South African Journal of Botany 109 (2017) 263–268

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Short communication

Baobabs (*Adansonia digitata* L.) are self-incompatible and 'male' trees can produce fruit if hand-pollinated

S.M. Venter [a,*], K.L. Glennon [a], E.T.F. Witkowski [a], D. Baum [b], G.V. Cron [a], R. Tivakudze [a], N. Karimi [b]

[a] School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa
[b] Department of Botany, 430 Lincoln Drive, University of Wisconsin Madison, WI 53706-1381, USA

ARTICLE INFO

Article history:
Received 21 November 2015
Received in revised form 25 October 2016
Accepted 11 January 2017
Available online 2 February 2017

Edited by OM Grace

Keywords:
Fruit set
Inbreeding
Microsatellites
Pollination
Self-incompatibility

ABSTRACT

The African baobab's (*Adansonia digitata*) breeding system has not been experimentally verified, and it remains unclear why such a stark disparity exists between 'male' trees that produce few, if any, fruit, and 'female' trees that produce many fruit consistently year after year. Combined results from gene flow analyses of 26 trees scored for nine microsatellite loci and hand-pollination experiments on five trees across three baobab populations in Mutale District, Limpopo Province, South Africa, investigated the breeding system, strength of incompatibility within trees, and if genetic differentiation was detectable between 'males' and 'females.' Our data suggest that *A. digitata* is largely self-incompatible. 'Male' and 'female' trees showed high heterozygosity and estimated outcrossing rates did not differ in degree of self-incompatibility, and showed no significant genetic differentiation. The ability of 'males' to produce fruit if cross-pollinated suggests that poor fruit production in male trees is most likely due to low rates of pollination.

1. Introduction

The African baobab (*Adansonia digitata* L.) is an iconic tree in much of continental Africa. Baobab trees are important to the livelihoods of African people (Sidibe and Williams, 2002; Venter and Witkowski, 2011), and are important components of many savannah and deciduous forest ecosystems. Baobab reproductive biology has been of interest for decades due to the trees' fruit production disparity. Baobabs have hermaphrodite flowers (Baum, 1995), yet across Africa trees are referred to as 'male' or 'female', which respectively produce, year after year, few versus many fruit (Assogbadjo et al., 2008; Venter and Witkowski, 2011, 2013). This pattern is also evident in northern South Africa, where poorly fruiting trees were named 'poor producers' and those producing many fruits 'producers' (Venter and Witkowski, 2011). Despite a number of studies on variation in fruit production across many tree species (Wheelwright, 1986; Wilson and Witkowski, 2003; Snook et al., 2005; Kainer et al., 2007; Helm et al., 2011), and causes of low fruit production such as pollen limitation, resource limitation, predation and genetic load, in hermaphroditic plants (Ayre and Whelan, 1989; Charlesworth, 1989) the causes of these large differences observed among baobab trees remain unresolved.

Previous work investigating differences between 'male' and 'female' baobabs in South Africa has shown few, if any, differences in floral morphology, number of flowers per tree, or length or timing of peak flowering (Venter and Witkowski, 2011). Likewise, environmental factors such as soil or climate at these same populations do not explain the difference (Venter, 2012). To explain fruiting differences, Venter (2012) suggested that poor fruit-set could be due to inadequate pollination or cryptic sexual dimorphism, and encouraged further pollination and breeding system studies. Likewise, after observing a lack of genetic differentiation between such categories of trees using AFLP data, Assogbadjo et al. (2009) suggested that 'male' trees might be self-incompatible individuals.

It is currently unknown if the mainland African baobab is self-compatible. Hand-pollination experiments that tracked fruit set in *Adansonia* have only been conducted on *A. gregorii* (previously known as *A. gibbosa*) and found the species is self-incompatible (Baum, 1995). Based on this result, it has been assumed that other *Adansonia* species are also likely self-incompatible.

This study had three main objectives, which were carried out across three populations in Mutale District, Limpopo Province, South Africa. First, a hand-pollination experiment was used to test if *A. digitata* is self-incompatible. Second, we tested if hand-pollinated 'male' tree flowers produce fruit as effectively as 'female' trees. Third, using genetic data, we tested for assortative mating between male and female trees, fine-scale spatial autocorrelation among the three sampled populations, and whether inbreeding was occurring within 'male'

* Corresponding author.
E-mail address: windwaai@mweb.co.za (S.M. Venter).

http://dx.doi.org/10.1016/j.sajb.2017.01.007
0254-6299/

trees relative to 'females'. Collectively, these objectives could help to identify causes behind differences in 'male' and 'female' tree fruit set in South Africa.

