RESEARCH ARTICLE

Botany American Journal of

Why honeybees are poor pollinators of a mass-flowering plant: Experimental support for the low pollen quality hypothesis

Carolina Diller 🅾 | Miguel Castañeda-Zárate 🅾 | Steven D. Johnson 🅾

Centre for Functional Biodiversity, School of Life Sciences, University of KwaZulu-Natal, P. Bag X01, Scottsville, Pietermaritzburg 3209, South Africa

Correspondence
Carolina Diller, Centre for Functional Biodiversity, School of Life Sciences, University of KwaZulu-Natal, P. Bag X01, Scottsville, Pietermaritzburg 3209, South Africa.
Email: dillercarolina@gmail.com

Abstract

Premise: Honeybees dominate the flower-visitor assemblages of many plant species, yet their efficiency in terms of the quality of pollen delivered to stigmas is largely unknown. We investigated why honeybees are poor pollinators of *Aloe ferox*, a self-incompatible succulent treelet with large numbers of flowers. Honeybees are very frequent visitors to flowers of this species, yet contribute very little to seed production.

Methods: We assessed pollen loads on honeybees, studied their visitation behavior, selectively excluded birds from plants to determine direct effects of bees on pollen deposition, seed set, and ovule abortion, and used a novel "split-pollinator" method to test whether honeybees deposit mainly low-quality self pollen. For the latter, we captured honeybees, and with their existing pollen loads, used them to either pollinate virgin flowers on the plant on which they were caught or to pollinate virgin flowers on different plants.

Results: Honeybees cumulatively deposit as much pollen on stigmas as do birds, but our experiments showed that the pollen deposited by honeybees is mostly low-quality self pollen that leads to substantial ovule discounting and depressed seed set.

Conclusions: Lack of movement among *A. ferox* plants during individual honeybee foraging bouts is the most likely explanation for their deposition of low-quality self pollen on stigmas. The "split-pollinator" method is a simple and cost-effective technique to test the quality of pollination.

KEYWORDS

Aloe ferox, *Apis mellifera*, flower constancy, geitonogamy, late-acting self-incompatibility, mass-flowering, ovule discounting, pollen grooming, pollen quality, pollinator effectiveness, single visit effectiveness

The importance of specific animals for pollination is usually assessed by their rates of visitation, amounts of pollen they deposit on stigmas, and their overall contributions to seed production (Castellanos et al., 2003; Reynolds et al., 2009; Stoepler et al., 2012; Wester and Johnson, 2017). However, it is also valuable to assess components of the quality of pollen delivered to stigmas by animals, such as the fractions of conspecific pollen (Stewart and Dudash, 2016), cross pollen (Matsuki et al., 2008), and mate diversity (Krauss et al., 2017). These components can be hard to measure. For example, the negative effects of self-pollination on fitness are not reflected in outcrossing rates of self-incompatible plants and need to be established through experimentation

(Waser and Price, 1991; Vaughton and Ramsey, 2010; Duffy et al., 2021).

The western honeybee, *Apis mellifera* L., is native to Europe, Africa, and the Middle East, but due to beekeeping it is now distributed around the world (Han et al., 2012). Honeybees are also the most widely used and economically valuable pollinator of crops in monoculture worldwide (Klein et al., 2007), but there is increasing evidence that they are not particularly effective pollinators in either agricultural or in natural environments (Garibaldi et al., 2014; Hung et al., 2018; Page et al., 2021). Just over 50 years ago, Free (1966a) published an important, but often overlooked, paper that used mark-recapture data to show that

honeybees tend to confine their activities to single apple trees, even during subsequent foraging bouts. At the species level, honeybees are considered generalist pollinators, but individual honeybees can show high levels of foraging constancy (Dupont et al., 2011). This behavior, particularly the tendency to return continuously to the same plant, is expected to increase within-plant self-pollination and thus diminish the quality of pollination (Brosi, 2016; Free, 1966a). Even in plants that are self-compatible, fertilization with self pollen generally leads to lower quality offspring (Dudash, 1990). All else being equal, pollinators that deliver a high fraction of self pollen to stigmas will generally be inferior to those that deliver a high fraction of cross pollen.

While single visit experiments (i.e., where pollinator effectiveness is measured after one visit from a pollinator) have been used to test the effectiveness of honeybees for pollination (Wilson and Thomson, 1991; Thomson and Goodell, 2001; Welsford and Johnson, 2012), these experiments have not generally been accompanied by treatments that parse out the various factors that contribute to pollination effectiveness of honeybees. There are formidable methodological challenges in distinguishing between cross and self pollen on stigmas, particularly for self-incompatible species. Using color-labeled pollen or pollen analogs is one possibility (e.g., Minnaar and Anderson, 2019), but color labeling of the entire self pollen complement of a plant to identify such pollen on stigmas is probably near-impossible for mass-flowering species. For self-compatible plants, genotyping of progeny can be used to assess the selfing rate after visits by particular pollinators (e.g., Brunet and Holmquist, 2009; Steenhuisen et al., 2012), but this may not be an accurate indicator of the fraction of self pollen deposited on stigmas if inbreeding depression manifests in early development of offspring. Genotyping pollen loads of flower visitors, such as in Matsuki et al. (2008), is very informative, but like any molecular technique, can be cumbersome and expensive.

