Title:

Authors: Ben Allgire, Ellen Welti

Author Affiliations: Smithsonian National Zoo and Conservation Biology Institute, Front Royal Virginia

What are the drivers of dung beetle body size? What drives body size variation in dung beetles?

1. Temperature (hump-shaped graph [temperature performance curves])
2. Nutrient Availability (positive)
3. Different grazers (This may be several hypotheses)
4. Insecticide (Reduce body size?)

Main question: What drives body size variation in dung beetles?

Hypothesis 1: There is a negative correlation between air temperature and body size. As temperature increases, body size will decrease.

Hypothesis 2: There is a positive correlation between nutrient availability and body size. As nutrient availability increases, so does body size.

Hypothesis 3: There is a positive relationship between the presence of large grazers and body size. Areas with large grazers will have larger beetles than areas without them.

Hypothesis 4: There is a negative relationship between insecticides and body size. Areas with insecticide will have smaller beetles than sites without insecticide.

**Abstract**

**Introduction**

\*\*\*Body size is an important life history trait that influences organismal fitness (Kingsolver and Huey 2008; Lighton, Quinlan, and Feener Jr 1994), mortality (Goatley and Bellwood 2016), and breeding success (Honek 1993). Global change, especially rising temperatures, altered biogeochemistry, and changes in land management, may cause shifts in animal body sizes. Insects are a well-suited taxa in which to examine body size shifts (Chown and Gaston 2010) because they have short generation times, often exist in high densities (Sources?), and play many key ecological roles (Elizalde et al 2020). Intraspecifically, key determinants of insect body size include temperature and nutrition. Higher temperatures can reduce insect body size (Horne, Hirst, Atkinson 2017; Macagno *et al*. 2018; Wonglersak *et al*. 2020; Davidowitz, Amico, and Nijhout 2003; Davidowitz, Amico, and Nijhout 2004), but there are exceptions to this trend (Walters and Hassall 2006; Wonglersak *et al*. 2020). Increasing diet quality is expected to increase insect body size (Chown and Gaston 2010; Thomas 1993; Davidowitz, Amico, and Nijhout 2003; Teder, Vellau, and Tammaru 2014; Pocas, Crosbie, and Mirth 2020). Insecticide use, which is common across many rangeland systems (Branson et al. 2006), may also influence body size in insects which do not suffer direct morality (Alexander, Heard, and Culp 2008). However, little is known about shifts in intraspecific insect traits in response to these chemicals in rangelands (Hayasaka et al 2012; Manning and Cutler 2020). Land management can indirectly affect factors controlling insect body size, including through altering temperature and nutrition. In rangelands, herbivory and excretion by large herbivores modify plant structure and provide manure, potentially affecting insect body sizes through changing ecosystem microclimate and resource availability.. Resulting shifts in insect body size have implications for both intraspecific population fitness and ecosystem function.

\*\*\*Dung beetles are an informal group of several genera of Coleoptera that use dung as their main food source during both adult and larval stages (Matthews 1963). They are a key indicator species (McGeoch, Rensburg, and Botes 2002) because of the many ecosystem services they provide, including nitrogen retention (Kazuhira, Hideaki, and Hirofumi 1991; Maldonado et al. 2019), livestock parasite reduction (Fincher 1973), and reduction of greehouse gas emissions (Slade et al. 2016). Dung beetles are are particularly critical members of the Earth’s grasslands, where large herbivore dung can pile up in the absence of this key taxa (Losey and Vaughan 2006). Grasslands are a globally threatened ecosystem, primarily from agricultural intensification (Aune, Bryn, and Hovstad 2018), and remaining untilled grasslands are primarily used for livestock grazing. The amount of individual dung beetles can bury increases with their body size (Hosler *et al*. 2021; Manning and Cutler 2020) and intraspecific body size of dung beetles can exhibit high variation in responses to environmental conditions (Emlen et al. 2007). Identifying drivers of dung beetle body size in complex field conditions is challenging but has important implications for retaining and managing the ecosystem services dung beetles provide.

