Marking Dung Beetles (Coleoptera: Scarabaeidae): Retrospective and a Reliable "Scarification" Method

Authors: Wuerges, Mariah, and Hernández, Malva I. M.

Source: The Coleopterists Bulletin, 74(1) : 168-171

Published By: The Coleopterists Society

URL: https://doi.org/10.1649/0010-065X-74.1.168

The Coleopterists Bulletin, 74(1): 168–171. 2020.

SCIENTIFIC NOTE

Marking Dung Beetles (Coleoptera: Scarabaeidae): Retrospective and a Reliable "Scarification" Method

Mariah Wuerges and Malva I. M. Hernández
Department of Ecology and Zoology, Federal University of Santa Catarina
Florianópolis, SC 88040-900, BRAZIL
mariahwuerges@gmail.com

DOI.org/10.1649/0010-065X-74.1.168

Scarabaeinae (Coleoptera: Scarabaeidae), with over 6,200 species described (Tarasov and Génier 2015), are widely studied beetles often used as ecological indicators in diversity studies (Halffter and Favila 1993). They also play important ecological roles in nutrient cycling, soil aeration, secondary seed dispersal, and control of parasites (Nichols *et al.* 2008). However, some aspects of the basic biology of these species are poorly investigated, such as life span, dispersal capacity, and other population parameters (Cultid-Medina *et al.* 2015; Hanski and Cambefort 1991; Villada-Bedoya and Cultid-Medina 2017). One way to access some of this information is through mark, release, and recapture studies (MRR) via permanent marks with the least possible effect on individuals' behavior and survival (Martínez-Quintero *et al.* 2013).

An MRR study with dung beetles was carried out by Peck and Forsyth (1982), which aimed to estimate population size for some species by marking them with enamel paint spots on the pronotum and elytra, with a five color-code. Following just one recapture event, with a recapture rate of 0.02%, they described distances covered by some species. Favila (1988) presented an individual marking method for laboratory and field studies, using paint and glue on the dorsum to protect the marking from soil friction, highlighting the pronotum as the best part of the beetle's body to mark. Using mark-release methods in fieldwork, Escobar and Chacón de Ulloa (2000) described the use of different habitats for some species, marking individuals of species greater than 10 mm by using different colors of paint. Their results provided information about movement patterns and distances covered by *Sulcophanaeus velutinus* (Murray, 1856) and *Dichotomius* cf. *quinquedens* (Felsche, 1910). Roslin (2000) provided results about distances covered for species of Aphodiinae by using a mark that consisted of two small holes in different positions in the elytra, using the tip of the thinnest available insect pin, which could only be seen under a microscope. Larsen and Forsyth (2005) performed a mark-release study with *Canthon acutus* Harold, 1868, a small diurnal ball-rolling beetle (body width = 4.1 mm), by using a silver pen. Each group was identified with a unique combination of dots on the elytra and pronotum and released once, performing recaptures every 24 hours for four days, which allowed them to propose a spatial design to avoid pseudoreplicates in ecological studies of biodiversity. To understand the effects of landscape structure on patterns of abundance and movement and the differences between male and female movement, Arellano *et al.* (2008) painted individuals of *Canthon cyanellus cyanellus* LeConte, 1859, *Phanaeus pyrois* Bates, 1887, and *Dichotomius amplicollis* (Harold, 1869) by using silver pens to make a unique combination of dots on the elytra and/or pronotum. Based on Favila (2008), each mark was covered with a fine layer of glue with cyanocrilate. Noriega and Acosta (2011) did a mark-release experiment for over a month with *Sulcophanaeus leander* (Waterhouse, 1891) by marking the ventral surface of the metasternum of this large bodied species with a fast-drying latex paint. The same mark was used for all individuals. They observed high fidelity to the habitat and dispersal between different environments and answered questions such as daily activity and sex ratio.

Recently, Martínez-Quintero *et al.* (2013) proposed a novel method for marking dung beetles, which consisted of a permanent and individual scarification mark. They used a Mototol Stylus 1100 Dremel® to scrape the dorsal surfaces of the elytra and pronotum, in companion with an emerald 84922 Dremel® stone that allowed a clean brand on the beetle's body. This mark was applied on individuals from 10 to 35 mm long because it was difficult to perform on individuals smaller than 10 mm, although they reported that they have been able to mark individuals as small as 6 mm as well. They reported no ecological, biomechanical, or reproductive implications on individuals, but, like previous studies, they did not test for such.

