Staining Method for Assessing the Ecological Function of Excrement Removal by Dung Beetles (Coleoptera: Scarabaeidae: Scarabaeinae)

Author(s): Moacyr Batilani-Filho and Malva I. M. Hernández
Source: The Coleopterists Bulletin, 70(4):880-884.
Published By: The Coleopterists Society
DOI: http://dx.doi.org/10.1649/0010-065X-70.4.880
URL: http://www.bioone.org/doi/full/10.1649/0010-065X-70.4.880

The Coleopterists Bulletin, 70(4): 880–884. 2016.

SCIENTIFIC NOTE

Staining Method for Assessing the Ecological Function of Excrement Removal by Dung Beetles (Coleoptera: Scarabaeidae: Scarabaeinae)

Moacyr Batilani-Filho and Malva I. M. Hernández
Department of Ecology and Zoology, Federal University of Santa Catarina
Florianópolis, SC, 88040-900, BRAZIL
malva.medina@ufsc.br

DOI.org/10.1649/0010-065X-70.4.880

Dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) perform several ecological functions that can be interpreted as environmental services, since they provide direct benefits to humans (Nichols *et al.* 2008). These benefits are associated with excrement and carcass removal, since these items are their primary food source, and this behavior has positive implications for matter decomposition and nutrient cycles (Yamada *et al*. 2007; Hanafy 2012), secondary seed dispersal (Vulinec 2002; Andresen 2003; Slade *et al.* 2007), soil aeration, and biological control of flies (Braga *et al.* 2012, 2013). Since these functions are related to dung beetle feeding habits, quantifying excrement removal has been a common approach in studies of ecological functions (*e.g.*, Klein 1989; Horgan 2001; Slade *et al.* 2007; Amézquita and Favila 2010; Braga *et al.* 2013). However, once dung beetles are classified into three functional guilds according to resource allocation for feeding or nidification, these guilds have a differentiated capacity to carry out these ecological functions. Among these three guilds, the paracoprids (tunnelers) are comprised of the beetles that allocate resources into subterranean galleries below the resource supply. The telecoprids (rollers) allocate resources into balls that will be used for nidification or feeding and roll these balls a certain distance from the source for subsequent burial. Lastly, the endocoprids (residents) consume food and lay eggs directly at the source (Halffter and Edmonds 1982). Of these guilds, the paracoprids and telecoprids are more effective at removing excrement, even if this removal will not always relate positively with other ecosystem functions (Braga *et al.* 2013).

To understand the dynamics of the dung beetles' ecological function, it is necessary to quantify resource partitioning among the functional guilds. However, this level of detail is difficult to obtain since the material relocated into galleries made by paracoprids loses its original color and has a low contrast with the dark ground. The use of cow excrement, green in color, as is done in some removal studies (*e.g.*, Klein 1989; Horgan 2001; Yamada *et al.* 2007; Amézquita and Favila 2010), provides greater contrast between soil and excrement; however, this resource is recommended for pasture environments, since dung beetles prefer omnivorous animal excrement in forest areas (Filgueiras *et al.* 2009, Bogoni and Hernández 2014). In the Atlantic Forest fragments of southern Brazil, the crab-eater fox, *Cerdocyon thous* (Linnaeus, 1766), is a common native omnivore (Graipel *et al.* 2001; Cherem *et al.* 2004) and tolerant of disturbed habitats (Dotta and Verdade, 2011). The excrement from *C. thous* is a highly attractive resource for dung beetles in the Atlantic Forest (Bogoni and Hernández 2014), but acquiring this material for experimental reasons to evaluate removal is not very feasible. A viable alternative is to use excrement from domestic dogs (*Canis lupus familiaris* Linnaeus, 1758) raised in animal facilities (bioterium) (Batilani-Filho *et al.* 2014). This type of resource has the advantage of being safe in regards to the spread of pathogens in the natural environment. To increase the contrast of omnivore excrement with the soil to allow faster quantification of the resource buried by the paracoprids separate from that used by the telecoprids, we present here a removal evaluation technique that involves prior staining of these excrements. For this technique, we used domestic dog excrement collected at the Central Animal Laboratory of the Federal University of Santa Catarina.

