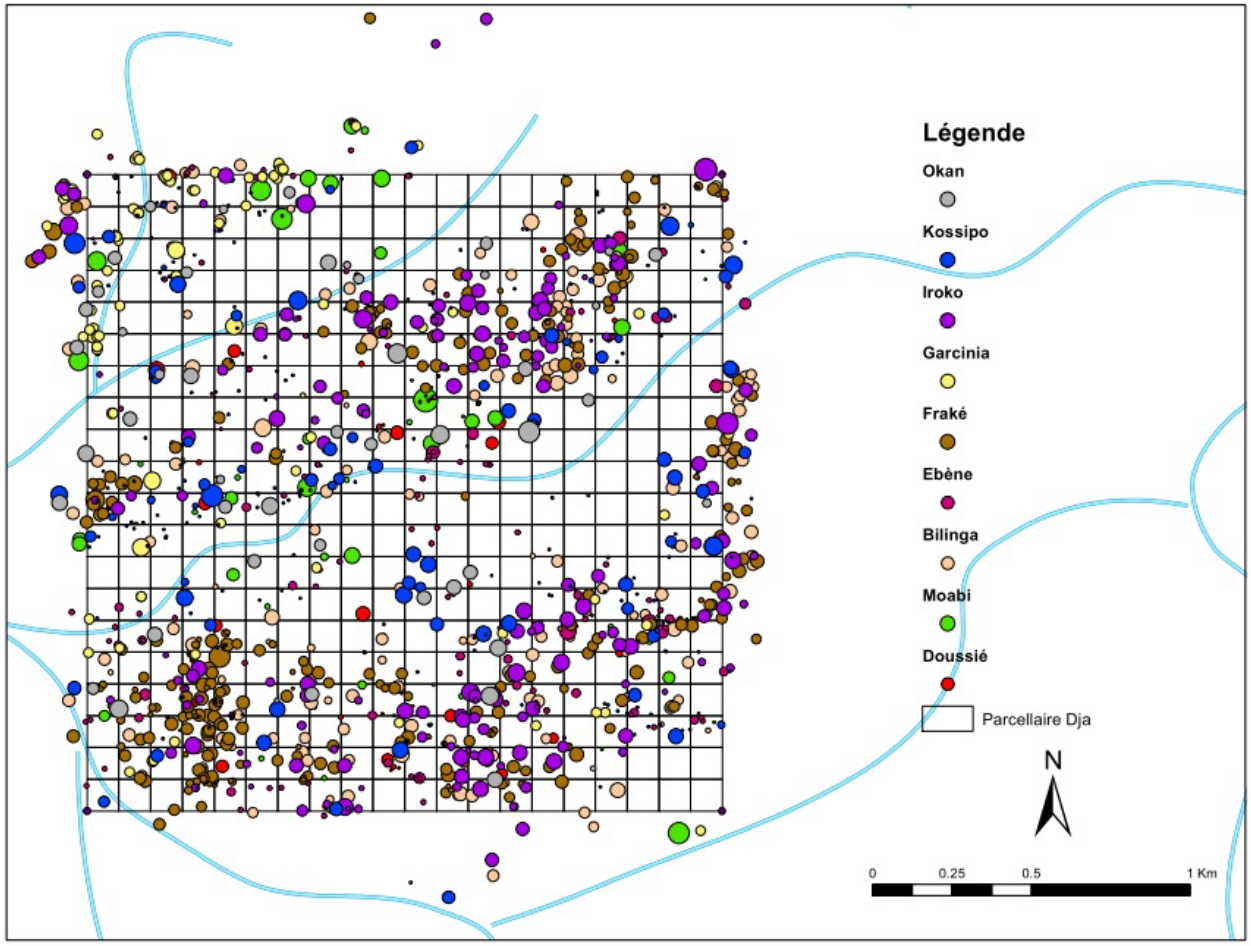


Figure 3. Répartition des espèces-cibles dans la parcelle de 400 ha de la Réserve de Faune du Dja.

3.2. Echantillonnage génétique : effectifs par espèce

Tous les arbres et juvéniles inventoriés ont été échantillonnés (feuille et/ou cambium prélevé) pour les analyses génétiques. Par ailleurs, des graines ont été récoltées pour les espèces ayant fructifié. Lorsque l'extraction de l'ADN était directement réalisable à partir des graines, un échantillon de celles-ci a été conservé à des fins d'analyses génétiques de paternité, tandis qu'un autre échantillon servait à évaluer les taux de germination en pépinière (point 3.3). Les plantules issues de ces tests de germination étaient également échantillonnées (fragments de feuille) à des fins d'analyses génétiques de paternité.

Le **Tableau 1** dresse le bilan de ces collectes par espèce cible. Un total de 4.603 échantillons a pu donc être constitué pour les analyses génétiques.

Tableau 1. Echantillonnage d'arbres, de juvéniles et de lots de familles pour les analyses de génétique. Les collectes aléatoires désignent des échantillonnages opérés au gré des rencontres, hors des deux principales parcelles de 400 et 900 ha.

Espèce	Effectif d'arbres (diamètre ≥ 10 cm)	Effectif de juvéniles (diamètre < 10 cm)	Effectif des lots de familles (plantules issues des tests de germination, ou graines) *	Site de collecte génétique
Assaméla	21	273	- 110 graines - 78 plantules	Collecte aléatoire
Doussié	241	103	169 graines	Pallisco 1, Pallisco 2 et Dja
Iroko	241	188	266 plantules	Pallisco 2, Dja et Collecte aléatoire
Moabi	457	730	300 plantules	Pallisco 2 et Dja
Sapelli	214	/	786 graines Plantules en cours d'obtention	Pallisco 1
Sipo	93	36	/	Pallisco 2
Tali	357	109	307 graines	Pallisco 1
Total par colonne	1.624	1.439	1.372 graines 644 plantules	/
Total général	**5.079**			/

3.3. Pluie de graines, disperseurs du pollen et des graines, et taux de germination

Les méthodologies de collecte des données, à l'échelle de l'individu, ont été les suivantes :

(i) *Quantification de la pluie de graines :* un échantillon aléatoire d'adultes plus ou moins isolés et atteignant le diamètre de fructification régulière de l'espèce, a été repéré avant le début de la fructification. Dès l'initiation de la fructification, ils ont été suivis à un rythme hebdomadaire pendant toute la période de fructification. L'ensemble des fruits sous le houppier a été récolté au sol lors de chaque visite. Les graines ont ensuite été comptées ;

(ii) *Identification des disperseurs du pollen :* les travaux se sont focalisés sur deux espèces dont les fleurs sont très différentes : le moabi et le doussié. Les insectes supposés effectuer la pollinisation ont été capturés dans deux types de piège et au filet. Des pièges photographiques (*camera-traps*) ont également été utilisés. La plupart des observations ont été effectuées directement dans la canopée. Des observations complémentaires ont été effectuées dans la cime d'arbres abattus (**Figure 4**).

(a) **(b)**

Figure 4. (a) Grimpe dans un moabi. **(b)** Piège à insectes placé dans la cime d'un doussié.

(iii) *Identification des disperseurs des diaspores et/ou des prédateurs* : pour les espèces zoochores (doussié, iroko, moabi et tali), les animaux consommant les graines ont été identifiés en utilisant des pièges photographiques placés au pied des arbres cibles (**Figure 5**). Ces mêmes caméras ont été utilisées pour quantifier la prédation (assaméla, tali), en sachant que celle-ci peut s'accompagner dans certains cas d'une dispersion secondaire. Pour l'iroko et le tali, des observations directes (à l'aide de jumelles) ont été également effectuées.

(a) **(b)**

Figure 5. (a) Installation d'un piège photographique près d'un arbre cible. **(b)** Exemple d'animal observé par le piège photographique : ici, un rat d'Emin mangeant des fruits d'iroko.

(iv) *Quantification des taux de germination* : les graines collectées pour l'étude de la pluie de graines ont été mises à germer en pépinière en veillant autant que possible à ce que les pots soient dans des conditions similaires (**Figure 6**). La germination a été suivie chaque semaine.

Figure 6. Tests de germination d'assaméla en pépinière ; les lots de graines par arbre mère sont distingués.

Compte tenu des contraintes logistiques, de la durée limitée du projet, du nombre important d'espèces et de l'irrégularité de leur fructification, ces activités n'ont pu être menées sur tous les taxons. Le **Tableau 2** synthétise les aspects étudiés pour chaque espèce.

Tableau 2. Aspects écologiques étudiés par espèce (cellules en foncé).

Espèce	Pluie de graines	Pollinisateurs	Disperseurs/prédateurs	Taux de germination
Assaméla				
Doussié				
Iroko				
Moabi				
Sapelli				
Sipo				
Tali				

4. Principaux résultats

Ce chapitre présente les résultats les plus importants. Etant donné l'ampleur des données collectées, de nombreuses valorisations complémentaires sont attendues dans les mois à venir.

Afin de disposer de l'ensemble des informations nécessaires à la bonne interprétation des données génétiques, nous présenterons d'abord la synthèse des connaissances obtenues sur l'écologie des espèces. Nous aborderons ensuite les distances de dispersion et terminerons par l'effet de l'isolement sur le succès reproducteur.

4.1. Synthèse de l'écologie des espèces

Le **Tableau 3** synthétise les principales informations relatives à l'écologie des espèces cibles. Aux sept espèces cibles prévues au départ, le movingui a été ajouté, car lors de travaux antérieurs, cette espèce a pu être étudiée en détail et a fourni d'intéressants éléments de comparaison. Le **Tableau 3** reprend :

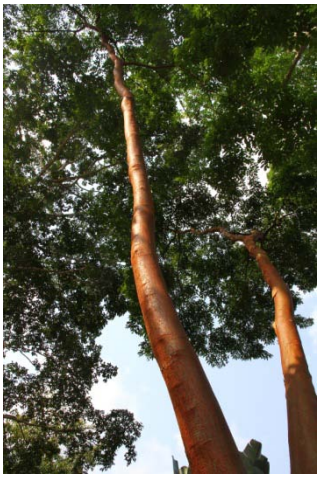
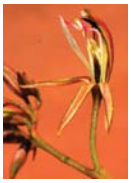
- le mode de reproduction (hermaphrodite : les organes mâles et femelles sont simultanément présents sur la même fleur ; dioïque : les fleurs mâles ou femelles se retrouvent sur des pieds séparés) ;
- le diamètre minimum de floraison ;
- le diamètre à partir duquel 50 % des pieds sont fertiles (Ouedraogo et al. soumis[4]) ;
- le mode de pollinisation ;
- le mode de dispersion des graines (ballochore : par explosion du fruit ; barochore : par chute du fruit suite à la pesanteur ; anémochore : par le vent ; zoochore : par les animaux) ;
- les taux de germination.

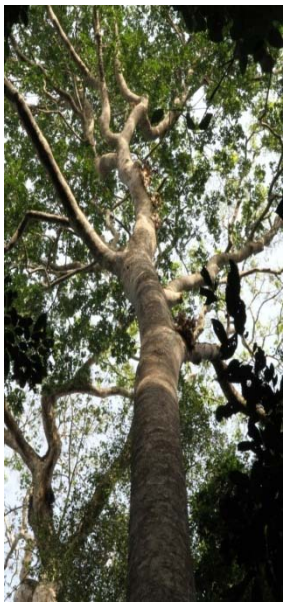
Tableau 3. Synthèse écologique des espèces étudiées.

Assaméla ou Afrormosia, *Pericopsis elata*, Fabaceae 	• Espèce hermaphrodite  • Diamètre minimum de floraison : 15 cm • 50 % de pieds fertiles dès 30 cm de dhp (diamètre à hauteur de poitrine) • Pollinisateurs probables : insectes • Dispersion des graines : anémochore (planeur lourd) • Prédation des graines : céphalophe bleu, rat d'Emin • Taux de germination : 55 – 69 %

[4] Ouédraogo D.-Y., Doucet J.-L., Daïnou K., Baya F., Biwolé A. B., Bourland N., Fétéké F., Gillet J.-F., Kouadio Y. L., Fayolle A., (soumis pour publication au journal **Biotropica)**. "The size at reproduction of canopy tree species in central Africa."

