Controlled pollinations reveal self-incompatibility and inbreeding depression in the nutritionally important parkland tree, *Parkia biglobosa*, in Burkina Faso

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Abstract—The socioeconomically important fruit tree *Parkia biglobosa* is becoming less abundant in the West African savannah, possibly due to poor regeneration. This decline can be self-enforcing if lower densities of fertile trees result in increasing self-pollination followed by increased abortion rates or poor regeneration due to inbreeding depression. Hence, we have studied the reproductive success and seedling viability of *P. biglobosa* after controlled self- and cross-pollination based on a full diallel crossing design with eight trees. Controlled cross-pollination tripled the pod set compared to open-pollinated capitula, suggesting that fruiting of *P. biglobosa* trees in the study area is already seriously pollen limited. Self-pollination and specific pairs of trees resulted in very few pods, suggesting a high level of self-incompatibility. Cross-pollination resulted in larger pods with more and heavier seeds than self-pollinated pods. The total amount of sugar in the fruit pulp was correlated with both the number of healthy and total seeds per pod. Growth rate of self-pollinated seedlings was lower than the cross-pollinated ones, suggesting significant inbreeding depression. Because the wild fruit trees play an important role in human nutrition, these results give rise to serious concerns. We recommend that future studies investigate how the level of cross-pollination can be increased and how the regeneration of *P. biglobosa*, whether natural or planted, can be improved.

Keywords: Controlled pollination; Fruit quality; Inbreeding depression; Pollen limitation; Self-incompatibility

Introduction

The majority of tropical tree species rely on animals for pollination (Ollerton et al. 2011), and many possess a system of self-incompatibility (Bawa et al. 1985; Ward et al. 2005). The high dependency on animal pollinators in the tropics (Ollerton et al. 2011), combined with pollination deficit due to historical decline of wild pollinators (e.g. Goulson et al. 2015) and limited alternative options for cross-pollination, may decrease fruit production and quality of important crop trees. In addition, anthropogenic impacts, such as farming (monoculture, pesticides), landscape fragmentation, and climate change are likely to reduce abundance and species diversity of pollinators (Kennedy et al. 2013). Understanding mechanisms of pollination and optimising fruit production in tropical fruit trees are crucial, given that many livelihoods depend on crop yield from animal-pollinated tropical trees.

Local decline of pollinators and/or increased distances between conspecific trees can reduce seed set due to pollen limitation, if fruit and seed set are limited by the supply of compatible pollen, rather than resource availability (Ashman et al. 2004; Knight et al. 2005; Aizen & Harder 2007).

Another potential consequence is increased selfing, which may decrease fruit production, and reduce the fitness of seedlings due to inbreeding depression (Husband & Schemske 1996). Hence, we expect a decrease in reproductive success, combined with reduction of seedling fitness when conspecific trees are more widely spaced in a disturbed landscape.

Parkia biglobosa (Jacq.) R. Br. ex G. Don (Fabaceae: Mimosoideae), is a West African parkland tree with high nutritional importance for the rural people due to its sweet fruit pulp and seeds with high protein content (Uwaegbute 1996; Hall et al. 1997). In Burkina Faso, pods from *P. biglobosa* are an important food source (Lykke et al. 2002), especially during periods of food scarcity (Nyadanu et al. 2017). Due to over-exploitation (Gaisberger et al. 2017), low regeneration (Ræbild et al. 2012), and reduced annual rainfalls (Maranz 2009; Funk et al. 2012), there is a high risk of seriously decreasing tree densities (Gaisberger et al. 2017) as already witnessed by the local population (Lykke et al. 2002). This may increase selfing (Lassen et al. 2017), but the potential effects of increased self-pollination on seedling growth and nutritional content of the fruits of *P. biglobosa* are unknown. Related species possess a self-incompatibility system (Hinata et al. 1993), and hence increased selfing is expected to reduce seed set. Studies of other plant species have documented positive correlations between seed number and fruit traits including fruit weight, size, and oil content (Hopping 1976; Roldán Serrano & Guerra-Sanz 2006; Abrol

2012). Thus, increased self-pollination, by causing reduced seed number, may also reduce the nutritional value of *P. biglobosa* fruits.

To understand productivity of this important crop tree species, it is important to determine if *P. biglobosa* is pollen limited, and how increased levels of self-pollination will affect fruit and seed set, nutrition contents and seedling fitness of this species. We addressed these questions by performing controlled self- and cross-pollinations of *P. biglobosa* capitula in order to compare 1) the reproductive success of selfed, crossed, and open-pollinated capitula, 2) the carbon and nitrogen contents of seeds and sugar content of fruit pulp from selfed and outcrossed pods, and 3) the germination percentage and seedling vigour of selfed and outcrossed seeds. We discuss the probability of *P. biglobosa* experiencing pollen limitation, self-incompatibility and inbreeding depression.

MATERIALS AND METHODS

Plant species

Parkia biglobosa is pollinated mainly by bees and bats (Baker & Harris 1957; Hopkins 1983; Ouédraogo 1995; Lassen et al. 2012; Lassen et al. 2017). It is predominantly outcrossing (Ouédraogo 1995; Sina 2006), although Lassen et al. (2017) found that selfing can occur in areas with low tree density. *Parkia biglobosa* flowers for around four weeks in the dry season in Burkina Faso from January to April, depending on latitude, and with year to year variation (pers. obs.).

Flowers are grouped in ball-shaped capitula with around 2,200 tiny bright red flowers packed closely on a bulbous receptacle hanging on a long peduncle (Hopkins 1983). Nectar is produced by sterile flowers close to the peduncle and accumulates in a nectar ring (Hopkins 1983). At the study site, buds opened during the afternoon. The capitula started producing nectar around 18:45 h (local time, UTC + 0 h) and shedding pollen around 19:30 h (pers. obs.). Each tiny flower has ten anthers and one style. *Parkia biglobosa* is andromonoecious, and the functionally male capitula have styles, which fail to elongate (Hopkins 1981). Pollen is shed in polyads with 32 pollen grains (pers. obs.) clumped together. The cup-shaped stigma can hold only one polyad; therefore all seeds per pod are full siblings (Lassen et al. 2014). According to our observations, based on $N = 15$ ovaries distributed on three trees in Burkina Faso, the ovary of a hermaphroditic capitulum contained a mean of 23 ovules (SE = 0.63, min-max: 16-29). The hermaphroditic capitula are protandrous (Ouédraogo 1995) and the female phase begins around 23:00-24:00 h when the stigmas have extended to reach the same level or above that of the anthers (pers. obs.). Furthermore, flowering within a capitulum is highly synchronized. Each capitulum blooms one night and during the morning both hermaphroditic and functionally male capitula start to wilt (pers. obs.). In the present study, each capitulum is treated as one unit.

One week after pollination and fertilisation, tiny green pods are visible, and after around two months the indehiscent,

brown pods are mature (pers. obs.). Even though each hermaphroditic flower has the potential of producing a pod, only a few pods per capitulum develop (Hopkins 1984).

