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DUNG BEETLE ECOLOGY: HABITAT AND FOOD PREFERENCE, HYPOXIA  
TOLERANCE, AND GENETIC VARIATION

by

Sean Doyle Whipple

A DISSERTATION

Presented to the Faculty of  
The Graduate College at the University of Nebraska  
In Partial Fulfillment of Requirements  
For the Degree Doctor of Philosophy

Major: Entomology

Under the Supervision of Professors W. Wyatt Hoback and John E. Foster

Lincoln, Nebraska

December, 2011

DUNG BEETLE ECOLOGY: HABITAT AND FOOD PREFERENCE, HYPOXIA  
TOLERANCE, AND GENETIC VARIATION

Sean Doyle Whipple, Ph.D.

University of Nebraska, 2011

Advisors: W. Wyatt Hoback, John E. Foster

Four studies were conducted on dung beetles (Coleoptera: Scarabaeidae), which are insects of great ecological and economic importance. Range management practices were found to impact dung beetle diversity and abundance. While sampling on organic and conventionally managed ranches in Nebraska, 93% (5,767) of total dung beetle capture was from the organically managed ranch. Only 480 dung beetles (7% of total) were collected from the conventionally managed ranch. Results indicated that organic ranching had increased numbers and diversity of dung beetles.

Comparison of the attractiveness of native and exotic herbivore, carnivore, and omnivore dung yielded 9,089 dung beetles from 15 species. Significant differences were observed in mean dung beetle capture and individual species preference among the dung of omnivores, herbivores, and carnivores. Omnivore dung and carrion were most attractive; however, preference for a specific dung type was not correlated with dung quality or mammalian diet.

Dung beetles are exposed to hypoxic conditions throughout much of their life cycle. Data on hypoxia tolerance of five species of adult dung beetle (*Aphodius haemorrhoidalis*, *Canthon pilularius*, *Melanocanthon nigricornis*, *Onthophagus hecate*, and *Phanaeus vindex*) yielded no differences in mean survival time (LT<sub>50</sub>) among behavioral groups, which ranged from 7-37 hours.

*Digitonthophagus gazella* (F.) has been intentionally released in numerous areas around the world. Using amplified fragment length polymorphism (AFLP) analysis, genetic variation was examined between two populations of *D. gazella* from South Africa and Vieques, Puerto Rico. Analysis of molecular variance (AMOVA) revealed 69% of genetic diversity to be within populations, while 31% of genetic diversity was between the populations indicating little gene flow. Genetic diversity was high in both South Africa and Vieques with no evidence of inbreeding depression on Vieques. These data are helpful in understanding the population dynamics of dung beetles through knowledge of the effects of agricultural practices, niche separation, and genetics.

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*This dissertation is dedicated to my mother, Pamela L. O'Neal.*

*Never give up, never back down.*

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## **Introduction and Literature Review**

Insect species composition and density have been shown to be a function of geography (Craig et al. 1999) and geographic features (Cigliano et al. 1995), with dung beetles being no exception (Edwards 1991, Hanski and Cembefort 1991, Scholtz et al. 2009). These differences are mostly a result of weather patterns, varying plant diversity, and soil characteristics (Kemp et al. 1990), which have been altered in North America since European settlement in the Great Plains region. Geographic features and weather patterns also affect the plant species composition, which impact mammalian herbivores and dung beetle habitat over a broad geographic range (Scholtz et al. 2009). Compounded with intentional geographic alterations for crop production, current dung beetle assemblages and niche partitioning in the Great Plains may be a direct result of our land use practices in the past 150 years. With current agricultural practices known to affect diversity (Morris 1979, Rushton et al. 1989, Madsen et al. 1990, Morris and Rispin 1993, Di Giulio et al. 2001, Holter et al. 2002, Kruess and Tschardtke 2002), research could aid in the conservation of dung beetles across the Great Plains.

The regions of Nebraska are divided by geology, climate, habitat, and soil type (Omernik 1987, 1995). Eastern, central, and western Nebraska receive different mean annual precipitation, and as a result have different vegetations (Johnsguard 2001). Eastern Nebraska receives the highest rainfall while the mixed-grass prairie of central Nebraska and short-grass prairie of western Nebraska receive considerably less precipitation (Johnsguard 2001). The tallgrass prairie now has less than 1% of the original prairie (Cully et al. 2003), resulting from conversion of prairie to cropland (White and Glenn-Lewin 1984, Cully et al. 2003). Considering the alterations to the

Nebraska landscape, including the Sand Hills and North Platte River Valley region of Nebraska (Ratcliffe and Paulsen 2008), dung beetles can be an excellent bioindicator of ecological changes.

### **Historic and Ecological Importance**

Dung Beetles are a relatively small group of Scarab beetles with approximately 7,000 species world wide. They occur on every continent but Antarctica, and are most diverse in Africa, where more than 2,000 species occur (Hanski and Cambefort 1991). Since the time of the ancient Egyptians, dung beetles have been revered as a representation of rebirth and rejuvenation (Hanski and Cambefort 1991). Dung beetles were a symbol of the sun-god Ra and it was believed that they rolled the sun across the sky each day and buried it in the evening, only to emerge again the following morning (Walters 2008). Egyptians were known to place “heart scarabs” within mummies in the belief that it would inhibit their heart from giving testament of the deceased’s transgressions (Hanski and Cambefort 1991).

Dung beetles are extremely important ecologically and are a major component of the biological removal of dung and control of pests and parasites which use dung for breeding (Fincher 1973). In the state of Nebraska, beef production is the most economically valuable industry, accounting for \$5.4 billion in sales in 2002 (Veneman et al. 2004). Dung beetles are estimated to save farmers and ranchers \$380 million annually in the United States (Losey and Vaughan 2006) based on yield loss, pesticide applications, and fertilizer use (Walters 2008). Not included in this estimation are health costs and environmental problems from pests and pesticides (Walters 2008). This is

especially important considering approximately \$800 million is spent annually to control livestock pests in the United States alone (Fincher 1981, Griffith 1997). Other than their role as decomposers, dung beetles are a likely candidate for use in the measure of biodiversity (Halffter and Favila 1993, Kremen et al. 1993), and are also involved in the pollination (Sakai and Inoue 1999) and seed dispersal of some plants (Andresen and Feer 2005, Nichols et al. 2008).

Individual cattle produce approximately 10-20 dung pats every day (Rougan 1987), with each pat covering a surface area of approximately 0.82 m<sup>2</sup> (Bornemissza 1960, Fincher 1981). Each pat may last up to four years without dung beetle activity (Walters 2008). While dung may be broken down by weathering (White 1960, Bastiman 1970) and by other organisms such as earthworms, ants, and termites (Denholm-Young 1978, Holter 1979), some areas in the southern United States rely more heavily on dung beetles (Merritt and Anderson 1977, Lumaret and Kirk 1987). Dung beetles significantly increase the rate of dung decomposition (Wratten and Forbes 1996), with larvae being able to consume up to 100% of their body weight per day until pupation (Holter 1974). One species, the tunneler *Onthophagus gazella* Fabricius, has been noted for its exceptional ability at dung removal (Bornemissza 1970, Young 2007). Additionally, cattle will not graze in close proximity to their own feces (Dohi et al. 1991) and it has also been concluded that the nutritional quality of the dung of grazing mammals is directly related to range health (Edwards 1991). Undegraded dung can prevent the growth of vegetation, resulting in an area that will remain ungrazed by cattle for up to two years (Anderson et al. 1984). Unfortunately, in feedlots, dung beetles often do not



have the opportunity to remove dung because of trampling by high densities of cattle as well as treatment with pesticides (Walters 2008).

Manure is a breeding ground for pests and parasites. The horn fly, *Haematobia irritans* L., causes an estimated \$730 million annual loss to the cattle industry (Drummond et al. 1981). Dung beetles reduce horn flies by 95%, bush flies by 80-100%, and result in nine times fewer parasites produced (Bornemissza 1970, 1976). This is staggering when it is considered that over 100 adult bush flies (*Musca vetustissima* Walker) can emerge from a 1000 cc. dung pat in Australia (Bornemissza 1970). Dung beetle activity has been shown to reduce numbers (Bryan 1973, 1976), resurgence (Reinecke 1960), and migration (Fincher 1973) of parasitic larvae within feces.

Up to 56% of cattle in the United States are treated with insecticides to control dipterans and internal parasites (Losey and Vaughan 2006, Scholtz et al. 2009). Some of these treatments have been known to affect dung beetle communities, with mixed results (Holter et al. 2002). Krüger and Scholtz (1996) observed that treating cattle with Ivermectin can hinder development of the dung beetle *Euoniticellus intermedius* (Reiche) for up to 28 days. Dung beetles are essential to range health and it is of the utmost importance that ranchers and range managers are informed of the value of dung beetles as well as of the repercussions of pesticide application. Taking into account the local environmental conditions, cattlemen should be aware of the affect that insecticides aimed at treatment of flies and parasites have on dung beetles (Holter et al. 2002, Floate et al. 2005, Kryger et al. 2005, Scholtz et al. 2009). Some veterinary pharmaceuticals can reduce survival and be fatal to dung beetle populations (Madsen et al. 1990, Krüger and

Scholtz 1996, Floate et al. 2005). If treatment is necessary, it should be done when dung beetles are inactive or by using dusts and sprays (Krüger and Scholtz 1996).

With overstocking and drought, as much as 85% of a pastures biomass can be consumed by herbivores (Olechowicz 1974). Hutton and Giller (2003) reported that dung beetle numbers, diversity, and species richness were significantly higher in Northern Ireland on organic rangelands compared to rangeland that was roughly and intensively grazed. Dung beetles efficiently cycle nutrients into the soil and create healthier rangelands and reduce greenhouse gas emissions (Halffter and Matthews 1966, Mittal 1993, Estrada et al. 1998, Walters 2008). If dung is not removed by the beetles, eighty percent of the nitrogen is lost to the atmosphere (Gillard 1967). In addition, burial of animal dung by the beetles increases soil aeration and the eventual leaching of water and nutrients into the soil (Bornemissza 1960, Bang et al. 2005). Soil aeration resulting from dung burial (Mittal 1993) enhances the role dung beetles play in nutrient cycling (Halffter and Matthews 1966, Mittal 1993, Estrada et al. 1998). Soil aeration lowers runoff of surface wastes and aid in reduction of water contamination and algal blooms. (Walters 2008).