Doussié, *Afzelia bipindensis*, Fabaceae 	• Espèce hermaphrodite  • Diamètre minimum de floraison : 28 cm • Pollinisateurs probables : lépidoptères et coléoptères (étude en cours) • Dispersion des graines : o primaire – ballochore o secondaire – zoochore (écureuil, rat d'Emin) • Prédation des graines : rat d'Emin, écureuils • Taux de germination : 54 – 83 %
Iroko, *Milicia excelsa*, Moraceae 	• Espèce dioïque • Diamètre minimum de floraison : 21 à 37 cm en fonction du site étudié • 50 % de pieds fertiles dès 44 cm de dhp • Pollinisateurs probables : insectes ou vent • Dispersion des graines : zoochore, chauve-souris (accessoirement perroquet, francolins, rongeurs, céphalophes) • Prédation des graines : perroquet  • Taux de germination : 44 % • Dormance et présence dans la banque de graines du sol

Moabi, *Baillonella toxisperma*, Sapotaceae 	• Espèce hermaphrodite  • Diamètre minimum de floraison : 65 cm • 50 % pieds fertiles dès 74 cm de dhp • Pollinisateurs probables : hyménoptères et diptères (étude en cours) • Dispersion des graines : o primaire – barochore o secondaire – Homme, rat d'Emin, athérure, éléphant • Prédation des graines : Homme, rat d'Emin, athérure, éléphant • Taux de germination : 20 – 30%
Movingui, *Distemonanthus benthamianus*, Fabaceae 	• Espèce hermaphrodite  • Diamètre minimum de floraison : 21 cm • Pollinisateurs probables : insectes • Dispersion des graines : o primaire – anémochore (planeur lourd) o secondaire – peut-être rongeurs et oiseaux • Dormance et présence dans la banque de graines du sol

Sapelli, *Entandrophragma cylindricum*, Meliaceae 	• Espèce hermaphrodite • Diamètre minimum de floraison : 17 - 44 cm en fonction du site • 50 % pieds fertiles : 36 – 93 cm de dhp en fonction du site • Pollinisateurs probables : insectes • Dispersion des graines : anémochore 
Sipo, *Entandrophragma utile*, Meliaceae 	• Espèce hermaphrodite • Pollinisateurs probables : insectes • Dispersion des graines : anémochore 

Tali, *Erythrophleum suaveolens*, Fabaceae 	• Espèce hermaphrodite  • Diamètre minimum de floraison : 26 – 55 cm en fonction du site • 50 % pieds fertiles dès 55 – 69 cm de dhp en fonction des sites • Pollinisateurs probables : insectes • Dispersion des graines : o primaire – autochore o secondaire – rongeurs, primates • Dormance et présence dans la banque de graines du sol

Toutes les espèces étudiées sont hermaphrodites, à l'exception de l'iroko. Certaines fleurissent à de faibles diamètres (≤ 30 cm), c'est le cas de l'assaméla, du doussié et du movingui (**Tableau 3**). Pour d'autres un effet "site" est perceptible **(Figure 7**). Le cas du sapelli est évocateur, alors qu'à un diamètre de 100 cm (diamètre minimum d'exploitation – DME – au Cameroun), la probabilité d'être mature est de 80 % dans le sud-est Camerounais, elle n'est que de 50 % dans le nord-Congo.

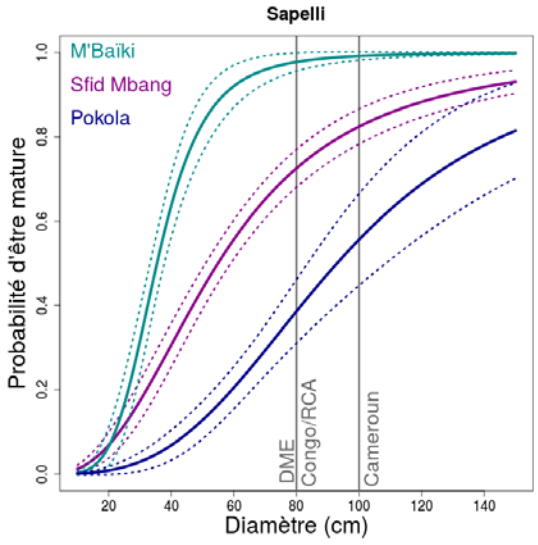


Figure 7. Influence du site sur la probabilité d'être fertile (Ouedraogo et al., soumis).

La plupart des espèces semblent pollinisées par les insectes, bien qu'une pollinisation par le vent soit également documentée pour l'iroko.

Les modes de dispersion des graines sont très variables (**Tableau 3**). En outre la dispersion peut se faire en deux temps : une dispersion primaire par la plante elle-même (barochorie et ballochorie) ou par le vent (anémochorie) pouvant être suivie d'une dispersion secondaire zoochore. Lors de celle-ci, une certaine prédation peut s'exercer (cas des rongeurs sur le doussié par exemple).

4.2. Estimation des distances de dispersion

a) Echantillons collectés

Les individus récoltés à Pallisco qui ont été génotypés sont repris dans le **Tableau 4**.

Tableau 4. Echantillons récoltés à Pallisco ayant fait l'objet d'extraction d'ADN.

Espèce	Adultes	Juvéniles établis	Graines ou plantules de lots de familles	Total
Assaméla	14	78	/	92
Doussié	178	/	132	310
Iroko	59	/	/	59
Moabi	95	95	/	190
Sapelli	95	/	/	95
Sipo	/	/	/	/
Tali	357	109	307	773
Total	**1519**			

A ceux-ci s'ajoutent :

- 417 assaméla (191 adultes et 226 juvéniles) de République Démocratique du Congo (Kisangani) ;
- 624 tali (adultes, juvéniles établis et lots de familles) de RDC (COTREFOR) et du Gabon (CEB-Precious Woods);
- 416 moabi (adultes, juvéniles établis et lots de familles) échantillonnés au Gabon (CEB-Precious Woods) ;
- 1287 sapelli (adultes, juvéniles établis et lots de familles) d'une concession proche de Pallisco (SCTB - Société Camerounaise de Transformation de Bois).

b) Génotypage des échantillons

Les extraits d'ADN ont été amplifiés (**Figure 8**) selon des protocoles spécifiques, à l'aide de jeux de marqueurs nucléaires microsatellites suivants :

- *Assaméla* : 11 marqueurs développés grâce aux collaborations de longue date entre les partenaires du projet (Micheneau et al. 2011)[5] ;
- *Doussié* : 11 marqueurs développés grâce aux collaborations entre les partenaires du projet (Donkpegan et al. 2015)[6] ;
- *Iroko* : 12 marqueurs ont été utilisés. Huit de ces marqueurs ont été développés par une équipe externe (Ouinsavi et al. 2006)[7], tandis que quatre ont été récemment mis au point à l'ULB (non encore publié) ;
- *Moabi* : 13 marqueurs développés par une équipe externe ont été utilisés (Ndiade-Bourobou et al. 2009[8]) et pour lesquels nous avons développé de nouveaux multiplexes permettant une amplification simultanée de plusieurs marqueurs ;
- *Sapelli* : 10 marqueurs développés par une équipe externe ont été utilisés (Garcia et al. 2004)[9]. Ces marqueurs sont également testés sur le sipo ;
- *Tali* : 9 marqueurs développés grâce aux collaborations de longue date entre les partenaires du projet (Duminil et al. 2011)[10].

Les fragments extraits et amplifiés ont été révélés à l'aide d'un séquenceur ABI 3730, et les résultats bruts ont été manuellement vérifiés et édités en utilisant GeneMapper ou Peak Scanner (Applied Biosystems). Les génotypes finaux obtenus ont été encodés pour les analyses suivantes.

[5] Micheneau, C., Dauby, G., Bourland, N., Doucet, J. L., & Hardy, O. J. (2011). Development and characterization of microsatellite loci in *Pericopsis elata* (Fabaceae) using a cost-efficient approach. *American journal of botany*, 98(10), e268-e270.

[6] Donkpegan, A. S., Doucet, J. L., Dainou, K., & Hardy, O. J. (2015). Microsatellite development and flow cytometry in the African tree genus *Afzelia* (Fabaceae, Caesalpinioideae) reveal a polyploid complex. *Applications in plant sciences*, 3(1).

[7] Ouinsavi, C., Sokpon, N., Bousquet, J., Newton, C. H., & Khasa, D. P. (2006). Novel microsatellite DNA markers for the threatened African endemic tree species, *Milicia excelsa* (Moraceae), and cross-species amplification in *Milicia regia*. *Molecular Ecology Notes*, 6(2), 480-483.

[8] Ndiade-Bourobou, D., Vaillant, A., Favreau, B., Gayrin, E., & Bouvet, J. M. (2009). Isolation and characterization of 15 nuclear microsatellite markers for *Baillonella toxisperma* Pierre (Sapotaceae), a low-density tree species of Central Africa. *Molecular Ecology Resources*, 9(4), 1135-1138.

[9] Garcia, F., Noyer, J. L., Risterucci, A. M., & Chevallier, M. H. (2004). Genotyping of mature trees of *Entandrophragma cylindricum* with microsatellites. *Journal of Heredity*, 95(5), 454-457.

[10] Duminil, J., Koffi, G. K., Debout, G., Sebastiani, F., Vendramin, G. G., Heuertz, M., Gonzalez-Martinez S. C., & Hardy, O. J. (2011). Isolation of SSR markers for two African tropical tree species, Erythrophleum suaveolens and E. ivorense (Caesalpinioideae). *American journal of botany*, 98(5), e106-e108.

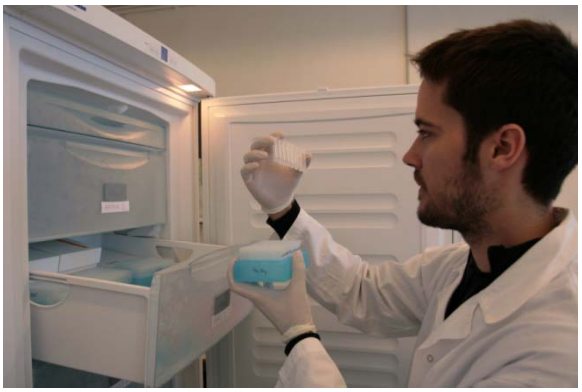



Figure 8. Illustrations d'étapes d'extraction et d'amplification d'ADN des échantillons collectés.

c) Distances de dispersion génique

Le **Tableau 5** synthétise les distances de dispersion génique des essences étudiées. Les distances n'ont pas pu être calculées pour toutes les espèces cibles, faute d'une fructification suffisante. En outre, ces distances dépendent des densités de population participant effectivement à la reproduction, et ces dernières sont estimées à partir des densités issues des inventaires d'aménagement dans les concessions étudiées.

Tableau 5. Distances de dispersion génique estimées pour les huit essences.

Espèce	Site	Distance moyenne dispersion pollen	Distance moyenne dispersion graines	Distance moyenne dispersion gènes (graines + pollen)
Assaméla	Biaro	*	50 m	200 m
Doussié	Pallisco	*	*	2700 m
Iroko	Pallisco	*	*	3500 m
Moabi	CEB	700 m	4000 m	8000 m
Sapelli	SCTB	540 m	420 m	1500 m
Sipo	Pallisco	*	350 m	> 1500 m
Tali	Pallisco	270 m	190 m	430 m
Movingui	CEB	1600 m	70 m	650 m

* Donnée non disponible, soit suite à une fructification insuffisante, soit parce que les analyses sont toujours en cours.

En termes de dispersion du pollen, les espèces dotées de petites fleurs (tali) connaitraient des événements de dispersion de moindre ampleur comparativement à celles ayant de grandes fleurs (movingui). La taille des insectes effectuant la pollinisation explique probablement cette relation. Les grandes fleurs sont effectivement plus attractives pour les insectes de grandes tailles (Lépidoptères, Hyménoptères) plus aptes à parcourir de grandes distances (**Figure 9**).