Study site

The present study took place in the village Pinyiri (syn. Kacheli) (11°14'34.89"N, 1° 8'1.73"W), eight km north of Pô, Nahouri province. The site is within the Sudanian climatic zone with a unimodal rainy season and an average precipitation (1981-2010) of 900-1,000 mm (Sanfo 2012). In 2011, preceding the fruiting season of *P. biglobosa* in 2012, the annual precipitation in Pô was 927 mm (Météo 2015).

Controlled pollination experiment

Prior to the experiment, swollen buds (expected to open the following night) were covered with cheesecloth with an inner band of chicken wire to keep the nettings from touching the flowers. We used inflorescences from the lowest part of the tree crown, which could be reached from a ladder. In the experiment, only the most apical bud within the compound inflorescence was used. During 8-18 March 2012, the crossing experiment was carried out as a diallelic cross (i.e. all trees were crossed with each other) of eight trees (mean DBH = 1.4 m, SD = 0.81), although one of the trees was not used as a pollen donor. Each treatment was replicated nine times per tree.

Treatments:

- Open = open-pollinated capitula (control, $N = 72$)

- Self = self-pollinated capitula ($N = 72$)

- Cross = cross-pollinated capitula ($N = 423$)

- 'Both' = half of a capitulum was pollinated with self-pollen and the other half with cross-pollen (only on six trees due to lack of capitula, $N = 54$)

Prior to the pollination treatments, we checked the sex (hermaphroditic or functionally male) of the flowers on each receiving capitulum, since only hermaphroditic capitula can develop pods. When in doubt, we measured the distance between stigmas and anthers, as this difference was suspected to influence the functional sex of the capitulum, due to the probability of the many densely packed anthers acting as a carpet keeping pollen away from the shorter stigmas. The dividing line between hermaphroditic and functionally male capitula was unknown at the time we carried out the controlled pollinations. Hence, these were carried out regardless of the assigned sex, from midnight until early morning (03-04 h) using capitula directly as pollen brushes by dabbing the donor capitula on the receiving capitula, which were previously protected by bags. No flowers were emasculated due to the high number of anthers per capitulum. To standardise the dose of pollen, one donor capitulum was used on three receiving capitula (1:3) for the treatments of self- and cross-pollinations (approximately one third of the donor on each of the receiving capitulum). To study the effect of self- and cross-pollination under identical conditions, we applied self and cross-pollen to flowers on the same capitulum (but not the same flowers). We refer to this treatment as

'both', and for this treatment we used two donor capitula (self and cross) for two receiving capitula (half self and half cross on each capitulum) (1:1). Half of the peduncle was marked with a black marker (for the cross-pollination) and we performed the two kinds of pollination by eye. After pollination, the capitula were re-bagged. The trees were visited daily for five weeks; dropped capitula were collected and examined for sex (hermaphroditic or functionally male) and signs of developing pods and/or predation.

After harvest, pods were counted and weighed. Their lengths were measured as the mean of each side of the pod, excluding the pedicel. We did not count the seeds of these pods (see below).

DNA extraction and genotyping

To assess the level of self-pollination (on purely self-pollinated capitula and on 'both' capitula), we extracted DNA, genotyped seeds ($N = 142$) and compared their genotypes with the genotype of the mother trees. Likewise, genotypes of the eight experimental trees were compared to genotypes of a sub-sample of pods, to test if the cross had been performed correctly. DNA was extracted directly from dehulled seed; endosperm is absent because the cotyledons provide resources to the embryo (Hopkins 1983). DNA extraction used the DNeasy 96 Plant Kit (QIAGEN, Hombrechtikon, Switzerland), following the manufacturer's protocol. The genotyping was based on ten microsatellite primer pairs developed for *P. biglobosa* (Lassen et al. 2014) with PCR reactions and fragment analysis following Lassen et al. (2017).

Comparison of self- and cross-pollinated pods

The self-pollinated capitula in the controlled pollination experiment yielded extremely few pods. Thus, in order to compare the quality of seeds in *P. biglobosa* after self- and cross-pollination we assessed the quality from 48 pods obtained from a previous experiment from the same study site (Lassen et al. 2017). These pods were collected from eleven trees and were randomly selected, although the selection was balanced with an equal number of pods per tree being self- and cross-pollinated. Seeds were categorised as 'healthy', 'eaten' (hole in the husk and/or seed remains due to predation by parrots and/or worms), 'aborted' (weight < 0.05 g and/or with a flat shape), 'missing' (empty cavity in the pulp), and from these four numbers a total number of seeds was established.

Moisture, carbon and nitrogen content of seeds

One seed from each of 48 pods (24 selfed and 24 outcrossed) was analysed for moisture, carbon and nitrogen content. The seeds were weighed with and without testa, placed in open 2 ml Eppendorf tubes and dried at 65°C for three days until stable weight. Moisture content of seeds without testa was calculated as fresh weight minus dry weight, divided by fresh weight and multiplied by 100 (%). Then the dehulled seeds were ground in a mortar and weighed into tin capsules with 5 mg in each sample. Total carbon (C) and nitrogen (N) content were measured using the Dumas principle: samples were combusted at 1,850°C on a FLASH 2000 NC Analyzer (Thermo Scientific) according to the manufacturer's manual. To calibrate the measurements, we

used a standard ('spruce needles' Forest Foliar Coordinating Centre, FFCC), and two reference samples ('maple leaves' FFCC, run twice, and 'corn gluten organic' Sercon, run for every nine samples of *P. biglobosa*). The FLASH 2000 Analyzer is comparable with the Kjeldahl method (Krotz & Giazzi 2014).

We have converted the amount of nitrogen (N) to crude protein by multiplying N with the commonly used factor 6.25 (AOAC 1990), although Ezeagu et al. (2002) found a lower nitrogen-to-protein conversion factor on 4.97 for Fabaceae seeds (mean for ten species) and Yeoh & Wee (1994) found an even lower conversion factor on 4.23 for leaves of *Parkia timoriana* (DC.) Merr. (syn. *P. javanica*).

Pod pulp analysis

We analysed the sugar content in the pulp of the same pods as above, except for two self-pollinated pods, which had no pulp (i.e. 22 self- and 24 cross-pollinated pods were used in this analysis). We kept the pulp from each pod separately. The pulp was dried (103°C for 3 h), ground and sifted, and two sub-samples of 100 mg per pod were used. The soluble sugars were extracted and analysed by HPLC according to the method described by Liu et al. (2004).

Germination rate and seedling vigour of selfed and crossed seeds

In order to compare seed viability under optimal conditions, we tested the germination by using healthy-looking seeds from the 24 self-pollinated pods (90 seeds) and 24 cross-pollinated pods (163 seeds) and assessed the seedling vigour by growth and dry weight. Each seed was weighed and scarified because of the hard seed coat (testa), which must be broken before the seeds can germinate (Etejere et al. 1982). The seeds were germinated in plastic boxes in a growth chamber in a 25°C day and night, and 12 h light/darkness regime. We defined the seeds as germinated when the radicle protruded for 3 mm, and we monitored the seed germination daily.