Bertone (2004) found the burial activity of dung beetles to have a positive impact on soil nutrients, pH, and cation exchange capacity, which positively influences forage growth (Bornemissza 1960, Macqueen and Beirne 1975, Kabir et al. 1985, Bang et al. 2005). A study by Bornemissza and Williams (1970) found that the burial of dung by *Onthophagus australis* Guerin resulted in significant increase in the yield of Japanese millet, *Echinochloa crus-galli* var. *frumentacea*. When dung alone was placed on the soil surface within the trials, yield was 17.3 g, compared to 31.3 g with beetles present

(Bornemissza and Williams 1970). Dung beetles are bio-indicators of a healthy ecosystem (Davis et al. 2001) and if there are no beetles present, the potential yield is not being met.

When stocking rates are high, dung beetles may become ineffective. Exceeding recommended stocking rates for livestock results in reduced forage, increased dung, and a decrease in livestock yield (Burton 1972). Overgrazing can result in reduced surface cover, increased surface temperature, increased runoff, reduced soil moisture, and an eventual change in plant community composition. Intensive and rough grazing has been observed to reduce dung beetle abundance (Hutton and Giller 2003).

### **Feeding and Niche Partitioning**

Dung beetles have adapted to fill numerous niches in a wide variety of ecosystems and many are highly specialized (Hanski and Cambefort 1991). The subfamilies Aphodiinae and family Geotrupinae have many specialist dung beetle species that feed on resources other than dung (Howden 1955, Halfpeter and Edmonds 1982). Depending upon species, dung beetles may have specific preference towards dung and dung condition (Doube 1987, Yasuda 1987), dung odor (Dormont et al. 2004). Dung beetles have also been shown to segregate based upon habitat and soil type (Peck and Forsyth 1982, Doube 1983). Species differ in their nocturnal or diurnal activity (Hanski and Cambefort 1991), as well as exhibit variances in seasonal activity (Hanski and Koskella 1977, Hanski 1980, Holter 1982, Doube 1991). Dung beetle numbers and species also vary depending upon light and light intensity (Halfpeter and Arellano 2002,

Ratcliffe and Paulsen 2008). Additionally, beetles respond differently to elevation, with higher temperatures at low altitudes being optimal (Medina et al. 2002).

While most dung beetles are generalist (coprophagous) feeders, specialization can occur as a result of reduced dung availability (Halffter and Matthews 1966, Howden and Young 1981, Young 1981, Hanski 1989, Davis and Sutton 1997). Feeding preference can also be influenced by factors such as predation and competition, which can directly alter feeding behavior (Schmitz et al. 1997). While predation and competition obviously impact a population, abiotic factors such as weather and climate have the potential to alter behavior, life cycles, and ultimate success of a species from year to year and location to location with little predictability (Scholtz et al. 2009). Numerous studies have focused on feeding preference and behavior, but the actual biology of dung beetle feeding is not well understood (Holter 2000).

Dung beetles likely evolved from detritus-feeding ancestors (Scholtz et al. 2009), so the switch to dung is not difficult to conceive. A dung resource contains all the nutrition that the beetles require. Adults will feed on a “dung slurpie”, which is primarily the moisture within the dung (Halffter and Matthews 1966, Hanski and Cambefort 1991). While adult beetles have filtering mouthparts, larvae have the advantage of biting mouthparts, and are able to feed on both moisture and the fiber of the dung within the brood balls (Halffter and Matthews 1966, Scholtz et al. 2009). The bacterial fauna of the larval gut increases markedly after ingestion, is capable of breaking down cellulose, and increases the nutritional gain for the developing larva (Goidanich and Malan 1962, Scholtz et al. 2009).

Dung beetles will feed on a variety of foods including dead or decaying plants and fruit, carrion, bones, and other invertebrates, although most species prefer dung (Halffter and Matthews 1966, Edmonds 1972, Young 1980, Cambefort 1984, Hanski and Cambefort 1991). Although most dung beetles utilize either herbivore or omnivore dung (Hanski and Cambefort 1991), human feces has been shown to be particularly attractive to many species of dung beetle (Hanski 1983, Howden and Nealis 1975). However, depending upon location and species, the preferred dung type can vary greatly (Halffter and Matthews 1966, Hanski and Cambefort 1991).

Nutritional content of dung will vary based upon numerous factors. However, dung beetles are extremely efficient in gaining adequate nutrition (Scholtz et al. 2009). The dung itself is comprised of cellulose, as well as gut fragments, epithelium, and microbes (Hanski and Cambefort 1991, Scholtz et al. 2009). Nutrition (carbohydrates and protein) is available from all portions of this mixture, but is highest in the portions from the animal that exuded the dung. For microbes and fungi that inhabit the dung, concentrations will increase as decomposition takes place, and add additional protein content to the dung resource (Hanski and Cambefort 1991, Scholtz et al. 2009). It has also been hypothesized that the lining of the brood chamber secreted by the female may serve as additional nutrition for the developing larvae (Halffter and Edmonds 1982).

During times of low dung availability, many dung beetles are capable of feeding upon other resources including rotting fruit and carrion, which are also highly nutritious (Hanski and Cambefort 1991, Scholtz et al. 2009). Carrion presents an opportunity for generalist dung beetles to obtain nutrition, be it from the decaying carcass or the gut contents of the carrion, for survival and reproduction (Halffter and Matthews 1966,

Scholtz et al. 2009). The reproductive rate of dung beetles may be directly related to an adequate supply of dung (Giller and Doube 1994).

Seasonality of dung beetle species is likely a function of dung quality, most notably nitrogen content, which is higher in the summer months (Edwards 1991, Holter and Scholtz 2007). Quality of dung is influenced by food intake, but because few dung beetle species are active in the winter (Scholtz et al. 2009), it may be of little consequence to adults or developing larvae. As a result of a changing diet, there will be seasonal variation in dung quality, which is directly correlated with the size of adult beetles (Emlen 1997, Scholtz et al. 2009). That being said, when dung quality is lower in early spring and fall, more dung may need to be provided to the larvae by the female (Emlen 1997, Scholtz et al 2009).

### **Competition and Behavior**

Dung beetles are divided into three behavioral groups; Rollers, tunnelers, and dwellers (Hanski and Cambefort 1991). Rollers are characterized by the female fashioning a brood ball which the male and female roll away from the dung pat. A single egg is deposited in the brood ball where the larva develops through its instars. Tunnelers bury dung into the soil directly beneath the dung pat to lay their eggs, while dwellers deposit their eggs into the main dung pat where they will develop into adults (Hanski and Cambefort 1991). Even though the three groups are distinct in their behavior, there is often both intraspecific and interspecific competition between adults and larvae for food and space (Anderson and Coe 1974, Cambefort 1982, Peck and Forsyth 1982, Hanski 1983, Janzen 1983, Doube 1987, Hanski 1989). Rollers and tunnelers are often more

adept at swift and efficient dung removal, but this depends on numerous factors including beetle size, burial speed, and depth (Scholtz et al. 2009).

Dung beetles are seemingly an exception to Gause's Axiom (competitive exclusion principle), which states that all things being constant, no two species can coexist while competing for the same limited resource. According to Scholtz et al. (2009), dung beetles have comparable ecological requirements, with the possibility existing for thousands of beetles of different species to arrive at a dung resource. The ability to coexist lies in the inherent specializations of a particular species and the niche they occupy. Although there is a great deal of competition, dung beetle species vary markedly in their utilization of dung, and often there is a competitive hierarchy (Doubé 1991, Scholtz et al. 2009).

Dung is a resource that presents many obstacles for dung beetles. Not only is it a limited resource in both spatial and temporal availability, but dung beetles must contend with competition and hypoxic environments once dung is located (Scholtz et al. 2009). Oxygen levels can be extremely low (1-2%) within dung (Holter 1991, Holter and Spangenberg 1997), with CO<sub>2</sub> and methane concentrations (20-50%) increasing the hypoxic conditions within the dung (Holter 1994, Holter and Spangenberg 1997, Scholtz et al. 2009). Additionally, dung beetles may spend months underground in low oxygen while brooding in closed burrows (Duncan and Byrne 2000, Scholtz et al. 2009). Small differences in oxygen availability may be detectable by dung beetles (Hoback 2011), although few direct tests of hypoxia tolerance have been conducted (Scholtz et al. 2009, Hoback 2011). Currently, there is little evidence to suggest that dung beetles conduct anaerobic metabolism (Holter and Spangenberg 1997), although it is evident in many

terrestrial insects including grasshoppers and tiger beetles (Hochachka et al. 1993, Wegener, 1993, Hoback et al. 2000).

In dung beetles, adaptations to avoid competition should impose variable selection pressures to adapt strategies to survive (Holter 1991, Holter and Spangenberg 1997, Duncan and Byrne 2000). While ball-rolling species may be in hypoxic conditions from minutes (Tribe 1976) to hours (Osberg 1988), tunneling and dwelling species may spend a much greater portion of their lives in low oxygen environments (Holter 1991, Holter and Spangenberg 1997, Scholtz et al. 2009). Research is needed to examine hypoxia tolerance in dung beetle species and over a broad geographic range (Hoback 2011).

### **Exotic Species and Biological Control**

The introduction of exotic livestock has caused numerous problems in the past. Australia is a prime example. In 1778, Europeans settled Australia and brought with them a variety of livestock and plant life (Hanski and Cambefort 1991). This led to overgrazing, as well as an excess of flies, midges, and parasites (Hanski and Cambefort 1991, Walters 2008). The native dung beetles, which had co-evolved with marsupials (Tyndale-Biscoe 1971), were not adapted to utilize bovine dung (Mathews 1972). Excessive dung caused obstructed plant growth, poor nutrient cycling, and unchecked fly and parasite populations (Hanski and Cambefort 1991, Walters 2008). Although many dung beetle species have adapted to quickly utilize large quantities of dung in a short period of time (Gillard 1967, Fincher 1975), some areas, including the southern United



States, do not have sufficient populations of dung beetles to contend with cattle numbers (Fincher 1975).

