**A test for competition between rolling and tunnelling dung beetles in an African tropical forest**

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**Abstract**

We conducted a series of differential exclusion experiments to test for interactions between functional groups of dung beetles in Kibale Forest National Park, Uganda. Using fresh elephant dung as bait at 17 replicate sites, we found that the exclusion of either group (rollers or tunnellers) did not affect the volume of dung removed by the other. Combining the volumes of dung removed by both groups when either one was excluded was not different from the total volume removed from the control where both groups were included (mean ± SE = 0.435 ± 0.017 L per 48-h trial period). This indicates that the physical disturbance of a dung pile by one group had no negative effect on the other’s use of the shared resource. This finding is contrary to assumptions in the literature, which have not previously been tested with controlled experiments. For dung beetles in closed-canopy rainforests we suggest that detection of and access to fresh dung are stronger limitations on resource acquisition than competition. We recommend that dung beetle assemblages be monitored as indicators of biodiversity risk in tropical forests that are being fragmented and invaded by agro-ecosystems.

**Keywords:** *Coleoptera*, exclusion, Uganda, resource acquisition, mixed models

**Introduction**

Dung beetles are an emerging model group for studies in ecology and evolution (Simmons and Ridsdill-Smith 2011) due to the important ecological functions they perform, such as nutrient cycling, soil turnover, secondary seed dispersal and parasite control (Nichols *et al.* 2008). Dung beetles are assumed to forage under conditions of high competition as they share the same food source with hundreds, or even thousands, of beetles per dung pile, and with little potential for resource partitioning (Finn and Gittings 2003). Nevertheless, typical dung beetle assemblages are highly speciose (Hanksi and Cambefort 1991) with their composition being both repeatable and stable at the landscape scale (Davis *et al.* 2001; Slade *et al.* 2007). Within this taxonomic diversity are four broad functional groups defined by the different ways that they utilise dung (Hanski and Cambefort 1991): (1) paracropids, ‘tunnellers’, bury dung directly below the dung pile; (2) telecropids, ‘rollers’, roll balls of dung away before burying them; (3) endocropids, ‘dwellers’, live within the dung pile; (4) kleptoparasites steal dung collected by tunnelers and rollers. Each group includes species that exploit the same class of resources in a similar way, thus matching Root’s (1967) original definition of a guild, and some authors (e.g. (Hanski and Cambefort 1991) consider dung beetle functional groups to be comparable to guilds. However, the same resource is exploited across all dung beetle groups and so the arena for interspecific competition extends across all groups too, so the guild concept is unhelpful in this case (Hawkins and MacMahon 1989). We thus refer to them as functional groups rather than guilds and our aim is to investigate whether tunnellers and rollers compete in exploiting shared dung piles in a tropical forest.

Tunnelers and rollers are the most important functional groups of dung beetle in terms of volume of dung removed and competition between them is assumed to be high (Hanksi and Camberfort 1991; Finn and Gittings 2003; Simmons and Ridsdill-Smith 2011). Surprisingly, however, the experimental approach has seldom been employed on this topic (Giller and Doube 1989; Slade *et al.* 2007). We have found no previously published experimental studies of competitive interactions between tunnelling and rolling dung beetles, yet it is an interesting topic for biodiversity research because of the implications for secondary seed dispersal and nutrient cycling (Andresen and Feer 2005). Where tunnelers are competitively dominant then most seeds in the dung remain concentrated under the dung pile, but where rollers are dominant then secondary seed dispersal should be more effective. The two groups could interact through scramble competition for dung as well as interference competition if tunnelers contaminate the dung with soil before the rollers are able form balls and roll them away. We tested for competition by measuring the removal (by volume) of fresh dung at replicated sites at which we experimentally blocked dung removal by either tunnelers or rollers for comparison with each other and a control. Our expectation was that the volume of dung removed by either group would be greater when the other (competing) group was blocked.

