Mammal pollinators lured by the scent of a parasitic plant

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To communicate with animals, plants use signals that are distinct from their surroundings. Animals generally learn to use these signals through associative conditioning; however, signals are most effective when they elicit innate behavioural responses. Many plant species have flowers specialized for pollination by ground-dwelling mammals, but the signals used to attract these pollinators have not been elucidated. Here, we demonstrate the chemical basis for attraction of mammal pollinators to flowers of the dioecious parasitic plant *Cytinus visseri* (Cytinaceae). Two aliphatic ketones dominate the scent of this species; 3-hexanone, which elicits strong innate attraction in rodents, and 1-hexen-3-one, which repels them in isolation, but not in combination with 3-hexanone. The aliphatic ketone-dominated scent of *C. visseri* contrasts with those of insect-pollinated plants, which are typically dominated by terpenoids, aromatic or non-ketone aliphatic compounds. 3-hexanone is also known from some bat-pollinated species, suggesting independent evolution of plant signals in derived, highly specialized mammal-pollination systems.

**Keywords:** *Cytinus visseri*; Cytinaceae; dioecy; floral syndrome; nectar; pollination

1. INTRODUCTION

Plants use a plethora of visual and olfactory signals to communicate with their animal pollinators [1,2]. These signals also provide cues that visitors either learn or instinctively associate with rewards, promoting the pollinator fidelity required for reliable pollen dispersal within populations. Scents serve these functions particularly well, because of the enormous diversity of volatile compounds and the focused sensitivity of the olfactory organs of many animals, especially those that frequent dimly lit environments [3,4]. There is a paucity of theory to predict what volatiles plants should deploy to attract their pollinators. One hypothesis proposes that floral scents are mainly learned through associative conditioning [5] and thus should associate only weakly with particular pollinator classes. An alternate hypothesis, supported here, is that selection favours volatiles that elicit innate responses by particular pollinators [6–8].

Scents cues are probably particularly valuable to plants pollinated by small ground-dwelling mammals, because, like most bat- and moth-pollinated species, they are usually visited at night when visual cues are less effective. Pollination by ground-dwelling mammals is uncommon, but widespread in angiosperms, occurring in at least 20 families [9]. Flowers pollinated by ground-dwelling mammals typically smell pleasantly ‘yeasty’ or ‘pungent’ to humans [10,11]. Nevertheless, in contrast to the scents of bat-pollinated flowers, which are often characterized by sulphur compounds [12–14], the chemical composition and attraction function of the floral scents of species pollinated by ground-dwelling mammals have remained unknown.

Here, we report the discovery of mammal pollination, including an unusual record of pollination by elephant shrews (Macroscelididae), in the dioecious, holoparasitic plant *Cytinus visseri* Burgoyne (Cytinaceae), as well as the chemical composition and functional role of compounds found in the scent of its flowers and nectar. In particular, we experimentally determined whether dominant scent compounds singly and in combination trigger innate behaviour of mammal pollinators.

2. MATERIAL AND METHODS

(a) Study species

*Cytinus*, which together with the central-American genus *Bdallophyton* comprise the Cytinaceae [15], has two centres of diversity; around the Mediterranean and in southern Africa and Madagascar [16]. Northern hemisphere *Cytinus* species are monocious with white-yellow flowers, whereas Southern hemisphere species are dioecious with white, red or purple flowers. The reproductive biology of the Cytinaceae is poorly known. Flowers of the European *Cytinus hypocistus* L. produce relatively limited nectar (ca 1 µl) and are visited
by insects [17], as is the related genus Bdallophyton [18], whereas bird-pollination has been inferred for the South African Cytinus capensis Marloth, which has red flowers and produces abundant nectar [19].

The recently described C. visseri [20] has floral features consistent with the floral syndrome of other southern African plants pollinated by small, ground-dwelling mammals [10,11], notably unusually robust flowers with a rigid central androecial or gynoecial column (figure 1b–d), copious nectar and pungent scent. As a holoparasite, C. visseri produces no aerial vegetative structures and so is apparent only from its dark maroon flowers and resulting fruits, which are produced at ground level, hidden under the dense canopy of host shrubs, Helichrysum reflexum (Asteraceae). Flowers of both sexes are usually closed, but the six petals have an unusual hinge formed by a folded constriction at the base petal, which allows the petals to be separated to access nectar at the base (figure 1c,d).

