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Notes on the flowering and pollination of the endemic grassland *Aloe reitzii* var. *reitzii* (Asphodelaceae)

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Background: *Aloe reitzii* var. *reitzii* is a succulent with a restricted distribution in the montane grassland of eastern South Africa. It is a summer (late January–March) flowering succulent that grows on rocky outcrops at 1000 m–1600 m, and the conspicuous inflorescences suggest a pollination system focused towards birds.

Objectives: To understand more about the pollination biology of *A. reitzii* var. *reitzii*.

Methods: Nectar standing crop (flower volume and concentration) and the proportion of plants flowering were recorded. Camera traps and observations were used to record visitors to *A. reitzii* var. *reitzii* inflorescences.

Results: Nectar volume was 36 µL ± 27 µL per flower (range 6 µL–93 µL; n = 27) and concentration was 16.5% ± 1.7% (range 13.5% – 19.5%). Camera trap observations, where 18.9% of all plants were observed flowering, recorded the three bird species Cape Weaver, *Ploceus capensis*, Malachite Sunbird, *Nectarinia famosa* and Greater Double-collared Sunbird, *Cinnyris afer* (60.4%, 27.1% and 12.5% of plant visits, respectively) visiting inflorescences.

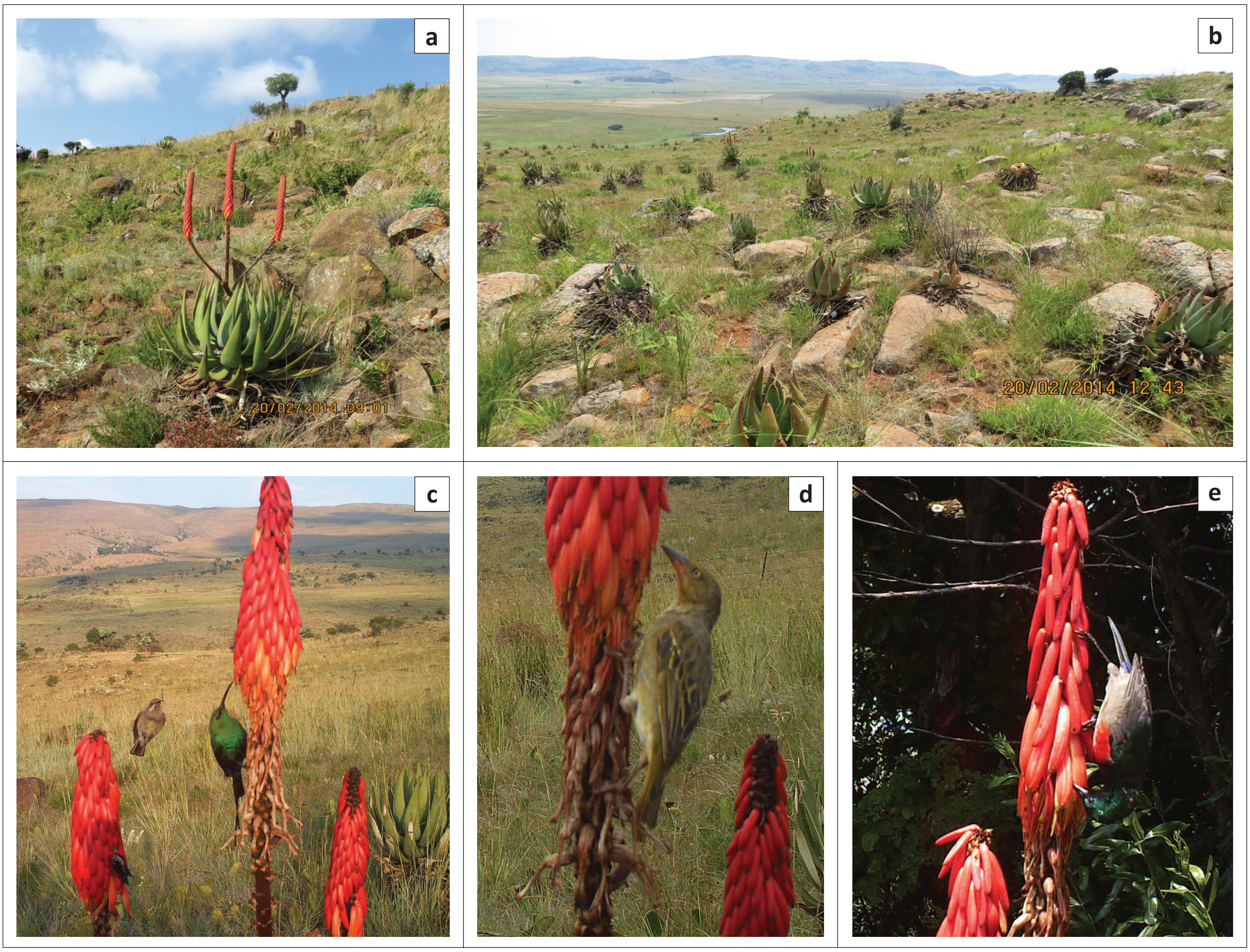
Conclusion: Because birds are important pollinators for many *Aloe* species, it is assumed that the bird species detected visiting *A. reitzii* var. *reitzii* are similarly important pollinators. At least 10 invertebrate species and sengi (*Elephantulus* sp.) were also recorded as visitors to flowers, but they may be less important pollinators than specialist and generalist avian nectarivores. This study provides further insight into the pollination biology of a diverse, and ecologically important, succulent genus in Africa.