The staining technique consisted of mixing edible, odorless liquid blue stain to domestic dog excrement (40 ml/kg). This stain is commonly used in cakes and has a neutral ethyl alcohol base mixed with organic colorants. The color blue was chosen instead of purple, yellow, or red because it provides a greater contrast with the ground after mixing, displaying a green color. We tested the effect of the stain on excrement attractiveness and manipulation by dung beetles in September 2013, in an area of Atlantic Forest in southern Brazil

(Environmental Conservation Unit Desterro, Florianópolis, Santa Catarina). We used ten pairs of collection and assessment arenas, which were installed and exposed for 48 hours (Fig. 1). Each arena consisted of a buried 1.5-L plastic container filled with sieved soil (maintaining a 5 cm height on the margins so that the relocated material and telecoprids remained in the arenas) and protected from rain with a suspended plastic cover. A spacing of 5 m was maintained between pairs of arenas and 30 m between the ten pairs. In each arena pair, one arena was baited with 50 g of unstained excrement and the other with 50 g of blue-stained excrement. For each treatment, four control arenas were installed, of which the value of excrement dissection was calculated, and subsequently this value was subtracted from the excrement removal data. The control arena differed from others in having its opening sealed with voile fabric, preventing the entry of insects.

Upon 48 hours in the field, arenas were inspected, and the insects that remained active on the surface of the area around the arenas were noted as showing various behaviors, such as feeding, rolling food balls, underground gallery construction, and fighting. The beetles on the ground were collected, and

