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Appearances can be deceiving: insect-pollinated *Aloe greatheadii* var *davyana* has an ornithophilous pollination syndrome

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Abstract

Aloe greatheadii var *davyana* (Asphodelaceae) is a winter flowering aloe with a widespread distribution in South Africa. It exhibits a characteristic bird pollination syndrome (conspicuous pinkish-red flowers, tubular corolla, copious, dilute and unscented nectar) and although visited by numerous occasional avian nectarivores, is an important plant in the bee industry. It is used by apiarists to build up honeybee colonies in winter and for honey production. Exclusion experiments were conducted to test pollinator efficiency during two flowering seasons. In both years fruit set indicated that honeybees were the most important pollinators. At least eleven species of birds; from the families Coliidae, Malaconotidae, Muscicapidae, Nectarinidae, Pycnonotidae, Ploceidae and Sturniidae were recorded visiting inflorescences, where they probed for nectar and ate flowers, but contributed very little to pollination success. Placement of honeybee hives at the flowering site in the second season significantly improved pollination success. Pollination was negligible when all pollinators were excluded, indicating no spontaneous pollination in *A. greatheadii* var *davyana*. Despite displaying the characteristics of a bird pollination syndrome, nectar characteristics measured in this study (volume 19.6 ± 6.9 µl and concentration $21.1 \pm 2.8\%$) are identified as the single characteristic responsible for successfully attracting bees *en masse* as successful pollinators.

Introduction

South Africa has the highest diversity of *Aloe* species with more than 100 species occupying a variety of habitats (Van Wyk & Smith, 1996). These succulent plants grow well in warm climates and flower mostly during the winter months. The genus *Aloe* L. consists of 26 Sections, of which the section Pictae, or spotted aloes (characterised by their spotted leaves), is the largest. *Aloe greatheadii* var *davyana* belongs to this section and may exist as either solitary individuals or in medium-sized groups (up to 15 individuals). *Aloe greatheadii* var *davyana* has a widespread distribution in the northern summer rainfall areas of South Africa (Glen & Hardy, 2000; Van Wyk & Smith, 1996).

The pinkish-red colour, tubular structure and lack of scent of *A. greatheadii* var *davyana* flowers, as well as the large amount of relatively dilute nectar (mean volume 14.7 ± 7.1 µl and concentration $18.6 \pm 2.7\%$, Chapter 4) typifies a bird pollination syndrome (Faegri & Van der Pijl, 1979). In South Africa at least 73 species of birds have been recorded visiting 14 species of aloes, as well as eight other flowering plants and trees, for nectar (Oatley & Skead, 1972). Sunbirds are closely associated with aloes, but bees and other insects also visit aloe flowers. From the few studies conducted it appears that sunbirds are important pollinators: in species such as *A. marlothii, A. ferox, A. mayottensis and A. divaricata* many opportunistic passerine birds have been recorded visiting flowers for nectar (Hoffman, 1988; Ratsirarson, 1995; Skead, 1967; Johannsmeier, 1976; Stokes & Yeaton, 1995; Pailler et al., 2002; Johnson et al., 2006; C.T Symes, pers. obs.). Birds visited *A. ferox*, although the most frequent floral visitors to flowers were honeybees (Hofman, 1988). Even though the red tubular flowers are suggestive of sunbird pollination honeybees were suggested to be important pollinators (Hoffman, 1988). According to Johnson et al. (2006) honeybees frequently visit *A. vryheidensis* but collect only pollen and do not attempt to collect nectar, due to its bitter taste. Sunbirds also find the nectar unpalatable, so that pollination of *A. vryheidensis* is carried out by short-billed birds.

Aloe greatheadii var *davyana* is commonly utilised by South African migratory beekeepers during winter months when few other nectar sources are available (Williams, 2002). The nutritious pollen (Chapter 1) and strong nectar flow (Chapter 4) are used to build up colonies, rear queens and obtain a substantial honey crop (Williams,

2002). Since *A. greatheadii* var *davyana* fits a typical bird pollination syndrome (see syndrome characters; Thomson et al., 2000), and is visited by honeybees and a host of bird species, the following questions are asked; 1) Is either of these pollinator guilds more effective than the other and if so what characteristics/ features determine any possible biased pollination success? 2) If honeybees are important pollinators is pollination success affected by changes in bee density?

Methods

Study site and plant species
Nectar secretion and pollination of *A. greatheadii* var *davyana* plants was studied during the peak flowering period at the Roodeplaat Nature Reserve (size 795 ha; 28º 39'E, 25º 66'S) in Gauteng Province, South Africa during the winter months (June and July) of 2003-2004. In the second year, six honeybee hives (*Apis mellifera scutellata*) were moved to the reserve prior to the onset of flowering. The hives were placed ± 2 m apart within 500 m of the study site with the exclusion cages (shown later).