**Methods**

Our study was conducted in Kibale Forest National Park in western Uganda, based at Makarere University Biological Field Station (0°33’39’’N 33°21’25’’E) during August 2015. Annual temperature there is fairly constant with a typical daily maximum of 27°C and minimum of 15°C at an elevation of 1400 m. The annual rainfall of ~1550 mm is bimodal with rainy seasons in March–May and August–December. The vegetation is mostly closed-canopy moist evergreen forest. The dung beetles of Kibale are well represented across functional groups but with apparently lower species richness than in some other African tropical forests (Nummelin and Hanski 1989).

Elephants in Kibale Forest occur in small and highly mobile herds consisting of savanna elephants, *Loxodonta africana*, and hybrids of savanna and forest elephants, *Loxodonta cyclotis*. Elephant dung was used as the resource for our experiments because it is well known to attract dung beetles of all types (Hanski and Cambefort 1991). Fresh dung is preferred (Krell-Westerwalbesloh et al. 2004) and so we collected fresh dung (warm, with a still-moist mucus layer) in the late afternoon when dung beetle activity is lowest, stored it in sealed plastic buckets overnight, and used it in our experiments the next morning. Depending on elephant movements the local availability of fresh dung varied and so we had to adjust the number of experimental piles set out each day.

Our experimental array consisted of 17 replicate sites located off alternating sides of forest trails, with 50-100 m between sites (Fig. 1). At each site we had three dung piles, each of 2 L in volume, placed ~5 m from one another. Dung was measured volumetrically for ease of measurement in the field; dung volume is less affected by moisture content than mass (Giller and Doube 1989) and 2 L was the typical volume of elephant dung piles we encountered in the field and thus our dung piles attempt to be ecologically relevant by mimicking natural piles. Our experimental dung piles were placed on cleared flat ground, away from trails to minimise disturbance and variation between samples (da Silva and Hernández 2014). Percentage canopy cover was recorded above every dung pile to account for variation in direct sunlight, which could affect the temperature and drying rate of the dung and therefore its attractiveness to dung beetles (Davis et al. 2001; Krell et al. 2003). At each site one dung pile was allocated to each of three treatments: a control being a dung pile placed directly on the soil with no further manipulation, allowing use by all dung beetles; a ‘plate’ treatment that blocked removal of dung by tunnelers with a plastic plate (40 cm diameter) placed between the dung and the soil; a ‘fence’ treatment that blocked removal of dung by rollers with a plastic mesh fence (30 cm high) erected around the dung pile (60 cm diameter, 0.5 x 0.5cm holes). These treatments allowed all dung beetles access to the dung pile but prevented dung removal by a blocked group, thereby permitting measurement of the volume removed by the unblocked group. The design was essentially that of a differential exclosure experiment in that we differentially excluded the effects of tunnellers and rollers although we did not exclude the beetles themselves.

Dung was left out in the experimental array for 48 h before being revisited, whereupon observations of dung beetle activity (presence/absence of burrows and dung balls) were noted and the volume of dung remaining was measured. The old dung was disposed of away from the study area and the experiment repeated in a new ‘round’ of fresh dung piles with different placement at each site. In total 96 dung piles were used in this study.

All statistics and data manipulation was performed in R version 3.2.2 (R core team 2015). A linear mixed model (LMM) was applied using the package ‘lme4’ (Bates *et al.* 2015) to analyse variation in volume lost across the control and two treatments. The model included both ‘site’ and ‘round’ as random factors in an attempt to control for the array of environmental variables that can affect dung beetle activity (Davis *et al.* 2001; Sánchez-de-Jésus 2015). Canopy cover was arcsine transformed and included along with its interaction with treatment. The model was simplified to find the best fitting model. To analyse pair-wise differences between treatments a post-hoc general linear hypothesis Tukey test was performed with the package ‘multcomp’ (Hothron *et al.* 2008).