Figure 9. Illustration du lien entre la taille de la fleur et du pollinisateur : une fleur du doussié et un de ses probables pollinisateurs

Certaines espèces sont confrontées à une faible dispersion spatiale de leurs graines. C'est le cas lorsque les fruits sont soit des gousses déhiscentes, soit des gousses indéhiscentes et papyracées. En effet, les gousses déhiscentes expulsent les graines à quelques dizaines de mètres de la cime, elles peuvent ensuite être amenées plus loin par exemple par des rongeurs parcourant de faibles distances (tali, doussié) **(Figure 10)**. Lorsque le fruit est grand et aliforme (movingui et assaméla), il est emporté par le vent mais seulement à de faibles distances (ne dépassant pas les 100 m). A l'inverse, lorsque le fruit est charnu et de grande dimension, il peut être transporté par des animaux de grande taille comme les primates ou l'éléphant (moabi). L'Homme pourrait aussi jouer un rôle crucial lorsque les fruits sont comestibles (moabi). Enfin, lorsque les graines sont ailées et légères, elles peuvent être emportées assez loin lorsque les vents sont violents (sapelli, sipo) et parcourent alors des distances dépassant les 400 mètres.

Figure 10. Les fruits du doussié sont des gousses dont les graines sont dispersées par éclatement du fruit. Des rongeurs, attirés par les graines très nourrissantes, les ramassent et les cachent pour les consommer ultérieurement. En oubliant certaines cachettes, ils participent ainsi à la dispersion secondaire.

Lorsque l'on combine les distances de dispersion du pollen et des graines, l'ampleur totale du flux de gènes est très variable en fonction des espèces. Pour l'assaméla, le tali et le movingui, l'isolement par la

distance est évident et les distances moyennes de dispersion génique ne dépassent pas les 800 m. Pour le sapelli et le sipo, elles sont proches de 1500 m. Pour le doussié et l'iroko, elles sont assez importantes et avoisinent les 3000 m. Enfin, elles sont très élevées pour le moabi puisqu'elles atteignent les 8000 m (Tableau 5).

Outre les vecteurs de dispersion, la densité du peuplement agit aussi de manière déterminante sur l'ampleur des flux. Il a ainsi été démontré que la distance de dispersion du pollen de tali augmente lorsque la densité des arbres diminue (Duminil *et al.*, 2016b ; **Figure 11**). Une plus faible densité d'arbres fertiles pourrait donc être compensée par une distance moyenne de pollinisation supérieure.

L'effet "site" peut agir de façon cruciale sur les caractéristiques de dispersion des espèces par au moins deux facteurs principaux : la densité d'arbres qui influence l'espacement entre les semenciers et l'abondance de la faune qui agit sur la qualité de la dispersion des graines.

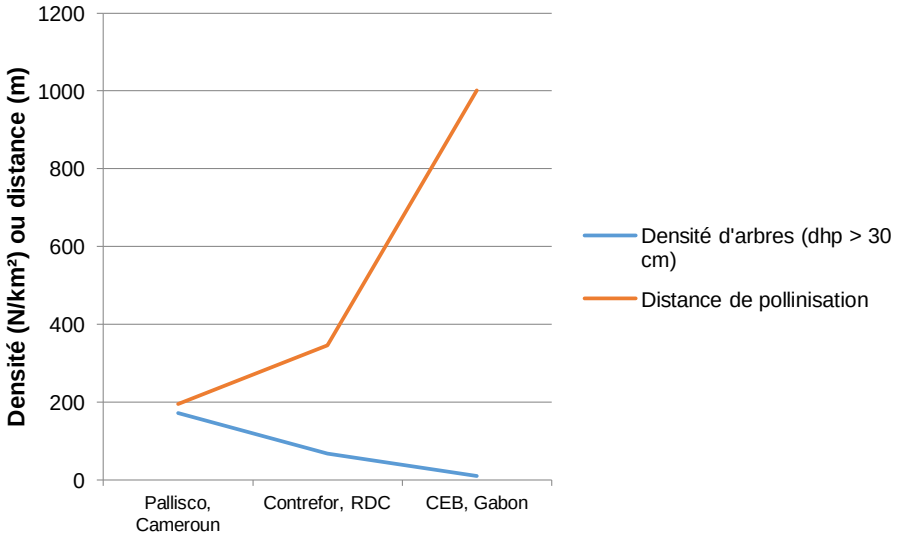


Figure 11. Relation entre distance de pollinisation du tali et densité d'arbres (Duminil et al. 2016 b[11]).

4.3. Effet de l'isolement sur le succès reproducteur

La proportion de graines issue de l'autofécondation est variable selon les espèces. Elle est bien entendu nulle pour la seule espèce dioïque étudiée, l'iroko. Elle est par contre très élevée pour l'assaméla puisque 50 % des graines sont concernées. Elle est modérée pour le tali et le moabi, et faible pour le doussié, le sapelli, le sipo et le movingui.

[11] Duminil, J., Daïnou, K., Kaviriri, D.K., Gillet, P., Loo, J., Doucet, J.-L., & Hardy, O. J. (2016b). Relationships between population density, fine-scale genetic structure, mating system and pollen dispersal in a timber tree from African rainforest. *Heredity*, 116, 295-303.

Tableau 6. Taux d'autofécondation et indice de dépression de consanguinité pour les huit espèces cibles.

Espèce	Site	Taux autofécondation (stade graine)	Dépression de consanguinité
Assaméla	RDC	50%	Oui
Doussié	Pallisco	*	*
Iroko	-	0% (sexes séparés)	Non
Moabi	CEB	25%	Oui
Sapelli	SCTB	14%	Oui
Sipo	Pallisco	<10%	*
Tali	Pallisco	25%	Oui
Movingui	CEB	10%	Oui

* Donnée non disponible pour l'instant.

A l'exception de l'iroko, les différentes espèces sont soumises à une dépression de consanguinité, le taux de consanguinité au sein de la population diminuant du stade graine au stade adulte. Cela signifie que les graines ou les plantules issues d'autofécondation sont moins vigoureuses, voire non viables, comparativement à celles issues d'une fécondation croisée (allofécondation). Cela pourrait expliquer les taux de germination très variables obtenus dans les pépinières forestières en fonction du semencier récolté. Des graines collectées sur un arbre très isolé, et donc peu enclin à être pollinisé par un voisin, seraient de moindre qualité. Cette notion d'isolement est toutefois relative, puisqu'il a été observé des mécanismes de compensation pour le tali, espèce pour laquelle la distance de pollinisation augmente avec l'isolement (voir 4.2).

La constatation d'une dépression de consanguinité chez la plupart des espèces étudiées est cohérente avec les données de littérature chez les arbres forestiers (principalement disponibles chez des espèces des forêts tempérées). D'une manière générale, cela implique que les mesures de gestion durable des ressources forestières doivent veiller à éviter les croisements consanguins. Les graines récoltées sur le terrain pour élevage en pépinière et replantation in situ doivent donc provenir d'individus non isolés afin d'augmenter la chance de ne pas avoir d'effets de consanguinité.

5. Recommandations en termes de gestion

5.1. Nécessité d'adapter les paramètres de gestion en fonction des espèces et des sites

Un résultat majeur de ce projet a été la variabilité des paramètres observés en fonction de l'espèce étudiée et du type de forêt. Tant les diamètres de fertilité que les distances de dispersion peuvent varier d'un site à l'autre.

Les diamètres de fertilité dépendent vraisemblablement des conditions environnementales (climat, fertilité du sol). Ils devraient en conséquence être étudiés pour les différents types forestiers d'Afrique Centrale[12] **(Figure 12).**

La distance de pollinisation dépend des insectes pollinisateurs, lesquels sont probablement aussi différents en fonction du type de forêt, les insectes étant de bons bioindicateurs du niveau de perturbation de l'environnement.

Il en est de même des disperseurs. Une forêt fortement perturbée par les activités anthropiques, dont la chasse, voit sa densité en grande faune, donc en grands disperseurs, se réduire au profit notamment des rongeurs. Bien que ceux-ci, principalement prédateurs, interviennent aussi dans la dispersion, celle-ci se fait sur de plus courtes distances et probablement en moindres quantités.

Il est donc probablement impossible, du moins à l'échelle d'un pays, de donner des recommandations précises en termes de nombre d'arbres à maintenir au-delà d'un certain diamètre en vue de garantir une régénération suffisante.

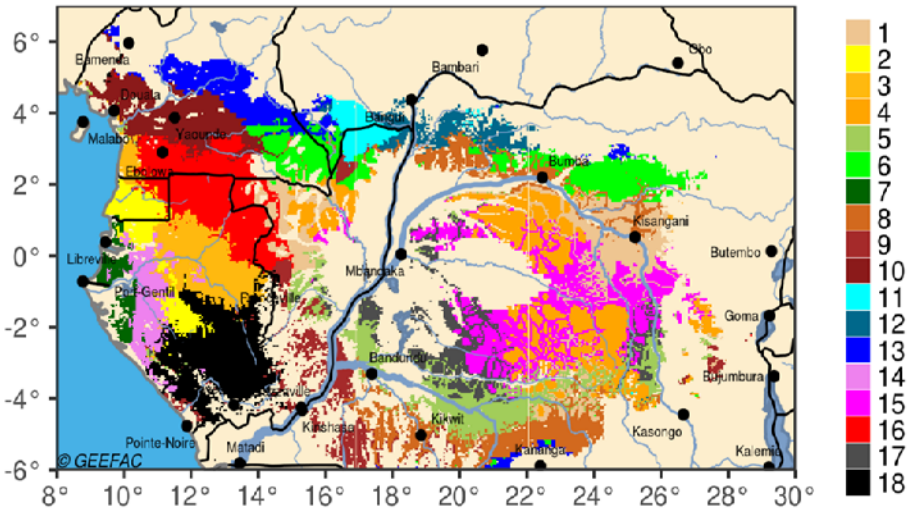


Figure 12. Les types de forêt d'Afrique centrale (Mortier et al., 2017).

[12] Mortier F., Réjou-Méchain M., Cornu G., Bayol N., Benedet F., Bry X., Doucet J-L. Fayolle A., Gourlet-Fleury S., Haurez B., Pelissier R. Trottier R., and the GEEFAC members (2017). Mapping of tree genus distribution at the Central African scale. Rapport du projet Forêts du Congo Changement Climatique (FCCC).

5.2. Maintien d'une proportion de semenciers suffisante

Maintenir des flux de gènes efficaces (croisements par allofécondation) est nécessaire afin de garantir une bonne régénération des essences commerciales et éviter la consanguinité. En termes de dispersion du pollen, il est indispensable de ne pas espacer les arbres matures d'une distance supérieure à la distance que peuvent parcourir les insectes pollinisateurs. Pour y arriver, deux solutions non exclusives sont envisageables : un diamètre minimum d'exploitation (DME) suffisamment élevé, un coefficient d'exploitation limité.

Les diamètres minimum d'exploitation utilisés dans les plans d'aménagement devraient au moins être supérieurs au diamètre à partir duquel 50 % de la population est apte à se reproduire (D50). Le **Tableau 7** reprend, pour les espèces étudiées, les DME prévus dans les différentes législations des pays étudiés ainsi que les D50 observés dans le présent travail.

Parmi les espèces étudiées, les DME légaux des assaméla, doussié et movingui ne semblent pas poser de problèmes particuliers. Par contre, *le DME du moabi en RDC (pays où l'espèce est très peu présente) est trop bas. Il devrait être porté à 80 cm*. *Le D50 du sapelli étant très variable, il est difficile de recommander une valeur minimale qui puisse s'appliquer partout. Par précaution, nous préconisons néanmoins 90 cm. Cette valeur devrait aussi être appliquée au sipo, essence présente toujours en faible densité et dont l'écologie demeure très peu connue. Enfin, nous recommandons la valeur de 70 cm pour le tali.*

Tableau 7. DME légaux par pays et D50 (diamètre à partir duquel 50 % de la population est fertile) ou à défaut Dmin (diamètre minimum de floraison).