Population density
Population density of *A. greatheadii* var *davyana* was determined in 2004 for Roodeplaat Nature Reserve by counting the number of plants occurring in 15 plots (each 5 x 5 m) over a distance of 7 km, the plots being 500 m apart from each other. Forty five plants were randomly picked and the number of inflorescences and racemes counted as well as the number of flowers on each raceme, to estimate the number of flowers available for bee foraging in the Reserve.

Nectar production
Four flower stages can be recognised in *A. greatheadii* var *davyana* flowers. Flowers in which the corolla is just opening represent stage 1 and open flowers with the long stamens exerted stage 2. Stage 3 flowers can be recognised when the floral tube reaches its maximum width and all 6 anthers are exerted, and stage 4 flowers when the floral tube starts to wilt but the style remains turgid and exerted (Chapter 4, see Fig. 2). Nectar volume increases with flower age and reaches a peak in stage 3 flowers, thereafter it decreases dramatically. Nectar concentration follows the same pattern but less dramatic differences are observed (Chapter 4). Therefore all nectar measurements in this study

were made on unscreened, stage 3 flowers. Nectar production in *A. greatheadii* var *davyana* was investigated over a period of one week. Ten plants were randomly chosen and nectar was collected daily between 10.00 and 12.00 h from all stage 3 flowers, in haematocrit tubes (length 75 mm/75 µl). Volumes of nectar were determined from column length and the concentrations measured as % w/w sucrose equivalents with a hand-held pocket refractometer (Bellingham & Stanley Ltd, 0-50%, Tunbridge Wells, UK). Temperature and relative humidity were measured at flower height with a portable thermohygrometer (Model TES 1365, TES Electrical Corp., Taiwan).

Bird observations

In 2006, during peak flowering, the study site was visited to assess the activity of avian visitors to flowering *A. greatheadii* var *davyana*. Mist nets (12 m) were used to catch birds in the vicinity of flowering aloes to determine pollen loads on the face of potential flower visitors. For all specimens captured a swab of the crown and throat was obtained using sticky tape and placed on a slide mount for further inspection in the laboratory.

Exclusion experiments

In order to determine which pollinators were the most effective, twenty plants, at least 10 m apart, were arbitrarily chosen prior to bud opening for exclusion experiments. The experiment consisted of three treatments per plant: exclusion of birds, exclusion of birds and bees, and a control. A cage made of steel wire, with sufficient space around the florets (at least 5 cm) to prevent sunbird bills from reaching flowers, was placed over one raceme and attached to a steel rod with cable ties (Fig. 1). These cages excluded birds but allowed bees to enter and visit flowers (H. Human, pers. obs). Gauze bags (2 mm mesh size) were placed over a separate raceme to exclude all pollinators. The control raceme was marked and allowed access to all visitors. Racemes at different positions on the inflorescence were selected for each treatment in order to avoid allocating the terminal raceme with the most flower buds to a certain treatment. Racemes were caged prior to any flower opening. The length of each raceme was measured and the total number of flowers counted prior to caging. After fruit maturation all fruits were collected for each treatment and counted. Five fruits from each treatment were opened, and the number of seeds that developed in each fruit determined. All damaged and aborted flowers were collected and recorded. The experiment was repeated the following season after six beehives were placed at the reserve. These

exclusion experiment methods were similar to those described by Stokes and Yeaton (1995) and Ratsirarson (1995).

Figure 1. Growth form of a typical *Aloe greatheadii* var *davyana* plant showing exclusion treatments; **(A)** mesh bags to exclude all pollinators (i.e. birds and bees), **(B)** wire cages to exclude birds and, **(C)** control left open to all pollinators. (Height of cage support rod = 1, 20 m).

Statistical analysis

Data did not meet the assumptions for parametric statistics; variances were not homogeneous and data did not conform to a normal distribution. Student's t-tests were used for comparisons of flower length, bulb width and flower opening as well as

between flower length and bird bill lengths: this data met the assumptions for parametric tests. Differences in fruit set and seed set between treatments, sites and season were assessed with Kruskal-Wallis ANOVA and the Mann-Whitney U-test was used for all comparisons (Zar, 1984). Bonferroni corrections were applied for multiple paired combinations of the same data and the level of significance adopted for these comparisons was 0.0125, for all other analysis the level of significance was 0.05. Values are given throughout as mean ± SD. Analyses were performed using Statistica 6.0 (1984-2004).

Results

Plant species
The colour of the tubular flowers varied from pale pink to bright red. Flowers have a conspicuous basal swelling, as do most of the aloes belonging to the section Pictae (Fig. 2 A) (Van Wyk & Smith, 1996). Flowers opened throughout the day and had an average lifespan of 4.0 ± 6.8 days. Fruits developed approximately 3 weeks later (Fig. 2A, B).

Population density
Aloe greatheadii var *davyana* grows equally well in the sun and under trees (Fig. 2C). Its density was estimated to be 104 ± 96 plants/100m^2 (n = 15). Plants had an average of 4 ± 2.1 racemes on each of 2 ± 0.5 inflorescences. Each raceme had 34.0 ± 16.9 flowers with approximately 340 flowers on each plant (n = 45).