On the 19th day after sowing, seedlings were weighed and planted individually in pots (Ø = 13 cm) with planting peat soil. The pots were placed randomly in a greenhouse at 28°C day and 20°C night and with a 12 h light/darkness regime from 08:00 h (local time, UTC + 1 h). The seedlings were watered daily with demineralised water without fertiliser for the first 2 months, and thereafter with fertiliser. Plant height (from soil level to top of main stem, or to the highest stem in case of more stems), stem diameter (measured with an electronic caliper) and number of pinnae (i.e. primary division of a bipinnate compound leaf) were measured on five occasions (43, 76, 104, 144, and 222 days after sowing). At the last measuring, we included fresh weight of the seedlings before drying them at 80°C (Osonubi & Fasehun 1987). After 24 hours (until stable weight), we recorded dry weight of the entire plant, shoots (stem plus leaves) only, and roots only, in order to calculate the shoot:root ratio.

Data analysis

In the controlled pollination experiment, reproductive success was evaluated as 1) numbers of immature pods

(reflecting the success of pollination) and mature pods (additionally reflecting available resources by the mother-tree) per hermaphroditic capitulum and 2) weight and length of these pods. Analysis of variance was performed based on average values per treatment and tree applying the statistical SAS software v.9.4 (SAS Institute 2011). The mean number of pods (immature and mature) per hermaphroditic capitulum included capitula without any pods. When testing differences between treatments for the controlled pollination experiment, we used the general linear model as implemented in the GLM procedure:

$$1.\ Y_{gh} = \text{Treatment}_g + \text{Mother-tree}_h + \mathcal{E}_{gh}$$

where Y_{gh} is the response variable, treatment g = (open, self, cross, 'both'), and mother-tree h = 1…8. Treatment$_g$ was considered a fixed effect, whereas Mother-tree$_h$ was considered a random effect with residual $\mathcal{E}_{gh}$ assumed independent and $N(0,\sigma_\varepsilon^2)$. We assessed and accepted the model assumptions by visual inspection of the residuals.

For testing differences among pairs of pollen donors (i.e. male parent) and mother-trees (i.e. female parent), we analysed number of pods per single capitulum (not averaged per tree), using the general linear model as implemented in the GLM procedure:

$$2.\ Y_{ij} = \text{Pollen donor}_i + \text{Mother-tree}_j + \text{Pollen donor}_i*\text{Mother-tree}_j + \mathcal{E}_{ij}$$

where Y_{ij} is the response variable (log transformed), pollen donor i = (T10, T14, T22, T76, T90, T92, and T93), mother-tree j = 1…8, and pollen donor$_i$*mother-tree$_j$ = the interaction between the pollen donor and the mother-tree. All effects were considered fixed with residual $\mathcal{E}_{ij}$ assumed independent and $N(0,\sigma_\varepsilon^2)$. We assessed and accepted the model assumptions by visual inspection of the residuals.

When testing differences between self- and cross-pollination for several parameters related to plant fitness, the results were averaged per type of pollination and tree, and we used a similar general linear model as above:

$$3.\ Y_{kl} = \text{Pollination type}_k + \text{Mother-tree}_l + \mathcal{E}_{kl}$$

where Y_{kl} is the response variable, pollination type k = (self, cross), and mother-tree l = 1…11. Pollination type$_k$ was considered a fixed effect, whereas Mother-tree$_l$ was considered a random effect with residual $\mathcal{E}_{kl}$ assumed independent and $N(0,\sigma_\varepsilon^2)$. Again, we assessed and accepted the model assumptions by visual inspection of the residuals.

When testing the difference between carbon and nitrogen contents of selfed versus outcrossed seeds, we used the following general linear model:

$$4.\ Y_{klm} = \text{Pollination type}_k + \text{Mother-tree}_l + \text{Seed weight}_m + \mathcal{E}_{klm}$$

where Y_{klm} is the response variable, pollination type k = (self, cross), mother-tree l = 1…11, and seed weight included as covariate. Seed weight varied from $0.0821 - 0.2733$ g per seed. Pollination type$_k$ was considered a fixed effect, whereas Mother-tree$_l$ was considered a random effect with residual $\mathcal{E}_{klm}$ assumed independent and $N(0,\sigma_\varepsilon^2)$. Again, we assessed and

accepted the model assumptions by visual inspection of the residuals.

We used Fisher's exact test (as implemented in SAS procedure FREQ) to test differences between self- and cross-pollination for number of germination seeds and surviving seedlings.

The relationship between number of seeds per pod and sugar content in fruit pulp was analysed by calculation of Pearson correlation coefficients (using SAS procedure CORR).

RESULTS

Assessment of the diallel crossing experiment

In the controlled pollination experiment, 451 capitula were hermaphroditic, 139 capitula were functionally male, and 45 capitula were 'mixed' (i.e. containing both hermaphroditic and functionally male flowers in different ratios). Of capitula with hermaphroditic flowers, 83% developed pods.

The measurement of distances between stigmas and anthers of the capitula (N = 245 capitula) coupled with the reproductive success (setting fruit or not) revealed that capitula having anthers protruding > 5 mm longer than stigmas were typically functionally male (91%). Hence, this measure could be used as a rule of thumb.

Of the four pollination treatments (open, self, cross, and 'both'), self-pollination led to a significantly lower proportion of capitula with at least one immature pod (100%, 19%, 96%, 97%, respectively). In addition, the mature pod set also differed, and the experiment yielded 2,643 pods for the purely cross-pollinated capitula (423 pollinated capitula) and only 2 pods for the purely self-pollinated capitula (72 pollinated capitula). Genotyping of a subset of pods confirmed that cross-pollinated pods indeed were results of cross-pollinations while a few matured 'self-pollinated' pods, turned out to be cross-pollinated, probably due to small amounts of 'carryover' pollen. For treatment 'both', in which 502 pods were matured, no pods developed in the self-pollinated halves of the capitula and the subset of genotyped pods showed no self-pollination (N = 133).

Effects of pairs of trees and pollen doses on fruit set

In the controlled cross-pollination treatments, we found highly significant effects of both mother-trees (female parent, $F_{(7,231)}$ = 9.1, P < 0.001) and pollen donors (male parent, $F_{(6,231)}$ = 4.7, P < 0.001) on the number of immature pods per hermaphroditic capitulum. However, the number of mature pods was only significantly affected by the mother-trees ($F_{(7,199)}$ = 13.5, P < 0.001). Two trees (P14 and P22) were equally good as mother-trees and pollen donors, whereas three trees had highest reproductive success as mother-trees (i.e. producing many pods, P76, P92, and P93) and two trees were best as pollen donors (i.e. fathering many pods, T10 and T90) (Tab. 1 and 2).

Interactions between mother-trees and pollen donors were highly significant for both immature ($F_{(34,231)}$ = 3.4, P < 0.001) and mature pods ($F_{(34,199)}$ = 3.4, P < 0.001), i.e. fruit set depended on the combination of mother-trees and pollen

TABLE 1. Mean number (± SE) of immature pods per hermaphroditic capitulum (i.e. small pods before maturation) of *Parkia biglobosa* (incl. capitula without pods). Self-pollination (grey colour) is shown in the last row. Tree P33 did not give pollen to the other trees.