Africa, India, and Europe have dung beetle species which evolved with ruminants and could utilize the dung from the livestock introduced to Australia (Waterhouse 1974, Bornemissza 1976). Numerous species of dung beetle were introduced to Australia including: *Onthophagus taurus* from Europe and *Euoniticellus intermedius*, *Onitis viridulus*, *Digitonthophagus gazelle*, *Onthophagus nigriventris*, and *Sisyphus spinipes* from Africa (Bornemissza 1976, Hanski and Cambefort 1991). Introduced species had varying activity patterns as well as dung and habitat preferences. This allowed the beetles to succeed in dung removal from numerous Australian habitats (Bornemissza 1976), which is critical considering there was estimated to be over half a million tons of cow dung deposited daily (Waterhouse 1974). A similar situation may occur in many areas of the United States, as well as island ecosystems, on which exotic mammals and dung beetles have been accidentally or intentionally introduced.

Normally there are risks associated with biological control. The control species often turns to a vulnerable native species or an unwanted target, such as the case of the cane toad (*Bufo marinus* L.) and thistle-head weevil (*Rhinocyllus conicus* Frölich) (Hoddle 2004). However, dung beetles have modified mouth parts that are specifically adapted to feed on dung (Hanski and Cambefort 1991, Holter 2000). Research by Waterhouse (1974) and Giller and Doube (1994) have indicated that if there is no dung available, there are few beetles present. Additionally, introduced dung beetles are often active at different seasons than the native beetles as observed in Australia (Hanski and Cambefort 1991). However, the ecology of the native Australian dung beetle fauna is not

well understood, largely because of attention given to the introduction of non-native biocontrol species (Doubt et al. 1991, Hill 1996).

Although currently there is no observed threat to biodiversity from introduced non-native dung beetles, this does not necessarily imply that native species in other reintroduction areas will be unaffected. Non-native dung beetles are introduced largely based upon their climatic requirements and habitat specialization (Hanski and Cambefort 1991). If a non-native species is introduced that has overlapping niche requirements with a native species, competition could lead to a reduction in biodiversity. This is especially important given the capability for rapid dispersal and reproduction of some species such as *Digitonthophagus gazelle*, which may out-compete the native dung beetle fauna in many areas of introduction (Ivie and Philips 2008).

Through the developments of new molecular techniques that allow the sequencing of entire genomes, conclusions can be drawn from data relating to speciation and life history (Hoy 2004). Whereas traditional conclusions about taxonomic relationships were drawn from morphological data, today more details about evolutionary relationships can be gained through the use of genomic, mitochondrial, and cDNA (Hoy 2004). This genetic information, combined with data taken from the field, has the potential to drastically improve our understanding of ecological relationships within and between species (Eisenberg et al. 2000).

One commonly used molecular technique that has revolutionized numerous scientific disciplines is polymerase chain reaction (PCR). PCR can isolate DNA fragments, which are then selectively amplified (Hoy 2004). This allows for numerous copies to be made from a relatively small amount of DNA. Using specific primers, the

PCR process uses a series of temperature changes to denature and anneal the primers to amplify DNA (Hoy 2004).

Amplified Fragment Length Polymorphism (AFLP) analysis relies on PCR amplification and differs from other fingerprinting methods in that it does not require previous knowledge of a specific sequence (Vos et al. 1995, Hoy 2004). It is a very useful technique for comparing individuals and populations (Mueller and Wolfenbarger 1999). This form of analysis utilizes the whole genome, which eliminates many problems associated with other methods (Vos et al. 1995, Hoy 2004). This kind of analysis could be useful in field studies of dung beetles because it, much like other restriction enzyme based techniques, can reveal gene flow, geographic variation, relatedness, taxonomic problems, and genetic bottlenecks (Hoy 2004).

### **Objectives**

In my dissertation work I combine facets of ecology, feeding biology, life history, physiology, and genetics to examine the effects of organic ranching, exotic mammal introduction, hypoxia exposure, and reproductive and geographic isolation on dung beetle species and abundance. This work had four main goals: 1) to determine the effect of organic ranching practices on dung beetle abundance and diversity in western Nebraska; 2) to compare the attractiveness and nutritional quality of native and exotic herbivore, carnivore, and omnivore dung to dung beetle species in Nebraska; 3) to evaluate hypoxia tolerance and survival within and among dung beetle behavioral groups; 4) to determine the degree of genetic variability within and between two populations of *Digitonthophagus gazella* from Vieques, Puerto Rico and South Africa using amplified fragment length polymorphisms (AFLP).

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**Chapter 2:****Range Management Practices Impact the Abundance and Diversity of Dung Beetles  
(Coleoptera: Scarabaeidae) in Western Nebraska**

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### Abstract

Dung beetles (Coleoptera: Scarabaeidae) play a major role in nutrient cycling, soil aeration, and the biological control of pests and parasites that breed in manure. Habitat fragmentation, pesticide usage, and conventional agricultural practices are threats to dung beetle diversity, and their conservation is of growing concern. Comparison of organic and conventional ranching practices is of great ecological and economic value to the agricultural industry in the Great Plains region of North America. Using baited pitfall traps, this study compared abundance, diversity, and seasonal activity of dung beetles on adjacent rangelands in western Nebraska that are certified organic or managed conventionally. Numbers and diversity of dung beetles were much higher on organically managed rangeland. The organic ranch accounted for 53% of total dung beetle capture with 3,287 total dung beetles, while the area between ranches yielded 40% (2,480 dung beetles). Only 480 dung beetles (8% of the total capture) were collected from the conventionally managed ranch. A total of 15 species were captured, and all commonly collected species (> 50 individuals) were found in higher numbers on the organic ranch ( $p < 0.05$ ). Based on these results, organic ranching in a short grass prairie ecosystem preserves and increases the number of dung beetles compared to conventional ranching practices in a similar habitat.

**Key Words:** Insect ecology, livestock grazing, nutrient cycling, ranching, range management

## Introduction

Dung beetles (Coleoptera: Scarabaeidae) are a major component of the biological control of dung, as well as the pests and parasites which use dung as a breeding ground (Fincher 1973). With dung beetles present, 95% fewer horn flies (*Haematobia irritans* L.), 80-100% fewer bush flies (*Musca vetustissima* Walker), and nine times fewer cattle parasite loads were reported (Bornemissza 1970, 1976). Dung beetle activity has also been shown to reduce numbers (Bryan 1973, 1976), resurgence (Reinecke 1960), and migration (Fincher 1973) of parasitic fly larvae in livestock feces. Not only do dung beetles benefit cattle production, but they also efficiently cycle nutrients into the soil and create healthier rangelands (Halffter and Matthews 1966, Mittal 1993, Estrada et al. 1998, Walters 2008). With beef production being the most valuable industry in the state of Nebraska (Veneman et al. 2004), range managers should be aware of the economic value of dung beetles, which is estimated at \$380 million annually in the United States (Losey and Vaughan 2006).

While dung may be broken down by weathering (White 1960, Bastiman 1970) and other organisms such as earthworms, ants, and termites (Denholm-Young 1978, Hanski and Cambefort 1991, Scholtz et al. 2009), many areas rely extensively on dung beetles (Merritt and Anderson 1977, Lumaret and Kirk 1987). As much as 85% of a pasture's biomass can be consumed by herbivores under conventional management practices and grazing systems (Olechowicz 1974); however, cattle will not graze in close proximity to their own feces (Dohi et al. 1991). By regularly removing cowpats, dung beetles improve range health and increase available acreage for cattle production by an estimated five to ten percent (Walters 2008).

Most ranches treat cattle with pesticides to reduce fly pest numbers and internal parasites. Up to 56% of cattle in the United States are treated with pesticides aimed at the control of dipterans and internal parasites, potentially leaving only 44% of cattle that produce dung able to be broken down by dung beetles (Losey and Vaughan 2006, Scholtz et al. 2009). Some of these treatments pass through the cattle and affect the dung beetle community, although the effects have been variable depending on the pesticide used and local environmental conditions (Holter et al. 2002, Floate et al. 2005, Kryger et al. 2005, Scholtz et al. 2009). In particular, slow-release avermectins can reduce dung beetle reproduction by acting as a larvacide (Ridsdill-Smith 1993). Krüger and Scholtz (1996) demonstrated that treating cattle with avermectins (specifically Ivermectin) hinders development of the dung beetle *Euoniticellus intermedius* (Reiche) for up to 28 days.

Hutton and Giller (2003) reported that dung beetle numbers, diversity, and species richness in Northern Ireland were significantly higher on organic rangelands compared to rangeland that was intensively grazed. With current agricultural practices known to affect insect diversity (Rushton et al. 1989, Madsen et al. 1990, Morris and Rispin 1993, Di Giulio et al. 2001, Kruess and Tschardt 2002, Anduaga 2004), further study of the effects of organic farming and free range grazing systems could aid in the conservation of dung beetles. This research was undertaken to compare dung beetle assemblages in western Nebraska on an organic ranch (uses no pesticides or antibiotics) to a conventional ranch. I hypothesized that there would be no difference in dung beetle numbers and species diversity between ranches sampled with baited pitfall traps.

## Materials and Methods

### Research Site

Sampling took place in the spring and summer (April through the end of August) of 2009 and 2010. The study site consisted of two large (> 4,000 ha) bordering cattle ranches in western Nebraska, one of which is conventionally managed (uses pesticides, antibiotics, etc), and the other is certified organic. For the control of internal and external pests and parasites, the conventional ranch treats their cattle with a Dectomax® (doramectin) injectable in October, and both Ivermectin pour-on solution and oral wormer in the spring and fall at recommended doses.

The study region is primarily a short-grass steppe, with scattered Ponderosa pine (*Pinus ponderosa* Lawson) covered hills. Elevation in the region is between 1,220 and 1,370 m and the climate is variable with annual precipitation averaging 43 cm. Mean annual summer temperature is 22.6°C and the mean annual winter temperature is -3.7°C (Ratcliffe and Paulsen 2008).

Two bordering ranches were selected for this study. The Wagon Box Ranch encompasses approximately 4,050 contiguous hectares of organically managed rangeland located in Banner, Morrill, and Cheyenne counties in western Nebraska (Latitude 41.469004, Longitude -103.340270). The bordering, conventionally managed ranch is also approximately 4,050 ha. The ranches are similar in elevation, plant communities, soil type, annual precipitation, and livestock stocking rate, with approximately 600 head of cattle per ranch. Visually, the vegetation and range condition is indistinguishable between the ranches. Besides not using pesticides and antibiotics, the organic ranch employs a rotational grazing system while the conventional ranch free-grazes their cattle.