Introduction

Pollination systems in the genus *Aloe* are diverse, with numerous recent studies identifying unique and often unexpected mutualisms (Arena, Symes & Witkowski 2013; Botes, Johnson & Cowling 2009a; Botes, Wragg & Johnson 2009b; Hargreaves, Harder & Johnson 2008; Johnson 2004). While the perception remains that many *Aloe* species are pollinated by birds, relatively few studies have quantified the contributions of different pollinator guilds. Furthermore, in cases where a specific pollinator guild (e.g. birds) is identified as the most important contributor to pollination, fewer studies have addressed the specific roles of each species in that guild.

Many *Aloe* species can potentially hybridise and given the diversity of the genus it is important to understand how the species integrity of co-occurring taxa is maintained. Botes, Johnson & Cowling (2008) proposed that the problem of *Aloe* species coexistence may be explained by a greater diversity of bird pollination systems in the genus than previously documented. In five co-occurring *Aloe* species that flower together during winter there was a partitioning of pollinators, with short-billed generalist nectar feeders pollinating species with large amounts of dilute nectar in short corolla tubes, and long-billed specialist nectar feeders, that is, sunbirds, pollinating species with small amounts of concentrated nectar in long corolla tubes (Botes et al. 2008).

Many species-specific studies have provided valuable data regarding the widely distributed Afrotropical genus *Aloe*, where the diversity of species, with respect to (1) nectar characteristics, including volume and concentration, (2) flowering season and duration, (3) inflorescence, raceme and flower arrangement, (4) floral morphology, including structure, colour and position, (5) distribution and habitat preferences and (6) population size, is important in determining plant–pollinator associations (Botes et al. 2008, 2009a, 2009b; Hargreaves, Johnson & Nol 2004; Hargreaves, Harder & Johnson 2012; Hoffman 1988; Johnson 2004; Pailler, Warren & Labat 2002;



Source: Photos taken by Craig Symes

FIGURE 1: (a, b) *Aloe reitzii* var. *reitzii* flowering in grassland (Site 1), with avian visitors to racemes; (c) Malachite Sunbird, *Nectarinia famosa* (male perched, female in flight); (d) Cape Weaver, *Ploceus capensis* (male); (e) Greater Double-collared Sunbird, *Cinnyris afer* (male) (see Table 1).

