

# Native pollen thieves reduce the reproductive success of a hermaphroditic plant, *Aloe maculata*

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**Abstract.** Pollen is unique among floral rewards in functioning as both a carrier of gametes and an attractant and nutritious resource for floral visitors. Animals that collect pollen without pollinating (pollen thieves) could reduce siring success of thieved plants and cause pollen limitation of seed set at the population level; however, such impacts on plant reproduction have not been demonstrated experimentally. To test these effects we added hives of native honey bees (*Apis mellifera scutellata*) to populations of a primarily bird-pollinated plant, *Aloe maculata*, in eastern South Africa. In field and aviary trials, bee addition increased pollen removal from anthers but decreased pollen deposition on stigmas, and so reduced both male and female pollination components. Further, total seed production decreased with hive addition in the aviary experiment and in three of four field populations, indicating that population-level pollen theft can also compromise reproductive success. In the field, naturally occurring allodapine bees also seemed to act as pollen thieves, outweighing the effect of honey bee hive addition at one of the four aloe populations. Our results highlight the importance of social bees as pollen thieves, even of plants that have evolved in their presence, and the role of dichogamy in promoting pollen theft. Given the commonness of both social bees and dichogamy, pollen theft is likely a much more common influence on floral ecology and evolution than suggested by the sparse literature.

**Key words:** *Aloe maculata*; antagonist; *Apis mellifera scutellata*; cheating; honey bee; pollen theft; pollination efficiency; South Africa; sunbirds.

## INTRODUCTION

More than 80% of angiosperm species rely on animals to transfer their pollen from anthers to conspecific stigmas (Eriksson and Bremer 1992), and most obtain this service by offering floral rewards, typically nectar and/or pollen. However, floral visitors vary widely in their morphology and behavior, and many are capable of “stealing” floral rewards without effecting significant pollination in return (Inouye 1980, Irwin et al. 2001, Hargreaves et al. 2009). Although the effects of nectar theft on plant reproduction have been considered extensively during recent decades (Irwin et al. 2001), the implications of pollen theft have been largely overlooked and seldom quantified, even though pollen theft bears greater fitness consequences for plants (Hargreaves et al. 2009).

Pollen is unique among floral rewards in functioning as both a carrier of gametes and an attractant and nutritious resource for pollinators. Pollen theft could therefore affect plant reproduction directly, by reducing the pollen available for dispersal, or indirectly, by

altering pollinator behavior. Direct effects of pollen theft are universally negative, and probably more common and stronger than indirect effects (Hargreaves et al. 2009). Pollen theft can directly reduce aspects of pollination associated with siring success, including the amount of pollen carried by pollinators (Paton 1993, do Carmo et al. 2004) and exported to stigmas (Gross and Mackay 1998), although the magnitude of such effects can depend on the abundance of efficient pollinators (Lau and Galloway 2004). Removal of pollen from the pool available for dispersal could also reduce mean female success, if insufficient pollen import subsequently limits seed production (do Carmo et al. 2004). Except for Lau and Galloway (2004), studies that demonstrate negative consequences of pollen theft for plants have examined theft by non-native animals (Hargreaves et al. 2009), which may be more prone to acting as thieves on plants with which they have not evolved.

The impact of pollen theft on reproduction by animal-pollinated plants can be difficult to assess, and few studies have attempted to do so experimentally. Pollen thieves are usually small insects, so excluding them without affecting pollinators is often impossible. Thus, studies of how pollen theft affects plant fitness have generally relied on correlative approaches (e.g., do Carmo et al. 2004). Alternatively, the abundance of pollen thieves could be increased experimentally. Honey

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TABLE 1. Flower-visitor abundance and pollen-supplementation effort at four *Aloe maculata* populations in South Africa under natural abundance (N) of native honey bees *Apis mellifera scutellata* and when honey bee hives were present (H).

Population	Order of treatment	No. flowering <i>A. maculata</i>	Visitor survey days		No. sunbirds/30-min transect†	No. honey bees/20 plants‡	
			N	H		N	H
Hilton	H → N	365	1	1	5.7 (1.0)	0	0
Ixopo	N → H	120	2	1	3.2 (1.0)	0.5 (2.7)	1.5 (1.9)
Klipfontein§	N → H	550	2	2	2.0 (0.80)	4.8 (1.5)	12.3 (1.5)
Umgeni¶	H → N	100	2	2	4.25 (0.88)	0	0

† Visitor abundances are least-squares means ( $\pm$ SE).

‡ Asymmetric lower and upper SE for abundance of small bees are based on ln-transformed data.

§ Visitor abundances after the experimental period (one survey day) were 2.5 ( $\pm$ 0.77) sunbirds, 1.5 ( $\pm$ 4.3) honey bees, and 1.5 ( $\pm$ 1.5) small bees (mean  $\pm$  SE).

¶ Visitor abundances before the experimental period (one survey day) were 5.5 ( $\pm$ 1.76) sunbirds (mean  $\pm$  SE), 0 honey bees, 2.0 (1.2, 3.1) allodapine bees (least-squares means with asymmetric lower and upper SE).

bees (*Apis mellifera* L.) are the most commonly documented pollen thieves (Hargreaves et al. 2009). Because foragers return to their hives each night, their numbers can be augmented for specific periods by adding commercial hives to a site. We used this approach to assess population-level effects of pollen theft for a South African plant, *Aloe maculata* Medic. Floral morphology suggests that this species is primarily pollinated by sunbirds (Nectariniidae), but native honey bees commonly collect its pollen without providing significant pollination (this study; S. D. Johnson, M. Vass, and J. Ågren, *unpublished manuscript*). Our study included two components: (1) we compared pollination and seed production in four *A. maculata* populations under normal and increased densities of honey bees, and (2) we compared pollination from visits by sunbirds alone with those by both birds and honey bees using potted *A. maculata* plants in an aviary. If honey bees act as pollen thieves, their increased abundance should increase pollen removal and decrease pollen deposition, which in turn should cause or exacerbate pollen limitation of seed production.

## METHODS

### *Study species and location*

*Aloe maculata* is a succulent monocot (van Wyk and Smith 2003) with branched inflorescences that typically produce one to four terminal racemes of 30–200 flowers each. Flowers are protandrous and herkogamous, orange to red, with long (~30 mm), narrow, fused corollas constricted at the base and opening. Flowers can open at any time of day, and anthers dehisce throughout daylight and to a lesser extent during night. The stigma lies within the corolla during most of pollen presentation (male phase), which lasts for 6 to >24 h. At the end of the male phase, anthers shrivel, the style elongates and the stigmatic papillae expand, initiating the 1–1.5 day female phase (Hargreaves 2007). *Aloe maculata* is visited frequently by nectar-feeding sunbirds and a variety of insects, predominantly pollen-collecting bees. The narrow corolla prevents most honey bees from accessing the nectar at the base of a flower, but smaller bees can easily

crawl into flowers. Honey bees and small bees (Halictidae and Apidae) sometimes consume nectar that seeps through holes pecked in corollas by nectar-robbing birds. *Aloe maculata* is self-incompatible (S. D. Johnson, M. Vass, and J. Ågren, *unpublished manuscript*).