					Female parent (i.e. mother-tree)					
	Treatment	P10	P14	P22	P33	P76	P90	P92	P93	Across trees
	Open[a]	4.1 (4.80)	5.6 (4.52)	1.3 (4.52)	2.8 (4.52)	7.1 (4.52)	2.9 (4.52)	2.8 (4.52)	4.3 (4.52)	3.9 (0.41)
Male parent (i.e. pollen donor)	T10	-	42.5 (5.54)	41.7 (7.83)	5.0 (7.83)	50.0 (7.83)	10.3 (5.13)	23.9 (4.80)	61.6 (4.80)	39.4 (4.74)
	T14	15.5 (4.80)	-	15.8 (4.52)	9.6 (4.80)	31.1 (4.52)	1.0 (6.07)	23.5 (5.54)	22.6 (5.13)	17.8 (2.08)
	T22	26.8 (5.54)	17.3 (5.13)	-	18.8 (6.07)	26.1 (4.52)	7.4 (5.13)	9.5 (5.54)	26.5 (4.80)	19.4 (2.23)
	T33	na	na	na	-	na	Na	na	na	na
	T76	10.7 (5.13)	15.3 (5.54)	15.4 (4.80)	na[d]	-	9.5 (5.54)	1.0 (7.83)	27.5 (5.54)	14.3 (1.70)
	T90	12.4 (6.07)	1.1 (5.13)	22.0 (6.07)	12.0 (4.80)	39.0 (4.52)	-	42.5 (6.78)	20.3 (7.83)	20.9 (3.11)
	T92	18.2 (5.54)	23.7 (5.54)	14.2 (6.07)	18.0 (13.57)	11.6 (5.13)	8.5 (5.54)	-	25.8 (6.78)	16.4 (1.97)
	T93	26.5 (5.54)	25.8 (4.52)	9.0 (6.07)	7.7 (7.83)	23.1 (4.52)	14.6 (5.13)	45.5 (9.59)	-	21.0 (2.48)
	Cross[b]	18.2 (2.27)	20.7 (2.46)	17.6 (2.01)	11.5 (1.64)	30.9 (3.56)	8.8 (0.93)	22.5 (3.78)	33.1 (3.77)	21.2 (1.12)
	Self[c]	0.0 (0.00)	0.0 (0.00)	0.4 (0.30)	0.3 (0.25)	0.4 (0.18)	0.1 (0.12)	0.5 (0.50)	0.0 (0.00)	0.2 (0.07)

[a]Open is open-pollination (control). [b]Cross denotes the mean of cross-pollination (i.e. across T10-T93 except self-pollination). [c]Self signifies self-pollination. [d]All nine P33-capitula pollinated with T76 were functionally male.

donors (Tab. 1 and 2). For instance, using P10 as a pollen donor resulted in more than twice as many immature pods per hermaphroditic capitulum on P93 compared to P92 (61.6 versus 23.9), while for P90 the result was vice versa (20.3 versus 42.5, Tab. 1).

Effects of pollen doses on fruit set (excluding self-pollination) and results of the open-treatment are shown in Tab. 3. The number of capitula with at least one immature pod was not influenced by the different pollen doses while the number of pods per hermaphroditic capitulum was significantly higher in hand-pollinated capitula (cross and 'both') compared to the open-pollinated capitula. The hand-pollinated capitula differ in that the 'both' treatment had around 3 times more cross-pollen than the cross treatment, but only on half of the capitulum. Hence the figures can be made comparable by multiplying those for the 'both' treatment with ⅔: (30.8 x ⅔ =) 20.5 for immature pods and (13.5 x ⅔ =) 9.0 for mature pods, which are close to the actual figures for the cross treatment on 20.4 and 9.8 for immature and mature pods, respectively (Tab. 3). However, with an increasing number of pods per capitulum the abortion rate (immature minus mature pods) also increased. For pod weight and length, the differences between pollen doses were non-significant (Tab. 3).

In one pair of trees (P14 and P90), we found that cross-pollination resulted in a similar low pod set as for self-pollination, independently of which tree was mother-tree and which was pollen donor, suggesting that these two trees were not compatible. Finally, we found another pair of trees (P76 and P92) with very few pods when P92 was the mother-tree, but only a reduced pod set when P76 was the mother-tree (Fig. 1, Tab. 1 and 2), which suggest an incompatibility system.

Comparison of self- and cross-pollinated pods

Because the diallelic crossing experiment resulted in only two matured self-pollinated pods, we used 24 self-pollinated and 24 cross-pollinated pods originating from another experiment at the same study site, as stated above. Self-pollinated pods were significantly shorter and weighed less than cross-pollinated pods (Tab. 4). Furthermore, although not significant, the self-pollinated pods had half as many healthy seeds, significantly more aborted seeds and significantly fewer total seeds compared to the cross-pollinated pods (Tab. 4). The amounts of eaten seeds and missing seeds were low, and showed no difference between selfed and outcrossed pods (Tab. 4).

Table 2. Mean number ($\pm$ SE) of mature pods per hermaphroditic capitulum (i.e. harvested pods) of *Parkia biglobosa* (incl. capitula without pods). Self-pollination (grey colour) is shown in the last row. Tree P33 was not used as a pollen donor.

| | Treatment | Female parent (i.e. mother-tree) | | | | | | | | |
		P10	P14	P22	P33	P76	P90	P92	P93	Across trees
	Open[a]	4.1 (1.93)	4.7 (1.93)	1.3 (1.93)	2.7 (1.93)	6.8 (1.93)	2.9 (1.93)	2.4 (1.93)	3.7 (1.93)	3.6 (0.39)
Male parent (i.e. pollen donor)	T10	-	7.3 (2.36)	16.0 (5.79)	2.0 (4.09)	13.4 (1.93)	2.3 (3.34)	15.7 (2.19)	23.8 (2.05)	13.7 (1.84)
	T14	8.3 (2.05)	-	12.0 (4.09)	4.8 (2.36)	15.6 (2.05)	0.8 (2.59)	17.5 (2.36)	10.2 (2.36)	10.1 (1.33)
	T22	9.2 (2.36)	5.2 (2.59)	-	7.0 (2.59)	7.3 (2.19)	4.3 (2.19)	6.8 (2.59)	17.1 (2.19)	8.4 (1.04)
	T33	na	na	na	-	na	na	na	na	na
	T76	7.9 (2.05)	9.0 (2.36)	8.0 (2.36)	na[d]	-	4.8 (2.36)	1.0 (3.34)	18.8 (2.36)	8.9 (1.09)
	T90	8.0 (2.36)	0.9 (2.19)	7.0 (2.59)	5.3 (2.36)	20.0 (2.05)	-	25.3 (3.34)	6.7 (3.34)	9.9 (1.54)
	T92	13.5 (2.36)	11.8 (2.36)	11.0 (2.59)	1.0 (5.79)	8.0 (2.19)	3.4 (2.59)	-	15.5 (2.89)	10.1 (1.20)
	T93	13.3 (2.36)	11.0 (1.93)	4.2 (2.59)	1.7 (3.34)	9.3 (1.93)	6.9 (2.19)	18.5 (4.09)	-	9.1 (1.00)
	Cross[b]	9.8 (0.85)	7.7 (0.86)	8.3 (1.27)	4.6 (0.76)	12.4 (1.26)	4.1 (0.60)	14.0 (2.22)	16.6 (1.65)	10.0 (0.50)
	Self[c]	0.0 (0.00)	0.0 (0.00)	0.3 (0.19)	0.0 (0.00)	0.3 (0.16)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.1 (0.05)

[a]Open is open-pollination (control). [b]Cross denotes the mean of cross-pollination (i.e. across T10-T93 except self-pollination). [c]Self signifies self-pollination. [d]All nine P33-capitula pollinated with T76 were functionally male.

