

Attraction of dung beetles to herbivore dung and synthetic compounds in a comparative field study

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Abstract Dung beetles use a variety of vertebrate dung to provision their offspring. To locate these resources, dung beetles use volatile substances emitted from dung as cues. Although it has been shown in laboratory tests that dung beetles are able to discriminate between different dung types using dung volatiles as kairomones, the attraction of particular dung volatiles and their potential role in resource partitioning of dung types have never been tested in field experiments. For the present study, we conducted field experiments in Austria and two regions in Argentina using pitfall traps baited with either herbivore dung types or synthetic compounds of the dung bouquet (butyric acid, 2-butanone, skatole, indole, and blends of these

compounds) to investigate which components or simple mixtures are cues for several taxa of dung beetles. Additionally, we analyzed the degree of specialization of dung beetle species and communities on particular scent types and herbivore dung. Our results show that butyric acid in particular is an important volatile cue for dung beetles. Dung beetles show a preference for some scent types, but turned out to be generalists. This finding is in congruence with the assumption that organisms living from ephemeral resources should rather be generalists instead of specialists.

Keywords Dung scent · Kairomones · Butyric acid · Network analysis · Specialization

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Introduction

Dung beetles feed predominantly on feces produced by vertebrates, mainly mammals, which present a rich source of nutrients for both imagines and beetle larvae (Philips 2011). Dung is a patchy and ephemeral resource that stands out in the surrounding environment as an “island of high quality resource”, and varies enormously in the landscape (Hanski 1991). Consequently, high levels of specialization by dung beetles to any particular kind of dung are not expected. Thus, polyphagy across dung types may prevail among dung beetles (Hanski 1991; Hanski and Cambefort 1991; Larsen et al. 2006), as the chance of a coprophagous insect encounter an adequate food source should be higher for generalist feeders. Despite this supposed generalism, preferences do exist, primarily among the major types of dung (dung produced by herbivores, carnivores, or omnivores) (Hanski 1991; Martín-Piera and Lobo 1996). Although some cases of species-specific adaptations to certain resources are

known, systematic investigations on dung-specificity are scant. More specific feeding preferences have been shown in some dung beetle species, which prefer dung produced by certain vertebrate species when given the choice of feces of producers with similar dietary habits (Estrada et al. 1993; Galante and Cartagena 1999; Finn and Giller 2002; Dormont et al. 2004; Wirta et al. 2010; Carpaneto et al. 2011). Dormont et al. (2007) suggest that more specialized species might be less selective when competition is strong, exemplified by *Aphodius consputus*. While the few individuals they found of this species exclusively occurred in traps baited with one type of dung (Dormont et al. 2007), *A. consputus* is known from previous studies to colonize various dung types (Lumaret and Kirk 1987; Errouissi et al. 2004). It is important to distinguish general preferences (e.g., the majority of all dung beetle species prefer herbivore dung; Hanski and Cambefort 1991) from species-specific preferences that may promote interspecific niche partitioning and thus reducing interspecific competition (Estrada et al. 1993; Galante and Cartagena 1999; Finn and Giller 2002; Dormont et al. 2004; Larsen et al. 2006; Wirta et al. 2010; Carpaneto et al. 2011; Whipple and Hoback 2012).

Both general and species-specific preferences across dung types can only evolve and persist if dung beetles are able to distinguish between types of dung. Detection of airborne odors of dung is achieved through olfactory sensillae in the antennae and usually followed by cruising flights towards the dung pat (Gill 1991). As early as 1931, Warnke suggested that volatile components emitted by dung might attract dung beetles. Dormont et al. (2004, 2007) were able to show in laboratory tests that volatile compounds are crucial, not only for locating, but also for the selection of food resources. Olfactometer bioassays used in their studies found that some species of dung beetles do exhibit a species-specific preference for the type of dung, to which they were attracted in the field when confronted with dung of different herbivores. The authors concluded that the odor bouquet allows the dung beetles to identify and choose certain types of dung. Additionally, the composition of volatiles in the dung's odor varies across the types of dung: a distinct profile of volatiles was found for the specific types of herbivore dung which comprises some components common to all types of dung as well as substances specific to particular dung types (Dormont et al. 2007, 2010). These volatiles may consist of metabolites of digested food and thus, should be influenced by food sources, digestive flora, health of the producer, and other factors. This variation in odor emissions facilitates the dung beetles' search for their preferred type of dung. Thus, even the odorous bouquets of dung produced by different species of herbivores can be distinguished by some specialist dung beetles. The foraging behavior of generalist dung beetles could be explained by

the volatile compounds common to most dung types acting as attractants for dung beetles.