Nectar production
The average length of the tubular flowers was 27.3 ± 1.7 mm, fitting the bill of one sunbird species but not other short-billed passerine bird species (see Table 1 and Fig. 3a). The average bulb width was 5.2 ± 0.7 mm and flower opening width 6.6 ± 0.8 mm, allowing honeybees to easily crawl into flowers, wedging themselves between the flower petals (Fig. 3B). The average daily volume of nectar (standing crop) was 19.6 ± 6.9 µl per flower (total n = 365) and the average concentration of nectar was 21.1 ± 2.8%. As a result of low ambient winter temperatures, bee foraging only started at approximately 09.00 h in the mornings and stopped after approximately 16.00 h. The average minimum daily temperature (between 10.00 and 12.00 h) measured was 10.6 ±

0.8°C and the maximum was 24.6 ± 1.5°C. Relative humidity decreased during the day; the maximum RH measured was 32.1% and the minimum was 8.7%.

Figure 2. (A) Flower development of *Aloe greatheadii* var *davyana,* stages 1-4, **(B)** cluster of plants, and, **(C)** stand of *Aloe greatheadii* var *davyana* at Roodeplaat Nature Reserve, in peak flowering during July (period of exclusion experiments).

Figure 3. (A) Male sunbird *Cinnyris talatala* collecting nectar from a flower of *A. greatheadii* var *davyana* and, **(B)** Honeybee *A. mellifera scutellata* nectar-forager crawling into the floral tube.

Avian visitors to Aloe greatheadii *var* davyana

We have no quantitative data for flower visitation by the birds and bees that visited A. greatheadii var davyana but did observe passerine birds being earlier visitors in the day. Bird species recorded at the study site, and visiting A. greatheadii var davyana, are summarised in Table 1. Bird species that probed aloe flowers for nectar did so by perching on the aloe pedicel and probing upwards into stage three flowers. A number of stage three flowers would be probed before the feeding bird moved on. Vigilance was maintained with flocks of birds (mixed species) concentrating feeding activity in a patch. Feeding evidence observed on birds was noted by the presence of pollen predominantly on the throat. Some pollen was also occasionally noted on the head. Evidence of flowers removed from inflorescences was observed although no flower feeding was recorded during observations.
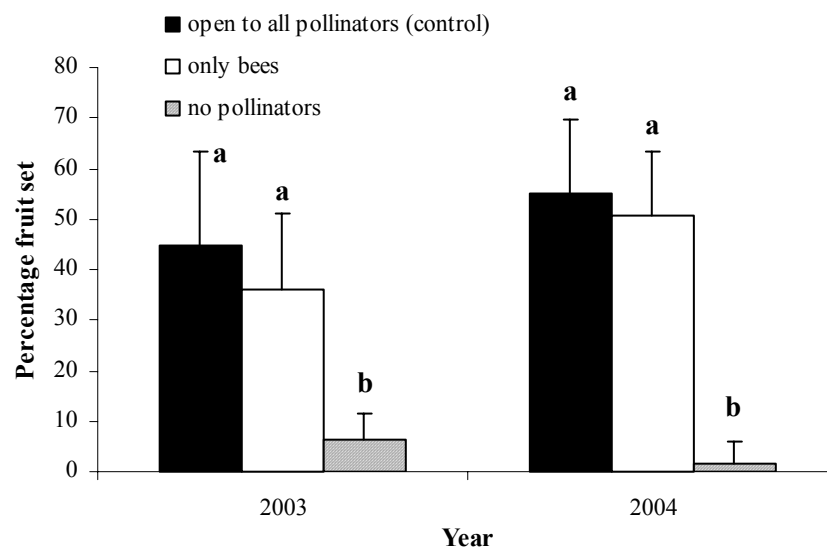
Exclusion experiments
Fruit set

Flowering of the dense population of *A. greatheadii* var *davyana* was immense during both seasons. Honeybees were observed to move freely in and out of the exclusion cages. Minimal other insects were observed visiting the flowers. Results of exclusion experiments indicate that both birds and bees contribute to fruit set (Fig. 4).

Table 1. Bird species at Roodeplaat Nature Reserve recorded feeding on *Aloe greatheadii* var *davyana* during July 2006, Feeding guilds of respective species indicated as, ins = insectivore; frug = frugiivore; nect = nectarivore; foli = folivore. Species previously recorded feeding on *Aloe* sp. indicated according to, 1 = Oatley (1964); 2 = Oatley & Skead (1972), * = recorded feeding on *A. greatheadii.* Bill length given as mean ± SD (n) (Symes unpubl. data). Bill length compared with average *A. greatheadii* var *davyana* flower length, 27.3 ± 1.7 mm. Significance is shown by italics. (t = test independent samples).

