DARK, BITTER-TASTING NECTAR FUNCTIONS AS A FILTER OF FLOWER VISITORS IN A BIRD-POLLINATED PLANT

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Abstract. Floral nectar is offered by plants to animals as a reward for pollination. While nectar is typically a clear liquid containing sugar and trace amounts of amino acids, colored nectar has evolved in several plant families. Here we explore the functional significance of the phenolic compounds that impart a dark brown color to the nectar of the South African succulent shrub *Aloe vryheidensis*. Flowers of this aloe are visited for their nectar by a suite of short-billed birds that are occasional nectarivores, including bulbuls, white-eyes, rock thrushes, and chats. Dark-capped Bulbuls were more likely to probe model flowers containing dark nectar than those containing clear nectar, suggesting a potential signaling function for dark nectar. However, the main effect of the phenolics appears to be to repel "unwanted" nectarivores that find their bitter taste unpalatable. Nectar-feeding honey bees and sunbirds are morphologically mismatched for pollinating *A. vryheidensis* flowers and strongly reject its nectar. However, the frugivorous and insectivorous birds that effectively pollinate this aloe are seemingly unaffected by the nectar's bitter taste. Thus the dark phenolic component of the nectar appears to function as a floral filter by attracting some animals visually and deterring others by its taste.

Key words: Aloe vryheidensis; bird pollination; flower models; honest signal; mutualism; nectar color; nectar palatability; phenolic; pollination syndrome; South Africa; specialization; sunbird.

INTRODUCTION

Plants that have flowers specialized for transferring pollen via particular animals can suffer reduced fitness if their flowers are also visited by animals that are ineffective as pollen vectors (Lau and Galloway 2004). This problem can be largely overcome if flowers act as filters, allowing only legitimate pollinators access to the floral rewards (Johnson and Steiner 2000, Castellanos et al. 2004). Floral traits that have been suggested to act as filters of animal visitors include colors that are inconspicuous to "nectar thieves" (Raven 1972); long, narrow corolla tubes (Alexandersson and Johnson 2001); unusual blends of compounds in floral fragrances (Schiestl et al. 1999); dilute nectar (Irwin et al. 2004); and toxic or unpalatable nectar (Baker and Baker 1975, Rhoades and Bergdahl 1981). Here we show that secondary compounds in nectar can function as effective visitor filters, resulting in a relatively specialized pollination system for a plant with open flowers and exposed nectar.

Nectar generally serves a straightforward function as an energy-rich enticement for animals to visit flowers (Baker and Baker 1983). The volume and sugar concentration of nectar are broadly correlated with particular classes of animal visitors (Baker and Baker

1983), but combinations of these two variables do not act as strict floral filters. Bees, for example, often feed on nectar in flowers that are adapted for pollination primarily by birds (Irwin and Brody 1999). It has been suggested that secondary compounds (e.g., alkaloids and phenolics) in nectar could play a more decisive role in filtering the visitors to flowers (Janzen 1977, Stephenson 1981, 1982, Hagler and Buchmann 1993). In a pioneering study, Stephenson (1981, 1982) found that iridoid glycosides in the nectar of *Catalpa speciosa* (Bignoniaceae) had an adverse effect on potential nectar thieves (ants and a skipper butterfly), while the regular bee pollinators were seemingly unaffected by these compounds.

Secondary compounds can change the appearance (Mione and Anderson 1996, Olesen et al. 1998, Weller et al. 1998), taste (Baker 1978, Hagler and Buchmann 1993, Adler 2000, Gardener and Gillman 2002), and digestibility (Tadmor-Melamed et al. 2004) of nectar. In one of the most striking cases, the nectar of a cliff-dwelling Mauritian plant *Nescodon mauritianus* (Campanulaceae) is rendered bright red by the presence of aurones, a product of flavonoid biosynthesis (Olesen et al. 1998). Although experiments were not conducted by Olesen et al. (1998), they did find a general association on Mauritius between colored nectar and visitation by birds, especially occasional nectarivores such as bulbuls and white-eyes. It was also recently shown that a Mauritian *Phelsumo* gecko prefers colored over clear

nectar (Hansen et al. 2006). Although this gecko has been observed to visit flowers of other Mauritian plants with colored nectar (Hansen et al. 2006), it is not yet known whether it plays any role in the pollination of *N. mauritianus*.

