

Seed dispersal by dung beetles in *Ceratocaryum pulchrum* (Restionaceae): Another example of faecal mimicry in plants

J.J. Midgley^{a,*}, J.D.M. White^a, C.H. Scholtz^b, S.D. Johnson^c

^aDepartment of Biological Sciences, University of Cape Town, P. Bag, Rondebosch 7701, South Africa

^bDepartment of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa

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

*Corresponding author. Jeremy.Midgley@uct.ac.za

Highlights

- We show a second plant species that deceives dung beetles into dispersing seeds by faecal mimicry.
- *Ceratocaryum pulchrum* seeds are similar in size, shape and scent to *C. argenteum* and herbivore dung.
- Both species produce mimetic seeds that attract generalist dung beetles.
- Speciation between these species is not disperser-driven.

Abstract

Faecal mimicry by seeds is a recently discovered phenomenon in plants. Here we demonstrate that seeds of *Ceratocaryum pulchrum* (Restionaceae) are dispersed and buried by dung beetles, making it the second species, after its sister species *C. argenteum*, known to exhibit this mode of dispersal. Seeds of *C. pulchrum* are similar in size, shape and scent to those of *C. argenteum* and the dung of various mammalian herbivores. The distribution range of *C. pulchrum* entirely overlaps with that of *C. argenteum*, but they flower and release seeds six months apart. Several dung beetle species, including both of those associated with *C. argenteum*, were found to be attracted to *C. pulchrum* seeds. Seeds were buried *in situ* by the tunnelling species *Copris victorini*. Since seeds of both plant species attract several generalist dung beetles, this suggests that speciation in these two species has not been due to evolution for different or specific dispersers but may instead be related to exploitation of temporal niche opportunities.

Keywords: Seed dispersal, Faecal mimicry, Dung beetles, *Ceratocaryum*

1. Introduction

Dispersal of seeds by animals that bury seeds underground offers many advantages to plants, including escape from predators, fire and germination within the soil (Moore and Vander Wall, 2015). The most familiar examples of dispersal associated with deliberate seed burial involve ants and rodents, especially in fire-prone Cape fynbos (Bond and Slingsby, 1983; White et al., 2017). Seeds of *Ceratocaryum argenteum* (Restionaceae) were recently shown to be dispersed and buried by two species of dung beetles in a deceptive process known as faecal mimicry (Midgley et al., 2015; Midgley and White, 2016). The seeds resemble the

scent, size and shape of the dung pellets of antelope but because they are hard, they cannot be used by dung beetles. The seeds even deceive sarcophagid flies that utilize animal dung (Midgley and White, 2016). The dung beetle species involved in dispersal of *C. argenteum* seeds, *Scarabaeus spretus* and *Epirinus flagellatus*, are not closely related (Davis et al., 2008), suggesting that the seeds deploy signals that are generally attractive to dung beetles and that they do not use a private signalling channel involving species-specific attraction to a specific scent molecule. Faecal mimicry has also been reported to occur in flowers and similarly involves non-specific attraction of a range of beetle and fly species primarily by means of volatile signals (Johnson et al., 2020; Johnson and Schiestl, 2016). Faecal mimicry, along with carrion mimicry, is different to most other forms of biological mimicry because the models are not living organisms, but rather their by-products (Johnson and Schiestl, 2016). This form of mimicry exploits the sensory bias of animal operators searching for oviposition sites (and, in some instances, food) and unlike classical Batesian mimicry does not depend on behaviour that is conditioned by experience with models.

Ceratocaryum pulchrum is sister species to *C. argenteum* (Linder, 2019) and because they share similar seed characteristics of uniquely large seeds with a tuberculate outer layer (Linder, 2001), we hypothesized that it is also likely to be dung beetle dispersed. Interestingly, *C. argenteum* and *C. pulchrum* co-occur for all of the latter's very limited distribution range (Linder, 1995) in Overberg Sandstone Fynbos (FFs12) of Mucina and Rutherford (2006). However, they have very different growth form (*C. pulchrum* is about 1.5 m+ shorter in stature) and flowering phenology as well as morphological differences such as tepal shape and numbers of fertile spikelets per culm (Linder, 1995). Also, *C. argenteum* releases seeds in mid-summer (January) whereas *C. pulchrum* releases seeds in spring (late August-early September; pers. obs.). It is possible that sympatric speciation in these two species has occurred to exploit different dung beetles, or the same species but at different times of the year or that these two species have speciated into two different phenological or life-history niches.