Ratsirarson 1995; Stokes & Yeaton 1995; Wilson et al. 2009). While species-specific predictions of pollinators can be made on the basis of pollination syndromes, it is clear that determination of pollinator assemblages requires thorough field study (Fenster et al. 2004; Geerts & Pauw 2009; Johnson & Steiner 2000). An important tool in this regard is the application of camera traps, which in recent years have provided important and detailed information on plant–pollinator interactions (Melidonis & Peter 2015; Steenhuisen et al. 2015; Zoeller et al. 2016).

Aloe reitzii Reynolds (1937) var. *reitzii* (Asphodelaceae) is a summer-flowering (late January–March) succulent with a restricted distribution in montane grassland around Belfast and Dullstroom, northeastern South Africa (Glen & Hardy 2000; Mucina & Rutherford 2006; Reynolds 1969; Van Wyk & Smith 2003) (Figure 1a and b). It grows on rocky areas, but is by no means confined to them (Glen & Hardy 2000; Reynolds 1969). It is known from 12 to 15 locations, with most populations confined to a small area (Raimondo et al. 2005). Plants reach a height of up to 1.0 m and produce multiple racemes that can reach 2.5 m in height (Glen & Hardy 2000; Reynolds 1969; Van Wyk & Smith 2003). The immature unopened flowers are dark red, becoming orange to pale yellow when mature (Van Wyk & Smith 2003), and are incurved and relatively long (32 mm–50 mm), suggesting pollination by birds (Figure 1a and b) (Glen & Hardy 2000; Reynolds 1969; Symes, Human & Nicolson 2009; Van Wyk & Smith 2003).

The objective of this study was to investigate potential pollinators of *A. reitzii* var. *reitzii* as inferred by visitation events captured by remote camera traps, as well as by field observations.

Materials and methods

Study site

Field work was conducted from 19 to 21 February 2014 at two localities near Dullstroom, approximately 4 km apart, and 10 km west of Verloren Vallei Nature Reserve: (1) Houtenbek farm (~1910 m–1950 m) and (2) Klipbankspruit farm (~1930 m–1945 m). Both localities are on privately owned farms and the *A. reitzii* var. *reitzii* sites on each are currently used for extensive beef farming (landowners, pers. comm.).

Nectar characteristics

Three mature flowers (Stage 2 flowers, see Symes & Nicolson 2008) were selected from each of nine plants (six plants at Site 1 and three plants at Site 2) and nectar standing crop volume (µL) and concentration (% w/w; hand-held refractometer, Bellingham & Stanley, Tunbridge Wells, UK) measured. Site 1 was sampled during the morning (08:15–09:30) and Site 2 during the early afternoon (13:05–13:25).

Flowering biology

A single meandering transect (~1.5 km), that avoided recounting the same individual, was walked through the areas where plants occurred at Site 1, and flowering recorded. The number of racemes was counted on 109 flowering plants.

Inflorescence visitors

Nine camera traps (Bushnell, model 119456, China) were set up on tripods (ht. ~1.5 m), at least 1 m distant from each plant, to record visitors to flowering plants. Cameras were set at high sensitivity to take three photographs when motion sensors were activated, with 3-s intervals between activations. Cameras were erected on the afternoon of 19 February and collected mid-morning on 21 February. A light rain fell on the night of 20 February. Any animal perched on the inflorescence, or photographed in flight close to the inflorescence, was defined as a flower visitor. If successive photographs captured what appeared to be the same individual, these were recorded as a single visit. Visits to plants ranged from an individual perched on a raceme and captured in only one photograph to an individual moving between racemes and photographed in successive images. While the presence of a visitor does not define its role as a pollinator, the records, together with observations at the site, do provide a basis from which to make interpretations regarding pollination of *A. reitzii* var. *reitzii*. Because different sized organisms may have different effects on the triggering mechanisms, and camera trapping is less likely to record all insects, no comparisons regarding visitation rates were made between birds and insects. For birds the proportion of visits by each species was calculated by dividing the total number of visits per species by the sum of all visits. For each plant, and for each hour during the day, the accumulative number of minutes (05:00–19:00) that cameras were set was determined. The number of visits for each plant was then used to calculate an hourly visitation rate (number of visits/plant/hour). To calculate visitation rates only daylight hours were considered although cameras were active during night hours. All values are given as mean ± SD.

