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Influence of dung volatiles on the process of resource selection by coprophagous beetles

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Summary. Most dung beetles colonize the faeces of several vertebrate species without much discrimination, and are thus often considered as polyphagous. Recent studies have provided evidence for clear feeding preferences in scarab beetles colonizing dung of herbivore species, but little is known about these insects' abilities to discriminate among odours from faeces of various herbivores. In this study, trophic preferences were examined using blocks of pitfall traps baited with dung from four different herbivore species, i.e., sheep, cattle, horse, and red deer, in a mountainous area of south-central France. 4941 coprophagous scarabs, belonging to 27 species, were captured. Beetles were more attracted to dung of sheep (2257 individuals) than that of cattle (1294 individuals), followed by deer dung (768 individuals) and horse dung (622 individuals). Eleven of the 27 beetle species collected had significant feeding preferences for one of the four dung types. For each insect species, trophic habits did not vary between the two different sites of trapping, an open pasture and a wooded habitat. In laboratory olfactometer bioassays, scarab beetles orientated preferentially towards the dung volatiles from the dung type they preferred in the field. *Trypocopris pyrenaeus*, *Anoplotrupes stercorosus,* and *Aphodius rufipes* were more attracted to volatile compounds from sheep dung, *Onthophagus fracticornis* significantly preferred horse dung volatiles, and *Aphodius haemorrhoidalis* responded positively to deer dung odours. The role of dung olfactory cues in the process of resource selection by dung beetles is discussed.

Key words. Dung beetles – herbivore faeces – trophic preferences – olfactometer – dung volatiles

Introduction

Numerous studies have focused on the process of resource selection by insects exploiting scarce and patchy food resources. In particular, specialized phytophagous insects have served as model systems for most of these investigations. Many plant insects feed only on one plant species (monophagous insect species), or on a limited range of plants (oligophagous species). Such feeding patterns are considered to result mainly from plant chemical and mechanical barriers (Bernays & Chapman 1994). How highly specialized insects locate and choose the appropriate host plant, which represents a patchy microhabitat, has been documented for many phytophagous species (Visser 1986; Dobson 1994; Grison-Pigé *et al.* 2002). In contrast, the process of colonization of dung resources by coprophagous insects has received very little attention, perhaps because these insects are commonly considered to be generalists. In most ecosystems, the droppings of mammals represent very patchy and ephemeral microhabitats. The faeces of vertebrates present a high degree of diversity in chemical composition (Nibaruta *et al.* 1980), but contrary to plants they do not "defend" themselves against insects. It is thus reasonable to expect that coprophagous insects which are polyphagous should be more efficient in locating and exploiting dung resources than would be insects specialized for a particular dung type. Most scarab beetles are indeed considered opportunistic and use a wide variety of dung types without much discrimination (Hanski & Cambefort 1991). The carcasses of vertebrates used by necrophagous insects represent a similar case of a scarce food resource, lacking chemical defenses. There are very few specialized species in burying beetle communities, and polyphagy is the most common feature (Scott 1998). For example, carrion beetles show no preference when given a choice between different bird or mammal carcasses (Scott 1998; Smith & Merrick 2001).

However, clear trophic preferences have been documented in some dung beetle species. These preferences mainly concern adaptation to faeces produced by a group of species with similar food habits, e.g., herbivore, carnivore, omnivore, or human faeces. Insect preferences within faeces produced by mammals of a single trophic type, e.g. faeces from different herbivorous animals, remain unclear. A few studies have provided evidence that dung beetles can display differences in colonisation activity among dungs of different herbivores (Lumaret & Iborra 1996). Field experiments

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conducted with dung of various herbivores showed significant differences in the abundance of beetles between dung types, both in the Mediterranean area (Martin-Piera & Lobo 1996; Galante & Cartagena 1999) and for north-temperate dung beetle communities (Gittings & Giller 1998; Finn & Giller 2002). In a previous study, we experimentally investigated the trophic preferences of Mediterranean dung beetles colonizing cattle and horse dung, showing that more than half of the species had clear feeding preferences for one of the two dung types (Dormont *et al.* 2004).