TABLE 3. Effect of open-pollination (unknown pollen doses) and two known pollen doses on the percentage of hermaphroditic capitula with at least one immature pod, number of immature and mature pods per hermaphroditic capitulum (incl. capitula without pods), and the pod weight and length of *Parkia biglobosa*, including *F*-tests and significance levels. Except in the open-pollinated treatment, capitula were bagged until hand-pollination and re-bagged following pollination treatment.

Type of pollination	Pollen doses	No. of capitula[a], N	Capitula with ≥ 1 immature pod[b], %	No. of immature pods/capitulum[b]	No. of mature pods/capitulum[b]	No. of pods[c], N	Pod weight[c], g	Pod length[c], cm
Open[d]	unknown	71	100.0 (1.15)	3.9 (2.87)	3.6 (1.02)	258	14.0 (0.62)	22.6 (0.74)
Cross[e]	1/3	292	95.7 (1.15)	20.4 (2.87)	9.8 (1.02)	2,643	11.9 (0.62)	20.7 (0.74)
'Both'[f]	1	40	98.0 (1.41)	30.8 (3.52)	13.5 (1.25)	502	12.8 (0.77)	21.2 (0.90)
$F(P)$ Pollination			$F_{(2,12)}=3.5$ (ns)	$F_{(2,12)}=18.8$ (***)	$F_{(2,12)}=20.7$ (***)		$F_{(2,12)}=2.9$ (ns)	$F_{(2,12)}=1.8$ (ns)
$F(P)$ Tree			$F_{(7,12)}=0.7$ (ns)	$F_{(7,12)}=2.3$ (ns)	$F_{(7,12)}=3.0$ (*)		$F_{(7,12)}=5.6$ (**)	$F_{(7,12)}=5.2$ (**)

[a]Number of capitula used per type of pollination. [b]Values are least squares (LS) means with the standard error (SE) in brackets of the LS estimate. Significance level: ***=$P<0.001$, **= $P<0.01$, *= $P<0.05$, and ns= $P>0.05$. [c]Number of pods harvested. [d]Open was open-pollinated capitula, which were neither bagged nor hand-pollinated (control). [e]Cross was cross-pollinated capitula with one capitulum giving pollen to three capitula (1:3 ratio). [f]'Both' was capitula, which were self-pollinated on one half and cross-pollinated on the other half, with one capitulum giving pollen to two half capitula (1:1 ratio), but only cross-pollinated pods developed.
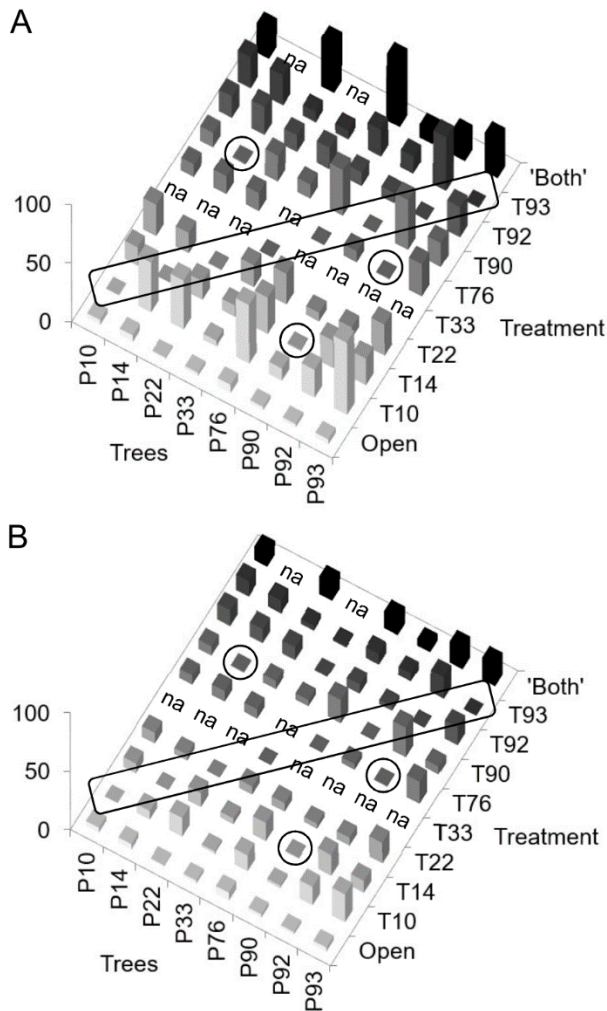
FIGURE 1. Overview of the number of immature (A) and mature (B) pods per hermaphroditic capitula for *Parkia biglobosa*, distributed on treatments (i.e. pollen donors) and trees. The box encompasses the events of self-pollination, which all resulted in very few pods, and the circles indicate low numbers of pods resulting from cross-pollinations. 'Open' indicate open-pollination (control) and 'Both' is a treatment in which capitula were pollinated with self-pollen on one half and cross-pollen on the other half. However, 'Both' yielded exclusively outcrossed pods. Pollen donor T33 was only applied to tree P33, pollen donor T76 was applied to only functionally male capitula on tree P33 and neither tree P14 or tree P33 had not enough capitula for the 'Both'-treatment.

Carbon & protein contents in seeds, and sugar contents in fruit pulp

Raw, dehulled seeds from cross-pollinated seeds had slightly more carbon compared to self-pollinated seeds (54.3% versus 53.4%), while percentage of protein (dry weight) was slightly lower (43.3% versus 45.2%, Tab. 4). The carbon content was highly dependent on seed weight ($F_{(1,35)} = 12.4$, $P < 0.001$) with larger seeds containing more carbon. This was not the case for amount of protein ($F_{(1,35)} = 1.0$, $P = 0.3$), hence larger seeds contained more carbon whereas protein content was constant, regardless of seed size.

Sugar content was higher in fruit pulp for the cross-pollinated pods, but only significantly so for glucose and fructose (Tab. 4). Numbers of healthy seeds and amounts of glucose, fructose and total sugars, respectively, were positively and significantly correlated (Tab. 5). No correlation was found between the amount of sucrose and number of seeds per pod (Tab. 5).

Germination and seedling growth of self- and cross-pollinated seeds

Seed weight and seedling growth was highly variable in both types of pollination (self and cross). The mean weight of cross-pollinated seeds was significantly higher than self-pollinated seeds, while germination percentage and germination speed did not differ significantly between pollination types (Tab. 4). Nine selfed and nine cross-pollinated seeds germinated but died before the first measurement of seedlings, and most seedlings, which died during the trial, perished before the second measurement. From the initial 90 self-pollinated and 163 cross-pollinated seeds, significantly fewer self-pollinated seedlings (69) than cross-pollinated (147) seedlings survived until the trial was terminated (Tab. 4). Initial fresh weights (19 days after sowing) and fresh and dry weights at harvest were significantly higher for the cross-pollinated seedlings compared to the self-pollinated ones. The shoot:root ratio was independent of the type of pollination (Tab. 4).