Results

Nectar characteristics

Mean nectar standing crop volume was 36 µL ± 27 µL per flower (range 6 µL–93 µL; n = 27 flowers, nine plants) and concentration was 16.5% ± 1.7%. Nectar standing crop in the early afternoon (Site 2) was lower (volume 12 µL ± 4 µL, n = 9 flowers, three plants) compared with the morning (Site 1; volume 47 µL ± 26 µL), but concentration was no different.

Flowering biology

A total of 726 plants (only sampled at Site 1) were recorded for the presence or absence of flowering. In total, 18.9% of plants were recorded flowering. The number of racemes per plant ranged from 1 to 10 (median = 3; mean = 2.9 ± 1.2; n = 109) and measured 44 cm ± 3 cm in length (n = 6 mature racemes). On all plants there were open flowers.

Inflorescence visitors and behaviour

A total of 48 visits were made by three bird species during 155 hours of camera trap observations. Most visits were by Cape Weaver, *Ploceus capensis* (60.4%), with fewer visits by Malachite Sunbird, *Nectarinia famosa* (27.1%) and Greater Double-collared Sunbird, *Cinnyris afer* (12.5%) (Table 1). Cape Weaver and Malachite Sunbird visited eight and six of the nine monitored plants, respectively. Greater Double-collared Sunbird only visited a single plant, located near a bush-clump in a rocky outcrop. The earliest bird visitor was at 05:40 and the latest at 18:26. Plants were visited by birds during all hours of the day except mid-afternoon.

The mean hourly visitation rate was 0.36 ± 0.19 visits/plant/hour. Cape Weaver, Malachite Sunbird and Greater Double-collared Sunbird visitation rates were 0.22 ± 0.15, 0.11 ± 0.11 and 0.03 ± 0.10 visits/plant/hour, respectively. These species were all observed probing flowers for nectar, by either perching below (Figure 1c) or above (Figure 1e) the open flowers.

At least nine invertebrate species were observed and photographed on camera traps (Figure 1) and visited throughout the day from mid-morning to late-afternoon (Table 1). The only nocturnal visitors recorded were moths (Lepidoptera) during 19:00–20:59. Invertebrates were observed drinking nectar (e.g. *Camponotus* sp.) or removing pollen (e.g. *Apis mellifera*) while others were observed simply perched on flowers (e.g. *Maura* sp.) or inflorescence stems (e.g. *Odontomutilla* sp.) (Table 1).

TABLE 1: Visitors recorded at *Aloe reitzii* var. *reitzii* flowers, Houtenbek farm, Dullstroom, from 19 to 21 February 2014, by camera trap (155 camera trap hours; n = 9 camera traps) and visual observations. '-' for number of visits indicates species not recorded on camera traps.

Order	Family	Species	Number of visits
Passeriformes	Nectariniidae	*Nectarinia famosa*	13
		Cinnyris afer	6
	Ploceidae	*Ploceus capensis*	29
Lepidoptera	-	-	2
Hymenoptera	Apidae	*Apis mellifera*	85
	Mutillidae	*Odontomutilla* sp.	-
	Formicidae	*Camponotus* sp.	-
Coleoptera	Cetoniidae	*Dischista* sp.	-
		Porphyronota sp.	-
Diptera	-	2 species	4
Orthoptera	Pyrgomorphidae	*Maura* sp.	4

