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HONEY BEES ENSURE THE POLLINATION OF *PARKIA BIGLOBOSA* IN ABSENCE OF BATS

Kristin Marie Lassen[1], Moussa Ouédraogo[2], Yoko Luise Dupont[3], Erik Dahl Kjær[1], Lene Rostgaard Nielsen[1]

[1]*Department of Geosciences and Natural Resource Management, University of Copenhagen, Rolighedsvej 23, 1958 Frederiksberg C, Denmark*
[2]*Centre National de Semences Forestières, Route de Kaya, 01 BP 2682 Ouagadougou, Burkina Faso*
[3]*Institute of Bioscience, Aarhus University, Vejlsøvej 25, 8600 Silkeborg, Denmark*

Abstract—One of the socio-economically important West African parkland trees, *Parkia biglobosa* yields pods consumed by people and animals. Being animal-pollinated, it is unknown how climate change will affect the species if the guild of pollinators changes. Here, we compare the pollinator guilds and the pod production at two climatically different study sites: the first site, Tiba was drier with lower tree density and fewer fruit bats, whereas the second site, Pinyiri was more humid, with higher tree density and more fruit bats. We carried out a pollinator exclusion trial with bags separating the flower-visitors and made observations of the flower-visitors. Furthermore, we calculated pollination distances based on paternity analysis of the seeds produced within the trial. The numbers of immature fruits were similar for the sites, but the drier site, Tiba experienced more abortion and decreased pod set. At both sites, exclusion of bats led to marginally reduced pod set, while exclusion of bats and honey bees led to significantly reduced pod set. We found a small effect of stingless bees and solitary bees but only at the more humid site, Pinyiri. Tiba experienced a higher level of self-pollination, fewer pollen donors per tree, and longer median distances of pollen flow, compared with Pinyiri. Implications of the results for management of the species and its pollinators in the face of climate change are discussed.

Keywords: Agroforestry parklands; Bats; Honey bees; Pollination ecology; Solitary bees; Stingless bees

INTRODUCTION

Animal pollination is a requirement for successful fruit set for more than 90% of the flowering species in the tropics (Ollerton et al. 2011), but both the species richness (Patiny et al. 2009) and composition of pollinator guilds (Franzén & Öckinger 2012) may be influenced by climate change through several mechanisms. The local presence of animal pollinators is affected by many environmental factors including temperature and annual precipitation (Fryxell 1957), which impact the quantity and quality of food and nesting places (Kearns et al. 1998). Furthermore, mismatching of periods of flowering and pollinator activity may occur (Parmesan 2007; Petanidou et al. 2014). Climatic changes are therefore expected to have consequences for the interactions between plants and pollinators (Abrol 2012; Settele et al. 2014), although different species of pollinators can be differentially affected. For instance, bats, honey bees (*Apis mellifera* ssp.) and some species of sunbirds are known to migrate when resources become scarce (Hepburn & Radloff 1998; Cheke et al. 2001; Monadjem et al. 2010), whereas e.g. stingless bees do not migrate (Roubik 2006). The yield of animal-pollinated plants may thus depend on whether important pollinating species can be substituted, e.g. honey bees for bats or stingless bees for honey bees.

In the present study we investigated if denied access of different types of animal pollinators led to reduced fruit set of an important fruit tree, the West African Sudano-Sahelian parkland tree *Parkia biglobosa* (Jacq.) R. Br. ex G. Don (English: African locust bean, French: néré). Specifically, we assessed the role of honey bees, because abundance of these pollinators can be increased by beekeeping and *P. biglobosa* is an important honey bee forage tree in the savannah (Dukku 2010).

Burkina Faso, where the present study took place, has a strong north-south increasing rainfall gradient, which divides the country into three climatic zones with different vegetation. From 1950 until the mid-1980s rainfall declined steeply but recovered in the 1990s and stabilised at a level 15% lower than that of 1920-1969. Since 1975, air temperatures have increased about 0.6°C through most of Burkina Faso (Funk et al. 2012), and this trend is projected to persist or even increase depending on the future concentration of greenhouse gasses in the atmosphere (Niang et al. 2014). Climate models are very uncertain regarding predictions for the future annual rainfall (Ibrahim et al. 2014). In connection with the declining rainfall, the composition of vegetation has changed in recent decades with xeric species replacing mesic species (Maranz 2009; Gonzalez et al. 2012). Although not documented, these changes may also impact pollination interactions of mesic species, including the legume *P. biglobosa* (Boffa 1999). Presently, the northern limit of the distribution of *P.*

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*Corresponding author: kmla@ign.ku.dk

biglobosa is in the Sudano-Sahelian climatic zone, and increased drought is therefore likely to move the border of the distribution further south (Ouedraogo et al. 2012).

Parkia biglobosa is animal-pollinated. The flowers yield nectar and pollen during night, and are pollinated by bats (Harris & Baker 1959; Hopkins 1983) in the period from dusk till dawn, after which several species of bees takes over in the early morning (Ouédraogo 1995; Lassen et al. 2012). Six species of frugivorous bats (Pteropodidae) are known from Burkina Faso (Kangoyé et al. 2012) and three of them are known to visit the flowers of *P. biglobosa* (Hopkins 1983). However, in the drier part of the distribution area of *P. biglobosa* in Burkina Faso, bats are rare, and different species of bees are expected to be the main pollinators (Ouédraogo 1995). It is likely that the widespread beekeeping activities in Burkina Faso (Schweitzer et al. 2013) act as a pollination buffer against the negative effects of low abundance of bats at the drier areas, especially if the bees are given access to water in order to limit their seasonal migration (B. Svensson 2014, pers. comm.). Generally, honey bees are believed to have smaller flight ranges than bats (Linhart 1995). Thus, pollination by honey bees may decrease distances of pollen transportation and hence limit gene flow across a fragmented landscape, relative to bat-pollination. Geitonogamous pollination is possible and any shift in pollinators that results in more selfing may reduce pod production because *P. biglobosa* is mainly outcrossing (Ouédraogo 1995; Sina 2006) and assumed to possess a partial self-incompatibility system (Sina 2006).

On the above background, the overall objective of the present study was to evaluate if pollinators can be substituted by others, when assessed by pod set in two different climatic zones in Burkina Faso. One study site, near the northern distribution limit of *P. biglobosa*, was characterised by a dry climate while the other site was characterised by higher precipitation and located within the core of the natural distribution area of *P. biglobosa*. More specifically, we studied: 1) differences between the sites in flower-visitors and pod yield, 2) the effect of excluding different pollinator types on yield and pollination distances, and 3) pod yield in relation to distances to bee hives. Based on the results, we have discussed the potential management of pollinators of *P. biglobosa* in a drier environment.