Species	Guild	Observed	Pollen swab	.loe	Bill length	Comparison with aloe (t-test independent samples)
COLIIDAE						
Speckled Mousebird *Colius striatus*	frug > foli > nect	yes	-		12.3 ± 0.6 (9)	*T = 28.3, df = 57, P < 0.0001*
MALACONOTIDAE						
Crimson-breasted Shrike *Laniarius atrococcineus*	frug > ins	no	no		20.7 (1)	
PYCNONOTIDAE						
Dark-capped Bulbul *Pycnonotus tricolour*	frug > ins	yes	-	, 2 *	16.7 ± 1.4 (35)	*T = 33.7, df = 83, P < 0.0001*
CISTICOLIDAE						
Neddicky *Cisticola fulvicapilla*	ins	suspect	-	, 2 *	10.2 ± 0.6 (13)	*T = 38.3, df = 61, P < 0.0001*
Black-chested Prinia *Prinia flavicans*	ins	yes	-	, 2 *	10.1 ± 0.7 (24)	*T = 50.9, df = 72, P < 0.0001*
MUSCICAPIDAE						
White-browed Scrub-Robin *Cercotrichas leucophrys*	ins > frug	suspect	no	, 2	13.7 (1)	
STURNIDAE						
Cape Glossy Starling *Lamprotornis nitens*	frug = ins		-	, 2 *	18.1 (1)	
NECTARINIIDAE						
Amethyst Sunbird *Chalcomitra amethystine*	nect > ins	yes	-		27.6 ± 1.3 (12)	T = 0.9, df = 60, P = 0.336
White-bellied Sunbird *Cinnyris talatala*	nect > ins	yes	yes		20.9 ± 1.1 (20)	*T = 17.7, df = 68, P < 0.0001*
PLOCEIDAE						
Cape Weaver *Ploceus capensis*	ins > frug > nect	yes	yes	, 2	21.4 ± 1.3 (20)	*T = 16.2, df = 68, P < 0.0001*
Southern Masked-Weaver *Ploceus velatus*	ins > frug > nect	yes	yes	, 2 *	16.2 ± 1.2 (126)	*T = 52.7, df = 174, P < 0.0001*

However, bees played a significantly greater role in flower pollination. Racemes open to all pollinators produced the highest fruit set while racemes where birds were excluded produced slightly less. Exclusion of all pollinators resulted in little to almost no fruit set. Fruit set was significantly different between the three treatments, in 2003 ($H_{2, 60}$ = 38.440, P < 0.001) and 2004 ($H_{2, 60}$ = 40.909, P < 0.001). A comparison between the three treatments in both 2003 and 2004 showed significant differences between the treatment with no pollinator and the one open to all (2003, U = 3.50, P < 0.001 and 2004, U = 0.00, P < 0.001) and the no pollinator treatment and bee treatment (birds were excluded (2003, U = 8.50, P < 0.001 and 2004, U = 0.00, P < 0.001). There was no significant difference between the open to all treatment and the treatment where birds were excluded (2003, U = 139.00, P = 0.1 and 2004, U = 194.50, P = 0.9). When the treatments of the two seasons were compared no significant difference was observed in fruit set in the open to all treatment (U = 156.00, P = 0.234) but significant differences were observed in treatments that excluded all pollinators (U = 74.00, P < 0.001) and birds (U = 99.00, P = 0.006).

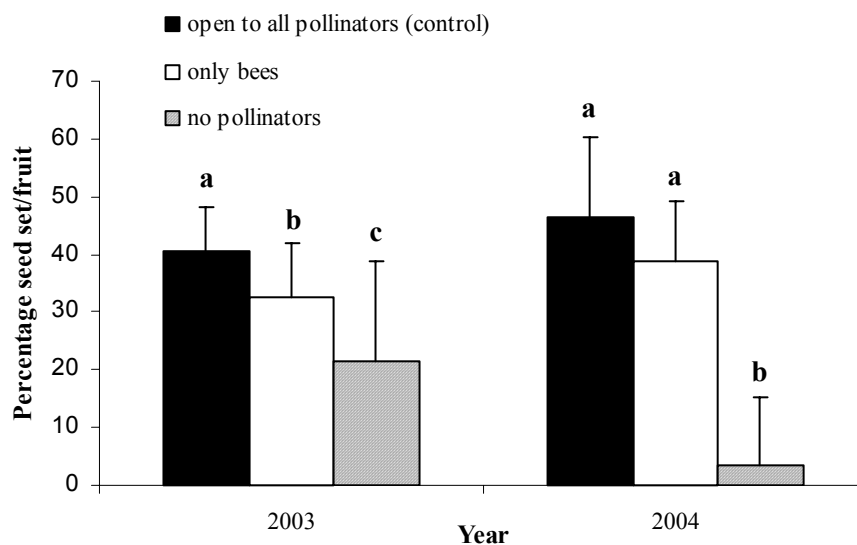**Figure 4.** The average percentage fruit set (± SD) per raceme (n = 20) for *A. greatheadii* var *davyana* from pollinator exclusion experiments for two seasons (2003 and 2004). Six beehives were introduced to Roodeplaat Nature Reserve in 2004.