Here, we introduce a novel "split-pollinator" method to address the issue of the quality of pollen deposited by pollinators. This method consists of capturing flower visitors on plants on which a subset of flowers had been bagged from the bud stage, and then using half the captured flower visitors to pollinate virgin flowers on the plant on which the animal was captured and the other half to pollinate virgin flowers on other plants of the same species. Any differences in seed set between the two treatment groups (among and within-plant crosses performed with a pollinator's existing pollen load) can be attributed to pollen quality. While similar to conventional single visit experiments assessed with pollen tubes or fruit and seed set, such as used by Free (1966b) and Brittain et al. (2013), our method introduces an important positive control—the application of among-plant crosses with a pollinator's existing pollen load—which allows for better interpretation of the results obtained.

We sought to clarify a long-standing puzzle about the contributions of honeybees (Apis mellifera subsp. scutellata

Lepeletier) to seed set of the self-incompatible South African treelet Aloe ferox Mill. This species produces numerous flowers that are attractive to both native honeybees and several bird species (Hoffman, 1988; Diller et al., 2019). Honeybees are far more frequent than birds as visitors to flowers of A. ferox (Hoffman, 1988; Hargreaves et al., 2012) and deposit pollen on stigmas (Diller et al., 2019), yet bird exclusion experiments indicate that honeybees are very poor pollinators when compared to birds, as assessed by resulting seed set (Strokes and Yeaton, 1995; Botes et al., 2009; Hargreaves et al., 2012). This is consistent with studies of many other aloes, which have also shown that honeybees make little contribution to seed set in comparison to birds, despite being frequent visitors (Botes et al., 2009; Hargreaves et al., 2010, 2012; Duffy et al., 2021, but see Patrick et al., 2018; Duffy et al., 2020).

We hypothesized that the reason why honeybees are poor pollinators of A. ferox is because they deposit mainly low quality self pollen on stigmas. To test this hypothesis, we (1) quantified the number of flowers per plant visited during foraging bouts by honeybees vs. birds, (2) used the split-pollinator technique to assess if pollen loads of honeybees are composed mainly of self pollen, and (3) investigated whether visits by honeybees lead to high levels of ovule discounting indicative of self-pollination.

MATERIALS AND METHODS

Study system

Aloe ferox Mill. (Asphodelaceae: Alooideae) is a tree-like succulent up to 5 m tall with a system of late-acting (ovarian) self-incompatibility (Appendix S1). Individuals at our study site produced up to 13 racemes with a median of 280 flowers per raceme, with ~33 flowers opening each day on a raceme. Flowers are protandrous, bright orange, and tubular with exserted stamen and pistils. Stigma receptivity peaks at 48 h after anthesis (Appendix S2) and pollen grain viability drops significantly after 48 h (Appendix S3). Voucher specimens are lodged in the Bews Herbarium (NU, University of KwaZulu-Natal, South Africa) (MCZ – 1587, 1588, 1589).

The main pollinators of A. ferox are opportunistic nectar feeding birds (e.g., *Pycnonotus tricolor* Hartlaub, *Ploceus* spp. and *Lamprotornis nitens* Linnaeus), but it is also visited by specialist nectar feeding birds, and very large numbers of honeybees, as well as some smaller Hymenoptera and occasional Diptera (Hoffman, 1988; Diller et al., 2019). The amount of pollen deposited on stigmas by honeybees during a single visit is lower than that deposited by opportunistic nectar feeding birds, but more than that deposited by specialist birds (Diller et al., 2019). The low per-visit pollen deposition by honeybees, relative to opportunistic birds, is due to low levels of stigma contact and not due to grooming or behavioral avoidance of the female phase flowers (Appendix S4). Individual flowers receive many visits by

honeybees and the cumulative amount of pollen deposited on stigmas by honeybees is approximately the same as that deposited by opportunistic birds (see below).

Study site

We studied a large population (>500 plants) of *A. ferox* in Ashburton, South Africa (−29.644115 S, 30.492922 E). This site is part of the Lower Mpushini Valley Conservancy Reserve, with a relatively pristine savanna thornveld vegetation and associated fauna. The experiments took place in both 2017 and 2018 during the flowering periods from July 3 through August 12 in each year.

Pollinator behavior

To estimate the potential for among-flower deposition of self pollen by honeybees vs. birds, we recorded the number of flowers probed on a single plant for each of these visitor groups. Because birds are shy and must be observed from a distance through binoculars and honeybees are too small to accurately observe behavior through binoculars, we observed honeybees and birds separately. We recorded behavior of 329 birds over a period of 31 hours encompassing 12 days, and 159 honeybee visits observed over a period of six hours encompassing six days. In each case, we observed one plant at a time and, for each pollinator we recorded the number of flowers probed during its entire visitation bout on the plant.