Inouchi et al. (1988) identified five substances in cow dung odor, namely 2-butanone, phenol, *p*-cresol, indole, and skatole, to trigger a reaction in single antennal olfactory cells of *Geotrupes auratus* (Coleoptera, Geotrupidae) which induce food searching behavior. An unpublished study by Inouchi (see Inouchi et al. 1988) suggests that these five components combined are able to attract dung beetles of this species in the field. Short chain carboxylic acids with particular high amounts of butyric acid have been reported from the headspaces of cattle and horse dung (Ohta and Kuwada 1988; Kimura 2001; Jeanbourquin and Guerin 2007; Krell and Schmitt in press). A more recent investigation performed a first analysis of volatiles emitted by dung produced by three different herbivores and one omnivore, in which only one common compound, *p*-cresol, was abundant (Dormont et al. 2010). Other volatiles like indole and skatole were observed in low amounts only but are known to produce a very strong smell nonetheless (Dormont et al. 2010). Yet, it remains unknown which volatile compounds from the headspace of dung have an attracting effect for coprophagous beetles.

The objective of this study is to investigate which volatiles commonly occurring in the headspace of dung act as general attractants (kairomones) for coprophagous beetles in the field. Four volatile substances, 2-butanone, butyric acid, indole, and skatole, were used, which were previously determined to be part of the headspace of various dung types and to be very efficient in attracting various species of dung beetles (see Pfrommer and Krell 2004). Using baited pitfalls, we compared the attractiveness of single compounds, various compound mixtures, and herbivore dung.

The studies were conducted in Austria and Argentina to compare the degree of preferences and specialization in different habitats and climate zones. In addition, we examined patterns of species-specific differences in volatile preferences, which may help to explain resource partitioning of dung types in a beetle community.

Materials and Methods

Study sites

In Argentina, the experiments were carried out in two regions in different climate zones. In December 2010 and January 2011, fieldwork took place in the Man and Biosphere Reserve of Ñacuñán (34°02'42"S, 67°54'32"W), which is located in the Mendoza Province. The reserve lies in the lowland Monte Desert of Argentina and is characterized by a semi-arid and strongly seasonal climate with hot,

humid summers and high annual and inter-annual variation in monthly precipitation (Ojeda et al. 1998). The traps were set up on five sequent days in mid-December and in early January.

In February 2011, trapping experiments were conducted in the National Park Mburucuyá (28°01'40"S, 58°04'02"W). This National Park is situated in the Corrientes Province in the Oriental Chaco, a region characterized by forests, palm groves, grassland, and swamps. The climate is subtropical with hot and humid summers (Rubio et al. 2007). In an area of patchy vegetation varying between small forests, palm groves, and grassland, two series of traps were set up in two different sites in the grassland (A & B) within close proximity. Trapping was done on the same five subsequent days in late February at both sites.

The trapping of dung beetles in Austria took place in the Austrian part of the National Park Neusiedler See—See-winkel at the biological station Neusiedler See close to the town Illmitz in July and August, respectively, in the years 2006, 2007, 2009, 2010, and 2011. The experiments were conducted on a meadow situated between the reed cover of the eastern shore of the Neusiedler See and the salty ponds close to the lake (47°46'14"N, 16°45'54"E). The area is affected by a dry, windy, and predominantly continental (pannonic) climate with hot summers and cold winters.

Dung beetle trapping

Dung beetle preferences for individual volatiles, volatile mixtures, and herbivore dung were studied using dung- and scent-baited pitfall traps. The traps consisted of plastic cups baited with different types of attractants (i.e., different types of dung, chemical components, and an empty control) and were deployed at-grade in the ground. A similar portion of fresh dung across dung types (15–30 g) was wrapped in tissue paper to minimize effects on attractiveness based on different dung quantities (Finn and Giller 2000, 2002). No antiparasitic treatment was applied to any of the animals the dung was collected from, as stated by the farmers and the veterinarian of the Mendoza zoo. About 0.5 ml of the pure liquid chemical components (2-butanone and butyric acid) and 0.5 ml of the pure powdery components (indole and skatole) were applied as liquids or powder without solvents, each wrapped in a separate piece of tissue paper. Note, that we did not bait with *p*-cresol as we were not aware of the potential significance of this compound when we started our first trapping experiments in Austria. Across synthetic compounds, similar amounts were used without taking differences in boiling points and vapor pressure of these compounds into account, hence the quantitative composition of the synthetic volatile blends changed over time during trapping. Declining concentrations in the headspace around the baits are also expected for single substances,

particularly for the liquid chemicals with higher vapor pressure. The control trap consisted of a single piece of tissue placed—like all baits—inside an attractant jar. Baits were set up between 11 am and 1 pm to lure dung beetles for 24 h before being replaced. The traps were arranged in a straight line with a distance of at least 20 m between traps. Each of the positions was marked and given a unique number. Attractants were randomly assigned to a new position every day. All captured dung beetles were preserved in alcohol (96%) or Scheerpeltz solution (65% ethanol, 5% acetic acid, and 30% distilled water) for later identification. The Austrian specimens are repositied in the Denver Museum of Nature & Science and in the Department of Evolutionary Biology and Animal Ecology at the University of Freiburg, the Argentinian specimens in the Argentine Institute for Dryland Research.

