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# Floral traits mediate the vulnerability of aloes to pollen theft and inefficient pollination by bees

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• *Background and Aims* Pollen-collecting bees are among the most important pollinators globally, but are also the most common pollen thieves and can significantly reduce plant reproduction. The pollination efficiency of pollen collectors depends on the frequency of their visits to female(-phase) flowers, contact with stigmas and deposition of pollen of sufficient quantity and quality to fertilize ovules. Here we investigate the relative importance of these components, and the hypothesis that floral and inflorescence characteristics mediate the pollination role of pollen collection by bees.
• *Methods* For ten *Aloe* species that differ extensively in floral and inflorescence traits, we experimentally excluded potential bird pollinators to quantify the contributions of insect visitors to pollen removal, pollen deposition and seed production. We measured corolla width and depth to determine nectar accessibility, and the phenology of anther dehiscence and stigma receptivity to quantify herkogamy and dichogamy. Further, we compiled all published bird-exclusion studies of aloes, and compared insect pollination success with floral morphology.
• *Key Results* Species varied from exclusively insect pollinated, to exclusively bird pollinated but subject to extensive pollen theft by insects. Nectar inaccessibility and strong dichogamy inhibited pollination by pollen-collecting bees by discouraging visits to female-phase (i.e. pollenless) flowers. For species with large inflorescences of pollen-rich flowers, pollen collectors successfully deposited pollen, but of such low quality (probably self-pollen) that they made almost no contribution to seed set. Indeed, considering all published bird-exclusion studies (17 species in total), insect pollination efficiency varied significantly with floral shape.
• *Conclusions* Species-specific floral and inflorescence characteristics, especially nectar accessibility and dichogamy, control the efficiency of pollen-collecting bees as pollinators of aloes.

**Key words:** Pollen theft, pollination efficiency, dichogamy, floral morphology, *Aloe*, Alooideae, Xanthorrhoeaceae, Asphodeloideae.

## INTRODUCTION

Bees are globally important pollinators of many plant species (Danforth *et al.*, 2006); however, because they collect considerable pollen to feed their larvae (Muller *et al.*, 2006), their flower visits can seriously impact plant reproduction if they provide little or no pollen transfer (Thomson and Thomson, 1992; Hargreaves *et al.*, 2009). The role of pollen-collecting bees as pollen vectors ranges from essential participants in specialized pollination systems, especially buzz pollination (Buchmann, 1983), through inefficient pollinators (Thomson, 2003; Johnson *et al.*, 2006), to pollen thieves that effect no pollination (Hargreaves *et al.*, 2009). In the latter cases, pollen collection by bees can directly reduce pollen transfer efficiency (Hargreaves *et al.*, 2010), siring success (Lau and Galloway, 2004) and seed production (Vaughton, 1996; Gross and Mackay, 1998; do Carmo *et al.*, 2004; Hargreaves *et al.*, 2010), with both evolutionary and ecological implications for plants (Hargreaves *et al.*, 2009).

Most pollen thieves pollinate some plant species (Hargreaves *et al.*, 2009), suggesting that the outcome of pollen collection depends primarily on plant, rather than animal, characteristics. Strong separation between pollen presentation and stigma receptivity in either time (dichogamy) or space (e.g. herkogamy, monoecy and dioecy) is a commonly cited mechanism causing pollen collectors to act as inefficient pollinators or pollen thieves (Hargreaves *et al.*, 2009). Herkogamy and dichogamy are common among hermaphroditic plants (Lloyd and Webb, 1986; Webb and Lloyd, 1986; Bertin, 1993; Bertin and Newman, 1993) and reduce interference between male and female function (Barrett, 2002). However, their beneficial effects may be compromised if they also reduce either visitation to female(-phase) flowers or stigma contact by pollen collectors.

Pollen theft could also result solely from animal characteristics, if visitors contact receptive stigmas but still do not effect pollination. Clear examples include pollen theft from stigmatic surfaces, whereby bees remove pollen previously deposited by legitimate pollinators (Gross and Mackay, 1998), and stigma contact by animals that carry insufficient or unavailable (e.g. in scopae) pollen. Alternatively, visitor behaviour may result in deposition of only poor-quality heterospecific and/or self-pollen (Aizen and Harder, 2007), resulting in inadequate, rather than inefficient, pollination.

Although pollen theft is probably common, with potentially significant consequences for plant reproduction, it has rarely been studied and its causes have not been investigated directly (Hargreaves *et al.*, 2009). We therefore assessed four non-exclusive hypotheses for why pollen collection might fail to result in seed set for some hermaphroditic flowers: (1) pollen collectors visit only male-phase flowers, as female-phase flowers do not present pollen (dichogamy); (2) pollen collectors visit receptive flowers, but do not contact stigmas (herkogamy); (3) pollen collectors contact stigmas, but do not deposit pollen; and (4) pollen collectors deposit only poor-quality pollen (Table 1). We addressed these hypotheses for ten *Aloe* species (Xanthorrhoeaceae, formerly Asphodelaceae; Angoiosperm Phylogeny Group, 2009) that represent the spectrum of floral forms within this genus of approx. 400 species (Heywood *et al.*, 2007). Most aloes share a basic tubular floral morphology, but species vary greatly in their relationships with pollen-collecting insects: some are entirely bee pollinated (Hargreaves *et al.*, 2008), whereas others are entirely bird pollinated, despite being visited frequently by pollen-collecting bees (Stokes and Yeaton, 1995; Botes *et al.*, 2009*a*). To test these hypotheses, we (1) experimentally compared pollen removal, pollen deposition and seed production resulting from insect visitation alone with that resulting from all visitors combined; (2) measured floral phenology to assess the temporal and spatial overlap between male and female function; and (3) observed insect visitors to characterize their foraging. Results of bird-exclusion experiments and some pollinator observations have been presented previously for *A. vryheidensis*, *A. inconspicua* and *A. maculata* (Johnson *et al.*, 2006; Hargreaves *et al.*, 2008, 2010); other data are presented here for the first time, enabling a novel examination of an infrageneric pattern of vulnerability to inefficient pollination and pollen theft.

## MATERIALS AND METHODS

### *Study species and location*

Aloes are succulent, perennial monocots, with flowers generally produced on vertical inflorescences. We studied ten *Aloe* species in KwaZulu-Natal, South Africa, during 2005 (for locations see Supplementary Data Table S1). These species represent the four main combinations of flower and inflorescence types found in the genus: (1) loose racemes of long, narrow flowers (*A. arborescens*, *A. boylei* and *A. maculata*; Fig. 1A, E); (2) dense racemes of medium length, narrow flowers with highly exserted anthers and stigmas (*A. ferox* and *A. marlothii*; Fig. 1B, C); (3) small to mid-sized inflorescences of short, tubular flowers with constricted corolla mouths (*A. dominella*, *A. inconspicua*, *A. kraussii* and *A. tenuior*; Fig. 1F, G); and (4) dense racemes of short, campanulate flowers (*A. vryheidensis*; Fig. 1D). Categories 1, 2 and 4 comprise putatively bird-pollinated species, whereas the third also includes insect-pollinated species (Botes *et al.*, 2008; Hargreaves *et al.*, 2008, 2010).