Selective exclusion experiment

To assess the quantity and quality of pollen deposited by birds vs. honeybees, we performed a selective exclusion experiment on 25 *A. ferox* plants and measured the levels of pollen deposition, fruit and seed set, and ovule discounting in flowers. Because *A. ferox* has a system of late-acting self-incompatibility, self-pollination increases the level of ovule abortion (e.g., Duffy and Johnson, 2011). We implemented four treatments, each randomly allocated to a different raceme, on each plant individual: "bird pollination" (honeybee exclusion), "bee pollination" (bird exclusion), "all pollinators" (open pollination), and "no pollinators" (bagged). For a subset of 21 of these plants, we quantified pollen grains on a sample of five to 10 flowers (at the end of anthesis) per raceme in each treatment group to assess the total number of pollen grains deposited by birds vs. honeybees over a flower's life span.

Exposing racemes to only birds is methodologically challenging. During the 2017 winter season, honeybee activity was restricted to the warm hours between mid-morning (~10:30 am) and early afternoon (~3:00 pm), as was also noted by Hoffman (1988). We took advantage of this pattern, and the racemes subjected to "bird pollination"

were uncovered late each afternoon after bee activity ended and were covered again each mid-morning before bees became active. The racemes were covered with insect-proof netting (1 mm^2 mesh) draped over plastic trellis cages. This procedure was repeated each day until all flowers for each treated raceme had finished flowering. Because flowers in the "bird pollination" treatment excluded all pollinators during the middle of the day, their stigmatic pollen loads (and proportion of flowers setting fruit, see below) represent a minimum estimate of pollen deposited only by birds. By observing bird visitation throughout the day, we estimate that racemes pollinated only by birds received ~50% fewer bird visits than those in the open pollination treatments (see Appendix S5).

Racemes in the "bee pollination" treatment were covered continuously with plastic trellis cages (aperture: 1.5 × 1 cm) that excluded birds but not honeybees and other small insects. Honeybees were not deterred by exclusion cages (Appendix S6). Racemes in the "all pollinator" treatment were left uncaged, while racemes in the "no pollinator" treatment were bagged continuously.

We collected fruits from each exclusion treatment and counted the total number of fruits set out of the total number of flowers per raceme. In addition, we counted the number of seeds for a sample of five fruits per raceme and classified the fate of ovules within a fruit as viable seed, aborted (swollen) ovules, and unfertilized ovules.

"Split pollinator" experiment

To further assess the quality of pollen carried by honeybees, we captured foraging honeybees and randomly assigned them to be used with their existing pollen loads to either pollinate previously bagged flowers on the plant (within-plant treatment) on which they were captured or to pollinate previously bagged flowers on other plants at least 10 m away (among-plant treatment). The prediction for this experiment was that the among-plant treatment would yield more seeds than the within-plant treatment if honeybees carry mainly the pollen of the plant on which they were caught.

Honeybees were captured randomly on a plant, i.e., neither sampled systematically at the beginning nor at the end of their bouts, which ensured that their pollen loads (ratio of self:cross pollen) would, on average, be typical of the honeybees when they interact with the flowers. Tracking individual honeybees after they leave a plant was not feasible, thus we cannot use direct observations to determine whether they moved on to a different plant or whether they returned to their hive.

Honeybees were either killed instantly with an electric zapper or captured live and immediately placed in an Eppendorf tube in a cool box with ice to reduce grooming and facilitate handling. Both live and dead honeybees were used evenly across treatments, which were assigned randomly. Pollinations were performed by holding a captured

bee in forceps and brushing its ventral side over a 24 h-old unvisited stigma (see Figure 4, photo inset). Flowers were covered again to avoid further pollinations. We also attempted to pollinate flowers with pollen originating from the corbiculae, but these crosses did not lead to seed production, consistent with previous evidence that corbiculate pollen on bees is generally not viable (Parker et al., 2015). We collected a total of 42 honeybees with which we did 24 among-plant and 18 within-plant pollinations across seven different plant individuals. Each honeybee was used to pollinate a single stigma. Fruits were collected and seeds were counted as described above. We analyzed both the proportion of flowers that set fruit and the proportion of ovules that developed into seeds.