Two slightly different trap set-ups were applied. In Argentina, one liter plastic bowls were half-filled with non-perfumed soap sud so that lured beetles which had fallen into the sud could not escape. The prepared attractant jar (50 ml) was attached to each trap using paper clips (Fig. 1a). For each trapping experiment, new jars and attractants were used to rule out contamination by volatiles from previous attractants. In Austria, plastic cups had a volume of about 150 ml, and instead of filling them with water, funnels were added on top of the empty jars to prevent the trapped beetles from escaping. The attractants were placed inside of a tea ball, which was hung from a metal post sticking in the ground at an angle of 45° so that the tea sewer came to hang roughly 5 cm above the plastic cup (Fig. 1b). Tea ball and plastic cup were used for only one type of attractant to avoid contamination with different chemicals. The equipment was moved to the new position, after discarding the previous days' bait, along with the type of attractant. After 24 h of exposition, the trapped beetles were transferred into ethanol or Scheerpeltz solution.

We focussed on herbivore dung as attractant in Argentina to allow a meaningful comparison with the data from Austria. We used dung of different families Equidae (*Equus ferus*, horse), Bovidae (*Bubalus arnee*, buffalo), Cavidae (*Hydrochoerushydrochaeris*, Capybara, and *Dolichotis patagonum*, Patagonian mara), and Camelidae (*Lama guanicoe*, Guanaco) collected from the zoological garden of Mendoza. The dung was kept frozen to preserve the same state of smell as possible for every trapping day. In Austria, we used dung of only two families, namely Equidae and Bovidae (Equidae: *Equus ferus*, horse and *Equus asinus*, donkey; Bovidae: *Bos taurus*, cow), which was collected freshly every day at surrounding pastures (Table 1).

In addition, nine different assemblies of the four chemicals butyric acid, 2-butanone, indole, and skatole applied on tissue were utilized as attractants. In Austria, compound mixtures varied marginally between the 5 years of

Fig. 1 Dung- or scent-baited pitfall traps. Traps consist of plastic bowls that are deployed at-grade in the ground. **a** Construction of the pitfall traps in Argentina. The jar containing the attractant is attached to the trap bowl. This bowl is filled with soap sud. **b** Construction of the pitfall traps in Austria. A tea sewer containing the attractant is attached to a metal post so that it comes to hang closely above the trap bowl. The bowl is closed by a funnel

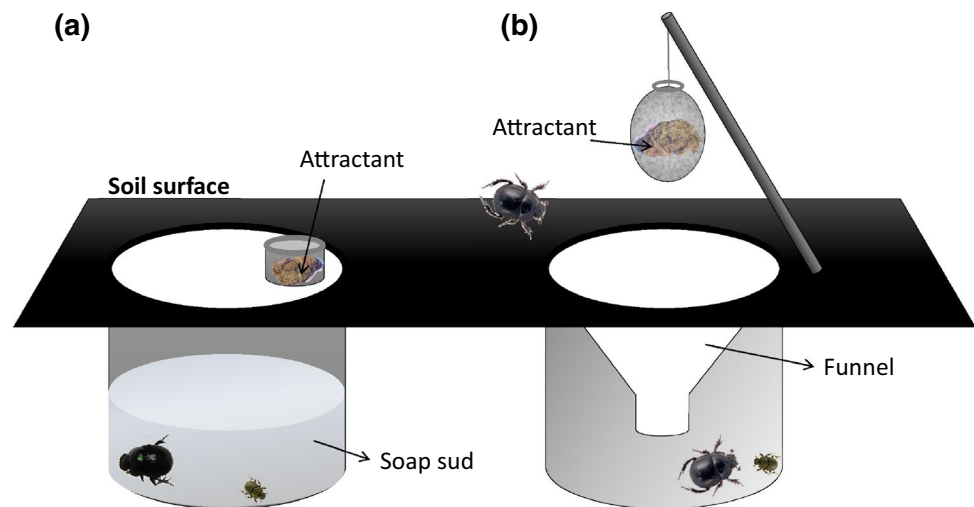


Table 1 Attractants used for baiting dung beetles in pitfall traps in Argentina and Austria

Type of attractant	Illmitz/Austria		Argentina	
	2011		Ñacuñán 2010/11	Corrientes 2011
Bovidae feces	60		10	10
Hydrochoerinae feces	–		10	10
Camelidae feces	–		10	10
Equidae feces	60, 60		10	10
Caviidae feces	–		10	10
2-Butanone	21		10	10
Butyric acid	53		10	10
Indole	21		10	10
Skatole	21		10	10
2-Butanone, butyric acid, indole & skatole	53		10	10
2-Butanone & butyric acid	53		10	10
2-Butanone & indole	21		10	10
2-Butanone & skatole	21		10	10
2-Butanone, indole & skatole	46		10	10
Butyric acid & indole	53		10	10
Butyric acid & skatole	53		10	10
Control	45		10	10

The number marks the count of days the type of attractant was applied at the particular location, while the dash (–) shows its absence. In Illmitz, two types of Equidae feces (horse and donkey dung) were used (labeled as 60, 60), while in Argentina only horse dung was applied. Two different types of Bovidae feces were applied, i.e., buffalo dung in Argentina and cow dung in Austria. In Argentina, dung of producers of three more families was used to attract beetles: Hydrochoerinae (capybara), Camelidae (guanaco), and Caviidae (mara)

sampling (Table 1). At all sites, a single untreated tissue in the attractant jar served as a control.