This study was conducted during May through August 2005 and 2008 in KwaZulu Natal Province, South Africa, in four *A. maculata* populations separated by 5–200 km: Hilton College private school grounds (Hilton; 29°29' S, 30°18' E), a private game reserve near Ixopo (Ixopo; 30°07' S, 30°09' E), Klipfontein ranch (Klipfontein; 28°50' S, 29°40' E), and Umgeni Valley Nature Reserve (Umgeni; 29°28' S, 30°17' E). All sites consisted of open grassland within larger natural areas and contained *A. maculata* populations of differing sizes (100–550 flowering plants; Table 1). During the experiment, the weather was hot and clear during daytime, with little precipitation at any site.

### *Field experiment*

To test the population effects of pollen theft, two commercial hives of ~30 000 *Apis mellifera scutellata* (the native subspecies) were placed in each population for approximately half the 2005 flowering period (9–14 d). This experimentally increased hive density is within the range of natural densities recorded in South Africa (Moritz et al. 2007). Hives were added and removed before sunrise or after sunset so that all worker bees were contained when hives were moved. To distinguish flowers exposed during the hive manipulation, we tied a string around each study inflorescence between open and unopened flowers when the hive treatment changed. Hive-treatment order was assigned randomly among populations to equalize effects of preferential resource allocation to early or late flowers within inflorescences (Corbet 1998): “no-hives” treatment, then “hives-present” at Klipfontein and Ixopo; hives-present then no-hives at Hilton and Umgeni. At the beginning of the Umgeni experiment we marked 10 plants that were halfway through flowering to measure pollination success prior to hive addition. At Klipfontein, flowering lasted long enough to allow a second no-hives treatment

TABLE 1. Extended.

No. small bees/20 plants†‡		No. days of pollen supplementation	
N	H	N	H
2	1	7.2 (3.1, 5.5)	36.0 (12.8, 19.8)
1	1	13.0 (7.0, 15.2)	15.3 (6.4, 11.0)
3	3	0	3.0 (1.2, 1.9)
2	2	35.5 (14.7, 25.2)	20.5 (8.6, 14.7)

after hives were removed. Thus, all populations could be considered in two-phase analyses (with or without hives), and Umgeni and Klipfontein could be considered in additional three-phase analyses (before, during, and after hive addition) of visitor abundance and seed and fruit production.

To assess the importance of bird pollinators and whether the effects of pollen theft differed in their absence, we applied one of three treatments to 55–82 randomly assigned *A. maculata* plants per population: (1) caged with rigid plastic mesh (20 mm diameter) to exclude birds, but not insects; (2) unmanipulated and open to all visitors; and (3) pollen supplemented, to determine whether pollen theft caused or aggravated pollen limitation of seed set. Pollen-supplemented plants were exposed to natural pollination and additional outcross pollen was applied by hand to as many flowers as possible (Table 1). Donor anthers were collected from plants >10 m from the recipient, <7 h prior to hand-pollinations. Flowers received pollen from  $\geq 2$  donors, applied with a toothpick to stigmas of all open flowers. Significantly higher seed production by pollen-supplemented vs. unmanipulated plants is considered evidence that insufficient pollen receipt limited seed set (Knight et al. 2005). We also bagged the inflorescences of 5, 10, and 26 plants at Hilton, Klipfontein, and Umgeni, respectively, to assess (self-)pollination in the absence of pollinators. In all cases, individual plants experienced the same pollination treatment for their entire flowering period, but could have experienced both hive treatments.

The abundances of sunbird and insect flower visitors were evaluated at each site during both bee treatments. During preliminary observations, sunbird activity peaked during midmorning (09:00–11:00 hours), whereas insect activity peaked during afternoon (14:00–16:00 hours), so we conducted morning and afternoon bird and insect surveys during these periods. To estimate insect visitation, we observed 20 randomly selected *A. maculata* plants long enough to count the insects on or flying around the inflorescences (~1 min per plant). Insects were categorized as honey bee, small bee, or other; specimens were collected and sent for identification at the South African Agricultural Research Council (Pretoria, South Africa). To estimate sunbird abundance, a fixed 30-min transect was walked through the study population, with five stops of 5 min each plus 5 min for walking between stops, which were 30–50 m

apart. Sunbirds seen or heard within 10 m of transects were counted and identified if possible. Flowering *A. maculata* plants on each transect were also counted, to estimate the abundance of pollinating birds per flowering plant.

We observed bees and sunbirds foraging on *A. maculata* to determine why they differ in pollination ability. Bees were observed opportunistically during 2005; observers noted which resource was collected (pollen or nectar), dichogamy phase (male or female), and whether the bee contacted the stigma. To test for a preference for pollen-bearing flowers, we conducted additional observations in 2008, recording bee visits to male (pollen remaining on anthers) or female (stigma exerted) flowers and the total male and female flowers available (two of the 82 flowers observed had both pollen in anthers and exerted stigmas and were counted as male and female). Birds were observed on 7 d during 2005; observers noted how many flowers they probed per plant.

We evaluated the effects of hive and pollination treatments on pollen removal and receipt at each site. Two recently wilted flowers were collected from every caged and open plant during both bee treatments, and anthers and stigmas were stored in 70% ethanol. Freshly opened anthers were also collected from one flower on each of nine bagged plants to assess pollen production. We later counted the pollen grains remaining on anthers using an Elzone 5380 particle analyzer (as per Harder 1990; Micromeritics Instrument Corporation, Norcross, Georgia, USA), and the pollen grains on stigmas using a compound microscope (100 $\times$ ) after soaking stigmas in basic fuchsin stain for at least 4 h, and squashing them in glycerin.

For every experimental plant we counted total fruit production and the seeds in three or four fruits per hive treatment. To account for differing flower abundance among treatments, we estimated the proportion of each raceme exposed to each hive treatment. These estimates were summed for every plant, and the resulting “flowering proportion” included in analyses of reproductive success. As a comprehensive measure of female fecundity, we calculated a plant’s total seed production during each hive treatment as the product of mean seeds/fruit and total fruit number. Results for comparisons of hive treatments for fruit production and seeds per fruit did not differ qualitatively from those for total seed production, so we present only the latter.

Flower and seed predation varied among and within sites during this study. Insect seed predators (mostly Diptera) were quantified by counting the cocoons, larvae, and adults inside fruits for which seeds were counted. If >10% of flowers per raceme were damaged (this occurred in <20% of racemes), we estimated the damaged proportion of the raceme and subtracted half of this amount (as roughly half of damaged flowers produced fruit) from the “flowering proportion” in the analysis of the relevant hive treatment.

### *Aviary experiments*

Insects could not be excluded from flowers in the field without also excluding birds, so we tested the effect of honey bees on *A. maculata* pollination in an aviary experiment, using two captive Amethyst sunbirds (*Chalcomitra amethystina* Shaw, the most common avian visitor to *A. maculata*), and a small nucleus hive of ~1000 honey bees. Arrays of 14 *A. maculata* inflorescences were arranged in two outdoor aviaries (3 × 1 × 2 m). One array was composed of potted plants and the second of cut inflorescences with their stems in water. The latter inflorescences flowered normally and produced nectar for more than a week. Inflorescences were given a unique identifier so they could be distinguished during observations.

Pollination trials were conducted by adding a sunbird to an *Aloe* array for 1 h during morning and 1 h during afternoon and allowing it to forage for nectar. During trials we recorded which plants the bird visited, the number of probes, and the duration of feeding. At the end of the hour, the bird was caught and pollen collected from its head and bill using fuschin-stained gel, which was then melted on a microscope slide (Beattie 1971). Pollen on these slides was counted under a light microscope (100×). At the end of each trial day, the pedicels of open flowers were marked with permanent marker to distinguish between flowers of different trials. Inflorescences were bagged between feeding trials to prevent visitation.