In South Africa, three aloe species belonging to section *Anguialoe* (*Aloe spicata* L.f., *A. castanea* Schonl., and *A. vryheidensis* Groenewald; Fig. 1A) have dark red-brown nectar with a distinctive bitter taste to humans (S. D. Johnson and A. L. Hargreaves, *personal observation*). The dark color and bitter taste of the nectar in these species are due to phenolic compounds (S. D. Johnson and F. van Heerden, *unpublished data*). Phenolics in the nectar of species belonging to *Aloe* section *Anguialoe* are present in unusually high concentrations (~1.2–1.5 mg/mL) compared to aloes with clear nectar (S. D. Johnson, *unpublished data*). *Aloe* leaves are well known as a source of phenolic compounds that are used medicinally and as an additive ("bitters") to various beverages (Reynolds 1985). Phenolics have been previously reported to occur in the nectar of *A. littoralis* Bak. and to deter feeding by honey bees in North America, where *A. littoralis* is cultivated as an ornamental plant (Hagler and Buchman 1993).

The functional significance of the unusual dark nectar in *Aloe* section *Anguialoe* has not been investigated previously, although anecdotal observations in the literature indicate that bees avoid drinking it (Reynolds 1950, Nicolson and Nepi 2005). In this study we ask (1) which flower visitors are effective pollinators of *A. vryheidensis*, (2) whether the dark color of the nectar is attractive to effective pollinators, and (3) whether the nectar deters ineffective pollinators because of its bitter taste.

METHODS

Floral traits

The study species, *Aloe vryheidensis*, is a succulent shrub ~2 m in height that grows in rocky habitats on mountain summits. Flowering takes place during the winter months of July and August. A natural population of ~200 flowering plants of *A. vryheidensis* was studied in the iGwala Gwala game reserve near Louwsberg in KwaZulu-Natal, South Africa, during August 2005. We arbitrarily selected 15 plants in this population for measurements of morphology, including the number of inflorescences per plant, number of flowers per inflorescence, style length, flower depth, and flower width. The bitter taste of the nectar in this population was

verified by applying small amounts (~0.5 μL) to the tip of our tongues. The standing crop of nectar was sampled from three flowers on each of 12 plants at 0900 and 1300 hours over two days, the first misty and cold (maximum <18°C) and the second sunny and hot (maximum 31°C). Volume and concentration were determined with 100-μL micropipettes and a handheld Bellingham and Stanley refractometer (Tunbridge Wells, Kent, UK), respectively. Data for nectar volume and concentration were analyzed using two-way ANOVAs with day and time of day as factors and individual plants as replicates (values for different flowers on a plant were averaged prior to the analyses). Production of nectar over a 24-h period was determined from 12 flowers that were initially drained and then bagged to exclude visitors.

Pollinator observations

Observations of pollinators were conducted between 0800 and 1600 hours over a three-day period. All avian flower visitors were identified and their behavior noted. Insect abundance was surveyed on 20–33 plants in the morning and afternoon, and additional observations were made whenever new insects were encountered. *A. vryheidensis* was the only plant producing orange pollen at the time of the study; thus large pollen loads on birds could be identified with binoculars. Pollen loads were further confirmed and quantified from birds captured in mist nets, by collecting pollen samples from their heads and comparing these to a reference pollen collection. Efficiency of the various bird species as pollen vectors in terms of their likelihood of coming into contact with the anthers and stigma was further gauged by inserting the heads of museum specimens (bulbuls, white-eyes, and sunbirds) into flowers of *A. vryheidensis*.

To assess the role of birds as pollinators, inflorescences with unopened buds were enclosed in cages constructed of rigid plastic mesh (aperture 20 mm diameter) that have been shown in studies of six other *Aloe* species to exclude birds without affecting the rate of visitation by bees (A. L. Hargreaves, *unpublished data*). Seed production in these caged inflorescences was later compared with that of adjacent unmanipulated control inflorescences on the same plant. To establish the extent to which *A. vryheidensis* is dependent on pollinators for reproduction, inflorescences on five plants were enclosed in fine gauze from the bud stage to exclude all pollinators, and later examined for fruit production. Although all aloe species studied to date are largely or completely self-incompatible (Hoffman 1988; A. L. Hargreaves, *unpublished data* for five *Aloe* species),