The total number of trap days varied between 21 and 180 for a given scent or dung type in Austria, while in Argentina sampling took place at 10 days per site.

Data analysis

The data analysis was performed using the program R version 3.0.0 (R Core Team 2013). Additionally, the packages “bipartite” (Dormann et al. 2009), “sna” (Butts 2013), “permute” (Simpson 2012) and “vegan” (Oksanen et al. 2012) were employed.

Our analyses distinguish between (1) general preferences for different scents apparent across all dung beetle species versus (2) species-specific preferences.

1. Variation in attractiveness of scents, i.e., general preferences, was reflected by total capture rates of different scents (and herbivore dung) per trap day. We used chi-squared tests of goodness of fit, applied to each of the three communities. The expected number of beetles for each scent, assuming that all scents are equally attractive, was determined according to the number of trap days at the site (since some scents or dung were exposed more often than others, Table 1). Expected values were compared against the observed total number of dung beetle individuals (of all species pooled) captured per scent. A more detailed analysis of the different attractiveness of scents was performed in two of the three communities (as number of trapped beetles was too low in Ñacuñán). We tested all pairwise combinations of scents with chi-squared tests, and corrected the significance for multiple comparisons based on the false discovery rate (Benjamini and Hochberg 1995). Furthermore, we compared the attractiveness of

synthetic scent compounds versus the dung using a chi-squared test for all baits with compounds and all baits with herbivore dung pooled.

- Interaction network analyses were employed to illustrate and quantify the species-specific preferences of dung beetle species across different scent compounds, controlling for the variation in general attractiveness. Two indices were used (Blüthgen et al. 2006): The network-level measure H_2' , the standardized two-dimensional Shannon entropy, or “complementary specialization,” characterizes the degree of scent partitioning across all dung beetle species in a community. It is suited for comparisons across different communities that may differ in the number of species and number of individuals captured per species and per scent type. The species-level measure d' , the standardized Kullback–Leibler distance, describes the specialization of each beetle species in comparison to the behavior of the entire community, i.e., its non-conformity. Both indices vary between 0 and 1, from the lowest to the highest possible degree of complementary specialization. For example, $H_2' = 0$ implies that all species are very similar in their choices of dung and different scents (although they may differ strongly in total number of individuals), while $H_2' = 1.0$ implies that each species rather performs exclusive choices. Both indices are standardized within the range of potential distributions of each species, keeping the total number of individuals per species fixed as well as the total capture per trap. The measures are thus unaffected by variation in species numbers, abundances, and differences in general trap attractiveness. In order to perform this analysis, we constructed an interaction matrix for each of the three communities with dung beetle species in rows and scent compounds and herbivore dung in columns, where each cell provides the total number of trapped individuals of the respective species by the respective compound. A null model analysis was performed to test whether the degree of species partitioning (H_2') differs from a random distribution of individuals, using Patefield’s null model, which is based on fixed row and column totals (Blüthgen et al. 2006). Whereas the network-level H_2' weights each species by their abundance, we confined our analysis of species-level d' only to species which had at least five individuals in total, given that the reliability of results increases with the number of observations.

In addition, chi-squared tests of goodness of fit were performed to identify those species, which were more attracted to scents than the average dung beetle at the particular location. As expected values of beetles per scents and dung, we used the total number of all dung beetles pooled minus the

individuals of the species tested. Those values were compared against the total number of individuals of one particular species also captured per scents and dung.

Results

Differential attractiveness of dung and scents

Both dung and chemical-baited traps attracted dung beetles in all locations. Although the number of species attracted to real dung sources was higher than to synthetic scent compounds, the overlap was high, and the most frequent species attracted to dung were also attracted to the synthetic scents. At all sites, the number of individuals attracted by dung was significantly higher than the number of dung beetles found in traps with chemical attractants [chi-squared test for the trapping results of every location: Corrientes ($\chi^2 = 144.7$, $df=1$, $p < 0.01$), Illmitz ($\chi^2 = 14079.7$, $df=1$, $p < 0.001$) and Ñacuñán ($\chi^2 = 195.6$, $df=1$, $p < 0.01$)]. Comparing the beetle abundance of the different trapping sites by determining the mean of all beetles trapped by herbivore dung per day showed that the highest density could be found in Illmitz (39.8 ± 16.8 beetles per day) followed Corrientes (6.3 ± 2.9 beetles per day) and Ñacuñán (2.4 ± 2.2 beetles per day).

In Illmitz (Austria), a total of 7155 dung beetles (nine spp.) were captured in traps baited with the three different dung types over 5 years, and 625 beetles (seven spp.) were attracted to chemical baits. Only five individuals were caught in the control traps in all years (Supplementary Fig. 1). The nine species belong to two families, Scarabaeidae (*Onthophagus furcatus*, *O. nuchicornis*, *O. ruficapillus*, *O. ovatus*, *O. joannae*, *O. taurus*, *O. illyricus*, and *Euoniticellus fulvus*) and Geotrupidae (*Geotrupes spiniger*). The two species *O. ovatus* and *O. joannae* were pooled due to their morphological similarity.