Seed set

Seed set was significantly different between the treatments in both years, for 2003 ($H_{2, 60}$ = 15.941, P = 0.0003) and for 2004 ($H_{2, 60}$ = 42.484, P < 0.001). In 2003 seed set was significantly different between the control treatment (open to all) and the treatment that allowed only bees (U = 100.50, P = 0.007) but there was no significant difference between the two treatments in 2004 (U = 131.00, P = 0.06). Seed set in treatments that excluded all pollinators was significantly lower than that of the control treatments (2003, U = 67.00, P < 0.001; 2004, U = 0.00, P < 0.001) and that of treatments that allowed only bees (2003, U = 127.00, P < 0.05; 2004, U = 0.00, P < 0.001). When seed set was compared between the two years significant differences were observed ($H_{2, 120}$ = 55.616, P < 0.001). Seed set was not significantly different between the control treatments (open to all) (U = 156.00, P = 0.234) but was significantly different for treatments that allowed only bees (U = 99.00, P < 0.05) and the treatments that excluded all pollinators (U = 74.00, P < 0.001).

Figure 5. The average seed set (± SD) per fruit (n = 5) for *A. greatheadii* var *davyana* from pollinator exclusions for two seasons (2003 and 2004). Six beehives were introduced to Roodeplaat Nature Reserve in 2004.

Discussion

Aloe greatheadii var *davyana* flowers conform well to the requirements characteristic of a bird pollination syndrome (Table 2). However the availability of relative constant nectar (volume and concentration) throughout the day may serve to attract pollinators from divergent pollinator groups. Nectar of *A. greatheadii* var *davyana* was found to be continuously available with a relatively constant volume and concentration, available to visitors throughout the day (Chapter 4). In this study the average volume and concentration of nectar produced in unscreened flowers was 19.6 ± 6.9 µl and $21.1 \pm 2.8\%$, a typical bird nectar (Nicolson, 2002). Plants may offer different rewards to pollinators; in *A. ferox* the chief reward for honeybees may be pollen while nectar may be the main reward for birds (Hoffmann, 1988). In *A. greatheadii* var *davyana* birds collect nectar while bees collect both pollen and nectar. This nectar flow contributes to a major part of the beekeepers' honey crop.

Just as birds sometimes visit flowers that are not typical bird flowers, other pollinators such as bees may visit ornithophilous flowers (Robertson et al., 2005). In spite of visitation by a wide variety of pollinator types only some of these visitors may actually be effective pollinators of the plant (Robertson et al., 2005). Aloes appear to be typical bird pollinated plants but bees and other insects may also pollinate them (Stokes & Yeaton, 1995). In this study, despite the typical bird pollination characteristics of *A. greatheadii* var *davyana* flowers, both birds and bees were responsible for its pollination; with the contribution of birds being significantly less than expected. The high percentage of fruit set in bird-excluded inflorescences suggests that bees are the primary pollinators of *A. greatheadii* var *davyana*, because only bees had access to flowers and the greater fruit set in the second season can be attributed to the increased density of bees at the study site, through the introduction of honeybee hives. However, these results do not show that birds are ineffective pollinators. This is in agreement with the findings of Tribe and Johannsmeier (1996) that considered both sunbirds and honeybees as the major pollinators of three species of tree aloes, *A. dichotoma, A. pillansii* and *A. ramosissima.* However, although stingless bees, *Trigona* species, were utilising pollen of *A. divaricata* they did not contribute to the pollination of this aloe while sunbirds, *Nectarinia souimanga* were considered to be the primary pollinators (Ratsirarson, 1995). Contrary to the results of this study, pollination of *A. vryheidensis*

is achieved by "occasional nectarivores" that use only a small quantity of the bitter tasting nectar in their daily diets while the contribution of bees to the pollination of this aloe is attributed to their numbers rather than their effectiveness (Johnson et al. in press). The primary pollinators of *A. candelabrum* (Stokes & Yeaton, 1995) and *A. divaricata,* an indigenous aloe from Madagascar (Ratsirarson, 1995), are birds.

Table 2. Comparison of bird pollination syndrome characters (Thomson et al., 2000) and flowers of *Aloe greatheadii* var *davyana.*

Bird pollination character	*Aloe greatheadii* var *davyana*
Red/orange coloured flowers	Flowers salmon pink to red
Absence of scent	No scent
Long floral tube	Flower length = 27.3 ± 1.7 mm; flower opening width = 6.6 ± 0.8 mm.
Exerted anthers and stigma	Anthers exserted before stigma, maximum nectar standing crop at male phase (stage 3)
Less pronounced landing platform	No landing platform
Inclined flower	Inclined at 120° at maximum nectar production (stage 3 flowers)
High volume of nectar	High volume- average 33.3 ± 9.8 µl (low compared to *A. ferox* and *A. marlothii*)
Low concentration of nectar	Average 21.4 ± 1.3% (high compared to *A. ferox* and *A. marlothii*)