To test the effect of honey bees, we compared pollination of flowers exposed to sunbird-only trials to that of flowers exposed while the nucleus hive was added to the aviary, 4 m from the aviaries containing the *Aloe* arrays. Honey bees could fly freely through the aviary cage mesh and so had easy access to both arrays and plants outside the aviary. Bees took 2 d to adjust to hive relocation and begin foraging on study plants, so bee treatments were alternated in blocks: potted plants experienced two no-bee trial days, two bee days (beginning 2 d after hive addition), and two more no-bee days; and cut inflorescences experienced two bee days followed by two no-bee days. Experiments were conducted every other day to ensure that flowers, which remained open for 2 d, experienced only one bee treatment. For bee trials, inflorescences were unbagged 1 h before a sunbird was added in the morning and left unbagged until the end of the afternoon trial. To assess pollen removal and deposition, anthers and stigmas were collected from all wilted flowers of the cut inflorescences, as per the field experiment. Pollen removal, pollen receipt, and seed production were compared between flowers exposed to sunbirds only and those exposed to both sunbirds and honey bees. Because the aviary plants represented a closed population, we could also estimate pollen-transfer efficiency (PTE) with and without bees. PTE was calculated as a percentage for each treatment as  $100(\text{mean pollen on stigmas})/(\text{mean pollen production of unvisited flowers} - \text{mean pollen remaining})$ . We

estimated standard errors and 95% confidence intervals based on the standard deviation and 0.025 and 0.0975 percentiles of estimates calculated for 1000 bootstrapped pseudosamples (Efron 1979).

### *Statistical analysis*

Most analyses involved generalized linear models (McCullagh and Nelder 1989; SAS 9.1, SAS Institute, Cary, North Carolina, USA). With four exceptions, the response variable was not normally distributed, so analyses considered negative-binomial distributions and a ln-link function (unless otherwise noted). Statistical analyses of independent observations involved likelihood-ratio ( $G_{df}$ ) tests, whereas repeated-measures analyses involved score statistics ( $T_{df}$ ). For analyses of multiple bee and/or pollination treatments on individual plants, correlated responses by flowers on individual plants were accounted for using generalized estimating equations and an exchangeable correlation matrix (Liang and Zeger 1986). Bird and honey bee abundance at each site and the number of flowers probed by birds in the field were analyzed using general linear models. Bee visitation to male vs. female flowers was compared to a null expectation of equal visitation given the abundance of each sexual phase using a likelihood-ratio test (Sokal and Rohlf 1995).

Models initially considered all possible interactions between independent variables, but nonsignificant interactions and factors were dropped from the models using backward elimination ( $\alpha = 0.05$ ). Interactions and factors that reflected the experimental design (i.e., pollination treatment × hive treatment) were retained in the model, even if not statistically significant. Significant factors and interactions were explored further using multiple-comparison tests, using  $\alpha = 0.05$  for a priori orthogonal contrasts (caged vs. open and open vs. pollen-supplemented plants) and the Dunn-Šidák procedure for all others (Kirk 1995). Analyses based on all four field sites considered data from the two primary hive treatments only. In addition to the overall models of seed production at all populations, separate analyses were conducted for Klipfontein and Umgeni that included the additional “no-hives” treatment at each of these populations. All least-squares means and standard errors based on ln-transformed data were back-transformed, resulting in asymmetric standard errors.

## RESULTS

### *Flower visitor abundance*

Three bird species visited *Aloe maculata* flowers legitimately: Amethyst Sunbirds (*Chalcomitra amethystina* Shaw), White-bellied Sunbirds (*Cinnyris talatala* Smith), and Malachite Sunbirds (*Nectarinia famosa* L.). Sunbird abundance varied significantly among *A. maculata* populations ( $G_3 = 7.82$ ,  $P < 0.05$ ; Table 1), but not between hive treatments ( $G_1 = 0.50$ ,  $P > 0.4$ ) or with the number of plants in flower ( $G_1 = 0.95$ ,  $P > 0.3$ ).

Sunbirds were relatively uncommon at Klipfontein and most common at Hilton. Streaky-headed Seedeaters (*Crithagra gularis* Smith) sometimes robbed nectar by pecking holes in the base of flowers, especially in the Hilton population.

Insects observed visiting flowers during the experiment included honey bees, halictid bees (Halictidae), and allodapine bees (*Allodapula variegata* Smith and *Braunsapis* sp., Apidae). The number of honey bees observed during instantaneous inflorescence sampling varied significantly among *A. maculata* populations ( $G_3 = 26.2$ ,  $P < 0.001$ ; Table 1), but not between hive treatments ( $G_3 = 2.43$ ,  $P > 0.1$ ), and site and hive treatment did not interact significantly ( $G_3 = 6.65$ ,  $P > 0.05$ ). Honey bees were more abundant at Klipfontein than the other sites ( $G_1 = 25.2$ ,  $P > 0.001$ ). Separate analysis for Klipfontein, where we studied pollination both before and after hive addition, found that honey bee abundance increased significantly during hive addition ( $G_1 = 11.74$ ,  $P < 0.001$ ), and tended to decrease when hives were removed ( $G_1 = 3.93$ ,  $P = 0.1$ ). Although honey bees foraged on *A. maculata* at Hilton and Umgeni when hives were present, there was noticeably less activity at entrances of hives at these sites than at Klipfontein, and no honey bees were observed feeding during insect surveys. The Hilton survey (one sample) is probably unrepresentative, because it was conducted the day after hives were added, when bees may not have begun foraging in their new environment (as seen in the aviary experiment).

The abundance of small bees (halictids and allodapines) also varied among populations ( $G_3 = 21.91$ ,  $P < 0.001$ ) and between bee treatments ( $G_1 = 5.70$ ,  $P < 0.05$ ), although these effects differed among populations (population  $\times$  bee interaction:  $G_3 = 8.72$ ,  $P < 0.05$ ; see Table 1). Small bees were significantly less abundant at Klipfontein than at other sites ( $G_1 = 20.86$ ,  $P < 0.001$ ). Small-bee abundance differed significantly between bee treatments only at Klipfontein, where more small bees were seen after hives were added. At Umgeni, allodapine bee abundance increased sharply from 1 to 81 for the two surveys during the hives treatment and remained high during most of the no-hives treatment (66 and 76 bees during two surveys). For all bees combined, abundance during surveys was higher during the hive treatment than the no-hives treatment at all sites except Umgeni, where the opposite was true, though the difference was statistically significant only at Klipfontein ( $T_1 = 11.68$ ,  $P < 0.001$ ).

#### *Behavior of flower visitors*

Field observations (and results from exclusion experiments; see *Results: Pollen removal and deposition*) indicate that sunbirds are effective pollinators of *A. maculata*. Individuals of all species carried orange pollen on their chins that could have come only from *A. maculata*. Sunbirds seemed to probe male- and female-phase flowers indiscriminately, and those in the aviary

contacted stigmas with their chins. Sunbird species did not differ significantly in the number of flowers probed per plant ( $G_2 = 0.89$ ,  $P > 0.6$ ), which varied from 1 to  $>20$  ( $7.9 \pm 1.2$  flowers, mean  $\pm$  SE,  $n = 21$  birds).