## **Sampling**

Pitfall traps consisting of 18.9 liter buckets with soil placed in the bottom to minimize dung beetle mortality were used for sampling. Per trap, 0.11 kg of human feces, which has been shown to be highly attractive to dung beetles (Hanski 1983, Howden and Nealis 1975), was wrapped in nylon tulle and used as bait. Fresh feces was collected, thoroughly mixed to ensure homogeneity, and frozen until sampling occurred. Four traps were placed approximately at the center of the organic ranch, at the center of the conventionally managed ranch, and at the border (edge) between the organic and conventional ranch, for a total of 12 traps. Each year, all traps were placed in close proximity to livestock (within 0.5 km) and spaced at least 50 m apart as recommended by Larsen and Forsyth (2005). Traps were baited for 24 h and checked for three consecutive days (re-baiting daily). Traps were opened every two weeks from the end of April to the end of August during each year. Dung beetles were counted and identified to species using Ratcliffe and Paulsen (2008), then subsequently released in an area approximately the same distance from trap locations on each rangeland. Voucher specimens of each species captured were deposited in the collection of the Department of Biology at the University of Nebraska at Kearney, NE. Aphodinae occasionally required collection and storage to identify under magnification.

## **Data Analysis**

Total numbers and species abundance data were compared by trap location using SigmaPlot 3.1 software (Jandel Scientific, Corte Madera, CA) with the Kruskal-Wallis one-way analysis of variance. A Tukey test was used as a post-hoc when differences

among treatments were detected. Shannon-Weiner and Simpson's diversity indices were also calculated for each trap location.

## Results

A total of 6,247 dung beetles were collected with 2,735 captured in 2009 and 3,512 captured in 2010 (Table 2.1). Over both years, 15 species were captured among all traps (Table 2.1). Significantly more dung beetles were collected from the organic ranch compared to both the edge and conventional ranch ( $p < 0.05$ ) (Fig. 2.1). The organic ranch accounted for 53% of total dung beetle capture with 3,287 total dung beetles, while the edge yielded 40% (2,480 dung beetles). Only 480 dung beetles (8% of the total capture) were collected from the conventionally managed ranch (Table 2.1). The means ( $\pm$  SE) for frequently captured species ( $> 50$  individuals) per range management type are presented in Table 2.2.

The most commonly collected species was *Onthophagus pennsylvanicus* Harold, which comprised 37% of all dung beetles captured. By range management type, *O. pennsylvanicus* was the dominant species on the organic ranch (36%) and at the border (edge) of the two ranches (42%). *Onthophagus hecate* (Panzer) was captured with the highest frequency on the conventional ranch and represented 40% of the total capture. Individuals of all species captured were collected on the organic ranch, while *Aphodius erraticus* (L.), *A. granarius* (L.), *A. prodromus* (Brahm), *A. testaceiventris* Fall, *A. walshii* Horn, *Melanocanthon nigricornis* (Say), and *Phanaeus vindex* MacLeay were never captured on the conventionally managed ranch (Table 2.1). *Aphodius erraticus*, *A.*



*granarius*, and *A. prodromus* were the only species not collected from the edge traps (Table 2.1).

All commonly collected species (> 50 individuals) were captured in statistically higher numbers on the organic ranch compared to the conventionally managed ranch ( $p < 0.05$ ) (Table 2.2). Mean capture of *A. coloradensis* Horn, *A. distinctus* (Müller), and *A. walshii* were also significantly different between the organic ranch and the edge ( $p < 0.05$ ) (Table 2.2).

Overall dung beetle capture was high for the region (Jameson 1989) and produced a Shannon-Wiener index of 4.33 and a Simpson's index of 4.42 (Table 2.3). The highest species diversity and evenness were found on the organic ranch with a Shannon-Wiener index of 4.43 and a Simpson's index of 4.78, followed by the edge with values of 3.52 and 3.64, respectively. Seven out of the 15 collected species were not captured on the conventional ranch, which had a Shannon-Weiner index of 2.38 and a Simpson's index of 3.29.

Seasonal activity varied greatly between species and genera captured (Figs. 2.2 – 2.4). Trends between years were similar, so only the 2010 season is reported. The *Aphodius* species were most active during the early season (Fig. 2.2), with *A. distinctus* and *A. coloradensis* being captured in the highest numbers. Dung beetles in the genus *Onthophagus* exhibited similar periods of activity between species, with numbers of *O. pennsylvanicus*, *O. hecate*, and *O. orpheus* all increasing in late July (Fig. 2.3). *Canthon pilularius*, *Copris fricator*, and *P. vindex* showed bimodal seasonal activity (Fig. 2.4). *Canthon pilularius* abundance peaked at the end of July, decreased until mid-August, and then increased into September. The highest numbers of *C. fricator* were observed

between late July until the end of August; however, there was also a slight increase in numbers during mid-June. Numbers of *P. vindex* steadily increased from the beginning of June until the end of July and then exhibited an apparent decrease in early August before rising again into late August (Fig. 2.4).

### **Discussion**

I found significantly higher ( $p < 0.05$ ) numbers and species of dung beetles on organically managed rangeland in western Nebraska compared to bordering conventional rangeland. Dung beetles are numerically responsive to abiotic conditions, including precipitation (Davis 1995), temperature, and geography (Edwards 1991, Hanski and Cambefort 1991, Scholtz et al. 2009). Additionally, beetles respond differently to elevation, with high temperatures at low altitudes being optimal (Medina et al. 2002). It has also been shown that dung beetle activity is dependent on season, soil type, and beetle size (Hanski and Cambefort 1991). Because geographic features and weather patterns also affect the plant species composition, which in turn affects mammalian herbivores, patterns in dung beetle occurrence will vary over a broad geographic range. However, at these ranches which are side-by-side and share a border, all these factors are similar. Thus, the substantial observed differences in numbers and species diversity appear most likely to be the result of organic ranching practices. Alternatively, because the ranches differ in grazing practices (rotational vs. free-graze), results could be influenced by these differences.

A potential alternative explanation of differences in dung beetle numbers between ranches is grazing practices. Reproductive rates of dung beetles are related to an

adequate supply of dung (Giller and Doube 1994). Although livestock stocking rates were similar between the two ranches, free-range grazing could produce less congregation of cattle, and thus greater patchiness of dung resources (Scholtz et al. 2009). Although the effects of rotational grazing and free-grazing should be directly tested in the future, the pronounced differences observed in this study suggest that grazing management did not affect results as much as treatment with pesticides. During sampling, all traps were located in close proximity to cattle (within 0.5 km), and in areas of heavy livestock traffic (i.e., to and from a water source). Although broad spatial and temporal distribution of mammalian herbivores and “patchy” distribution of dung resources may be a factor in certain areas (especially non-agricultural settings) (Hanski and Cambefort 1991, Scholtz et al. 2009), it does not appear to explain the results of this study.

Jameson (1989) found differences in dung beetle diversity on grazed vs. ungrazed prairie in Nebraska, neither of which used internal pesticides to treat livestock. The data of Jameson (1989) indicated a Shannon-Wiener Diversity of 1.49 on grazed rangeland and 0.91 on the ungrazed. My study yielded a Shannon-Wiener index of 4.43 on the organic ranch and 2.38 on the conventional ranch (Table 2.3), which is a much larger difference than observed by Jameson (1989). This indicates that even though the organic and conventional ranches in my study differ in grazing practices, the substantial differences in numbers and diversity is unlikely to be explained by this factor alone.

Other studies have focused on the benefits of organic farming and ranching practices with observed increases in the diversity of invertebrates and vertebrates alike (Blackburn and Arthur 2001, Freemark and Kirk 2001, Hutton and Giller 2003). Total

numbers (Table 2.1), mean capture (Fig. 2.1; Table 2.2), as well as species diversity and evenness (Table 2.3) were all significantly higher on the organic ranch than the conventionally managed ranch ( $p < 0.05$ ). All commonly collected species ( $> 50$  individuals) were captured in greater numbers on the organically managed ranch ( $p < 0.05$ ) (Table 2.2). Additionally, mean capture of *Aphodius* species on the organic ranch showed a statistical difference between both the edge and the conventional ranch ( $p < 0.05$ ) (Table 2.2). This is likely a result of pesticide effects overlapping from the conventional ranch. This result may be best observed in the Aphodinae because of their dung-dwelling nature and breeding behavior that may leave them more vulnerable to exposure and potential negative effects than other dung beetle genera (Errouissi et al. 2001, Wardhaugh et al. 2001, Hutton and Giller 2003).

Dung beetles have adapted to fill numerous niches in a wide variety of ecosystems, and many are highly specialized (Hanski and Cambefort 1991, Scholtz et al. 2009). Depending on species, dung beetles may have preference towards dung type and condition (Doube 1987, Yasuda 1987, Al-Houty and Al-Musalam 1997, Plewinska 2007), habitat and soil type (Peck and Forsyth 1982, Doube 1983), as well as exhibit variance in seasonal dung utilization (Hanski and Koskella 1977, Hanski 1980, Holter 1982, Yasuda 1984, Doube 1991). This study utilized human feces as bait, which has been shown to be attractive to many species of dung beetle (Hanski 1983, Howden and Nealis 1975). Typically, omnivore dung is highly odiferous compared to herbivore dung, and dung beetles rely on quickly locating the ephemeral dung resource prior to its desiccation or colonization by flies (Scholtz et al. 2009). Because a standardized quantity and condition of human feces was used as bait, differences observed in dung beetle

abundance and diversity among traps are not likely to be a result of variance in attraction to the dung resource.

Notable variation in seasonal activity was observed among dung beetle species. *Aphodius* dung beetle species were observed to be most active from April to mid-June (Fig. 2.2), while *Onthophagus* species did not become highly active until the end of July (Fig. 2.3). This is especially important considering the highest numbers of dung beetles collected in this study belong to the genus *Onthophagus* (*O. pennsylvanicus* and *O. hecate*). However, sampling was not continued into the months of September and October, when many species of Aphodinae are known to be active (Ratcliffe and Paulsen 2008). Larger species, such as *Canthon pilularius*, *Copris fricator*, and *Phanaeus vindex*, showed bimodal activity during the months of June, July, and August (Fig. 2.4). A bimodal seasonal activity would likely also be observed for many *Aphodius* species if sampling was continued into September and October (Ratcliffe and Paulsen 2008). Larger species of dung beetles (rollers and tunnelers in particular) are more adept at dung removal (Hanski and Cambefort 1991, Hutton and Giller 2003, Scholtz et al. 2009), and the lack of these species in a given area (Tables 2.1 and 2.2) tends to suggest that dung could be processed more adequately with their addition, thus increasing forage yield (Anduaga 2004, Walters 2008, Scholtz et al. 2009).

