

SHORT COMMUNICATION

Interspecific and intraspecific variation in diet preference in five Atlantic forest dung beetle species

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Abstract. 1. Dung beetles are commonly assumed to be generalist feeders, but there has been limited work in identifying whether there is interspecific variation in feeding preference. Equally, there has been no work exploring whether generalist feeding behaviour in a species is a result of within-species specialisation.

2. This study identified the individual and species-level feeding preferences of five dung beetle species towards human, jaguar and pig dung using a choice experiment.

3. It was found that species varied in their preference for the dung types, but there was no evidence that within a species, individual beetles varied in their dung choice. These findings were similar to results from field experiments that are more typically used to assess feeding preferences in dung beetles.

4. The results suggest that individual specialism in feeding may not be common in dung beetles. However, there is variation in feeding preferences among species, which is often overlooked and can have implications for the ecosystem functions they provide. It is suggested that choice arenas can be used to assess feeding preferences in dung beetle species that are not abundant enough to reliably estimate dietary choice from field studies.

Key words. Choice experiment, coprophagy, dietary specialism, niche variation, Scarabaeinae, tropical.

Introduction

Resource use can vary at the level of the species (interspecific variation) or of the individual (intraspecific variation) (Bolnick *et al.*, 2003; Violle *et al.*, 2012). For example, within-species variation is common in insects such as pea aphids (Sandström & Pettersson, 1994). Intraspecific variation in diet is based on functional trade-offs that prevent an individual from exploiting all available resources (Araújo *et al.*, 2010; Araújo *et al.*, 2011). Identifying niche variation in feeding is of particular relevance for taxa where resource use is related to ecosystem functions, such as dung beetles that contribute towards dung removal and secondary seed dispersal (Nichols *et al.*, 2008).

The majority of dung beetle species are thought to be generalists, able to utilise a wide range of dung from naturally co-occurring vertebrates (Howden & Nealis, 1975; Frank *et al.*,

2018), as well as non-native species (Whipple & Hoback, 2012; Stavert *et al.*, 2014). Although species can vary in their preference for certain dung types (Bogoni & Hernández, 2014), interspecific feeding variation is often overlooked in studies of dung beetle ecology. Equally, there has been no research investigating whether apparently generalist resource use in dung beetles could be a result of individual specialist feeding and interindividual variation.

Bacterial symbionts present in the gut are thought to play a role in nutrient acquisition in dung beetles (Holter, 2016), and have been found to vary in composition between dung beetle species with different diets (Franzini *et al.*, 2016; Shukla *et al.*, 2016). Variation in gut microbiota between individuals may affect the ability to digest nutrients in the dung that would be otherwise unavailable, and could result in interindividual diet selection in dung beetles (Schwab *et al.*, 2017). We investigated within- and between-species variation in diet preference for five dung beetle species using a choice experiment and three dung types representative of the omnivore and carnivore feeding guilds. This was compared with field experiments to identify whether

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Table 1. Dung beetle species, number of individuals trialled, and diet preference as indicated from the literature

| Species | No. of individuals | Diet preference |
|--|--------------------|---|
| <i>Dichotomius mormon</i> Ljungh | 33 | Generalist (Falqueto <i>et al.</i> , 2005) |
| <i>Dichotomius sericeus</i> Harold | 14 | Necrophage (Silva & Hernández, 2007) Coprophage (Bogoni & Hernández, 2014) |
| <i>Coprophanaeus dardanus</i> MacLeay | 15 | Necrophage (Feer & Pincebourde, 2005) |
| <i>Coprophanaeus saphirinus</i> Strum | 12 | Coprophage (Bogoni & Hernández, 2014) Necrophage (da Silva <i>et al.</i> , 2012) |
| <i>Phanaeus splendidulus</i> Fabricius | 19 | Generalist/necrophage (Da Silva & Di Mare, 2012) Coprophage (Da Silva & Di Mare, 2012; Bogoni & Hernández, 2014) Generalist (da Silva <i>et al.</i> , 2012) |

the preference of individuals is equivalent to that detected at the population level – the standard method for assessing dietary specialism in dung beetles.