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FIG. 1. *Aloe vryheidensis* and its flower visitors. (A) flowering plants of *A. vryheidensis* (scale bar 50 cm). (B) Black-capped Bulbuls feeding from flowers (scale bar 100 mm). (C) Cape White-eye carrying visible pollen of *A. vryheidensis* under its chin (scale 50 mm). (D) Flower being probed by a Cape White-eye (scale 50 mm). (E) Large load of *A. vryheidensis* pollen on the head of a Buff-streaked Chat (scale 20 mm). (F) Cape Rock Thrush carrying large amounts of *A. vryheidensis* pollen (scale 20 mm). (G) Honey bees collect pollen from flowers but ignore the copious black nectar. Arrows show the difference in nectar color between newly opened (upper) and older (lower) flowers (scale 10 mm). (H) Model flowers used to test the responses of birds to dark nectar (scale 10 mm).
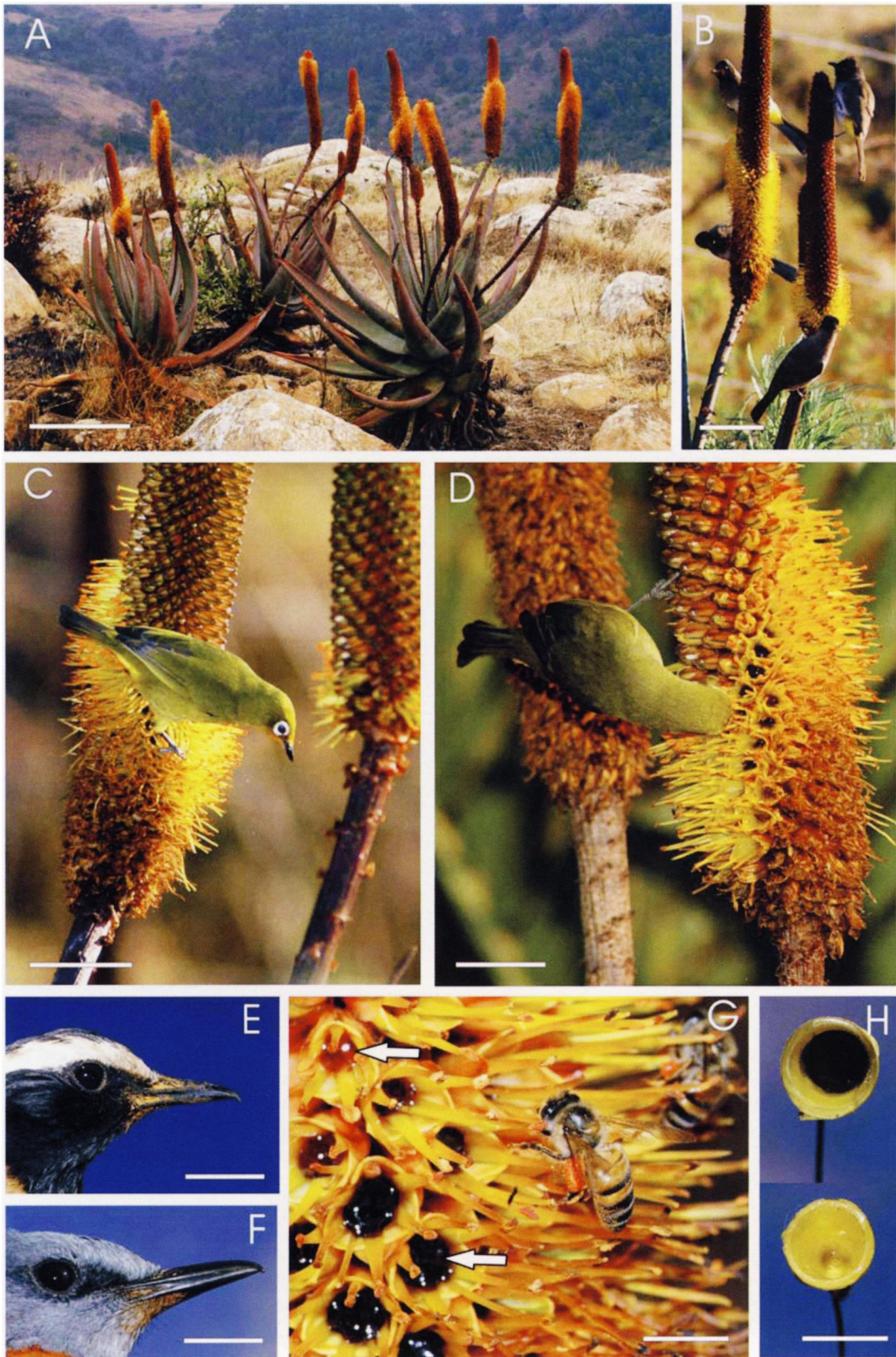
we carried out a small pilot study of the compatibility system in *A. vryheidensis* using 320 flowers that reached anthesis on one of the bagged plants. Flowers on this plant were randomly assigned to be either self-pollinated (to test for self-compatibility) or cross-pollinated (as a control). Fruits and seeds produced by flowers in these treatment groups were counted at the end of the flowering season.