In addition to local climatic conditions and dung beetle life cycle, seasonality of dung beetle species is likely to be influenced by dung quality, most notably nitrogen content, which is higher in the summer months (Edwards 1991, Emlen 1997, Holter and Scholtz 2007, Scholtz et al. 2009). When dung quality is low, more dung may need to be provided to the larvae by the female (Emlen 1997, Scholtz et al. 2009), and beetles may

have evolved activity patterns that support phenotypes through quality of dung (Scholtz et al. 2009). Variation in size and activity among species reinforces the importance of grazing and range management techniques as it pertains to the conservation of dung beetles (Anduaga 2004).

As others have shown, ranchers and range managers must be aware of the effect that insecticides aimed at the treatment of flies and parasites have on dung beetles. Acaracides, as well as chemicals such as Avermectin and Ivermectin, can reduce survival and be fatal to dung beetle larvae (Madsen et al. 1990, Krüger and Scholtz 1996, Scholtz et al. 2009). Organic ranching utilizes no pesticides or antibiotics that can pass through the system of livestock and potentially affect dung beetle communities. The non-target effects of veterinary pharmaceuticals will vary depending upon numerous factors (Kryger et al. 2005), and results from one ecosystem cannot necessarily be applied to other habitats and grazing systems (Scholtz et al. 2009). Based on beetle activity patterns, my results support previous findings that if treatment with parasiticides is necessary, it should be done at colder times of year during periods of dung beetle inactivity (Figs. 2.2 – 2.4). Additionally, ranchers should use products that are safer to dung beetle communities, such as dusts or sprays (Krüger and Scholtz 1996, Scholtz et al. 2009), whenever possible. For a thorough review of the non-target effects of various veterinary parasiticides, see Floate et al. (2005).

My results show that dung beetle abundance and species diversity are significantly higher on organically managed rangeland in western Nebraska. With all the observable benefits that can be attained, dung beetle conservation is of great importance to the agricultural community. Previous studies have shown that dung beetle abundance

and diversity can be increased by utilizing sound range management techniques (Anduaga 2004, Novelo et al. 2007, Giraldo et al. 2011). Because a congregated dung resource can increase dung beetle numbers and diversity (Hanski and Cambefort 1991, Barbero et al. 1999, Scholtz et al. 2009), I recommend utilizing organic farming and ranching techniques whenever possible, as well as implementing a monitored rotational or holistic resource management grazing system to maximize dung beetle abundance and diversity.

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**Table 2.1.** Total number of each dung beetle species captured by year and range management type. Org. = Organic, Conv. = Conventional.

Species	2009			2010			Both Years		
	Org.	Edge	Conv.	Org.	Edge	Conv.	Org.	Edge	Conv.
<i>Aphodius coloradensis</i>	56	4	2	100	10	0	156	14	2
<i>Aphodius distinctus</i>	92	8	1	273	51	3	365	59	4
<i>Aphodius erraticus</i>	10	0	0	18	0	0	28	0	0
<i>Aphodius fimetarius</i>	0	0	0	2	8	6	2	8	6
<i>Aphodius granarius</i>	13	0	0	19	0	0	32	0	0
<i>Aphodius prodromus</i>	1	0	0	7	0	0	8	0	0
<i>Aphodius testaceiventris</i>	0	3	0	3	4	0	3	7	0
<i>Aphodius walshii</i>	17	0	0	46	3	0	63	3	0
<i>Canthon pilularius</i>	18	13	0	35	21	1	53	34	1
<i>Copris fricator</i>	127	103	18	139	81	11	266	184	29
<i>Melanocanthon nigricornis</i>	4	16	0	9	14	0	13	30	0
<i>Onthophagus hecate</i>	293	341	76	423	338	115	716	679	191
<i>Onthophagus orpheus</i>	23	85	69	25	76	89	48	161	158
<i>Onthophagus pennsylvanicus</i>	525	518	36	650	533	53	1,175	1,051	89
<i>Phanaeus vindex</i>	153	110	0	206	140	0	359	250	0
Totals	1,332	1,201	202	1,955	1,279	278	3,287	2,480	480

**Table 2.2.** Mean capture ( $\pm$  SE) (per trap, per day) by range management type for dung beetle species with  $> 50$  individuals captured. Different letters indicate significant differences ( $p < 0.05$ , Kruskal-Wallis ANOVA, Tukey test post-hoc) among mean capture of each species.

Species	Organic	Edge	Conventional
<i>Aphodius coloradensis</i>	0.91 $\pm$ 0.21a	0.08 $\pm$ 0.03b	0.11 $\pm$ 0.01b
<i>Aphodius distinctus</i>	2.12 $\pm$ 0.35a	0.34 $\pm$ 0.08b	0.02 $\pm$ 0.01b
<i>Aphodius walshii</i>	0.37 $\pm$ 0.07a	0.02 $\pm$ 0.01b	0.0 $\pm$ 0.0b
<i>Canthon pilularius</i>	0.31 $\pm$ 0.05a	0.20 $\pm$ 0.03a	0.01 $\pm$ 0.01b
<i>Copris fricator</i>	1.55 $\pm$ 0.22a	1.07 $\pm$ 0.19a	0.17 $\pm$ 0.04b
<i>Onthophagus hecate</i>	4.16 $\pm$ 0.56a	3.94 $\pm$ 0.52a	1.11 $\pm$ 0.16b
<i>Onthophagus orpheus</i>	0.28 $\pm$ 0.04a	0.94 $\pm$ 0.16ab	0.92 $\pm$ 0.12b
<i>Onthophagus pennsylvanicus</i>	6.83 $\pm$ 0.90a	6.11 $\pm$ 0.89a	0.52 $\pm$ 0.09b
<i>Phanaeus vindex</i>	2.09 $\pm$ 0.19a	1.45 $\pm$ 0.16a	0.0 $\pm$ 0.0b

**Table 2.3.** Shannon-Weiner and Simpson's Diversity Indices for each range management type including the dominant species and percent of total capture.

Range Management Type	Shannon-Wiener Diversity Index	Simpson's Diversity Index	Dominant Species	% of Total Capture
Overall	4.33	4.42	<i>O. pennsylvanicus</i>	37%
Organic	4.43	4.78	<i>O. pennsylvanicus</i>	36%
Edge	3.52	3.64	<i>O. pennsylvanicus</i>	42%
Conventional	2.38	3.29	<i>O. hecate</i>	40%

### Figure Legend

**Fig. 2.1.** Mean ( $\pm$  SE) dung beetle capture (per day) by range management type during 2009 and 2010. Mean capture was statistically higher on the organic ranch than both the edge\* and the convention ranch\*\* ( $p < 0.05$ , Kruskal-Wallis ANOVA, Tukey test post-hoc). The edge\* was also statistically greater than the conventional ranch\*\* ( $p < 0.05$ ).

**Fig. 2.2.** Seasonal activity of *Aphodius* species dung beetles in 2010.

**Fig. 2.3.** Seasonal activity of *Onthophagus* species dung beetles in 2010.

**Fig. 2.4.** Seasonal activity of other collected dung beetle species in 2010.

Fig. 2.1.

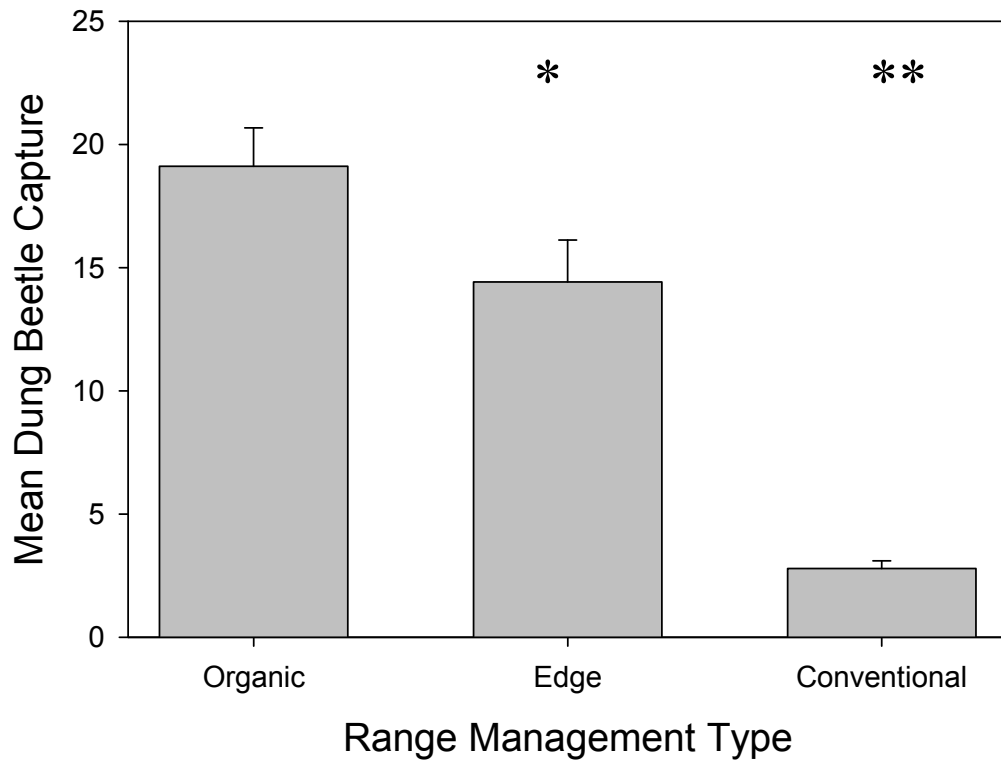




Fig. 2.3.