Statistical analysis

Unless otherwise stated, data were analyzed using generalized linear models implemented in SPSS 25 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY, USA). Count data (e.g., pollen loads, number of flowers visited) tended to have a variance that exceeded the mean and were thus analyzed using models that incorporated a negative binomial distribution and log link function, while analyses of proportion data (e.g., proportion of flowers setting fruit) incorporated a binomial distribution and logit link function. For studies that involved repeated sampling of the same individual, we used generalized estimating equations (GEEs) with an exchangeable correlation matrix to control for potentially correlated observations (Fitzmaurice et al., 2011). We treated the plant as the subject for GEE analyses of stigma loads on flowers and seeds per fruit (five per raceme) in the exclusion experiments, honeybee behavior in the visitation observations, and within- vs. among-plant pollinations using different captured bees. For analyses of the differences in pollen loads among bee body parts, we treated the individual bee as the subject. Model significance was assessed using likelihood ratios, except in the case of GEEs for which we used Wald statistics. Marginal means and standard error (SE) values were back-transformed from the scales of link functions used in the analyses, resulting in asymmetric lower and upper SE values (hereafter, LSE and USE, respectively). All post hoc pairwise comparisons were performed with sequential Šidák correction. Pearson correlation was used to test for a relationship between the number of viable seeds and aborted ovules per fruit across all pollination treatments from the selective exclusion experiments.

RESULTS

Pollinator behavior

Honeybees probed significantly fewer flowers (mean [LSE, USE]: 10 [9, 11]) than did both opportunistic (23 [21, 24])
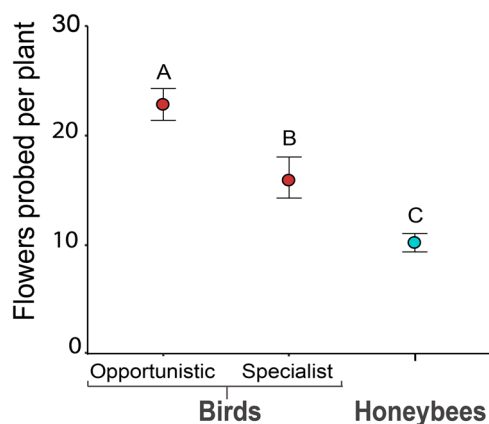

FIGURE 1 The number of flowers on *Aloe ferox* plants probed during animal foraging bouts. Means that do not share letters are significantly different ($P < 0.01$).

and specialist (16 [14, 18]) nectar feeding birds during a visitation bout ($\chi^2 = 56.2$, $P < 0.001$, post hoc comparisons: $P < 0.001$ and $P = 0.003$, respectively; Figure 1).

Selective exclusion experiment

Flowers subjected to different exclusion experiments differed significantly in their accumulated stigma pollen loads ($\chi^2 = 29.9$, $P < 0.001$), and post hoc comparisons showed that these differences were only between the control (i.e., no pollination/bagged) and the rest of the exclusion treatments. Hence, cumulative stigma pollen loads did not differ between flowers exposed exclusively to either bees or birds, nor to racemes exposed to all pollinators (Figure 2A).

Fruit set differed significantly across exclusion treatment ($\chi^2 = 116.7$, $P < 0.001$; Figure 2B). The number of viable seed and aborted ovules also differed across exclusion treatments ($\chi^2 = 17.27$, $P < 0.001$ and $\chi^2 = 82.63$, $P < 0.001$, respectively), but unfertilized ovules did not ($\chi^2 = 1.47$, $P = 0.479$). The number of viable seeds was significantly higher for both open and bird pollination treatments when compared to bee pollinated flowers ($P < 0.001$ and $P = 0.001$, respectively). Conversely, the number of aborted ovules was significantly higher for bee pollinated flowers in comparison to open pollination and bird pollinated flowers ($P < 0.001$, Figure 2C). We observed a significant negative correlation between viable seed and aborted ovules (Pearson $r = -0.428$, $P < 0.001$; Figure 3).