Behavioral experiments

Behavioral responses of birds and bees to the nectar of *A. vryheidensis* were determined in experiments conducted in large outdoor aviaries (4 m²) and in the laboratory. The bird species used were Dark-capped Bulbuls (*Pycnonotus tricolor*; $n = 16$), Cape White-eyes (*Zosterops pallidus*; $n = 20$), and Amethyst Sunbirds (*Chalcomitra amethystine*; $n = 2$). The birds were divided among seven aviaries (three with bulbuls, two with white-eyes, and two with one sunbird each). Due to the limited availability of sunbirds for the aviary experiments, additional observations were made of the responses of sunbirds to three *A. vryheidensis* inflorescences translocated into a patch of the sunbird-pollinated species *Aloe maculata*.

To establish whether birds discriminate visually between flowers with dark vs. clear nectar, birds (bulbuls, white-eyes, and sunbirds) were offered a choice between two yellow model flowers (base sections of 1.5-mL Eppendorf tubes cut in half at the 0.5-mL mark and wrapped in yellow plastic insulation tape), identical in all respects except that the base of one was wrapped in black plastic insulation tape to give the nectar a dark appearance (Fig. 1H). The model flowers were filled with 50 μL of a 12% hexose solution (glucose and fructose in a 50:50 mixture, corresponding to the known composition of *Aloe* nectar; van Wyk et al. 1993) and were placed 5 cm apart above a suitable perch using a wire framework. The first model flower to be probed by a bird was recorded in each trial. After each trial the sugar solution was replaced and the position (with respect to left and right) of each model was randomized, to avoid any learning of positions or imitation among birds. The identity of individual birds could be determined from their unique color bands and was recorded for each trial.

Palatability of *A. vryheidensis* nectar to birds and honey bees was established in feeding trials involving choices among solutions of hexose, sucrose, and *A. vryheidensis* nectar (taken from flowers that had been open for at least one day). The sugar solutions and nectar consisted of 12% sugar by mass and were offered to birds simultaneously in three yellow model flowers (constructed as described in this section). Each trial was terminated after a bird had probed all three of the model flowers, after which the amount of sugar solution and nectar consumed were recorded, and the model flowers were replenished. We also recorded whether the bird showed an adverse response, such as head shaking, after

probing a model flower. The positions of the three model flowers were randomized after each trial.

Honey bees ($n = 12$) were captured and kept in individual containers (20 cm²) for up to 20 minutes prior to the start of behavioral experiments. They were then offered sugar solution and nectar in the form of 5-μL droplets on a 5 cm diameter yellow plastic disk. In one set of trials, honey bees were offered a choice between two 5-μL droplets of sucrose solution and two 5-μL droplets of *A. vryheidensis* nectar, while in the second set of trials a hexose solution was substituted for the sucrose solution. Each trial was terminated after an individual bee had probed all four droplets on the disk. The volume of each droplet that remained at the end of the experiment was determined using a calibrated micro-pipette. The identity of individual bees was recorded in each trial.

RESULTS

Floral traits

Aloe vryheidensis plants ($n = 15$; all values given as mean ± SE) had 1.9 ± 0.22 inflorescences, each bearing 483 ± 29.9 flowers with a depth of 13.7 ± 0.38 mm, a width of 11.3 ± 0.35 mm, and a style length of 21.8 ± 0.95 mm. Flowers produced copious amounts of nectar, replacing nectar removed from open flowers at an average rate of 9.2 ± 1.6 μL/d ($n = 12$). The average standing crop of nectar per flower varied between 27 and 70 μL, depending on day ($F_{1,43} = 64.1$; $P < 0.001$), but not time of day ($F_{1,43} = 0.09$; $P = 0.76$; Appendix A). Average sugar concentration varied between 5.6% and 17% by mass, depending on day ($F_{1,43} = 62.7$; $P < 0.0001$) and time of day ($F_{1,43} = 10.5$; $P = 0.002$; Appendix A).