MATERIALS AND METHODS

Plant species

Parkia biglobosa is a medium sized leguminous tree widespread in the savannah woodlands in a belt from Senegal to Uganda (Hopkins & White 1984). It is limited by 500-700 mm rainfall but prospers with up to 1,200 mm annual precipitation (Haq 2008). In Burkina Faso, flowering takes place in the dry season between January and May. The bright red, tiny flowers are closely packed in capitula (with around 2,000 flowers), borne on long peduncles. Close to the peduncle are about 85 staminodial flowers, and below are around 250 sterile flowers that produce nectar, which accumulates in a nectar ring (Hopkins 1983). Each capitulum flowers only for one night (Hopkins 1983; Ouédraogo 1995), and is here treated as a single unit.

The hermaphroditic flower consists of a bract, calyx, corolla, ten stamens, and an ovary of one carpel ending in a style with a cup-shaped stigma. *Parkia biglobosa* is generally protandrous and pollen is shed in the evening in polyads of around 32 pollen grains (Nombré, 2003; pers. obs.). The styles elongate during the night of flowering, but in some flowers the styles do not elongate and these flowers are thus functionally male. A pilot trial with controlled cross-pollination showed that the stigmas were receptive and polyads fertile from at least 21:30 to 07:30 (Lassen 2016). An ovary contained 16-29 ovules (mean = 23, SE = 0.63, $N = 15$). At our study sites, pods matured in around two months, producing mostly a few pods per capitulum, and rarely up to 20 pods per capitulum.

Study sites and sampled trees

A pollinator exclusion trial of *P. biglobosa* was carried out at two sites in Burkina Faso in 2012. A relatively dry northern site was located at two neighbouring villages, Tiba and Gam (12°42'26.26"N, 1°18'2.04"W) in the Sudano-Sahelian climatic zone with a mean annual precipitation (1981-2010) of 600-700 mm (Sanfo 2012), which henceforth will be called Tiba for convenience. Another more humid southern site was located at the village Pinyiri (syn. Kacheli) (11°14'34.89"N, 1°8'1.73"W) in the Sudanian climatic zone with a mean annual precipitation (1981-2010) of 900-1,000 mm (Sanfo 2012). The distance between the two sites is approximately 160 km. In 2011, the annual rainfall (preceding the fruiting season of *P. biglobosa* in 2012) was 566 mm for the dry site (nearest weather station was at Guilongou 9 km from Tiba) whereas the more humid site obtained 927 mm (measured at Pô, 8 km from Pinyiri) (Météo 2015). Mean annual temperatures (2012) were 29.3°C and 28.4°C for Tiba and Pinyiri, respectively (Météo 2015).

The sites were 360 ha (Tiba) and 220 ha (Pinyiri), respectively. Tree characteristics were similar between the two sites (diameter, height, crown area and age), but tree density was higher at the more humid site, Pinyiri (1.23 trees/ha relative to 0.26 trees/ha). Further, only around half of the trees flowered at the drier site, Tiba, giving a density of 0.14 flowering trees/ha. GPS coordinates of trees were recorded, leaves were sampled for DNA extraction, and flowering periods were recorded. A total of 93 and 271 trees were sampled at Tiba and Pinyiri, respectively. These included mother-trees, which were used for experimental pollination studies.

Pollinator observations

At both study sites, flower-visitors were observed in different periods between dusk and dawn, during five nights at Tiba (total of 11 hours) and six nights at Pinyiri (total of 16 hours). Capitula were observed from the ground using a Yukon Ranger 5x42 digital night vision monocular (model 28041VK) with a mobile recorder and a compact camera (Canon PowerShot S95). Insect observations were supplemented by video recordings, which we paused regularly (every ten seconds) and counted the number of insects on these frames. Based on these counts we estimated mean visitation number per capitulum per site during early morning. Samples of insects were preserved in alcohol for later identification. Visiting bats and birds were registered, but it was not possible to identify the species. At both study sites, we registered the GPS coordinates of colonies of the most abundant flower-visitors, namely, bats and honey bees (both hived and feral honey bees).

Pollinator exclusion trial

Flower-visitors were separated according to their size by bags with different mesh size. Four treatments were applied with means of 18 and 20 replicates of each treatment on each tree, respectively for Tiba (12 trees) and Pinyiri (16 trees). All treatments were distributed randomly in the tree crown but applied only to the most apical capitulum (bud) on the compound inflorescences (consisting of many capitula), since these have the greatest chance of developing into hermaphroditic capitula (Lassen 2016). The treatments consisted of an open treatment, T0, where capitula were accessible to all visitors, two semi-open treatments, T1, where capitula were protected by a bag of chicken wire with ca. 10 mm × 10 mm holes, excluding bats and birds, and T2, where the capitula were protected by bags of mosquito netting with ca. 1.8 mm × 2.0 mm holes, only accessible to small bees and ants. Furthermore, a closed treatment, T3, excluded all potential visitors by bags of cheesecloth with ca. 200 µm × 350 µm holes. The bags in treatments T2 and T3 were kept in shape by an inner band of chicken wire, in order to keep the fabric from touching the flowers and the flower-visitors from reaching the flowers from the outside of the bags.

Trees were tended on a daily basis during the experiment, and abscised capitula were collected and examined for sex (hermaphroditic or functionally male) and signs of pods, pests, and diseases. Based on earlier results (Lassen 2016), we considered a capitulum as functionally male if the lengths of the anthers in the dissected flowers were more than 5 mm longer than the lengths of the corresponding stigmas.

The bags were removed around one week after flowering, and the number of developing pods was counted. At maturity we harvested the complete infructescenses from the trees. The pods were weighted and length measured excluding the pedicel. Two pods per receptacle (one if single) were randomly chosen and the number of healthy seeds, aborted seeds, and seeds consumed by birds (creating shallow holes on one side of the pods) were assessed. A seed was registered as aborted when the weight was below 0.05 g and/or the shape was flat.