Figure 1. Habitat, morphology and pollinators of C. visseri. (a) Habitat on the summit of the Long Tom Pass, South Africa. (b) Male inflorescence. scale bar, 10 mm. (c) Cross section of male flower. A = androecium, N = nectar chamber. Scale bar, 5 mm. (d) Cross section of female flower. S = stigma. Scale bar, 5 mm. (e) A short-snouted elephant shrew E. brachyrhynchus feeding on nectar in C. visseri flowers. The tongue entering the flower is visible below the snout. Scale bar, 10 mm. (f) A striped field mouse R. pumilio feeding on nectar in male C. visseri flowers. Scale bar, 10 mm.
(b) Study site
Field components of this study were conducted at Mauchsberg (25°08′29″S, 30°36′09″E; elevation, 2143–2160 m), the highest point of Long Tom Pass in Mpumalanga Province, South Africa, which is occupied by the largest known C. visseri population. Temperatures during flowering of C. visseri (March–May) can drop below freezing at night. Plants in this population grow among rocky outcrops (figure 1a) at a density of ca 120 plants per 1000 m² [20]. The observations and experiments reported here were conducted over 12 days during March–May of 2002, 2005 and 2006. Little flowering occurred during 2003 and 2004, probably owing to unusually dry conditions.

(c) Sex ratios, floral morphology and nectar, and fruit set
We quantified the relative abundance of flowering male and female plants while walking transects through the population. Male and female inflorescences tend to be well-separated (>5 m), suggesting that a particular host shrub (typical diameter <1 m) is seldom, if ever, infected by more than one parasite individual [19].

We selected a representative sample of male and female plants to measure floral traits. For these plants we counted flowers, petals and anthers per flower, and measured flower width, petal length and width, nectar-chamber length and width, height of the conuate androecium and gynoecium, anther length, stigma height and diameter with digital callipers. The volume of the nectar standing crop in flowers was measured with 100 μl pipettes at ca 16.00. Sugar concentration of nectar was determined using a 0–50% handheld refractometer (Bellingham and Stanley). Sexual differences in morphological and nectar traits were tested with Student’s t-tests.

Natural fruit set was quantified for nine plants during 2005 and eight plants during 2006. Plants were marked with steel stakes when flowers were counted, and fruits were counted approximately eight weeks later.

(d) Floral volatiles
Volatiles emitted from female and male flowers were collected from intact plants in the field using a dynamic head-space method [21]. Clusters of 1–15 flowers were enclosed in polyester bags, and sampled using the headspace extraction technique described above. Subsequently blotted onto Whatman no. 1 filter paper [22], extracted from each flower using a 100 μl pipette (length, 15 mm; inner diameter, 2 mm) containing a 1 : 1 mixture of 3 mg 10/C2 and 1 m/l solution (except during 27–29 April 2005 when clear sellotape was used) to remove pollen [25]. Fuchsin-gel cubes were then placed into the probe, which was then inserted into the modified GC injector. The injector split vent was opened (1/20) and the injector heated to 40°C to flush any air from the system. The split vent was closed after 2 min and the injector was heated at 200°C for 4.2 min, after which the split vent was opened (1/10) and the injector cooled.

A ZB-5 column (5% phenyl polysiloxane) was used for the analyses (60 m long, inner diameter 0.25 mm and film thickness 0.25 μm, Phenomenex). Electronic flow-control maintained the flow of helium carrier gas at 1.8 ml min⁻¹. The GC oven temperature was held for 7 min at 40°C, then increased by 6°C min⁻¹ to 250°C and held for 1 min. The mass spectrometer (MS) interface was held at 260°C and the ion trap worked at 175°C. The mass spectra were taken at 70 eV (in EI mode) at 1 scan s⁻¹ and m/z 30–350. The GC-MS data were processed using Saturn Software 5.2.1. Component identification was conducted with the NIST 02 mass spectral database, or MassFinder 3, and confirmed by comparison of retention times with published data [24]. Identification of individual components was confirmed by the comparison of mass-spectrum and GC-retention data with those of authentic standards. The masses of individual compounds in scent samples were quantified by recording the mean response of known masses of different terpenoids, fatty-acid derivatives and benzenoids.

(e) Identification of animal pollinators
Almost all potential mammalian pollinators are nocturnal, and so are difficult to observe directly. During April 2005 and May 2006, we surrounded the bases of 12 and 20 inflorescences, respectively, with cardboard discs that had been covered finely with black soot from a smoky paraffin lamp to record the footprints of animals that approached the inflorescences.