In Corrientes, a total of 189 dung beetle individuals (15 spp. plus an undetermined number of *Aphodius* spp.) were captured in traps using the five dung types as attractants, compared to a total of 77 beetles (11 spp. plus an undetermined number of *Aphodius* spp.) caught by synthetic chemicals (Supplementary Fig. 2, for particular types of attractant see Table 1). Here, 15 different species were identified but not all of them could be identified to species. Among these, the following fourteen belong to the Scarabaeidae: *Trichillum* sp.1, *Canthon puncticollis*, *Ontherus appendiculatus*, *Canthidium* sp.1, *Canthidium* sp.2, *Anomiopus* sp.1, *Sulcophanaeus menelas*, *Onthophagus hirculus*, *Malaganiella puncticollis*, *Deltochilum cupreicolle*, *Canthon lituratus*, *Canthon* sp.2, *Canthon* sp.4, and *Canthon* sp.6. Regarding Aphodiinae, *Aphodius lividus* was distinguished

from the remaining individuals of this genus; the latter were pooled as *Aphodius* spp.

In Ñacuñán, fewer dung beetles were trapped using the same experimental setup, attractants, and timeframe. A total of 145 individuals (nine spp. plus an undetermined number of *Aphodius* spp.) were captured by traps baited with the five different dung types and only 20 individuals (two spp. plus an undetermined number of *Aphodius* spp.) in traps provided with chemicals (Supplementary Fig. 3, for particular types of attractant see Table 1). The following nine species of the subfamily Scarabaeinae were identified: *Malagoniella argentina*, *Malagoniella puncticollis*, *Eucranium arachnoides*, *Vulcanocanthon seminulum*, *Canthon ornatus bipunctatus*, *Eutrichillum hystrix*, *Nunoidium argentinum*, *Sulcophanaeus imperator*, and *Scybalophagus plicatipennis*. Dung beetles of the subfamily Aphodiinae belonged to the genus *Aphodius* and were pooled.

In Corrientes, synthetic scents differed in general attractiveness ($\chi^2 = 56.6$, $df=8$, $p<0.01$) and Illmitz ($\chi^2 = 287.7$, $df=10$, $p<0.01$). In Ñacuñán, the total number of dung beetles captured by the different chemical components was too low for statistical analyses, but the variation was similar to the other two sites (Fig. 2 c). In Illmitz, the attractiveness of all mixtures containing butyric acid and butyric acid alone were significantly more attractive than other compounds ($p<0.05$; complete list of statistical values of pairwise comparisons see Supplementary Table 2) (Fig. 2a). Furthermore, the three mixtures of butyric acid with any of the three other volatiles were significantly more attractive than butyric acid alone. In Corrientes, butyric acid also attracted significantly more dung beetles than any other individual substance ($p<0.05$; complete list of statistical values of pairwise comparisons see Supplementary Table 3). However, other comparisons were less clear, reflecting the lower sample size in the other field sides than Illmitz (Fig. 2b).

Species-specific preferences

The dung beetle—scent networks show a rather opportunistic distribution of dung beetle species across the various attractants and thus a generally low degree of specialization H_2' (Fig. 3). The highest value of overall specialization was detected in Corrientes ($H_2' = 0.145$, $p<0.001$), followed by Ñacuñán ($H_2' = 0.099$, $p=0.56$) and Illmitz ($H_2' = 0.076$, $p<0.001$), hence two of the three networks differed from a random distribution (p values according to Patefield null model).

Corresponding to low overall specialization H_2' , the species-level specialization d' was found to be lowest for the Austrian species, ranging from 0.005 in *Onthophagus furcatus* to 0.05 in *O. ovatus & joannae*. In Ñacuñán, d' was similarly low, ranging from 0.02 (*Vulcanocanthon*