Bees of all types visited male-phase flowers with freshly dehisced anthers predominantly, and generally ignored female-phase flowers from which pollen had been removed. During 2005, we observed visits by 57 honey bees, of which 55 collected pollen, two drank nectar from holes pecked in corollas by birds, and one drank nectar legitimately by pushing her head into flowers. Hundreds of small bees (halictids and allodapines) gathered pollen from *A. maculata* flowers, but pollen- vs. nectar-collecting individuals were not counted. Of the 29 small bees observed closely, only one individual visited a female-phase flower. Additional bee observations during 2008 showed that honey bees visited male-phase flowers significantly more often than expected, given the abundance of male and female flowers ( $G_1 = 20.9$ ,  $P < 0.001$ ,  $n = 16$  bees). Two individual allodapine bees collected pollen and visited male flowers exclusively, but small samples precluded analyses. Small bees collected pollen from one anther at a time and did not contact stigmas while doing so, although contact during nectar collection cannot be ruled out.

#### *Pollen removal and deposition*

The amount of pollen remaining on anthers of wilted flowers differed significantly among populations ( $T_3 = 10.25$ ,  $P < 0.025$ ) and hive treatments ( $T_1 = 5.65$ ,  $P < 0.025$ ), but was not affected by bird exclusion or interactions between factors ( $P > 0.1$ ). *Aloe maculata* anthers produce a mean of 18300 pollen grains each (upper and lower SE, USE = 1250, LSE = 1342). Overall, pollen removal was highest at Umgeni and lowest at Klipfontein (Fig. 1a). The effect of hive addition on the pollen remaining in anthers differed significantly between Umgeni and the other populations (population  $\times$  bee treatment interaction,  $T_3 = 17.17$ ,  $P < 0.001$ ); whereas, similar amounts of pollen remained at all populations when hives were present, much less remained at Umgeni than at other sites when hives were not present (Fig. 1a, c). At Klipfontein, Hilton, and Ixopo, hive addition almost halved the pollen remaining in caged and open flowers, compared to that without hives ( $T_1 = 8.3$ ,  $P < 0.01$ ; Fig. 1a). In contrast, at Umgeni significantly more pollen remained on anthers during hive addition than during the following period of natural bee densities ( $T_1 = 5.72$ ,  $P < 0.05$ ; Fig. 1a). The amount of pollen remaining before hives were added at Umgeni (mean = 460 grains/anther, LSE = 56, USE = 64) was intermediate to that during and after hive addition and did not differ statistically from either ( $P > 0.3$ ). This pattern of pollen availability among populations was reflected in our ability to find adequate pollen for hand pollinations. At Klipfontein, Hilton and Ixopo, pollen was available on non-study plants until 11:00 h under natural conditions, but was completely depleted

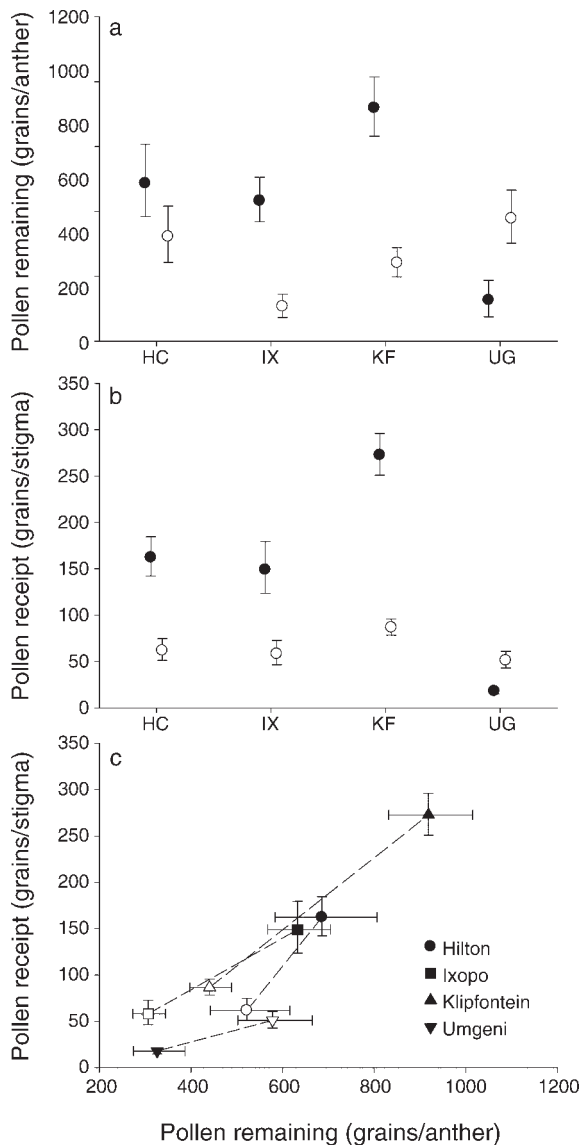


FIG. 1. Least-squares mean ( $\pm$ SE) pollen transfer to caged and open plants (*Aloe maculata*) under natural conditions (solid circles) and with two added native honey bee (*Apis mellifera scutellata*) hives (open circles), including (a) pollen remaining on anthers of wilted flowers, (b) pollen receipt, and (c) the relation between receipt and removal for each study population in South Africa (abbreviations are HC, Hilton; IX, Ixopo; KF, Klipfontein; and UG, Umgeni). This figure is based on analyses of ln-transformed data; the results in this figure have been back-transformed.

by 09:00 h when hives were present. In contrast, pollen at Umgeni became even less available after hives were removed, probably due to the many allodapine bees that appeared at the end of the hives treatment.

Pollen receipt varied with bird-exclusion and hive addition. Stigmas of caged plants received fewer pollen grains than those of open plants in all populations ( $T_1 = 8.26$ ,  $P < 0.005$ ), and this effect did not differ between hive treatments (pollination  $\times$  hive treatment,  $T_1 =$

0.00002,  $P > 0.99$ ), or populations (population  $\times$  pollination treatment,  $T_1 = 6.51$ ,  $P > 0.05$ ). However, the effect of hive addition differed significantly between Umgeni and the other populations (population  $\times$  hive treatment interaction,  $T_3 = 12.85$ ,  $P < 0.005$ ; Fig. 1b, c). At Klipfontein, Ixopo, and Hilton, stigmas received almost a third less pollen when hives were present than under natural conditions ( $T_1 = 9.45$ ,  $P < 0.005$ ; Fig. 1b), whereas at Umgeni the opposite was true (see data later in this section). Stigmas of 41 plants from which we excluded pollinators by bagging received a mean of 83 grains (LSE = 8.8, USE = 9.9) autonomously, which exceeded deposition on stigmas of open plants during hive addition at all sites (see Fig. 1b).

Stigmas of Umgeni plants received significantly less pollen than those in other populations ( $T_1 = 19.52$ ,  $P < 0.001$ ), primarily because of low deposition after hives were removed (Fig. 1b). Mean pollen deposition at Umgeni was 75% higher before hives were present (mean = 115 grains, LSE = 13.1, USE = 14.8) than with hives, but the difference was not statistically significant ( $T_1 = 2.71$ ,  $P = 0.1$ ). Pollen deposition during the final no-hives treatment was significantly lower than when hives were present ( $T_1 = 5.74$ ,  $P < 0.05$ ) or before hive addition ( $T_1 = 7.04$ ,  $P < 0.025$ ), and was lower than during any treatment in any other population (Fig. 1b). Overall, higher abundance of bees (*Apis* and small bees) coincided with increased pollen removal from anthers and reduced pollen deposition on stigmas for all populations, though this did not coincide with the hive treatment at Umgeni (Fig. 1c).