Methods

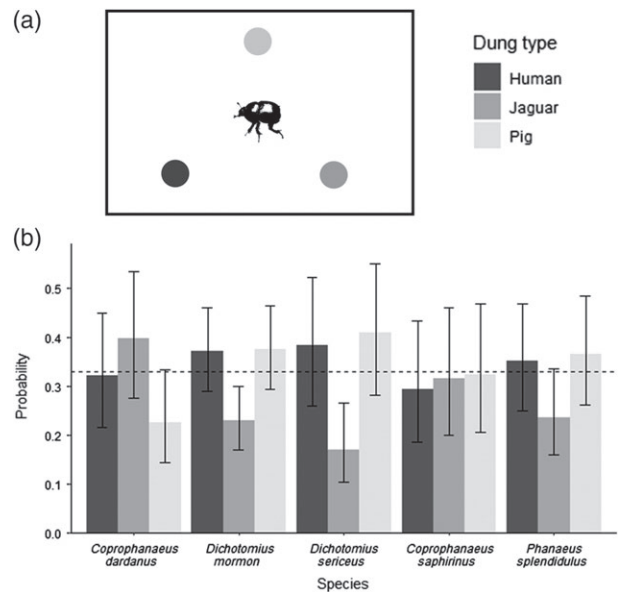
The study was conducted in an area of continuous lowland tropical forest in the Atlantic forest, Paraná state, Brazil at each of three sites separated by 500 m to 1 km (see Supporting information, Fig. S1) (25°27'11"S, 48°52'57"W). Jaguar dung was provided by Curitiba Zoo, and pig dung was collected from nearby farms. Both of these dung types and human faeces were frozen upon collection to ensure they remained fresh, and to kill any beetles or beetle predators.

Collection of dung beetles

Five large-bodied tunnelling species, chosen due to their abundance in the area, were used in the experiment (Table 1). Beetles were collected along the three transects in February 2016 using live pitfall traps baited with 50 g of human dung. Dung beetles were kept in cool ventilated containers with damp soil. Beetles were exposed to normal daylight hours and deprived of food for 5 days before trials.

Choice experiment

The diet preference arena was a 43 × 30-cm tray with a mesh top, lined with 4 cm of fresh compact soil and used to assess the choice of omnivore (human, pig) and carnivore (jaguar) dung. A quantity of 30 g of fresh dung of each type was added to the arena, positioned 20 cm apart in an equilateral triangle. In all, 93 beetles were tested (Table 1). Each trial consisted of a beetle placed in the centre of the triangle (Fig. 1a). Diet choice

**Fig. 1.** (a) Choice arena design; (b) model estimates and 95% confidence intervals for the probability of each dung type being chosen. The dashed line is for reference, and represents no preference between dung types.

was recorded for those that showed movement within 5 min. Choice was determined by the observation of feeding for 30 s. If no feeding occurred after 10 min, despite movement, the trial ended. Each beetle individual was tested five times with a minimum of 1 h between trials. The tray was rotated between trials.

Field data

Sampling was carried out monthly (December 2015–March 2016) using traps placed at 200-m intervals along the transects. Pitfall traps consisted of a 1-litre cup buried flush with the soil surface and half-filled with water, with a small amount of detergent and salt. The traps were baited with natural volumes of human (c. 90 g), pig (c. 210 g), or jaguar dung (c. 74 g). Natural dung volumes were used to attract a realistic dung beetle community that would feed on a dung pat. Dung beetles were identified by a taxonomist (F. Vaz-de-Mello) and voucher specimens were deposited at the Universidade Federal de Mato Grosso (UFMT) and Embrapa Florestas, Paraná.

Statistical analysis

We tested whether there was variation both between and within species in preference for the three dung types using a linear mixed-effect model. We excluded trials where no feeding took place. For each individual beetle, the proportion of times each dung type was chosen was logit-transformed to meet model assumptions and used as the response variable (Warton & Hui, 2011). Interactions between species and dung type were included as an explanatory variable to identify whether feeding preference varied by species for each dung type, and a random effect for beetle individual was included to assess how much

variation in choice can be attributed to the beetle individual. We also tested whether there were differences among species in the proportion of times an individual was observed to feed, using a generalised linear mixed-effect model with a binomial error distribution. Species and trial number were included as explanatory terms, and the beetle individual as a random effect.