Patterns of resource partitioning in dung beetles depend on the insects' abilities to detect and select different resource types. The influence of volatile compounds emitted by dung in the long- and short-range attraction of insects has received very little attention, although adult beetles are commonly supposed to rely heavily on dung odours to locate dung pats (Hanski & Cambefort 1991). Martin-Piera and Lobo (1996) considered that "everything suggests an undifferentiated attraction towards effluents and the volatile components of the different types of herbivore faeces". However, we recently showed in laboratory olfactometer bioassays that dung beetles are capable of making a choice between volatiles emitted from cattle faeces versus horse faeces, suggesting that dung odours are likely to be involved in the process of dung selection by beetles (Dormont *et al.* 2004). No information exists on the behavioural responses of coprophagous insects to various dung volatiles, nor on the ability of dung beetles to discriminate among qualitative or quantitative differences in dung odours from faeces of different herbivore species.

The purpose of this study was to understand how dung beetles select resources when several dung types are simultaneously available. In mountainous areas of the French Mediterranean region, cattle, horse, sheep and deer faeces are among the most important resources for dung beetle communities. Our objectives were thus: (i) to examine possible feeding preferences in dung-feeding scarab beetles colonizing the four kinds of dung during field experiments, and (ii) to test the behavioural responses of adult beetles to dung volatiles in laboratory olfactometer bioassays.

Materials and Methods

Field tests of feeding preference

The experiments were carried out in a mountainous area of south-central France, 70 km north of Montpellier, near Mont Aigoual, in the Cévennes National Park (44°08'N, 3°34'E, 1490 m altitude). The area consisted of a mosaic of open and wooded patches where domestic (cattle, *Bos taurus* L., sheep, *Ovis aries* L., and horse, *Equus caballus* L.) and wild ungulates (such as red deer, *Cervus elaphus* L.) co-exist. Two different sites were selected within this area, in order to test whether the number of insects trapped may vary according to both food (dung from cattle, horse, sheep, and deer) and habitat (open and wooded habitats). The first site consisted of an open pasture dominated by herbaceous plants (*Deschampsia flexuosa* L., *Festuca* spp., *Nardus stricta* L.). The second site was located 1 km from the first site, and consisted of a woodland habitat of approximately 40 ha, with beech (*Fagus silvatica* L.) and silver fir (*Abies alba* Mill.).

Dung samples were collected from cattle, horse, and sheep grazing in pastures close to the study site. Dung was taken from untreated animals because antiparasitic drugs administered to herbivorous mammals possibly influence the attractiveness of their dung to insects (Wardhaugh & Mahon 1991; Floate 1998). The dung was collected fresh from several pats (immediately after defecation, before arrival of any coprophagous animals) and was thoroughly mixed together to homogenise it and to avoid possible differences in physical and chemical composition among individual pats. Fresh dung from deer was collected from adjacent clearings used for pastoralism and regularly grazed by deer. Deer dung samples were individually examined to verify that no insects had already colonised the samples, and were then mixed and homogenised as above. Additional dung samples from cattle, horse, sheep and deer were simultaneously collected for further experiments, i.e., behavioural bioassays.

Beetles were collected using dung-baited pitfall traps (CSR type following Lobo *et al.* [1988]). Each trap consisted of a plastic basin 21 cm in diameter and 18 cm high, buried to its rim in the soil. The top of the basin was covered by a conical plastic funnel, with the spout leading into the basin, permitting entry but not exit of adult beetles. A wire grid covered the top of the basin and the funnel cone. Three hundred grams of fresh dung was deposited on the grid. In both sites, the trapping design consisted of 20 traps, including five blocks of four traps. In each block, the four traps were placed at the four corners of a 1 m × 1 m square: in each block, one trap was baited with each of the four dung types. The five blocks were placed at random in the pasture, separated by 10 m intervals. Traps were left out for 48h. After 48h, each basin and its associated pat were collected and examined for insect colonization. Beetles were counted and identified to the species level. All individuals were then put in plastic boxes, three-quarters filled with moist soil, and without any dung resource. Insects were grouped by species in separate boxes, and were kept alive for future behavioural experiments. Two trapping experiments were performed, one in mid-May 2002 and one other in mid-May 2003.

Laboratory bioassays

Behavioural bioassays were carried out in 2002 and 2003 to test the responses of adult beetles to volatiles from different kinds of dung. Tests were performed using adults freshly collected from the pitfall trap experiments described above. Insects collected from both open and wooded habitats were used. The species selected for the olfactory tests were those for which large numbers of live individuals were available, and that showed clear feeding preferences in field experiments. For each species, the field-trapped individuals were placed in individual boxes without reference to the type of dung-baited trap from which they came: no attempt was made in the behavioural tests to take into account dung preferences of individual beetles observed in the field. However, insects collected from open and wooded habitats, as well as males and females, were tested separately. Five species were finally used in behavioural tests: *Trypocopris pyrenaeus*, *Anoplotrupes stercorosus, Onthophagus fracticornis*, *Aphodius rufipes*, and *A. haemorrhoidalis*.