2. Materials and methods

2.1. Study area

The hand-pollination experiment was conducted in Mutale District, Limpopo Province, South Africa (S22° 31 and E30° 38), in an area commonly known as 'northern Venda' near Muswodi and Tshipise villages (Fig. 1). It is a semi-arid, summer rainfall area with an average annual precipitation of 334–423 mm. The vegetation type is Musina Mopane Bushveld in which baobabs are common (Mucina and Rutherford, 2006).

2.2. Study species

Adansonia digitata is deciduous, bearing leaves in the wet season (October–March) and follows a steady-state flowering pattern—producing 10–50 flowers per night from October to April (Venter, 2012). Peak flowering occurs in November of each year. Flowers are large (ca. 150 mm in diameter), white and borne singly on a pendulous stalk (Fig. 2A, D). Each flower opens around dusk; releases pollen and stigmatic lobes are receptive for just one night (Baum, 1995). Bats are considered to be the main pollinators (Baker, 1961; Baum, 1995), but visits by hymenoptera (bees, bumble bees, and wasps) and hawk moths (*Sphingomorpha chlorea*, *Nephele comma*) have been reported (Baum, 1995, Venter, 2012; Fig. 2B, D). *Adansonia digitata* is a known autotetraploid based on flow cytometry and chromosome squashes (Baum and Oginuma, 1994; Cron et al., 2016).

2.3. Sampling and measurement

Five maternal trees were chosen to conduct the hand-pollination experiment. Two of the maternal trees were known 'female' trees with many fruit beneath them during the experiment. A further three known 'male' maternal trees were chosen and identified as such by the local villagers. Two of these 'male' trees (Trees C and D; Table 1)