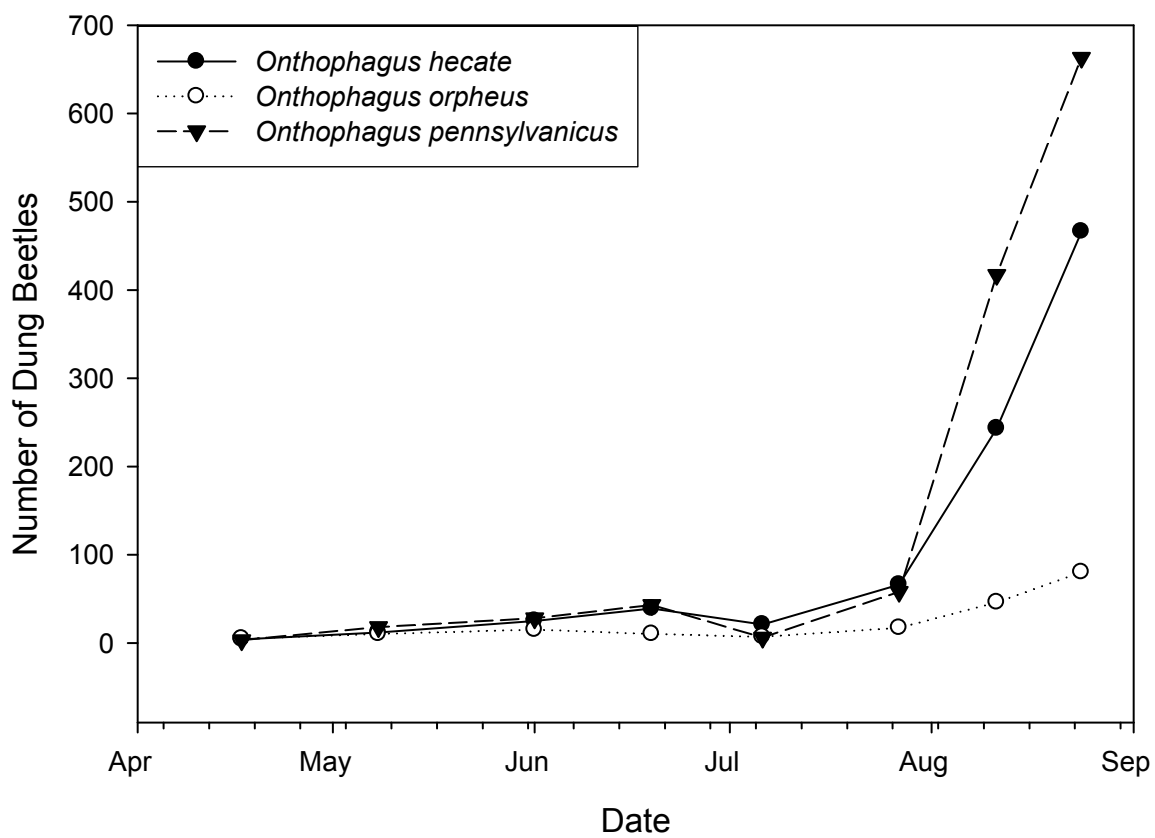
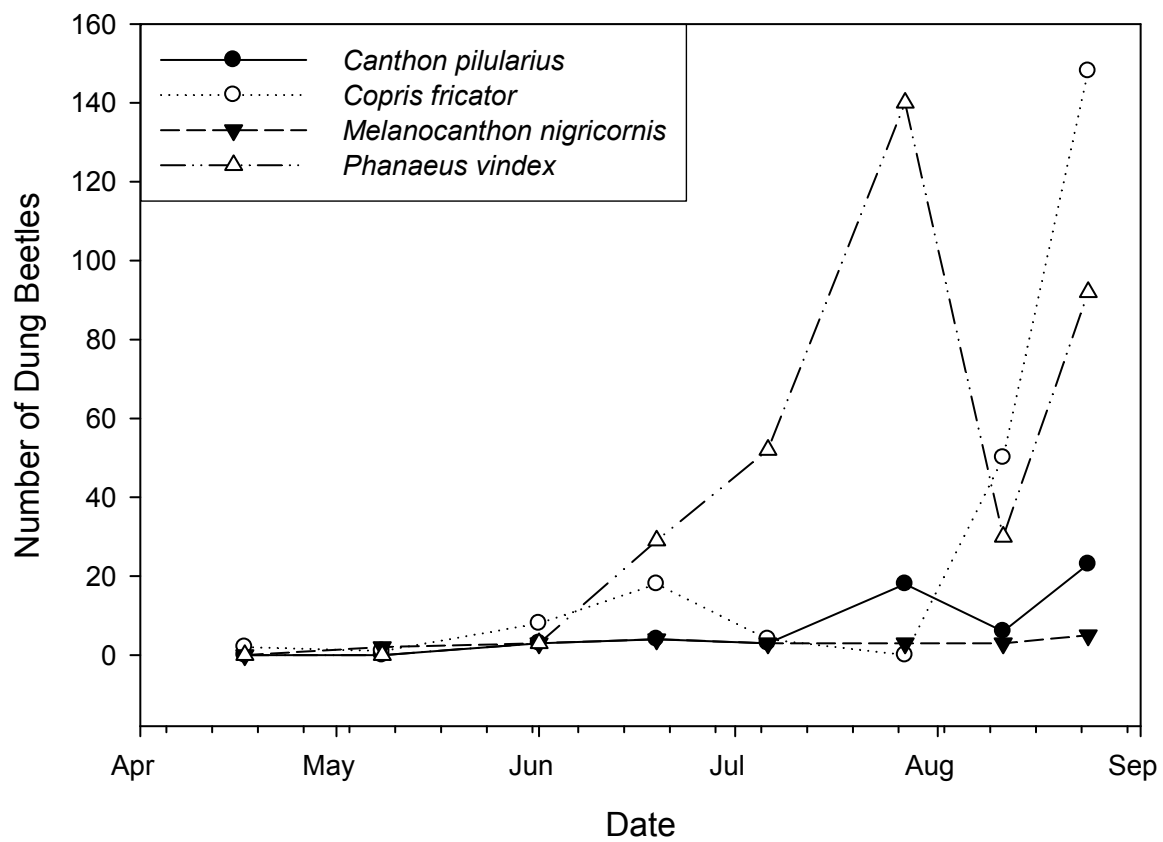


Fig. 2.4.





### **Chapter 3:**

## **A Comparison of Dung Beetle (Coleoptera: Scarabaeidae) Attraction to Native and Exotic Herbivore, Omnivore, and Carnivore Dung**

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### Abstract

Although the preference of dung beetles (Coleoptera: Scarabaeidae) for specific types and conditions of dung has been given substantial attention, little has been done to investigate the potential effects of exotic mammal introduction for game farms or re-wilding projects. I used pitfall traps baited with various native and exotic herbivore, carnivore, and omnivore dung to evaluate dung beetle preference. Additionally, I analyzed the nutrient quality of each dung type. A total of 9,089 dung beetles from 15 species were captured in two years of sampling. I found significant differences ( $p < 0.05$ ) in mean dung beetle capture among omnivore, herbivore, and carnivore dung, as well as differences in individual species preference for dung type. Omnivore dung was the most attractive with chimpanzee and human dung having the highest mean capture ( $291.1 \pm 27.6$  and  $287.5 \pm 28.5$  respectively). Carrion was also highly attractive with a mean of  $231.9 \pm 20.6$ . My results suggest definitive local preference of carrion in *Phanaeus vindex* Macleay and *Onthophagus hecate* (Panzer) ( $p < 0.05$ ), while the congener, *O. pennsylvanicus* (Harold), was rarely captured in carrion and highly preferred omnivore dung. Preference for a specific bait type does not appear to be correlated with dung quality or mammalian diet. Results suggest possible niche segregation between dung beetles in the Great Plains.

Key Words: Exotic species, feeding preference, niche partitioning, *Onthophagus*, *Phanaeus*

## Introduction

While most dung beetles are generalist dung feeders, specialization can occur as a result of competition and scarcity of dung resources (Halffter and Matthews 1966, Howden and Young 1981, Young 1981, Hanski 1989, Davis and Sutton 1997). Previous research indicates that dung beetles differ in their preference towards the condition (Doube 1987; Yasuda 1987) and odor (Dormont et al. 2004) of dung. Dung beetle fauna differ by habitat and soil type (Peck and Forsyth 1982, Doube 1983), as well as regional and seasonal activity pattern (Hanski and Koskella 1977, Hanski 1980, Holter 1982, Doube 1991). Although numerous studies have investigated feeding preference, the reasons for preferential feeding is not well understood (Holter 2000).

According to Scholtz et al. (2009), dung beetles have comparable ecological requirements, with the possibility existing for thousands of beetles from multiple species to arrive at a dung resource. Although there is a great deal of competition (Hanski and Cambefort 1991), dung beetle species vary markedly in their utilization of dung, and often there is a competitive hierarchy (Doube 1990, Scholtz et al. 2009). When native dung beetle faunas encounter dung from exotic animals, they may not respond. For example, European colonization of Australia in 1778 brought a variety of non-native herbivores and plant life (Hanski and Cambefort 1991). Overgrazing, as well as an excess of flies, midges, and parasites resulted (Bornemissza 1976, Hanski and Cambefort 1991). The native dung beetles, which had co-evolved with marsupials, did not adequately utilize bovine dung (Mathews 1972). Although many dung beetle species have adapted to quickly utilize large quantities of dung in a short period of time (Gillard 1967), some areas may not have sufficient populations of dung beetles to contend with

rising cattle numbers (Fincher 1975). Introduction of exotic mammals to North America as a result of exotic game farms may affect dung beetle assemblages. However, the identification variable dung attractiveness has received little attention in the Great Plains region.

It is possible that the introduction of exotic mammals has resulted in a shift in dung beetle species composition in the past 150 years. Historically, the Great Plains were largely inhabited by nomadic people, following herds of Bison and moving their living quarters to better hunting grounds (Van Every 1964). By the end of the 19<sup>th</sup> century, European settlers had colonized, bison were nearly exterminated, and much of the grassland was fragmented by agriculture (Jones 1968). The eradication of bison and prairie dogs has had negative impacts on insect communities because these mammals directly alter the habitat through their activity (Benedict et al. 1996, 2000). Herds of large mammals, such as bison, would normally congregate in an area, and after a limited stay, move on to new grounds (Scholtz et al. 2009). Thus, dung resources, both from bison and the nomadic humans who followed their movements, were different than those generated by practices for cattle and other domestic animals. Work by Barbero et al. (1999) revealed that land occupied by numerous species of livestock contained greater numbers and diversity of dung beetles, and the attraction of exotic dung types in the Great Plains region is of interest.