Behavioural tests were done using an olfactometer design derived from those described by Dormont & Roques (2001). The design consisted of a plastic rectangular arena (30 × 12 × 8 cm) with two holes cut on the arena floor. The holes were 2.5 cm in diameter, spaced 20 cm apart, and covered by a small circular wire mesh. A circular plexiglass container (6 cm in diameter, 12 cm high) was placed under each hole and pierced at the bottom in order to allow air entry. Airflow was generated by a pump connected to the olfactometer at the center of the floor, which provided a continuous movement of air from outside through each container's grid holes, as well as within the arena. The airflow rate, measures using an air flowmeter and olfactometer, was maintained at 500 ml min^{-1}. Airflow movements within the arena were assessed using chemical smoke (a mixture of ammonia and hydrochloric acid, 1:1), and airflow rate was adjusted so that insects at the center of the arena could perceive both odour sources without any air turbulence within the arena. The tests were done in a darkened room equipped with two red lights (40 Lux) placed 50 cm above the arena. The source of volatile compounds consisted of two different fresh dung samples (50 g each) placed in the different containers. In order to record insect movements, the arena floor

Table 1. Dung beetle species collected from pitfall traps baited with sheep, cattle, horse or deer faeces, in 2002 in the open pasture site (Mont Aigoual, south-central France). For each insect species and dung type, the number of insects gives the total number of individuals captured from the different traps (five dung-baited traps for each dung type). Insect species showing no significant preference for any of the four dung types, or insect species represented by very few specimens, are not presented in the table*.

	Dung-baited pitfall traps			
Insect species	Sheep dung	Cattle dung	Horse dung	Deer dung
Geotrupes stercorarius	119 a**	88 b	15 d	46 c
Trypocopris pyrenaeus	419 a	170 b	46 c	165 b
Anoplotrupes stercorosus	33 a	21 a	1 b	25 a
Onthophagus fracticornis	3 b	6 b	37 a	0 b
Onthophagus vacca	15 a	13 a	4 b	16 a
Aphodius fimetarius	22 b	62 a	0 c	5 c
Aphodius sphacelatus	81 a	2 b	69 a	0 b
Total	692	363	165	257

**Onthophagus lemur* (total number of individuals: 4), *O. similis* (3), *O. joannae* (2), *Aphodius luridus* (7), *A. haemorrhoidalis* (3), *A. fossor* (16), *A. granarius* (3), *A. constans* (13), *A. erraticus* (1).
**For a species, values in the same row followed by the same letter are not significantly different (Kruskal-Wallis test, $p<0.05$). For a given dung type, the test was applied on the mean number of insects collected from the five traps.

was completely covered with a white paper sheet that was divided in four equal parts designated A, B, C, and D. Sections A and D included the holes connected to the containers with dung samples. Following each test, the paper sheet was replaced and the entire assembly was washed using a solvent mixture of ethanol-acetone (1:1). Room temperature was held at 21 ± 2 °C during the experiments.

One hour prior to each experiment, the individual plastic boxes containing the beetles were transferred to the testing room. Fresh samples of two different dungs were placed in the separate containers. Dung samples were collected each morning from pastures close to the study site of field trappings (see above). Each experiment then consisted of releasing an individual in the center of the arena. Each beetle's position was continuously observed, and the total length of time (in seconds) spent by an individual in each part of the olfactometer was summed over a 10-min period. All beetles of each species were submitted in a random order to the two following series of tests, which were applied successively: (i) no dung sample in either of the two containers, in order to record beetle activity in the absence of an odour source; (ii) a dung sample vs. a dung sample from another mammal species, in order to test for olfactory preferences among volatiles from two different dung types. Four dung types (from cattle, sheep, horse, and deer) were used for the test. Each individual insect was successively submitted to the six different tests, each test consisting of sampling two different dung types among the four different kinds of dung. Both males and females were used for behavioural tests: *T. pyrenaeus* (24 and 11, respectively), *A. stercorosus* (17 and 13), *O. fracticornis* (14 and 14), *A. rufipes* (10 and 16), *A. haemorrhoidalis* (16 and 9).