### *Floral morphology and intrafloral phenology*

Floral traits and phenology were examined for 5–20 flowers on 3–13 plants of all species, except *A. ferox* and *A. marlothii*, for which data were obtained from Hoffman (1988) and Reynolds (1950), respectively. Measurements were made in the field, when possible, and on cut inflorescences for *A. boylei*, *A. dominella* and *A. kraussii*, which flower and produce nectar normally when kept hydrated. For *A. vryheidensis* we conducted

TABLE 1. *Hypotheses about why insects act as inefficient pollinators or pollen thieves (hypotheses 1–3) or as poor-quality pollinators (hypothesis 4) for some hermaphroditic plant species. (A) Summary of the predictions generated by each hypothesis. (B) Details of which predictions were supported for six bird-pollinated* Aloe *species studied here, for which insect visitation alone resulted in incomplete to negligible seed production*

| | | Floral phenology‡ | | Insect behaviour | | | |
|---|---|---|---|---|---|---|---|
| | Deposition cage < open | Dichogamy insect < bird | Herkogamy insect < bird | Collect pollen only | No stigma contact | Less interplant movement than birds | Supported hypotheses |
| (A) Hypothesis | | | | | | | |
| (1) Dichogamy: do not visit female-phase flowers | Yes | Yes | | Yes | | | |
| (2) Herkogamy: do not contact receptive stigmas | Yes | No | Yes | | Yes | | |
| (3) Do not deposit pollen | Yes | No | No | | No | | |
| (4) Deposit only poor-quality pollen | No | No | No | | No | Yes | |
| (B) Species | | | | | | | |
| *A. arborescens* | No | Yes | No | Yes | No* | | 4 |
| *A. boylei* | Yes | Yes | No | No | No | | 3 |
| *A. ferox* | No | No* | No* | Yes | No† | Yes | 4 |
| *A. maculata* | Yes | Yes | No | Yes | | | 1 or 3 |
| *A. marlothii* | | | | Yes | | | |
| *A. vryheidensis* | | No | No | Yes | No | | 4 |

* Hoffman (1988).
† No stigma contact observed, but stigmas of caged flowers received considerable pollen.
‡ Floral phenology predictions compare insect- vs. bird-pollinated species.

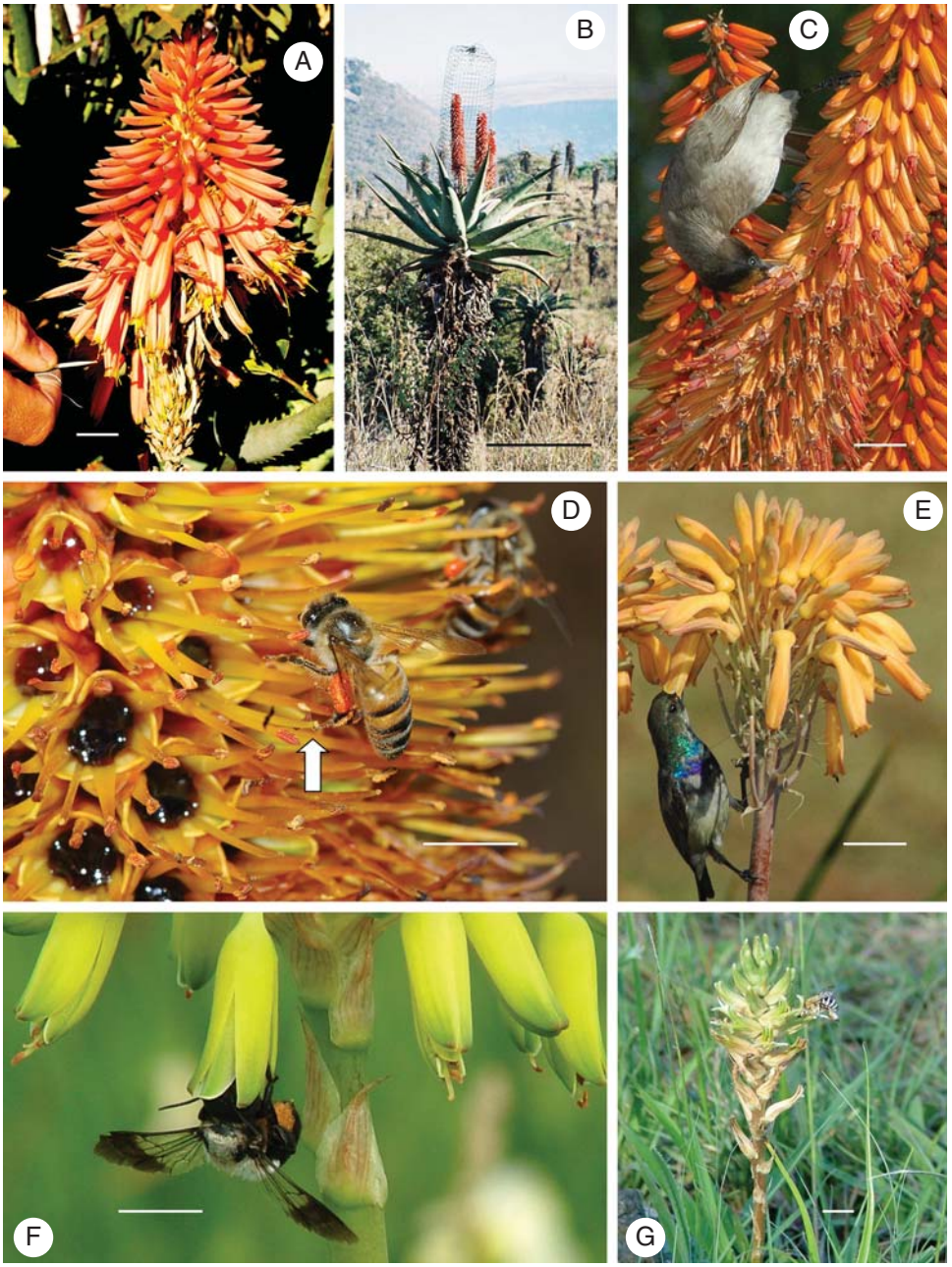


FIG. 1. Representative *Aloe* species and their visitors. (A) Hand-pollination of female-phase *A. arborescens* flowers (scale bar = 20 mm). (B) *Aloe ferox* with one raceme caged to exclude birds (scale bar = 50 cm). (C) Black-capped bulbul feeding on *A. ferox* and carrying visible pollen on its forehead (scale bar = 20 mm). (D) Honey-bees collecting pollen from *A. vryheidensis* (arrow shows stigma at stage 3 with pollen: scale bar = 10 mm). (E) White-bellied sunbird on *A. maculata* (scale bar = 20 mm). (F) Megachilid bee probing for nectar from *A. kraussii* with pollen clearly visible on its abdomen. Note exserted stigma with pollen in the flower to the right of the bee (scale bar = 10 mm). (G) *Amegilla fallax* probing *A. inconspicua*. Note pollen in the scopae (scale bar = 10 mm). Photographs: (A, B), A. L. Hargreaves; (C–E) S. D. Johnson; (F, G) G. T. Langston.

a less-detailed assessment of floral phenology using high-resolution photographs, from which we assessed anther dehiscence, the state of stigmatic papillae and whether pollen was visible on stigmas (Fig. 1D). As indicators of nectar accessibility, we measured the width of the corolla mouth, and corolla depth [corolla opening to the top of the ovary, the location of the septal nectaries (Schnepf and Pross, 1976), where nectar could be collected using microcapillary tubes]. To assess herkogamy we measured the maximum and minimum exsertion of dehisced anthers (corolla mouth to the tip of the longest and base of the shortest open anthers, respectively) and stigma exsertion; negative exsertion indicates that sexual organs were included within the corolla tube. We counted the dehisced anthers and recorded whether they retained pollen. Stigma age was scored developmentally: 0, papillae not or half expanded, stigma white; 1, papillae expanded, stigma white; 2, papillae expanded or maximally expanded, stigma turning translucent; 3, papillae maximally expanded, stigma translucent and often moist; 4, stigma drying,

style turning brown; and 5, papillae shrunken, stigma dry and brown, flower closing or closed. The volume and sugar concentration of nectar standing crop were measured from flowers of various ages using microcapillary tubes and a hand-held Bellingham and Stanley refractometer, respectively. To assess floral phenology, we marked buds and took a series of measurements every 2 h during daylight (from 0700 to 1700 h; phenology slowed greatly nocturnally).