The growth of the seedlings is shown in Fig. 2 (A, B, and C) and the final height, stem diameter and number of pinnae 222 days after sowing in Tab. 4. Means of height, diameter, and numbers of pinnae were always lower for self-pollinated seedlings compared to cross-pollinated ones, and these differences in growth increased with time (Fig. 2). Seven months after sowing (222 days), the effect of type of pollination was significant for plant height and stem diameter, but not for number of pinnae ($F_{(1,7)} = 4.3$, $P = 0.08$) (Tab. 4).

DISCUSSION

Parkia biglobosa is known to be mainly outcrossing (Ouédraogo 1995; Sina 2006; Lassen et al. 2017), and this study showed that self-pollination reduced the number of pods produced, pod size, number of seeds, sugar content in pulp, seed weight, and weight of seedlings (Tab. 1, 2, and 5). In addition, the diallel cross revealed that some combinations of mother-trees and pollen donors were more productive than others. The findings of variation in the success of male and female reproductive organs, i.e., an individual plant being good at either setting pods or at fathering pods on other conspecifics, has also been reported in other plant species such as the self-incompatible Trumpet creeper *Campsis radicans* (L.) Seem. (Bignoniaceae) (Bertin 1982) and the self-incompatible Crested dogstail grass *Cynosurus cristatus* L. (Poaceae) (Ennos & Dodson 1987).

Pollen limitation in Parkia biglobosa

Controlled pollinations with different doses of cross-pollen conducted in the present study yielded significantly more immature and mature fruits than in open-pollinated

TABLE 4. Influence of self- versus cross-pollination on the fitness of various parameters of pods and seeds of *Parkia biglobosa* including *F*-tests, Fisher's exact tests, and their significance levels.

	Type of pollination					Inbreeding depression[b], %
	Self, *N*	Cross, *N*	Self[a]	Cross[a]	$F(P)_{Pollination}$	
Pod length, cm	24	24	15.8 (1.16)	20.0 (1.16)	$F_{(1,10)} = 6.4$ (*)	21.0
Pod weight, g	24	24	7.9 (1.03)	12.5 (1.03)	$F_{(1,10)} = 10.1$ (**)	36.8
Husk weight, g	24	24	4.0 (0.41)	5.5 (0.41)	$F_{(1,10)} = 7.5$ (*)	27.3
Pulp[c] weight, g	22	24	2.5 (0.40)	4.2 (0.37)	$F_{(1,9)} = 8.7$ (*)	40.5
Seed weight, g	24	24	1.6 (0.29)	2.6 (0.26)	$F_{(1,9)} = 6.9$ (*)	38.5
Seeds per pod:						
Healthy seeds, *n*	24	24	6.6 (1.87).	12.4 (1.87).	$F_{(1,10)} = 4.8$ (ns)	46.8
Eaten seeds, *n*	24	24	1.4 (0.70)	0.7 (0.70)	$F_{(1,10)} = 0.6$ (ns)	-100.0
Aborted seeds[d], *n*	24	24	2.8 (0.46)	1.3 (0.46)	$F_{(1,10)} = 5.2$ (*)	-115.4
Missing seeds[e], *n*	24	24	1.1 (0.29)	1.5 (0.29)	$F_{(1,10)} = 0.9$ (ns)	26.7
Total seeds, *n*	24	24	11.9 (0.99)	15.9 (0.99)	$F_{(1,10)} = 8.1$ (*)	25.2
Moisture in seeds, %	24	24	3.5 (0.14)	3.1 (0.14)	$F_{(1,10)} = 3.3$ (ns)	-12.9
C and N in seeds, dry weight:						
Carbon in raw seeds, %	24	24	53.4 (0.29)	54.3 (0.29)	$F_{(1,10)} = 4.8$ (ns)[f]	1.6
Nitrogen in raw seeds, %	24	24	7.2 (0.10)	6.9 (0.10)	$F_{(1,10)} = 5.0$ (*)	-4.4
Protein in raw seeds[g], %	24	24	45.2 (0.60)	43.3 (0.60)	$F_{(1,10)} = 5.0$ (*)	-4.4
Sugars in fruit pulp, dry weight:						
Sucrose[c], %	22	24	28.5 (0.81)	29.5 (0.74)	$F_{(1,9)} = 0.9$ (ns)	3.4
Glucose[c], %	22	24	3.5 (0.68)	5.6 (0.62)	$F_{(1,9)} = 5.6$ (*)	37.5
Fructose[c], %	22	24	4.2 (0.51)	5.9 (0.47)	$F_{(1,9)} = 5.6$ (*)	23.8
Total sugar[c], %	22	24	36.2 (1.81)	41.3 (1.66)	$F_{(1,9)} = 4.3$ (ns)	12.3
Seed germination:						
Seed weight, g	117	196	0.16 (0.006)	0.20 (0.005)	$F_{(1,8)} = 32.9$ (***)	20.0
Seed germination[h], %	117	196	76.9	83.2	$P < 0.2$ (ns)	7.6
Days to germination	90	163	4.4 (0.28)	4.3 (0.23)	$F_{(1,8)} = 0.1$ (ns)	-2.3
Seedlings:						
Fresh weight 19 days[i], g	88	161	0.85 (0.04)	1.02 (0.03)	$F_{(1,8)} = 10.2$ (*)	16.7
Survival 222 days[h,i], %	90	163	76.7	90.2	$P < 0.005$ (**)	15.0
Height 222 days[i], cm	69	147	20.3 (0.97)	23.2 (0.74)	$F_{(1,7)} = 5.8$ (*)	12.5
Stem diameter 222 days[i], mm	69	147	3.9 (0.20)	4.6 (0.15)	$F_{(1,7)} = 6.2$ (*)	15.2
Number of pinnae 222 days[i]	69	147	35.0 (2.13)	40.5 (1.61)	$F_{(1,7)} = 4.3$ (ns)	13.5
Fresh weight 222 days[i], g	69	147	17.0 (2.03)	23.4 (1.53)	$F_{(1,7)} = 6.3$ (*)	27.4
Dry weight 222 days[i], g	69	147	6.1 (0.72)	8.4 (0.54)	$F_{(1,7)} = 6.5$ (*)	27.4
Shoot, dry weight g	69	147	2.8 (0.41)	4.0 (0.31)	$F_{(1,7)} = 4.9$ (ns)	30.0
Root, dry weight g	69	147	3.3 (0.48)	4.5 (0.37)	$F_{(1,7)} = 3.6$ (ns)	26.7
Shoot:root ratio (dry weight)	69	147	1.0 (0.15)	0.9 (0.11)	$F_{(1,7)} = 0.1$ (ns)	-11.1