Data analyses

In each trapping experiment, and for each beetle species, the mean number of insects collected from the five pitfall traps baited with one dung type and those baited with the three other dung types were compared using the non-parametric Kruskal-Wallis test (Statistica 6.0 Microsoft) ($p<0.05$). With regard to the beetle behavioural responses to dung volatiles, the mean cumulative time spent by insects in each part of the olfactometer was compared between the two olfactory situations (test with no odour source vs. test with two different dung odours) using the Mann-Whitney test (Statistica 6.0 Microsoft) ($p<0.05$). For each beetle species, a ranking of preferences over the four dung types was assessed by using Wilcoxon matched-pairs signed ranks tests (Siegel and Castellan, 1988).

Results

Feeding preferences observed in the field

A total of 1924 beetles belonging to 16 species were captured in 2002 (Tables 1 and 2). In 2003, 3017 beetles from 20 species were captured (Tables 3 and 4). For most beetle species, individuals were found in traps baited with three or four dung types. If we exclude beetle species represented by very few specimens (fewer than 5 individuals), only one species was found exclusively in traps baited with one type of dung: *Aphodius consputus* (55 individuals), collected only on deer faeces, in open and in wooded habitats. *Aphodius aestivalis* was attracted only by two dung types, and was only observed in traps baited with cattle or deer dung.

For most species, there was considerable variation in the distribution of individuals between dung types and between years of trapping. Four species exhibited a significant feeding preference for sheep dung (*Geotrupes stercorarius*, *Trypocopris pyrenaeus*, *Anoplotrupes stercorosus, Aphodius rufipes*). A few species were significantly more attracted to cattle dung-baited traps (*Aphodius fimetarius*), or horse dung-baited traps (*Onthophagus fracticornis*), while two other species were found in significant larger numbers in traps baited with deer faeces (*Aphodius consputus, Aphodius haemorrhoidalis)*. Considering the total number of insects collected in both open and wooded sites and in both years, pitfall traps baited with sheep dung attracted significantly more insects than traps baited with other faeces: 2257 insects trapped with sheep dung vs. 1294 insects with cattle dung, followed by deer dung (768 insects) and horse dung (622 insects) ($\chi^2 = 41.6$; df = 9, $p<0.001$).

There was a significant difference in the abundance of insects between the open and the wooded habitat. The total number of individuals trapped in 2002 was four times higher in open habitat (1535 insects) than in the wooded site (389

Table 2. Dung beetle species collected from pitfall traps baited with sheep, cattle, horse or deer faeces, in 2002 in the wooded habitat site (Mont Aigoual, south-central France). For each insect species and dung type, the number of insects gives the total number of individuals captured from the different traps (five dung-baited traps for each dung type). Insect species showing no significant preference for any of the four dung types, or insect species represented by very few specimens, are not presented in the table*.

	Dung-baited pitfall traps			
Insect species	Sheep dung	Cattle dung	Horse dung	Deer dung
Geotrupes stercorarius	16 a**	9 b	0 c	7 b
Trypocopris pyrenaeus	85 a	35 b	2 d	16 c
Anoplotrupes stercorosus	126 a	41 b	2 c	39 b
Total	227	85	4	62

**Aphodius fimetarius* (total number of individuals: 1), *A. constans* (8), *Onthophagus similis* (1), *O. fracticornis* (1).
**For a species, values in the same row followed by the same letter are not significantly different (Kruskal-Wallis test, $p<0.05$). For a given dung type, the test was applied on the mean number of insects collected from the five traps.

Table 3. Dung beetle species collected from pitfall traps baited with sheep, cattle, horse or deer faeces, in 2003 in the open pasture site (Mont Aigoual, south-central France). For each insect species and dung type, the number of insects gives the total number of individuals captured from the different traps (five dung-baited traps for each dung type). Insect species showing no significant preference for any of the four dung types, or insect species represented by very few specimens, are not presented in the table*.