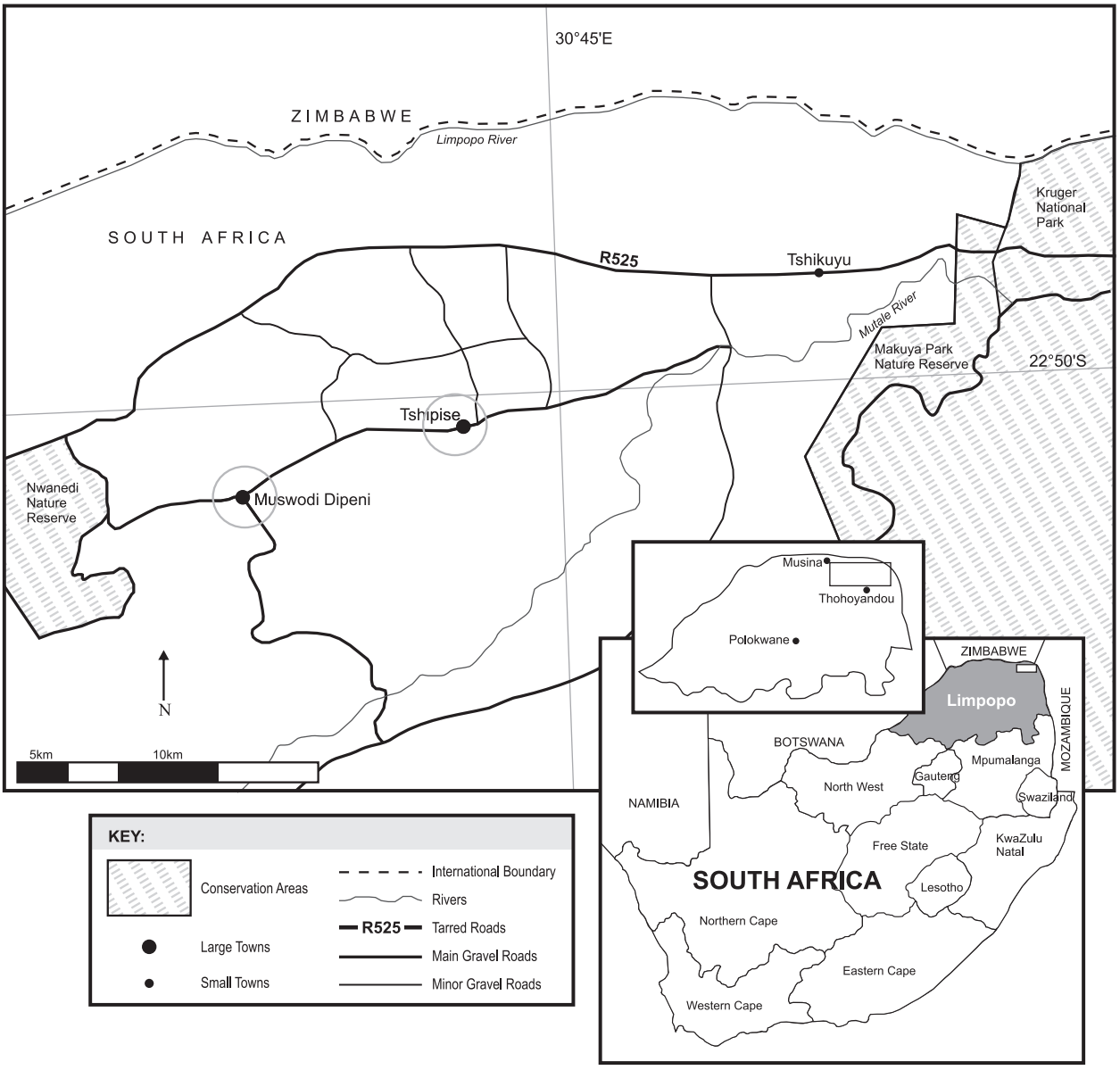

Fig. 1. Map of *Adansonia digitata* study sites, indicated with grey circles, across the Venda region, South Africa.

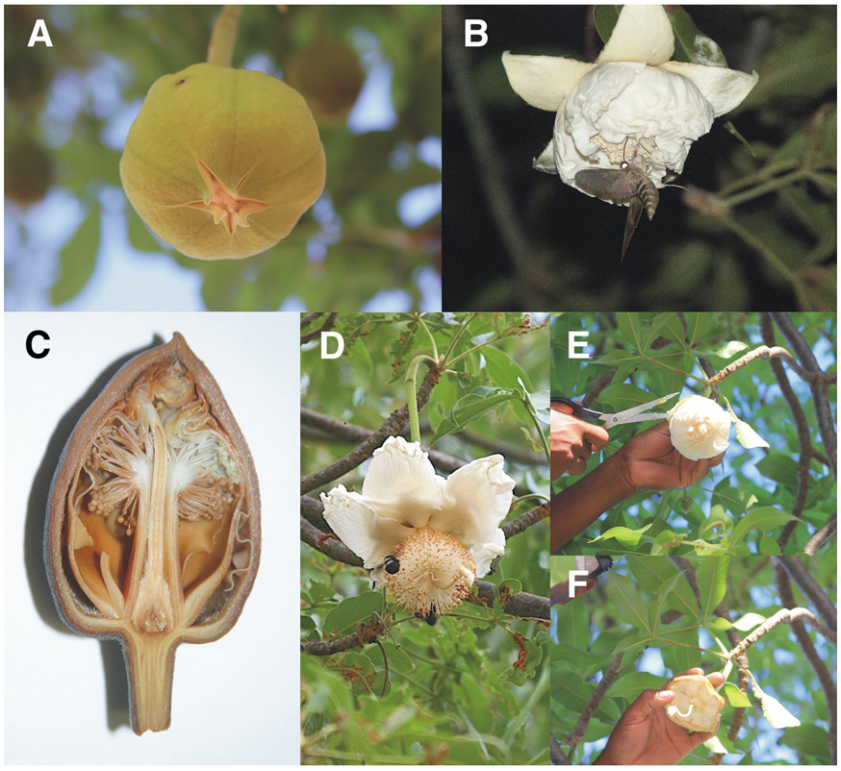

Fig. 2. Flowers of *Adansonia digitata*. A) Flower fully enclosed by calyx in bud; B) Hawk moth (*Nephele comma*) visiting baobab flower at night; C) Gynoecium (LS) – 5–10-lobed stigma on single terminal style leading to syncarpous ovary; D) Bees are frequent visitors to mature flowers in northern Venda; E) and F) Sepals, petals and stamens removed from 'treatment' flowers.

had been confirmed as 'males' by an 8-year fruit production dataset (Venter and Witkowski, 2013). Treatments took place at the end of November 2014 and fruit-set results were collected, one month later, at the end of December 2014. One year later, an additional 14 flowers were cross-pollinated using the same methods, on Tree D. This was done to increase the cross-pollination sample size for this individual.

For all treatments, mature flowers were emasculated a few hours before bud opening. These were identified by their size and by the formation of small cracks in the calyx. Using a pair of sharp scissors, the calyx, corolla (petals) and androecium (staminal tube, filaments and anthers) were removed, leaving only the gynoecium (stigma, style and ovary) (Fig. 2E, F). The emasculated flowers were enclosed in a large (30 × 20 × 12 cm) paper bag.