Dung beetle species' diet preferences were compared with the results of population-level assessments of feeding preference from the field. We calculated the total proportion of trials per species that a dung type was chosen, and the total proportion of individuals captured from the field experiment per species. This was then compared using Spearman's coefficient. Models were fitted using the *lme4* package (Bates *et al.*, 2015) and confidence intervals calculated with *emmeans* (Lenth, 2018) for R 3.4.3 (R Core Team, 2017).

Results and discussion

The mammalian dung type chosen varied significantly depending on the dung beetle species ($F_{8,264} = 2.25$, $P = 0.025$). There was no evidence of strong preference for a particular dung type by any of the five species, but jaguar dung was chosen less regularly than expected at random for two species, *Dichotomius mormon* and *Dichotomius sericeus* (Fig. 1b). However, beetle individual explained very little of the variation in choice (SD = 0, 95% CI: 0–0.13).

Dung beetle species differed in the proportion of times they chose to feed versus not to feed ($\chi^2 = 28.28$, d.f. = 4, $P < 0.001$), which was also significantly different across the trials ($\chi^2 = 29.40$, d.f. = 4, $P < 0.001$), with all species choosing dung less frequently as the trials progressed. Four species preferentially chose to feed on the dung, whereas *C. dardanus* showed no preference. There was also a large variation among beetle individuals in whether they chose dung (SD = 1.6, 95% CI: 0.95–2.07).

The field experiment identified 432 individuals of the five species attracted to the three dung types (Table S1), and the proportion that each dung type was chosen by each species did not differ between the laboratory and the field experiment ($t = 1.58$, d.f. = 13, $P = 0.14$).

Individual beetles within a species accounted for little variation in dung choice, which suggests that, within species, beetles do not vary in the type of dung they choose to feed on. *Coprophanæus dardanus* was the only dung beetle species that did not show a preference for feeding on dung. This could be accounted for by its necrophagous feeding habits (Table 1), but that it did feed sometimes shows that when resource-deprived it may still utilise dung.

Jaguar dung was the least preferred dung type by two species in the choice experiment, and field studies have also found carnivore dung to be the least attractive (Bogoni & Hernández, 2014; Hewavithana *et al.*, 2016). Adult dung beetles require liquid to filter-feed high-nutrient particles from dung, and the low moisture content of carnivore dung could explain the avoidance by some species (Al-Houty & Al-Musalam, 1997; Frank *et al.*, 2017). Pathogenic bacteria in carnivore dung may also deter dung beetles from feeding (Mansourian *et al.*, 2016).

Previous work has shown that feeding preferences vary among dung beetle species (Dormont *et al.*, 2007; Santos-Heredia *et al.*, 2010; Filgueiras *et al.*, 2011). Similarly, these results suggest that there is a variation in preference for types of dung among dung beetle species from the Atlantic forest. This has implications for identifying the robustness of dung beetles to environmental change, and, by extension, the ecosystem functions they provide.

The comparison of findings between the field and the choice experiment suggests that the estimates of dietary choice made across individual beetles are similar to those from population-level assessments, which are frequently used to classify dung beetle diet preference. Should this assessment hold true for other species, feeding trials could be used as a valid way of assessing dietary preference for rarer dung beetle species. This provides a new potential for assessing dietary specialism in dung beetles, as the extent to which these specialisms are a relic of undersampling is unknown (Larsen *et al.*, 2006).

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. White lines represent site extent and yellow lines represent transect locations for pitfall traps in each of three sample sites in continuous lowland tropical forest in the Atlantic forest, Paraná state, Brazil (25°27'11"S, 48°52'57"W). The numbering refers to the site identities in Table S1.

Table S1. Abundance of dung beetles of five species collected from dung-baited pitfall traps. The numbering refers to the site identities from Fig. S1.

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