	Dung-baited pitfall traps			
Insect species	Sheep dung	Cattle dung	Horse dung	Deer dung
Geotrupes stercorarius	93 a**	93 a	7 c	29 b
Trypocopris pyrenaeus	663 a	369 b	219 c	116 d
Anoplotrupes stercorosus	48 a	51 a	10 c	25 b
Onthophagus fracticornis	0 b	7 b	44 a	2 b
Aphodius fimetarius	2 b	14 a	8 a	10 a
Aphodius fossor	7 a	11 a	0 b	6 a
Aphodius haemorrhoidalis	1 c	26 b	0 a	89 a
Total	814	576	251	277

* *Onthophagus similis* (total number of individuals: 7), *O. vacca* (2), *O. verticicornis* (2), *Copris lunaris* (1), *Aphodius rufipes* (10), *A. scrutator* (2), *A. aestivalis* (14), *A. rufus* (3), *A. consputus* (6).
** For a species, values in the same row followed by the same letter are not significantly different (Kruskal-Wallis test, $p<0.05$). For a given dung type, the test was applied on the mean number of insects collected from the five traps.

Table 4. Dung beetle species collected from pitfall traps baited with sheep, cattle, horse or deer faeces, in 2003 in the site of wooded habitat (Mont Aigoual, south central France). For each insect species, and for a dung type, the number of insects gives the total number of individuals captured from the different traps (five dung-baited traps for each dung type). Insect species showing no significant preference for any of the four dung types, or insect species represented by very few specimens, are not presented in the table*.

	Dung-baited pitfall traps			
Insect species	Sheep dung	Cattle dung	Horse dung	Deer dung
Geotrupes stercorarius	24 a**	9 b	8 b	2 b
Trypocopris pyrenaeus	104 a	49 b	56 b	40 b
Anoplotrupes stercorosus	278 a	146 b	62 c	45 c
Onthophagus fracticornis	1 b	0 b	21 a	0 b
Aphodius consputus	0 b	0 b	0 b	49 a
Aphodius rufipes	85 a	16 b	0 c	0 c
Total	460	252	137	136

* *Onthophagus similis* (total number of individuals: 3), *O. verticicornis* (4), *Euoniticellus fulvus* (1), *Aphodius fimetarius* (6), *A. haemorrhoidalis* (1), *A. fossor* (1), *A. rufus* (5), *A. equestris* (1), *A. corvinus* (2), *A. reyi* (1).
** For a species, values in the same row followed by the same letter are not significantly different (Kruskal-Wallis test, $p<0.05$). For a given dung type, the test was applied on the mean number of insects collected from the five traps.

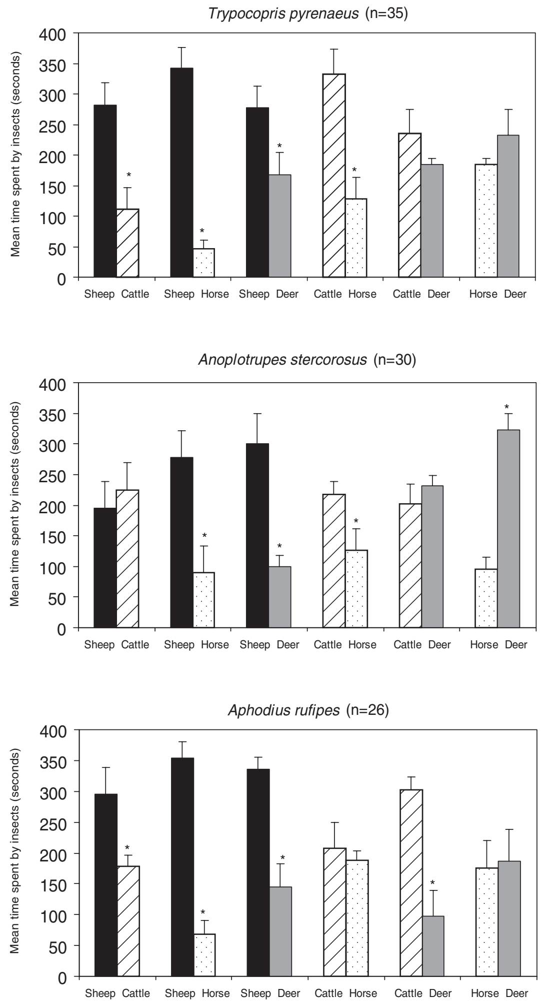

Fig. 1 (*Continued*)

insects), and two times higher in the open site (1997 individuals) than in the wooded site (1020 individuals) in 2003 ($\chi^2 = 27.4$; df = 3, $p<0.001$). Eight species were significantly more abundant in the open pasture ($p<0.01$ for each species): *G. stercorarius*, *T. pyrenaeus*, *O. fracticornis*, *O. vacca, A. fimetarius*, *A. sphacelatus*, *A. haemorrhoidalis, A. fossor*. Only three species were significantly more abundant in the wooded habitat: *Anopl. stercorosus* (in 2002 and 2003), *A. rufipes* and *A. consputus* (in 2003 only). All the species that showed significant feeding preferences for a dung type in one habitat were either significantly more attracted to the same dung type in the other habitat as well, or showed no significant preference.