Aloe ferox and *A. maculata* are examples of aloes with flowers suggestive of a bird pollination syndrome that are pollinated by both birds and bees (Hoffmann, 1988; S.D. Johnson, pers. comm.). The percentage fruit set for the different treatments in *A. greatheadii* var *davyana* was much higher (45-55% for all pollinators) than observations on other species (Hoffman, 1988; Ratsirarson, 1995; Stokes & Yeaton, 1995). Pailler et al. (2002) reported only 30% for *A. mayottensis,* which has only one bird pollinator. There may be various explanations for the low effect of birds on fruit set in *A. greatheadii* var *davyana*. The low effect can be attributed to the fact that the more slender inflorescence stalks of *A. greatheadii* var *davyana* plants may not be as suitable for larger perching birds (e.g. > 35g bulbuls) as those of, for example, *A. marlothii*. The

nectar may furthermore be less accessible to larger passerine birds due to the downward orientation of flowers. But the rigid stems allow a place to perch to access these downward facing flowers – even more suggestive of ornithophily. Flower length of *A. greatheadii* var *davyana* allows sunbirds to get pollen on their throats when probing for nectar, thereby making them legitimate nectar feeders (Maclean, 1990). Subsequently only sunbirds (legitimate nectarivores) are able to contribute to pollination while other birds may remove and eat whole flowers (robbers) in order to get to the nectar (Oatley & Skead, 1972). This was noted during feeding observations, with bulbuls and mousebirds often seen removing whole flowers during visits to *A. greatheadii* var *davyana* inflorescences (Human & Symes, pers. obs).

Caging in this experiment did not negatively affect bee behaviour and subsequent fruit set, as was suggested by Celebrezze and Paton (2004). The differences in behaviour of pollinators and timing of their visits may have contributed to differences in their effectiveness. Foraging bees only started foraging at 09.00 h and stopped at 16.00 h compared to birds that were early morning visitors when bees were inactive. Birds may also have been more active on cooler days when bees were less active. Taking into account the number of foraging bees (20 000+), the amount of flowers they were visiting and the time spend on foraging compared to the number of birds may also have resulted in reduced effectiveness of subsequent visits by birds. Variation in the effectiveness of pollinators may be the result of the inability of the pollinator to pick up or deposit pollen onto the stigmas of a plant and may be influenced by flower shape and size as well as the fit of the visitor (Robertson et al., 2005).

Although nectar volume and concentration, in general, may be broadly associated with certain classes of visitors, Johnson et al. (in press) showed that secondary compounds in nectar, resulting in a dark colour and bitter taste of nectar, may serve to filter visitors from flowers. A few studies have tested whether ornithophilous pollination syndromes correctly predict the floral visitors and primary pollinators of plant species, other than *Aloes*. Varying results were obtained. Hingston and McQuillan (2000) concluded that pollination syndromes are mostly unreliable predictors of Tasmanian floral visitors. On the other hand, as predicted by its ornithophilous characteristics, *Protea roupelliae* Meisn is pollinated exclusively by birds (Hargreaves et al., 2004). Contrary to the study by Hargreaves et al. (2004) and its ornithophilous syndrome the major pollinators of

A. greatheadii var *davyana* flowers are honeybees. This is in agreement with Robertson et al. (2005) who observed both bees and birds to be the pollinators of two New Zealand mistletoes, *Peraxilla colensoi* and *P. tetrapetala*, which displayed a classical ornithophilous syndrome.

In this study the assigned floral syndrome could have led to inaccurate predictions about the plant's primary pollinator if it was not critically evaluated and investigated. The primary pollinator predicted by the syndrome only played a minor role. This confirms Hargreaves et al.'s (2004) conclusion that pollination syndromes are valuable for the development of testable hypothesis about pollination systems but should not be accepted as evidence on pollinators of a plant. Appearances can thus be deceiving.

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GENERAL CONCLUSION

Throughout the preceding chapters we have attempted to evaluate the floral resources of *Aloe greatheadii* var *davyana* utilised so keenly by honeybees and beekeepers. We have analysed the pollen for its nutritional value and investigated the effect of its exceptionally high protein content on the ovarian development of honeybees. In addition we have determined extraction efficiency during digestion of the pollen by honeybees. In order to assess the nectar resource available to honeybees, we have examined the nectary structure of *A. greatheadii* var *davyana* as well as the variation in nectar volume and concentration on various levels, from within individual flowers to across the northern summer rainfall region of South Africa. The ability of honeybees to deal with the excess water derived from utilising such dilute nectar was investigated, together with the effect of the dilute nectar on regulation of nest humidity. The floral characteristics of *A. greatheadii* var *davyana* are suggestive of bird pollination but little was previously known about its pollination. We determined the primary pollinators of this aloe through exclusion experiments. Here we summarise the key findings of each chapter and bring together the bees and the aloes, highlighting areas for future research.