Agricultural practices are known to also affect dung beetles (Morris 1979; Rushton et al. 1989, Madsen et al. 1990, Morris and Rispin 1993, Di Giulio et al. 2001, Holter et al. 2002, Kruess and Tschardtke 2002). The nutritional quality of the dung from grazing mammals is also directly related to range health (Edwards 1991). Investigation

of the nutritional content of exotic mammal dung may help us understand how dung beetles react to novel potential food sources.

Nutritional content of dung will vary based upon numerous factors. Dung of herbivores is mainly comprised of cellulose, gut fragments, epithelium, and microbes (Hanski and Cambefort 1991, Scholtz et al. 2009). The concentration of microbes and fungi that inhabit the dung will increase as decomposition takes place (Hanski and Cambefort 1991, Scholtz et al. 2009). Because dung beetles are adapted to feed on liquid and small particles within the dung (Halfpter and Matthews 1966, Halfpter and Edmonds 1982), during times of low dung availability, they are also capable of feeding on other resources such as rotting fruit and carrion, which is also highly nutritious (Hanski and Cambefort 1991, Scholtz et al. 2009). Resources such as carrion present an opportunity for generalist dung beetles to obtain nutrition, be it from the decaying carcass or the gut contents, for survival and reproduction (Halfpter and Matthews 1966, Scholtz et al. 2009).

Dung quality, usually associated with nitrogen content, is influenced by an animal's food intake (Scholtz et al. 2009) and will likely be highest for temperate herbivores during the summer months (Edwards 1991, Holter and Scholtz 2007). As a result of a changing diet, there will be variation in dung quality, which is directly correlated with the size of larvae and resulting adult beetles (Emlen 1997, Scholtz et al. 2009). Beetles may compensate when dung quality is low by providing more dung to the larvae (Emlen 1997, Scholtz et al. 2009). Thus, if nutritional quality varies between native and exotic herbivores, carnivores, and omnivores, and attractiveness also varies between these dung types, the resulting preference for novel food sources could influence dung beetle community composition.

In this study, I used field sampling to determine the attractiveness of dung from native and exotic herbivores, carnivores, and omnivores, and provide nutritional analysis of the tested dung types. I tested the null hypotheses that there would be no difference in attraction between dung types or nutrient content among different types of dung.

## **Materials and Methods**

### **Field Study**

Field sampling took place from April through August of 2010 and 2011. The study site consisted of a large (> 4,000 ha) organic cattle ranch on the border of Banner, Morrill, and Cheyenne counties in western Nebraska (Latitude 41.469004, Longitude - 103.340270). At the time of study, the ranch was stocked with approximately 600 head of cattle, and a small number (< 20) of horses.

Pitfall traps (19 liter buckets) with soil in the bottom were baited using 113 grams of dung from various species of native and exotic mammalian herbivore, carnivore, and omnivore dung. Tested dung was from American bison (*Bison bison* L.), domestic pig (*Sus scrofa domestica* L.), Shiras moose (*Alces alces* L.), chimpanzee (*Pan troglodytes* Blumenbach), Bengal tiger (*Panthera tigris* L.), African lion (*Panthera leo* L.), cougar (*Felis concolor* L.), zebra (*Equus burchellii* Gray), waterbuck (*Kobus ellipsiprymnus* Ogilby), donkey (*Equus asinus* L.), and human (*Homo sapiens*) feces. Carrion, which preliminary data indicate to be highly attractive (Appendix A), was also used as a bait type and consisted of a whole rat (*Rattus norvegicus* L.) rotted in the sun for four days in a dark container. Rats weighed approximately 227 grams. Dung from animals was collected from Riverside Discovery Center in Scottsbluff, Nebraska. Feeding regiments

of the animals are reported in Appendix A. Only fresh dung (defecation observed) was used, and all dung was mixed and frozen, then thawed before use. Four traps per dung type (48 total) were randomized then baited for 24 hours and checked for three consecutive days biweekly starting in late April and ending in early August. All traps were spaced a minimum of 100 meters apart. All dung was replaced daily, and carrion was not replaced during a trap session.

Beetles were counted and identified to species (Ratcliffe and Paulsen 2008), and then released at a location approximately equidistant from all traps. Members of the Aphodinae occasionally needed collection and storage to identify under magnification. Voucher specimens were placed in the collection of the Department of Biology at the University of Nebraska at Kearney. Total capture and numbers of each species were compared across years by bait type (N=8) using SigmaPlot 3.1 software (Jandel Scientific, Corte Madera, CA) with the Kruskal-Wallis one-way analysis of variance, which analyzes differences in median values. A Tukey test was used when differences were detected among treatments.

### **Dung Quality**

Once dung was collected from each mammal and mixed to ensure homogeneity, frozen 113 gram sub-samples of each dung type were sent to Ward Laboratories in Kearney, Nebraska for nutrient analysis of pH, moisture, nitrogen, organic matter, ash content, sodium, zinc, iron, magnesium, manganese, copper, soluble salts, phosphorus, potassium, sulfur, calcium, and carbon to nitrogen ratio. Carrion was not included in analysis.

## Results

### Field Study

Sampling in 2010 and 2011 yielded a total capture of 9,089 dung beetles from 15 species. There were significant differences (Kruskal-Wallis ANOVA,  $p < 0.05$ ) in mean dung beetle capture among omnivores, herbivores, and carnivores (Table 3.1, Figure 3.1). Omnivore dung was the most attractive with chimpanzee, human, and pig dung having higher mean capture ( $291.1 \pm 27.6$ ,  $287.5 \pm 28.5$ , and  $75.9 \pm 9.6$ , respectively) than all other bait types ( $p < 0.05$ ) except tiger ( $42.0 \pm 10.4$ ), African lion ( $59.5 \pm 3.9$ ), zebra ( $44.9 \pm 5.3$ ), and carrion ( $231.9 \pm 20.6$ ) (Table 2.1). Carrion was more attractive than all types of herbivore dung except zebra ( $p > 0.05$ ). Carnivore dung was more attractive than dung from many herbivore species (Table 3.1, Figure 3.1), although mean capture of all three types of carnivore dung did not differ statistically from zebra dung. Bison dung was the least attractive; with only 38 beetles (mean  $4.8 \pm 0.9$ ) being captured from all samples (Table 3.1).

Although no differences were found when comparing overall mean capture within the native and exotic omnivore, carnivore, and herbivore groups, individual dung beetle species showed a high degree of variation in their attraction to dung types, as well as carrion (Table 3.1, Figure 3.2). All *Aphodius* dung beetles were captured in the highest numbers in omnivore dung, with *A. coloradensis* Horn and *A. distinctus* (Müller) both being statistically greater with chimpanzee and human dung ( $p < 0.05$ ) than moose, bison, and carrion bait types (Table 3.1). However, no differences in attraction were observed among dung types for *A. fimetarius* (L.), *A. granarius* (L.), *A. prodromus* (Brahm), or *A. testaceiventris* Fall.



*Canthon pilularius* (L.), *Copris fricator* (Fabricius), *Onthophagus hecate* (Panzer), *Melanocanthon nigricornis* (Say), and *Phanaeus vindex* MacLeay all were all caught in the highest numbers in carrion, with chimpanzee or human dung being second most attractive (Table 3.1). In the case of *P. vindex*, carrion was more attractive than all herbivore dung types ( $p < 0.05$ ); with 354 individuals captured on carrion while the next highest capture was chimpanzee dung accounting for only 92 total beetles. In contrast, *Onthophagus pennsylvanicus* Harold was more attracted to chimpanzee and human dung than carrion, as well as the dung of cougar, waterbuck, moose, donkey, and bison ( $p < 0.05$ ). Only 21 *O. pennsylvanicus* were captured in carrion traps compared to 1,108 in chimpanzee dung and 954 in human dung (Table 3.1, Figure 3.3). *Onthophagus orpheus pseudorpheus* (Howden and Cartwright) was also more attracted to human and chimpanzee dung than carrion ( $p < 0.05$ ) (Table 3.1).

### **Dung Quality**

Dung composition differed among herbivores, carnivores, and omnivores, as well as between exotic and native species. Nitrogen content (%) ranged from as low as 1.1 in zebra dung to 5.5 in human dung (Table 3.2). Organic carbon was also highest in human dung at 52.0%, while pig dung contained the lowest at 33.5% organic carbon. All omnivores and carnivores had lower C:N ratios than the herbivores tested. The ratio of carbon to nitrogen (C:N) varied from values of 9.1 in human dung to 33.1 in zebra. All measures of nutritional value and content of dung types are listed in Table 3.2.

## Discussion

This study reveals variation in the attraction of dung beetles to native and exotic omnivore, herbivore, and carnivore dung. My results support previous findings that omnivore dung is highly attractive when compared to that of herbivores and carnivores (Hanski and Cambefort 1991, Scholtz et al. 2009), although degree of attraction varied greatly between species (Table 3.1). This can largely be attributed to omnivore dung being more odiferous in comparison to that of a herbivore (Scholtz et al. 2009).

Although differences in nutrient content are apparent among dung types (Table 3.2), no trends in capture appear to be correlated with nutritional value (Table 3.1, Figure 3.1).

Dung nutrient content differed greatly between mammals tested (Table 3.2). Nitrogen content is typically viewed as an estimation of dung quality in mammalian herbivores (Edwards 1991, Holter and Scholtz 2007). Human feces had the highest percent nitrogen (5.74%), which would be expected given the attractiveness (Table 3.2). However, zebra dung, which was more attractive than other herbivores, had the lowest nitrogen concentration at 1.18%. Because nitrogen is influenced by ash content, nutritional value may be better approximated by carbon to nitrogen ratio (Holter and Scholtz 2007, Scholtz et al. 2009). Holter and Scholtz (2007) showed that ratios should be between 10-20 in order to be most advantageous for dung beetles. The lowest C:N ratio was observed in human dung at 9.1. However, no correlation can be drawn between nutritional quality and attractiveness in this study, as the next lowest ratio was African lion dung at 9.5 (Table 3.2).

Given the broad spatial and temporal distribution of a dung resource, as well as intense competition for food and space (Anderson and Coe 1974, Cambefort 1982, Peck

and Forsyth 1982, Hanski 1983, Janzen 1983, Doube 1987), utilization by dung beetles relies upon quickly locating a limited resource (Scholtz et al. 2009). Nearly all species collected were caught in the highest numbers in chimpanzee and human dung, or carrion bait types (Table 3.1), which is likely a function of odor. Human feces is known to attract many species of dung beetle (Hanski 1983, Howden and Nealis 1975). However, this does not explain the observed differences in dung beetle attraction between omnivore dung and carrion, or among carnivores and herbivores with similar diets (Table 3.1; Figures 3.1 and 3.2). Halffter and Mathews (1966) noted that carnivore dung was much less sought after than the feces of herbivores and omnivores. Although omnivore dung was generally most attractive, carnivore dung resulted in higher mean capture than nearly all herbivore dung types (Table 3.1, Figure 3.1).