Here we ask; i) is *C. pulchrum* dung beetle dispersed and ii) does it have different or unique scent and/or dung beetle dispersers, compared to *C. argenteum*?

2. Methods

2.1. Dung beetle behaviour

We used field bioassays to determine whether dung beetles are attracted to seeds of *C. pulchrum*. In August 2018 we placed 10 seeds in five large (10 cm diameter and deep) pit-fall traps and had nearby (50 cm away) paired controls without seeds at a site located at 34 38 41.19 S and 19 42 22.04 E. Traps remained in the field for two weeks. It was not expected that controls would trap any dung beetles as most beetles can fly and are attracted by scent. Although we observed numerous individuals of the large flightless dung beetle *Circellium bacchus* in the study site, none of this species, nor any other dung beetle species were captured in the control traps, whereas many dung beetles were captured in traps with seeds (see Results). Given that the 2018 experiment clearly showed that dung beetles are attracted to seeds, we did not use empty control traps in 2019, but instead used a further 20 pitfall traps (5 cm diameter) each baited with 10 freshly collected *C. pulchrum* seeds in order to gain a better understanding of the spectrum of beetles attracted to seeds. We also placed out five

seeds tagged with brightly coloured thread at 10 of the pit-fall traps. The brightly coloured threads allowed us to track removed seeds and confirm whether they were buried or not. Missing seeds were searched for in a 1 m radius around where tagged seeds were initially placed. The traps and tagged seeds were left out in the field for two weeks in September and checked every 1–3 days.

2.2. Scent analysis

To determine the chemical composition and rate of emission of scent emitted from seeds of *C. pulchrum*, we used headspace sampling in conjunction with gas-chromatography mass-spectrometry (GCMS). Freshly collected seeds were placed in two polyacetate sampling bags (12 seeds per bags) and a third empty bag was treated as a control sample. Using a PAS-500 personal air sampler (Spectrex, Redwood City, California), air was pumped from each of the bags at 250 ml/min for a period of four hours and volatiles were trapped on micro-filters consisting of 1.5 mg carbotrap® B (20–40 mesh; Sigma-Aldrich Co.) activated charcoal and 1.5 mg tenax® TA (60/80; Supelco™).

Volatiles were analysed using a Varian CP-3800 GC coupled to a Bruker 300 quadrupole MS. The GC was fitted with a Varian 1079 PTV injector port modified with a ChromatoProbe thermal desorption device. Volatiles samples were separated using an Alltech EC-WAX SGE polar capillary column (30 m x 0.25 mm ID, 0.25 µm film thickness). The GC temperature program was 40 °C for 3 min, then increased to 240 °C at 10 °C min⁻¹ and then kept at 240 °C for 12 min. The injector temperature program was 40 °C for 2 min (with a 20:1 split), then increased to 200 °C at 200 °C min⁻¹ and held for 2 min (splitless) for thermal desorption, and finally increased to 250 °C at 200 °C min⁻¹ and held at 250 °C (with a 1:100 split) for the remainder of the run. Helium (1 ml min⁻¹) was used as a carrier gas. The mass spectrometer was operated in electron-impact ionisation mode at 70 eV with the detector voltage continually adjusted by the Extended Dynamic Range (EDR) function. Compounds were identified using Varian MS Workstation (version 7.0) and NIST MS Search (version 2.3) with the NIST 2017 mass spectral library. Library identifications were confirmed by comparison of calculated linear (non-isothermal) *n*-alkane Kováts retention indices with published values in the NIST library and, and where possible, comparison of retention times and mass spectra with those of synthetic standards injected under identical conditions to samples. Absolute emission rates (in methyl benzoate equivalents) were calculated from comparison to peak areas resulting from the injection of known amounts of methyl benzoate.

To compare the chemical scent profile of *C. pulchrum* seeds with those previously published for *C. argenteum*, various herbivore species dung and other Restionaceae species seeds (Midgley et al., 2015), we square-root transformed the percentages that each compound contributed to the overall scent and calculated a similarity matrix among samples using the Bray–Curtis method and visualized relative similarities using non-metric multidimensional scaling (NMDS) using Primer 6.1.6.

3. Results

3.1. Dung beetle behaviour

Eight dung beetle species were attracted to *C. pulchrum* seeds and caught in pitfall traps (Table 1). Fourteen seeds were found to have been buried and these ranged from 2 cm to 15 cm in depth below soil surface. During excavations of the buried seeds, we observed six individuals of *Copris victorini*, each at a seed and we observed two individuals of this species actively burying seeds *in situ*. Six marked seeds were not relocated within the 1 m search radius. This movement indicates possible dung beetle dispersal by rolling for *C. pulchrum* rather than removal by granivores, as Midgley et al. (2015) found negligible rates of predation of *C. argenteum* seeds.