#### Seed production

The effects of bird exclusion and honey bee addition on total seed production differed among populations (Table 2, Fig. 2). Overall, bird exclusion lowered seed production significantly at Hilton ( $T_1 = 6.20$ ,  $P < 0.05$ ) and Ixopo ( $T_1 = 12.73$ ,  $P < 0.001$ ), but not in the other populations ( $P > 0.1$ ; Fig. 2), suggesting that birds were more effective or insects were less effective pollinators in these populations than at Klipfontein or Umgeni. Considering the two primary experimental periods only (i.e., excluding the additional “no-hives” treatments at Klipfontein and Umgeni), hive addition significantly lowered seed production of caged and open plants at Ixopo ( $T_1 = 12.67$ ,  $P < 0.001$ ; Fig. 2a) and Klipfontein ( $T_1 = 8.55$ ,  $P < 0.01$ ; Fig. 2c), but did not affect seed production at Hilton ( $T_1 = 0.39$ ,  $P < 0.5$ ; Fig. 2b). Conversely, Umgeni plants set more seeds when hives were present than after their removal ( $T_1 = 4.11$ ,  $P < 0.05$ ; Fig. 2d).

Pollen supplementation had inconsistent effects on seed production among populations and hive treatments (population  $\times$  pollination  $\times$  hive interaction, Table 2). At Klipfontein, pollen supplementation increased seed production when hives were present ( $T_1 = 7.80$ ,  $P < 0.01$ ), but not before ( $T_1 = 1.91$ ,  $P < 0.1$ ; Fig. 2g). In contrast, supplementation increased seed production

TABLE 2. Score statistics ( $T$  values) for generalized models of the effects of pollination treatment (caged, open, or pollen supplemented), bee treatment (natural or hives present), and population on the total reproductive success (fruit production  $\times$  mean seeds per fruit per plant during each bee treatment) of *Aloe maculata*, considering all populations together.

Independent variable	df	Caged vs. open	Open vs. pollen supplemented
Population	3	24.58***	20.21***
Pollination treatment	1	13.89***	14.55***
Bee treatment	3	2.09	10.33**
Population $\times$ pollination treatment	1	11.44**	14.20**
Population $\times$ bee treatment	3	9.13*	17.29***
Pollination treatment $\times$ bee treatment	1	0.17	3.63
Population $\times$ pollination treatment $\times$ bee treatment	3	6.45	10.06*

Note: Additional "no hives" treatments at Klipfontein and Umgeni are not included.

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

under natural conditions at Hilton ( $T_1 = 5.46$ ,  $P < 0.05$ ), but not when hives were present ( $T_1 = 0.95$ ,  $P > 0.3$ ; Fig. 2f), probably because of greater supplementation effort during the first treatment when roughly twice as many flowers were supplementally hand pollinated (Table 1). Supplementation did not affect seed production during either bee treatment at Ixopo ( $P > 0.05$ ; Fig. 2e), but it increased seed production during both treatments at Umgeni ( $T_1 = 7.41$ ,  $P < 0.001$ ; Fig. 2h).

Additional analyses of fruit and seed production by open plants were conducted for Klipfontein and Umgeni to consider the additional no-hives treatment for each population. At Klipfontein, where prolonged flowering allowed a second no-hives treatment, honey bee addition did not affect fruit production ( $T_2 = 0.05$ ,  $P > 0.9$ ), but significantly affected seed set ( $T_2 = 9.95$ ,  $P < 0.01$ ). Specifically, seed set decreased after hives were added ( $T_1 = 8.80$ ,  $P < 0.01$ ) and increased again after their removal, although this was only marginally significant ( $T_1 = 3.18$ ,  $P = 0.07$ ; Fig. 3a). At Umgeni, both fruit production ( $T_1 = 19.71$ ,  $P < 0.001$ ; Fig. 3b) and seed production ( $T_1 = 10.97$ ,  $P < 0.01$ ; Fig. 3c) declined significantly throughout the experiment.

The number of seed predators per fruit followed a Poisson distribution, indicating that predators were distributed randomly among flowers. Seed-predator abundance did not differ between fruits of caged and open plants ( $T_1 = 0.15$ ,  $P > 0.7$ ), but was lower overall for fruits of flowers exposed when hives were present ( $T_1 = 5.02$ ,  $P = 0.025$ ). Thus, seed predators cannot account for lower seed production during hive treatments. Seed predation differed between sites ( $T_3 = 32.59$ ,  $P < 0.0001$ ), primarily due to higher predation at Klipfontein and Hilton than at Ixopo and Umgeni ( $T_1 = 23.23$ ,  $P < 0.001$ ).

#### Aviary experiment

Sunbird behavior varied with plant characteristics, but not between birds or with the presence of bees, whereas hive addition affected the number of foraging bees. The two individual sunbirds used in the aviary experiment carried similar pollen loads and effected similar pollination ( $P > 0.1$ ). Birds probed more flowers per visit on racemes with many flowers ( $G_1 = 6.35$ ,  $P =$

0.012,  $n = 83$  plant-trial observations). Honey bees were the only insects seen visiting arrays during 1-h trials; 3–12 bees collected pollen during every trial at which the hive was present, but none visited plants during no-hive trials. The presence of bees did not change the duration of sunbird feeding bouts ( $G_1 = 0.57$ ,  $P > 0.4$ ) or the number of flower probes per trial ( $G_1 = 0.003$ ,  $P > 0.95$ ).

Honey bee foraging increased pollen removal, but reduced the pollen carried by sunbirds, pollen deposition on stigmas, pollen-transfer efficiency, and seed set per fruit compared to trials when sunbirds foraged alone (Table 3). Indeed, addition of honey bees reduced pollen-transfer efficiency by 77% compared to bird pollination alone. Because the aviary population was a closed system, reduced pollen deposition also indicates reduced mean siring success. The effect of bees on fruit set depended on the number of flowers open per raceme (bee  $\times$  ln(flower) interaction,  $T_1 = 6.52$ ,  $P < 0.01$ ; Fig. 4). Bees did not affect fruit set by inflorescences with few open flowers, which had consistently low fruit set, but significantly reduced fruit set by inflorescences with many open flowers (Fig. 4).