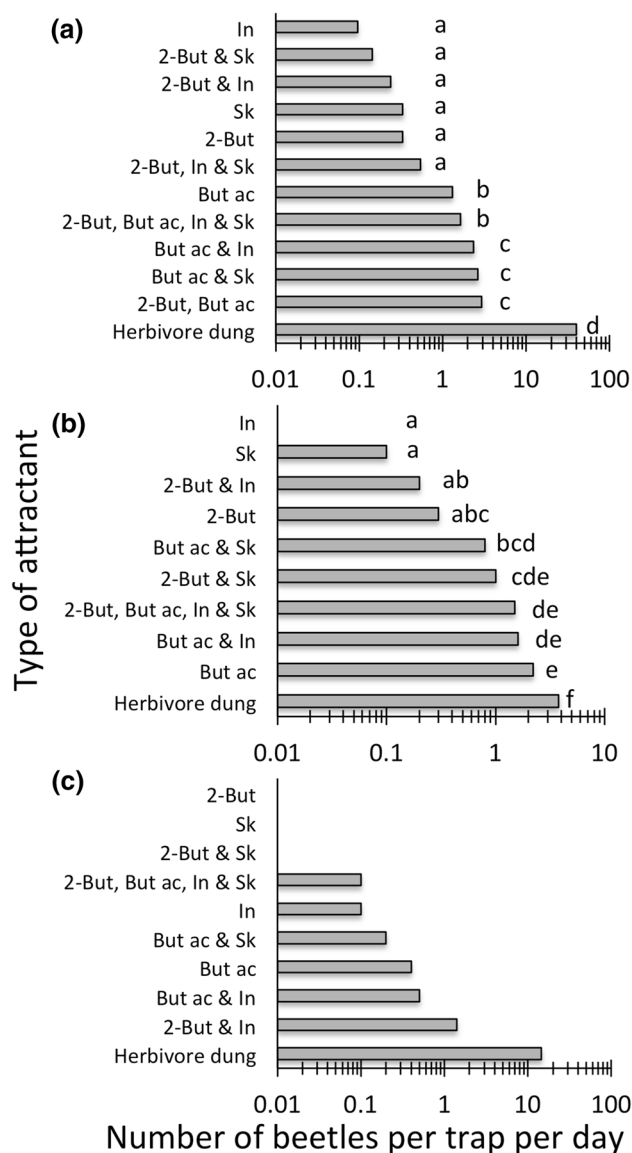


Fig. 2 Dung beetle capture rates of different attractants in **a** Illmitz (Austria), **b** Corrientes (Argentina), and **c** Ñacuñán (Argentina). Standardized numbers of dung beetles per trap day shown. Scents labeled with different letters were significantly different in FDR-corrected pairwise chi-squared tests ($p<0.05$). 2-But—2-Butanone, But ac—Butyric acid, In—Indole, Sk—Skatole, Herbivore dung in a and b=dung produced by herbivores of the families Bovidae (*Bubalus arnee*, buffalo), Equidae (*Equus ferus*, horse), Hydrochoerinae (*Hydrochoerus hydrochaeris*, Capybara), Caviidae (*Dolichotis patagonum*, Patagonian mara), and Camelidae (*Lama guanicoe*, Guanaco) in c=dung produced by herbivores of the families Equidae and Bovidae (Equidae: *Equus ferus*, horse and *Equus asinus*, donkey; Bovidae: *Bos primigenius taurus*, cow)

seminulum) to 0.06 (*Canthon ornatus bipunctatus*). Species-level specialization in Corrientes was slightly higher, with d' values from 0.05 (*Canthidium* sp.1) to 0.14 (*Canthidium* sp.2). For specific d' values, see Appendix table A.1.

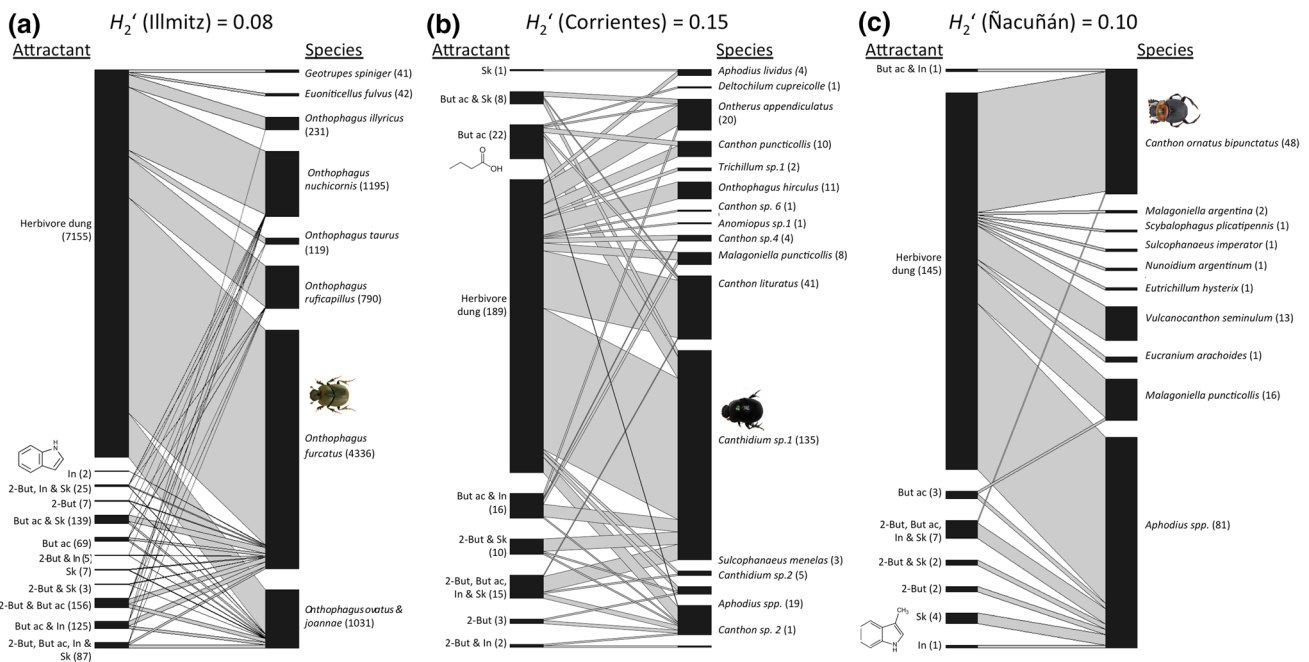


Fig. 3 Dung beetle-attractant network figures for Illmitz, Corrientes, and Ñacuñán. Widths of links are scaled by the number of dung beetle individuals or by attractant visited by each beetle species, bar sizes to their respective totals for each dung beetle species, and attractant type. Species are sorted to minimum overlap of lines leading to a centralization of common dung beetle species. *2-But*—2-Butanone, *But ac*—Butyric acid, *In*—Indole, *Sk*—Skatole, *Herbivore dung* in a and b=dung produced by herbivores of the families Bovidae (*Bubalus arnee*, buffalo), Equidae (*Equus ferus*, horse), Hydrochoerinae