Split pollinator experiment

Flowers pollinated by honeybees captured on the same plant (within-plant hand pollination) set fewer fruits and seeds in comparison with those pollinated by honeybees captured on different plants (among-plant hand pollinations; Figure 4). These differences were marginally nonsignificant in the case
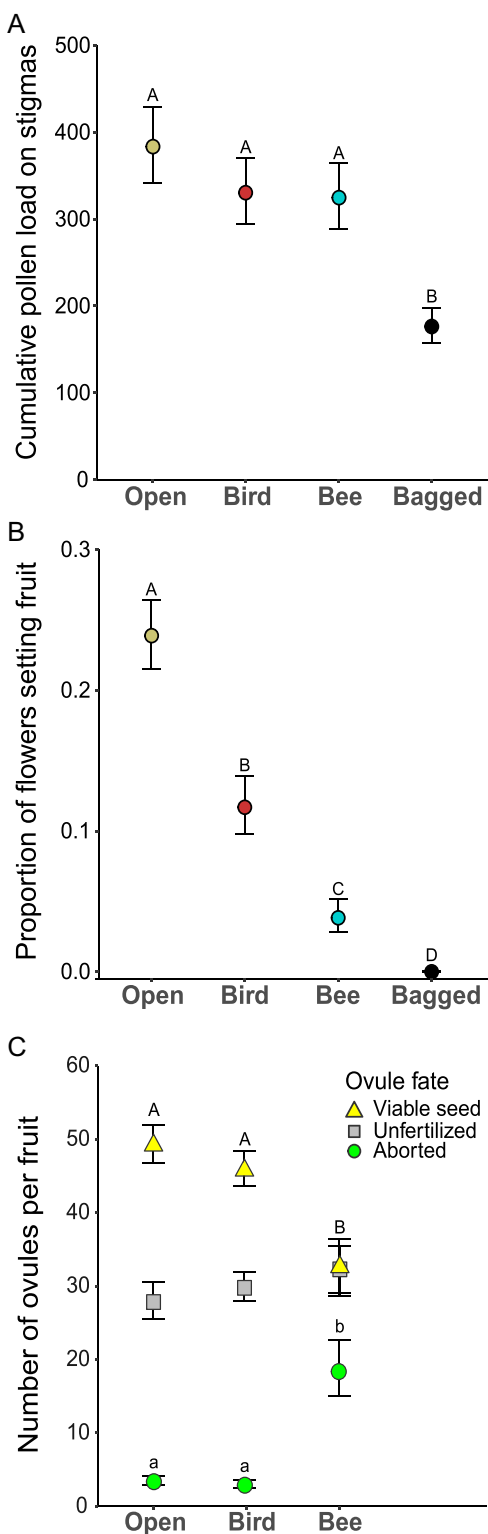
FIGURE 2 Selective exclusion experiments on *Aloe ferox*. Mean (±SE) values for the (A) accumulated pollen load on stigmas, (B) proportion of flowers setting fruit per raceme, and (C) fate of ovules per fruit. Open: racemes were exposed to all pollinators; Bird: racemes were covered during the honeybee activity period; Bee: racemes were covered with cages with a mesh size that allowed access to honeybees (and other insects) but not birds; Bagged: racemes were covered and excluded from all pollinators. Means that do not share letters are significantly different ($P \leq 0.001$).
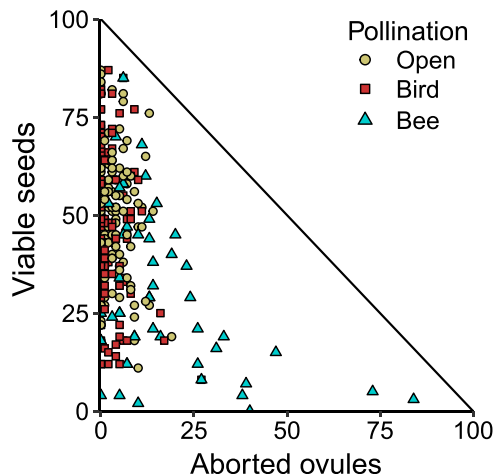

FIGURE 3 The trade-off between viable seed and aborted ovules per fruit in *Aloe ferox* (Pearson $r = -0.428$, $P < 0.001$).

of fruit set ($\chi^2 = 3.8$, $P = 0.051$; Figure 4A), but were significant in the case of seed set ($\chi^2 = 5.53$, $P = 0.019$; Figure 4B).

DISCUSSION

Our results indicate that pollen deposited by honeybees on *Aloe ferox* stigmas is mostly low quality self pollen that translates into very poor fruit and seed set. Honeybees deposit less pollen than do birds on a single visit basis (Diller et al., 2019), but cumulatively deposit as much pollen as do birds (Figure 2A). The results of the analysis of aborted ovules following selective exclusion of birds (Figure 2C) and the "split-pollinator" experiment (Figure 4) are consistent with our hypothesis that honeybees carry mostly low quality self pollen. This pollen is likely to arise from geitonogamous self-pollination among flowers on the same plant (Free, 1966a; Paton, 1993; Brittain et al., 2013; Mallinger and Gratton, 2015). However, the difference in quality of pollination between honeybees and birds cannot be explained solely by geitonogamy because birds actually probe significantly more flowers per plant than do honeybees (Figure 1). Therefore, the differences in quality of pollen deposited by the two groups of flower visitors is likely to reflect a smaller fraction of cross pollen on honeybees because of limited movements between plants during foraging bouts. Honeybees probably often fill their scopae and crop with pollen and nectar from a single *A. ferox* plant and subsequently return to their hives and perhaps even return to the same plant individual on the next foraging trip. This would explain the evidence for a high fraction of self pollen on their bodies and also explains the sharp reduction of fruit and seed set when birds, but not bees, are excluded from flowers (Figure 2B,C; Strokes and Yeaton, 1995; Botes et al., 2009; Hargreaves et al., 2012). Birds do not return to any particular place between bouts
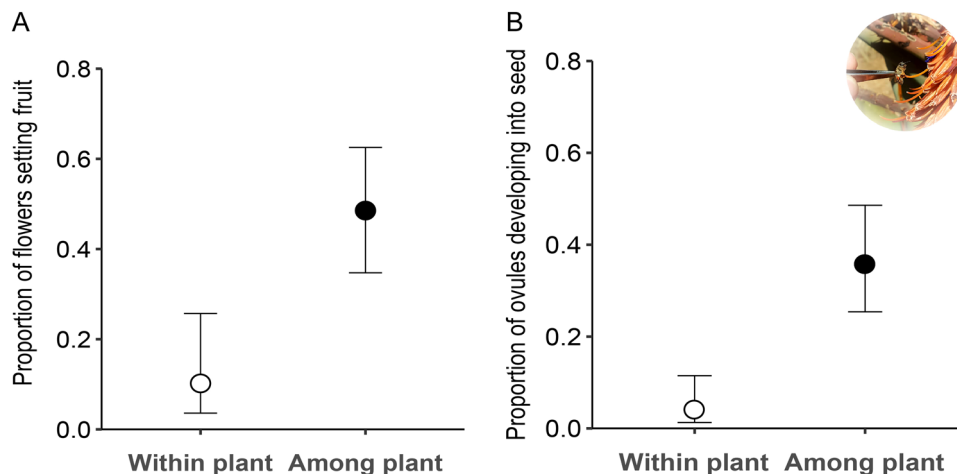
FIGURE 4 Mean (±SE) values for the (A) proportion of *Aloe ferox* flowers setting fruit, and (B) proportion of ovules developing into viable seed for flowers in the "split-pollinator" experiment. Among-plant crosses were done with honeybees captured while foraging on a plant different to the one that was hand-pollinated. Within-plant crosses were done with honeybees captured while foraging on the same plant that was hand-pollinated. Inset: manual pollination with a captured honeybee.