Pollen of *Aloe greatheadii* var *davyana*: a rich floral reward

This study is the first, to our knowledge, to compare the changes that occur in pollen of a single species after its collection by honeybees. We have shown that the composition of pollen is indeed altered through the addition of nectar and glandular secretions by honeybees (Winston, 1987; Roulston, 2005). This is reflected in the increase in water and carbohydrate content of the pollen and the resultant decrease in its protein and lipid content. For meaningful comparisons of the nutritional value of pollens, standardised methods are needed. Although it is time consuming to collect fresh pollen compared with the ease of obtaining bee-collected pollen, it will give a better reflection of the true nutritional composition of pollen. The protein content of fresh *A. greatheadii* var *davyana* pollen (51% dry mass) is, with the exception of *A. zebrina*, the highest recorded yet for South African pollens. The comparison between the amino acid composition of pollen and royal jelly (De Groot, 1953) demonstrates the overall excellent nutritional content of this pollen.

Apis mellifera scutellata bees have a very low threshold response to intruders and become particularly aggressive and defensive during the aloe flow. In addition the number of bees available for colony defence greatly increases as a result of the availability of abundant pollen and nectar (Johannsmeier, 2001). Doull (1979a) suggested that the aggressive behaviour of bees may be due to chemical properties of the pollen of *A. greatheadii* var *davyana*. However, apart from the high protein content and high concentration of gadoleic acid, there is no obvious chemical explanation for the observed aggression in bees. The excellent nutritional quality of the pollen may contribute to increased egg production by the queen, resulting in a large amount of sealed brood present in the colonies. Winter weather delays foraging and results in short working days for forager bees (foraging only starts around 09.00 h in the morning), and hence overcrowding of the hive. Therefore the observed aggression may be the result of natural behaviour patterns in a situation of abundant food and several thousands of brood, all worth defending.

It is well known that protein is essential for the normal growth and development of bees (Moritz & Crailsheim, 1987) and the effect of dietary protein on ovarian development is well documented (Hoover et al., 2006; Lin & Winston, 1998; Pernal & Currie, 2000). Pollen is the main dietary source of protein for honeybees (Grogan & Hunt, 1979; Pernal & Currie, 2000), and protein concentrations vary widely among pollens of different plant species. The extent of pollen digestion also varies between plant species (Roulston & Cane, 2000) and is influenced by the type of consumer and the method of digestion. Extraction efficiency reflects the efficiency with which consumers extract pollen contents, but the proportion of empty pollen grains in fresh pollen is not always quantified in these calculations even though it can be substantial; according to Law (1992) up to 18% of fresh *Banksia* pollen grains are empty. Therefore in calculations of extraction efficiency one needs to adjust for the proportion of initially empty grains. Pollen digestion is furthermore influenced by pollen wall thickness and grain size (Roulston, 2005). Pollen consumers may be able to extract enough nutrients from grains even if they don't completely empty them. Less effort is needed to digest thin-walled pollen compared to ornamented pollen grains, and this is illustrated by the work of Suarez-Cervera et al. (1994) and Human and Nicolson (2003) as well as the present study.

The greater ovarian development in queenright worker bees sustained on aloe than on sunflower pollen may be attributed to the higher extraction efficiency (80%) during digestion and the exceptionally high protein content of bee-collected *A. greatheadii* var *davyana* pollen (31%). The contradictory results of workers with more developed ovaries in induced queenless groups sustained on sunflower pollen compared to aloe pollen, and the higher mortality for bees fed aloe pollen, may be explained by the detrimental effects of proteins available in high concentrations (Herbert et al., 1977; D. Raubenheimer, pers. comm.). This leads to an interesting question about the effect of high protein diets in laboratory experiments. Worker bees sustained on diets known to promote ovarian development will facilitate investigations on the correlation between ovarian development and production of mandibular gland pheromones.

Nectar of *Aloe greatheadii* var *davyana*: a water source in dry winter atmospheres

In assessing the nectar reward we also looked at the nectary structure and nectar presentation of an aloe species with open flowers belonging to a different section of the genus *Aloe*. However, anatomical differences between the nectaries of *A. castanea* and *A. greatheadii* var *davyana* were not correlated with the greater nectar secretion in *A. castanea* (Nicolson & Nepi, 2005). Variation in nectar (volume and concentration) of *A. greatheadii* var *davyana* was investigated on various levels, from within flowers to across its distribution range. The distinct basal swelling (bulb) at the bottom of the tubular flowers of *A. greatheadii* var *davyana* is a characteristic feature of the section Pictae (spotted aloes) (Glen & Hardy, 2000). The observed gradient in nectar concentration between the bulb and the tube can be explained by slight evaporation that may occur in the floral tube. The microclimate in the tubular flowers delays evaporation (Plowright, 1987), ensuring a constant concentration of the nectar available to foragers throughout the day, in spite of extremely low ambient humidities during the flowering period. The nectar, which from a bee perspective may be dilute (Southwick & Pimentel, 1981), is more concentrated than nectar measured in other *Aloe* species. Although the standing crop volume of nectar (15 µl) is lower than the mean residual nectar measured at two study sites after bee visitation (17 and 23 µl respectively), a large proportion of this nectar (10-12 µl) is inaccessible to honeybees and remains in the bulb of the flowers. The observed gradient between floral bulb and tube nectar adds a new level of variation in nectar studies.