Essence	DME (cm)					D50
	Cameroun	*Congo*	*Gabon*	*RCA*	*RDC*	
Assaméla	90	60	*	*	60	30
Doussié	80	60	70	80	60	(Dmin : 28)
Iroko	100	70	80	70	80	21-37
Moabi	100	80	90	*	60	74
Movingui	60	50	70	*	*	(Dmin : 21)
Sapelli	100	80	90	80	80	36-93
Sipo	80	80	90	80	80	
Tali	50	60	70	80	50	55-69

* espèce absente du pays

Ces valeurs sont celles que nous recommandons au niveau national en suivant un principe de précaution. Idéalement, elles devraient être adaptées en fonction du type de forêt, de même que les autres paramètres d'aménagement (croissance, mortalité...). Toutefois, l'état actuel des connaissances ne permet pas encore de l'envisager.

Par ailleurs, l'adoption de ces paramètres ne devrait pas dispenser l'aménagiste d'adapter ces DME légaux en fonction des structures des populations de la concession. En effet, dans le cas d'une

population vieillissante (courbe en cloche), des augmentations supplémentaires pourraient être recommandées comme l'illustre la **Figure 13**.

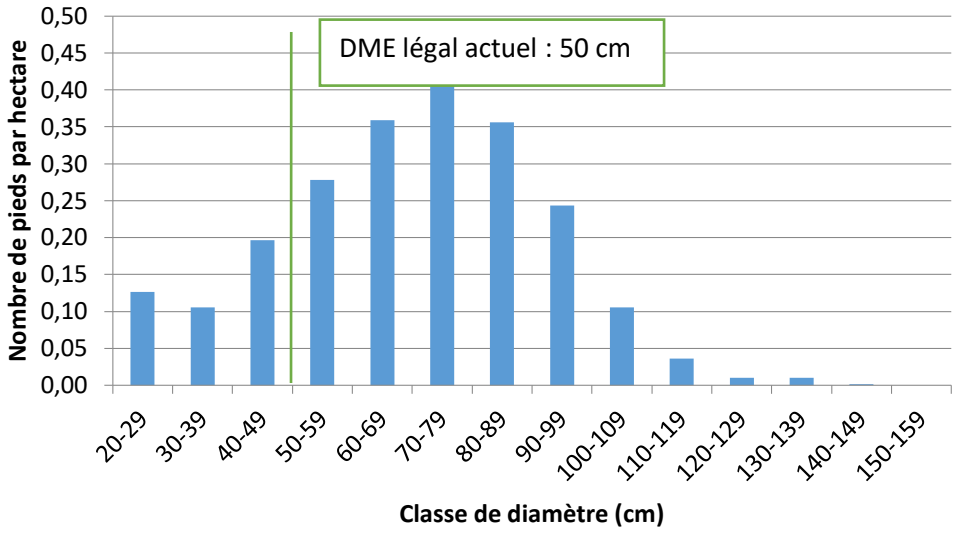


Figure 13. Structure d'une population du tali dans une UFA du Cameroun. Dans l'exemple, faire passer le DME légal de 50 cm à 70 cm ne suffira pas nécessairement pour une préservation de 50 % des semenciers du fait des faibles effectifs des tiges de diamètres intermédiaires.

Une alternative pourrait alors consister à limiter le prélèvement (c'est-à-dire le coefficient d'exploitation) en vue de garantir le maintien d'une population d'arbres fertiles équivalente à 50 % de la population initiale (à la première rotation).

Enfin, pour une espèce dont la structure de population suggère un important ralentissement de la régénération (**Figure 13**), seule la mise en œuvre de techniques sylvicoles d'enrichissement à partir de plants produits en pépinière devrait permettre le maintien de l'espèce sur le long terme.

5.3. Mise en place d'une réelle sylviculture

Les différentes espèces étudiées subissent une dépression de consanguinité. Cette observation a deux implications très pratiques :

1. *Il convient d'éviter de produire des plants en pépinière à partir de graines récoltées sur un arbre très isolé des autres arbres fertiles de la même espèce.*
2. *Il est indispensable de diversifier autant que possible les sources (pieds mères) sur lesquelles les graines sont récoltées pour produire les plants en pépinière. Une pépinière qui s'approvisionnerait sur moins de 10-20 pieds mères par espèce ferait courir le risque qu'apparaisse une forte consanguinité à la génération suivante, en particulier chez des espèces présentant un déficit de régénération naturelle.*
3. *Lors des activités d'enrichissement et de plantation, il est nécessaire d'espacer les plantations d'individus issus d'une même mère, et de mélanger au niveau d'une parcelle d'enrichissement des plants issus de mères différentes, ce qui suppose une parfaite traçabilité depuis la collecte des graines jusqu'à la plantation.*

Idéalement, les semences devraient être produites par une structure nationale veillant à la qualité du matériel produit (comptoir à graines). L'absence de dormance des graines de la plupart des essences commerciales réduit toutefois les possibilités de leur conservation.

Il serait aussi souhaitable d'installer des vergers à graines qui sont des plantations de matériel végétal très diversifié destinées à produire des graines de grande qualité.

6. Formation

Le projet a permis la formation de nombreux chercheurs africains, notamment : Franck Monthe, Armel Donkpegan, Sandra Owusu, Ebenezer Ofori, Dieumerci Assumani, Félicien Tosso, Fructueux Houngbegnon (**Figure 14**).

Figure 14. Le renforcement des capacités de chercheurs africains sur l'approche d'écologie moléculaire a été une priorité du projet.

7. Diffusion des résultats

Les résultats du projet ont été exposés lors de l'atelier du comité scientifique consultatif du MINFOF qui s'est tenu le 26 avril 2017 à Yaoundé (**Figure 15**) . La soixantaine de participants a été particulièrement réceptive aux recommandations formulées.

Figure 15. Les participants au comité scientifique consultatif du MINFOF

Deux articles scientifiques ont été publiés dans le cadre du projet ; ils sont repris en annexe :

- Duminil, J., Abessolo, D. M., Bourobou, D. N., Doucet, J. L., Loo, J., & Hardy, O. J. (2016). High selfing rate, limited pollen dispersal and inbreeding depression in the emblematic African rain forest tree Baillonella toxisperma–Management implications. *Forest Ecology and Management*, *379*, 20-29.

- Duminil, J., Daïnou, K., Kaviriri, D. K., Gillet, P., Loo, J., Doucet, J. L., & Hardy, O. J. (2016). Relationships between population density, fine-scale genetic structure, mating system and pollen dispersal in a timber tree from African rainforests. *Heredity*, *116*(3), 295-303.

D'autres articles sont en cours de finalisation sur le sapelli, le tali et le movingui.

Enfin des articles de vulgarisation à destination de l'ensemble des parties prenantes sont en cours de finalisation, ils seront soumis à la Lettre de l'ATIBT et la Lettre Verte du MINFOF.

ANNEXE

Articles publiés

OPEN

Heredity (2016) 116, 295–303
Official journal of the Genetics Society
www.nature.com/hdy

ORIGINAL ARTICLE

Relationships between population density, fine-scale genetic structure, mating system and pollen dispersal in a timber tree from African rainforests

J Duminil[1,2,3], K Daïnou[2,3,4], DK Kaviriri[1,5], P Gillet[2,3], J Loo[6], J-L Doucet[3] and OJ Hardy[2]

Owing to the reduction of population density and/or the environmental changes it induces, selective logging could affect the demography, reproductive biology and evolutionary potential of forest trees. This is particularly relevant in tropical forests where natural population densities can be low and isolated trees may be subject to outcross pollen limitation and/or produce low-quality selfed seeds that exhibit inbreeding depression. Comparing reproductive biology processes and genetic diversity of populations at different densities can provide indirect evidence of the potential impacts of logging. Here, we analysed patterns of genetic diversity, mating system and gene flow in three Central African populations of the self-compatible legume timber species *Erythrophleum suaveolens* with contrasting densities (0.11, 0.68 and 1.72 adults per ha). The comparison of inbreeding levels among cohorts suggests that selfing is detrimental as inbred individuals are eliminated between seedling and adult stages. Levels of genetic diversity, selfing rates (~16%) and patterns of spatial genetic structure ($Sp \sim 0.006$) were similar in all three populations. However, the extent of gene dispersal differed markedly among populations: the average distance of pollen dispersal increased with decreasing density (from 200 m in the high-density population to 1000 m in the low-density one). Overall, our results suggest that the reproductive biology and genetic diversity of the species are not affected by current logging practices. However, further investigations need to be conducted in low-density populations to evaluate (1) whether pollen limitation may reduce seed production and (2) the regeneration potential of the species.
Heredity (2016) **116,** 295–303; doi:10.1038/hdy.2015.101; published online 23 December 2015

INTRODUCTION

The population density of reproductive individuals ('population density' for short) is central in the context of sustainability of tree resources under selective logging practices. Harvesting implies, among other effects, a reduction of population density with potential prejudicial consequences on reproduction, regeneration and genetic diversity (Degen *et al.*, 2006; Sebbenn *et al.*, 2008; Wernsdörfer *et al.*, 2011; de Lacerda *et al.*, 2013). Plant reproduction, and in particular the extent of gene flow (defined here as the sharing of alleles through mating among individuals), depends on the mating system (from selfing to outcrossing) and the pollen and seed dispersal abilities (Wilcock and Neiland, 2002; Levin *et al.*, 2003; Duminil *et al.*, 2009). A reduction of population density may limit the amount of pollen available for outcrossing, which could reduce seed production (pollen limitation) in obligate outcrossing species and/or increase selfing rate in self-compatible hermaphrodite species (Naito *et al.*, 2008). In the latter case, the quality of seeds would be reduced if inbreeding depression occurs, a phenomenon commonly reported in tree species (Charlesworth, 2003; Duminil *et al.*, 2009). However, the impact of population density reduction on animal-pollinated species is highly variable as it depends on a number of factors, including the behaviour of pollinators (Karron *et al.*, 1995; Ghazoul, 2005). In a number of species, a reduction of population density has been shown to be counterbalanced by a change in pollinator behaviour that allows higher pollen dispersal distance (Hardy *et al.*, 2006; Carneiro *et al.*, 2011). Another potential long-term impact of a demographic reduction is a higher rate of loss of rare alleles, reducing the population adaptive potential (Beardmore *et al.*, 2014). Here again, this impact might be counterbalanced if effective pollen dispersal distances increase at lower density. We could imagine, however, that pollen dispersal distances are smaller below some density threshold where a disruption of pollen flow between individuals would occur because of the unavailability of mates (Forsyth, 2003; de Waal *et al.*, 2015).

The effect of population density reduction on plant species' reproductive biology and genetic diversity has notably been addressed by studying the impact of logging on timber tree species. Indeed, logging results in a reduction of population density. However, logging may also affect reproductive biology through the environmental changes it induces (for example, canopy openings may affect wind movements and pollinators' population size or behaviour, cause stress or, alternatively, favour the regeneration of light-demanding species). Studies addressing the impact of logging have provided contradictory

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results. Most studies have demonstrated a limited impact of logging on timber species reproductive biology and genetic diversity after one event of logging only (one cutting cycle) (Cloutier *et al.*, 2007; Silva *et al.*, 2008; Carneiro *et al.*, 2011). However, modelling approaches have demonstrated that multiple cycles of logging can have a detrimental impact on genetic diversity and demography of harvested tree species (Degen *et al.*, 2006; Sebbenn *et al.*, 2008; Wernsdörfer *et al.*, 2011; de Lacerda *et al.*, 2013). Overall, these studies demonstrate that each species is unique. Accordingly, sustainable forest management practices must be species specific, taking into account the complex relationship between tree density and species reproductive biology. Unfortunately, in Africa, the biology of most tropical species is poorly documented because of the large number of species and logistical difficulties in conducting fieldwork in tropical forests.

Ideally, the impact of logging on population dynamics should be evaluated by a diachronic approach characterizing patterns of gene flow and genetic diversity several years before and after logging (Cloutier *et al.*, 2007; Silva *et al.*, 2008; Carneiro *et al.*, 2011). Alternatively, the impact of logging can be investigated indirectly through a synchronic approach by comparing gene flow patterns of populations with contrasting densities (Piotti *et al.*, 2012; Inza *et al.*, 2012; Ojeda-Camacho *et al.*, 2013; Fageria and Rajora, 2013). Although indirect, the latter approach has the advantage of focussing on one factor (population density) that is affected by logging. In contrast, the synchronic approach should be less affected by such confounding factors when assessing the impact of population density on processes (rather than patterns) such as selfing and pollen dispersal.