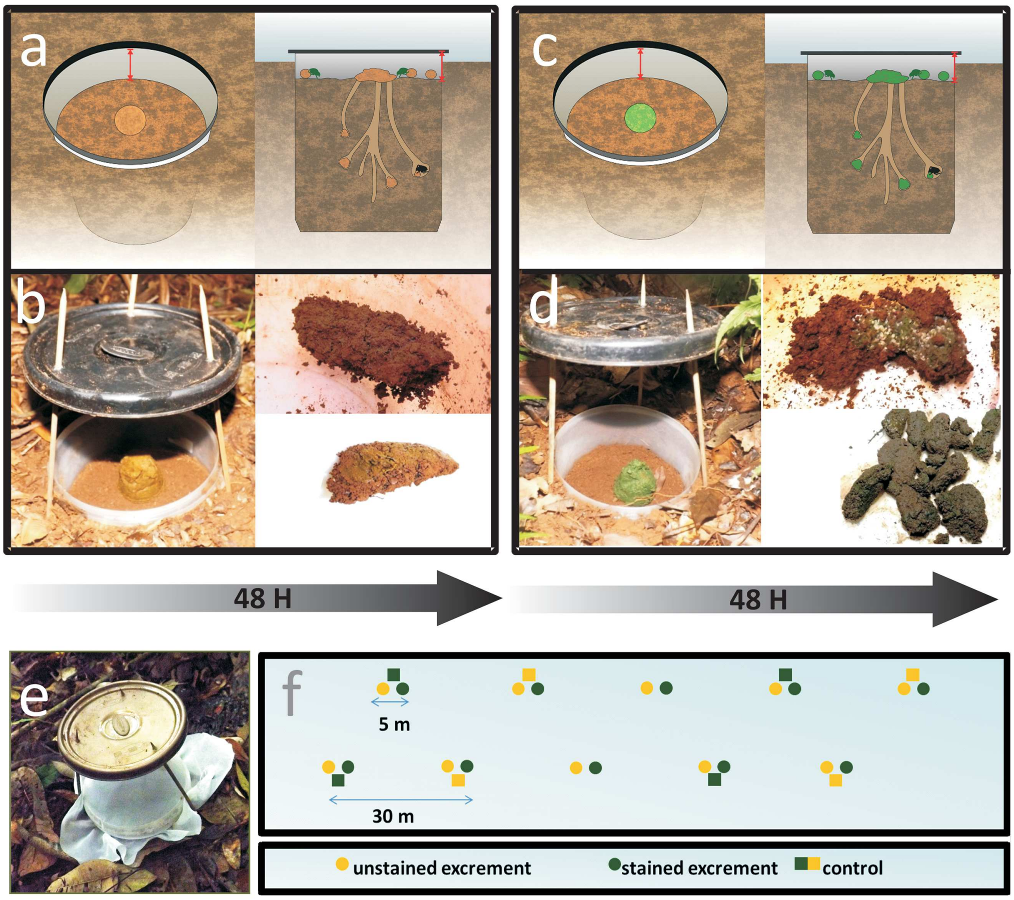

Fig. 1. a) Illustration of the arena used for collecting dung beetles and assessing unstained excrement removal with 5 cm of height on the margin (double-pointed arrow) and demonstration of the possible unstained excrement removed during the 48 hours of assessment (telecoprids on the surface and/or paracoprids in subterranean galleries), b) Photographs of the arena with a plastic cover protection and unstained material removed by paracoprids, c) Illustration of the arena used for collecting dung beetles and assessing stained excrement removal with 5 cm of height on the margin (double-pointed arrow) and demonstration of the possible stained excrement removed during the 48 hours of assessment (telecoprids on the surface and/or paracoprids in subterranean galleries), d) Photographs of the arena with a plastic cover protection and stained material removed by paracoprids, e) Photograph of control arena; f) Sample design to evaluate the effect of the stain on excrement attractiveness and manipulation by dung beetles.

the ground surface material was separated into non-removed resource (that stayed within the arena) and resource allocated by telecoprids, which were the rolled food balls made by this guild, but were not buried. After this procedure, the arenas were capped and taken to the laboratory for screening and measurements, and differentiation of removed excrement was conducted. During this process, a brush and a spoon were used to carefully remove 1 cm of soil at a time in order to find the material buried by paracoprids and possible balls buried by telecoprids, as well as beetles that were buried. For arenas with unstained excrement, the soil was removed with a spoon when it presented a pasty consistency; during the screening process, this was observed under a magnifying glass due to the low contrast with the soil, in order to isolate excrement using small brushes and forceps. Triage in arenas with stained excrement followed the same protocol. In both cases, when the material appeared to form a spherical shape, the allocation was attributed to telecoprids, and material with an amorphic shape was attributed to paracoprids (Fig. 1).

Species richness, beetle abundance, and amount of excrement removed (g) were analyzed with t-tests to compare treatment means. Community structure similarity was assessed using a Bray-Curtis distance in an ANOSIM similarity analysis (Clarke 1993; Warton *et al.* 2012).

The arenas with unstained excrement captured 100 dung beetles representing ten species, and the arenas with stained excrement captured 142 individuals of nine species. No differences in species richness [t = 1.6; p = 0.5], abundance [t = 0.9; p = 0.3], or community structure attracted by the different treatments [ANOSIM R = 0.05; p = 0.7] were detected. The mean removal of 33.1 g of unstained excrement did not differ from the mean removal of 33.3 g of stained excrement [t = 0.05; p = 0.9] (Fig. 2). The use of the stain had no effect on the removal of material depending on the functional guild. On average, 21.7 g of unstained excrement were buried by the paracoprids, which did not differ significantly from the average of 15.1 g of removed stained excrement [t = 1.08; p = 0.3] (Fig. 2). The telecoprids relocated an average of 6.8 g of unstained excrement, which also did not differ from an average of 12.4 g of relocated stained excrement [t = 1.72; p = 0.1] (Fig. 2).

Our results contribute to dung removal studies by demonstrating that using stains facilitates visualization of removed and accelerates reocated excrement quantification. Both the triage and identification of stained excrement allocation was faster and more effective than unstained excrement, where the use of brushes, magnifying glasses, and forceps were necessary to separate the excrement from the soil. Moreover, when the buried material was found, it was easier to isolate stained excrement from the soil to measure the excrement's true weight due to its elevated contrast, therefore, the proportion of stain used (40 ml/kg) was considered effective.