Aloe greatheadii var *davyana* flowers in dry winter months when little else is flowering and when the water needs of honeybee colonies may increase (Johansson & Johansson, 1978). Typical winters in the summer rainfall area are characterised by pronounced diurnal temperature changes and very low relative humidity. Leaf succulence enables *A. greatheadii* var *davyana* to provide abundant dilute nectar that thus rewards honeybees with both energy and water, compensating for the low relative humidity and the evaporative costs associated with flying in dry air.

Utilisation of dilute nectar will require elimination of a greater amount of excess water by honeybees and it is possible that this process can begin in the field prior to unloading in the hive. Studies by Park (1932) and Oertel et al. (1951) contributed to the common assumption that the concentration of nectar is either slightly diluted or unchanged en route to the hive and only concentrated after unloading in the hive. Contrary to this assumption, we observed dramatic increases in nectar concentration between the flowers and the crops of bees captured at the flowers, with a further increase in returning bees captured at the hive entrance. Obviously, using the concentration of nectar in the forager's crop as an accurate indication of the nectar concentration of the flowers it has been visiting and therefore as a method of sampling nectar (see Roubik & Buchmann, 1984; Roubik et al., 1995) needs careful reconsideration.

The dilute nectar of *A. greatheadii* var *davyana* is not a problem for water balance at a colonial level, since low ambient humidities during the flowering season facilitate evaporation. Honeybees are highly efficient at regulation of hive temperature but little is known about the regulation of humidity in spite of its supposedly important role in brood development (Lindauer, 1955; Büdel, 1960; Doull, 1976b). Earlier measurements of humidity were made in hives with large monitoring devices such as hygrothermographs (e.g. Oertel, 1949) and led to the conclusion that humidity in beehives simply follows variations in temperature without active regulation (Lindauer, 1955; Simpson, 1961). Our use of miniaturised data loggers to measure humidity in different parts of the nest showed that bees are also able to adjust nest humidity within sub-optimal limits. The daily fluctuations in humidity that we observed could be the result of the honey ripening process (and timing thereof) and may also be influenced by the number of workers inside the hive at a given time. The regulation of humidity is influenced by trade-offs with regulation of temperature and respiratory gas exchanges

(e.g. Seeley, 1974; Korb & Linsenmair, 1998; Kleineidam & Roces, 2000). Further investigation into the regulation of humidity (actively or passively) is underway.

Pollination of *Aloe greatheadii* var *davyana* does not fit the pollination syndrome

Most of the characteristic floral features associated with bird pollination (Thomson et al., 2000) are exhibited by *A. greatheadii* var *davyana* flowers, such as conspicuous pinkish-red flowers, long tubular corollas, copious, dilute and unscented nectar. Even though these aloes are visited by at least 11 species of birds, exclusion experiments showed that honeybees are the primary pollinators. This is in contrast with other *Aloe* species that are pollinated by sunbirds and other passerine birds (Chapter 7) but similar to *A. ferox* which is, in spite of the expected sunbird pollination, pollinated by both birds and bees (Hoffman, 1988). Although it is common for bird-pollinated flowers to have flowers with a downward floral orientation (Aizen, 2003), the low effect of birds on fruit set in this study can be attributed to the combination of a downward orientation of the flowers on inflorescence stalks that may not be suitable for larger perching birds such as bulbuls thereby making the nectar less accessible to these birds and the eating of flowers by birds (pers. obs.). The slender inflorescence stalks of *A. greatheadii* var *davyana* are however, not a problem for sunbirds therefore they may be contributing to the pollination of this aloe. One should be aware that appearances could be deceiving. The usefulness of pollination syndromes in predicting pollinators has been questioned in recent literature (see Hargreaves et al., 2004). A critical evaluation and investigation of assigned floral syndromes is therefore needed in pollination studies.

This broad study of the interactions between honeybees and *A. greatheadii* var *davyana* serves to highlight the beneficial two-way interaction and simultaneously the paradox of *A. greatheadii* var *davyana* and bees. Honeybees are entirely dependent on the flowers of *A. greatheadii* var *davyana* in winter when little else is available. These aloes offer copious amounts of pollen that provides all the nutritional requirements of larval and adult honeybees and results in extensive brood production. Beekeepers depend on the aloe flowering season to increase their colony numbers by division. *Aloe greatheadii* var *davyana* flowers in an arid setting, but offers dilute nectar that serves as both water and energy rewards. In spite of the apparent ornithophilous syndrome and the fact that a large proportion of the nectar remains inaccessible to bees, hidden in the bulb, these

aloes ensure a constant availability of nectar at a concentration ideally suited to the needs of honeybees and that enables beekeepers to harvest a substantial honey crop. In return for the floral rewards they utilise, bees are the primary pollinators of the aloe.

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