Species reproductive biology can be characterized by genetic markers, using either direct or indirect approaches. Direct methods (see, for example, Marshall *et al.*, 1998) estimate contemporary gene flow by conducting parentage analysis, whereas indirect methods estimate either contemporary pollen flow by characterizing the spatial genetic structure of pollen clouds (Robledo-Arnuncio *et al.*, 2007) or historical gene flow by characterizing the spatial genetic structure of adults (see, for example, Vekemans and Hardy, 2004). Although direct methods are generally preferable because they do rely on fewer model assumptions than the indirect approaches (Vekemans and Hardy, 2004), they are also more difficult to set up (availability of highly polymorphic markers, difficulties associated with exhaustive sampling in natural tropical forests and so on).

We applied both indirect and direct methods to assess gene flow and mating patterns on three different populations of *Erythrophleum suaveolens*, an important timber tree species in Central Africa, commonly known as 'tali'. Here we compare the reproductive biology and genetic diversity of populations that present contrasting population densities. As outlined before, this methodology allows investigation of one aspect of the potential impact of logging on population dynamics: population density reduction. We can thus expect lower genetic diversity in a low-density population if gene flow does not compensate for an increase in local genetic drift. In the present paper, we analysed whether a lower population density was associated with: (1) lower genetic diversity, (2) stronger spatial genetic structure due to higher local genetic drift, (3) higher selfing rate and inbreeding, (4) fewer pollen donors contributing to the pollination of each mother tree and (5) a change of pollen dispersal distances. Theoretically, our expectations (1) to (4) should hold if the extent of pollen dispersal is unaffected by population density, or decreases at low density, whereas an increase in pollen dispersal distances under low population density (relationship (5)) might result in a lack of evidence to support any of the other expectations.

MATERIALS AND METHODS

Species description

E. suaveolens (Guill. et Perr.) Brenan (syn. *E. guineense* G. Don.) is an important timber species (up to 40 m in height) that belongs to the Fabaceae–Caesalpinioideae. It has widespread distribution in tropical Africa, occuring east–west from Senegal to Sudan and Kenya, and southward to Mozambique and Zimbabwe (Aubréville, 1959,1970). Within its Central Africa distribution, the species inhabits inland semi-evergreen and evergreen rainforests (Duminil *et al.*, 2010). Individuals are hermaphrodites (Aubréville, 1970), producing racemes up to 12-cm long that bear small-size yellowish white to greenish yellow flowers. The mean flowering time of *E. suaveolens* individuals is ca. 2 months, whereas the mean flowering time of the population is ca. 4 months (F Feteke, Gembloux Agro-Bio Tech, unpublished results). Pollen dispersal is probably assisted by small insects as suggested by the size of the flowers and by observations in the congeneric species *Erythrophleum fordii* (Zhu *et al.*, 2009). Pods do not present any morphological device for dispersal, suggesting predominant barochory. Seeds in fresh pods are surrounded by a mucilage (Guion, 2011) that might have a nutritive value leading to secondary seed dispersal by animals. This is further supported by the presence of seeds in the faeces of primates (Poulsen *et al.*, 2001), including gorilla (Petre *et al.*, 2015). The soil seedbank of the species is important and seeds probably remain viable for $\geqslant 2$ years (Daïnou *et al.*, 2011). The species presents a bell-shaped distribution of diameter size classes (Supplementary Material 1 online), with little representation of low- and high-diameter classes (Kouadio *et al.*, 2014), suggesting reduced natural regeneration in mature closed-canopy forests.

As for all rainforest timber species from Central Africa, *E. suaveolens* is harvested by selective logging (Ruiz Pérez *et al.*, 2005). Logging carried out in Cameroon, Democratic Republic of Congo (DRC) and Gabon does not follow exactly the same standards (Perthuisot and Durrieu De Madron, 2008).The rate of logging is calculated taking into account: (1) a cutting cycle (20 to 30 years according to the country or the logging company); (2) a minimum recovery rate (ratio of the number of trees that will reach the minimum diameter-felling limit at the end of the cutting cycle to the number of trees that already attained the diameter-felling limit before the cutting cycle; the threshold for recovery rate per species was fixed at 40% in Gabon and DRC and 50% in Cameroon); and (3) a minimum diameter-felling limit (fixed at 70 cm diameter at breast height (DBH) in Gabon and 50 cm DBH in Cameroon and DRC).

Sampling

Adult trees are defined as individuals that can contribute to pollination (DBH above 30 cm according to Kouadio, 2009). Leaves or cambium of adult trees and offspring (seeds and leaves of seedlings) were collected in three different populations from Central Africa (Figure 1 and Supplementary Material 2 online). The first population is located in Cameroon within the Forest Stewardship Council (FSC)-certified 'Pallisco' logging concession (East province; mean coordinates: 14.34°E, 3.28°N). The second population is located in DRC within the 'Compagnie de Transport et d'Exploitation Forestière (COTREFOR)' logging concession (Orientale province; mean coordinates: 25.48°E, 1.00°N). The last population is located in Gabon within the FSC-certified 'Precious Woods' logging concession (Ogooué-Lolo province; mean coordinates: 13.03°E, 0.79°S). Sampling was done in January–March 2012 in Cameroon and Gabon and in August 2013 in DRC.

These populations present contrasting tree densities (*D*, population density measured for DBH >30 cm), with the highest density found in Cameroon (1.72 ind ha^{-1}), an intermediate density in DRC (0.68 ind ha^{-1}) and the lowest density in Gabon (0.11 ind ha^{-1}). In Cameroon and DRC we sampled a maximum number of individuals (adults, offspring) in 47.5 and 100 ha plots respectively and along three or four 4.5 to 11 km long transects departing from each plot (Figure 1). These two populations were never logged. In contrast, the population from Gabon has been logged twice, first, 15 years ago primarily targeting *Aucoumea klaineana*. Although *E. suaveolens* was not logged at that time, the opening of the canopy induced by this logging event has probably favoured the regeneration of *E. suaveolens* that would explain the relatively higher representation of low diameter classes in this population compared with the other two populations (Supplementary Material 1 online). Second, 60 to 70% of individuals of *E. suaveolens* with a DBH >70 cm were logged in the

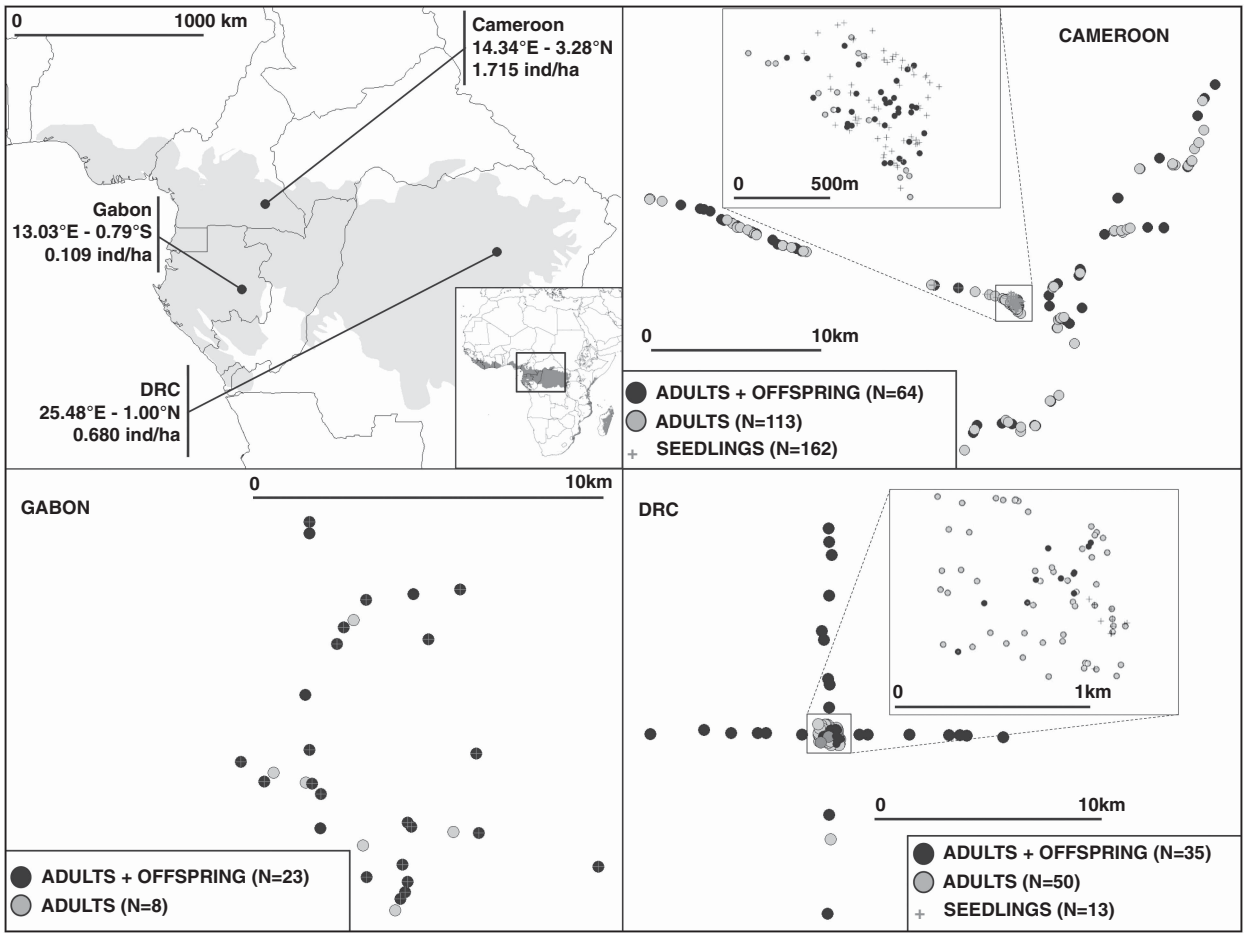

Figure 1 Localization of the three populations and sampling scheme in each population. Upper left: localization of the three studied populations of *Erythrophleum suaveolens* in the Central African forest (grey shaded area represents the potential rainforest area). Upper right: sampling scheme in Cameroon. An exhaustive sampling was conducted in a 47.5-ha plot as represented in the embedded figure. Lower left: sampling scheme in Gabon. Lower right: sampling scheme in DRC. An exhaustive sampling was conducted in a 100-ha plot as represented in the embedded figure.

sampling zone between 2010 and 2011. This recent logging event has favoured species regeneration as attested by the high number of available seedlings (see below) in contrast to the other two populations.

In Gabon, where population density was very low, we sampled as many individuals as possible in a zone of ~7000 ha. In total 177, 88 and 31 adult trees were sampled respectively in Cameroon, DRC and Gabon, among which 72, 33 and 23 were mother trees from which offspring families were also sampled. Seeds or seedlings' leaves were collected on the ground below mother trees (Supplementary Material 2 online). Seeds were predominantly available in Cameroon and DRC, whereas seedlings were predominantly available in Gabon. Most progeny families were composed of seven or eight offspring (Supplementary Material 3 online). Note that this difference in sampling (seeds versus seedlings) could have consequences for the estimation of selfing rate if an elimination of selfed individuals occurs between the seed and seedlings stage.

Genetic diversity and consanguinity characterization

DNA extraction and genotyping of nine microsatellite markers were carried out as described in Duminil *et al.* (2011). Using SPAGeDi v.1-5 (Hardy and Vekemans, 2002), we estimated for each population and cohort (adults, seedlings, seeds) (1) the effective number of alleles (NA_E) following Nielsen *et al.* (2003); (2) the allelic richness expressed as the expected number of alleles among k gene copies ($A_R(k=24)$); (3) the expected heterozygosity (gene diversity) corrected for sample size (H_E) (Nei, 1978); (4) the observed heterozygosity (H_O); and (5) the inbreeding coefficient (F_{IS}).