Nectar at anthesis is reddish brown, but darkens through oxidation of phenolics as flowers age (Fig. 1G). Similar color changes over a period of one to two days were observed in nectar from newly opened flowers that was stored in open vials. Fresh and aged nectar tasted equally bitter to us.

Pollinator observations

Flowers of *A. vryheidensis* were visited by eight bird species during the observation periods (Appendix B). The most common visitors, in order of decreasing frequency, were Dark-capped Bulbuls (Fig. 1B), Cape White-eyes (Fig. 1C, D), Streaky-headed Canaries, Buff-streaked Chats (Fig. 1E), and Cape Rockthrushes (Fig. 1F). The most striking commonality among these species is that none are specialized nectarivores. Individuals of each of these species were observed carrying appreciable quantities of aloe pollen. Bulbuls and white-eyes observed arriving at *A. vryheidensis* plants with no visible pollen on their faces picked up copious pollen loads in less than one minute. All birds caught in mist nets (one chat, one rockthrush, one bulbul, and five white-eyes) were carrying *A. vryheidensis* pollen, and the two with visible pollen loads carried massive quantities:
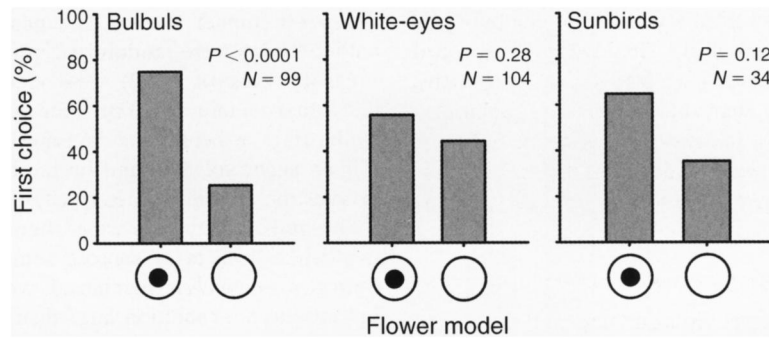
FIG. 2. Choices between dark-centered and all-yellow model flowers (shown as diagrams) for three bird species. Sample sizes represent the number of choices as tests showed homogeneity of responses among individual birds (see *Results: Behavioral experiments* for details).

152 000 and 10 800 grains on the chat and rockthrush, respectively (Fig. 1E, F). By inserting the heads of stuffed birds into flowers of *A. vryheidensis*, we confirmed that the faces of bulbuls and white-eyes make effective contact with the anthers and stigmas, while this is precluded by the longer, narrower bills of the sunbirds (Appendix B, C).

Although three sunbird species were abundant at the study site, we observed only one individual Greater Double-collared Sunbird feeding at an *A. vryheidensis* plant, and then only briefly. Native honey bees were frequent visitors to *A. vryheidensis* flowers (Fig. 1G), especially in the morning, when we recorded 1.7 ± 0.29 bees per surveyed plant ($n = 33$). In the afternoon, small halictid bees were even more abundant, averaging 6.5 ± 1.22 bees per plant ($n = 20$). However, both types of bees gathered pollen exclusively and did not attempt to drink the nectar.

The seed production per flower in inflorescences from which birds were excluded ($n = 9$) was significantly lower than in adjacent open-pollinated inflorescences (2.9 ± 0.7 vs. 6.0 ± 1.4; $P = 0.016$, paired t test). No seeds were produced by bagged control flowers ($n = 400$ flowers on four plants), indicating that *A. vryheidensis* is fully dependent on pollinator visits for seed production. Self-

pollination of flowers yielded few fruits relative to cross-pollination (35% vs. 92%; $G = 122.2$, $P < 0.001$). In addition, the mean number of seeds in fruits arising from self-pollination was 10-fold lower than in fruits arising from cross-pollination (2.2 ± 1.4 vs. 26.5 ± 0.6; $t = 16.1$, $P < 0.01$).