DNA extraction and genotyping

We extracted genomic DNA from dried leaf samples of mother-trees (i.e. providers of pods for the study, *N* = 12 and 16 for Tiba and Pinyiri, respectively), potential pollen donors (*N* = 81 and 255 respectively), and from a sub-sample of seeds from the randomly chosen pods from the exclusion trial (*N* = 61 and 498 from Tiba and Pinyiri, respectively). Only one seed per pod was selected as we had previously found that all seeds per pod were sired by a single pollen donor (Lassen et al. 2014). The raw de-shelled seeds were utilised without prior germination. For the extraction, we used the DNeasy 96 Plant Kit (QIAGEN, Hombrechtikon, Switzerland) following the manufacturer's protocol.

The extracted DNA samples were genotyped using ten microsatellite markers developed for *P. biglobosa* (Lassen et al. 2014), and divided into three primer mixes (Mix 1: PbL03, PbL04, PbL15. Mix 2: PbL09, PbL11, PbL21. Mix 3: PbL02, PbL05, PbL12, PbL22). PCR reactions were done with Qiagen Multiplex PCR Kit in 10 µl reactions including 1 µl of extracted DNA. PCR conditions, fragment separation and genotype scoring followed Lassen et al. (2014).

Paternity analysis

Paternity analysis was performed on seeds from the exclusion trial treatments with Cervus v3.0.3 (Kalinowski et al. 2007), where we assigned the most likely father-trees to offspring given the known genotype of the mother-tree. Only individuals with a minimum of eight scored loci were included in the analysis. Selfing was allowed in the model. The critical values for assignment with 95% confidence were estimated by 100,000 simulations assuming a genotyping error-rate of 0.01 and sampling of 75% of the potential pollen donors. Only trees assigned with confidence level of 95%, positive trio (mother-father-offspring) LOD scores, and a maximum of two mismatching loci were accepted as fathers.

For each assigned paternity, the pollen transfer distance was calculated as the distance between the known mother-tree and the assigned father-tree based on their GPS coordinates.

For the adult trees at the two study sites, we tested deviations from expected genotype frequencies under Hardy-Weinberg equilibrium using Genepop v4.3 (Rousset 2008). The level of genetic differentiation between the two sites was estimated using AMOVA (Analysis of Molecular Variance) (Excoffier et al. 1992) as implemented in GenAlEx v6.501 (Peakall & Smouse 2006; Peakall & Smouse 2012) including test of significance based on 999 permutations.

Statistical analyses

For numerical variables (Y_{ijk}), the average value was calculated per tree and treatment excluding non-flowering trees. The differences between treatments, trees or sites were tested based on a general linear model as implemented in the GLM procedure in the SAS software v.9.4 (SAS Institute 2011) corresponding to the model:

$$Y_{ijk} = \text{Treatment}_i + \text{Site}_j + \text{Tree}_k(\text{Site}_j) + \varepsilon_{ijk}$$

, where Y_{ijk} is the response variable, treatment i = (T0, T1, T2, T3), site j = (1, 2) and tree k = (1 - 28). Treatment$_i$ and site$_j$ were considered fixed effects while Tree$_k$(Site$_j$) was considered a random effect with residual ε_{ijk} assumed independent and N(0,σ_e^2). The model assumptions were assessed and accepted by visual inspection of the residuals.

For T0 data (open-pollinated flowers) and T1 data (vertebrate excluded flowers), the relationship between fecundity variables (immature and mature fruits) and number of bee colonies within a certain range of each tree were analysed. We tested this for number of colonies within 9 different distances from the studied trees; d = 200, 300, 400, 500, 600, 700, 800, 900, and 1,000 m. The significance levels were sequential Bonferroni corrected with table-wide N = 18 (9 distance classes at each of the two sites).

RESULTS

Pollinator observations

The first animals visiting capitula with accessible pollen were nocturnal animals, such as bats and moths. During the first hour after sunrise we observed mainly honey bees, different species of smaller bees, and birds.

At Tiba, we observed two small colonies of bats of about ten individuals each. At Pinyiri we registered two small colonies of around five and fifteen individuals and a large colony of about 500 individuals. However, bats from roosting sites outside the study sites may also visit the trees during the night. At both sites, we observed bats visiting *P. biglobosa* capitula in the period from ca. 20:00 until ca. 06:00. At Tiba, bats were observed visiting *P. biglobosa* in three out of five nights and at Pinyiri in four nights out of six. Around 45 min of video recordings at Tiba captured no bats visiting the capitula, and only 5 bats were recorded under the crowns of *P. biglobosa.* In comparison, about one hour video recordings at Pinyiri showed three bats at the capitula and 53 bats under the crowns. The bats moved quickly and silently, and visitation rates of bats were difficult to assess. Nevertheless, we observed more bats visiting capitula of *P. biglobosa* at the more humid site, Pinyiri, and they also stayed longer in the trees, compared to Tiba.

Honey bees were common visitors of *P. biglobosa.* At the study sites, the honey bees belong to the species *Apis mellifera jemenitica* Ruttner (Apidae, tribe Apini) (Al-Ghamdi et al. 2013). We registered 69 colonies of honey bees at Tiba and 65 colonies at Pinyiri. However, at the drier site, Tiba, the area was larger and the population of flowering *P. biglobosa* trees was smaller compared with the more humid site, Pinyiri. A few honey bee colonies were feral (without hive) while the majority were managed by beekeepers. The honey bees arrived at *P. biglobosa* around dawn. The number of visiting honey bees peaked around 15 minutes after the first honey bee had arrived (with up to 24 honey bees simultaneously on one side of a capitulum), and after another 15 minutes only very few honey bees remained. Despite the differences between sites, the mean visitation numbers were very similar with 1.9 honey bees per capitulum (SD = 1.64, N = 137 counts) at Tiba compared with 1.6 honey bees per capitulum (SD = 1.66, N = 101 counts) at Pinyiri during the early morning.

We observed two species of stingless bees (Apidae, tribe Meliponini) at Pinyiri; *Hypotrigona* sp. 1 (large type) and *Hypotrigona* sp. 2 (small type), while at Tiba, only *Hypotrigona* sp. 1 was found. Additionally, one species of solitary bees, *Compsomelissa borneri* Alfken (Apidae, tribe Allodapini) was observed at both study sites. The small bees generally arrived after the peak of honey bees but stayed longer and peaked in numbers around one hour after the honey bees had left. Up to 15 small bees were observed collecting pollen simultaneously on one side of a capitulum. The mean visitation number was 1.1 small bees per capitulum (SD = 0.55, N = 14 counts) at Tiba and 6.5 small bees per capitulum (SD = 3.16, N = 85 counts) at Pinyiri. However, at both study sites we observed large differences among trees in numbers of small bees visiting the capitula.