Considering the total number of individuals, dung beetles in open pastures were dominated by *T. pyrenaeus*, which represented more than half of the total insects trapped in both 2002 (800 individuals caught/total of 1528 insects) and in 2003 (1367/1965). In the wooded site, *Anopl. stercorosus* was the most abundant species, yielding more than 50% of the total number of insects trapped in both 2002 (208 individuals caught/total of 389 insects) and 2003 (531/1010).

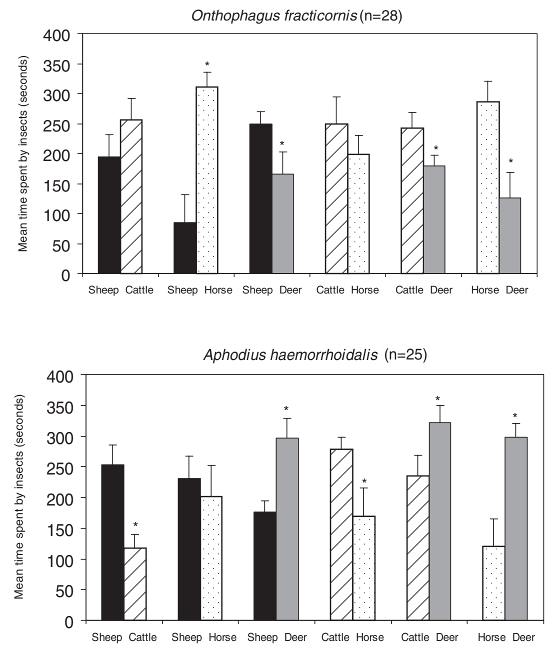

Fig. 1 Olfactory responses of beetle species to dung volatiles: mean time spent by insects in two different parts of the olfactometer when submitted to two different dung odour stimuli, from sheep ■, cattle ▨, horse ⬚, or deer ▪. The arena floor was divided into 4 equal parts designated A, B, C, and D. For a test, individual insects were left in the olfactometer over a 10 min period (the number of insects tested for each species is given in brackets). Each pair of columns summarizes one behavioural test, for which insects were submitted to odours from two different dung types. The first column presents the average number of seconds (± s.e.) spent by insects in the presence of one odour source in section A (left column), the other column presents the mean time spent by insects in the presence of odours from another dung type in the opposite section D (right column). The mean time spent by beetles in the two medium parts (B and C) of the olfactometer is not shown on the graph. For a species, the mean time spent by beetles was compared between two situations (test with no odour source vs. test with dung odours) for each part of the olfactometer, using a Mann-Whitney test ($p<0.05$). An asterisk indicates significant differences for both columns of a pair. *Trypocopris pyrenaeus*, *Anoplotrupes stercorosus,* and *Aphodius rufipes* were observed to prefer sheep faeces in field experiments, *Onthophagus fracticornis* was observed to prefer horse faeces, and *Aphodius haemorrhoidalis* was observed to prefer deer faeces.

Behavioural responses of beetles to dung volatiles

Five insect species were used in olfactory tests (Fig. 1). Three of these were observed to prefer sheep dung in field trapping experiments (*T. pyrenaeus*, *Anopl. stercorosus, A. rufipes*), one species was observed to prefer horse dung (*O. fracticornis*), and the last species significantly preferred deer dung (*A. haemorrhoidalis*).