and thus likely move among plants more often than honeybees do, as reflected in the higher fruit and seed set for the racemes from which bees, but not birds, were excluded from flowers (Figure 2B,C).

Pollen grooming (Appendix S4) and the low levels of among-plant visitation by honeybees can render them as pollen thieves (Hoffman, 1988; Hargreaves et al., 2009, 2010). Here, we also show that self-pollination by honeybees can also severely affect the female reproductive success of *A. ferox* through ovule discounting. This is demonstrated by the overall negative relationship between the number of viable fertilized ovules and the number of aborted ovules (Figures 2C, 3). This trend, which is particularly evident in fruits arising from honeybee pollination, is a consequence of the late-acting self-incompatibility system present in *A. ferox*. Self pollen not only germinates but also penetrates ovules that will later degenerate (Appendix S1). Hence, pollinator-mediated self-pollination has a negative female fitness cost by usurping ovules that otherwise could be fertilized by outcross pollen grains and develop into viable seeds (Barrett et al., 1996). This is one of very few studies to show that visits by different animal species lead to differing rates of ovule discounting (e.g., Duffy and Johnson, 2011; Duffy et al., 2020).

A paradox in this study was that fruit set, but not cumulative pollen loads on stigmas or seed set, was lower in the bird-only treatment compared to the open treatment (Figure 2A,B). This could reflect saturation of stigmas or that flowers in the open treatment received more pollen, and honeybees collected some of this pollen from their stigmas (Gross and Mackay, 1998). The lower fruit set and equivalent seed set in the bird-only treatment compared to the open treatment likely reflects racemes in the bird-only treatment received about 50% fewer bird visits than did open-pollinated racemes (Appendix S5). Hence, it is likely that stigmatic pollen deposition, fruit set, and seed set

arising from bird visits are higher than were estimated in this study.

Recent meta-analyses showed that honeybees are generally less efficient when compared to the most efficient non-*Apis* pollinators for various plant species (Hung et al., 2018; Page et al., 2021), and this is particularly true for when honeybees are compared to birds (Page et al., 2021). It also applies both where honeybees are native (e.g., Brown et al., 2009) and where they are introduced (e.g., Celebrezze and Paton, 2004). The introduction of honeybees to continents and islands where they are not native has raised concerns about their effects on native pollinators and local flora (Goulson, 2003). The potential negative repercussions of honeybees on native plant fecundity include pollen theft, both from anthers and stigmas, and ovule and seed discounting (this study, Gross and Mackay, 1998; England et al., 2001), as well as disruption of other plant-pollinator interactions (e.g., Valido et al., 2019). Our study highlights the potential negative effects of introduced honeybees on native mass flowering plants such as shrubs and trees, resulting from their tendency to develop extreme floral constancy on a single plant.

CONCLUSIONS

We demonstrate that the quality of pollen deposited by honeybees can be a key factor in determining their pollination effectiveness. This may help explain why relatively few plants are specialized for pollination by honeybees in regions where they are native (Stanley et al., 2020). These findings raise additional concerns about the potential effects of introduced honeybees on native flora around the world. Finally, in agricultural contexts, our results underscore the importance of management practices

that foster among-plant movement by honeybees to improve yields of mass-flowering tree or shrub crops.

COMPETING INTERESTS

We declare we have no competing interests.

DATA AVAILABILITY STATEMENT

Data and coding available at Figshare: https://doi.org/10.6084/m9.figshare.20110787.