Several species of moths were observed. During the already mentioned video recordings, we counted a total of 17 moths visiting capitula at Tiba, whereas only 1 moth was observed at a capitulum at Pinyiri.

Other occasional visitors observed were: eight species of beetles, four species of flies, three species of ants, one dragonfly, one true bug, two spiders, in addition to a few sunbirds.

Effect of pollinator exclusion on pod and seed production

Three of the 12 flowering mother-trees at the drier site, Tiba, did not produce any pods, whereas all mother-trees at the more humid site, Pinyiri, produced pods. Predation by birds affected 6% of the pods within the trial at Pinyiri compared to 79% at Tiba, and one of the nine pod-producing trees at Tiba had all its pods eaten by birds. An additional 14% of the harvested pods at Tiba were flat and with aborted seeds and no pulp.

The average numbers of immature and mature pods per hermaphroditic capitulum (incl. capitula without pods) varied significantly among treatments and trees (Tab. 1). For both sites, the number of pods per capitulum in treatments T0 and T1 were statistically similar. For Pinyiri, we found a trend towards fewer pods per capitulum with smaller mesh size in bags for T2 and T3, whereas for Tiba, treatment T2 and T3 resulted in the same low number (Tab. 1). The number of mature pods per hermaphroditic capitulum was significantly lower at Tiba compared to Pinyiri, in particular for T0, which resulted in around five times more pods being harvested at Pinyiri compared to Tiba (Tab. 1).

Pod size at Tiba was not analysed because of the heavy predation. At Pinyiri, pod weight and pod length ranged from 0.4 g to 24.5 g and from 1.8 cm to 35.4 cm, respectively, while the number of healthy seeds per pod varied from 0-27. The pods from T2 were heavier and longer compared with T0 and T1 (Tab. 2), but T2 also had fewer pods per capitulum as mentioned above. The numbers of healthy seeds per pod followed the trend but were not statistically different (Tab. 2).

Paternity analysis

The paternity analysis assigned most of the genotyped seeds (each representing one pod) to a potential father-tree

TABLE 1. Average number of immature and mature pods per hermaphroditic capitulum (incl. capitula without pods) for *Parkia biglobosa*, and total number of pods shown per treatment and site, including F-tests and their significance levels.

Treatment	No. of immature pods/ hermaphroditic capitulum[a]		No. of mature pods/ hermaphroditic capitulum[a]		Total no. of mature pods	
	Tiba	Pinyiri	Tiba	Pinyiri	Tiba	Pinyiri
T0, open	2.52 (0.37) a	3.31 (0.22) a	1.16 (0.26) a	2.88 (0.21) a	97	579
T1, chicken wire	1.84 (0.40) a	2.73 (0.22) a	1.16 (0.28) a	2.32 (0.21) a	107	441
T2, mosquito net	0.02 (0.35) b	0.39 (0.22) b	0.01 (0.25) b	0.28 (0.21) b	1	56
T3, closed	0.02 (0.35) b	0.03 (0.22) c	0.01 (0.25) b	0.01 (0.21) c	1	1
All treatments	1.11 (0.16)	1.61 (0.13)	0.61 (0.14)	1.37 (0.11)	206	1077
$F_{\text{Treatment}}$	F = 57.0 (***)		F = 40.8 (***)			
$F_{\text{Tree(Site)}}$	F = 1.7 (*)		F = 1.7 (*)			
F_{Site}	F = 3.8 (ns)		F = 10.6 (**)			

[a] Values are least squares (LS) means with the standard error (SE) of the LS estimate in brackets. Values within a column followed by different letters are significantly different at $P < 0.05$. Significance level: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, and ns = $P > 0.05$.

TABLE 2. Pod weight and length, and the number of healthy seeds per pod for *Parkia biglobosa* shown per treatment at Pinyiri, including F-tests and their significance levels (excluding treatment T3 due to a single pod).

Treatment	Pod weight, g	Pod length, cm	Healthy seeds/pod
T0, open	10.5 (0.53) a	19.0 (0.63) a	12.9 (0.64) a
T1, chicken wire	11.9 (0.53) b	20.3 (0.63) a	13.6 (0.64) a
T2, mosquito net	13.5 (0.68) c	23.0 (0.82) b	14.9 (0.83) a
T3, closed	8.0 (2.42) -	19.7 (2.90) -	3.2 (2.92) -
Treatments T0,T1,T2	12.0 (0.34)	20.8 (0.42)	13.8 (0.41)
$F_{\text{Treatment}}$	F = 6.4 (**)	F = 7.3 (**)	F = 1.8 (ns)
F_{Tree}	F = 4.3 (***)	F = 4.5 (***)	F = 2.3 (*)

Values are least squares (LS) means with the standard error (SE) of the LS estimate in brackets. Values within a column followed by different letters are significantly different at $P < 0.05$. Significance level: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, and ns = $P > 0.05$.

TABLE 3. Percentage of assigned pods (with number of assigned pods in brackets) for *Parkia biglobosa*, percentage of self-pollination (of all genotyped pods, including the unassigned), mean number of pollen donors per tree (incl. self-pollination), and the average distance to pollen donors (without selfing), shown per treatment and site. Treatments with single pods are excluded. F-tests and their significance levels are shown for number of pollen donors and average distance to pollen donors.

Treatment	Assigned pods, % (No. pods)		Self-pollination, %		No. of pollen donors per tree[a]		Average distance to pollen donors[a], m	
	Tiba	Pin[b]	Tiba	Pin[b]	Tiba	Pin[b]	Tiba	Pin[b]
T0, open	70 (23)	84 (195)	21	2	1.8 (0.37) a	7.0 (0.60) a	155 (70.5) a	238 (19.1) a
T1, chic[c]	96 (25)	93 (207)	23	4	2.2 (0.33) a	7.5 (0.60) a	275 (64.1) a	183 (18.9) a
T2, mos[d]	- (1)	98 (41)	-	19	- (na) -	2.0 (0.77) b	- (na) -	173 (43.8) a
T3, clos[e]	- (1)	- (1)	-	-	- (na) -	- (na) -	- (na) -	- (na) -
All treatments	82 (50)	89 (444)	23	4	0.5 (0.69)	5.5 (0.36)	212 (55.8)	198 (18.7)
$F_{\text{Treatment}}$					F = 19.2 (***)		F = 2.0 (ns)#	
$F_{\text{Tree(Site)}}$					F = 2.9 (**)		F = 3.3 (***)	
F_{Site}					F = 19.0 (***)		F = 0.1 (ns)	

[a] Values are least squares (LS) means with the standard error (SE) of the LS estimate in brackets. [b] Pin means Pinyiri. [c] T1, 'chic' is chicken wire. [d] T2, 'mos' is mosquito net. [e] T3, 'clos' is closed. Values within a column followed by different letters are significantly different at $P < 0.05$. Significance level: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, and ns = $P > 0.05$. # Treatment T0 versus T1 is significantly different ($P < 0.04$) at Pinyiri, when excluding treatment T2.

