

Aloe inconspicua: The first record of an exclusively insect-pollinated aloe

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Received 2 November 2007; received in revised form 26 January 2008; accepted 25 February 2008

Abstract

Most *Aloe* species are wholly or partly bird-pollinated, but a suite of seven *Aloe* species and two genera (*Haworthia* and *Chortilirion*) that likely belong within the *Aloe* clade (Asphodelaceae, subfamily Alooidea) share morphological characteristics suggestive of insect pollination. Field studies of one of these species, *Aloe inconspicua*, revealed that it is effectively and exclusively pollinated by insects, especially females of *Amegilla fallax* (Apidae) that visit its flowers for nectar and pollen. The small ($7.9 \text{ mm} \pm \text{SD} = 2.0$) white flowers produce a standing nectar crop of $0.097 \pm 0.10 \mu\text{l}$, much less than that of bird-pollinated aloes. Unlike other aloes studied to date, birds did not visit *A. inconspicua*, and bird exclusion had no effect on fruit or seed production. Visiting individuals of *A. fallax* typically contacted stigmas and anthers with their heads while accessing nectar, and single visits by them and a halictid bee resulted in seed set. Recent molecular evidence suggests that insect-pollination is the ancestral state for the Alooidea. If similar floral morphology indicates similar pollination systems, shifts from insect- to bird-pollination and possibly reversions back to insect pollination have occurred repeatedly within the *ALOE* clade.

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Keywords: *Aloe*; Alooideae; *Amegilla fallax*; Entomophily; Pollinator shifts

1. Introduction

The subfamily Alooideae (Asphodelaceae) is a large, primarily African clade of succulent plants. Recent molecular phylogenetic analysis (Treutlein et al., 2003a,b) suggests that within this clade the traditionally recognised genus *Aloe* (Dagne et al., 2000) is polyphyletic and should include species currently classified as *Chortilirion* (1 species), *Haworthia* (ca. 70 species; Bayer, 1999) and *Gasteria* (ca. 20 species; Van Jaarsveld, 1994). Both the species traditionally included in *Aloe* and the newly expanded generic concept show remarkable morphological diversity, from tree-like species that reach more than 15 m, to diminutive plants vegetatively similar to the grasses they grow amongst. Floral form also varies greatly, from the reddish, tubular flowers typical of *Aloe* species, through the yellow, cup-shaped flowers of *Aloe* section *Anguialoe*, to white, nocturnally-open flowers of *Aloe suzanneae*.

Aloes are characteristic plants in many South African floras, but their pollination systems have been investigated experimentally only recently, with accounts published for few South African species (*A. ferox*: Hoffman, 1988; Stokes and Yeaton, 1995; *A. vryheidensis*: Johnson et al., 2006). The most visible and therefore well-known *Aloe* species, such as *A. ferox* and *Aloe marlothii*, grow in dense populations and produce large inflorescences of brightly coloured flowers, which attract diverse communities of nectar-foraging birds (e.g., Hoffman, 1988; Stokes and Yeaton, 1995; Nepi et al., 2006; Symes et al., 2008). Recent studies have revealed divergent pollination systems within bird-pollinated aloes, which exhibit floral adaptations for pollination by either specialised (long-billed), or generalist (short-billed) nectarivores (Johnson et al., 2006; Johnson, 2007; Johnson and Nicolson, 2008). However, the abundance and conspicuousness of bird-pollinated species, and the evidently mistaken separation of small, white-flowered clades into separate genera (Treutlein et al., 2003a, 2003b) have led to an under-appreciation of the true diversity of aloe flowers and their pollinators.

In addition to birds, most, if not all, aloe species are visited by a variety of insects, most commonly nectar and/or pollen-

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collecting bees (e.g., Ratsirarson, 1995; Paillet et al., 2002; Craib, 2005; Hargreaves, 2007). The role of bees differs extensively among aloes. Although bees contribute significantly to seed set in some “ornithophilous” species (Johnson et al., 2006; Hargreaves, 2007), they steal pollen from other aloes without effecting pollination (Stokes and Yeaton, 1995; Hargreaves, 2007). To date most experimental studies have found bees to be poor pollinators of aloes (Ratsirarson, 1995; Stokes and Yeaton, 1995; Johnson et al., 2006), but they have considered only ‘typical’ aloes with large inflorescences of brightly coloured flowers.