ORCID

Carolina Diller http://orcid.org/0000-0002-7145-3299
Miguel Castañeda-Zárate http://orcid.org/0000-0002-3179-358X
Steven D. Johnson http://orcid.org/0000-0002-5114-5862

REFERENCES

Barrett, S. C. H., D. G. Lloyd, and J. Arroyo. 1996. Stylar polymorphisms and the evolution of heterostyly in *Narcissus* (Amaryllidaceae). *In* S. C. H. Barrett and D. G. Lloyd [eds.], Floral biology: studies on floral evolution in animal-pollinated plants, 339–376. Chapman & Hall, New York.

Botes, C., S. D. Johnson, and R. M. Cowling. 2009. The birds and the bees: using selective exclusion to identify effective pollinators of African tree Aloes. *International Journal of Plant Sciences* 170: 151–156.

Brittain, C., N. Williams, C. Kremen, and A.-M. Klein. 2013. Synergistic effects of non-*Apis* bees and honey bees for pollination services. *Proceedings of The Royal Society B* 280: 1–7.

Brosi, B. J. 2016. Pollinator specialization: from the individual to the community. *New Phytologist* 210: 1190–1194.

Brown, M., C. T. Downs, and S. D. Johnson. 2009. Pollination of the red hot poker *Kniphofia caulescens* by short-billed opportunistic avian nectarivores. *South African Journal of Botany* 75: 707–712.

Brunet, J., and K. G. A. Holmquist. 2009. The influence of distinct pollinators on female and male reproductive success in the Rocky Mountain columbine. *Molecular Ecology* 18: 3745–3758.

Castellanos, M. C., P. Wilson, and J. D. Thomson. 2003. Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. *Evolution* 57: 2742–2752.

Celebrezze, T., and D. C. Paton. 2004. Do introduced honeybees (*Apis mellifera*, Hymenoptera) provide full pollination service to bird-adapted Australian plants with small flowers? An experimental study of *Brachyloma ericoides* (Epacridaceae). *Austral Ecology* 29: 129–136.

Diller, C., M. Castañeda-Zárate, and S. D. Johnson. 2019. Generalist birds outperform specialist sunbirds as pollinators of an African *Aloe*. *Biology Letters* 15: 1–5.

Dudash, M. R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution* 44: 1129–1139.

Duffy, K. J., and S. D. Johnson. 2011. Effects of pollen reward removal on fecundity in a self-incompatible hermaphrodite plant. *Plant Biology* 13: 556–560.

Duffy, K. J., Z. M. Mdlalose, and S. D. Johnson. 2021. Sexual conflict in hermaphroditic flowers of an African aloe. *International Journal of Plant Sciences* 182: 238–243.

Duffy, K. J., K. L. Patrick, and S. D. Johnson. 2020. Outcrossing rates in a rare "ornithophilous" aloe are correlated with bee visitation. *Plant Systematics and Evolution* 306: 1–9.

Dupont, Y. L., K. Trøjelsgaard, and J. M. Olesen. 2011. Scaling down from species to individuals: a flower–visitation network between individual honeybees and thistle plants. *Oikos* 120: 170–177.

England, P. R., F. Beynon, D. J. Ayre, and R. J. Whelan. 2001. A molecular genetic assessment of mating-system variation in a naturally bird-pollinated shrub: Contributions from birds and introduced honey-bees. *Conservation Biology* 15: 1645–1655.

Fitzmaurice, G. M., N. M. Laird, and J. H. Ware. 2011. Applied Longitudinal Analysis. Second. John Wiley & Sons, New Jersey.

Free, J. B. 1966a. The foraging areas of honeybees in an orchard of standard apple trees. *Journal of Applied Ecology* 3: 261–268.

Free, J. B. 1966b. The pollinating efficiency of honey-bee visits to apple flowers. *Journal of Horticulture Science* 41: 91–94.

Garibaldi, L. A., I. Steffan-Dewenter, R. Winfree, M. A. Aizen, R. Bommarco, S. A. Cunningham, C. Kremen, et al. 2014. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* 339: 1608–1611.

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.

Han, F., A. Wallberg, and M. T. Webster. 2012. From where did the western honeybee (*Apis mellifera*) originate? *Ecology and Evolution* 2: 1949–1957.

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.

Hargreaves, A. L., L. D. Harder, and S. D. Johnson. 2010. Native pollen thieves reduce the reproductive success of a hermaphroditic plant, *Aloe maculata*. *Ecology* 91: 1693–1703.

Hargreaves, A. L., L. D. Harder, and S. D. Johnson. 2012. Floral traits mediate the vulnerability of aloes to pollen theft and inefficient pollination by bees. *Annals of Botany* 109: 761–772.

Hoffman, M. T. 1988. The pollination ecology of *Aloe ferox* Mill. *South African Journal of Botany* 54: 345–350.

Hung, K.-L. J., J. M. Kingston, M. Albrecht, D. A. Holway, and J. R. Kohn. 2018. The worldwide importance of honey bees as pollinators in natural habitats. *Proceedings of the Royal Society B: Biological Sciences* 285: 1–8.

Klein, A.-M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of The Royal Society B* 274: 303–313.