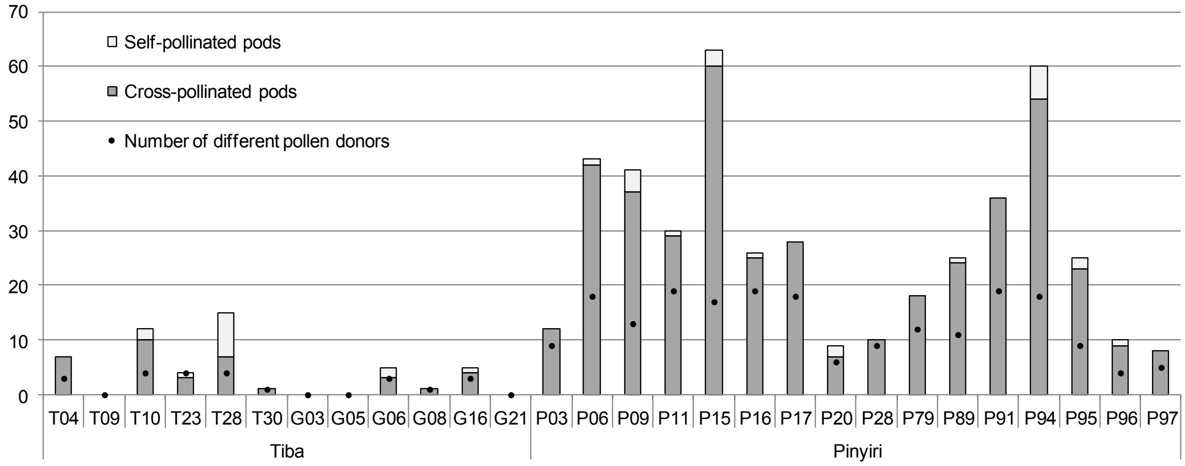

FIGURE 1. Number of genotyped self- and cross-pollinated pods across treatments in *Parkia biglobosa* shown per tree and site. The points show the total number of dissimilar pollen donors (one father-tree counts only once) across treatments per mother-tree.

incl. self-pollination (Tab. 3). Some flat pods contained only aborted seeds, but these did also yield DNA suitable for the paternity analysis. A father-tree was assigned to 82% of genotyped seeds (50 pods) for Tiba and 89% (444 pods) for Pinyiri (Tab. 3). The majority of unassigned pods came from the open (T0) treatment (10 pods from Tiba and 38 pods from Pinyiri). At the drier site, Tiba, all unassigned pods had negative trio LOD scores (i.e. pollen most likely came from outside the study site), whereas it was 76% at the more humid site, Pinyiri.

Self-pollination in T0 and T1 was significantly higher ($X^2 = 28.3$; $P < 0.001$) at Tiba (21-23%) compared to Pinyiri (2-4%). At Pinyiri, 19% of the pods from T2 were self-pollinated (Tab. 3). Self-pollinated pods were found on five trees at Tiba and on ten trees at Pinyiri (Fig. 1).

The number of different pollen donors (incl. self-pollination) per studied mother-tree in Tiba was only 1-4 (based on 50 assigned pods from 8 mother-trees) compared to 4-19 (based on 444 assigned pods from 16 mother-trees) at Pinyiri (Tab. 3, Fig. 1). At Tiba, 55% (= 11 capitula) of the capitula with two studied pods revealed more than one pollen donor, while the corresponding fraction was 74% (= 164 capitula) in Pinyiri. The number of pollen donors per tree differed significantly among treatments, with treatments T0 and T1 resulting in a higher number of pollen donors compared to T2 where the honey bees were excluded (Tab 3).

Genetic diversity

The microsatellite genotyping of the adult trees revealed genotypic distributions corresponding to Hardy-Weinberg proportions at both sites, which suggests a history of random mating. Only limited genetic differentiation between the two study sites was revealed by the AMOVA ($\Phi_{st} = 0.011$, $P < 0.001$).

Spatial pollen dispersal at the two study sites

The distances between the studied mother-trees and their nearest flowering *P. biglobosa* neighbour ranged from 16 m to 263 m (mean 98 m, SD = 65 m) at Tiba and from 10 m to 68 m (mean 32 m, SD = 18 m) at Pinyiri. Still, the average distance of realised cross-pollination per tree across treatments did not differ significantly between the sites (Tab. 3). A specific comparison of treatments T0 against T1 at Pinyiri showed significant longer mean cross-pollen transport within the open treatment T0 compared to T1 at this site ($P < 0.04$).

The median distances of pollen transport (excluding selfing) including all treatments were 143 m and 115 m for Tiba and Pinyiri, respectively. About half of the pollen donors were within a distance of 200 m from the mother-trees, while approximately 20% of the donors were more than 300 m away (Fig. 2). Long distance pollen movement (> 500 m) were estimated as 6% for treatment T0 and 5% for T1 at Tiba, whereas at Pinyiri long distance pollination occurred four times more frequently for T0 (8%) compared to T1 (2%). However, many of the non-assigned pollen donors (mostly found in T0) are likely to be trees outside the sampled area and therefore reflect additional long distance pollen movements.

The DNA documented pollination events at the two sites are presented in Fig. 3. At Tiba, only a small fraction of trees were identified to serve as pollen donors and self-pollination was relatively frequent (Fig. 3 A). At Pinyiri, the pollination events involved many trees and often included movement of pollen across the main road that divides the village (Fig. 3 B).