To further test for an interaction between the two functional groups, the volumes of dung lost from plate and fence treatments at each site were summed. A second LMM then tested the difference in volume lost between the combined and control totals including both site and round as random factors. Plots were constructed using the package ggplot2 (Wickham 2009). P-values for mixed models were calculated via Wald-tests by the package ‘aod’ (Lesnoff and Lancelot. 2012). Least-squares means along with standard errors were calculated with the package ‘lsmeans’ (Lenth 2016)

**Results**

Dung beetles were present in all 96 dung piles but the action of each target functional group was blocked in the exclusion treatments as inferred by the presence of abandoned balls of dung around the edge of the fence treatment and a collection of tunnellers trapped at the bottom of the plate treatment. Some of the dung piles in the plate treatment were disturbed by birds such as francolins (*Pternistis squamatus*), which do not eat the dung but the seeds within it.

The volume of dung lost over 48 hours varied across the control and treatments (F2,73 = 89.7, p < 0.001; Fig. 2). The post-hoc Tukey test showed this was due to the greater volume (p < 0.001) lost in the control (mean ± SE = 0.435 ± 0.017 L) over both the fence and plate treatments respectively (fence, 0.222 ± 0.017 L; plate, 0.235 ± 0.017 L), which did not differ (p = 0.743). There was no effect of canopy cover on volume of dung lost (F1,72= 0.01, p = 0.92) or any interaction between treatment and canopy cover (F1,70 = 0.006, p = 0.94). Site and round accounted for 4.4% and 9.4% respectively of the total residual variance.

The second model, testing the difference in volume lost between the combined and control volumes showed that there was no difference between the volume of dung lost in the control compared with the other treatments combined (F1,39 = 0.16, p = 0.69; Fig. 2). Site accounted for 5.6% and round 2.3% of the residual variance in the model. Three outliers were removed due to disturbance by birds (francolins) which scattered the dung in search of seeds.

**Discussion**

Our highly replicated experiment, involving almost a hundred dung piles, found no evidence of competition between the two main functional groups of dung beetles. When dung removal by one group was blocked we expected that would relieve competitive pressure upon the other group, as has previously been found for slow- and fast-tunneling dung beetles in cattle dung (Giller and Doube 1989). In both fence and plate treatments we expected the volume of dung removed to be similar to that removed in the control, but we found both to be about half the amount removed in the control (Fig. 2). If competition had been in effect then dung removed by one group might not have fully compensated for that lost to the other, but it should still have responded to the blockage of the competitor so that the combined volume of dung lost from the plate and fence treatments should have been more than that lost from the control. But that did not occur in our experiments, which is at odds with predictions from the literature (Simmons and Ridsdill-Smith 2011).

Most studies of dung beetle community ecology are conducted in open habitats such as savannas and other rangeland ecosystems (for examples see Hanski and Cambefort 1991) where the dung deposited by herds of cattle and wild grazing ungulates is rapidly detectable and accessible to dung beetles. In African tropical forests, however, where grazing herds are absent and the only bulk-providers of dung are elephants, the foraging environment for dung beetles is very different. Our study aimed to fill an important knowledge gap of dung beetle functional group interactions in a previously untested system. Here, we suggest the detection of and access to fresh dung is a stronger constraint than competition within the dung beetle assemblage. Elephants in tropical forests are highly mobile and so spatiotemporal variability in the occurrence of fresh dung is higher than in open habitats where grazing herds congregate near surface water. Also, the abilities of dung beetles to detect and follow olfactory cues through dense forest vegetation, where odour plumes and flight paths are impeded, must be compromised in relation to open habitats. Only 22% of the dung volume in our controls was removed by dung beetles in 48 hours, which contrasts with the often complete removal of fresh dung often exhibited in tropical savannas to the extent that other insects (even flies) can be excluded from the resource (Cambefort 1991).

In heed of calls for experimental research on competition in dung beetle assemblages (Hanski and Cambefort 1991; Finn and Gittings 2003) we offer our study as evidence that the strength of competition in structuring such assemblages should be expected to vary considerably across ecosystem types. We also suggest that facilitation could be an important interaction if the physical action of the large rollers, with their powerful appendages, facilitates access by tunnellers to compact dung balls. We were unable to test for facilitation in our experiment, which was designed to differentially block dung removal, not access, by each functional group.