To determine the local fauna of small mammals at the study site and whether they carried C. visseri pollen, we conducted four trapping sessions between 2002 and 2006 (247 trap nights: March 2002, 20 Willan-type traps, 10 baited with Cytinus flowers and 10 unbaited; 11–13 April 2005, 50 Sherman-type traps baited with peanut butter and rolled oats; 27–29 April 2005, 39 Sherman-type traps baited with peanut butter, rolled oats and fortified red wine; 3 May 2005, 10 Sherman-type traps baited with peanut butter and rolled oats). All animals were captured using the peanut butter and oats as bait, except for a single Rhodontomys pumilio captured in a trap baited with Cytinus flowers. Because of the brief trapping periods, recaptures probably represent a small fraction of the total captures. During each session, traps were placed ca 10 m apart along a transect through the population. Captured mammals were placed temporarily in a plastic bag and photographed. Their snouts protruded from a small hole in the corner of the plastic bag and were swabbed with cubes of fuchsin gel (except during 27–29 April 2005 when clear sellotape was used) to remove pollen [25]. Fuchsin-gel cubes were then melted onto microscope slides and sellotape was dipped in aqueous methylene-blue stain, rinsed, mounted under a large coverslip and viewed at 100× magnification. Cytinus pollen is highly distinctive as grains are released as tetrads. We also collected any faecal pellets from traps. In the laboratory, we softened two to four faecal pellets from each animal captured in a trap baited with Cytinus flowers in 1 ml of water for 12 h, then added 10 μl of aqueous methylene blue, ground this mixture, and placed a 10 μl subsample on a microscope slide for pollen counting. The animal captured in a trap baited with Cytinus flowers was not subjected to pollen analysis.

Three R. pumilio and one Elephantulus brachyrhynchus captured in the Cytinus population were housed temporarily in...
glass tanks (300 × 200 × 200 mm) to record their responses to *C. visseri* inflorescences. The base of the tank was covered with soil from the study site and we added large stones to provide shelter. Male and female inflorescences of *C. visseri* were introduced to the tanks during late afternoon and we then observed each animal for 180 min.

(f) Tests of rodent responses to floral volatiles

We used choice experiments to test the responses of rodents to the main volatiles in the scent of *C. visseri* flowers, 1-hexen-3-one, 3-hexanone and ethyl butyrate. This experiment involved four male and two female *R. puntillo*, which we live-trapped in grassland near Pietermaritzburg, South Africa, outside the range of *C. visseri*. These animals, which would not have encountered *Cytinus* plants previously, were placed in separate cages and fed seeds and vegetables in the animal facilities at the University of KwaZulu-Natal, Pietermaritzburg. During an experimental trial, a mouse was placed in a small glass tank (20 × 30 × 20 cm) attached to a y-maze olfactometer, which consisted of a proximal 20-cm section of 5-cm diameter clear Plexiglas tubing that split into two distal 20 cm arms (50° between arms) that each terminated in a compartment. Each compartment included a space that a mouse could enter directly, a wire-mesh chamber into which a vial-containing scent could be introduced, and a fan that blew air (and scent if present) through the scent chamber into the attached arm of the y-maze at ca 1000 ml min⁻¹.

The scent introduced to the test arm of the y-maze comprised a solution of either 1-hexen-3-one, 3-hexanone or ethyl butyrate diluted in paraffin oil (1:50 000 dilution by volume), or a 50:50 (vol:vol) mixture of 1-hexen-3-one and 3-hexanone, diluted 1: 25 000 by volume in paraffin oil. Analysis of the headspace of this mixture detected a ratio of 60: 40 (mass:mass) 1-hexen-3-one:3-hexanone, which is very similar to the headspace of *C. visseri* flowers (see below). Despite the extreme dilution of the pure compounds, these test solutions were quite detectable to the human nose. To minimize contamination of ambient air around the test apparatus, we pipetted 1 μl of dilute scent solution onto a small piece of Whatman no. 1 filter paper inside a vial and closed it while outdoors and then carried the vial into the experimental room, placed it inside the test arm of the y-maze and opened it again to release scent. Although paraffin oil is odourless to humans and does not produce detectable GC peaks in headspace samples, we ran additional trials with pure paraffin oil as the test substance to determine whether it influenced the behaviour of small mammals or not.