## DISCUSSION

### *Reproductive consequences of pollen theft*

The results of this study indicate that pollen theft can reduce the reproductive success of plant populations. Our aviary experiment demonstrated that addition of pollen-collecting honey bees increased pollen removal from anthers, but decreased pollen deposition on stigmas, a pattern that also occurred after hive addition at three of the four *Aloe maculata* populations. Thus, honey bees acted as pollen thieves as defined by Hargreaves et al. (2009), reducing mean pollen export, which in turn decreased overall seed production in both artificial arrays and wild populations. The clearest field evidence of detrimental effects of honey bees on pollination came from the Klipfontein population, in which seed production decreased with hive addition, but rebounded after hive removal (Fig. 3a). Correspondingly, seed production by non-supplemented Klipfontein plants decreased significantly in the presence of hives,

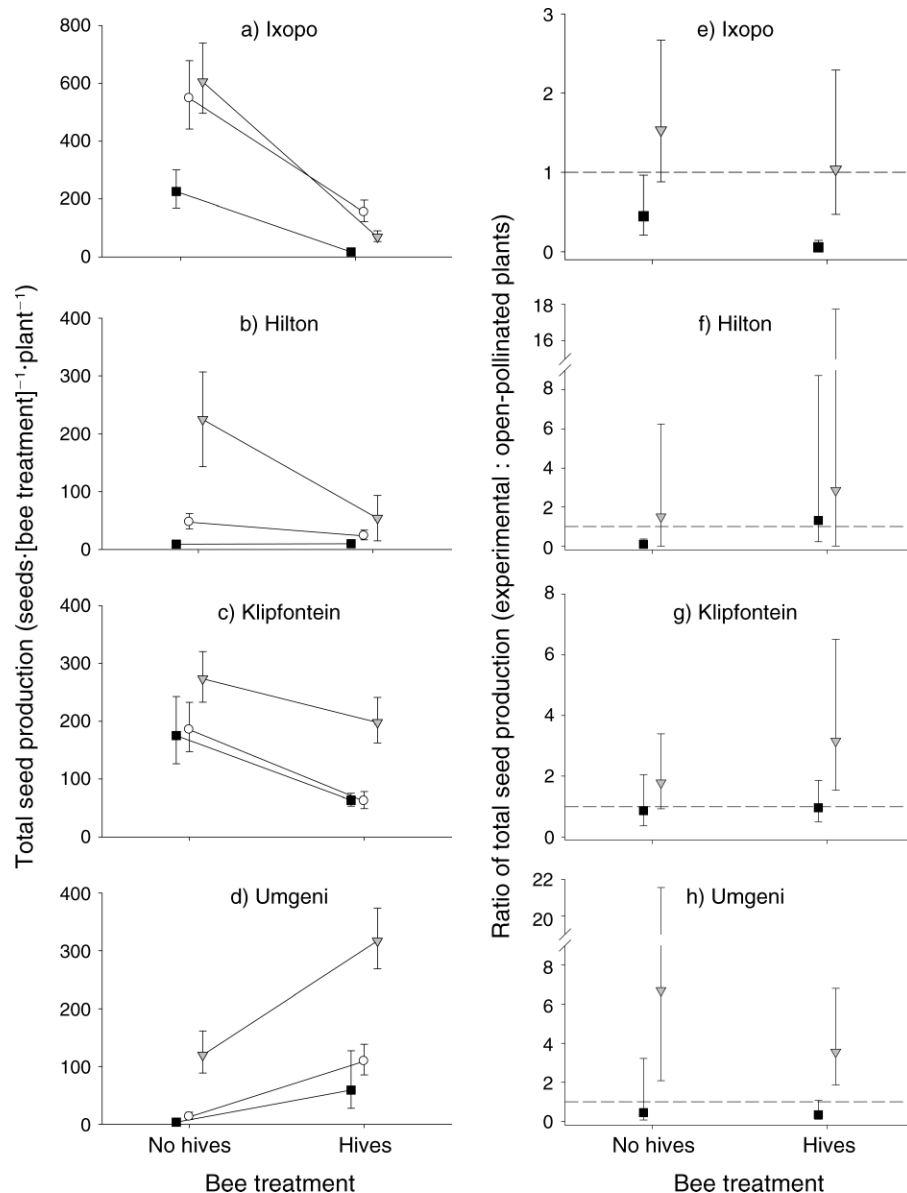


FIG. 2. Overall effects of honey bee addition on reproductive success of *Aloe maculata* plants subject to caging (solid squares), open pollination (open circles), and pollen supplementation (gray triangles) in four populations (note that hive treatments are not presented chronologically for Hilton and Umgeni). (a–d) Least-squares mean ( $\pm$ SE) seed production (note the different y-axis for Ixopo). (e–h) Ratio ( $\pm$ 95% confidence intervals) of total seed production by plants subject to experimental manipulation vs. open-pollinated plants. A ratio of 1 (dashed reference line) represents equal seed production; thus confidence limits that do not include 1 indicate a significant decline in seed production with bird exclusion (squares, caged : open-pollinated) or significant pollen limitation (triangles, pollen-supplemented : open-pollinated). These results are based on analyses that did not consider the additional “no hives” treatments at Klipfontein and Umgeni. The effects of flower abundance per treatment were controlled for in the analyses by including the proportion of flowers open during each hive treatment as a covariate (see *Methods*). This figure is based on analyses of ln-transformed data.

whereas pollen-supplemented plants achieved seed production similar to that prior to hive addition, indicating that this population became pollen limited when hives were present (Fig. 2).

Although the aviary experiment established unambiguously that honey bees act as pollen thieves on *A. maculata*, our field-experiment results were not without

anomalies. First, thief-induced reduction in pollen export and import should increase pollen limitation, as detected by pollen-supplementation experiments. That this did not occur consistently in this study largely reflects supplementation effort and the difficulty of synchronizing hand-pollination with the brief stigma receptivity of *A. maculata* flowers (S. D. Johnson, M.

Vass, and J. Ågren, *unpublished manuscript*). At Klipfontein, where pollen was supplemented most often (Table 1), we detected pollen limitation in response to hive addition, as expected (Fig. 2b). At Hilton, pollen limitation seemed to decrease when hives were present, probably because pollen was supplemented less often during the hives treatment (Table 1) as exposed donor plants were too depleted of pollen. The lack of response to pollen addition at Ixopo was likely also due to insufficient supplementation, which was applied only once during each bee treatment.

Second, insect surveys failed to detect honey bees during either hive treatment at Hilton and Umgeni. As honey bees were observed on *A. maculata* at all sites during fieldwork (but not during surveys) when hives were present, this must partially reflect the inadequacy of instantaneous insect surveys for assessing visitor abundance. However, hives at Umgeni and Hilton also seemed less active than those at other sites, and contained noticeably fewer bees after the experiment (W. Ercket, *personal correspondence*). Thus, insect surveys likely reflect differing abundance of foraging honey bees, suggesting that our experimental manipulation of pollen-thief abundance was less effective at Umgeni and Hilton than at Ixopo and Klipfontein. Interestingly, small-bee abundance was always highest during the treatment in which pollen export and seed production were lowest (i.e., with-hives treatment at all sites except Umgeni; Table 1).

Indeed, the anomalous results from the Umgeni population, where pollen receipt and seed production were higher when honey bee hives were present than after their removal (Fig. 3b, c), is likely explained by the sudden appearance of many pollen-collecting allodapine bees near the end of the hives-present treatment. These bees stripped anthers of pollen by 08:00 hours, visited primarily male-phase flowers, did not contact stigmas, and so acted as pollen thieves. Insect surveys were too infrequent to identify the beginning and end of high allodapine abundance, but mean abundance suggests it was highest during the no-hives treatment. Furthermore, in the Umgeni population, pollen removal was higher and deposition lower during the no-hives treatment than when hives were present, suggesting more intensive pollen theft after hive removal. Thus, pollen-thief abundance probably increased during the experiment, first with hive addition and then with the natural increase in small-bee abundance, such that this population experienced the highest pollen theft during the final (no-hives) treatment. If so, the apparent exception to the negative effect of pollen-collecting honey bees on *A. maculata* reproduction can in fact be explained by pollen theft, if not by the experimental manipulation.