Distance to honey bee colonies

The average distance from a mother-tree to the closest honey bee colony was 402 m (SD = 323 m) for Tiba and 108 m (SD = 59 m) for Pinyiri. Within a radius of 1,000 m from a given mother-tree, Tiba had a mean of 4 colonies while Pinyiri had a mean of 35 colonies. However, average number of honey bee colonies per flowering *P. biglobosa* trees was higher at Tiba compared to Pinyiri (1.3 versus 0.2), due to the low density of flowering *P. biglobosa* trees (Fig. 3). We found no correlation between the number of honey bee colonies within a given radius and the pod

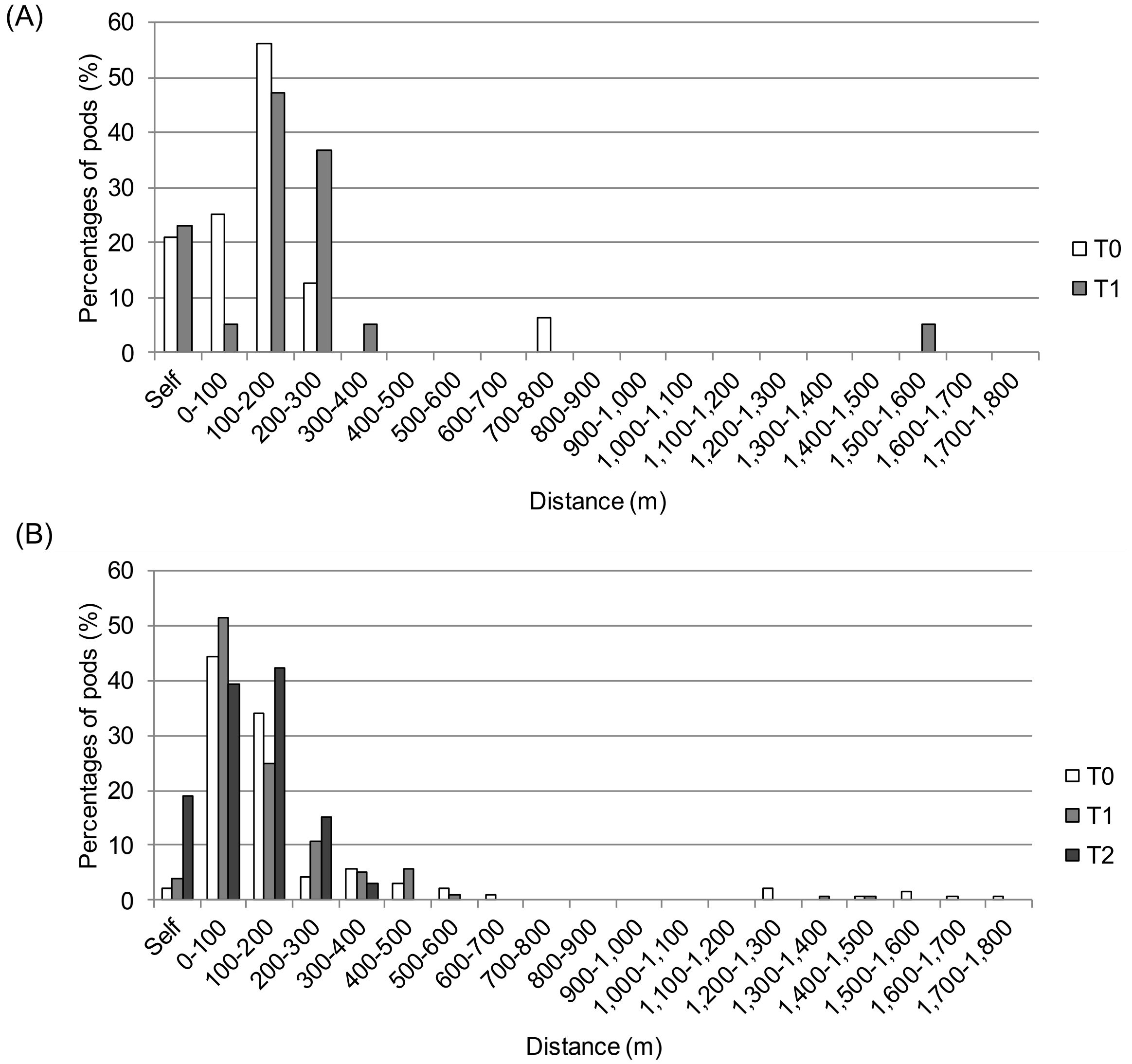

FIGURE 2. Percentages of self-pollination and distances of cross-pollen transport from donor tree to mother-tree in *Parkia biglobosa* divided on treatments and study sites (A) Tiba (drier site); (B), Pinyiri (more humid site).

production for neither the open T0-treatment nor the chicken wire T1-treatment at any of the two sites.

DISCUSSION

In the current study, pollinator exclusion trials showed that pod and seed set were only slightly reduced when excluding vertebrates, while pod and seed set were highly reduced when also excluding larger insects such as honey bees from visiting the flowers. Genetic analysis of seeds revealed a higher level of selfing, fewer pollen donors per tree, and longer median pollen transport distances at Tiba, compared to the population at Pinyiri.

Differences in flower-visitors and pod yield between the sites

We found the same functional groups of flower-visitors at the two study sites. However, bats were more frequent visitors of *Parkia biglobosa* at the more humid site, Pinyiri, compared to the drier site, Tiba, while honey bee visitation rates were similar despite differences in tree densities between sites. Visitation rates by smaller bees varied among individual trees.

The number of mature pods per hermaphroditic capitulum was significantly lower at Tiba, but the difference in number of immature pods per hermaphroditic capitulum was less noticeable and higher abortion rate rather than pollen limitation thus seemed to be the major factor behind the lower pod production per capitulum at the drier site. However, the higher level of self-pollination and fewer pollen donors per tree at Tiba compared to Pinyiri, suggest that poor conditions for cross-pollination played a role at Tiba, and might have contributed to the high abortion rate and therefore poor fruit set. Similar findings of low tree density increasing self-pollination rates have been observed in other studies (Franceschinelli & Bawa 2000; Eckert et al. 2010).