Source: Author's own work

Discussion

While this study considers visitation data from only three days of a flowering season, camera traps and field observations provided information on the most likely pollinators of *A. reitzii* var. *reitzii*. In the grassland environment in which *A. reitzii* var. *reitzii* occurs there are a limited number of grassland bird species (Hockey, Dean & Ryan 2005), including specialist (e.g. sunbirds) and generalist avian nectarivores. Only a few potential bird-pollinators that are likely to visit flowering plants occur within *A. reitzii* var. *reitzii* grassland habitat and those recorded for the period of study are not unexpected (Hockey et al. 2005), given that flower and inflorescence structure in *A. reitzii* var. *reitzii* strongly suggests pollination by birds. Furthermore, nectar characteristics suggest that *A. reitzii* var. *reitzii* is more likely to be pollinated by sunbirds. Generalist avian nectarivores are predicted to prefer flowering plants with dilute (8%–12% w/w) and large volumes (40 µL–100 µL) of nectar, while specialist passerine nectarivores (i.e. sunbirds) prefer nectar of higher concentration (15%–25% w/w) with lower volumes (10 µL–30 µL) (Johnson & Nicolson 2008). With values for *A. reitzii* var. *reitzii* at the lower end of the concentrations predicted for specialist nectarivores, and volumes extending into the range expected for generalist nectarivores, it is not surprising that the majority of floral visits were by Cape Weavers, which are generalist nectarivores and are adept at feeding on nectar (Craig 2014; Johnson & Nicolson 2008). Malachite Sunbirds were recorded on as many study plants as Cape Weavers, suggesting that these two species may both be important pollinators of *A. reitzii* var. *reitzii*. Malachite Sunbirds are ubiquitous throughout southern Africa and have been identified as primary pollinators of an entire guild of plant species – the 'Malachite Syndrome' (Geerts & Pauw 2009).

Many of the study plants received significant visitation from invertebrates. While it is possible that several of these were nectar robbers (Richardson 2004), honeybees were recorded in this study and have been shown as important pollinators of other *Aloe* species, (Botes et al. 2009a; Symes et al. 2009). They made up by far the greatest proportion of recorded insect visitors so, like in *A. greatheadii* var. *davyana*, they may be important pollinators when they occur in large numbers (Symes et al. 2009). Interestingly, observations of a wide variety of insects visiting study flowers contrasts findings in winter-flowering *Aloe* species which, unless they are visited by honeybees, are less likely to be visited by insects (Botes et al. 2009a; Hoffman 1988; Kuiper et al. 2015; Payne, Symes & Witkowski 2016; Symes et al. 2009).

Aloe peglerae, an endemic grassland *Aloe* of the Magaliesberg Mountain Range, is pollinated mainly by Cape Rock Thrush *Monticola rupestris*, a diurnal generalist nectarivore, with > 60% of pollination made by this species (Arena et al. 2013; Payne et al. 2016). However, pollination is also supplemented by nocturnal mammals such as Namaqua Rock Mouse *Micaelamys namaquensis* and Eastern Rock Sengi *Elephantulus myurus* (Payne et al. 2016). Anecdotal reports from the landowner at Site 2 suggest that sengi (*Elephantulus* sp.) have been observed visiting flowers during the day (B. Struwig, pers. comm.). However, no photographs were obtained of small mammals visiting flowers during this study. Thus, while visits by small mammals, such as sengi (Wester 2010), may be more common in rocky areas at our study sites, this mammal–plant association remains to be investigated.

Conclusion

In conclusion, *A. reitzii* var. *reitzii* is visited primarily by generalist and specialist avian nectarivores, as well as by invertebrates. Although the latter cannot be confirmed as legitimate pollinators, the fact that (1) birds and insects have been shown to be successful pollinators of numerous *Aloe* species and (2) fruit set at the site is abundant each year (landowners, pers. obs.) suggests that these organisms are important role players in the pollination of *A. reitzii* var. *reitzii.* A generalist pollination system might contribute to fitness of *A. reitzii* var. *reitzii*, a range-restricted, summer-flowering endemic occurring in fire-dominated grasslands; this remains to be investigated further. Furthermore, this study shows the usefulness of remote camera traps in testing predictive floral syndromes in the diverse genus *Aloe*.

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Competing interests

The author declares that there are no financial or personal relationships that may have inappropriately influenced the writing of this article.

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