During a trial, one of the three scents was emitted from one randomly selected arm of the y-maze. We recorded whether the mouse entered the y-maze within 10 min after being introduced to the apparatus and, if so, whether it entered the arm containing the scent. We also recorded the latency period between a mouse’s entry to the y-maze and its arrival at the end of one arm. Each mouse experienced each scent during up to six trials, with mice and scents used in a random order (i.e. 36 trials per scent).

We assessed sources of variation in the behaviour of mice during scent trials with repeated-measures ANOVA [26] that included scent and sex as fixed factors. Each observation for an individual mouse represented either the proportion of trials during which it entered one arm of the testing apparatus (i.e. proportion of responses), the proportion of responses in favour of the scented arm, or the latency period before a mouse reached the end of one arm of the y-maze. The proportions represent binomial, rather than normal, variables, so we applied the logit transformation, ln(p/[1−p]), where p is the proportion of positive responses [26]. This transformation is undefined if p = 0 or p = 1, so we used p = n/2 and p = 1−(n/2), respectively, in these cases, where n is the total number of trials involved [26]. The latter adjustment moderates the effect of unanimous responses by individual mice to specific scents and so results in conservative statistical tests. Latency periods were also not normally distributed, which we rectified by applying the inverse transformation, y' = 1/y. To account for the lack of independence of responses by individual mice to the different scents, we adjusted the denominator degrees of freedom using the method of Kenward & Roger [27], which can result in fractional degrees of freedom. This approach incorporated a variance–covariance model of compound symmetry for overall responses and of heterogeneous compound symmetry for scent preference and latency. The latter model accounted for heterogeneous variances in responses among scent treatments. We used Dunn–Šidák’s method to control the experiment-wise type I error rate for *a posteriori* contrasts [26].

3. RESULTS

(a) Sex ratios, floral morphology and nectar, and fruit set

In the population we studied, male inflorescences (figure 1b,e) were twice as common as female inflorescences (figure 1: males = 47, females = 20, G₁ = 11.1, p < 0.001). Males also produced approximately twice as many flowers per inflorescence than did females (electronic supplementary material, table S1). Other than primary sexual differences, female and male *C. visseri* flowers exhibit limited sexual dimorphism in overall dimensions and nectar properties, although male flowers have significantly deeper nectar chambers (electronic supplementary material, table S1). During late afternoon, individual flowers contained copious nectar, averaging about 50 μl (maximum of 134 μl in a male flower) with a sugar concentration of ca 30 per cent (electronic supplementary material, table S1). On average (95% CI), about half of female flowers produced fruits (2005: 50.1%, 40.9–64.6%; 2006: 47.8%, 37.1–58.6%).

(b) Floral volatiles

Flowers of *C. visseri* emit a strong, unusual plastic-like odour at about 90 ng h⁻¹. The scent comprised 30 compounds, especially fatty-acid derivatives, mono- and sesquiterpenoids (figure 2 and electronic supplementary material, table S3). Female and male flowers emitted a similar mixture of compounds, except for some terpenoids emitted only by male flowers. Most compounds were present in small relative amounts, and only three compounds constituted greater than 5 per cent of the mass of scent chemicals: 1-hexen-3-one in flower and nectar samples, 3-hexanone in flower samples, and ethyl butyrate in male flowers (electronic supplementary material, table S3). Scents of nectar were strongly dominated by 1-hexen-3-one (figure 2), and the only other compound detected in nectar was ethyl butyrate in trace amounts (electronic supplementary material, table S3). GC-sniffing revealed that 1-hexen-3-one imparts the plastic-like scent.

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(a) Cytinus pollen was carried by R. pumilio, E. brachyrhynchos and M. minutoides, indicating that they could act as pollinators. Although we found a few (<6) Cytinus pollen tetrads on the snouts of six of 11 R. pumilio and none on other species (electronic supplementary material, table S2), the faecal pellets of 10 of 14 R. pumilio, three of three E. brachyrhynchos and one of two M. minutoides contained many (>5000) C. visseri tetrads (electronic supplementary material, table S2), with Cytinus pollen comprising 30–50% of the solid material of some pellets.