The implication of our study for biodiversity conservation is that the ubiquity of dung beetles, and the reliability of the ecological services they provide, should not be over-estimated for tropical forests. In particular, secondary seed dispersal by dung beetles has an important effect on tree regeneration in tropical forests (Andresen and Feer 2005). However, as we found to the detriment of some of our experimental replicates, seeds in elephant dung are vulnerable to various seed predators such as birds (e.g. francolins and Guinea fowl, *Guttera pucherani*) feeding on the forest floor. The fate of such seeds is thus dependent on a race between secondary dispersers and predators, and if the local dung beetle assemblage is impacted by anthropogenic factors then the regeneration of forest tree populations could ultimately be affected. Conversion of tropical forests to agro-ecosystems is a global phenomenon and is very evident around Kibale Forest, where clearing for cropland and grazing pastures has created a ‘hard edge’ along the park boundary. In such agro-ecosystems it is increasing common for livestock to be treated with pesticidal compounds for controlling ectoparasites and endoparasites, but the residues voided in dung are toxic to dung beetles in both adult and larval stages (Davis et al. 2004). Our study thus endorses the importance of monitoring the dung beetle assemblage, as an early-warning indicator of biodiversity risk, in tropical forests that are becoming fragmented and invaded by agro-ecosystems.

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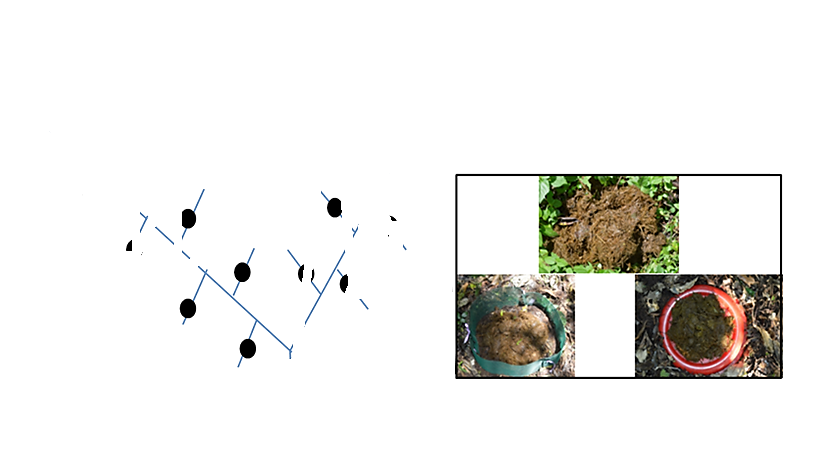
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**Fig. 1** – Diagram of the layout of the sites. The Y-shaped lines represent major forest paths paths while the shorter lines perpendicular to these are smaller trails cut by guides which are over grown with vegetation. The black dots represent the location of the sites used. Those on the left hand side of the Y were set up on one day and sites on the right the next before returning to the left side after 48 hours. This allowed each group of sites to be left undisturbed for the length of the experiment while maximising the number of sites that could be used in this area of the forest. Diagram is not to scale and the minimum distance between sites was. The inset shows the three experimental treatments, from top clock-wise: control, plate, fence. These were replicated as a trio at each site. Pictures by B. Makori.

**Fig. 2** – Volume of dung removed (Litres) over 48-hours from each treatment (control, fence and plate) plus the combined value of fence and plate treatments. The middle line on each box corresponds to the median value of the treatment while the outer lines of the box represent the 25 and 75% quartiles. Black dots represent outliers which fall out with the expected distribution maximum and minimum values of the data. The plot shows that the two exclusion treatments resulted in less dung being removed from the pile over 48-hours compared to the control (F2,73 = 89.7, p < 0.001). The combined value shows that a similar amount of dung was removed as in the control ((F1,39 = 0.16, p = 0.69).

**Fig. 1**



**Fig. 2**