Flowers on each tree were assigned randomly to one of three treatments: cross-pollination, self-pollination and no-pollination. Between 5 and 27 flowers per tree were treated. After sunset, the bags were removed from the emasculated flowers destined for cross- or self-pollination. Pollen was obtained from either another flower on the maternal tree (self-pollination) or a freshly collected flower from a nearby 'female' tree (cross-pollination). By using flowers from 'female' trees, we hoped to verify male function of the female trees. Unpollinated flowers did not receive any pollen.

Table 1
Proportion of flowers forming fruits for each treatment across the five trees with number of flowers used per tree in parentheses.

Tree	Tree category	Unpollinated	Self	Cross
A	Female	0% (9)	0% (9)	78% (9)
B	Female	14% (7)	14% (7)	100% (7)
C	Male	0% (4)	0% (5)	100% (5)
D	Male	0% (1)	0% (2)	69% (16)
E	Male	0% (5)	0% (5)	100% (5)
	Total	4% (26)	4% (28)	83% (42)

Pollination was achieved by dabbing the anthers of the donor flower onto the stigma of the recipient flower. After hand pollination, bags were retied around the treated flower. A tag was attached to the branchlet above each treated flower to identify the flower and treatment given. The bags from un-pollinated flowers were not removed, thus should not have received any pollen. Bags were removed 48 h later, at which point the stigma and style were brown and dry. One month later, at the end of December, the presence of developing fruit was noted for each treated flower and taken as indicating successful fruit-set. The absence of fruit was taken to indicate fruit abortion.

2.4. Microsatellite data collection

Young leaf tissue was collected from 26 individuals from three populations in Mutale District, Limpopo province. The three populations are Muswodi Village (most westerly locality), near Tshipise village (most northern), and near Tshikuyu village (most eastern; Fig. 1). Individuals sampled were the same trees where Venter and Witkowski (2011) indicated fruit set, but two of the trees used for our pollination experiments were not sampled for genetic analyses. 'Male' (n = 12) and 'female' (n = 14) trees were found intermingled in all three populations. One young leaf, composed of five leaflets, was collected from each tree and placed immediately in a resealable plastic bag with silica gel.

DNA was extracted using a Qiagen DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions, except that the volume of the buffers, AP1 and P3, was doubled. We used nine published primers for *Adansonia digitata* (Larsen et al., 2009) to amplify microsatellites. PCR reactions consisted of a 10 μl final volume and included the following reagents: 2 μl of nuclease free water, 0.5 μl of 1 mg/ml Bovine Serum Albumin, 0.5 μl each of 10 μM forward and reverse primers, and 5 μl Phusion Master Mix (ThermoScientific; Inqaba Biotech, Pretoria, South Africa). PCR conditions were as follows: an initial denaturation step at 98 °C for 10 s,

followed by 30 cycles at 98 °C for 10 s, 58 °C for 5 s, 72 °C for 15 s, and a final extension at 72 °C for 1 min. The PCR products were visualized on 1% agarose gels stained with SYBRSafe gel stain (BioRad). After verification of the presence of a band within the correct size range, successful PCR products from different individuals of up to three loci that were labelled with different dyes (e.g., FAM, VIC, and NED) were pooled and sent to the Central Analytical Facility (CAF) at Stellenbosch University for fragment analysis on an ABI 3130. Chromatographs were examined and microsatellite alleles scored manually and sized using PeakScanner v1 (Applied Biosystems, www.appliedbiosystems.com).

2.5. Microsatellite data estimates of genetic diversity and selfing rates

We evaluated whether the loci were in Hardy–Weinberg equilibrium (HWE; the populations were pooled given the low genetic differentiation). We then calculated observed and expected heterozygosity and compared inbreeding coefficients between 'males' vs. 'females' using GenoDive (Meirmans and Van Tienderen, 2004). GenoDive assumes full tetrasomic inheritance to estimate HWE and can calculate heterozygosity for polyploids, using a maximum likelihood method to correct allele dosage (Meirmans and Van Tienderen, 2004), which is necessary for autotetraploid *A. digitata* (Baum and Oginuma, 1994). We converted the codominant data to a dominant data set to calculate estimated heterozygosity within populations and total heterozygosity.