Behavioral experiments

Preferences for dark-centered vs. all-yellow model flowers did not differ among birds in three different cages in the case of bulbuls ($\chi^2 = 2.9$; $P = 0.2$) or among individual birds in the case of 22 white-eyes ($\chi^2 = 28.3$; $P = 0.12$) and two sunbirds ($\chi^2 = 0.05$; $P = 0.8$). We thus pooled data for individuals (within each species only) in subsequent analyses of choice experiments using the binomial statistic. These analyses showed that bulbuls were more likely to probe yellow model flowers with a dark center than those that were all yellow (Fig. 2). White-eyes and sunbirds, on the other hand, did not discriminate between the two kinds of model flower (Fig. 2).

Feeding trials showed that the three bird species differed markedly in their response to the taste of *A. vryheidensis* nectar (Fig. 3). Individual birds of a particular species, on the other hand, showed near-
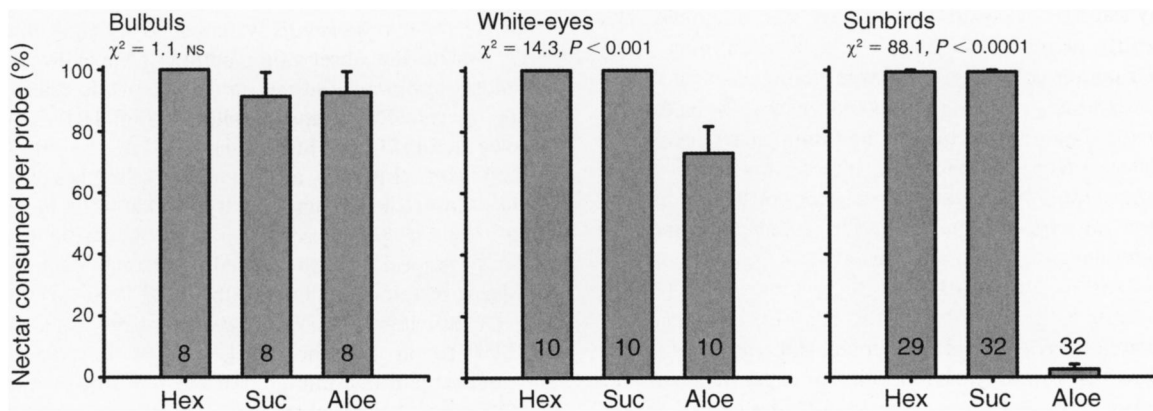

FIG. 3. Preferences of three bird species when offered a choice among sucrose (Suc) and hexose (Hex) solutions and *A. vryheidensis* (Aloe) nectar (all solutions 12% sugar by mass).
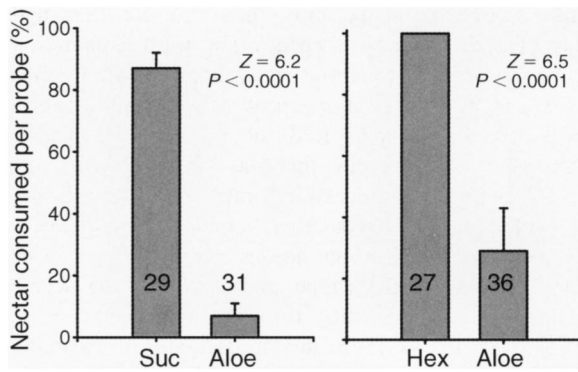
FIG. 4. Preferences of honey bees when offered a choice among sucrose and hexose solutions and *A. vryheidensis* nectar (all solutions 12% sugar by mass). Abbreviations are as in Fig. 3.

identical responses. Thus statistical analyses (Kruskal-Wallis test) were conducted using pooled data within species. The amount of *A. vryheidensis* nectar consumed by bulbuls did not differ from the hexose and sucrose controls, while white-eyes significantly preferred the sugar solutions but still consumed 73% of the *A. vryheidensis* nectar offered (Fig. 3). Sunbirds, on the other hand, strongly rejected the *A. vryheidensis* nectar, leaving it almost untouched in every trial (Fig. 3). Sunbirds reacted to *A. vryheidensis* nectar by always (100% of instances) withdrawing their bills as soon as they probed it and shaking their heads violently ($n = 32$). White-eyes usually (70% of instances) withdrew and shook their heads on first tasting *A. vryheidensis* nectar, but then proceeded to drink it anyway ($n = 10$). Bulbuls did not show an adverse reaction while drinking *A. vryheidensis* nectar ($n = 8$).