A suite of species in the *ALOE* clade share morphological characteristics suggestive of insect pollination. These include seven South African *Aloe* species (Table 1), *Chortolirion angolense* (the only species of that genus), and the polyphyletic genus *Haworthia*. Six of the putatively insect-pollinated *Aloe* species are grass aloes (the taxonomic affiliations of the unusual *A. bowiea* remain unclear: Smith, 1990; Craib 2005), an apparently closely related group (Viljoen et al., 1998) to which *Chortolirion angolense* bears the closest phylogenetic affiliation (Treutlein et al., 2003b). These species are small (<50 cm tall), inconspicuous, and produce single inflorescences of small, pale flowers, ≤ 2 cm in length (Table 1). To test the insect-pollination hypothesis we studied the pollination of one of these species, *A. inconspicua* Plowes. This paper describes our observations of floral characteristics, pollinator visitation, and pollination and seed production in plants from which birds were excluded experimentally. We demonstrate that *A. inconspicua* is pollinated exclusively by insects, suggesting a broader diversity of pollination systems among aloes than previously appreciated.

2. Methods

2.1. Study species and location

The aptly named *A. inconspicua* is a diminutive aloe known from only a few localities in the midlands of KwaZulu–Natal, South Africa (Van Wyk and Smith, 2003). Its thin leaves grow up to 15 cm long and, although succulent, are not readily

distinguishable from the surrounding blades of grass (Fig. 1C). Flowering plants produce a single vertical inflorescence, 8–20 cm tall, with up to 50 small, white-green flowers that open from the bottom (Fig. 1C). Flowers are protandrous, and stigmas do not become receptive until all six anthers have dehisced (Hargreaves, 2007). Flowers produce no scent discernable to humans at any time.

This study was conducted on a farm near Estcourt (28°53' S, 29°58' E; 1000 masl), which is the type locality of *A. inconspicua* (described by Plowes, 1986). Fieldwork was conducted during November 2005, which encompassed the flowering season of *A. inconspicua* at this site. During this period *A. inconspicua* was the only *Aloe* in flower at this site, and the only plant producing orange-yellow pollen. Daily temperatures generally ranged from 20 to 30 °C, and weather was typical for this area during November (D. Green, personal communication).

2.2. Plant traits

We quantified the size, reward characteristics and colour of *A. inconspicua* flowers. Inner corolla depth (distance from ovary to corolla mouth) and diameter of the corolla mouth (where petals first separate) were measured on 35 flowers from six plants to the nearest mm. Nectar volume was measured for 40 flowers from six unbagged plants using a 20- μ l microcapillary tube, but even bagged flowers produced too little nectar to measure concentration using a hand-held Bellingham and Stanley refractometer (0–50%). Spectral reflectance of one flower from each of two plants was measured over the UV–visible range (300–700 nm) using an Ocean Optics S2000 spectrophotometer and fibre optic reflection probe (UV/VIS 400 μ m) as described by Johnson et al. (2003), and compared to the reflectance of blades of surrounding grass to determine whether flowers contrasted in colour with their background. Study plants were visited during the morning of each field day to count open flowers and those that appeared to have receptive stigmas. Stigmas were presumed to be receptive when papillae were maximally expanded and exudate became visible. To assess the timing of anther

Table 1

Putatively bee-pollinated grass aloes (sections Graminaloe and Leptoaloe; Reynolds 1982) and their closest affiliate *Chortolirion* with similar floral morphology to that of *A. inconspicua*

<i>Aloe</i> and <i>Chortolirion</i> species	Flower			Inflorescence		Location	Flowering season
	Colour	Corolla length (mm)	Protruding lower tepal?	Structure	Max. height (cm)		
<i>A. albida</i>	W	18	Yes	Cluster	18	MP, SZ *	Feb–Mar
<i>A. bowiea</i>	G-W	11	Yes	Vertical	25	EC *	Nov–Jan
<i>A. minima</i>	P	12	No	Cluster	50	KZN, MP, SZ	Feb–Mar
<i>A. myriacantha</i>	W-P	20	Yes	Vertical	30	EC, KZN, TA	Mar–Apr
<i>A. parviflora</i>	P-W	8	No	Cluster	40	KZN *	Jan–Mar
<i>A. saundersiae</i>	P	12	No	Cluster	18	KZN *	Feb–Mar
<i>A. inconspicua</i>	W-G	15	Yes	Vertical	20	KZN *	Nov
<i>C. angolense</i>	W	14	No	Vertical	36	SnA	Sep–Dec Feb–Mar