Stigma receptivity was determined by cross-pollinating virgin flowers during the five stages of stigma development. Styles were collected 24 h after pollination and kept in 70 % ethanol. Styles were later rinsed in water, softened in 0·8 M NaOH for 6–12 h, rinsed again and stained using 0·1 % aniline blue for 24 h. Stained styles were mounted in glycerine and viewed at ×100 using UV microscopy (Leica Aristoplan epifluorescence microscope with filter system A) to count the pollen grains attached to the stigma and the pollen tubes that reached the base of the style (Kearns and Inouye, 1993). We define initial stigma receptivity as the earliest stage during which pollen adhered to and germinated on stigmas, such that pollen tubes could be seen in the distal third of collected styles. Too few *A. inconspicua* flowers were available to assess receptivity this way. Instead, we compare the seed set of flowers hand pollinated during stigma stages 2–3 from another study (Hargreaves *et al.*, 2008) with that of open-pollinated flowers.

We collected limited data for *A. dominella* and *A. tenuior*. In the sole *A. dominella* population we found, all 30 plants were damaged by florivorous beetles. We measured phenology of undamaged flowers from the least damaged inflorescences. This species is included despite incomplete data, because it is one of few aloes with discernibly scented flowers, and so provides an interesting addition to a comparison of bird- vs. insect-pollinated species. We were unable to study *A. tenuior* in the wild, but include morphological data from plants in the University of KwaZulu-Natal Botanical Garden (outside its natural range), as this species belongs to a putatively basal *Aloe* lineage (Holland, 1978; Treutlein *et al.*, 2003).

### *Flower visitors*

To evaluate the abundance and effectiveness of floral visitors, we observed wild plants of each species for ≥4 h during ≥3 d, except for *A. dominella*, which was observed for only 2 h as plants were too damaged and uncommon to attract visitors, and *A. tenuior*, which was observed in the botanical garden. Bird and insect visitors were counted during morning (0900–1100 h) and afternoon (1400–1600 h) surveys at each site. To estimate insect visitation, we observed 20 randomly selected plants long enough to count the insects on or flying around the inflorescences (approx. 1 min per plant). Insects were categorized into groups (e.g. honey-bee, megachilid bee), and specimens were collected for identification and to quantify pollen loads. Some insects were observed more closely to determine whether they collected nectar and/or pollen, whether they contacted stigmas, and the sexual stage of the flowers they visited (number of freshly dehisced anthers and/or stigma exsertion).

Bird visitors were counted using transect surveys or, for *A. kraussii* and *A. inconspicua*, patch observations. We walked transects through populations for 30 min, with five observation stops of 5 min each plus 5 min for walking between stops (15–30 m). Patch observations involved observing a pre-defined group of flowering aloes from a hidden location for 10–30 min. All birds seen or heard in patches or within 10 m of transects were counted and identified.

### *Self-compatibility*

To determine the importance of outcross pollination, we compared seed set of hand-pollinated out-crossed, hand-pollinated selfed, and autogamous (unmanipulated) flowers on bagged inflorescences, following the methods detailed in Johnson *et al.* (2006). Styles were collected and viewed using UV microscopy to determine whether self- and outcross pollen tubes could be distinguished (Kearns and Inouye, 1993). Self-compatibility data were obtained from Hoffman (1988) for *A. ferox*; we have no self-compatibility information for *A. dominella*, *A. marlothii* and *A. tenuior*.

### *Pollination system*

The contribution of insect visitors to pollination was assessed experimentally by excluding birds from racemes (*A. arborescens*, *A ferox*, *A. marlothii* and *A. vryheidensis*) or whole plants (*A. boylei*, *A. inconspicua*, *A. kraussii* and *A. maculata*) with rigid plastic mesh cages that allowed insects to pass freely (Fig. 1B). Seed production was calculated as seeds/flower or seeds/raceme, and compared with that of unmanipulated plants or racemes that were open to all visitors. Swollen ovaries that were visually indistinguishable from fruits, but which contained no seeds, were counted as fruits for measures of fruit set, and their seed count (0) was included in seed set calculations.

To distinguish between the pollen quality and other hypotheses (Table 1), we compared pollen deposition and removal from caged and open flowers for five putatively bird-pollinated species (*A. arborescens*, *A. boylei*, *A. ferox*, *A. kraussii* and *A. maculata*). Stigmas and anthers were collected from two wilted flowers per treatment per plant from caged and open inflorescences and stored in 70 % ethanol. Freshly opened anthers were also collected from one virgin flower on each of 6–10 plants per species to assess pollen production. We counted the pollen grains on anthers using an Elzone 5380 particle analyser (as per Harder, 1990) or by hand using a light microscope (×100) in the case of some depleted anthers. We counted pollen grains on stigmas using a compound microscope (×100) after soaking stigmas in basic fuschin stain for at least 4 h, and squashing them in glycerin. Pollen transfer efficiency (PTE) was calculated, for open plants only, as a percentage: $100S/(P - R)$, where $S$ and $R$ are the site-specific pollen receipt and pollen remaining, respectively, and $P$ is the species' average pollen production. We estimated standard errors and 95 % confidence intervals from the standard deviation and 2·5 and 97·5 percentiles, respectively, of estimates calculated for 1000 bootstrapped pseudosamples (Efron, 1979).

We further explored the relationship between successful insect pollination and flower type by searching the literature for bird-exclusion experiments and incorporating all *Aloe* species for which such data were available (17 species). In addition to the species studied here, we included results for:

*A. minima* and *A. linearifolia* (Botes *et al.*, 2009*b*), *A. pluridens*, *A. lineata*, *A. africana* and *A. speciosa* (Botes *et al.*, 2009*a*), *A. greatheadii* var. *davyana* (Symes *et al.*, 2009), *A. divaricata* (Ratsirarson, 1995) and *A. pruinosa* (Wilson *et al.*, 2009). Based on our own observations or descriptions provided by Reynolds (1950) or van Wyk and Smith (2003), species were categorized into one of the four floral forms described above: short tubular (*A. inconspicua*, *A. kraussii*, *A. minima* and *A. linearifolia*), mid-length tubular with corolla entrances clogged by highly exserted anthers and stigma (*A. ferox*, *A. marlothii* and *A. speciosa*), long tubular (*A. arborescens*, *A. boylei*, *A. maculata*, *A. pluridens*, *A. lineata*, *A. africana*, *A. greatheadii* var. *davyana*, *A. divaricata* and *A. pruinosa*), and short open (*A. vryheidensis*). As a measure of insect pollination we calculated the ratio of mean reproductive success for caged plants relative to open-pollinated plants for each species. We used mean seeds/flower when available, mean seeds/raceme for *A. arborescens*, *A. ferox* and *A. marlothii* (this study), and mean fruit set for *A. divaricata*, for which seed set was not counted (Ratsirarson, 1995).

### *Statistical analysis*

Species comparisons involved general linear models (Kutner *et al.*, 2005: proc mixed SAS 9·2) for pollen remaining in anthers (after log transformation) and floral morphology, and generalized linear models (McCullagh and Nelder, 1989: proc genmod SAS 9·2) for seed production. Generalized linear models considered negative binomial distributions (seed production) or gamma distributions (seed production ratio) and a ln-link function. Models initially considered all interactions between independent variables, but non-significant interactions were excluded using backward elimination ($\alpha = 0{\cdot}05$). Significant main effects and interactions were explored further using multiple comparison tests and the Dunn–Šidák procedure (Kirk, 1995). Estimates of least-squares means and standard errors derived from log-transformed data were back-transformed for presentation.

We used randomized general linear mixed models to compare pollen receipt, as the distributions of response variables differed among species. These tests compared the *F*-statistics from the linear model for the original data with those calculated for linear models conducted on 1000 randomizations of the original data (Manly, 1997: SAS 9·2). The test probabilities that we report for these analyses represent the proportion of randomized *F*-tests that exceeded the *F*-tests for the original data.