[a]Values are least squares (LS) means with the standard error (SE) in brackets of the LS estimate. Significance level: ***= $P<0.001$, **= $P<0.01$, *= $P<0.05$, and ns= $P>0.05$. [b]Inbreeding depression is calculated as: (Cross-self) /cross*100. [c]Missing data for two self-pollinated pods (no pulp). [d]Aborted seeds: weight<0.05 g and/or with a flat shape. [e]Missing seeds: empty cavity in the pulp. [f]$P = 0.054$. [g]Nitrogen-to-protein conversion factor = 6.25 (AOAC, 1990). [h]Fisher's exact test. [i]Days after sowing.

capitula (Tab. 3). These results document pollen limitation of fruit set in *P. biglobosa* during the study year, indicating that fruit set could be increased by increasing pollen load above the natural level of pollination (but see discussion below). Freely exposed capitula attracted up to 50 honey bees foraging simultaneously per capitulum. As honey bees have been found to be good pollinators of *P. biglobosa* (Lassen et al. 2017), pollen limitation may be due to insufficient deposition of compatible cross-pollen compared to deposition of self- and incompatible cross-pollen. The density of *P. biglobosa* was relatively high (1.2 trees/ha), and seven of the eight mother-trees were separated by less than 60 m to the nearest *P. biglobosa* tree. However, the mother-trees were large (mean crown area = 472 m², SD = 170) with many capitula, and this profuse blooming may have increased geitonogamy of the open-pollinated capitula. Higher fruit production of controlled cross-pollination compared to controlled self-pollination and open-pollination has also been reported for other tropical tree species e.g. the Coligallo palm, *Calyptrogyne ghiesbreghtiana* H. Wendl. (Cunningham 1996) (Arecaceae) and five species of neotropical *Inga* trees (Koptur 1984) (Fabaceae), where it has been linked to limitation of compatible pollen of self- and open-pollinations.

TABLE 5. Correlation between number of seeds per pod and amount of sugar (sucrose, glucose, and fructose) in pulp for 46 pods (22 self-pollinated and 24 cross-pollinated) of *Parkia biglobosa* illustrated by Pearson correlation coefficients and significance levels.

	Sucrose	Glucose	Fructose	Total sugars
No. of healthy seeds	0.19 (ns)	0.48 (***)	0.38 (**)	0.48 (***)
No. of aborted seeds	0.08 (ns)	-0.48 (***)	-0.42 (**)	-0.28 (ns)
No. of total seeds	0.15 (ns)	0.26 (ns)	0.19 (ns)	0.31 (*)

Significance level: ***=$P<0.001$, **= $P<0.01$, *= $P<0.05$, and ns= $P>0.05$.
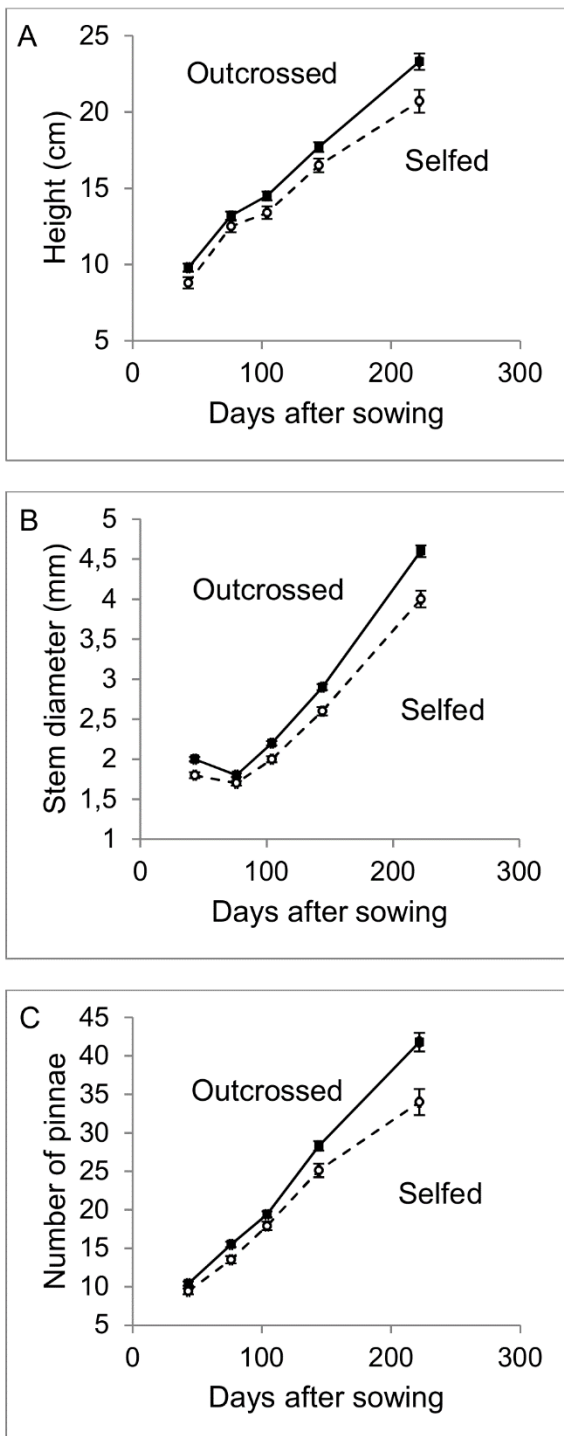
FIGURE 2. Growth of seedlings of *Parkia biglobosa* originating from 69 self-pollinated and 147 cross-pollinated seeds, showing the temporal development (days after sowing) of A) mean height, B) mean stem diameter, and C) mean number of pinnae. Error bars indicate $\pm$ 1 standard error of the mean. Only seedlings, which survived until harvest at 222 days after sowing are included.

Although half of the immature pods were aborted in controlled cross-pollinations, pollen dose was highly important for number of mature pods in *Parkia biglobosa*. The converting calculations between treatments with different pollen doses ('cross' and 'both') proposed a linear relationship between pollen dose and fruit set, suggesting that even more pods could have been initiated and matured, if the high dose of the cross-pollen in the 'both'-treatment (1:1) had been applied to the whole capitulum, and not only to half of it, or if the pollen dose had been even higher (e.g., 3:1). Most likely, the increased abortion of pods (i.e., immature pods minus mature pods) with higher pollen doses were due to lack of resources, suggesting that there is an upper limit for the number of produced pods per capitulum. However, we found no differences in pod weight or pod length between pods from open-, cross- or 'both'-treatments. The experiment had some limitations, and hence we cannot conclude whether the trees were truly pollen limited. First, the experiment lasted only one night at each tree; second, only a part of the blooming capitula per tree was included in the experiment; third, the experiment was only performed in one season. It is possible that the increased fruit set of hand-pollinated capitula came at the cost of open-pollinated capitula within the same tree (Obeso 2002). Nevertheless, some plant species show pollen limitation both on a whole plant level and in subsequent years (Ashman et al. 2004). More detailed hand-pollination experiments are needed to test whether a higher fruit production can be obtained in *P. biglobosa* by supplying cross-pollen night after night and year after year.