Cultid-Medina *et al.* (2015), based on the same method, carried out a population study of two dung beetle species, *Oxysternon conspicillatum* (Weber, 1801) and *Dichotomius* cf. *alyattes* (Harold, 1880), which were chosen for being abundant, large species (10 to 35 mm body length) with different habitat preferences. They made a general marking on the elytra and pronotum, which allowed them to identify sex, site, and type of vegetation in which individuals were captured. They proposed that their data were valid based on the consequence of the permanent mark not adversely affecting the beetles. Using a similar permanent marking technique, Silva and Hernández (2015) redesigned the trap spacing protocol proposed by Larsen and Forsyth (2005). They used the broken tip of a needle to scarify the cuticle of the pronotum and elytra and used a dot scheme resulting in a unique mark. Villada-Bedoya and Cultid-Medina (2017) also used the scarification method of Martínez-Quintero *et al.* (2013) and carried out a population study for seven months with *Dichotomius* cf. *satanas* (Harold, 1867) and *D.* cf. *alyattes*, obtaining a recapture rate of 0.04%.

Our goal was to test if the scarification technique affects any component of individual fitness, by assessing survival, behavior, and reproduction of marked and unmarked dung beetles. In addition, we assessed if markings could be applied individually and permanently on species of various sizes, and thus whether this technique can be used in laboratory experiments and in the field without harming these insects.

We conducted a laboratory experiment with 26 pairs of *Canthon rutilans cyanescens* Harold, 1868, a Neotropical dung beetle about 4 mm in length. Thirteen pairs marked pairs and 13 unmarked pairs were tested to determine if markings affect survival, behavior, and reproduction. The marking was performed with a dental micromotor (Beltec LB100) with a 0.5 mm spherical drill and the aid of a magnifying glass or a stereoscope. We scraped the elytra of the beetles by drawing a scheme of points and lines in a numbering system used to provide a unique identifier to each individual (Fig. 1). Each point represented one unit, and each line corresponded to five units. The right elytron corresponded to units, the left elytron to tens, the right half of the pronotum to hundreds, and the left half of the pronotum to thousands (Fig. 2). For example, the identifier 8 (Fig. 1A) resulted from one line and three points in the right elytra. The identifier 13 (Fig. 1B) is created by a point in the left elytron and three points in the right elytron.

The beetles were collected with baited pitfall traps in September 2017 at the Serra do Tabuleiro State Park (27°43′S, 48°48′W, 300 m elevation), an area of Atlantic rainforest located in southern Brazil, and taken to the Laboratory of Terrestrial Animal Ecology at the Federal University of Santa Catarina. To avoid weight and age as confounding effects in the experiment, we formed pairs of similar weight and age and distributed them between two

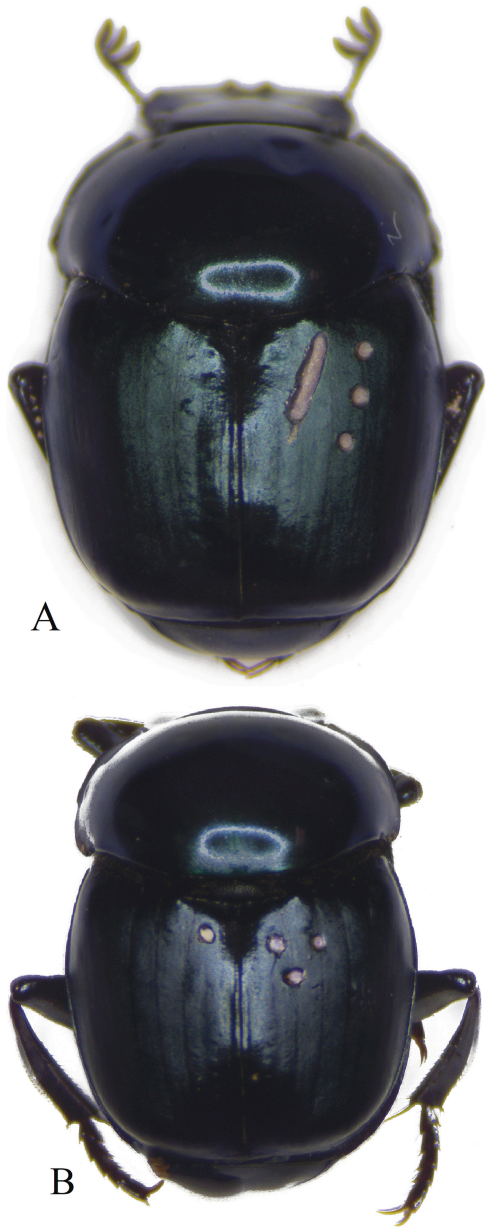

Fig. 1. Scarification marks on the elytra of *Canthon rutilans cyanescens*. A) Marks on the right elytron corresponding to the number 8, in which was made one line (value = 5) and three dots (value = 3, one dot = 1), B) Marks for number 13, in which a point was made in the left elytron (value = 10) and three points in the right elytron (value = 3).