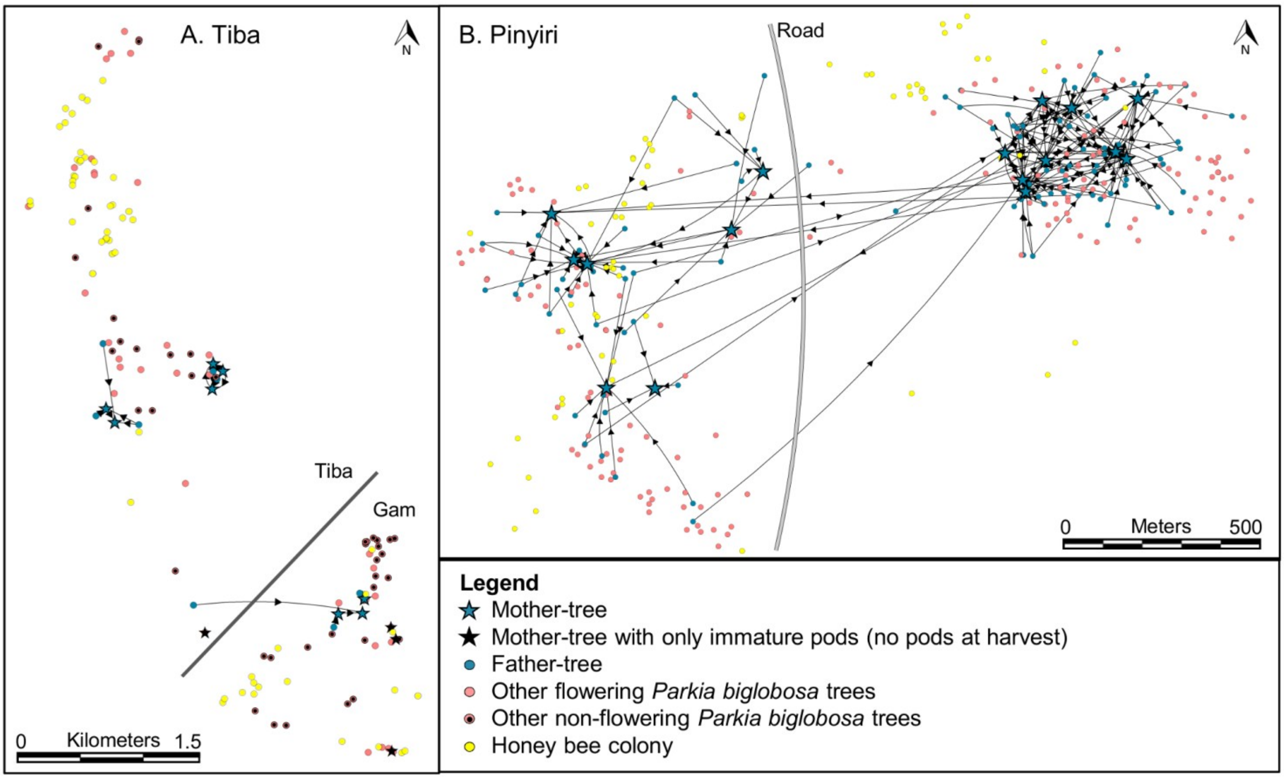

FIGURE 3. Illustration of the cross-pollination events for *Parkia biglobosa* across treatments (A, Tiba (drier site, also showing Gam); B, Pinyiri (more humid site)). The arrows show the direction of the pollen transport. One arrow may represent more than one pod. Events of self-pollination are not shown.

Additionally, differences in climate at the two sites may have contributed to fewer flowering *P. biglobosa* trees and higher abortion rate of pods at the drier site, Tiba, which is located close to the northern border of the natural distribution area of the species (Ouédraogo 1995). Here, the annual precipitation in 2011 leading up to the fruiting season of *P. biglobosa* in 2012 was below average (only ca. 570 mm). Furthermore, many *P. biglobosa* trees found north of the 700 mm isohyet are in bad condition due to water stress (Timmer et al. 1996). Thus, the growth conditions at Tiba were less favourable for the species compared with those at Pinyiri. The paternity analysis revealed that half of the underdeveloped pods with large, flat seeds and no pulp at Tiba was cross-pollinated, which indicate lack of resources as cause of aborted seeds (Stephenson 1981; Lee 1988). In comparison, we found no flat pods at Pinyiri.

The trend towards fewer but larger pods with decreasing mesh size (T0<T1<T2) observed at Pinyiri suggests the presence of competition for maternal resources for the development of the pods and seeds in line with results from other studies (Lloyd 1980; Weinbaum et al. 2001; Minchin et al. 2010).

Effects of excluding certain types of pollinators

The result that the closed treatment (T3) inhibited fruit set confirms the need of animal pollinators for *P. biglobosa* as previously found in a similar study in The Gambia (Lassen et al. 2012). However, a major finding of the present study was that excluding bats and birds had no or little effect on the yield of *Parkia biglobosa,* whereas excluding larger insects such as honey bees reduced the yield significantly and resulted at the drier site, Tiba, in as few pods as the closed treatment.

As the open treatment T0 allowed all pollinators to visit the capitula, while the chicken wire in treatment T1 excluded larger visitors (bats and birds), any difference between the two treatments will reveal the effect of bats and birds as pollinators. We suspect that mainly bats were responsible for the few differences between T0 and T1. Sunbirds were mostly interested in the nectar ring and therefore considered to be insignificant pollinators (Hopkins 1983), and during field work we only observed birds visiting *P. biglobosa* on very few mornings. In general the differences between T0 and T1 were small at Tiba, and although the mean cross-pollen transport distance was much higher for T1 than T0 the difference was statistically non-significant. However, it was not possible to assign fathers for 30% of the pods from T0 while this was the case for only 4% of the pods from T1, thus indicating that the fathers of T0 pods more often were found outside the study site compared with the insect-pollinated pods (T1). At Pinyiri, the cross-pollen transport occurred over significantly longer distances for the open treatment T0 compared with treatment T1. This fits well with the observations of considerably more bats at Pinyiri than at Tiba. At Pinyiri, the percentage of pods from T0 and T1 that had been sired by a father outside the study site was smaller than at Tiba. However, this may be due to the difference in densities of *P. biglobosa* at the two sites.

The differences between treatments T1 and T2 are assumed mainly to be due to the absence of honey bees in T2. Pod and seed set measures were significantly larger for T1 than for T2 at both sites suggesting that honey bees were important for proper pollination of *P. biglobosa* flowers. This result is also in line with a similar trial performed in The Gambia, where a comparable T2 treatment yielded more aborted seeds than a comparable T1 treatment (Lassen et al. 2012).

Our observations at both sites showed that the smaller bees were responsible for the main pollination in treatment T2. At the drier site, Tiba, it is likely that the smaller bees carried out relatively more self-pollination due to the lower density of flowering *P. biglobosa*. The distances of pollen transport for T2 were similar to the findings from the tropical tree species yellow mombin (*Spondias mombin* L.) and fig (*Ficus* spp.), where different small insect pollinators regularly transported pollen 100-200 m among flowering trees (Nason & Hamrick 1997).

Influence by pollinator type on the number of pollen donors has also been observed for Japanese chestnut (*Castanea crenata* Siebold & Zucc.), where analysis of individual pollen grains from different pollinators revealed that the average effective number of pollen donors was around three times higher for bumblebees than for small bees (Hasegawa et al. 2015).