Individuals of R. pumilio and E. brachyrhynchos captured at the study site fed eagerly on nectar in C. visseri flowers (figure 1e,f), sometimes within seconds of flowers being introduced to the observation tank, but they did not attempt to eat flowers or their pollen. Animals of both species sniffed the air (snout wiggling) when C. visseri flowers were introduced to the tank, even when the flowers were concealed behind rocks. Such olfaction was usually followed immediately by visits to the C. visseri flowers (electronic supplementary material, video). By contrast, these animals showed no interest when flowers of other species were introduced. Mammals accessed C. visseri flowers by bending the hinged petals back with their snouts (figure 1e,f) or front paws. They systematically moved around each flower, probing all six nectar chambers and consumed all available nectar by rapid licking. While probing flowers to ingest nectar, individuals of R. pumilio inadvertently brushed their snouts against the anthers (male flowers) or stigma (female flowers) (figure 1f). In contrast, E. brachyrhynchos contacted the sexual organs of flowers with only the sides of the snout tip (figure 1e). Large accumulations of pollen were visible on the snouts of animals after these visits (figure 1f), but these had been groomed off after a few hours.

(b) A detailed list of compounds is given in electronic supplementary material, table S3. Black bars, nectar; grey bars, whole flower.

(c) Identification of animal pollinators
Despite extensive observations (ca 35 h over 12 days) during daylight, we did not observe insects or birds visiting C. visseri flowers: instead, small nocturnal mammals pollinate this species. Rodent footprints and tail-drag marks were found on eight of the 32 smoked plates placed adjacent to C. visseri inflorescences. We trapped 35 small mammals representing five species from three orders (electronic supplementary material, table S2): Rodentia—striped fieldmouse R. pumilio (n = 22), pygmy mouse Mus minutoides (n = 2) and an unidentified dormouse (n = 3); Macroscelidea—short-snouted elephant shrew E. brachyrhynchos (n = 3); and Insectivora—unidentified shrews (n = 5). Cytinus pollen was carried by R. pumilio, E. brachyrhynchos and M. minutoides, indicating that they could act as pollinators. Although we found a few (<6) Cytinus pollen tetrads on the snouts of six of 11 R. pumilio and none on other species (electronic supplementary material, table S2), the faecal pellets of 10 of 14 R. pumilio, three of three E. brachyrhynchos and one of two M. minutoides contained many (>5000) C. visseri tetrads (electronic supplementary material, table S2), with Cytinus pollen comprising 30–50% of the solid material of some pellets.

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(d) Tests of rodent responses to floral volatiles
Responses of R. pumilio mice captured outside the range of C. visseri during scent trials differed strongly between compounds (figure 3a). During 66 trials with 3-hexanone, either alone or mixed with 1-hexen-3-one, mice always entered the y-maze, except for one trial during which a male mouse burrowed into the sawdust on the cage floor and hid. By contrast, mice participated less consistently during trials involving 1-hexen-3-one alone, with significant differences between the sexes (figure 3a: scent × sex interaction, F2,8.1 = 5.26, p < 0.05). In particular, male mice responded during trials with 1-hexen-3-one as frequently as during trials with 3-hexanone alone or in mixture (Dunn–Šidák comparisons, p > 0.1 in all cases), whereas female mice entered the y-maze less often when one arm presented 1-hexen-3-one (p < 0.01). During trials with ethyl butyrate alone, mice responded as frequently (28 of 36 trials) as when 1-hexen-3-one was presented alone (F1,4 = 0.62, p > 0.45).

When mice entered the y-maze, they exhibited contrasting responses to 1-hexen-3-one alone versus 3-hexanone alone or in mixture (figure 3b: scent effect, F2,6,2 = 51.93, p < 0.001), with similar responses by both sexes (sex, F1,6,2 = 2.70, p > 0.1; scent × sex, F2,6,2 = 6.21, p > 0.05). Mice were repelled by 1-hexen-3-one, as all mice that responded entered the arm without scent during 22 trials. By contrast, mice entered the scented arm during all but five of the 65 trials with 3-hexanone alone or in mixture. The frequency of entry into the scented arm did not differ significantly between the two treatments involving 3-hexanone (F1,5,2 = 0.25, p > 0.5). During trials with ethyl butyrate alone, mice entered the scented arm as frequently (26 of 28 trials) as when 1-hexen-3-one was presented alone (F1,29,6 = 0.26, p > 0.6).

The latency period before a mouse exhibited a choice differed among scents (F2,21,6 = 3.47, p < 0.05). On average, mice responded most quickly during trials involving 3-hexanone alone or in mixture (figure 3c); however, the latency period varied extensively (range 2–165 s) when mice were confronted with a mixed scent. As a result, the longer average latency period during trials...
scent/C2

characteristic for dioecious species, which typically produce
This is the only dioecious species known to be mammal
Reproduction by

4. DISCUSSION

Female and male mice (sex, F_{1,9.1} = 0.46, \( p > 0.5 \)).

with 1-hexene-3-one (figure 3c) differed statistically only
from that for trials with pure 3-hexanone (\( p < 0.025 \)).
Latency periods did not differ significantly between
female and male mice (sex, \( F_{1,0.1} = 0.83, \ p > 0.25 \);
scent \( \times \) sex, \( F_{2,21.6} = 0.46, \ p > 0.5 \)).