Differences in genetic diversity parameters between cohorts and populations were tested using an analysis of variance procedure in R (R Core Team, 2013). The different genetic parameters (NA_E, A_R, H_E, H_O, F_{IS}) were estimated for each locus, cohort and population. The parameters were compared among populations for adults only accounting for the locus effect. Then, for each population, the parameters were compared among cohorts accounting for the locus effect.

The presence of null alleles has previously been demonstrated (Duminil *et al.*, 2011). As null alleles cause a bias in the proportion of heterozygotes used to estimate Wright's inbreeding coefficient F, we also used INEST (Chybicki and Burczyk, 2009) under a Population Inbreeding Model to estimate for each cohort and population $F_{(null)}$, an estimator of inbreeding coefficient that removes the bias caused by null alleles. In each population we tested whether the level of inbreeding was significantly different between cohorts by applying unpaired t-tests on (H_0) per individual (proportion of heterozygous loci) in each cohort, considering only individuals genotyped for at least seven out of nine loci. Under inbreeding depression, if inbred (that is, less heterozygous) individuals are counter selected at early life stages, we expect to observe more heterozygosity in adults than in seeds and/or seedlings.

Fine-scale spatial genetic structure

Spatial genetic structure (SGS) of adult trees was assessed for each population. Pairwise kinship coefficients (F_{ij}) between individuals (Loiselle *et al.*, 1995) and 95% confidence intervals were estimated at regular geographical distance intervals using SPAGeDi v.1-4c (Hardy and Vekemans, 2002). SGS was tested by permuting randomly the position of the individuals (10 000 randomizations). Estimates of the *Sp* statistic (a synthetic measure of SGS strength) were obtained for each population from the slope of the regression of pairwise

kinship coefficient on ln(distance) and the mean pairwise kinship coefficient measured at the first distance class (F_1), following Vekemans and Hardy (2004).

Additionally, assuming drift–dispersal equilibrium, we estimated the neighbourhood size and the gene dispersal distance σ_g for each population relying on SGS patterns following the procedure described in Hardy *et al.* (2006). The principle of the method is that F_{ij} is expected to decay linearly with the ln (distance) at a rate inversely proportional to the product $D_E.\sigma_g^2$, at least for a distance range between σ_g and ca. 20 σ_g, where D_E is the effective density of reproductive individuals and σ_g^2 is the axial variance of gene dispersal distance between two generations. As the regression must be performed on a distance interval depending on the parameter to estimate (σ_g), an iterative procedure is implemented in SPAGeDi and should converge only if data were sampled at an adequate spatial scale (Hardy *et al.*, 2006). Different values of D_E were tested, considering 1/2, 1/4 and 1/8 of the adult densities to account for the lifetime variation in reproductive success among adult trees (Hardy *et al.*, 2006). Thus, we tested respectively for Cameroon, DRC and Gabon the following effective densities: 0.850/0.425/0.212, 0.340/0.170/0.085 and 0.054/0.027/0.013 (ind ha^{-1}). Approximate standard errors are obtained by jackknifing over loci.

Assignments of offspring to mothers

As a prerequisite to mating system and pollen pool analyses, given the potential for secondary seed dispersal, we first tested whether the genotypes of candidate mothers and seeds/seedlings that were collected below them were compatible with a mother–offspring relationship. We conducted a maternal analysis using CERVUS (Marshall *et al.*, 1998). CERVUS uses a maximum likelihood approach and assigns maternity according to the highest 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 adults in a population were considered to be candidate mothers; individuals were typed at a minimum of five loci; 90% of candidate mothers were sampled; and a genotyping error rate of 0.1. Only offspring correctly assigned to the expected mother (mother localized above collected offspring) were used in the following steps. When seeds or seedlings were not assigned to the expected mothers, presumably because of secondary dispersal, we did not tentatively reassign them.

Mating system analyses

For each population, we estimated the outcrossing rate (t) in four ways. First, we can rely on $F_{(null)}$ seeds (Cameroon and DRC) or $F_{(null)}$ seedlings (Gabon) obtained from INEST to estimate the outcrossing rate. The outcrossing rate can be calculated from $F_{(null)}$ through the relation $t=(1-F_{(null)})/(1+F_{(null)})$ (Fyfe and Bailey, 1951) that assumes that inbreeding results only from selfing, there is no inbreeding depression and that the inbreeding of adults is at equilibrium. However, as we detected strong inbreeding depression so that adults were non-inbred (see below), we used the relation $t=1-2.F_{(null)}$ that assumes they are in Hardy–Weinberg equilibrium despite selfing because seeds or seedlings resulting from selfing never reach the adult stage.

Second, outcrossing rate per population was also estimated by leading paternity analyses in CERVUS (Marshall *et al.*, 1998). Assigned mothers (see maternity analysis above) were fixed for each offspring and the paternity analysis was conducted using the self-fertilization option. The following simulation parameters were applied to define the confidence level of paternity analysis assignment: 10 000 simulated mating events; all adults of the given population as candidate father plants; individuals typed at a minimum of five loci; 0.5 as the proportion of candidate fathers sampled; genotyping error rate of 0.1. The outcrossing rate was estimated as the number of observed outcrossing events over the total number of tested offspring.

The per-family self-fertilization rate was estimated for each individual of each family using a Bayesian approach implemented in the MSF software (Chybicki, 2013a, b,). The method is based on the mixed mating model, with rates of self-fertilization treated separately for each maternal individual. Markov Chain Monte Carlo analyses were run for 1 million cycles with sampling of the parameters every 1000 steps. The per-family selfing rate was then estimated using a burn-in of 10%. The difference of these selfing rates between populations was tested using unpaired t-tests.

Finally, the multilocus outcrossing rate (t_m) and the single-locus outcrossing rate (t_s) were estimated using MLTR v.3.2 (Ritland, 2002) from progeny arrays ($N=58$ families for Cameroon, 32 for DRC and 23 for Gabon). Mating among relatives (biparental inbreeding) was estimated by the difference (t_m-t_s). In case of biparental inbreeding, it is expected that $(t_s)<(t_m)$, and the difference is a minimum estimate of the apparent selfing because of biparental inbreeding (Shaw *et al.*, 1981). Standard deviation of these estimators was evaluated through a bootstrap procedure (1000 repetitions).

Spatial structure of pollen pools

Relying on mapped mother–offspring genotypic data, contemporary pollen dispersal was inferred using the so-called KINDIST and TWOGENER methods as implemented in the software POLDISP (Robledo-Arnuncio *et al.*, 2007). We followed recommendations from POLDISP's user manual in the preparation of the data sets per population: no mismatch between mother and offspring (loci presenting mismatches were transformed as missing data in offspring), no missing data for the mothers, no seeds resulting from selfing (we used the results from the CERVUS analysis to removed selfed seeds) and a minimum of two offspring per mother. We first used KINDIST to estimate the correlation of paternity of outcrossed progenies within families and between maternal families (separately for each pair of families) from the mapped genotypes of mother–offspring data. The mean number of effective pollen donors (N_{ep}) that participate to cross-pollination was estimated from the within-sibship correlated paternity (r_p) as $N_{ep}=1/r_p$. For comparison (r_p) was also calculated with MLTR (Ritland, 2002) using as input files all correctly-assigned-to-mother offspring (including those resulting from selfing).

To test whether the among-sibship correlated paternity was inversely correlated with the distance between mother trees, we used a Mantel test procedure as implemented in the zt software (Van de Peer, 2002). The slope was negative and significant in each population. We then tested the fit of our data with the different pollen dispersal distribution models available in POLDISP (normal, exponential, exponential power). We used 1000 m (Cameroon and DRC) and 2000 m (Gabon) as reference threshold distances to define unrelated pollen pools, because there was no decrease of the among-sibship correlated paternity beyond these threshold distances. For each population, the best dispersal distribution was chosen by comparing the least-square residuals obtained for each of these distributions. Finally, we used TWOGENER to estimate the effective male population density (D_{Ep}) having as input the pollen dispersal distribution parameters estimated with KINDIST. The ratio (D_{Ep}/D) provides an indication of the proportion of adult trees that have contributed to reproduction as pollen donors within the population for one given year.

RESULTS

Genetic diversity and consanguinity

None of the genetic diversity parameters calculated for the adult cohort differed significantly among populations (data not shown). Only the population in Gabon presented differences in genetic diversity among cohorts (for NA_E, A_R and H_E; see Table 1), but this can be attributed to a sampling effect (low representation of the seed cohort with 23 seeds coming from only 4 mother trees). The (uncorrected) inbreeding coefficient (F) was significantly higher than zero in all populations and cohorts. Estimates correcting for the presence of null alleles, $F_{(null)}$, showed a decreasing trend from seeds to adults: F was slightly lower in seedlings than in seeds and strongly decreased from seedlings to adult stage. In adults, $F_{(null)}$ never departed significantly from zero, indicating that they are not inbred. The unpaired t-test on observed heterozygosity per individual was significant ($P<0.05$) only between adults and seeds in Cameroon and DRC, and between adults and seedlings in Cameroon. The non-significant result in Gabon between adults and seedlings may be due to the low sample size of adult individuals in this population because

mean heterozygosity values showed the same trends as in the other populations.

Fine-scale SGS and inference of gene dispersal distances

In all three populations a signal of isolation by distance was observed with pairwise kinship coefficients decreasing significantly with distance (Figure 2 and Table 2). In all populations, kinship for the first distance class (ca. 100 m for Cameroon and DRC, ca. 500 m for Gabon) ranged from 0.04 to 0.06, and quickly dropped with distance, indicating that spatially close individuals are more related. The kinship-distance curve for the low-density Gabonese population was similar to the ones of the medium- to high-density populations but shifted towards larger distances (Figure 2), whereas the *Sp* statistics, which quantify the strength of SGS, were similar for all three populations, ranging from 0.0053 to 0.0073 (Table 2). The procedure to estimate gene dispersal parameters converged only for the Cameroonian population. For $D_E = 0.820$ ind ha^{-1}, $\sigma_g = 379 \pm 67$ m (mean ± s.e.), and neighbourhood size Nb = 148 ± 54. For $D_E = 0.410$ ind ha^{-1}, $\sigma_g = 483 \pm 64$ m, and neighbourhood size Nb = 120 ± 33. No convergence was reached for $D_E = 0.205$ ind ha^{-1}.

Table 1 Parameters of genetic diversity of the different cohorts of the *Erythrophleum suaveolens* populations

Population	*Cohort*	N	NA_E	A_R	H_E	H_O	F	$F_{(null)}$
Cameroon	Adults	177	3.63	5.03	0.628	0.531	0.155	0.003
	Seedlings	162	3.53	4.94	0.606	0.488	0.195	0.122
	Seeds	499	3.51	5.02	0.624	0.498	0.202	0.157
Difference among cohorts[a]			NS	NS	NS	NS*	NS	—
DRC	Adults	88	3.39	5.07	0.581	0.512	0.119	0
	Seedlings	13	3.07	4.38	0.535	0.465	0.130	NA
	Seeds	238	3.30	4.95	0.590	0.470	0.203	0.110
Difference among cohorts[a]			NS	NS	NS	NS*	NS	—
Gabon	Adults	31	4.11	6.08	0.658	0.515	0.217	0
	Seedlings	175	4.51	5.98	0.648	0.489	0.246	0.116
	Seeds	23	3.19	4.68	0.600	0.461	0.232	0.132
Difference among cohorts[a]			*	***	*	NS	NS	—

Abbreviations: A_R, Allelic richness ($k = 24$); DRC, Democratic Republic of Congo; *F*, Inbreeding coefficient (potentially biased by null alleles); $F_{(null)}$, Corrected inbreeding coefficient under a population inbreeding model (unbiased by null alleles); H_E, Gene diversity corrected for sample size; H_O, Observed heterozygosity; *N*, Sample size; NA, not available; NA_E, Effective number of alleles; NS, not significant.
[a]Differences of genetic diversity among cohorts as tested through a two-way analysis of variance (ANOVA) procedure (*$P<0.05$; ***$P<0.001$); NS* indicates test that were not significant with the two-way ANOVA but that were significant using the paired *t*-test (see text for details); '—' indicates that no test was done for the corresponding parameter as estimates of $F_{(null)}$ per loci cannot be estimated.