Flower colours are white (W), green (G) or very pale, dull pink (P) with the predominant colour noted first. Inflorescences are either vertical or contracted with clustered flowers. Location codes refer to South African provinces (EC=Eastern Cape, MP=Mpumalanga, KZN=KwaZulu-Natal), Swaziland (SZ), southern Africa including South Africa, Lesotho, Swaziland and Zimbabwe (SnA) and tropical Africa north to Kenya (TA). Based on Reynolds (1966, 1982), Smith and Van Wyk (1990), Smith (1995), Van Wyk and Smith (2003), and Craib et al. (2004).

* Grows in a restricted area.

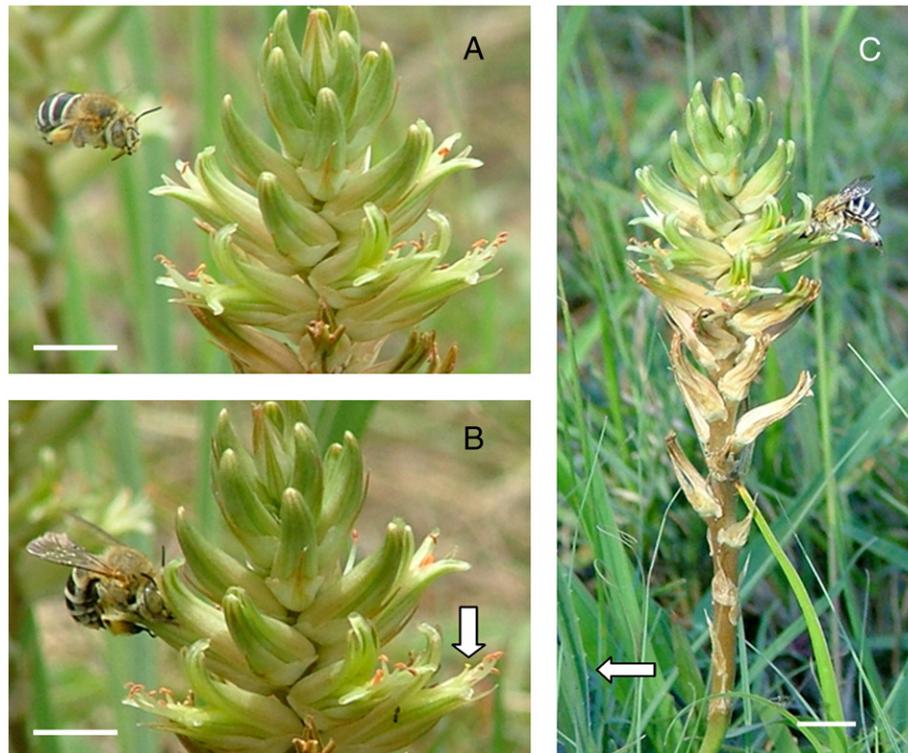


Fig. 1. Female *Amegilla fallax* visiting *Aloe inconspicua*. A. Nectar-foraging *A. fallax* approaching *A. inconspicua* with partially extended proboscis and *A. inconspicua* pollen visible in its scopae. B and C. *Amegilla fallax* probing for nectar. The arrow in panel B indicates a receptive stigma tinged yellow with deposited pollen, whereas the arrow in panel C indicates an *A. inconspicua* leaf, which is similar in size and colouration to surrounding grass. All scale bars = 10 mm. Photos: Gregory Langston.

dehiscence, four plants were visited every 2 h from dawn to dusk for 3 d, and the dehisced anthers counted.