Plants were sampled repeatedly during all experiments, so that different observations for individual plants are not independent. For floral phenology, we averaged observations for each plant prior to analysis. For other variables, we used repeated-measures analyses that incorporated a variance–covariance model of compound symmetry to account for correlated responses, using the methods of either Kenward and Roger (1997: general linear models), or Liang and Zeger (1986: generalized linear models). In the latter case, significance tests involved score statistics ($T$) generated using generalized estimating equations.

## RESULTS

### *Floral characteristics*

The ten *Aloe* species differed extensively in both qualitative and quantitative floral characteristics. Flowers of *A. arborescens*, *A. maculata* and *A. boylei* were significantly deeper than those of other species (Table 2). Corollas of *A. ferox* and *A. marlothii* were intermediate in depth, but congestion of the corolla mouth by exserted anther filaments and styles nonetheless precluded access by insects (Fig. 1C). Among species with short flowers, *A. vryheidensis* flowers

TABLE 2. *Flower and inflorescence characteristics of ten* Aloe *species*

| Flower shape and *Aloe* species | Flower colour | Corolla depth (mm): mean (s.e.) | Corolla width (mm): mean (s.e.) | Nectar volume (μL): mean | Nectar concentration (% sugar): mean | Pollen per flower (1000 grains): mean (s.e.) | Flowers per raceme: median | Racemes per plant: median (max.) |
|---|---|---|---|---|---|---|---|---|
| Long, tubular | | | | | | | | |
| *A. arborescens* | Orange | 32·1 (0·25)[a] | 3·7 (0·07)[b] | 39·6 | 12·1 | 108 (13·4)[b] | 168 | 5 (16) |
| *A. boylei* | Orange | 30·3 (0·71)[a] | 3·8 (0·44)[b] | 19·4 | 22·1 | 180 (12·0)[a] | 40 | 1 |
| *A. maculata* | Orange | 31·0 (0·78)[a] | 4·3 (0·16)[b] | 32·2* | 16·6* | 101 (9·3)[b] | 34 | 3 (7) |
| Mid-length, tubular | | | | | | | | |
| *A. ferox* | Orange | 25·5 (0·62) | 3·8 (0·17) | 81·3* | 8·6* | 215 (19·0)[a] | 400 | 5 (12) |
| *A. marlothii* | Yellow-orange | 23·5[†] | | 46·6* | 8·3* | | 300 | 6 (13) |
| Short, tubular | | | | | | | | |
| *A. dominella* | Yellow | 14·3 (0·17)[b] | 2·6 (0·10)[c] | 4·5 | 20·5 | | 45 | 1 |
| *A. inconspicua* | Whitish green | 7·8 (0·63)[c] | 1·8 (0·09)[c] | Trace[‡] | | | 23 | 1 |
| *A. kraussii* | Yellow | 10·7 (0·19)[c] | 1·6 (0·08)$_c$ | 2·2 | 19·1 | 75·3 (13·5)[b] | 67 | 1 (2) |
| *A. tenuior* | Orange | 14·1 (0·75)[b] | 2·2 (0·15)[c] | 1·2 | 29·6 | | 56 | |
| Short, open | | | | | | | | |
| *A. vryheidensis* | Yellow | 13·7 (0·37)[b] | 11·3 (0·35)[a] | 41·1 | 11·6 | | 500 | 2 (4) |

* Unpublished data from S. W. Nicholson.
† Reynolds (1950).
‡ Even bagged *A. inconspicua* flowers produced too little nectar to allow measurement of its concentration.
Superscript letters denote significant differences among species for a given trait (Dunn-Šidák procedure, $\alpha = 0{\cdot}05$).

were distinguished by their wider corolla opening and abundant nectar (Fig. 1D, Table 2). *Aloe inconspicua* produced the smallest flowers of the species studied, which were also notable for their white-green colour. Pollen production varied significantly among species for which it was measured, ($F_{4,30} = 23{\cdot}08$, $P < 0{\cdot}0001$), being three times greater in *A. ferox* than in *A. kraussii* (Table 2).

### *Flower visitors*

*Birds.* Bird visitors to *Aloe* species ranged from sunbirds (Nectariniidae) alone, to a mixture of sunbirds and short-billed birds (Dicruridae, Ploceidae, Pycnonotidae and Timaliidae), to short-billed birds alone (visitor abundance is given in Supplementary Data Table S2). *Aloe inconspicua* was never visited by birds, even though species known to visit aloes were abundant at the site. Too few observations were made of *A. dominella* and *A. tenuior* to be certain that birds do not visit them.

Long-billed sunbirds effectively pollinated several *Aloe* species. *Aloe* pollen was visible on sunbirds foraging at *A. ferox* and *A. arborescens*, and aviary experiments confirmed that sunbirds transferred significant quantities of pollen from anthers to receptive stigmas for *A. maculata* (251 grains/stigma, lower s.e. = 29·1, upper s.e. = 33·0; Hargreaves *et al.*, 2010) and *A. kraussii* (mean ± s.e. = 79·2 ± 9·5 grains/stigma; A. L. Hargreaves, unpubl. res.). Per plant visit, sunbirds probed 2·9 ± 0·25 flowers on *A. kraussii* (14 birds), 7·6 ± 2·6 flowers on *A. ferox* (three birds), 7·9 ± 1·2 flowers on *A. maculata* (21 birds) and 9·3 ± 2·2 flowers on *A. arborescens* (18 birds).

Various short-billed birds, including bulbuls (*Pycnonotus tricolor*), drongos (*Dicrurus adsimilis*), white-eyes (*Zosterops pallidus*) and several weaver species (Ploceidae) visited *A. ferox*, *A. marlothii* and *A. vryheidensis* regularly (Supplementary Data Table S2) and often carried >100 000 pollen grains (Johnson *et al.*, 2006; Hargreaves, 2007). Short-billed birds probed 6·7 ± 4·6 flowers per plant on *A. ferox* (seven birds) and 13·4 ± 6·1 flowers on *A. vryheidensis* (eight birds). Several short-billed species robbed nectar from the bases of *A. arborescens* flowers, and streaky-headed seedeaters (*Crithagra gularis*: Fringillidae) robbed nectar from *A. maculata* and *A. marlothii*, sometimes damaging the style and/or ovary.

*Insects.* Bees (Apoidea) commonly visited all *Aloe* species (Supplementary Data Table S2), whereas other insects visited rarely and were unlikely to act as pollinators. Honey-bees (*Apis mellifera*) were the most common insect visitors to the primarily bird-pollinated species, except *A. maculata* and *A. vryheidensis*, which were visited even more often by small (halictid or allodapine) bees. Per plant, honey-bees visited 16 ± 10·1 flowers on *A. ferox* (four bees) and 2·4 ± 0·24 flowers on *A. kraussii* (eight bees). Although many honey-bees foraged on nearby species, none visited *A. inconspicua* during >25 h of observation. Instead, *Amegilla fallax* (Apidae) was the primary visitor to this

TABLE 3. *Characteristics of bee visits to* Aloe *flowers, including: the ratio of nectar- (N) to pollen-collecting (P) flower visits, the ratio of visits to female- (F) vs. male-phase (M) flowers, and percentage of visits during which the stigma was or was not contacted*

| Flower shape and *Aloe* species | Bee type (*n*) | N : P visits | F : M flowers[‡] | % flower visits with stigma contact (*n*)[#] |
|---|---|---|---|---|
| Long tubular flowers | | | | |
| *A. arborescens* | Honey-bee (7) | 8 : 23 | 14 : 22 | 41 % (17) |
| *A. boylei* | Honey-bee (3) | 3 : 4 | – | 0 % (1) |
| | Small bee (1) | 0 : 1 | 0 : 1 | 0 % (1) |
| | *Amegilla* (4) | 12* | 8 : 8 | Likely |
| *A. maculata* | Honey-bee (30) | 1 : 55 | Only M | Unlikely |
| | Allodapine (14) | 0 : 14 | Only M | – |
| Mid-length tubular flowers | | | | |
| *A. ferox* | Honey-bee (4) | 3 : 107 | 2 : 104 | 5 % (21) |
| *A. marlothii* | Honey-bee (4) | 0 : 10 | 0 : 10 | – |
| Short, tubular | | | | |
| *A. inconspicua* | *Amegilla* (31) | 244 : 9 | All | 89 % (27) |
| *A. kraussii* | Honey-bee (8) | 3 : 30 | 2 : 36 | 9 % (22) |
| | Megachilid (3) | 12[†] | Both | 100 % (12) |
| | *Amegilla* (4) | 6 : 0 | Both | 75 % (4) |
| *A. tenuior* | Allodapine | 72 : 11 | 10 : 19 | 50 % (8) |
| Short, wide | | | | |
| *A. vryheidensis* | Honey-bee (61) | All P | Only M | Likely |
| | Halictid (133) | All P | Only M | – |
| | *Xylocopa* (3) | All P | Only M | 100 % (30) |

* Bees moved too quickly to observe distinct pollen-collecting behaviour, but they carried *A. boylei* pollen.