Self-pollination rates and pollination distances

In a controlled pollination trial with *P. biglobosa* in Burkina Faso, Ouédraogo (1995) found around 5% fruit set after self-pollination compared with around 37% fruit set after cross-pollination. Sina (2006) estimated about 5% self-pollination in open-pollinated *P. biglobosa* based on enzyme electrophoresis at two sites close to Ouagadougou, Burkina Faso, which is similar to our findings of 2% for the open treatment at Pinyiri. It is thus likely that *P. biglobosa* possesses a self-incompatibility system (SI), which is also supported by a controlled pollination trial with *P. biglobosa* in Burkina Faso (Lassen et al., *in prep.*). Asian species of *Parkia* (*P. speciosa* and *P. timoriana*) have also been reported as self-incompatible (Bumrungsri et al. 2008). The relatively high percentages (21% and 23%) of self-pollination in both the open (T0) treatment and the chicken wire (T1) treatment at Tiba, however, imply that selfing is important and possible under certain conditions. The difference in selfing between sites cannot simply be explained by genetic differentiation as the two populations were very similar. Nor can it be explained by the limited number of bats at Tiba, since treatment T1 (exclusion of bats) also had significantly more self-pollination at Tiba compared with Pinyiri. Hence, the differences in percentages of self-pollination are most likely due to limited mating opportunities allowing some selfing to occur.

In animal-pollinated populations of neo-tropical tree species, pollen dispersal occurs over longer distances in low-density populations compared to high-density populations (Ward et al. 2005). We found a similar tendency for *P. biglobosa* for cross-pollination (medians were 143 m and 115 m for Tiba and Pinyiri, respectively). Hence, a lower density may impact the reproduction in two opposite directions; one is a higher percentage of self-pollination and the other is an increased distance of pollen flow due to lack of close neighbours (Dick et al. 2003; Ward et al. 2005). Nevertheless, the pollen dispersal distributions (Fig. 2) are very similar for the two study sites. In several cases the assigned father-trees were more distantly placed than the neighbouring potential pollen donors (Fig. 3), in contrast to the findings by Levin & Kerster (1974). However, other studies have also reported random pollen movements (Finkner 1954; Larsen 2010; Stingemore 2014). The patterns may be driven by pollinator movements (Thomson & Plowright 1980; Ward et al. 2005) but also by the presence of a SI system, which may blur the real pollen flow since a given self- or cross-pollination event due to genetic SI may fail to result in fertilisation and pod production. The reduced but continuous outcrossing despite increased distances among flowering trees is likely to counteract potential negative effects of inbreeding (Breed et al. 2015).

Increased selfing as a consequence of low mating possibilities despite SI has been observed for other species (e.g. Willi, 2009). Breakdown of an SI system is common in pioneer species and marginal populations where compatible mates are lacking or where pollinators are scarce (Baker 1955; Barrett 2002; Xiong et al. 2013). This will ensure seed set, although severe consequences may occur due to inbreeding depression (Charlesworth & Charlesworth 1987; Whisler & Snow 1992). A controlled pollination trial with *P. biglobosa* reported around half the number of healthy seeds for the self-pollinated pods compared with the cross-pollinated ones (Lassen et al., *in prep.*).

Distances to honey bee colonies and pod yield

In spite of the importance of honey bees in both The Gambia and Burkina Faso, we did not find a correlation between distances to honey bee colonies and yield, which perhaps was because the distance to any colony was much shorter than the normal flight range of the honey bees (Abrol 2012). Furthermore, we have neither inspected the strength of the colonies (i.e. estimated the numbers of individuals) or registered competing flower species at the two study sites, both of which may have interfered with a given correlation.

Management of pollinators of P. biglobosa in face of climate change

The present study found that honey bees are capable of securing the pollination of *Parkia biglobosa* in the absence of bats, at least with distances between flowering trees as found in this study. However, fruit bats are still important pollinators of *P. biglobosa* especially due to their longer flight range (Linhart 1995; Monadjem et al. 2010; Fahr et al. 2015), which may connect scattered populations. Still, honey bees are almost equally good as bats in pollinating *P. biglobosa* due to their large numbers and relatively long flight range.

Besides being hunted as bushmeat (Kamins et al. 2011), fruit bats are influenced by fluctuations in the climate (Welbergen et al. 2008) and the availability of food (pollen, nectar, fruits) (Kalko 1997; Richter & Cumming 2006). Furthermore, the different bat species have different requirements to roosting sites (caves, trees, bushes etc.) (Kangoyé et al. 2012). The acknowledgement of their value as creating gene flow both as pollinators (e.g. Acharya et al., 2015) and as seed dispersers (e.g. Djossa et al., 2008) is important and likewise is their protection (Fujita & Tuttle 1991; Kunz et al. 2011).

If the climate change proceeds as some models foresee and the number of bats available for pollination is reduced in West Africa, it is important that honey bees are present and maintained in good conditions, so that the honey bees can supplement the needed pollination services. However, climate change will also impact the populations of honey bees (Le Conte & Navajas 2008) and other pollinating insects, and it may be necessary to provide them with water in order to reduce migration and increase survival. In spite of this, pod yield decline may be unavoidable, since reduced rainfall may hamper the blooming of *P. biglobosa* and increase the pod abortion, while birds may predate on what little is produced. However, a reduced pod set will have adverse effects for the rural people, because both the sweet pulp of the pods and the protein-rich seeds are widely used (Hall et al. 1997). The pods mature at the end of the dry season, where food supplies may be low (Longhurst 1985) and many women gain income from fermenting the seeds into a popular condiment known as 'soumbala' (Kessler 1994; Gausset et al. 2005).

Maintaining genetic variation in the future is important due to the challenge of adaptation to new situations (climate, pollinators, etc.). Another reason is the potential for breeding the species. *Parkia biglobosa* is considered semi-domesticated due to selective logging of trees when clearing new farm land (Kreike 2013). Hall et al. (1997) list examples of countries where different ethnic groups practice sowing of *P. biglobosa* seeds. However, due to the low natural regeneration (Ræbild et al. 2012), it is possible that more farmers will have to begin planting this species (Nikiéma 1993), despite problems with land tenure (Etongo et al. 2015), and cultural beliefs (Hall et al. 1997; Buchmann et al. 2009). Seeds or seedlings from high yielding trees producing pods with sweet pulp and many seeds will probably be in demand (Hall et al. 1997).

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