(*Hydrochoerus hydrochaeris*, Capybara), Caviidae (*Dolichotis patagonum*, Patagonian mara), and Camelidae (*Lama guanicoe*, Guanaco), in c=of the families Equidae and Bovidae (Equidae: *Equus ferus*, horse and *Equus asinus*, donkey; Bovidae: *Bos primigenius taurus*, cow). The numbers put in parentheses give the total number of dung beetle individuals per attractant or dung beetle species. **a** Dung beetle-attractant network at Illmitz (Austria). **b** Dung beetle-attractant network at Corrientes (Argentina). **c** Dung beetle-attractant network at Ñacuñán (Argentina)

Dung beetles differ in their response to chemical baits in comparison to dung baits. In Corrientes, two species could be captured only in traps baited with scents (*Deltotichilum cupreicolle* and *Canthon* sp. 2 with one individual each). Species of the genus *Aphodius* were over-represented in chemical traps and were marginally more common than all species together ($\chi^2 = 2.9$, $df=1$, $p=0.088$). A similar result was found for *Aphodius* in Ñacuñán: more individuals of this genus were captured in traps with scents compared to the capture rate for all remaining species ($\chi^2 = 71.3$, $df=1$, $p<0.01$). While no specimens of *Aphodius* were trapped in Illmitz, *Onthophagus ovatus* & *O. joannae* were over-proportionally common in traps with chemicals ($\chi^2 = 387.8$, $df=1$, $p<0.01$).

Discussion

Attractiveness of chemical components

The results of our trapping experiments confirmed that volatiles emitted by herbivore dung are attractive to several species of dung beetles in three regions (one in

Europe, two in South America). Using traps baited with four synthetic substances (2-butanone, butyric acid, indole, and skatole) which have been proven to be components of dung odor, we showed for the first time that synthetic dung volatiles are attractive to dung beetles. We used these four components to mimic a very simplified dung bouquet, including expected uncontrolled temporal changes in quantitative composition due to different volatility in such multiple-component baits. Interestingly, all compounds are known to occur in floral scents from several plant families as well as from other sources, such as human sweat and cheese. (Knudsen et al. 2006; Hassan et al. 2013; Dormont et al. 2013) and can therefore be considered as common compounds emitted by many sources and organisms. The attractiveness of the artificial odors was significantly lower than the attractiveness of herbivore dung at all locations. This was expected, considering the fact that the bouquet of dung comprises a large number of volatile components (Dormont et al. 2010), while we tested either single compounds or mixtures of up to four substances. Besides, as the true quantitative proportions of applied volatiles could not be mimicked with the synthetic scents due to a lack of data

available on the proportion of dung volatiles, the attractiveness of our multi-component baits is likely to be reduced compared to natural dung bouquets. The applied scents thus represent minimal mixtures of volatiles required to attract dung beetles.

The results of the scent-baited traps show that single components with the exception of butyric acid were not or less attractive than mixtures of these compounds. As a single substance, butyric acid was significantly more attractive than 2-butanone, skatole, and indole alone. Thus, butyric acid might be an important cue for generalist dung beetles to locate their resources because it is common to the odor of a large variety of dung types (Krell and Schmitt in press). This hypothesis is supported by the higher attraction of beetles to synthetic mixtures that include butyric acid compared to baits without this component. This effect was consistent in Austria as well as in Argentina (Corrientes).

Interestingly, butyric acid was found to be a constituent of the sex-attracting secretion of the male dung beetle *Kheper subaeneus* and as such to trigger an EAD response in antennae of male and female *K. subaeneus* (Burger et al. 2002). We predict that it will be identified as one of the volatiles common in many more dung types, because scents including butyric acid had the highest capture rates and because dung beetle antennae at least in *K. subaeneus* and *Geotrupes auratus* are able to detect this component. If butyric acid was a cue for the smell of dung in general, a variety of the generalist dung beetles responding to various dung types should be attracted of this substance. In this case, males including this substance in their pheromone could be using an already existing sensitivity to attract more females and gain a selective advantage according to the sensory trap model. Interestingly, butyric acid is not only known to attract dung beetles. It has also been proven as attractant for two mosquito species, namely *Aedes aegypti* and *Anopheles gambiae*, as well as for the nymphalid butterflies, *Kaniska canace* and *Vanessa indica* (Davis 1988; Ômura et al. 2000; Smallegange et al. 2009).