Krauss, S. L., R. D. Phillips, J. D. Karron, S. D. Johnson, D. G. Roberts, and S. D. Hopper. 2017. Novel consequences of bird pollination for plant mating. *Trends in Plant Science* 22: 395–410.

Mallinger, R. E., and C. Gratton. 2015. Species richness of wild bees, but not the use of managed honeybees, increases fruit set of a pollinator-dependent crop. *Journal of Applied Ecology* 52: 323–330.

Matsuki, Y., R. Tateno, M. Shibata, and Y. Isagi. 2008. Pollination efficiencies of flower-visiting insects as determined by direct genetic analysis of pollen origin. *American Journal of Botany* 95: 925–930.

Minnaar, C., and B. Anderson. 2019. Using quantum dots as pollen labels to track the fates of individual pollen grains. *Methods in Ecology and Evolution* 10: 604–614.

Page, M. L., C. C. Nicholson, R. M. Brennan, A. T. Britzman, J. Greer, J. Hemberger, H. Kahl, et al. 2021. A meta-analysis of single visit pollination effectiveness comparing honeybees and other floral visitors. *American Journal of Botany* 108(11): 2196–2207.

Parker, A. J., J. L. Tran, J. L. Ison, J. D. K. Bai, A. E. Weis, and J. D. Thomson. 2015. Pollen packing affects the function of pollen on corbiculate bees but not non-corbiculate bees. *Arthropod-Plant Interactions* 9: 197–203.

Paton, D. C. 1993. Honeybees in the Australian environment. Does *Apis mellifera* disrupt or benefit the native biota? *BioScience* 43: 95–103.

Patrick, K. L., S. D. Johnson, and K. J. Duffy. 2018. High levels of fecundity in small and isolated populations of a self-compatible *Aloe* pollinated by opportunistic birds and bees. *Plant Biology* 20: 780–788.

Reynolds, R. J., M. J. Westbrook, A. S. Rohde, J. M. Cridland, C. B. Fenster, and M. R. Dudash. 2009. Pollinator specialization and pollination syndromes of three related North American *Silene*. *Ecology* 90: 2077–2087.

Stanley, D. A., S. M. Msweli, and S. D. Johnson. 2020. Native honeybees as flower visitors and pollinators in wild plant communities in a biodiversity hotspot. *Ecosphere* 11: e02957.

Steenhuisen, S. L., H. van der Bank, and S. D. Johnson. 2012. The relative contributions of insect and bird pollinators to outcrossing in an African Protea (Proteaceae). *American Journal of Botany* 99: 1104–1111.

Stewart, A. B., and M. R. Dudash. 2016. Differential pollen placement on an Old World nectar bat increases pollination efficiency. *Annals of Botany* 117: 145–152.

Stoepler, T. M., A. Edge, A. Steel, and R. L. O'Quinn. 2012. Differential pollinator effectiveness and importance in a milkweed (*Asclepias*, Apocynaceae) hybrid zone. *American Journal of Botany* 99: 448–458.

Strokes, C. J., and R. I. Yeaton. 1995. Population dynamics, pollination ecology and the significance of plant height in *Aloe candelabrum*. *African Journal of Ecology* 33: 101–113.

Thomson, J. D., and K. Goodell. 2001. Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. *Journal of Applied Ecology* 38: 1032–1044.

Valido, A., M. C. Rodríguez-Rodríguez, and P. Jordano. 2019. Honeybees disrupt the structure and functionality of plant-pollinator networks. *Scientific Reports* 9: 1–11.

Vaughton, G., and M. Ramsey. 2010. Floral emasculation reveals pollen quality limitation of seed output in *Bulbine bulbosa* (Asphodelaceae). *American Journal of Botany* 97: 174–178.

Waser, N. M., and M. V Price. 1991. Reproductive costs of self-pollination in *Ipomopsis aggregata* (Polemoniaeae): Are ovules usurped? *American Journal of Botany* 78: 1036–1043.

Welsford, M. R., and S. D. Johnson. 2012. Solitary and social bees as pollinators of *Wahlenbergia* (Campanulaceae): single-visit effectivenes, overnight sheltering and responses to flower colour. *Arthropod-Plant Interactions* 6: 1–14.

Wester, P., and S. D. Johnson. 2017. Importance of birds versus insects as pollinators of the African shrub *Syncolostemon densiflorus* (Lamiaceae). *Botanical Journal of the Linnean Society* 185: 225–239.

Wilson, P., and J. D. Thomson. 1991. Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. *Ecology* 72: 1503–1507.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Late-acting self-incompatibility in *Aloe ferox*.

Appendix S2. Determination of the female phase in *Aloe ferox* flowers.

Appendix S3. Determination of pollen longevity in *Aloe ferox* flowers.

Appendix S4. Quantitative assessment of honeybee pollination service to *Aloe ferox*.

Appendix S5. Estimate of the proportion of bird visits excluded in the bee exclusion experiments.

Appendix S6. Cage effect on honeybee visitation.