4. DISCUSSION

Reproduction by C. visseri is unusual in several respects.
This is the only dioecious species known to be mammal
pollinated and its large, nectar-rich flowers are uncharacter-
Cytinus visseri is also
highly unusual in being pollinated by elephant shrews
(Macroscelididae), a phenomenon that has been reported
in only one other plant species, the South African lily
Whiteheadia biolila [29]. Previous studies have suggested
that elephant shrews visit inflorescences to feed on seeds
[30]. This was clearly not the case for the elephant
shrews that visited C. visseri: not only are inflorescences
devoid of insects, but also a captive E. brachyrhynchus
clearly lapped nectar from flowers with its long tongue
(figure 1c; electronic supplementary material, video).

3-hexanone, one of the two main compounds in the
floral scent of C. visseri, strongly attracted its primary
small mammal pollinator, the striped field mouse. Our
experimental animals were captured outside the range of
C. visseri and received no rewards during the choice exper-
iments, suggesting that 3-hexanone is innately attractive to
them, although we cannot completely exclude the possi-


t of conditioning prior to capture. This compound
may also function in mammalian communication, as it is
a volatile urinary compound of some mice [31] and activ-
vates the posterior olfactory bulb glomeruli of rats [32].
One possibility is that the presence of 3-hexanone in C. vis-
seri scent may represent the co-opting of animal
semiochemicals during the evolution of floral scent, as
has been demonstrated for sexually deceptive orchids
[7,8]. Alternatively, the compound may be effective for
attraction of small mammals because it is indicative of
certain foods. Interestingly, although 3-hexanone is not
a component of most floral scents [3], it occurs in the
scents of a variety of neotropical bat-pollinated
species, including as a major component of members of the
Bromeliaceae and Bignoniaceae [12] and as a minor
component of members of the Amaryllidaceae [12] and
Cactaceae [33].

The other main volatile produced by C. visseri flowers, 1-
hexen-3-one, repulsed experimental animals on its own, but
had no negative effects on the attractiveness of 3-hexanone
when offered in combination with that compound
(figure 2b). A similar phenomenon, whereby compounds
are repellents when offered alone and neutral or attractive
in a mixture, was recently described in insects [34]. The
occurrence of 1-hexen-3-one, but not 3-hexanone, in C. visseri
nectar (electronic supplementary material, table S1) is an intriguing feature of this system, as both compounds
are soluble in water [35], so their contrasting presence in
nectar suggests different secretion processes. Although 1-
hexen-3-one was repellent to naive mice when offered
alone, it may still function as an honest advertisement of
the nectar reward [22]. Whether 1-hexen-3-one in nectar
serves additional functions, such as enhancing taste, repelling
insect nectar thieves, inhibiting yeast growth and/or acting as
an anti-freeze, remains to be determined.

The behavioural effectiveness of the compounds pres-
ent in the scent as trace amounts, among them
silhipherfolene sesquiterpenoids, which have not been
reported previously as a component of floral scent [3],
remain to be investigated. However, our results show
that 3-hexanone, alone or in mixture with 1-hexen-3-
one, or ethyl butyrate alone are sufficient to account for
attraction of naive small mammals in this system.
The scent of C. visseri differs markedly from that of most insect-pollinated plants, which have scents dominated by aromatic, terpenoid and non-ketone aliphatic compounds [36]. Accumulating information on the scent profiles of diverse angiosperms reveals striking associations with particular pollination systems [36,37]. Whether aliphatic ketones are a general feature of plants pollinated by ground-dwelling mammals, perhaps reflecting peculiarities of their olfactory modalities and communication systems, awaits analysis of additional species that rely on these animals as pollen vectors.

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REFERENCES


28 Wester, P. 2010 Sticky snack for sengis: the Cape rock elephant-shrew Elephantulus edwardsi (Macroscelidea), as a pollinator of the pagoda lily, Whiteheadia bifolia (Hyacinthaceae), Naturwissenschaften 97, 1107–1112. (doi:10.1007/s00114-010-0723-6)