In contrast, the other three volatiles tested as single substances (2-butanone, indole, and skatole) have been less attractive compared to butyric acid. However, blends of butyric acid with one or more additional substance were either more attractive (Illmitz) or similar attractive (Corrientes) than butyric acid alone. This result might be explained by a synergistic effect of 2-butanone, indole, and skatole in combination with butyric acid. However, like butyric acid, all additional tested compounds are not highly specific for dung. Indole and skatole are already known as compounds in floral scents proved to be active in luring pollinators (Knudsen et al. 2006; Bischoff et al. 2015). 2-butanone is also described as floral scent in various plant species (Robertson et al. 1993; Mottram and Flament 1996; Baraldi et al. 1999), but has been shown to be active as cue

for the attraction of several mosquito species as well (Kline and Mann 1998).

Besides considering the attractiveness for dung beetles in general, we examined the species-specific reaction to traps baited with scents. In Illmitz, individuals of *Onthophagus ovatus* and *Onthophagus joannae* could be trapped more often by scents than other dung beetles. Beetles of these species were mainly observed in traps containing butyric acid. If butyric acid is indeed a general cue for coprophagous beetles, it may contribute to a high generalization in dung type choices in such species.

Additionally, at both Argentinian sites, individuals of the genus *Aphodius* were observed far more often than individuals of other species in scent-baited traps. The component that best explained the occurrence of individuals of this genus in traps was not butyric acid. Pitfall traps containing butyric acid did not show higher capture rates of *Aphodius* than scents lacking this component. Even though individuals of this genus have been found in scent-baited traps lacking either indole or skatole, there appears to be a bias towards traps baited with scents including those two substances, suggesting a preference for indole and skatole. Feeding preferences for a specific type of herbivore dung by members of the genus *Aphodius* have been described in earlier studies (Galante and Cartagena 1999; Dormont et al. 2007). Our results suggest that such preferences may be driven by particular volatile substances such as indole and skatole. Both volatiles are nitrogenous components derived from tryptophan in the mammalian digestive tract and as such occur mainly in either carnivore or omnivore but less in herbivore dung. If indole and skatole were more specific cues for particular dung types, dung beetles orienting themselves towards their smell could hint at the existence of a feeding preference for dung of either carnivores or omnivores or both.

Network analysis

Dung is a patchy and ephemeral resource so that a low level of specialization to a particular type should be expected for coprophagous insects and, accordingly, polyphagy is considered as the predominant feeding pattern of dung beetles (Hanski and Cambefort 1991). Therefore, dung beetles are expected to be generalist feeders, and species should exhibit little difference in their choices of dung and different scents (low H_2' values). Accordingly, the partitioning of dung types across beetle species is low at all three locations with H_2' values ranging from 0.076 (Illmitz) over 0.099 (Ñacuñán) up to 0.145 (Corrientes). In comparison with other biological interaction networks, the specialization of dung–beetle interactions is lower than the values reported for ant–nectar plant interactions (median $H_2' = 0.23$) and for seed–dispersal

interactions (0.28), and much lower than plant–pollinator networks (0.55) or ant–myrmecophytes (0.80) (Blüthgen et al. 2007). In such mutualistic or trophic networks, the level of specialization may vary according to species-specific traits such as morphological barriers, but also due to competition and evolutionary drivers, e.g., the plants' benefits from specialized pollen transfer agents (Blüthgen et al. 2007).

The variation in specialization (H_2') across the three regions may reflect differences in dung diversity, dung availability, and competition between dung beetles (higher in tropical ecosystems, Hanski 1987). As discussed above, species-specific responses to dung volatiles (such as the preference of *Aphodius* for indole and skatole) may contribute to a higher partitioning of dung types across species. Moreover, the dung beetle community in Austria (Illmitz) and elsewhere in temperate ecosystems is typically much less heterogeneous, which may contribute to a low level of differentiation in dung choices. The community in Illmitz was dominated by species of one single genus *Onthophagus*, whereas the two Argentinian sites had a variety of genera of the family Scarabaeidae. The higher degree of complementary resource use in the subtropical sites was thus paralleled by a higher taxonomic differentiation, a hypothesis that needs to be tested with additional surveys of dung beetle networks based on multiple dung types. Complementary resource use by different beetle species is also relevant for an increased decomposition rate of dung (Dangles et al. 2012).

Conclusions

Although it has been shown that dung odor bouquets consist of 50 to almost 400 compounds (Amann et al. 2014) we showed that dung beetles can be lured in the field with a much reduced number of volatiles and even single substances. Testing 2-butanone, butyric acid, indole, and skatole as single substances and in various combinations revealed that butyric acid is attractive to dung beetles as a single component albeit it is, like the other tested components, emitted by various plant and animal species and not specific to dung odor. Although our trapping set-up did not allow us to control the quantitative composition of volatile bouquets in our multi-component traps, skatole, indole, and 2-butanone seem to have a synergistic effect at least for some dung beetle species. Since dung beetles use these more general kairomones for locating their resources, specialization to specific dung types is unlikely, confirmed by our network analysis. This finding is in congruence with the assumption that organisms living from ephemeral resources profit from being generalists rather than specialists.

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