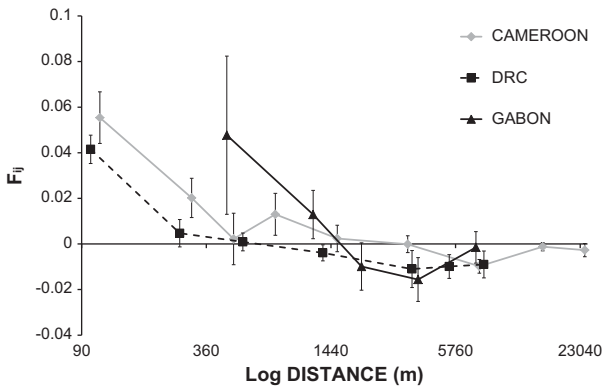

Figure 2 Average kinship coefficients F_{ij} between pairs of individuals at different geographical distance intervals (log scale) in each population. Vertical bars are s.e.m. The continuous grey line, the continuous black line and the dashed grey line correspond respectively to the Cameroonian, the Congolese and the Gabonese populations.

Assignment of offspring to mothers

For the Cameroonian population, 384 out of 499 seeds (~77%) were assigned to the expected mother. In this population, we did not tentatively assign collected seedlings to mothers. For the Congolese and Gabonese populations, respectively 200 out of 254 seeds and seedlings (~79%), and 179 out of 199 seeds and seedlings (90%) were assigned to the expected mother.

Mating system analyses

All three populations of *E. suaveolens* presented a mixed mating system (mixtures of selfed and outcrossed pollination). We found a linear trend between selfing rate and population density with one of the methods used (MSF software, Table 2), which was contrary to our expectations as selfing increases with increasing population density. However, the unpaired *t*-test between populations (based on the per-family self-fertilization rates) demonstrates that estimates of outcrossing rates are only significantly different between the Gabonese ($t_m = 0.850$) and Cameroonian ($t_m = 0.782$) populations. This is potentially only because of a methodological bias as the selfing rate could have been underestimated in the population from Gabon where seedlings had to be used instead of seeds. The Gabonese population also presented a higher biparental inbreeding signal as measured by ($t_m - t_s$) than the two other populations.

Spatial structure of pollen pools

In all three populations, the among-sibship correlated paternity was negatively and significantly correlated with distance between mother trees (Supplementary Material 4 online). MLTR and POLDISP provided similar estimates of correlated paternity within maternal sibships (r_p) for each population (Table 3). The differences between populations were not significant ($P > 0.05$ for all comparisons, unpaired *t*-test). Despite a more than twofold higher population density (*D*) in Cameroon than in DRC, this latter population presented only a slightly lower proportion of individuals participating in pollination (D/D_{Ep}) (Table 3). However, the number of effective pollen donors (N_{EP}) was higher in the Congolese population than in the Cameroonian one. The Gabonese population presented the lowest D_E and N_{EP}. In the absence of confidence intervals around these measures, we were not able to test whether differences were statistically significant. The best fit of the dispersal distribution was obtained with the exponential dispersal kernel in all three populations. The average pollen dispersal distance estimates decreased from 1001 m in the low-density population to 195 m in the high-density population (Table 3).

DISCUSSION

We analysed patterns of genetic diversity and structure, mating system and gene flow in three populations of the African timber tree species *E. suaveolens*. The three populations present different population densities (for trees with DBH > 30 cm). The study of the relationship between contrasting population densities and reproductive biology or genetic diversity parameters can be used to predict one aspect of the potential impact of logging. The Cameroonian population presented

Table 2 Mating system and fine-scale spatial genetic structure parameters of the *Erythrophleum suaveolens* populations

	Cameroon	*DRC*	*Gabon*
F_1 (mean ± s.e.)[a]	0.0554* (0.0113)	0.0415* (0.0062)	0.0477* (0.0347)
Sp statistic (mean ± s.e.)	0.0061 (0.0016)	0.0053 (0.0012)	0.0070 (0.0081)
t ($F_{(null)}$)[b]	0.690	0.780	0.740
t (CERVUS)[c]	0.800 (0.166–1.00)	0.830 (0.571–1.00)	0.780 (0.428–1.00)
t_m[d] (s.d.)	0.782 (0.103)	0.818 (0.145)	0.850 (0.177)
t_m[e] (s.d.)	0.816 (0.030)	0.885 (0.029)	0.874 (0.046)
t_m–t_s[f] (s.d.)	0.098 (0.020)	0.109 (0.023)	0.161 (0.033)

Abbreviation: DRC, Democratic Republic of Congo.
Mating system parameters are based on the genotypes of seeds (Cameroon and DRC) or seedlings (Gabon), whereas fine-scale spatial genetic structure estimates are based on the genotypes of adults.
[a]F_1: mean kinship coefficient between individuals at the first distance class.
*Significant F_1 values ($P<0.001$).
[b]Outcrossing rate t as calculated through the relation $t=1-2F_{(null)}$.
[c]t as estimated through a paternal analysis in CERVUS, numbers in brackets refer to the range of t observed in the population using progeny array with a minimum size of six offspring.
[d]t_m: multilocus population outcrossing rate (MSF).
[e]t_m: multilocus population outcrossing rate (MLTR).
[f]t_m-t_s: indirect estimation of the presence of biparental inbreeding (MLTR).

Table 3 Parameters of pollen dispersal in each population

	Cameroon	*DRC*	*Gabon*
D: density of adults per ha (diameter >30 cm)[a]	1.72	0.68	0.11
D_{Ep}: estimated effective density of pollen donors per ha[b,c]	0.40	0.13	0.01
D_{Ep}/D: proportion of individuals that participate to the pollination (%)	23.3	19.1	9.0
Global ϕ_{ft}[b,c]	0.061	0.059	0.094
r_p: average within-sibship correlated paternity[d] (N: sample size/s.e.)	0.112 (58/0.045)	0.087 (32/0.051)	0.164 (23/0.057)
r_p: average within-sibship correlated paternity[e] (N: sample size/s.e.)	0.110 (57/0.034)	0.084 (28/0.039)	0.153 (22/0.044)
N_{EP}: number of effective pollen donors[c]	9	12	7
Average pollen dispersal distance (in m)[e]	195	346	1001

Abbreviation: DRC, Democratic Republic of Congo.
[a]As measured in the field.
[b]Obtained by TWOGENER.
[c]Confidence intervals cannot be derived for these estimators.
[d]Obtained by MLTR.
[e]Obtained by KINDIST using an exponential dispersal distribution model.

the highest density followed by the population from DRC (ca. 40% of the Cameroonian one; Table 3) and finally the population from East Gabon with the lowest density (ca. 6% of the Cameroonian one). Importantly, these results concern only *E. suaveolens* and not *E. ivorense*, both commonly named as tali, but that actually correspond to two clearly differentiated parapatric species (Duminil *et al.*, 2010).

Lower population density is not associated with lower genetic diversity

No relationship between genetic diversity and population density is supported by our results. Levels of genetic diversity are equivalent across the three populations and across cohorts within each population (Table 1). A previous study indicated that the Cameroonian and the Gabonese populations are located in two different gene pools with equivalent levels of genetic diversity (Duminil *et al.*, 2013). Our data, acquired at a fine-geographical scale, support this result, highlighting that historical factors did not lead to different levels of genetic diversity. Estimates of genetic diversity of the seed cohort also depend on pollen dispersal distance (pollen diversity). The larger the pollen distance, the greater the degree seeds will 'capture' the pollen diversity of surrounding individuals. We have demonstrated that pollen dispersal distance is inversely related to population density. This indicates that the absence of relationships between population density and genetic diversity in the case of *E. suaveolens* actually corresponds to a drift/migration balance: local genetic drift would be expected to be higher in the low-density population (the strength of drift is inversely proportional to population size), but it is prevented by long-distance pollen dispersal (see below), maintaining genetic diversity.

Nevertheless, we need to be careful about extrapolation of such a conclusion. Despite the absence of a relationship between genetic diversity and population density in this study and others (Cloutier *et al.*, 2007; Fageria and Rajora, 2013), it is not certain that genetic diversity is secure. Indeed, long-term impacts of selective logging across multiple cutting cycles investigated through modelling have led to detrimental effects (Degen *et al.*, 2006; Sebbenn *et al.*, 2008; Wernsdörfer *et al.*, 2011; but see Vinson *et al.*, 2014). One of these models particularly emphasized the impact of juvenile mortality on genetic diversity (Wernsdörfer *et al.*, 2011). We do not have specific data on *E. suaveolens* juvenile mortality, but the population structure is clearly truncated at small-diameter classes in the two nonlogged populations (Cameroon and DRC; Supplementary Material 1 online), indicating the poor regeneration capacity of the species under closed canopy as expected for light-demanding species. This suggests that the future replacement of seed trees after logging is not ensured at the same level, unless intensive enrichment planting is carried out as done by some FSC-certified logging companies of Central Africa. This effect has to be carefully modelled before drawing any clear conclusion on the long-term impact of logging on *E. suaveolens* genetic diversity. Further information on the impact of cutting on species' regeneration is needed to propose recommendations. *E. suaveolens* is a light-demanding species in its early stages and opening the canopy by logging might have a positive influence on its regeneration (Kouadio,

2009) if the openings are large enough. Moreover, important parameters such as cutting diameter, growth rates, natural mortality rate and time between two cutting cycles have to be integrated for accurate interpretation of results.

Lower population density is not associated with stronger spatial genetic structure

The strength of SGS depends on a migration–drift equilibrium. Overall, the strength of *E. suaveolens* SGS as measured by the *Sp* statistic is typical of tree species (Table 2; Vekemans and Hardy, 2004) and suggests that seed-mediated dispersal is relatively efficient in this species (Dick *et al.*, 2007). It is expected that lower-density populations present stronger SGS as local drift would be more important (Vekemans and Hardy, 2004). Here, we can only test this relationship between the population from Cameroon and the population from DRC as the SGS estimation for the population from Gabon lacks precision because of a small sample size. The population from DRC does not seem to present a stronger SGS than the population from Cameroon, suggesting that the strength of SGS is not related to population density (large overlap of s.e.m. values; Table 2). This suggests that the potentially larger drift effect expected at lower densities is compensated by increased dispersal distance (see below). Such a pattern has already been observed in another African tropical tree species, *Aucoumea klaineana* (Born *et al.*, 2008).

Lower population density is not associated with higher selfing rate and inbreeding

There is no clear relationship between population density and mating system at least for the range of densities observed in the current study (Table 2). The only significant difference, between the lowest and the highest population density (between Gabon and Cameroon) is potentially only due to a methodological bias. Altogether, all three populations seem to present similar levels of selfing, regardless of population density (between 13 and 19% based on MLTR analyses; Table 3). The same result has already been obtained for a set of timber species, where the selfing rate has been demonstrated to remain unchanged after selective logging (Cloutier *et al.*, 2007; Lourmas *et al.*, 2007; Ojeda-Camacho *et al.*, 2013). However, in these studies, the outcrossing rate was close to one that tends to support the existence of a strong auto-incompatibility system. Here, we also observed similar levels of selfing despite different population densities. The stability of this mixed mating system is puzzling. It has been proposed that the evolutionary stability of mixed mating systems is determined by the timing and relative amount of self- and outcross-pollination (Holsinger, 1991). In other words, if outcross pollen supply is limited, the selfing rate will be higher. We can expect higher pollen limitation in low-density populations, but this is probably not the case for *E. suaveolens*, as pollen distance increases with decreasing density (see below). The compensation of population density reduction by larger pollen flow would explain that selfing rate is relatively stable in populations that present different levels of density. Alternatively, the relative stability of the mixed mating system could be explained by post-pollination mechanisms, such as partial self-incompatibility systems (Goodwillie *et al.*, 2005).