Although other processes could explain the anomalous results from the Umgeni population, they are inconsistent with the results of this study. Umgeni plants could have been pollinator limited, so that added honey bees increased overall pollination service, despite their

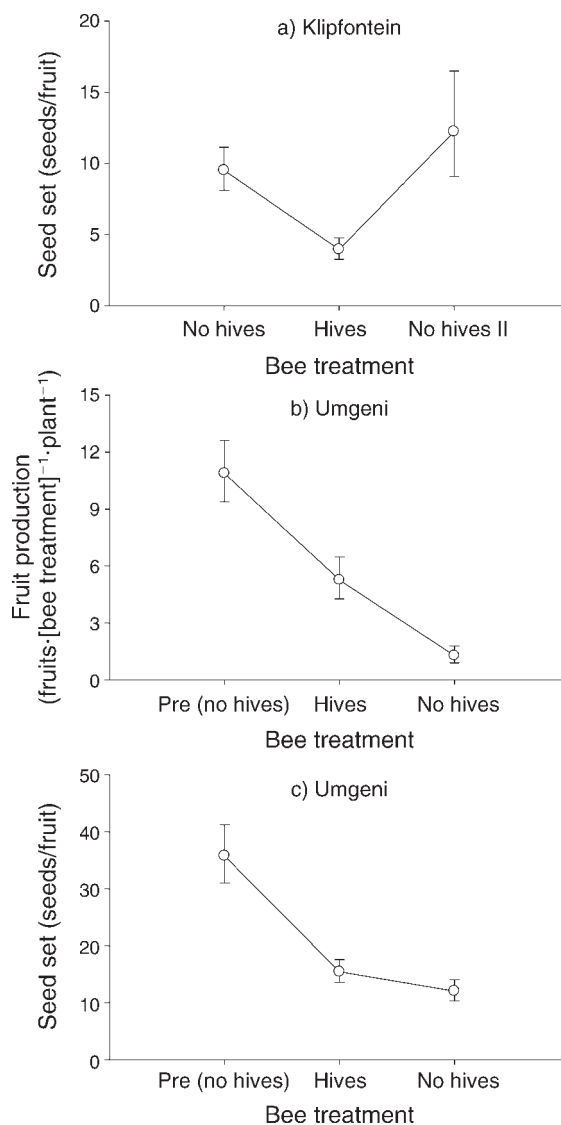


FIG. 3. The effects of honey bee addition on female reproductive success of open-pollinated plants at (a) Klipfontein and (b, c) Umgeni: populations that were observed for an additional period without hives, either before (pre hives) or after (no hives II) the main experimental treatments. Fruit and seed production (least-squares mean  $\pm$  SE) are based on analyses of ln-transformed data. Hive treatments are presented in chronological order. Data have been back-transformed.

relative inefficiency as pollinators. However, pollen receipt, fruit production and seed set at Umgeni were all highest before hive addition and declined significantly after hives were added (Fig. 3), thus pollinator limitation cannot explain the declining seed production. A second explanation for the continuously declining seed production at Umgeni is resource limitation, if flowers pollinated before hive addition depleted limited resources for fruit and seed production (Corbet 1998). However, this possibility is inconsistent with the

TABLE 3. *Aloe maculata* pollination and seed production during aviary experiments in the presence and absence of honey bees.

Variable	No bees	With bees	Repeated subject	Test statistic
Pollen remaining	5832 (553, 612)	3185 (387, 441)	plant	$T_1 = 7.35^{**}$
Pollen deposited	251 (29.1, 33.0)	39 (9.7, 12.1)	plant	$T_1 = 4.56^*$
Pollen-transfer efficiency†	0.42% (0.164%)	0.01% (0.028%)	...	...
Sunbird pollen load	9118 (1654, 2021)	3080 (644, 815)	none	$G_1 = 9.03^{**}$
Seeds per fruit	26.8 (3.3, 3.7)	20.0 (1.6, 1.8)	plant	$T_1 = 4.73^*$

Note: Least-squares means (and lower and upper SE) are back-transformed from ln-transformed data for all variables except pollen-transfer efficiency.

\*  $P < 0.05$ ; \*\*  $P < 0.01$ .

† Based on nonoverlapping 95% confidence intervals, pollen-transfer efficiency in the absence of bees (0.260–0.587%) significantly exceeded that in the presence of bees (0.070–0.126%).

significant increase in seed production in response to pollen supplementation during both bee treatments (Fig. 2b).

Finally, our observations that high bee densities reduced seed production in the field and aviary might be explained by ovule discounting, rather than pollen theft, if bees elevated self-pollination. For example, on *Aloe ferox* individual honey bees visit up to twice as many flowers per plant as birds (Hargreaves 2007), and so could transfer pollen geitonogamously. Although *A. maculata* is self-incompatible, self-pollen applied to 12 stigmas grew pollen tubes into the ovary (A. L. Hargreaves, unpublished data), suggesting a late-acting, ovarian incompatibility system like that reported for *Gasteria* (Brandham and Owens 1978, Naaborgh and Willemse 1992), a genus closely allied with *Aloe* (Treutlein et al. 2003). Such incompatibility involves abortion of ovules penetrated by self-pollen tubes (Naaborgh and Willemse 1992), which reduces opportunities for cross-fertilization (ovule discounting: Barrett et al. 1996). However, the observation that bee addition reduced pollen receipt contradicts the expectation of increased (geitonogamous) pollination from the ovule-discounting hypothesis. Thus, our results are most consistent with the explanation that bees reduce pollination quantity, rather than quality.

#### General patterns

Our finding that social bees acted as significant pollen thieves is consistent with the broader literature in which eusocial bees (which include *Apis*, *Trigona*, and some halictid and allopapine species) are the most commonly documented pollen thieves (Hargreaves et al. 2009). This suggests that as pollen collectors, they can be especially detrimental for plants, which could occur for several non-exclusive reasons. First, the behavioral flexibility of these bees allows them to exploit unfamiliar floral resources, and may impede floral adaptation to co-opt them into pollinating (Westerkamp 1991). Second, when eusocial bees act as pollen thieves, their large colonies and, in some cases, ability to communicate the location of food resources would make their effect on plant reproduction particularly severe. Finally, honey bees

have been introduced around the world (Huryn 1997, Goulson 2003), bringing them into contact with many plants not adapted to them. However, this study demonstrates that native bees at realistic densities (Moritz et al. 2007) can also reduce reproductive success of plants with which they have evolved.