Codominant data was used to calculate G_{IS}, which compares the observed heterozygosity within subpopulations (here, male vs female) to the expected heterozygosity. We used G_{IS} to estimate the selfing-rate using the following formula: $S = (2 \times G_{IS}) / (1 + G_{IS})$ (Ritland, 1984) as it accounts for the potential effects of inbreeding depression. It should be noted that selfing rates could be over-estimated if biparental inbreeding occurs, or could be under-estimated if selection acts against homozygous genotypes (Tedder et al., 2015).

Lastly, we used genetic data to test for patterns of assortative mating, and whether genetic data indicated spatial autocorrelation. An analysis of molecular variance was conducted between male and female trees to test for assortative mating, using 1000 permutations to test for significance. Variance estimates are reported as ρ_{ST} due to the polyploid nature of the dataset. To test for patterns of fine-scale genetic variance among trees, two analyses were conducted. First, AMOVA was used to test for genetic variance among populations. Second, a Mantel test was conducted using genetic and geographic distance matrices, and then again using a geographic distance matrix divided into distance classes. Individual tree GPS coordinates were used to calculate geographic distances among trees. Genetic distances between individuals were calculated using Bruvo's distance (Bruvo et al., 2004), which accommodates polyploidy. Due to the proximity of our three populations and the trees within populations, geographic distances were divided into four classes to test if individual distance classes were associated with pairwise genetic distances. All three analyses were conducted in GenoDive.

3. Results

3.1. Pollination experiment

Summing across all trees, cross-pollinated flowers resulted in much greater fruit set (83%) than self-pollinated (4%) or unpollinated (4%) flowers. Applying a Chi-square (with Yates correction) test, cross-pollinated flowers gave a significantly higher probability of yielding fruit than self-pollinated flowers ($X^2 = 39.7$, df = 1, $P < 0.0001$) or unpollinated flowers ($X^2 = 37.6$, df = 1, $P < 0.0001$). Despite the fact that the only selfed flower that set fruit was on a female tree, this single observation is not sufficient to conclude that 'male' and 'female' trees differ in the proportion of aborted fruit following self-pollination (Fisher's exact test, $P = 0.62$).

3.2. Genetic diversity estimates, inbreeding, and assortative mating

Microsatellite allele sizes across loci ranged from 94 to 301 base pairs (bp), which correspond with previous estimates for *A. digitata* (Larsen et al., 2009). Allele number ranged from 8 to 18 alleles per locus and 1–4 distinct alleles per individual (usually 2 or 3). Eight of the nine loci were found to violate Hardy–Weinberg equilibrium among the three populations. This is potentially due to the small sample size (n = 26 trees, across three populations that are likely not completely isolated). The average observed heterozygosity (H_O) across all trees was high ($H_O = 0.909$; Table 2). Both the 'female' and 'male' groups showed high levels of observed heterozygosity, ($H_O = 0.931$, $H_O = 0.888$, respectively; Table 2) and high gene diversity ($H_S = 0.839$, 0.801, respectively). Dominant data analyses reported lower estimates of heterozygosity within the groups (female $H_S = 0.354$, male $H_S = 0.305$).

Male and female groups showed very similar estimates of inbreeding (females $G_{IS} = -0.076$; males $G_{IS} = -0.058$; Table 2). These data correspond to a marginally lower estimate of the effective selfing rate in 'males' ($S = -0.123$) than in 'females' ($S = -0.164$; Table 2). Given that inbreeding can lead to aborted fruits through purging of deleterious recessive alleles (Husband and Schemske, 1996), or lower fruit production, these data suggest that inbreeding does not explain the lower fruit production of 'male' trees. In addition, AMOVA results indicate little genetic variance between male and female trees ($\rho_{ST} = 0.007$, $P = 0.303$), suggesting that assortative mating is not occurring between males and females.

The overall Mantel test suggests a very slight positive correlation between genetic and geographic distances across all trees ($r = 0.042$, $P = 0.276$; Table 3). The two intermediate distance classes (2 and 3) showed significant correlations (Table 3); class 2 showed a positive correlation, while class 3 showed a negative correlation. Class 1 showed a positive and class 4 a negative correlation, both non-significant. AMOVA results showed some genetic variance among the three populations in different geographic areas ($\rho_{ST} = 0.048$, $P = 0.005$).