Like most tree species, *E. suaveolens* is subject to inbreeding depression. Indeed, we observed a trend of decreasing inbreeding from seed to adult stage (Table 1), suggesting that individuals resulting from selfing die before reaching the adult stage (Barrett and Charlesworth, 1991). This trend was significant only for two out of the three populations (Cameroon and DRC). Selfing is generally detrimental in tree species because of their sensitivity to inbreeding depression, a likely consequence of their long generation time (Charlesworth, 2003; Duminil *et al.*, 2009). This expression of inbreeding depression has important consequences in terms of species population dynamics in relation to selective logging. Adult individuals that become genetically isolated after logging will have a very low probability of producing viable descendants. Though we have demonstrated that selfing rate is somewhat independent from population density, studying the relationships between degree of genetic isolation associated with greater distances than observed in the current study and mating system/seed set of trees in low-density populations of *E. suaveolens* will be necessary to further test the existence of this detrimental effect.

Biparental inbreeding seems to be more pronounced in the low-density population than in the high-density one (Table 2). The main cause of biparental inbreeding might be the low efficiency of seed-mediated gene flow as we demonstrated that pollen-mediated gene flow decreases with population density. If distance of seed dispersal is similar (and low) in all three populations, it is expected that the lowest density population is also the one most prone to biparental inbreeding. Here we did not find any clear relationship between population density and level of SGS, which tends to contradict this hypothesis. However, the absence of an accurate estimation of SGS for the low-density population due to small sample size limits our capacity for interpretation. Alternatively, the presence of a biparental inbreeding signal could suggest preferential mating between related individuals as would be the case if phenological asynchrony has a genetic origin. In the absence of phenological data, we cannot further interpret our results.

Lower population density is not associated with fewer pollen donors contributing to the pollination of each mother tree

Levels of correlated paternity within maternal sibships are similar in all three populations (Table 3). This result is well in line with an increase in the average distance of pollen dispersal with decreasing density and further provides information on the quality of mating events (similar number of pollen donors in different population densities). One can expect that this qualitative estimation can also indicate the quantity of pollen received, but this is actually not necessarily the case. The estimations that have been obtained in this study provide a picture of mating events based only on collected seeds or seedlings. These observations are independent from the actual level of seed production. We can obtain similar patterns of correlated paternity in the presence or in the absence of pollen limitation (with respectively low and high seed production). Measures of seed production through direct observations at different classes of physical isolation to the nearest neighbour need to be obtained to get a clearer view of the quantitative aspects of mating events.

Our results suggest that the effective density of pollen donors might be more reduced in low-density populations than in the high-density ones. This result needs to be interpreted with caution as we were not able to test the statistical significance of this trend. This result is, however, in line with our expectation and with other studies (Mimura and Aitken, 2007; Tani *et al.*, 2012; Masuda *et al.*, 2013).

Lower population density is associated with a change of pollen dispersal distances

The extent of gene flow is correlated with population densities as pollen dispersal distances have been shown to decrease with population density. The average distance of pollen dispersal varies from 200 m (Cameroon) to 1000 m (Gabon; Table 3). In other words, these results suggest that at lower population densities, trees are still

connected as a result of larger pollen dispersal distances. If pollinating species are identical in all three populations, this can outline their capacity to travel longer distances when flowering trees are more distant. Increased pollen dispersal distances in the case of lower tree densities have already been reported for other tropical species (White *et al.*, 2002; Dick *et al.*, 2003; Born *et al.*, 2008). This pattern probably explains the absence of relationships between population density and previously discussed factors (genetic diversity, SGS, mating system, inbreeding depression) within *E. suaveolens*.

Logging implications

Norms differ among the three studied countries on the minimum cutting diameters. Logging intensity of *E. suaveolens* is theoretically lower in Gabon (minimum cutting diameter of 70 cm) than in Cameroon and DRC (minimum cutting diameter of 50 cm). Our results suggest that such logging intensities in populations with the same characteristics as those studied in Cameroon and DRC would not affect a population's viability in a single cutting cycle. Given logging norms in these countries, we can estimate that population density of adult individuals would be reduced to 0.47 and 0.30/ha respectively in Cameroon and DRC. Such population densities are still higher than the population density in Gabon. However, models need to be developed to investigate the impact of multiple cutting cycles. It is difficult to predict the impact of logging on the population from Gabon as we have no indication of pollen dispersal distances at lower densities. This study needs to be completed by additional data on pollen limitation, notably through the investigation of the relationships of seed set, fruit phenology, seed–tree distance to the nearest neighbour and selfing rate. We recommend conducting such a study in populations that present densities equivalent to or lower than the population from Gabon. Such studies require exhaustive sampling, detailed cartography of the population structure and long-term monitoring of the flowering and fruiting phenology, which are challenging to organize in the field.

CONCLUSION

The comparison of three different populations with contrasting densities of the timber species *E. suaveolens* provides indications of the relationships between population density, mating pattern and genetic diversity. Equivalent levels of genetic diversity, selfing rates and correlated paternity suggest that these population parameters are resilient to a decrease of population density, one of the key consequences of selective logging. In particular, although the species exhibits inbreeding depression, there is no evidence of a loss of quality of the seed produced at low densities. This stability can be explained by the negative relationship observed between pollen dispersal distances and population density, a phenomenon that may be prevalent in plant populations. Although these results are reassuring regarding the risk that selective logging may impede the regeneration of timber tree populations, our study did not allow us to test whether seed production was affected by a decrease in density, nor to address all the other consequences of logging, notably those related to habitat perturbations, which were not the focus of the study. Additional work is thus necessary to provide logging recommendations, such as the definition of a maximum distance to be kept between seed trees to allow the qualitative and quantitative reproduction of the species. We suggest that investigations need to be conducted in low-density populations, which are more threatened by population reduction.

DATA ARCHIVING

Genotype data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.b78fb.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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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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Timber species

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.

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

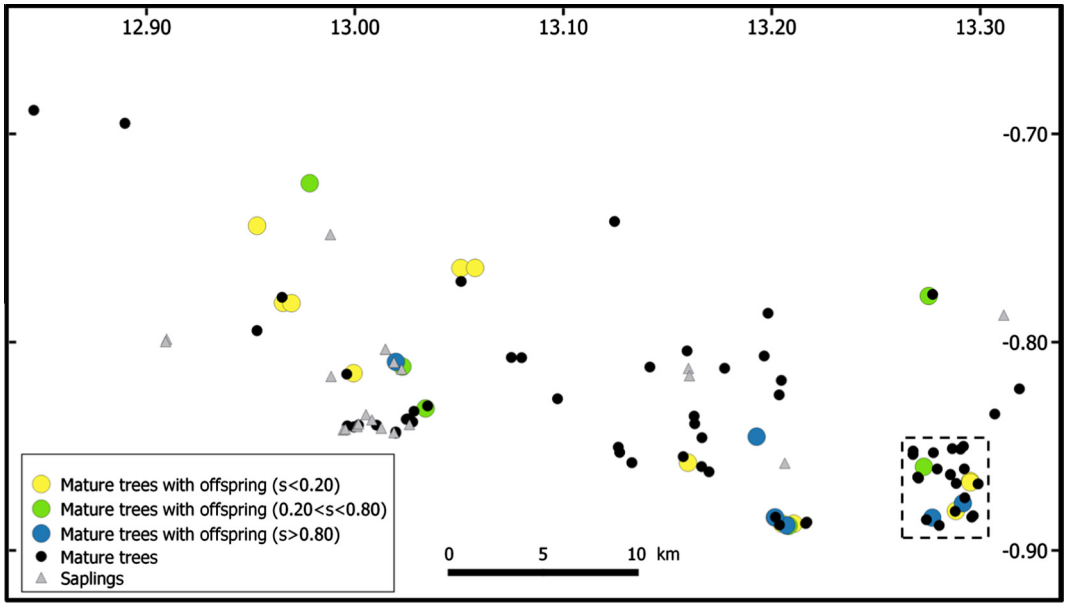

Fig. 1. Sampling of individuals of *B. toxisperma* in East Gabon and assignation of selfing rates for some mature trees (= adults). Yellow, green and blue dots respectively represent seed trees with low (s < 0.20), intermediate (0.20 < s < 0.80) and high rates of selfing (s > 0.80). An exhaustive sampling 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-

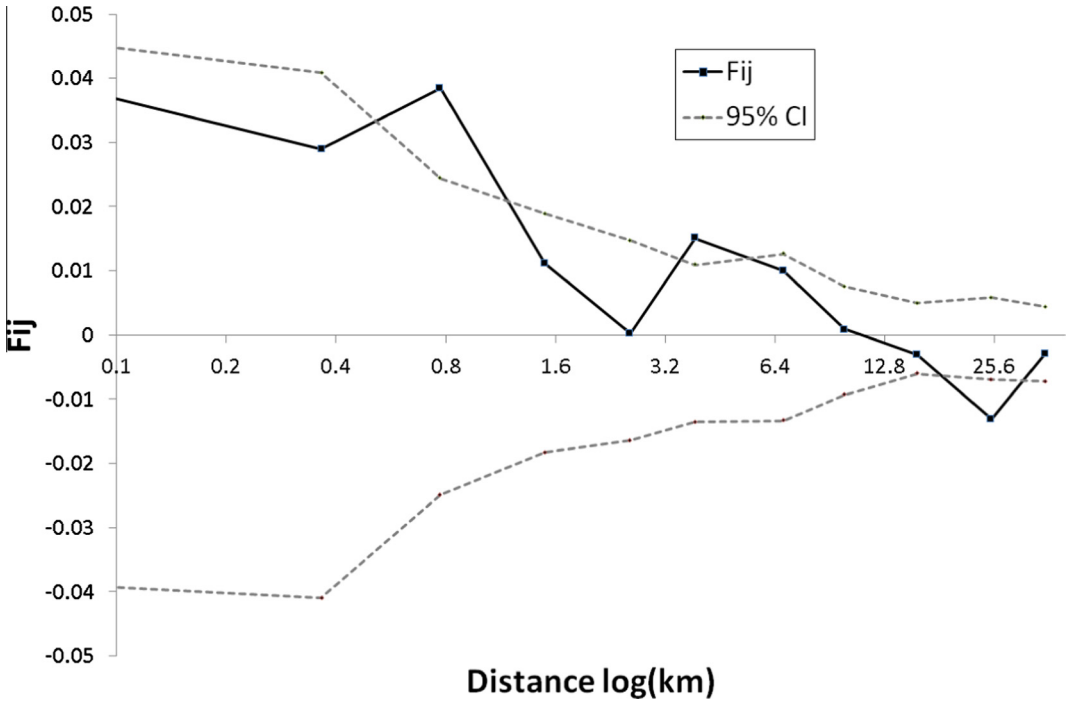

Fig. 2. Average kinship coefficients Fij between pairs of individuals plotted against the logarithm of geographical distance in the whole population.

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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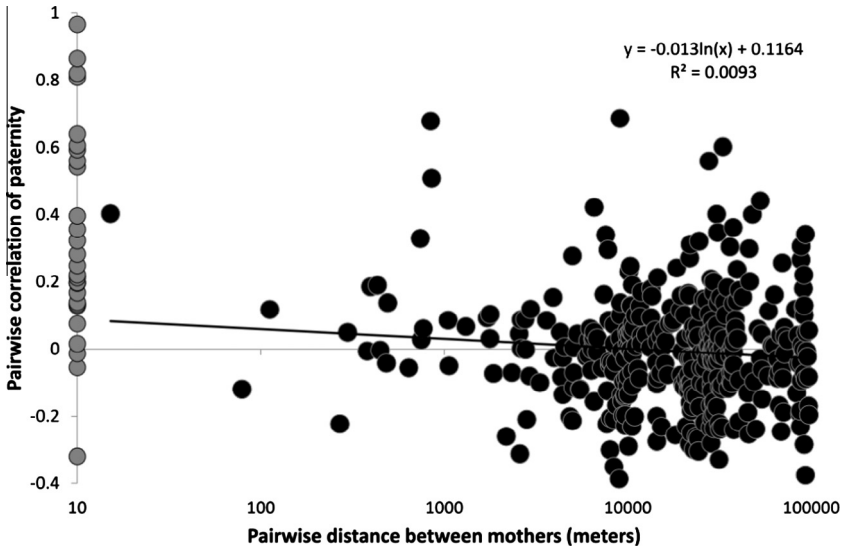
Appendix A

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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