2.3. Breeding system

To assess the degree of self-compatibility of *A. inconspicua* and the species' dependence on pollinators for seed production, inflorescences of six plants were enclosed in fine-mesh fabric to exclude all visitors, and one of three treatments was applied randomly as flowers became receptive: 1) hand-pollination with outcross pollen from plants >1 m away, 2) hand-pollination with self-pollen, and 3) unpollinated to test for autonomous self-pollination (i.e., without visitation). Each plant received all three treatments and there were 2–6 flowers per treatment per plant. Pollen from donor flowers was collected in a plastic vial <1 h prior to its use in hand pollination, and it was applied to stigmas using a toothpick. We later noted whether flowers had set a fruit, and counted the seeds in up to three fruits per plant.

2.4. Pollinator observations

To determine the relative abundance and behaviour of *A. inconspicua* visitors we conducted a total of 25 h of pollinator observations during two weeks. These observations included 21.5 h of patch observations, during which all visitors to a group of two to seven *A. inconspicua* plants were recorded

during a set period (0.5–1 h), and an additional 3.5 h of opportunistic observations. Patch-observation effort was spread evenly throughout the day, from 0530 to 2100. Evening observations ceased after no insects had been seen flying for 20 min (c. 2100). Visitation rates were calculated as the number of visitors/flowering plant/h observation. When possible, we recorded the number of plants and flowers per plant visited by insects, whether visitors contacted anthers and stigmas, and whether they collected pollen (determined by scraping and packing movements of legs over anthers) and/or nectar (i.e. pushed their head into the corolla, and/or were seen approaching or leaving flowers with proboscis extended). Reference insects were collected for identification and voucher specimens were deposited in the National Collection of Insects, Pretoria. We collected all pollen from these insects using gelatine stained with basic fuschin, which was melted onto a microscope slide (Beattie, 1971). Stained pollen was later identified and counted under a light microscope (100x) to determine the total pollen load.

2.5. Pollinator effectiveness

To evaluate the effect of insect visitors on *A. inconspicua* reproduction, we selected 15 plants with unopened inflorescences and randomly assigned five plants to each of three treatments. To assess the natural incidence of pollination, five plants were left exposed to natural pollination. To test whether

birds contributed to pollination we caged five plants with 30-mm diameter wire mesh that allowed most insects (but not large Lepidoptera) to pass freely, while excluding birds and other vertebrate visitors. The mesh was painted matte green to blend in with the background vegetation. Finally, to test whether stigmas received enough pollen for maximum seed production, alternate flowers on the remaining five plants were assigned to either open pollination (as above) or open pollination plus supplemental cross-pollination. At the end of the experiment we counted the flowers in each treatment that produced a fruit capsule, and the seeds per fruit in up to three randomly selected fruits per treatment per plant.

To confirm the effectiveness of the most common flower visitors as pollinators of *A. inconspicua*, we tested whether single visits were sufficient to effect pollination. Visitors were excluded from four *A. inconspicua* plants by enclosing inflorescences with flower buds in fine mesh bags. We exposed inflorescences when at least one flower had opened and observed them until they received a single insect visit or until time constraints forced us to end observations. However, the most common visiting bee species, *A. fallax*, failed to locate newly exposed inflorescences, even after visiting *A. inconspicua* plants within a metre. We therefore caught one bee, confined it for 10 min in a fine mesh bag, and then placed it in a 3-L clear plastic jar over an *A. inconspicua* inflorescence with three open unvisited flowers, one of which appeared to have a receptive stigma. This bee eventually visited each open flower, after which it was collected for identification, the visited flowers were marked and the inflorescence was rebagged. Fruit and seed set on the marked flowers were later counted.

2.6. Statistical analyses

All statistical analyses assessed generalized linear models (McCullagh and Nelder, 1989) and were conducted with the Genmod procedure of SAS 9.1.3 (SAS Institute Inc., 2004). Analyses of seed set (seeds/flower) considered negative binomial distributions using a ln-link function, whereas those of insect visitation considered normal distributions. Plants and most insects were sampled repeatedly, so different observations for individual plants and insects may not be independent. Consequently, descriptive statistics for flower traits and insect behaviours are based on averages for each plant. Furthermore, when analyzing influences on reproductive success we used a variance-covariance model of compound symmetry and generalized estimating equations to account for correlated responses among repeated measurements (Liang and Zeger, 1986).