[†] Bees collected both nectar and pollen during observed visits.

[‡] 'Both' indicates that bees did not seem to discriminate among male and female flowers, whereas 'all' indicates that bees visited all open flowers on a plant.

[#] When stigma contact frequency could not be observed directly (e.g. stigma enclosed), it is noted as 'likely' or 'unlikely' based on visitor behaviour; *n* = flower visits for which the occurrence or absence of stigma contact could be observed.

Sample sizes differ among columns depending on the ease of observing the phenomenon while following a bee. Note that observation effort varied among species, so visitor abundance cannot be compared directly.

species (Hargreaves *et al.*, 2008). Only pollen-collecting allodapine bees visited *A. tenuior* in the botanical garden.

Resource collection by bees varied among *Aloe* species, largely according to floral form (Table 3). *Aloe vryheidensis* was the only species visited exclusively for pollen, as bees avoided consuming the readily available, but phenolic-rich, nectar (Johnson *et al.*, 2006). Flowers of species with long corollas were also visited mostly for pollen (Table 3), except for those of *A. boylei*, which *Amegilla natalensis* probed for nectar. These large bees moved too quickly to observe distinct pollen-collecting behaviour, but two captured individuals carried 990 and 2750 grains of *A. boylei* pollen, mostly on their scopae. Bees readily consumed nectar that had seeped from the mouths of *A. ferox* corollas and from holes made by nectar-robbing birds in *A. maculata* and *A. arborescens* flowers, so their lack of nectar collection from these species probably reflects difficulty accessing it. Aloes with short, tubular corollas were visited most often by nectar-collecting insects, which could access nectar legitimately via the corolla mouth. Pollen collection from these species generally occurred while insects probed for nectar.

The likelihood that bees contacted stigmas also varied among *Aloe* floral forms (Table 3). The exserted anthers and stigmas on dense *A. vryheidensis* inflorescences facilitated pollen transfer by pollen-collecting honey-bees as they walked between flowers (Fig. 1D, Table 3). Although *A. marlothii* and *A. ferox* also have dense racemes, anthers and stigmas remain tightly clustered when exserted (Fig. 1C) and honey-bees generally flew between flowers instead of walking. Bees thus largely avoided pollenless female-phase flowers (Table 3), but would nonetheless have visited receptive stigmas if receptivity began during anther dehiscence, as reported for *A. ferox* (Hoffman, 1988). Nectar-collecting bees visited male- and female-phase flowers indiscriminately and pushed their heads into corollas while probing (e.g. Fig. 1F, G), probably contacting even enclosed stigmas of all species they visited.

### *Self-compatibility*

Examination of pollen tubes from flowers of *A. arborescens*, *A. boylei*, *A. dominella*, *A. kraussii*, *A. maculata* and *A. tenuior* revealed that self-pollen germinated successfully on stigmas and that self-pollen tubes were indistinguishable from outcross pollen tubes, growing normally and reaching the base of the style. However, self-pollinated flowers set $<5\,\%$ as many seeds as cross-pollinated flowers, and this difference was highly significant for all species, except those with few samples (Supplementary Data Table S3). Thus, these aloes appear to be self-incompatible, owing to a mechanism that acts in the ovary.

### *Pollination system*

Bird exclusion did not significantly affect pollen removal for any *Aloe* species (pollination treatment × species interaction, $F_{4,\ 430} = 1{\cdot}4$, $P > 0{\cdot}2$; Fig. 2A), but it did alter pollen receipt for some species (pollination treatment × species interaction, $F = 4{\cdot}310$, randomized $P < 0{\cdot}01$; Fig. 2B). Pollen receipt did not differ significantly between caged and

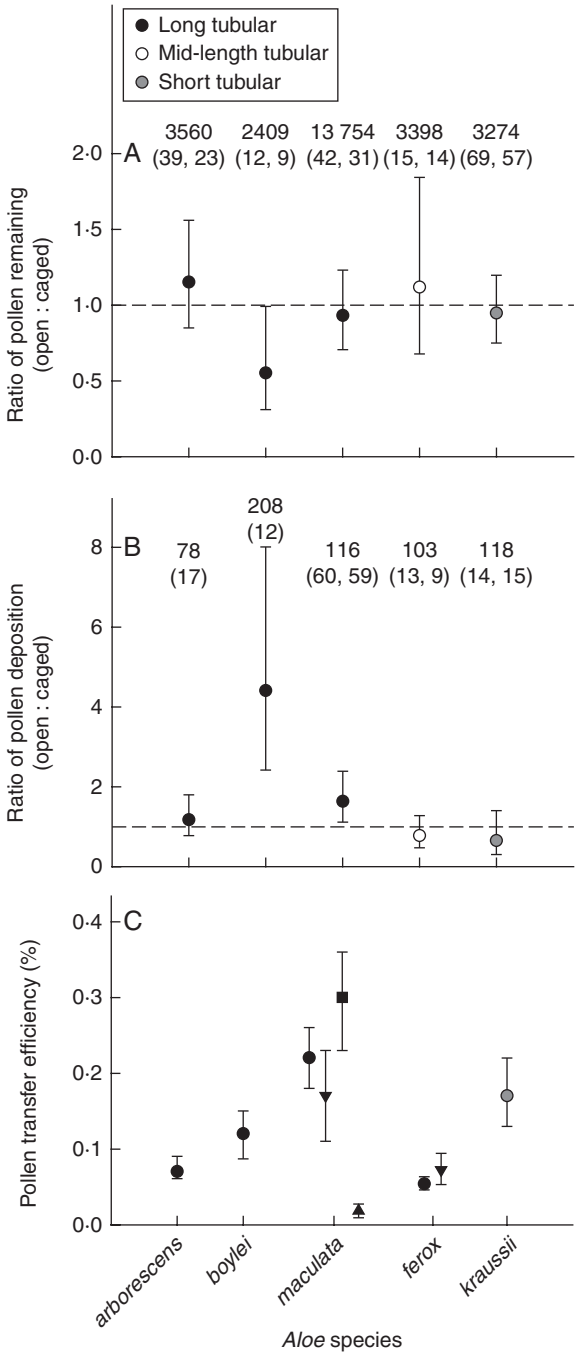


FIG. 2. Pollen transfer characteristics of *Aloe* species with long, mid-length or short tubular flowers, as indicated. (A, B) Ratio (±95 % confidence interval) of pollen remaining in anthers of wilted flowers (per flower; A) and pollen deposited on stigmas (B) by plants from which birds were excluded experimentally vs. open-pollinated plants. A ratio of 1 (dashed line) represents equality; thus confidence limits that do not include 1 indicate a significant difference with bird exclusion. Values above the bars indicate the mean number of pollen grains remaining in anthers (A) and deposited per stigma (B) for open plants; sample sizes are given in brackets, listed as 'caged, open' when sample sizes differed between treatments. (C) The site-specific percentage (±95 % confidence interval) of pollen removed from anthers that was found on stigmas of open-pollinated flowers (pollen transfer efficiency, PTE). For species studied at more than one site (*A. arborescens*, *A. maculate* and *A. ferox*), symbol shapes differentiate sites: Hilton (circle), Ixopo (inverted triangle), Klipfontein (square), Umgeni (upright triangle). Non-overlapping confidence intervals indicate significant differences.