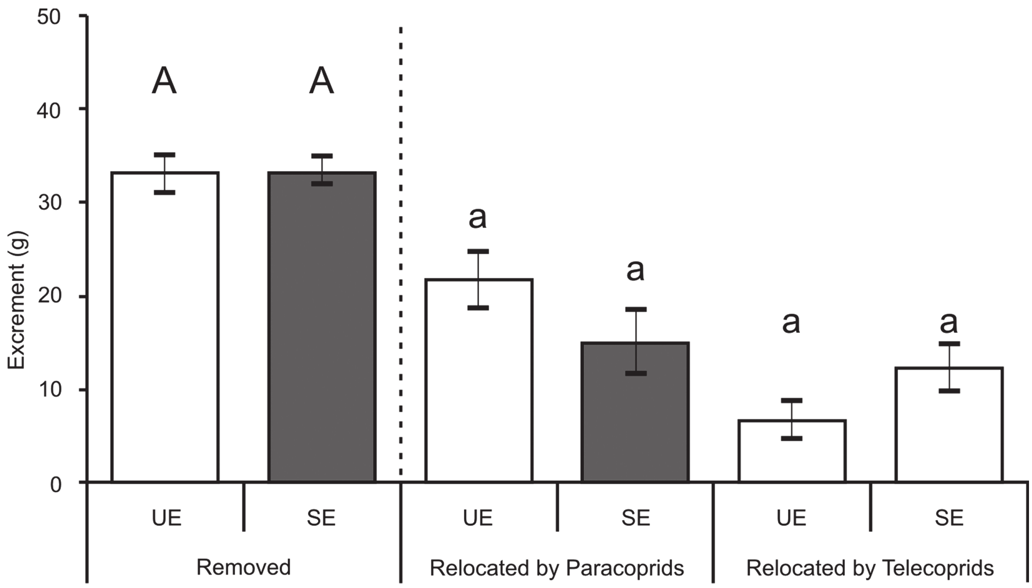

Fig. 2. Mean (± SD) amount of excrement removed and amount relocated between treatments according to two functional guilds. The same letters indicate no significant differences (p > 0.05) between treatments within a variable. UE = unstained excrement; SE = stained excrement.

In using this technique, it is possible to differentiate and quantify the removed resource by functional guild, whether it is paracoprid or telecoprid. This approach is conducted without having to manipulate the density or restrict organism access to exclusively assess its participation, as was done in similar studies (Horgan 2001; Yamada *et al.* 2007; Slade *et al.* 2007; Dangles *et al.* 2012). This is a more realistic approach, since paracoprid and telecoprid species compete for resources at the same time. Another advantage of using this staining technique is that it is possible to calculate the resource quantity that dung beetles are actually using, since the difference between what is removed by the community and the sum of the allocation refers to the resource quantity that was immediately consumed or fractioned by beetles.

In using this methodology, it is possible to simultaneously acquire data for community and ecological function analysis during the same period and in the same arena. In regards to the type of resource used, due to the high attractiveness of omnivore excrement, the use of domestic dog excrement in removal studies in forest areas is a good alternative, since there is no difference in removal when compared to native omnivore excrement (Batilani-Filho *et al.* 2014). Additionally, this resource is easy to acquire, and since they are from a laboratory animal facility, their use is safer in regards to the presence of pathogens, allowing handling and staining.

Lastly, excrement staining, with quantitative detailing by functional guild, may be used in studies that investigate other ecological functions associated with removal, such as secondary seed dispersal (Vulinec 2002; Andresen 2001; Slade et al., 2007), soil aeration, and biological control of flies (Braga *et al.* 2012, 2013). The staining method can be used with any type of excrement according to the research objective.

Acknowledgments

We appreciate the contributions made by Dr. Mario E. Favila, Dr. Pedro G. da Silva, Msc. Renata C. Campos, and Msc. Juliano A. Bogoni in the development of the study, and we appreciate manuscript suggestions made by Dr. Rodrigo F. Braga. We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the MSc scholarship granted to the first author and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financing the project "*Population dynamics and behavioral ecology of decomposer beetles from the Scarabaeinae subfamily: the importance of ecosystem functioning in Atlantic Forest fragments*" (479203/2010-5) and granting a research productivity grant to the second author for the project "*Dung beetle behavior and its role in organic matter decomposition cycle*" (303800/2010-0).

References Cited

Amézquita, S., and M. E. Favila. 2010. Removal rates of native and exotic dung by dung beetles (Scarabaeidae: Scarabaeinae) in a fragmented tropical rain forest. Environmental Entomology 39: 328–36.

Andresen, E. 2001. Effects of dung presence, dung amount and secondary dispersal by dung beetles on the fate of *Micropholis guyanensis* (Sapotaceae) seeds in Central Amazonia. Journal of Tropical Ecology 17: 61–78.

Andresen, E. 2003. Effect of forest fragmentation on dung beetle communities and functional consequences for plant regeneration. Ecography 26: 87–97.