Fig. 2. Marks on a specimen of *Canthon rutilans rutilans*, corresponding to the number 1428, whereas 1 point at the left side of the pronotum (= 1,000), 4 points on the right side of the pronotum (= 400, 1 point = 100), 2 points on the left elytron (= 20, 1 point = 10), and 1 line (= 5) plus 3 points (= 3) on the right elytron.

treatments, systematically allocating similar pairs of different ages and sizes between the treatments. Age categorization was performed according to the opacity of the elytra and the wear of the tarsal spur of the anterior tibia, thereby classifying individuals as new, mature, and old. Very old or damaged individuals were not used. All 26 pairs were placed in pots (8 × 11 × 6 cm) with 400 g of soil from the collection site; before use, the soil was heated in a microwave for five minutes to avoid contamination, especially by mites. The beetles were fed weekly with 10 g of feces from domestic dogs bred with animal feed at the Laboratory Animal House of the Federal University of Santa Catarina. The experiment started in November 2017 and lasted until the beginning of March 2018, completing four months. During this period the beetles were kept in the laboratory under ambient temperature and controlled luminosity (12 hours light and 12 hours dark). Deaths and births were counted weekly, and the number of nest balls produced by the pairs was counted once in December 2017 and once in January 2018. Statistical analysis included t test and ANOVA for normal data and GLM with Poisson distribution for counting data, all performed with R software.

Scarified markings did not affect the survival of the individuals under laboratory conditions. No significant difference was detected between sexes (t = 0.51, df = 48.63, p = 0.61), as males lived on average 62.0 days and females lived on average 67.8 days. Nor was there a significant difference between treatments (t = 0.33, df = 50, p = 0.74), with marked individuals living a mean of 66.8 days and unmarked individuals a mean of 63.1 days. Moreover, the mark did not affect behavior, as measured by the production of nesting balls. Marked pairs produced a mean of 2.3 nest balls, and unmarked ones produced on average 1.6 nest balls, with no difference between treatments (t = 1.25, df = 34.98, p = 0.22). Scarification also did not affect the reproduction of marked pairs, since the number of offspring per pair was statistically the same in both treatments (z = 1.48, df = 11, p = 0.77). The total number of offspring was 10 females and six males for all marked pairs, and four females and three males for all unmarked pairs. Furthermore, larval developmental times were not significantly different between treatments (F = 0.55, df = 1, p = 0.46), with an average of 86.6 days and 78.7 days for larvae from marked and unmarked parents, respectively. The fertility rates (calculated as the ratio between the number of developed offspring per pair divided by the maximum number of nest balls produced by that pair) for the marked and unmarked pairs were 0.53 and 0.54, respectively, with no significant difference detected (F = 0.001, df = 1, p = 0.97).

MRR experiments with insects are quite challenging tasks, yet they are improving with technological advances, although still requiring creativity and dexterity of the researcher. Beyond the difficulties of application, mainly because of the great abundance and small size of these animals, an ideal mark must be durable, cheap, non-toxic, and easy to apply and observe (Hagler and Jackson 2001). Paintings and tattoos have been a common technique for dung beetles (Arellano *et al.* 2008; Escobar and Chacón de Ulloa 2000; Favila 1988; Larsen and Forsyth 2005; Noriega and Acosta 2011; Peck and Forsyth 1982). Markings of this type are mainly used on large insects because of the logistical difficulties in applying the mark on small individuals (Hagler and Jackson 2001), although some studies managed to apply it on small dung beetles (<10 mm) (Arellano *et al.* 2008; Larsen and Forsyth 2005). Moreover, the use of ink makes it possible to recapture marked dung beetles only over short periods, since the markings have a limited duration due to friction with the soil during the beetle's burial behavior (Martínez-Quintero 2013). Although Favila (1988) and Arellano *et al.* (2008) applied a drop of glue to fix the paint, this technique is difficult to perform because of the waxy epicuticle on the beetle's integument and the small size of some species. Roslin (2000) was able to mark small individuals with elytral perforations visible only with a dissecting scope. Although it was a permanent mark, perforations in the elytra can expose individuals to parasites (Martínez-Quintero 2013) and solar radiation, and the needs to be tested regarding individual fitness.

Scarification marks, more recently performed, are durable, cheap, non-toxic, easy to identify, and do not create perforations. This method has been mostly applied on large individuals (>10 mm length) (Cultid-Medina 2015; Martínez-Quintero 2013; Villada-Bedoya and Cultid 2017), and more rarely applied to small individuals (<10 mm length) (Silva and Hernández 2015), although it was not tested for potential negative effects it might have on beetle fitness. We have shown that labeling with a "scarification mark" has no adverse influence on dung beetle survival, behavior, and fertility. The marks are permanent and easy to see with the naked eye, and the method can be applied to species of different sizes and shapes. Furthermore, a marking machine can be added to a small mobile battery and used in the field. Therefore, we propose that scarification can be a reliable method for field and laboratory MRR studies.