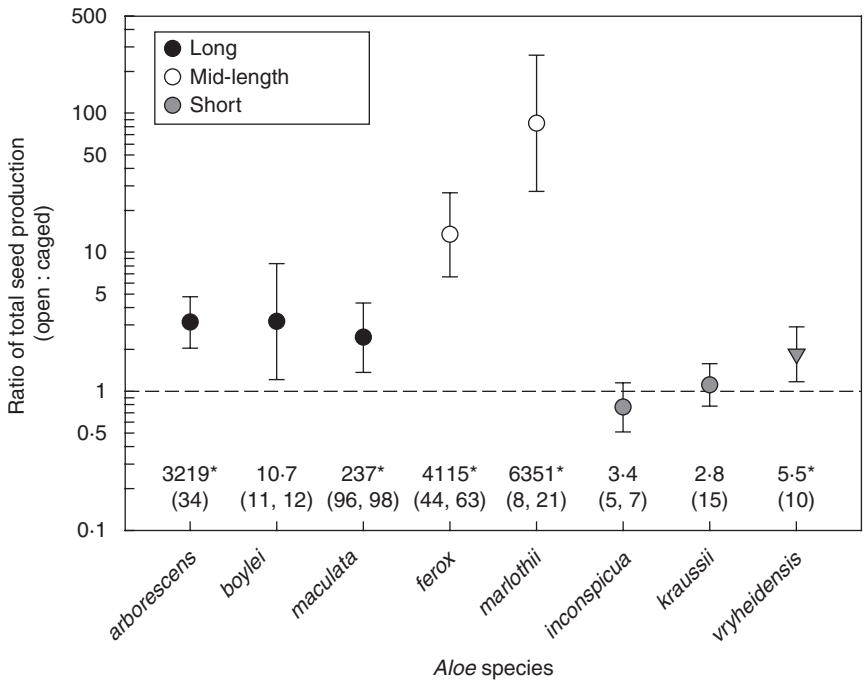


FIG. 3. Effect of experimental bird exclusion on seed production. Values are ratio (±95 % confidence intervals) of mean seed production by open-pollinated plants to mean seed production by caged plants. A ratio of 1 (dashed line) represents equality; thus confidence limits that do not include 1 indicate a significant reduction in seed production with bird exclusion. Values below the bars are means for open-pollinated plants, measured as seeds/flower (no asterisk) or seeds/raceme (*). Symbol shapes indicate whether corollas were tubular (circles) or open (triangle), and shading indicates whether corollas were long, mid-length or short, as shown in the key. Sample sizes (number of plants) are given in parentheses, listed as 'caged, open' when they differed between treatments.

open inflorescences of *A. arborescens*, *A. ferox* and *A. kraussii* (Fig. 2B), even though caged flowers of the first two species produced significantly fewer seeds than open flowers. Bird exclusion significantly reduced pollen receipt by flowers of *A. boylei* ($F_{1,\ 234} = 8{\cdot}33$, $P < 0{\cdot}01$) and *A. maculata* ($F_{1,\ 234} = 6{\cdot}45$, $P < 0{\cdot}05$; Fig. 2B). Aloes uniformly experienced inefficient pollination, as <0·5 % of the pollen removed from anthers reached stigmas (Fig. 2C). Pollen transfer efficiency varied widely among species and, for *A. maculata*, among populations (Fig. 2C).

The effect of bird exclusion on seed production varied significantly among *Aloe* species (pollination treatment × species interaction, $T_7 = 43{\cdot}11$, $P < 0{\cdot}001$; Fig. 3), but not among populations within species ($P > 0{\cdot}25$ in all cases; data are therefore averaged over conspecific populations). Bird exclusion reduced seed production for species with medium to long, tubular flowers by 69–98 %, and for *A. vryheidensis*, which has short campanulate flowers, by 45 % (Fig. 3). In contrast, bird exclusion did not significantly affect seed production by species with short, tubular flowers (*A. kraussii* and *A. inconspicua*; Fig. 3), indicating that bees effectively pollinate these species. Whereas *A. inconspicua* is entirely bee pollinated, sunbirds frequently visited *A. kraussii* in the field and successfully transferred its pollen in the aviary (A. L. Hargreaves, unpubl. res.), thus *A. kraussii* seems to have a dual pollination system. For all *Aloe* species for which bird-exclusion results are available, the extent of successful insect pollination was best predicted by floral shape category alone ($F_{3,\ 13} = 29{\cdot}61$, $P < 0{\cdot}0001$), rather than by corolla length ($F_{1,\ 12} = 2{\cdot}29$, $P > 0{\cdot}1$). Insects effected full seed production for species with short, tubular corollas, but almost no seed production for species with mid-length corollas congested by highly exserted anthers and stigmas (Fig. 4). The

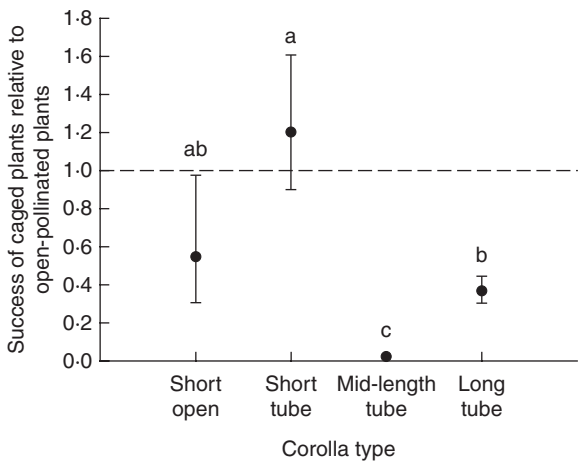


FIG. 4. Differences in insect-mediated seed production among flower-type categories, based on bird-exclusion experiments for 17 *Aloe* species. The ordinate represents the mean (± s.e.) ratio of reproductive success by caged plants (i.e. only insects contributed to pollination) to that by open-pollinated plants; a ratio of 1 (dashed line) represents equality. Differing letters above the error bars indicate significant differences among floral forms ($\alpha = 0{\cdot}05$). Based on data collected during this study and those reported by Ratsirarson (1995), Botes *et al.* (2009*a*, *b*), Symes *et al.* (2009) and Wilson (2009) (for details see Materials and Methods: Pollination systems).

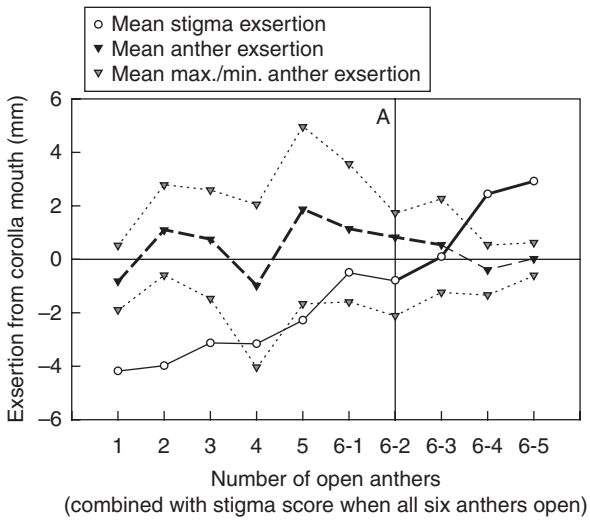


FIG. 5. Mean anther and stigma positions for *Aloe maculata* during a flower's life, as measured by the progression of anther dehiscence (1–6 open anthers), and subsequent stigma receptivity (see text). Mean stigma exsertion, mean anther exsertion, and mean minimum and maximum anther exsertion are indicated. Entirely recessed anthers are represented by an exsertion of −1. Thick lines for mean anther and stigma exsertion represent the (mean) period during which anthers bear pollen and the stigma is receptive, respectively. Reference line A marks the average occurrence of initial stigma receptivity.

contribution of insects to seed production for long-tubed species was intermediate between these extremes (Fig. 4).