4. Discussion

Chi-square tests of hand-pollination results show that *A. digitata* is self-incompatible. However, one out of sixteen self- and one out of sixteen un-pollinated flowers, both on 'female' trees produced fruit. These instances likely reflect pollen contamination rather than limited self-compatibility or apomixis, respectively. Flowers on 'male' trees successfully set fruit when cross-pollinated, which indicates that the widespread observation of poor fruit production in 'male' trees is not due to poor female fertility. Similarly, given that all cross-pollinations used pollen from 'female' trees, and these usually yielded fruit, shows that these trees have full male function. Therefore, 'male' vs. 'female' distinction is biologically unjustified and we recommend that it be replaced with 'producer' and 'poor producer'. (However, for continuity we will continue to use this designation for the remainder of this paper.)

Our genetic diversity estimates showed high heterozygosity and gene diversity (Table 2). These estimates are not unexpected given our finding of self-incompatibility, and suggest a predominantly outcrossing mating system. The use of Nei's gene diversity estimate, H_S, allowed us to compare our estimates to other baobab studies. Interestingly, our diversity estimate ($H_S = 0.820$) is highly relative to studies elsewhere in Africa that estimated gene diversity in a dominant framework ($H_S = 0.12$ to 0.18; Munthali et al., 2013; $H_S = 0.22$ to 0.37, Assogbadjo et al., 2009; Kyndt et al., 2009). The codominant nature of microsatellite markers can overestimate within population genetic diversity (Nybom, 2004). A reanalysis of our data in a dominant framework shows that our gene diversity indices are comparable to the other studies ($H_S = 0.330$, $H_t = 0.329$, data from combined study site populations).

Tests for patterns of genetic variance due to geographic distance indicate that there is some genetic similarity between trees that are

Table 2
Summary of genetic diversity indices* between 'female' (n = 14) and 'male' (n = 12) baobab trees, and across all *Adansonia digitata* trees sampled in the Venda region of South Africa.

Grouping	N	N_e	H_o	*Hs*	*Ht*	*H't*	G_{ST}	G_{IS}	*S*
Female	9.22	5.66	0.931	0.839	0.839	n/a	n/a	−0.076	−0.164
Male	10	6.328	0.888	0.801	0.801	n/a	n/a	−0.052	−0.123
All trees	12.56	6.74	0.909	0.820	0.825	0.829	0.003	−0.067	n/a

Note: *N shows the number of alleles, N_e is the effective number of alleles, H_o is the observed heterozygosity/gametic heterozygosity for polyploids (e.g., Moody et al., 1993), *Hs* is the expected heterozygosity within subpopulations (also 'gene diversity'), *Ht* is the expected heterozygosity over all populations, and *H't* is the total expected heterozygosity with a correction for bias that is due to sampling a limited number of populations, G_{ST} is the measure of the amount of fixation in subpopulations (males vs females) relative to the whole group of baobab tress, and is analogous to F_{ST}, G_{IS} is an inbreeding coefficient that relates the observed heterozygosity within the male and female subpopulations to the expected heterozygosity and is analogous to F_{IS}, lastly *S* is the estimated effective selfing rate calculated using G_{IS} (see text for details).

closer together, but the relationship is not significant across all populations, with varied relationships across distance classes. The AMOVA results somewhat coincided with intermediate distance class findings in that some genetic variance was detected between the three populations studied, albeit low (~5). Together, these results suggest that genetic clustering may occur within a couple of distance classes, but there is no strong evidence for spatial autocorrelation in the genetic data. This lack of a strong trend might be due to the low number of individual trees sampled within certain geographic areas.

Our results show that 'male' trees are fully capable of setting fruit when crossed, have similar estimated selfing rate to 'female' trees, and show no significant genetic differentiation from 'females.' Therefore, low fruit production likely results from inadequate cross-pollination due to pollinator visitation patterns. In order to be effective pollinators, floral visitors would need to carry pollen from tree-to-tree, which may not apply to bees except in areas of high baobab density. In this study area, reproductively mature baobab trees occur with mean densities of less than 2 trees/ha (Venter and Witkowski, 2010), which may be too sparse for bee or moth pollination to be effective. Alternatively, pollinators (e.g., bats) might be avoiding flowers on certain trees due to unpalatable or low-reward nectar (e.g., Gould, 1978; Johnson et al., 2006). Future work determining nearest-neighbour tree distances, bee and hawk moth foraging ranges and comparing 'male' and 'female' nectar reward and scent are needed to see if these factors could drive differences in visitation rate.

Collectively, our work confirms that baobabs in South Africa are self-incompatible and that further investigations into baobab pollination biology may help to identify the factors promoting bi-modal fruit production patterns among trees.