Here we asked how habitat conditions drive variation in body size using two dung beetles in the North American Northern Great Plains. Throughout an entire growing season we collected 18,068 specimens and measured body segments on 4,646 individuals of two common dung beetles in northeastern Montana, USA: *Canthon pilularius*, a large native species, and *Onthophagus nuchicornis*, a smaller non-native species. We investigated four main hypotheses: (1) higher air temperatures reduce dung beetle body size, (2) increased nutrient availability results in increased dung beetle body size, (3) temperature and nutrient effects on dung beetle body size are mediated by the presence and density of large mammalian grazers, and (4) insecticides reduce dung beetle body size.

**Methods**

***Study species***

Dung beetles include species from the Scarabaeidae and Geotrupidae beetle families that use dung as a food source for adults and larvae (Matthews 1963). Dung beetles can be grouped into three functional groups: dwellers, tunnellers, and rollers (Floate *et al*. 2017). Dwellers live within the dung, tunnellers bury portions of the dung directly below the original dung pat, and rollers process the dung extensively, removing pieces of dung pats, rolling them away as balls, and then burying dung (Floate *et al*. 2017).

We collected body size measurements on two species of dung beetle. The first is *Canthon pilularius*, a widespread species native to North America (Matthews 1963). *C. pilularius* is a relatively large species of dung beetle (10-19 mm in length), a roller, and has several color phases (black, blue, bronze, and green) (Matthews 1963). The second species is *Onthophagus nuchicornis*, a tunneling Eurasian species that has been in the United States for over a century (Floate *et al*. 2017; Manning and Cutler 2020). This species is considerably smaller in size (6-8mm) than *C. pilularius*, and has yellow or brown elytra with black spots (Hoebeke and Beucke 1997). Males of this species have a single horn on their head making them easily distinguishable from the females.

***Field site and environmental data***

\*\*\*The study was conducted in shortgrass prairie from late May to mid September of 2022 in Phillips county, Montana, USA on land owned or leased by American Prairie, Bowdoin National Wildlife Refuge, Charles M Russell National Wildlife Refuge, and the Bureau of Land Management. We sampled dung beetles on 24 total sites with 5 treatment levels: bison grazed, cattle grazed, ungrazed, prairie dog town in the bison area with insecticide treatment, and prairie dog town in the bison area without insecticide treatment. Sites included 15 core sites (3 replicates per treatment level) where corresponding data on temperature and dung counts, and 9 supplemental sites (3 additional replicates of bison grazed, cattle grazed, and ungrazed treatments) where only beetles and no environmental data was collected. Land owned by American Prairie or leased by BLM was formerly plowed and used for crop plants and cattle ranching. Bison stocking densities varied from 0.012613-0.017616 (bison/area), while cattle stocking densities varied from 0.024984-0.029740 (cattle/area). In bison areas, the conversion from cattle grazing to bison grazing took place 7 to 20 years before our study began.

In core sites, temperature data was collected with Onset HOBO data loggers. These devices recorded temperature and humidity every 6 hours. At the end of the field season, the loggers were collected and their data was downloaded and analyzed. Dung was quantified in order to estimate the type and number of herbivores passing through a particular site. Dung was counted at every core site in column 9 in 10 m X 10 m sections and differentiated between patties (left by large herbivores such as cows or bison), piles of small dung pieces (made by animals such as deer, pronghorn antelope, or coyotes), and prairie dog pellets. The dung was then tallied into these three distinct groups.