### *Intrafloral phenology*

The timing of initial stigma receptivity differed among species. Stigma receptivity began during stigma stage 1 for *A. arborescens*, *A. boylei* and *A. kraussii*, and during stage 2 for *A. maculata*. Stigma appearance did not reliably predict receptivity for *A. dominella*, as pollen germinated on stigmas of all stages, even as flowers were closing and drying. Pollen did not adhere to *A. inconspicua* stigmas until they reached stage 1, whereas seed set of flowers hand-pollinated at stage 3 (mean = 14·5 seeds/fruit, s.e. = 1·17) was comparable with that of naturally pollinated flowers visited throughout stigma receptivity (12·6 seeds/fruit, s.e. = 0·73; $F_{1,\ 39} = 2{\cdot}95$, $P > 0{\cdot}09$). Stigmas of *A. inconspicua* were therefore assumed to become receptive during stage 2.

Among tubular-flowered species, stigmas were generally enclosed within corollas when the first anther opened (e.g. Fig. 5). As a flower matured, stamens elongated and anthers dehisced sequentially, eventually wilting and receding, and the style grew gradually, so that the stigma eventually surpassed the anthers (Fig. 5). In contrast, *A. vryheidensis* flowers open widely and all anthers and the stigma were exserted before anthers began dehiscing. By late female-phase in all species, anthers had wilted and receded and were ignored by pollen-collecting insects. During daylight, anthers were usually depleted of pollen within 4 h, although this varied depending on visitor abundance and the weather.

*Dichogamy.* Bird-pollinated species generally exhibited greater dichogamy than bee-pollinated species (Table 4), although the average number of anthers open at initial stigma receptivity did not differ significantly among species ($T_5 = 6{\cdot}29$, $P < 0{\cdot}2$; analysis does not include *A. vryheidensis* or *A. dominella* for which the exact timing of initial receptivity is unknown), perhaps owing to few samples. Stigmas of primarily bird-pollinated species with tubular flowers became receptive only at the end of pollen presentation (male phase), generally after at least five of six anthers had dehisced (Table 4). Late receptivity increased the likelihood of complete pollen removal before stigmas became receptive for *A. maculata*, but not for *A. arborescens* (Table 4). In contrast, pollen adhered to *A. vryheidensis* stigmas after as few as three anthers had dehisced and anthers retained pollen when stigmas appeared fully receptive (stages 2 and 3). Thus,

TABLE 4. *Temporal and spatial separation of male and female functions for flowers of eight* Aloe *species*

| Flower shape and *Aloe* species | *n* | Initial stigma receptivity: No. of open anthers: mean (s.e.) | Initial stigma receptivity: Hours since last anther dehisced: mean (s.e.) | Initial stigma receptivity: Proportion of flowers with depleted anthers (no. of flowers) | Initial stigma receptivity: Stigma–anther separation (mm): mean (s.e.) | Maximum receptivity Stigma–anther separation (mm) during maximum receptivity: mean (s.e.) | Throughout receptivity Proportion of adichogamous flowers throughout receptivity (no. of observations) |
|---|---|---|---|---|---|---|---|
| Long, tubular | | | | | | | |
| *A. arborescens* | 3 | 5·7 (0·22) | 0·79 (0·31) | 0·20 (10) | −0·91 (0·052) | 0·42 (0·27) | 0·48 (95) |
| *A. boylei* | 5 | 5·2 (0·35) | 0·86 (0·53) | | −0·47 (0·50) | | 0·31 (45) |
| *A. maculata* | 6 | 6·0 (0·07) | 5·8 (1·2) | 0·81 (37) | −1·91 (0·84) | −1·1 (1·1) | 0·32 (111) |
| Short, tubular | | | | | | | |
| *A. dominella* | 4 | 2 + | 0 | 0 | −1·7 (0·89) | | 0·42 (224) |
| *A. inconspicua* | 4 | 5·4 (0·26) | 2·0 (1·4) | 0·5 (10) | −1·0 (0·68) | −0·15 (0·21) | 0·43 (122) |
| *A. kraussii* | 13 | 3·4 (1·1) | 0 | 0 | −0·5 (0·42) | | 0·27 (124) |
| *A. tenuior* | 4 | 3·9 (0·79) | 0·3 (0·3) | 0 | −4·3 (1·4) | −2·0 (0·99) | 0·36 (150) |
| Short, open | | | | | | | |
| *A. vryheidensis* | 4 | 4 + | 0 | 0 | | | 1·0 (24) |

Initial receptivity is the earliest stigma stage (see text) during which pollen adhered to the stigma and/or germinated successfully, whereas maximum receptivity was determined with hand pollination (blank if maximum pollen adhesion and germination did not correspond to an observed stigma stage). Sample size (*n*) is the number of plants. Negative stigma–anther separation indicates that the anthers exceeded the stigma. Adichogamy indicates that flowers had ≤1 unwilted, undepleted anther while the stigma was receptive.

male and female functions apparently overlap more for this species than for bird-pollinated species with tubular flowers. Stigmas of bee-pollinated species generally became receptive relatively early during a flower's life, before all anthers dehisced and thus before all pollen was removed. Of the six *A. inconspicua* flowers for which stigma state could be assessed before all anthers had opened, only one was receptive before the last anther dehisced. Thus, stigmas of this species seem to become receptive later than those of the other bee-pollinated species.

*Herkogamy.* None of the three measures of herkogamy differed obviously between bee- and bird-pollinated species (Table 4). On average, stigmas were adjacent to anthers during initial and maximum receptivity for all species. Receptive stigmas of most species were level with pollen-bearing anthers during fewer than half of the observations (Table 4), although *A. vryheidensis* stigmas were level with the splayed anthers throughout pollen presentation.

## DISCUSSION

The ten *Aloe* species that we studied represent the full range of interactions with flower-visiting insects (Fig. 3). At one extreme, *A. inconspicua* was pollinated exclusively by bees, which collected nectar and pollen simultaneously. Indeed, pollen supplementation of this species did not increase seed production above that occurring naturally (Hargreaves *et al.*, 2008), indicating that bees provide a complete pollination service. Similarly, bird exclusion demonstrated that *A. kraussii* can be fully pollinated by nectar- and pollen-collecting bees, although sunbirds also visit it extensively, and transfer pollen successfully (A. L. Hargreaves, unpubl. res.). Given the consistent pollination role of bees for aloes with small, tubular flowers (Fig. 4), we expect that bees probably also pollinate *A. tenuior* and *A. dominella* effectively, although not necessarily exclusively. At the other extreme, species with dense racemes of tubular flowers (*A. ferox* and *A. marlothii*) set almost no seeds when birds were excluded, despite high bee visitation. Pollen-collecting bees were also negligible pollinators of these species at other sites (Stokes and Yeaton, 1995; Botes *et al.*, 2009*a*; Symes *et al.*, 2009), and for two similar species (*A. speciosa* and *A. africana*), whose corolla mouths are also congested with highly exserted anthers and stigmas (Botes *et al.*, 2009*a*; Fig. 4). Thus, pollen collectors consistently fail to effect seed set for aloes with this floral morphology, and instead act as highly ineffective pollinators (depositing pollen without effecting seed set) or even as pollen thieves (no deposition). Surprisingly, the *Aloe* species whose flowers best conform to the classic bird-pollination syndrome, with loose racemes of long, narrow, orange to red flowers, received some insect pollination, although not enough for full seed set (Figs 3 and 4). Previous studies of sunbird-pollinated aloes (*A. pruinosa*, Wilson *et al.*, 2009; *A. maculata*, Hargreaves *et al.*, 2010) suggest that nectar-collecting bees probably pollinate effectively, whereas exclusively pollen-collecting bees act as highly inefficient pollinators or pollen thieves.