In this study, bees acted as pollen thieves due to their strong preference for pollen-bearing flowers and their subsequent avoidance of female-phase flowers. Bees readily consume nectar from pierced *A. maculata* flowers, so their focus on pollen collection likely results from their exclusion from nectar resources by long, narrow corollas, and the ease of accessing pollen from slightly exerted anthers. This flower morphology is common among flowers pollinated by strict nectarivores (e.g., birds, butterflies, moths), and may promote exclusive pollen collection by bees with short proboscises (Wilson et al. 2004). Conversely, bee-pollinated flowers generally promote contact with receptive stigmas via accessible nectar rewards and/or morphology that manipulates visitor movement (e.g., keel-blossoms, poricidal anthers). Thus, while nectarivore-pollinated

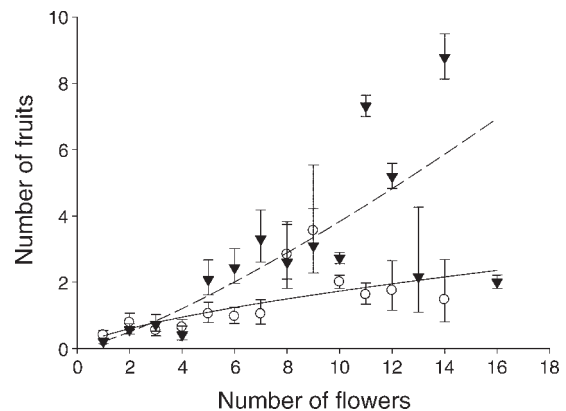


FIG. 4. The interacting effects of the presence (circles, solid curve) or absence (triangles, dashed curve) of honey bee hives and the number of open flowers on *A. maculata* inflorescences on least-squares mean ( $\pm$ SE) fruit production during the aviary experiment. This figure is based on analysis of ln-transformed observations. Data have been back transformed.

species may successfully exclude bees from nectar resources, they may be more vulnerable to pollen theft than species pollinated primarily by pollen-collectors.

Pollen-collecting bees avoided flowers lacking pollen, and so rarely visited female-phase flowers. Such avoidance may be common among pollen collectors, as pollen acts as a visual and olfactory cue for many floral visitors (Dobson and Bergstrom 2000, Lunau 2000). If so, plants whose female-phase flowers do not bear pollen, including dichogamous and dioecious species, would be much more vulnerable to pollen theft. Indeed, nectar inaccessibility and dichogamy were both positively associated with vulnerability to pollen theft among aloes (Hargreaves 2007). Given the commonness of bees as floral visitors (Danforth et al. 2006) and dichogamy in angiosperm flowers (Bertin and Newman 1993), pollen theft is likely much more common than suggested by the relatively sparse literature.

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#### LITERATURE CITED

- Barrett, S. C. H., D. G. Lloyd, and J. Arroyo. 1996. Patterns of style length variation in *Narcissus*: implications for the evolution of heterostyly. Pages 339–376 in D. G. Lloyd and S. C. H. Barrett, editors. *Floral biology: studies on floral evolution in animal pollinated plants*. Chapman and Hall, New York, New York, USA.
- Beattie, A. J. 1971. A technique for the study of insect-borne pollen. *Pan-Pacific Entomologist* 47:82.
- Bertin, R., and C. Newman. 1993. Dichogamy in angiosperms. *Botanical Review* 59:112–152.
- Brandham, P. E., and S. J. Owens. 1978. Genetic control of self-incompatibility in genus *Gasteria* (Liliaceae). *Heredity* 40:165–169.
- Corbet, S. A. 1998. Fruit and seed production in relation to pollination and resources in bluebell, *Hyacinthoides non-scripta*. *Oecologia* 114:349–360.
- Danforth, B. N., S. Sipes, J. Fang, and S. G. Brady. 2006. The history of early bee diversification based on five genes plus morphology. *Proceedings of the National Academy of Sciences USA* 103:15118–15123.
- Dobson, H. E. M., and G. Bergstrom. 2000. The ecology and evolution of pollen odors. *Plant Systematics and Evolution* 222:63–87.
- do Carmo, R. M., E. V. Franceschinelli, and F. A. da Silveira. 2004. Introduced honeybees (*Apis mellifera*) reduce pollination success without affecting the floral resource taken by native pollinators. *Biotropica* 36:371–376.
- Efron, B. 1979. Bootstrap methods: another look at the jackknife. *Annals of Statistics* 7:1–26.
- Eriksson, O., and B. Bremer. 1992. Pollination systems, dispersal modes, life forms, and diversification rates in angiosperm families. *Evolution* 46:258–266.
- Goulson, D. 2003. Effects of introduced bees on native ecosystems. *Annual Review of Ecology, Evolution, and Systematics* 34:1–26.
- Gross, C. L., and D. Mackay. 1998. Honeybees reduce fitness in the pioneer shrub *Melastoma affine* (Melastomataceae). *Biological Conservation* 86:169–178.
- Harder, L. D. 1990. Pollen removal by bumble bees and its implications for pollen dispersal. *Ecology* 71:1110–1125.
- Hargreaves, A. L. 2007. The ecological effects of pollen-stealing insects on plant reproductive success. M.Sc. Thesis. University of Calgary, Calgary, Alberta, Canada.
- Hargreaves, A. L., L. D. Harder, and S. D. Johnson. 2009. Consumptive emasculation: the ecological and evolutionary consequences of pollen theft. *Biological Reviews* 84:259–276.
- Hury, V. M. B. 1997. Ecological impacts of introduced honey bees. *Quarterly Review of Biology* 72:275–297.
- Inouye, D. W. 1980. The terminology of floral larceny. *Ecology* 61:1251–1253.
- Irwin, R. E., A. K. Brody, and N. M. Waser. 2001. The impact of floral larceny on individuals, populations, and communities. *Oecologia* 129:161–168.
- Kirk, R. E. 1995. *Experimental design: procedures for the behavioral sciences*. Third edition. Brooks/Cole Publishing Company, Pacific Grove, California, USA.
- Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, R. J. Mitchell, and T. L. Ashman. 2005. Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics* 36:467–497.
- Lau, J. A., and L. F. Galloway. 2004. Effects of low-efficiency pollinators on plant fitness and floral trait evolution in *Campanula americana* (Campanulaceae). *Oecologia* 141:577–583.
- Liang, K. Y., and S. L. Zeger. 1986. Longitudinal data analysis using generalized linear models. *Biometrika* 73:13–22.
- Lunau, K. 2000. The ecology and evolution of visual pollen signals. *Plant Systematics and Evolution* 222:89–111.
- McCullagh, P., and J. A. Nelder. 1989. *Generalized linear models*. Chapman and Hall, London, UK.
- Moritz, R., F. Kraus, P. Kryger, and R. Crewe. 2007. The size of wild honeybee populations (*Apis mellifera*) and its implications for the conservation of honeybees. *Journal of Insect Conservation* 11:391–397.
- Naaborgh, A. T., and M. T. M. Willemse. 1992. The ovular incompatibility system in *Gasteria verrucosa* (Mill.) H. Duval. *Euphytica* 58:231–240.
- Paton, D. C. 1993. Honeybees in the Australian environment. *BioScience* 43:95–103.
- Sokal, R., and F. Rohlf. 1995. *Biometry: the principles and practice of statistics in biological research*. Third edition. W. H. Freeman, New York, New York, USA.
- Treutlein, J., G. F. Smith, B. E. van Wyk, and M. Wink. 2003. Phylogenetic relationships in Asphodelaceae (subfamily Alooioideae) inferred from chloroplast DNA sequences (rbcL, matK) and from genomic fingerprinting (ISSR). *Taxon* 52:193–207.
- van Wyk, B., and G. Smith. 2003. *Guide to the aloes of South Africa*. Second edition. Briza Publications, Pretoria, South Africa.
- Westerkamp, C. 1991. Honeybees are poor pollinators—why? *Plant Systematics and Evolution* 177:71–75.
- Wilson, P., M. Castellanos, J. Hogue, J. Thomson, and W. Armbruster. 2004. A multivariate search for pollination syndromes among penstemons. *Oikos* 104:345–361.