Inflorescences of *A. vryheidensis* translocated into a patch of the sunbird-pollinated species *A. maculata* were visited by three individual White-bellied Sunbirds and two Amethyst Sunbirds, each of which probed just a single flower. This elicited the same violent head-shaking behavior that we had recorded in the aviary experiments, and although individual birds remained in the patch, they subsequently avoided the *A. vryheidensis* inflorescences.

Honey bees showed clear rejection of the nectar of *A. vryheidensis* in feeding trials (Fig. 4). This response did not vary among individuals, and data were thus pooled for statistical analysis using the Mann-Whitney test. Sucrose and hexose solutions were consumed readily, while only small amounts of *A. vryheidensis* nectar were consumed (Fig. 4).

DISCUSSION

At first, bitter-tasting nectar seems highly paradoxical, given that nectar's primary function is to attract animal pollinators. However, our results suggest that the paradox can be resolved if the plant's effective pollinators are undeterred by the bitter taste, while

animals that could potentially deplete the plant's nectar without being effective as pollinators find the nectar unpalatable. The nectar of *A. vryheidensis* is clearly distasteful to specialist nectarivores (honey bees and sunbirds), yet palatable to a suite of birds that do not rely on nectar as the main component of their diet. The flowers of *A. vryheidensis* are effectively pollinated by these short-billed birds, as evidenced by the large pollen loads these birds carry, direct contact made with stigmas during probing, and significantly reduced fruit set in inflorescences from which birds, but not bees, were excluded.

Because of the length and narrow diameter of their bills, sunbirds would be poor vectors of *A. vryheidensis* pollen (Appendix C). This was confirmed by the insertion of heads of stuffed sunbirds into flowers of *A. vryheidensis*. Field observations and aviary experiments (Fig. 3) show that sunbirds find the nectar of *A. vryheidensis* highly unpalatable. To the best of our knowledge, this is the first example of a plant excluding ineffective bird pollinators from flowers by means of secondary compounds in nectar.

The bird-exclusion experiments showed that pollen-collecting bees do make some contribution to seed production in *A. vryheidensis*. This is probably due more to their sheer abundance (bees were several hundred-fold more abundant than birds as visitors to flowers of *A. vryheidensis*) than to their per-visit effectiveness as pollen vectors. As the anthers and stigmas of *A. vryheidensis* flowers are both exserted the same distance from the corolla, bees make contact with the stigmas when they collect pollen (Fig. 1G), but would not do so while feeding on nectar. Hence the unpalatability of the nectar to bees may actually enhance their efficiency as secondary pollinators of this aloe species.

The tolerance shown by occasional nectarivores toward the bitterness of *A. vryheidensis* nectar is a key element of this pollination system. It has been suggested that pollinators may "overcome" toxins in nectar through a process of coevolution (Rhoades and Bergdahl 1981). This seems highly unlikely in the case of the birds that feed on *A. vryheidensis* nectar, as nectar forms only a very small fraction of their diet. It is much more likely that these birds are "preadapted" for this pollination system in that bitter-tasting items, such as insects and phenolic-rich fruits, form the bulk of their everyday diet (Maclean 1993).

Alternative explanations for the rejection of *A. vryheidensis* nectar by honey bees and sunbirds in the feeding trials include that its color, low sugar concentration, or sugar composition have a deterrent effect. We consider it very unlikely that the color of nectar, rather than its taste, would have influenced the amount consumed per probe, especially given that sunbirds did not discriminate between dark-centered and yellow model flowers, but this possibility cannot be excluded completely as we did not artificially darken the nectar of the control sugar solutions. Nicolson and Nepi (2005)

suggested that nectar of *A. castanea* is too dilute (~9%) to be attractive to honey bees. In the case of *A. vryheidensis*, this explanation can be rejected out of hand for the following reasons. In the aviary and laboratory experiments, sunbirds and honey bees rejected *A. vryheidensis* nectar, but freely consumed equally dilute (12%) sugar solutions. In addition, both honey bees and sunbirds at our study site fed freely on the nectar of *Greyia sutherlandii*, a sympatric species with nectar that was even more dilute (~9%) than that of *A. vryheidensis* (14%) when measured at the same time on the same day (S. D. Johnson, A. L. Hargreaves, and M. Brown, *unpublished data*). Comprehensive surveys have shown that the nectar of *Aloe* (including members of section *Anguialoe*) is dominated by equal percentages of the hexose sugars glucose and fructose, with sucrose present only in trace amounts (van Wyk et al. 1993). As sunbirds and honey bees freely consumed both hexose and sucrose sugar solutions, yet rejected *A. vryheidensis* nectar, sugar composition of *Aloe* nectar is unlikely to play a significant role in deterring nectarivores.