3. Results

3.1. Plant traits

The flowers of *A. inconspicua* differ in many respects from those of other *Aloe* species, being more similar to *Haworthia* flowers. The flowers are relatively small, with an average inner

corolla depth of 7.9 mm (\pm SD=2.0) and diameter of 1.7 mm (\pm 0.38, $n=35$ flowers, 6 plants). Unlike the radially symmetrical flowers of most *Aloe* species, the lower tepal of *A. inconspicua* flowers is slightly extended, so that it provides a small landing platform for foraging insects (Fig. 1B). The spectral reflectance of *A. inconspicua* corollas was similar to that of surrounding grasses at wavelengths in the visible spectrum (below 700 nm; Fig. 2), in contrast to the yellow or red corollas typical of aloes. Finally, the average standing nectar crop was only 0.097 μ l (SD=0.10, $n=40$ flowers, 6 plants), compared to 40–70 μ l for bird-pollinated species (Hoffman, 1988; Nicolson and Nepi, 2005; Johnson et al., 2006).

A. inconspicua flowers and anthers opened throughout the day, and flowers lasted for three days. Once flowering started, inflorescences displayed two to seven flowers simultaneously (mean \pm SD=4.1 \pm 1.2 flowers, $n=30$ plants), of which one to three appeared to have receptive stigmas (mean \pm SD=1.4 \pm 0.67 flowers, $n=19$ plants). Seed set following hand pollination confirmed that stigmas with highly expanded papillae and visible exudate were receptive, although initial receptivity may precede the appearance of exudate, as in some other aloes (Hoffman, 1988; Hargreaves, 2007).

3.2. Breeding system

Like most aloes (Hoffman, 1988; Johnson et al., 2006; Hargreaves, 2007), *A. inconspicua* is largely self-incompatible. None of the bagged flowers that were not subject to hand-pollination ($n=23$) and only 4% (1 of 25) of self-pollinated flowers set fruit. This contrasts with the 72% (18 of 25) of flowers that set fruit after hand-pollination with outcross pollen, although the difference in fruit set between hand-pollinated self- and cross-pollinated flowers was not quite statistically significant (score statistic, $T_1=3.51$, $P=0.06$). The single self-pollinated fruit produced 4 seeds, whereas outcrossed fruits produced 15.0 seeds on average (lower SE=2.3, upper SE=2.8, based on ln-transformed data), but this difference was not significant ($T_1=1.06$, $P=0.3$), perhaps because of limited statistical power.

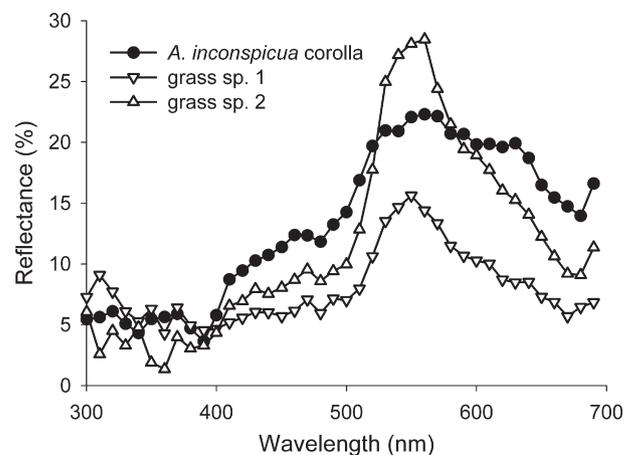


Fig. 2. Spectral reflectance of *A. inconspicua* flowers and blades of two grass species (Poaceae) collected beside *A. inconspicua* plants.

3.3. Pollinator observations

Aloe inconspicua seems to be pollinated exclusively by solitary bees. Many sunbirds and honey bees were active in the area, but none visited *A. inconspicua* flowers. Similarly, 24 moths were seen during evening observations, but none visited *A. inconspicua*.