In the current study, a cross-pollinated capitulum was pollinated with only one cross-pollen donor, and hence the pod abortion was not due to selection between pollen donors, as has sometimes been suggested when explaining high rates of abortion (Bookman 1984). These patterns suggest a general lack of maternal resources to mature all or most of the initiated cross-pollinated pods.

Self-incompatibility in Parkia biglobosa

Self-incompatibility of *P. biglobosa*, as suggested by our study, is supported by other studies: Using controlled self- and

cross-pollination (N = 15 trees), Ouédraogo (1995) concluded that *P. biglobosa* is largely a self-incompatible species: self-pollination was possible, but outcrossing was more successful. Likewise, Sina (2006) found high values of multi-locus outcrossing (N = 238 trees), also consistent with partial self-incompatibility. *Parkia* is a pantropical genus of around 35 species, of which most are believed to be bat-pollinated and the rest insect-pollinated (Hopkins 1998). The breeding system of most *Parkia* species has not been investigated (Bumrungsri et al. 2008), but their high pollen:ovule ratios are in the range characteristic of outcrossing (Cruden 1977; Hopkins 1984). Piechowski (2007) has tested *Parkia pendula* in Brazil for selfing, and as no pods were produced in spontaneous or controlled self-pollinations, he concluded that this species was self-incompatible. Furthermore, a study of two Asian species of *Parkia*, *P. speciosa* and *P. timoriana*, involving spontaneous (i.e. bagged capitula) and controlled self-pollination treatments, suggested that both species were self-incompatible (Bumrungsri et al. 2008). Finally, our finding of two cross-incompatible tree pairs (P14 & P90, and P76 & P92) fit with the self-incompatibility being controlled by a few specific loci (de Nettancourt 1977; Seavey and Bawa 1986).

In spite of the high pollen dose in the 'both'-treatment, no self-pollinated flowers developed into pods in this treatment. Since only one polyad pollinates one flower in *P. biglobosa* (Lassen et al. 2014), competition and selection between pollen donors is likely to take place between flowers rather than within flowers (Bawa & Buckley 1989). Pod set after self-pollination of the entire capitulum was rare. The 24 selfed pods contained significantly more aborted seeds and fewer total seeds, perhaps due to late acting self-incompatibility and/or early acting inbreeding depression. The 'missing' seeds were thought to be seeds that aborted very early, leaving only the empty cavity in the pulp, but we found few cavities per pod, and no differences between types of pollination.

Inbreeding depression in Parkia biglobosa

Initial seedling growth has been shown to depend on seed size, possibly due to the size of the cotyledons (Blackman 1919; Howe & Richter 1982; Boot 1996). Considering the correlation between carbon content and seed weight, and the higher seed weight of cross-pollinated seeds, we hypothesise that the nutritional differences between selfed and outcrossed seeds were due to relatively larger cotyledons in the cross-pollinated seeds. Because the differences in growth between self- and cross-pollinated seedlings increased with time in this study, we expect that the self-pollinated seedlings suffered from inbreeding depression. Few cases of inbreeding depression in early germination stages have been documented in other plant species while inbreeding depression at later life stages ('seed production of parent' and 'growth and reproduction') has more often been reported (Husband & Schemske 1996; Hardner & Potts 1995). A study by Mašková and Herben (2018) showed that larger-seeded species consistently had lower root:shoot ratios, explained by an advantage of faster development of shoots in asymmetric above-ground competition. We found no difference between shoot:root ratios of selfed and outcrossed seedlings, perhaps

due to lack of competition in the greenhouse. In the present study, the test of germination and growth took place under presumably optimal growth conditions (available water, light, nutrition and no competition), but the survival of seedlings was significantly lower for self-pollinated seeds. Walters & Reich (2000) observed that for ten tree species survival of seedlings in low light and/or low levels of N increased with seed weight. Therefore, it is likely that under natural conditions, inbreeding depression may have been more evident.

We expect that increased selfing in natural populations of *P. biglobosa* will negatively affect propagation by seeds, resulting in decreasing densities of adult trees in the future.

Quality of seeds and pod pulp from self- and cross-pollinated capitula

Pollination has been shown to impact the quality of fruits in different species (IPBES 2016). Because seeds and pulp from *P. biglobosa* are important food resources consumed by people and animals, it is highly relevant to understand the impact of self- versus cross-pollination on fruit and seed quality.

The content of protein in raw *P. biglobosa* seeds without testa (dry weight) of 43%- 45% (for outcrossed and selfed seeds, respectively) is similar to 43% found by one study (Ekpenyong et al. 1977), but much higher than reported by other studies: 27% (Esenwah & Ikenebomeh 2008), 30% (syn. *Parkia filicoidea* Welw.) (Fetuga et al. 1974) and 34% (Ijarotimi & Keshinro 2012). We found that self-pollinated seeds were more protein-rich but weighed less than cross-pollinated seeds. Hence, the increase in protein content can probably be explained by a simple concentration effect as found for various species grown under stress (Wang & Frei 2011). Inverse relationships of protein content and starch content of grains and yield, respectively, have been documented in maize hybrids (*Zea mays* subsp. *mays* L.) (Poaceae) (Idikut et al. 2009). Likewise, other studies of maize have found significantly higher protein content in self-pollinated kernels and significantly higher starch content in cross-pollinated kernels (Letchworth & Lambert 1998; Sulewska et al. 2014). Total seed protein produced by self-pollinated pods was much lower than for crossed pods, as self-pollinated pods contained much fewer healthy seeds (Tab. 4).

The positive correlation between seed number per pod and sugar content in pulp observed in the current study was also found in a similar study of *P. biglobosa* in The Gambia (Lassen et al. 2012). However, the percentages of sugars (dry weight) were much higher in the fruit pulp from The Gambia compared with the pulp from Burkina Faso (total sugar: 60% versus 36-41%) (Lassen et al. 2012). In the literature, carbohydrate content (dry weight) in fruit pulp of *P. biglobosa* is reported to be from around 40% in Nigeria (Nadro & Umaru 2004) to around 85% in Mali (Nordeide et al. 1996). The positive correlation between number of healthy seeds and total amount of sugar in the pulp in the current study may be due to seeds acting as sinks during pod development, attracting nutrients to their own growth and to that of the surrounding pod (Stephenson 1981; Lee 1988; Marcelis & Hofman-Eijer, 1997). Similarly, Valantin-

Morison et al. (2006) found a less-pronounced sweetness of the flesh in fruits with few filled seeds compared to fruits with a normal number of filled seeds in cantaloupe melon (*Cucumis melo* L.). In self-compatible, sweet orange (*Citrus sinensis* var. Red Junar) (Rutaceae), Partap (2000) found a higher number of seeds and more juice with a higher sugar content after honey bee pollination compared to wind-pollination.

Overall, our results suggest that fruit production, nutritional value of *P. biglobosa* pulp and seeds, and the fitness of seedlings, will decrease with increased levels of self-pollinations and affect the rural human populations negatively. We propose more research into how to increase the regeneration (natural or planted) of *P. biglobosa*. Our results could also be expanded by testing combinations of some of the acknowledged plus-trees (i.e. the superior trees) of *P. biglobosa* and by grafting the best combinations together, making it easier for the pollinators to bring about more cross-pollination with highly compatible pollen.

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