Acknowledgements

We thank the National Research Foundation (NRF) of South Africa's Centre of Excellence in Tree Health Biotechnology (CTHB), and the NRF's Integrated Biodiversity Information Programme (Grant 86959) for funding. KLG was supported by grant B8749.R01, Carnegie Corporation of New York to the Global Change and Sustainability Research Institute at the University of the Witwatersrand. D. Baum acknowledges support from the National Science Foundation (DEB-1354793). Thanks to Samuel Phaswana and Prince Musekwa for ongoing assistance with fieldwork and Colin Schoeman for hawk moth identification.

Table 3
Results from Mantel tests where geographic distances among sampled baobab trees in Venda were divided into four distance classes as well as a Mantel test across all trees. Bold values indicate significant relationships.

Class	Mantel's *r*	P-value
1 (≤12.4 km)	0.041	0.217
2 (≤24.73 km)	−0.148	**0.045**
3 (≤37.1 km)	0.180	**0.019**
4 (≤49.46 km)	−0.098	0.137
Overall	*0.042*	*0.276*

Appendix 1. Genetic diversity indices* of *Adansonia digitata* individuals for each microsatellite locus where maximum likelihood methods were used to correct for unknown dosages of the alleles.

Locus	N	N_e	*Hs*	*Ht*	*H't*	G_{IS}
Ad01	11	6.152	0.862	0.863	0.864	**−0.094**
Ad02	10	4.203	0.795	0.834	0.873	0.307
Ad04	14	7.356	0.894	0.89	0.886	**−0.002**
Ad08	8	6.16	0.859	0.863	0.867	**−0.148**
Ad09	7	4.281	0.788	0.784	0.78	**−0.243**
Ad12	11	5.33	0.843	0.843	0.842	**−0.056**
Ad14	7	3.153	0.705	0.71	0.715	**−0.250**
Ad17	11	5.633	0.849	0.849	0.848	**−0.042**
Ad18	7	4.133	0.785	0.784	0.784	−0.113
Overall	9.556	5.156	0.82	0.825	0.829	−0.067

Note: *N shows the number of alleles, N_e is the effective number of alleles, H_o is the observed heterozygosity/gametic heterozygosity for polyploids (e.g., Moody et al., 1993), *Hs* is the expected heterozygosity within subpopulations (also 'gene diversity'), *Ht* is the expected heterozygosity over all populations, and *H't* is the total expected heterozygosity with a correction for a bias that is due to sampling a limited number of populations, G_{IS} is an inbreeding coefficient that relates the observed heterozygosity within the male and female subpopulations to the expected heterozygosity and is analogous to F_{IS} (see text for details). Bolded values indicate that the locus rejects HWE ($P < 0.001$).

References

Assogbadjo, A.E., Kakai, R.G., Chadare, F.J., Thomson, L., Kyndt, T., Sinsin, B., Van Damme, P., 2008. Folk classification, perception and preference of baobab products in West Africa: consequences for species conservation and improvement. Economic Botany 62, 74–84.

Assogbadjo, A.E., Kyndt, T., Chadare, F.J., Sinsin, B., Gheysen, G., Eyog-Matig, O., Van Damme, P., 2009. Genetic fingerprinting using AFLP cannot distinguish traditionally classified baobab morphotypes. Agroforestry Systems 75, 157–165.

Ayre, D.J., Whelan, R.J., 1989. Factors controlling fruit set in hermaphroditic plants: studies with the Australian Proteaceae. Tree 4, 287-272.

Baker, H.G., 1961. The adaption of flowering plants to nocturnal and crepuscular pollinators. The Quarterly Review of Biology 36, 64–73.

Baum, D.A., 1995. The comparative pollination and floral biology of baobabs (*Adansonia* – Bombacaceae). Annals of the Missouri Botanical Garden 82, 322–348.

Baum, D.A., Oginuma, K., 1994. A review of chromosome numbers in Bombacaceae with new counts for *Adansonia*. Taxon 43, 11–20.

Bruvo, R., Michiels, N.K., D'Souza, T.G., Schulenberg, H., 2004. A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. Molecular Ecology 13, 2102–2106.

Charlesworth, D., 1989. Evolution of low female fertility in plants: pollination limitation, resource allocation and genetic load. Tree 4, 289–293.

Cron, G.V., Karimi, N., Glennon, K.L., Udeh, C.A., Witkowski, E.T.F., Venter, S.M., Assogbadjo, A.E., Baum, D.A., 2016. One African baobab species or two? Synonymy of *Adansonia kilima* and *A. digitata*. Taxon 65 (5), 1037–1049.

Gould, E., 1978. Foraging behavior of Malaysian nectar-feeding bats. Biotropica 10, 184–193.