Batilani-Filho, M., M. Batista, B. Romeu, E. Bécker, M. I. M. Hernández, and M. E. Favila. 2014. Eficiência de escarabeíneos tuneleiros e roladores na remoção de fezes de mamíferos em Floresta Ombrófila Densa [pp.105-113]. *In*: Ecologia de Campo: Abordagens no Mar, na Terra e em Águas Continentais (M. Batilani-Filho, L. Zanette-Silva, B. Segal, and M. I. M. Hernández, editors). UFSC, Florianópolis, SC, Brazil.

Bogoni, J. A., and M. I. M. Hernández. 2014. Attractiveness of native mammal's feces of different trophic guilds to dung beetles (Coleoptera: Scarabaeinae). Journal of Insect Science 14(1): 299 (dx.doi.org/10.1093/jisesa/ieu161).

Braga, R. F., V. Korasaki, E. Andresen, and J. Louzada. 2013. Dung beetle community and functions along a habitat-disturbance gradient in the Amazon: a rapid assessment of ecological functions associated to biodiversity. PLoS One 8: e57786.

Braga, R. F., V. Korasaki, L. D. Audino, and J. Louzada. 2012. Are dung beetles driving dung-fly abundance in traditional agricultural areas in the Amazon? Ecosystems 15*:* 1173–1181.

Cherem, J. J., P. C. Simões-Lopes, S. Althoff, and M. E. Graipel. 2004. Lista dos mamíferos do estado de Santa Catarina, sul do Brasil. Mastozoología Neotropical 11: 151–184.

Clarke, K. R. 1993. Non-parametric multivariate analysis of changes in community structure. Australian Journal of Ecology 18: 117–143.

Dangles, O., C. Carpio, and G. Woodward. 2012. Size-dependent species removal impairs ecosystem functioning in a large-scale tropical field experiment. Ecology 93(12): 2615–2625.

Dotta, G., and L. M. Verdade. 2011. Medium to large-sized mammals in agricultural landscapes of south-eastern Brazil. Mammalia 75: 345–352.

Filgueiras, B. K. C., C. N. Liberal, C. D. M. Aguiar, M. I. M. Hernández, and L. Iannuzzi. 2009. Attractivity of omnivore, carnivore and herbivore mammalian dung to Scarabaeinae (Coleoptera, Scarabaeidae) in a tropical Atlantic rainforest remnant. Revista Brasileira de Entomologia 53: 42–427.

Graipel, M. E., J. J. Cherem, and A. Ximenez. 2001. Mamíferos terrestres não voadores da Ilha de Santa Catarina, sul do Brasil. Biotemas 14: 109–140.

Halffter, G., and W. D. Edmonds. 1982. The Nesting Behavior of Dung Beetles (Scarabaeinae): An Ecological and Evolutive Approach. Instituto de Ecología, Mexico.

Hanafy, H. E. M. 2012. Effect of dung beetles, *Scarabaeus sacer* (Scarabaeidae: Scarabaeinae) on certain biochemical contents of leaves and fruits of tomato and squash plants. Journal of Applied Sciences Research 8(10): 4927–4936.

Horgan, F. G. 2001. Burial of bovine dung by coprophagous beetles (Coleoptera: Scarabaeidae) from horse and cow grazing sites in El Salvador. European Journal of Soil Biology 37: 103–111.

Klein, B. C. 1989. Effects of forest fragmentation on dung and carrion beetle communities in Central Amazonia. Ecology 70: 1715–1725.

Nichols, E., S. Spector, J. Louzada, S. Amézquita, and M. E. Favila. 2008. Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. Biological Conservation 141: 1461–1474.

Slade, E. M., D. J. Mann, J. F. Villanueva, and O. T. Lewis. 2007. Experimental evidence for the effects of dung beetle functional group richness and composition on ecosystem function in a tropical forest. Journal of Animal Ecology 76: 1094–104.

Vulinec, K. 2002. Dung beetle communities and seed dispersal in primary forest and disturbed land in Amazonia dung beetle communities and seed dispersal in primary forest. Biotropica 34: 297–309.

Warton, D. I., T. W. Wright, and Y. Wang. 2012. Distance-based multivariate analyses confound location and dispersion effects. Methods in Ecology and Evolution 3: 89–101.

Yamada, D., O. Imura, K. Shi, and T. Shibuya. 2007. Effect of tunneler dung beetles on cattle dung decomposition, soil nutrients and herbage growth. Grassland Science 53*:* 121–129.

(Received 15 October 2015; accepted 3 July 2016. Publication date 18 December 2016.)