Of the four hypotheses for inefficient pollination by pollen-collecting insects, only the possibility that extensive herkogamy limits pollen-collector contact with stigmas seems irrelevant for all ten *Aloe* species studied here (Table 1). All species exhibited strong herkogamy late during flowers' lives, but stigmas were not widely separated from anthers during either initial or maximum receptivity (Table 4), so pollen collectors could contact receptive stigmas during some flowering stage. In contrast to Botes *et al.*'s (2009*a*) proposal that herkogamy prevented pollen-collecting bees from depositing pollen on young (i.e. pollen-bearing) *A. ferox* flowers, we found equivalent deposition for caged (bird-excluded) and exposed flowers of this species (Fig. 2b). It is unlikely that pollen was deposited on stigmas of caged flowers either autonomously, given the intense pollen removal, or primarily by nectar-collecting insects, which were rare; thus, pollen-collecting bees seem to contact stigmas, despite herkogamy. Although herkogamy is frequently identified as the primary reason that pollen collectors act as pollen thieves (Hargreaves *et al.*, 2009), the limited herkogamy of these aloes was insufficient to explain their contrasting relationships with pollen-collecting bees.

Dichogamy did influence the role of pollen-collecting insects, although not without exception (Table 4). Among species with tubular flowers, primarily bird-pollinated species generally exhibited stronger dichogamy than those pollinated effectively by insects. However, insects deposited many pollen grains on *A. arborescens* stigmas and were the only pollinators of *A. inconspicua*, despite strong protandry in both species. Strong dichogamy does not prevent insect pollination of *A. inconspicua*, because insects visit female-phase flowers for nectar. Indeed, dichogamy is a common feature of bee-pollinated plants that are visited for nectar (Harder *et al.*, 2004). The explanation for insect-mediated pollen deposition on *A. arborescens* is less clear, as insects rarely visited this species for nectar.

Nectar accessibility partially determines whether insect foragers visit female-phase flowers, thus influencing their pollination efficiency (also see Ashman, 2000). Of the *Aloe* species studied to date, those pollinated most successfully by insects have short corollas (*A. kraussii*, *A. inconspicua*, this study; *A. minima*, *A. linearifolia*, Botes *et al.*, 2009*b*; Fig. 4) and/or are visited regularly by nectar-feeding bees (these species and *A. pruinosa*; Wilson *et al.*, 2009). The contribution of insects to reproductive success was strongly associated with flower type, and species with flowers that excluded most insects from nectar collection (i.e. corolla entrances were blocked by anthers and stigmas or long narrow corollas) were pollinated least effectively by insects (Fig. 4). Thus, we conclude that nectar collection promotes bee pollination of aloes. If individual bees collect pollen and nectar simultaneously (e.g. *Amegilla* on *A. inconspicua*), they will visit both male- and female-phase flowers and so probably act as pollinators. In contrast, if individual bees collect only one resource at a time (e.g. honey-bees on *A. kraussii*), conspecific pollen and nectar collectors may act as pollen thieves and pollinators, respectively (Ish-Am and Eisikowitch, 1993).

Pollen collectors could effectively pollinate species with pronounced dichogamy, but weak herkogamy, if pollen adheres to immature stigmas and remains viable until stigmas become receptive. Although young stigmas of some species did not retain pollen owing to lack of stigmatic

exudate, pollen germinated on stigmas of *A. dominella* and *A. ferox* before exudate was apparent (Hoffman, 1988). The role of dichogamy in reducing pollination by pollen collectors therefore depends partially on pollen longevity. This contrasts with Thomson and Thomson's (1992) proposal that visitors who remove much pollen but deposit little are better pollinators when pollen is short lived, because 'low viability puts a premium on pollen removal' (p. 13). However, their simulations considered adichogamous plants, such that pollen longevity determined how long pollen could 'wait' before removal from anthers, rather than the period during which stigmas could receive it.

In addition to the role of floral characteristics, the behaviour of pollen collectors seems to inhibit their effectiveness at pollinating some *Aloe* species. For *A. boylei*, bird exclusion reduced pollination despite regular visits to female-phase flowers by large nectar-collecting bees (*Amegilla natalensis*). Although *Amegilla* appeared to contact stigmas, little of the pollen they carried may have been available pollen to pollinate (hypothesis 3, Table 1); two collected individuals carried ample *A. boylei* pollen, but primarily in their scopae and therefore unavailable to stigmas. Alternatively, these bees may have been too infrequent to effect full pollination, especially if their foraging at this high altitude location is inhibited by the relatively cold, windy weather (Cruden, 1972; Kromer *et al.*, 2006). Thus, the ability of birds to be active during adverse weather and the better location of pollen on their bodies may increase their relative effectiveness as pollinators in such conditions.

Evidence from several *Aloe* species suggests that insects may deposit lower quality pollen than birds. Flowers on caged inflorescences of *A. arborescens* and *A. ferox* received equivalent pollen loads to those on open inflorescences (eliminating hypotheses 1–3 that propose that insects do not deposit pollen, Table 1), but produced significantly fewer seeds. Given that both *A. ferox* and *A. arborescens* plants are self-incompatible and produce thousands of flowers, and that honey-bees visited twice as many *A. ferox* flowers per plant as birds, the low fruit production by caged inflorescences suggests that insects primarily caused self-pollination. Low-quality pollination by insects may also have been a factor for *A. maculata* and *A. vryheidensis*. Bird exclusion reduced pollen deposition on *A. maculata* stigmas, but seed set was reduced proportionally more (compare Fig. 2B and Fig. 3). Although pollen receipt was not measured for *A. vryheidensis*, pollen-collecting insects carried abundant pollen on their abdomens and regularly contacted stigmas (Fig. 1D), but nonetheless effected less seed production than birds, despite being more frequent visitors. In *Aloe*, as in closely related genera *Gasteria* and *Bulbine*, which also have late-acting self-incompatibility systems, self-pollination is likely to be especially costly because it leads to ovule discounting, which can strongly reduce seed set (Naaborgh and Willemse, 1991; Vaughton and Ramsey, 2010).

This survey of *Aloe* pollination revealed that multiple factors determine whether pollen-collecting insects serve as pollen thieves or as pollinators of varying quality, and that the relative importance of these factors can differ among species with similar floral morphology and visitor assemblages. As predicted, plant characteristics, in this case nectar accessibility and dichogamy, largely governed whether insects visited female-phase flowers, and therefore whether they acted as pollinators or pollen thieves. The importance of plant characteristics is especially clear in this study, as all *Aloe* species interacted with the same major insect visitors (honey-bees, halictids and allodapine bees). The study of pollination efficiency and pollen theft would benefit from more studies that quantify both dichogamy and pollen longevity, as the intersecting roles of these traits have yet to be explored empirically. However, even when plants successfully facilitate stigma contact, insect behaviour can limit the quantity and quality of the pollen deposited; insects deposited lower quality, probably self-pollen, on flowers of several species, and preliminary evidence suggests they move less frequently between plants than do birds. Thus, assessing pollen deposition, as well as fruit and seed production, is critical in determining whether pollen collectors act as pollinators, thieves or ineffective pollinators. Because low-quality, insect-mediated pollen deposition was a particular problem for species with large inflorescences, plant characteristics may again ultimately determine patterns of pollen theft and inefficient pollination.

## SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: locations and elevation of study sites for the ten *Aloe* species considered in this study. Table S2: total abundance of floral visitors observed during surveys and pollinator observations for each *Aloe* species (excluding nectar-robbing birds). Table S3: comparison of seed production of bagged flowers following either autonomous self-pollination, or hand-pollination with outcross pollen or self-pollen.

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