Female *Amegilla fallax* Smith (Hymenoptera, Apidae) were the most common visitors to *A. inconspicua* during this study (54 bees observed). These bees invariably probed for nectar during flower visits ($n=31$ bees, >350 visits), and visited an average of 68.8% of open flowers per inflorescence (SD=32%, $n=38$ bees). *Amegilla* usually landed on the lower tepal to probe (81.8% of visits, $n=24$ bees), closely contacting the exerted anthers and stigmas, but they sometimes probed while hovering, which may have resulted in less contact with floral organs. Nine of 11 *A. fallax* individuals that we captured or photographed carried the yellow-orange pollen characteristic of *A. inconspicua* in their scopae. Microscopic inspection of the pollen carried by one bee confirmed that it had been collected from *A. inconspicua* flowers. This bee carried >600 grains of *A. inconspicua* pollen and c. 600 grains of non-aloe pollen, mostly in its scopae. Only four of 54 *A. fallax* individuals were observed actively grooming *A. inconspicua* pollen to their scopae while visiting flowers. However, pollen collection probably involved buzzing, as the bee that visited flowers on a hand-held, cut *A. inconspicua* inflorescence vibrated each flower while probing for nectar. This bee did not manipulate pollen with her legs, but removed most of the pollen visible on anthers.

Other insects visited *A. inconspicua* infrequently. A few small, unidentified bees (7 bees, 14 visits) seemed unable to access nectar from *A. inconspicua* flowers, but occasionally visited them to collect pollen. On five occasions an unidentified species of *Pseudapis* (Halictidae) visited a total of 37 *A. inconspicua* flowers for nectar in a single small patch of three plants. A collected individual of this species carried >6500 grains of *A. inconspicua* pollen and 2000 grains of non-aloe pollen.

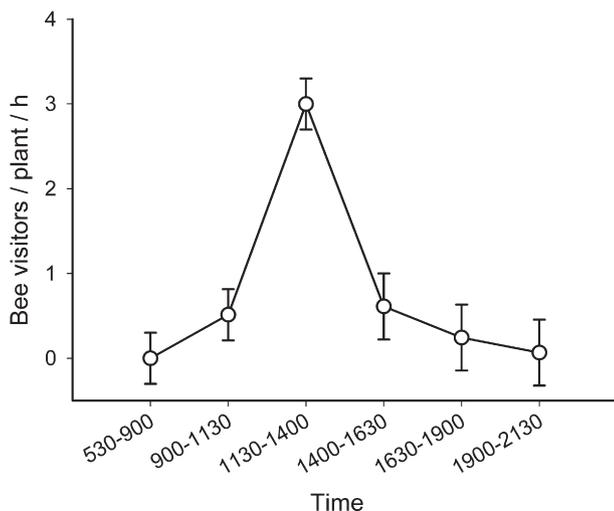


Fig. 3. Mean (\pm SE) rates of bee visits to *A. inconspicua* flowers throughout the day.

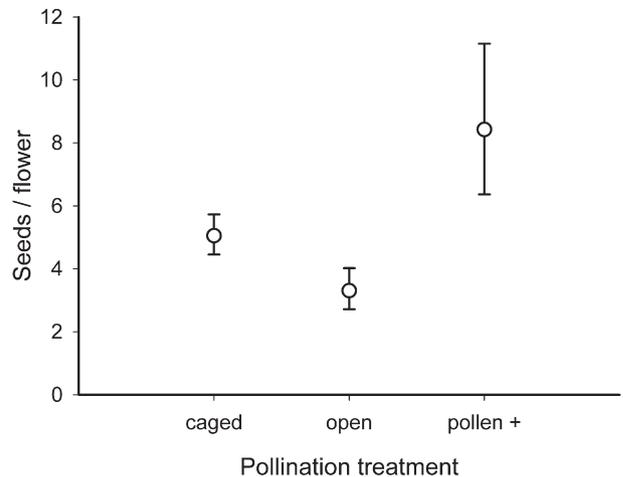


Fig. 4. Mean (\pm SE) seed set of flowers that received insect visits only (caged), natural pollination (open) and natural pollination plus additional hand-pollination with outcross pollen (pollen +). The observation for open pollination includes open-pollinated plants in the exclusion experiment and open-pollinated flowers in the pollen-limitation experiment, which did not differ significantly. No means differed significantly ($\alpha=0.05$).