Acknowledgments

We appreciate the contributions made by Eloisa Alves, Maristela Carpintero, and Luiz Fernando Ferreira in the development of the laboratory experiment, and we appreciate the initial suggestion by Dr. Jorge Lobo of the method involved in this work. We thank the other members of the Laboratory of Terrestrial Animal Ecology for help and support and the biologist Fernando Brüggemann for providing access to the collection site. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and in part by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for a research productivity grant to the second author for the project "Diversidade beta de assembleias de besouros Scarabaeinae: Efeito da temperatura na distribuição espacial e temporal" (307437/2017-5). This research was funded by the "PVE" project MEC/MCTI/CAPES/CNPq/FAPs No 09/2014 of the Ministry of Education of Brazil: "Efeito comparado do clima e das mudanças no uso do solo na distribuição espacial de um grupo de insetos indicadores (Coleoptera: Scarabaeinae) na Mata Atlântica" (88881.068089/2014-01).

References cited

Arellano, L., J. León-Cortés, and O. Ovaskainen. 2008. Patterns of abundance and movement in relation to landscape structure: a study of a common scarab (*Canthon cyanellus cyanellus*) in southern Mexico. Landscape Ecology 23: 69–78.

Cultid-Medina, C. A., B. G. Martínez-Quintero, F. Escobar, and P. Chacón de Ulloa. 2015. Movement and population size of two dung beetle species in an Andean agricultural landscape dominated by sun-grown coffee. Journal of Insect Conservation 19(4): 617–626.

Escobar, S. F., and P. Chacón de Ulloa. 2000. Distribución espacial y temporal en un gradiente de sucesión de la fauna de coleópteros coprófagos (Scarabaeinae, Aphodiinae) en un bosque tropical montano, Nariño - Colombia. Revista de Biología Tropical 48(4): 961–975.

Favila, M. E. 1988. Un método sencillo para marcar escarabajos. Folia Entomológica Mexicana 75: 117–118.

Hagler, J. R., and C. G. Jackson. 2001. Methods for marking insects: Current techniques and future prospects. Annual Review of Entomology 46: 511–543.

Halffter, G., and M. E. Favila. 1993. The Scarabaeinae (Insecta: Coleoptera) an animal group for analyzing, inventorying and monitoring biodiversity in tropical rainforest and modified landscapes. Biology International 27(27): 15–21.

Hanski, I., and Y. Cambefort. 1991. Resource partitioning [pp. 330–349]. *In*: Dung Beetle Ecology (I. Hanski and Y. Cambefort, editors). Princeton University Press, Princeton, NJ, xii + 481 pp.

Larsen, T. H., and A. Forsyth. 2005. Trap spacing and transect design for dung beetle biodiversity studies. Biotropica 37(2): 322–325.

Martínez-Quintero, B. G., C. A. Cultid-Medina, and J. C. Rudas-Grajales. 2013. Marking method for dung beetles (Coleoptera: Scarabaeinae) and its implementation in Colombian Andes. Acta Zoológica Mexicana (n. s.) 29(2): 448–451.

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

Noriega, J. A., and A. Acosta. 2011. Population size and dispersal of *Sulcophanaeus leander* (Coleoptera: Scarabaeidae) on riverine beaches in the Amazonian region. Journal of Tropical Ecology 27(1): 111–114.

Peck, S. B., and A. Forsyth. 1982. Composition, structure, and competitive behavior in a guild of Ecuadorian rainforest dung beetles (Coleoptera; Scarabaeidae). Canadian Journal of Zoology 60(7): 1624–1634.

Roslin, T. 2000. Dung beetle movements at two spatial scales. Oikos 91: 323–335.

Silva, P. G., and M. I. M. Hernández. 2015. Spatial patterns of movement of dung beetle species in a tropical forest suggest a new trap spacing for dung beetle biodiversity studies. PLoS ONE 10(5): 1–18.

Tarasov, S., and F. Génier. 2015. Innovative Bayesian and parsimony phylogeny of dung beetles (Coleoptera, Scarabaeidae, Scarabaeinae) enhanced by ontology-based partitioning of morphological characters. PLoS ONE 10(3): e0116671.

Villada-Bedoya, S., and C. A. Cultid-Medina. 2017. Population structure of two species of *Dichotomius* Hope, 1838 (Coleoptera: Scarabaeinae) in a coffee landscape of the western Andes of Colombia, Risaralda. Boletín Científico del Centro de Museos 21(1): 188–198.

(Received 31 August 2018; accepted 2 January 2020. Publication date 25 March 2020.)