Table 1. Details of dung beetles trapped in two separate years of field-based bioassays.

SPECIES	NUMBER TRAPPED	
	2018	2019
<i>CIRCELLIUM BACCHUS</i> (FABRICIUS, 1781)	1	2
<i>EPIRINUS RUGOSUS</i> SCHOLTZ & HOWDEN, 1987	5	1
<i>EPIRINUS FLAGELLATUS</i> (FABRICIUS, 1775)	1	0
<i>SCARABAEUS SPRETUS</i> ZUR STRASSEN, 1962	5	0
<i>COPRIS ANCEUS</i> (OLIVIER, 1789)	1	5
<i>COPRIS VICTORINI</i> BOHEMAN, 1857	11	9
<i>ONTHOPHAGUS GIRAFFA</i> (HAUSMANN, 1807)	3	1
APHODIINAE SPP1	0	4

3.2. Scent analysis

We recorded seven volatile compounds in the headspace samples of *C. pulchrum* seeds (Table 2). Five of these compounds were previously reported to occur in the scent of *C. argenteum* seeds and antelope dung (Table 2) (Midgley et al., 2015). The overall chemical composition of the scent of the seeds was very similar to that of herbivore dung and that of *C. argenteum* and unlike that of other Restionaceae species (Fig. 1).

Table 2. Relative and total amount of volatiles detected in headspace samples of seeds of *Ceratocaryum pulchrum*. KRI = Linear Kováts retention indices calculated relative to retention times of n-alkanes.

KRI	COMPOUND	RELATIVE AMOUNT (%)	
		Sample 1 (12 seeds)	Sample 2 (12 seeds)
	Empty Cell		
	Benzenoids		
1545	4-Ethylanisole	26.92	26.69
1632	Acetophenone*#	13.58	17.76
1785	1-phenylethanol*#	18.63	10.27
1969	Phenol*#	1.24	1.38
2174	4-ethylphenol*#	7.17	17.48
	Sulphur compound		
1871	Dimethyl sulfone*#	2.93	3.62
	Unknown		
1702	m/z: 121,93,123,79,154,81,41,139	29.53	22.81
	Total emissions (ng/seed/h)	9.15	10.28

*= compounds previously detected in headspace samples of *Ceratocaryum argenteum* by Midgley et al. (2015).

#= compounds previously detected in headspace samples of antelope dung by Midgley et al. (2015).