Visitation rates varied throughout the day, and were higher during midday than during any other period ($T_1=30.98$, $P<0.001$; Fig. 3). We did not observe any visits to *A. inconspicua* before 0900, and the latest visitor (an *A. fallax*) was seen just after 1900, at dusk.

3.4. Pollinator effectiveness

Insects were highly effective pollinators of *A. inconspicua*. Seed set by caged plants did not differ significantly from that of exposed plants ($T_1=2.36$, $P>0.1$; Fig. 4). Supplemental pollination tended to increase seed set compared to control flowers on the same inflorescences, but the difference was not statistically significant ($T_1=2.46$, $P>0.1$; Fig. 4), suggesting that pollen receipt did not limit seed set in this population. Seed set did not differ significantly between flowers on open-pollinated plants in the exclusion experiment and open-pollinated flowers in the pollen-limitation experiment ($T_1=0.02$, $P>0.9$). Single-visit trials showed that *A. fallax* deposited pollen (45 grains) and visits by both *A. fallax* and *Pseudapis* sp. induced seed set (11 and 4 seeds/fruit, respectively).

4. Discussion

A. inconspicua is the first aloe species shown to be exclusively insect-pollinated, with *A. fallax* being its primary pollinator. *Amegilla* bees were frequent visitors to both male- and female-phase flowers, and made effective contact with anthers and receptive stigmas when they pushed their heads into corollas to access nectar. We did not measure proboscis lengths, but *A. fallax* proboscises at sites to the north (Mpumalanga) and southwest (Western Cape) ranged from 4–6 mm (Goldblatt et al., 1998), a length that would enable bees to access nectar at the base of *A. inconspicua* flowers only by pushing their heads into corollas, ensuring contact with sexual floral organs. *A. inconspicua* flowers

provide much less nectar than those of bird-pollinated aloes (e.g., Hoffman, 1988; Nicolson and Nepi, 2005; Johnson et al., 2006; Johnson and Nicolson, 2008), but the small nectar standing crop attracted sufficient visitation to satisfy at least female function, as seed production was not obviously pollen limited in this population.

The most complete Alooideae phylogeny to date (Treutlein et al., 2003b) suggests that insect pollination in *A. inconspicua* could represent either maintenance of the ancestral pollination system in the subfamily, or a reversion from bird-pollination. Reversions to bee-pollination are unusual, and may be unlikely if bees do not visit flowers with long corollas from which they cannot obtain a reward, as suggested by the directional evolution of elongated nectar spurs (Whittall and Hodges, 2007). However, many bird-pollinated aloes with long corollas are visited for pollen by bees, who will also consume nectar opportunistically if it drips out of the flower (Hargreaves, 2007). The maintenance of bee visitors via easily accessible pollen could help bridge a selective trough, as plants make the evolutionary transition from long to short corollas.

Six other South African *Aloe* species (Table 1), as well as *A. bowiea* and all species of *Chortolirion* (Table 1) and *Haworthia*, resemble *A. inconspicua*, with diminutive leaf rosettes and small, whitish flowers. If similar morphology reflects similar pollination systems, the *Aloe* clade likely includes more than 70 species pollinated exclusively by insects. If no reversions to bee-pollination have occurred and these putatively insect-pollinated species all represent the conserved ancestral pollination system, the current phylogenetic tree (Treutlein et al., 2003b) indicates that bird-pollination must have evolved independently at least five times within the aloe clade. Identifying the most likely evolutionary history will require phylogenetic analysis of the entire clade based on more thorough taxon sampling than that of Treutlein et al. (2003b). Whether aloes have undergone rare reversions to bee-pollination or repeated pollinator shifts to bird-pollination, they present an excellent opportunity to study patterns in pollinator shifts and floral evolution.

Acknowledgements

We dedicate this paper to the late David Green, who discovered *Aloe inconspicua* and dedicated himself to its recognition as a species and protection on his farm. This study would not have been possible without the help and hospitality of David and Dorothy Green. Thanks to Greg Langston for invaluable help in the field, C. Eardley at the South African Agricultural Research Council for insect identification, and the Natural Sciences and Engineering Research Council of Canada (ALH and LDH) and the National Research Foundation of South Africa (SDJ) for funding.

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