The first three species, *T. pyrenaeus*, *Anopl. stercorosus,* and *A. rufipes*, were all significantly more attracted to sheep dung odours in the laboratory bioassays. When submitted to dung odour stimuli, these beetles spent significantly more time in the section A or D (which included the hole corresponding to the container with sheep dung) than during tests in which no odour source was present. During the tests including odours from sheep dung vs. odours from another dung type, these three insect species all significantly preferred sheep dung odours. One exception was the case of *Anopl. stercorosus* during the test including sheep vs. cattle dung volatiles, where no preference was observed. For these three species, *T. pyrenaeus*, *Anopl. stercorosus, A. rufipes,* volatiles from sheep dung were the most attractive odour (Wilcoxon matched-pairs signed ranks tests, T=78, N=35, P<0.01, T=55, N=30, P<0.01, T=43, N=26, P<0.01, respectively), followed by cattle dung volatiles, deer dung volatiles, and horse dung volatiles. The one species which preferred horse dung in the field, *O. fracticornis*, was also significantly attracted to volatiles from this dung type in laboratory bioassays. Adult beetles of this species spent significantly more time in the section of the olfactometer from which horse dung volatiles emanated, except during the test comparing horse dung and cattle dung, where no significant preference was noted. For this species, volatiles from horse dung were the most attractive odour (Wilcoxon matched-pairs signed ranks tests, T=62, N=28, P<0.05), followed by cattle dung volatiles, sheep dung volatiles, and deer dung volatiles. The sole species that preferred deer dung in the field, *A. haemorrhoidalis*, also showed a significant preference for deer dung odours compared to other dung types (sheep, cattle or horse) in laboratory bioassays. In the tests including volatiles from other dung types, *A. haemorrhoidalis* was significantly more attracted to emissions from sheep dung than to those from cattle dung, though this species was observed to prefer cattle dung over sheep dung in one field trapping (Table 3). Volatiles from deer dung were the most attractive odour (Wilcoxon matched-pairs signed ranks tests, T=28, N=25, P<0.05), followed by sheep dung volatiles, cattle dung volatiles, and horse dung volatiles.

For all five species, no difference in response to dung odours was observed between males and females, nor between individuals collected from open and wooded habitats.

Discussion

In field experiments, about half of the beetle species showed a significant preference for one of the four dung types, which was sheep dung in most cases. In a recent study, we had already documented significant differences in the abundance of dung beetles between series of pitfall traps baited with cattle or horse faeces (Dormont *et al.* 2004). The results presented here clearly indicate that feeding preferences also occur when a greater diversity of dung types is considered. Moreover, most of the beetles showing no preference in Tables 1-4 were represented by very few individuals, so that the apparent lack of preference in these species may be simply due to the limited sample size. In other studies, sheep dung also tended to attract the greatest number of beetles during experimental field trappings (Finn and Giller, 2002; Errouissi *et al.* 2004a).

Another important conclusion emerging from the trapping experiments stems from the apparent absence of specialist insects with a diet restricted to one kind of dung. One species, *A. consputus*, was surprisingly found only in deer faeces. This species is known from previous studies to colonize various dung types in the Mediterranean region, including cattle, sheep, horse and even human faeces (Lumaret & Kirk 1987; Errouissi *et al.* 2004b). In our study, some other beetle species were found only in one dung type, but all of these were represented by very few individuals. Beetle species that appeared linked exclusively to one kind of dung have been reported in a very few cases in Mediterranean areas (Lumaret & Iborra 1996; Galante & Cartagena 1999). Because of the scarcity and the spatially distributed nature of the dung resource, extreme specialization is considered unlikely to occur in coprophagous insects (Hanski & Cambefort 1991; Finn and Giller 2002).

Other experimental comparisons of the colonisation of different dung types by coprophagous beetles, have provided evidence for clear feeding preferences in dung beetle communities (Lumaret & Iborra 1996; Martin-Piera & Lobo 1996; Barbero *et al.* 1999; Galante & Cartagena 1999; Gittings & Giller 1998; Finn & Giller 2002). However, noticeable variation in trophic habits emerged from these studies, suggesting that dung beetles may show geographic variation in their food preferences. Barbero *et al.* (1999) concluded that trophic preferences are not constant species traits in dung beetles, and stressed the fact that habitat preferences of dung beetles, depending mainly upon temperature, soil type, and humidity, seem to be more important than specialisation on different dung types. Other authors have postulated that the choice of a particular dung type by insects may be conditioned by the habitat predilection of the source species (Martin-Piera & Lobo 1996; Galante *et al.* 1995; Verdú & Galante 2002). In our study, some beetle species showed a significant predilection for open pasture or wooded habitat, but their feeding patterns did not vary between the two habitats. For example, the myco-coprophagous beetle *Anopl. stercorosus* was predominantly collected from dung-baited traps placed in the wooded habitat, and has already been shown to prefer such habitat in other studies (Lumaret, 1990; Barbero *et al.* 1999). However, in all trapping experiments, this species was more attracted to sheep and cattle dung than to faeces from deer, a wild ungulate associated with wooded habitats. The extent to which food preferences of dung beetle species vary in relation to vegetation cover or altitude, or vary geographically, remains an open question. Further experimental comparisons of feeding preferences among faeces of different herbivore species offered simultaneously in both different habitats and distinct geographical sites are needed before any conclusions can be drawn.