Helm, C.V., Scott, S.L., Witkowski, E.T.F., 2011. Reproductive potential and seed fate of *Sclerocarya birrea subsp. caffra* (marula) in the low altitude savannas of South Africa. South African Journal of Botany 77, 650–664.

Husband, B.C., Schemske, D., 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50, 54–70.

Johnson, S.D., Hargreaves, A.L., Brown, M., 2006. Dark, bitter-tasting nectar functions as a filter of flower visitors in a bird-pollinated plant. Ecology 87, 2709–2716.

Kainer, K.A., Wadt, L.H., Staudhammer, C.L., 2007. Explaining variation in Brazil nut fruit production. Forest Ecology and Management 250, 244–255.

Kyndt, T., Assogbadjo, A.E., Hardy, O.J., Kakaï, R.G., Sinsin, B., Van Damme, P., Gheysen, G., 2009. Spatial genetic structuring of baobab (Adansonia digitata, Malvaceae) in the traditional agroforestry systems of west Africa. Am. J. Bot. 95, 950–957.

Larsen, A.S., Vaillant, A., Verhaegen, D., Kjær, E.D., 2009. Eighteen SSR-primers for tetraploid *Adansonia digitata* and its relatives. Conservation Genetics Resources 1, 325–328.
Meirmans, P.G., Van Tienderen, P.H., 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Molecular Ecology Notes 4, 792–794.
Moody, M.E., Mueller, L.D., Soltis, D.E., 1993. Genetic variation and random drift in autotetraploid populations. Genetics 134, 649–657.
Mucina, L., Rutherford, M.C. (Eds.), 2006. The Vegetation of South Africa, Lesotho and Swaziland. South African Botanical Institute, Pretoria, pp. 439–539 (Chapter 9).
Munthali, C.R.Y., Chirwa, P.W., Changadeya, W.J., Akinnifesi, F.K., 2013. Agroforestry Systems 87, 117–130.
Nybom, H., 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Molecular Ecology 13, 1143–1155.
Ritland, K., 1984. The effective proportion of self-fertilization with consanguineous matings in inbred populations. Genetics 106, 139–152.
Sidibe, M., Williams, J.T., 2002. Baobab *Adansonia digitata*. International Centre for Underutilised Crops, South Hampampton, United Kingdom.
Snook, L.K., Camara-Cabrales, L., Kelty, M.J., 2005. Six years of fruit production by mahogany trees (*Swietenia macrophylla* King): patterns of variation and implications for sustainability. Forest Ecology and Management 221–235.
Tedder, A., Helling, M., Pannell, J.R., Shimizu-Inatsugi, R., Kawagoe, T., Van Campen, J., Sese, J., Shimizu, K.K., 2015. Female sterility associated with increased clonal propagation suggests a unique combination of androdioecy and asexual reproduction in populations of *Cardamine amara* (Brassicaceae). Annals of Botany 115, 763–776.
Venter, S.M., 2012. Are There Male and Female Baobabs (*Adansonia digitata* L.)? A Study of Phenology, Flowering and Fruit-Set Patterns in Southern Africa. The Ecology of Baobabs (*Adansonia digitata* L.) in Relation to Sustainable Utilization in Northern Venda, South Africa. (PHD Thesis). University of the Witwatersrand, Johannesburg.
Venter, S.M., Witkowski, E.T.F., 2010. Baobab (*Adansonia digitata* L.) density, size-class distribution and population trends between four land-use types in northern Venda, South Africa. Forest Ecology and Management 259, 294–300.
Venter, S.M., Witkowski, E.T.F., 2011. Baobab (*Adansonia digitata* L.) fruit production in communal and conservation land-use types in southern Africa. Forest Ecology and Management 261, 630–639.
Venter, S.M., Witkowski, E.T.F., 2013. Fruits of our labour: contribution of commercial baobab (*Adansonia digitata* L.) fruit harvesting to the livelihoods of marginalized people in northern Venda, South Africa. Agroforestry Systems 87, 159–172.
Wheelwright, N.T., 1986. A seven-year study of individual variation in fruit production in tropical bird-dispersed tree species in the family Lauraceae. In: Estrada, A., Fleming, T. (Eds.), Frugivores and Seed Dispersal. Springer, Netherlands, pp. 19–35.
Wilson, B.G., Witkowski, E.T.F., 2003. Seed banks, bark thickness, and changes in age and size structure (1978–1999) of the African savanna tree, *Burkea africana*. Plant Ecology 167, 151–162.