Plant clippings were taken from every site 10 m apart from each other in columns 2, 3, 6, and 7. Plant clippings were separated into grasses, forbs, woody plants, and dead plant matter. These clippings were ground up using a coffee grinder and dumped into individual envelopes. All samples from a core site were then combined by their plant type (grass, forb, woody, or dead plant matter) and placed in a new envelope that corresponded to the core site, vegetation type, and sampling month. This process was repeated for every vegetation type in every sampling month for every core plot. These samples were then sent to the Cornell Nutrient Analysis Laboratory. These samples were analyzed using…to get…\*\*\* (Put in document on 6/30/2023)

The study was conducted in the shortgrass prairies from late May to mid September of 2022 in Phillips county, Montana, USA on land owned or leased by American Prairie, Bowdoin National Wildlife Refuge, Charles M Russell National Wildlife Refuge, and the Bureau of Land Management. We sampled dung beetles on 24 total sites with 5 treatment levels: bison grazed, cattle grazed, ungrazed, prairie dog town in the bison area with insecticide treatment, and prairie dog town in the bison area without insecticide treatment. Sites included 15 core sites (3 replicates per treatment level) where corresponding data on temperature and dung counts, and 9 supplemental sites (3 additional replicates of bison grazed, cattle grazed, and ungrazed treatments) where only beetles and no environmental data was collected. Land owned by American Prairie or leased by BLM was formerly plowed and used for crop plants and cattle ranching. Bison stocking densities varied from XX- XX, while cattle stocking densities varied from XX- XX. In bison areas, the conversion from cattle grazing to bison grazing took place 7 to 20 years before our study began. In core sites, temperature data was collected with HOBOs …. Dung was quantified… Plant elemental chemistry …

***Dung beetle sampling***

Dung beetles were collected in four pitfall traps per site, with traps arranged in a 50 m x 50 m square ordinated by cardinal directions. Dung beetle pitfall traps were baited using one tablespoon of homogenized pig dung rolled into balls and bound by 4” X 4” pieces of cheesecloth. Pig dung was sourced from the Swine Teaching and Research Center operated by the Department of Animal Sciences and Industry at Kansas State University and was frozen before deployment. The traps were made of 0.65 L plastic cups (9cm diameter, 15 cm depth) and were filled ¼ full with soapy water, baited with pig dung using binder clips to attach cheesecloth balls to pitfall trap cups, and left open for 48 hours during each trapping period. Following exposure, the traps were collected and samples were washed three times using plain water. The specimens were then stored in 99.7% ethanol.

***Sorting and size measurements***

We measured 10 specimens of *C. pilularius*, 10 *Onthophagus nuchicornis* males, and 10 *O. nuchicornis* females from each sample. If fewer than 10 individuals of any group were found in a trap, all specimens available were measured. On each specimen, we measured head length, forearm length, central thorax length, and central elytra length using electronic calipers (0.01 mm accuracy). For *O. nuchicornis* males, we additionally measured horn length.

***Statistics***

We first calculated body size estimates of each of the three groups of *C. pilularius, O. nuchicornis* females, and *O. nuchicornis* males for each treatment and collection month. Next we examined environmental driver effects on body size of each of the three groups. For all analyses, we used Bayesian linear models fitted with the R package brms (Bürkner 2021). Models were run using four chains for 5000 iterations (50% burn-in) and default brms priors. The form of the body size model was:

XX

The form of the driver model was:

XX

Code for all analyses is available at: https://github.com/Ewelti/AmongTheDung/tree/main/R

**Results**

**Across all samples, we collected 13,628 individual C. pilularius and 4,440 O. nuchicornis. Body size measurements were taken on 2,100 individual C. pilularius (non sexually dimorphic), 1,344 female O. nuchicornis, and 1,202 male O. nuchicornis.**

**Discussion**

Summary of Results. Start the Discussion with a statement or paragraph that summarizes the main results of the study. The last sentence of this section should be a topic sentence that outlines the major points that will be considered in the remainder of the Discussion.

Interpretation. When interpreting the Results, try to be even-handed. Do not make conclusions that the data do not support or fail to address. Present alternative explanations if caveats are appropriate. Being self-critical takes this option away from a reviewer. Keep in mind that sample sizes and the size of the differences between your treatments may be small.

Broader perspective. Conclude the Discussion by addressing the broader implications of the research. This can include: questions that remain unanswered, suggestions of areas where further research is necessary, implications of the results for problems in other taxa or areas of theory, development of new hypotheses, or implications for management and conservation.