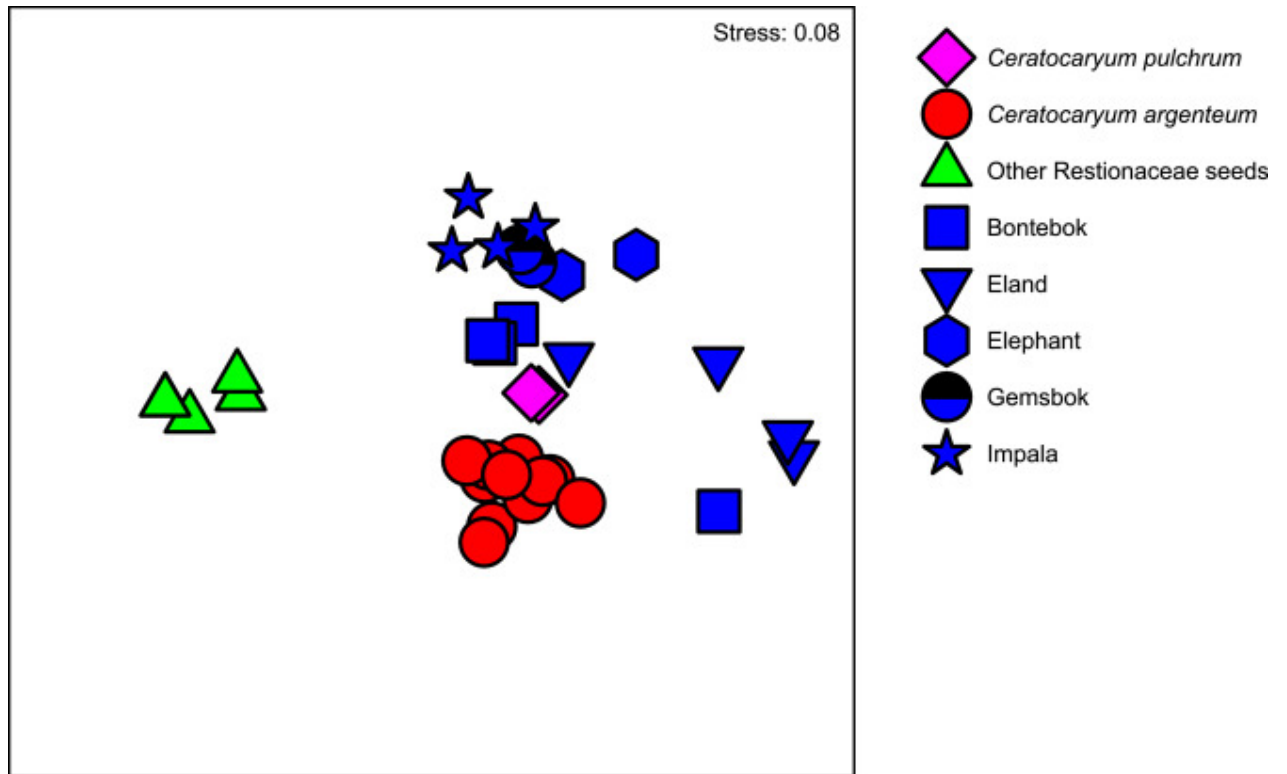


Fig. 1. Visual representation of overall similarities of volatile profiles of seeds of two *Ceratocaryum* species, other Restionaceae species, and herbivore dung based on non-metric multidimensional scaling (NMSD) using data from this study and the previously published study by Midgley et al. (2015).

4. Discussion

These results provide confirmation of a second case of dung mimicry by seeds of species in the Restionaceae. As several dung beetle species were attracted to the seeds, we infer that the mimicry system is generalised and not targeted at a specific dung beetle species. The two dung beetle species, *E. flagellatus* and *S. spretus*, associated with *C. argenteum* at Potberg field site (Midgley et al., 2015), were also attracted to *C. pulchrum* seeds, although numbers of captures were small. Since these two beetle species were captured in both summer and winter, and many beetles were captured in winter (Table 1), it seems as though there is no seasonality in their occurrence in fynbos. During the period of this study, *Copris victorini* was found to be the most numerous species trapped and the only species we observed burying seeds. This species is a tunneller (bury dung *in situ*) whereas *E. flagellatus* and *S. spretus* are both rollers (Davis et al., 2008; Midgley and White, 2016). *Copris victorini* will thus effect short dispersal distances. This is similar to short distance dispersal by ants, which occurs in c. 1000 fynbos species (Bond and Slingsby, 1983) and is not a problem in fynbos because germination takes place after potentially competitive maternal plants have been killed or damaged by fire. Fire thus reduces the unequal resource conflict between established parents and their seedlings. Burial of such large seeds is important to escape fire damage. However, we noted that some seeds were buried quite deep and thus further research is needed to determine whether these seeds are potentially buried too deeply to be able to receive the fire signal or even to emerge.

The lack of strong scent differences, the attraction of several dung beetle species to seeds of both species and the nested distribution of *C. pulchrum* within *C. argenteum* (Linder, 1995), suggests that speciation in *Ceratocaryum* is unlikely to have been due to evolution of differences in species-specific attraction of dung beetle dispersers.

The chemical composition of scent of *C. pulchrum* seeds is very similar to that of the closely related species *C. argenteum* and the dung of various herbivore species (Fig. 1). They share several benzenoid compounds as well as the sulphur compound dimethyl sulfone (Table 2). However, we detected just seven compounds in the scent of seeds of *C. pulchrum* as opposed to a mean of 18 compounds recorded for *C. argenteum* seeds by Midgley et al. (2015). The total amount of scent emitted per fresh *C. pulchrum* seed per hour was c. 10 ng which is comparable to that of older *C. argenteum* seeds (c. 28 ng), but is less than that of fresh *C. argenteum* seeds (c. 200 ng) and greater than that of seeds of other Restionaceae species (c. 1.4 ng) (Midgley et al., 2015).