The results obtained with olfactometer bioassays provided clear evidence that volatile compounds emitted by faeces are involved in the process of resource location and selection by dung beetles. In most cases, beetles responded positively to dung volatiles in laboratory experiments, and orientated preferentially towards the volatiles from the dung type they preferred in the field. Regarding the insect species observed to prefer sheep in field trappings, beetles were systematically more attracted to sheep dung volatiles in laboratory olfactory tests than to any other dung source. Olfactory tests thus confirmed the results obtained during field trappings, which was not surprising with regard to the collecting method we used, i.e. dung-baited pitfall traps, to compare beetle abundance in the field. In contrast to other collecting methods, e.g. dung pats (naturally-dropped pats or artificially aggregated pats), beetles attracted to pitfall traps cannot escape. Insect abundances recorded with this method thus reflect the pattern of initial colonisation by adult beetles. Because we used pitfall traps in our study, insects should not have first tested or fed on another dung-baited trap type before falling into the trap, and beetle abundances in the different types of traps thus probably reflect a choice primarily based on emission of volatile compounds by dung. Finally, field trappings, as well as bioasssays, clearly suggested that dung beetles are capable of making choices between odours from faeces of different herbivore, and thus are adapted to exploit preferentially a particular dung type. Because the dung quality of herbivorous animals can vary greatly depending on the animal's foraging behavior (Barth *et al.* 1994; Gittings & Giller 1998), further investigations will have to address the possible differences in dung volatiles in relation to animal diet, and must also consider the geographic origins of the individuals (both mammals and dung beetles) studied. Variations in dung emissions over dung degradation and over dung colonization by insects also remain to be surveyed. Fecal residues of veterinary parasiticides may also modify the composition of dung volatiles. Veterinary parasiticides, such as ivermectin, may also modify the chemical composition of dung volatiles (Bernal *et al.* 1994). The effects of fecal residues of antiparasitic drugs on the process of colonization by dung-feeding insects remain unclear (Floate *et al.* 2005). Cattle dung from treated animals has been shown to be either more attractive (Wardhaugh & Mahon, 1991; Holter *et al.* 1993; Lumaret *et al.* 1994) or much less attractive than those from untreated animals (Floate 1998) to dung beetles in the field.

A comparison of the chemical composition of dung odours revealed notable differences between the content of volatile compounds in dung of sheep, cattle, horse and deer (Dormont, unpublished). Each dung type was characterized by a distinct profile of volatiles consisting of more than twenty components, including compounds common to all dung types as well as a few compounds specific to each dung type. Considering the differences in the profiles of volatile compounds in dung from sheep, cattle, horse and deer, it is not surprising that dung beetles are able to select the appropriate resource on the basis of olfactory cues, as was confirmed by olfactometer bioassays. The process of dung selection by beetles is thus probably mediated by the emission of volatile compounds by dung, although other factors related to dung quality (moisture content, physical parameters, nutritional quality of the dung, etc) are probably also involved in this process (Hanski & Cambefort 1991; Gittings & Giller 1998). Other inhabitants of the dung (diptera larvae, earthworms, bacteria...) may also reduce the suitability of the resource for coprophagous beetles.

Whether or not olfactory cues dominate in the factors used by insects in choosing dung types remains an open question. Some dung beetle species have been reported to have locally variable preferences for particular dung types, i.e. these species significantly attracted to one dung type in a country were found more frequently in a different dung type in another country (Barbero *et al.* 1999). Local feeding preferences have been considered to depend upon various ecological parameters, such as habitat characteristics, nesting strategies, or even the size of the dung pat (Peck & Howden 1984; Lumaret & Kirk 1987; Gittings & Giller 1998; Finn & Giller 2002; Errouissi *et al.* 2004a). Further olfactory tests with populations of dung beetle species showing distinct, geographically variable preferences are needed to assess whether olfactory responses to dung volatiles may vary among populations of a same species.

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