The phenolics found in *Aloe* nectar are related to those found in aloe leaves (S. D. Johnson and F. van Heerden, *unpublished data*). A nonadaptive explanation for their presence in nectar would be that they diffuse passively into the nectar from the phloem vasculature that supplies the nectaries. Similar arguments have been advanced to explain the presence of secondary compounds in animal-dispersed fruits (Ehrlén and Eriksson 1993, Eriksson and Ehrlén 1998). However, pleiotropy is implausible as the sole explanation for phenolics in *A. vryheidensis* nectar, as many *Aloe* species with similarly high levels of leaf phenolics have clear, sweet-tasting nectar (A. L. Hargreaves, *unpublished data*). A more likely hypothesis is that selection by pollinators has modified the amount of phenolics initially present in *Aloe* nectar as a pleiotropic consequence of their production in leaves, resulting in sweeter nectar in sunbird-pollinated species and bitter nectar in some species specialized for non-nectarivore pollination. It has been suggested that phenolics in nectar may also play an antimicrobial role (cf. Hagler and Buchmann 1997), but this would not explain why the concentration of phenolics in nectar is so variable among *Aloe* species.

There are four possible explanations for the evolution of the striking color of *A. vryheidensis* nectar. The first is that it has been selected for by pollinators that find it visually attractive. Indeed, naïve bulbuls showed a highly significant preference for model flowers with apparently dark nectar (Fig. 2). It is possible that these birds have an innate preference for small, dark objects resembling the fruits that comprise the major portion of their diet. The second possibility is that birds learn to associate the distinctive color of the nectar with its presence in newly opened flowers, so that it effectively acts as an honest signal that increases pollination efficiency. We have no data to address this possibility, other than the anecdotal observation that birds in the field tended to probe only those flowers that had conspicuous droplets of nectar. The third explanation is that the color functions as a warning signal, thus reducing the likelihood of repeat visits by sunbirds and bees that have experienced the bitter-tasting nectar. Anecdotal evidence for this was obtained from the translocation experiments in which we observed that individual sunbirds avoided *A. vryheidensis* inflorescences after probing just one flower. However, these birds may have used the shape and color of the whole inflorescence, rather than the color of the nectar, as visual cues. Furthermore, individual sunbirds repeatedly probed artificial flowers with dark aloe nectar in consecutive feeding trials, which doesn't support the warning hypothesis. Finally, the dark color may be a simple by-product of selection for nectar that is bitter in taste, as some of the phenolic compounds found in aloes are darkly colored. We suspect that all of these explanations have some validity and that phenolics in nectar have been selected for their effects on both taste and color.

Anecdotal evidence scattered throughout the ornithological literature suggests that the results we obtained for *A. vryheidensis* may also apply to other aloe species with dark nectar. Birds previously recorded as visitors to these aloes belong to the guild of "occasional nectarivores," including bulbuls (recorded on *A. spicata* and *A. castanea*), Streaky-headed Canaries (on *A. castanea*), and Buff-streaked Chats (on *A. vryheidensis*) (Oatley and Skead 1972). Sunbirds are conspicuously absent from these lists. Previous authors have also noted that honey bees avoid the nectar of *A. vryheidensis* (Reynolds 1950) and *A. castanea* (Nicolson and Nepi 2005), despite being frequent floral visitors.

In this study, the effects of nectar taste and color on the reproductive success of *A. vryheidensis* are inferred indirectly from the effects they have on the behavior of animals that differ in their morphological suitability as pollen vectors. It remains a challenge to demonstrate direct effects of these traits on plant fitness by manipulation of nectar traits at the whole-plant level.

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APPENDIX A

Nectar volume and concentration in flowers of *Aloe vryheidensis*, as measured in the field over two days at two times of the day (*Ecological Archives* E087-163-A1).

APPENDIX B

Identity, pollen loads, behavior, and bill lengths of birds observed to feed on nectar of *Aloe vryheidensis* and *Greyia sutherlandii* at the study site (*Ecological Archives* E087-163-A2).

APPENDIX C

Relative dimensions of *Aloe vryheidensis* flowers, Buff-streaked Chats, and Amethyst Sunbirds (*Ecological Archives* E087-163-A3).