Originally, Midgley et al. (2015) thought that seed faecal mimicry was highly specific as only one dung beetle species buried *C. argenteum* seeds. However further research, including the present paper, has shown that this specificity is not the case. A later survey showed that another beetle also dispersed *C. argenteum* seeds (Midgley and White, 2016). Based on isotopic evidence, Midgley and White (2016) speculated that dung from eland (*Taurotragus oryx*) a C3 mixed-feeder was preferred over dung of bontebok (*Damaliscus pygargus*) a C4 short-grass grazer, by *S. spretus* and *E. flagellatus*; the two dispersers of *C. argenteum* seeds. These were the two main large herbivores at the Potberg site. Over the course of many visits to our *C. pulchrum* site, we did not see any eland dung, suggesting that the seeds do not specifically mimic the scent of eland dung, though the co-occurrence of these herbivore species may have been altered in the Anthropocene. A more compelling reason to doubt the association is that the seeds of both plant species are smaller than eland dung pellets. Finally, given that *C. argenteum* co-occurs at this site with *C. pulchrum* also suggests that eland dung is not necessary to maintain this faecal deception. Some of the beetles attracted to *Ceratocaryum* seeds, such as *Scarabaeus spretus*, are widespread and are even attracted to carnivore (e.g. dog) dung (J. Midgley, Table Mountain National Park). It thus appears that the seeds elicit general responses to the scent of dung by several dung beetles.

No other Restionaceae species have seeds similar to those of these two *Ceratocaryum* species. Why the whole system of faecal mimicry is restricted to these two rather rare, southern Cape, deep sand fynbos species is unanswered. Possibly these nutrient-poor systems favoured the selection of very large seeds and thus a shift from the small seeds of closely related species that are dispersed by ants (Linder, 1995). The phenomenon of faecal seed mimicry also implies the historical presence of large herbivores in nutrient-poor fynbos. It could also be that the chemical mutations required for the evolution of dung mimicry are exceedingly rare and occurred only in this particular lineage of the Restionaceae.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the land-owner Mr Ric Kuisis for access to his land, for assistance and for Mr Pietie Mintoer for checking our traps, NRF Grant 46372 (SDJ) and UCT Science Faculty Research Committee (JJM) for funding.

References

- Bond, W.J., Slingsby, P., 1983. Seed dispersal by ants in shrublands of the Cape Province and its evolutionary implications. *South African Journal of Science* 79, 231–233.
- Davis, A.L.V, Frolov, A.V., Scholtz, C.H., 2008. *The African Dung Beetle Genera*. Protea Book House, Pretoria.
- Johnson, S.D., Schiestl, F., 2016. *Floral Mimicry*. Oxford University Press.
- Johnson, S.D., Sivechurran, J., Doarsamy, S., Shuttleworth, A., 2020. Dung mimicry: the function of volatile emissions and corolla patterning in fly-pollinated Wurmbea flowers. *New Phytologist*. <https://doi.org/10.1111/nph.16791>.
- Linder, H.P., 2019. Rare species, restionaceae, and the cape flora. *J. Biogeogr.* 46, 2637–2650. <https://doi.org/10.1111/jbi.13709>.
- Linder, H.P., 2001. Two new species of ceratocaryum (restionaceae). *Kew Bulletin* 56, 465–477. <https://doi.org/10.2307/4110968>.
- Linder, H.P., 1995. Ceratocaryum pulchrum, a new restioid from the Bredasdorp plains. *South African Journal of Botany* 61, 222–225. [https://doi.org/10.1016/S0254-6299\(15\)30518-4](https://doi.org/10.1016/S0254-6299(15)30518-4).
- Midgley, J.J., White, J.D.M., 2016. Two dung beetle species that disperse mimetic seeds both feed on eland dung. *South African Journal of Science* 112, 7–9. <https://doi.org/10.17159/sajs.2016/20160114>.
- Midgley, J.J., White, J.D.M., Johnson, S.D., Bronner, G.N., 2015. Faecal mimicry by seeds ensures dispersal by dung beetles. *Nature Plants* 1, 15141. <https://doi.org/10.1038/nplants.2015.141>.
- Moore, C.M., Vander Wall, S.B., 2015. Scatter-hoarding rodents disperse seeds to safe sites in a fire-prone ecosystem. *Plant Ecology* 216, 1137–1153. <https://doi.org/10.1007/s11258-015-0497-1>.
- Mucina, L., Rutherford, M.C., 2006. *The Vegetation of South Africa, Lesotho and Swaziland*. Strelitzia, Pretoria.
- White, J.D.M., Bronner, G.N., Midgley, J.J., 2017. Camera-trapping and seed-labelling reveals widespread granivory and scatter-hoarding of nuts by rodents in the fynbos biome. *African Zoology* 52, 31–41. <https://doi.org/10.1080/15627>.