My results support that most of the species captured are generalists of all dung (Ratcliffe and Paulsen 2008). Although many of the dung beetle species collected are known to be associated with carrion (Shea 2005, Price 2006, Ratcliffe and Paulsen 2008, Scholtz et al 2009), my results suggest local preference of carrion in *Phanaeus vindex* and *Onthophagus hecate* (Table 3.1). For *O. hecate*, this is contrary to findings by Price (2006), who noted a significant preference for dung over carrion in New Jersey. While *O. hecate* and *O. orpheus* were readily captured in carrion (Table 3.1, Figure 3.2), the congener, *O. pennsylvanicus*, was rarely captured in carrion (Table 3.1, Figure 3.2). These results suggest possible niche partitioning between *Onthophagus* species dung beetles.

Considering native and exotic dung, no definitive conclusions can be drawn from these data. Although the exotic dung from zebra was generally more attractive than other

herbivores (Table 3.1), it was not statistically more attractive than donkey feces, which is in the same genus (*Equus*) and common livestock in the Great Plains. Additionally, bison dung, which would have been exceedingly common in the region less than 150 years ago had the lowest capture of nearly all species collected (Table 3.1). It was surprising that native dung beetles, which coevolved with bison in this region (Van Every 1964, Jones 1968, Benedict et al. 1996; 2000, Ratcliffe and Paulsen 2008), showed little attraction to this dung type (Table 3.1). This suggests either adaptation to resource availability, or preference for a novel food source. This information holds further importance when considering that dung beetles can act as an indicator of change in an ecosystem (Davis et al. 2001).

It is also worth mentioning that because dung was collected from zoo animals, diet was nearly identical among herbivores and among carnivores from which dung was collected (Appendix B). Additionally, omnivores (chimpanzee and pig) were fed a mostly herbivorous diet. This reinforces that overall dung quality and attractiveness are also a function of inherent physiology, digestion, and bacterial microflora present within the mammal (Scholtz et al. 2009), not a result of food type alone.

My data indicate that dung beetle species in Nebraska differ in their attraction to mammalian dung and carrion, with many exhibiting strong preferences (Table 3.1; Figure 3.2). With exotic game ranches on the rise and further introduction of exotic megafauna being proposed in order to restore Pleistocene ecological potential (Rubenstein et al. 2006), dung beetle communities may be affected. More research is needed to identify specific preference for native and exotic dung types, and future studies should be aimed

at directly testing the effects of diet, nutritional value, and the correlation with dung beetle attraction.

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**Table 3.1.** Mean capture of each dung beetle species across all traps in 2010 and 2011 by bait type (N=8). Different letters indicate significance between dung types (Kruskal-Wallis ANOVA,  $p < 0.05$ ).

	Chimpanzee	Human	Pig	Tiger	African Lion	Cougar	Zebra	Waterbuck	Moose	Donkey	Bison	Carrion
<i>Aphodius coloradensis</i>	5.9±1.1ab	8.1±1.4a	3.3±1abcd	0.9±0.4bcd	2.8±0.5abc	0.4±0.2cd	1.0±0.4bcd	1.4±0.5abcd	0.3±0.2cd	0.5±0.2bcd	0.0±0.0d	0.8±0.3bcd
<i>Aphodius distinctus</i>	53.0±2.6ab	34.3±3.6a	11.4±2.1abc	4.4±1.3abcde	7.3±1.2abcd	5.0±1.3abcde	5.4±0.8abcde	1.9±0.5cde	2.1±0.5cde	3.9±0.8abcde	0.4±0.2e	0.6±0.3e
<i>Aphodius erraticus</i>	2.5±0.6a	1.4±0.5ab	0.4±0.2ab	0.3±0.2ab	0.5±0.3ab	0.3±0.2ab	0.1±0.3b	0.4±0.3ab	0.5±0.2ab	0.5±0.2ab	0.0±0.0b	0.4±0.2ab
<i>Aphodius fimetarius</i>	0.0±0.0a	0.3±0.2a	0.5±0.3a	0.0±0.0a	0.0±0.0a	0.0±0.0a	1.5±0.3a	0.1±0.1a	0.1±0.1a	1.9±0.5a	0.3±0.2a	0.0±0.0a
<i>Aphodius grenarius</i>	1.0±0.3a	0.9±0.4a	0.4±0.3a	0.5±0.3a	0.1±0.1a	0.5±0.3a	0.0±0.0a	0.4±0.2a	0.0±0.0a	0.4±0.2a	0.0±0.0a	1.0±0.3a
<i>Aphodius prodromus</i>	0.8±0.3a	1.1±0.3a	0.0±0.0a	0.3±0.2a	0.3±0.2a	0.0±0.0a	0.6±0.3a	0.0±0.0a	0.4±0.2a	0.1±0.1a	0.0±0.0a	0.5±0.2a
<i>Aphodius testaceiventris</i>	0.5±0.3a	0.8±0.3a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
<i>Aphodius walshii</i>	3.6±0.9a	3.0±0.7ab	0.8±0.5abc	0.3±0.2abc	1.1±0.6abc	0.3±0.2abc	0.3±0.2abc	0.0±0.0c	0.3±0.2abc	0.4±0.3abc	0.3±0.2abc	0.5±0.4abc
<i>Canthon pilularius</i>	7.4±1.4abc	7.5±1.4ab	1.9±0.5abcd	1.6±0.6bcd	2.4±0.9abcd	1.3±0.6bcd	1.0±0.3bcd	0.1±0.1d	0.4±0.2d	0.5±0.2d	0.1±0.1d	20.3±1.8a
<i>Copris fricator</i>	14.4±2.6ab	13.0±2.5abc	3.5±0.7abcde	2.5±0.8abcde	5.2±1.0abcd	2.1±1.0bcde	1.3±0.6de	0.1±0.1e	0.8±0.2de	2.6±0.8abcde	0.1±0.1e	17.5±3.1a
<i>Melanocanthon nigricornis</i>	6.9±1.5ab	9.4±2.6abc	2.1±0.7abcde	1.8±0.6abcde	2.9±0.4abcd	1.9±0.6abcde	1.3±0.3bcde	0.8±0.4de	0.6±0.3de	1.1±0.3bcde	0.0±0.0e	15.0±2.4a
<i>Onthophagus hecate</i>	61.0±6.6abc	74.3±7.1ab	21.4±3.0abcde	16.5±3.8bcde	21.5±2.1abcd	13.6±2.8bcde	12.9±2.0bcde	8.9±1.6de	4.4±1.2de	7.1±2.0de	2.9±0.8e	128.3±8.8a
<i>Onthophagus orpheus</i>	4.3±1.2abc	4.6±0.9ab	3.0±0.8abcd	1.0±0.5abcd	0.9±0.4bcd	0.1±0.1d	5.3±0.6a	0.4±0.2cd	0.9±0.2abcd	2.3±0.9abcd	0.2±0.2d	0.2±0.2d
<i>Onthophagus pennsylvanicus</i>	138.5±15.5a	119.3±17.2ab	25.1±3.7abc	10.6±3.1abcdef	11.9±0.7abcd	7.6±1.6cdef	13.9±2.7abcde	6.5±1.4cdef	4.1±0.9def	6.8±1.7cdef	0.5±0.3f	2.6±0.7def
<i>Phanaeus vindex</i>	11.5±1.9ab	9.6±1.7abc	2.3±1.1abcd	1.5±0.7bcd	2.9±0.4abcd	1.9±0.7abcd	0.5±0.3d	0.1±0.1d	0.0±0.0d	0.1±0.1d	0.0±0.0d	44.3±7.0a
total	291.1±27.6a	287.5±28.5ab	75.9±9.6abcd	42±10.4abcdfg	59.5±3.9abcdf	34.9±5.6cdefg	44.9±5.3abcdfg	21.0±3.6efg	14.8±1.9efg	28.1±5.4efg	4.8±0.9g	231.9±20.6abc

**Table 3.2.** Nutritional analysis of dung types used in field sampling.

Dung Type	Moisture (%)	Total N (%)	Organic N (%)	Organic C (%)	C:N ratio	Ash (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Sodium (%)	Zinc (PPM)	Iron (PPM)	pH	Dry matter (%)
Human	75.71	5.74	5.54	51.96	9.1	10.69	3.28	1.33	2.31	0.73	0.14	796.9	391.1	5.8	24.29
Chimpanzee	79.29	3.33	3.25	43.71	13.1	17.88	4.78	2.77	2.88	0.48	0.03	366	487.1	6.2	20.71
Pig	62.23	2	1.96	33.49	16.7	38.65	3.19	0.75	2.86	0.54	0.2	442	2449.3	6.7	37.77
Bengal Tiger	40.17	3.12	3	37.2	11.9	44.43	16.85	0.24	13.71	0.81	0.36	1240.2	2377.3	6.2	59.83
African Lion	69.43	4.15	4.03	39.48	9.5	32.77	4.96	0.46	5.37	0.43	0.49	427	2616.2	6.2	30.57
Moose	70.37	2.28	2.21	45.75	20.1	13.81	2.76	0.26	2.68	0.92	0.06	424	1172.4	6.9	29.63
Bison	74.14	1.44	1.41	37.84	26.3	28.96	1.24	0.45	2.99	0.46	0.09	71.8	1694.3	8.1	25.86
Zebra	76.88	1.12	1.08	37.23	33.2	29.55	1.27	2.11	0.64	0.2	0.23	126.2	2198.2	7.1	23.12
Waterbuck	70.39	1.88	1.84	42.89	22.8	19.55	2.85	1.99	1.65	0.41	0.21	231.5	932.9	6.8	29.61

### Figure Legend

**Figure 3.1.** Mean capture ( $\pm 1$  S.E.) of dung beetles during field sampling across all dates by dung type.

**Figure 3.2.** Mean capture of *Onthophagus hecate*, *O. orpheus*, and *O. pennsylvanicus* by bait type across traps in 2010 and 2011 (N=8).

Fig. 3.1.

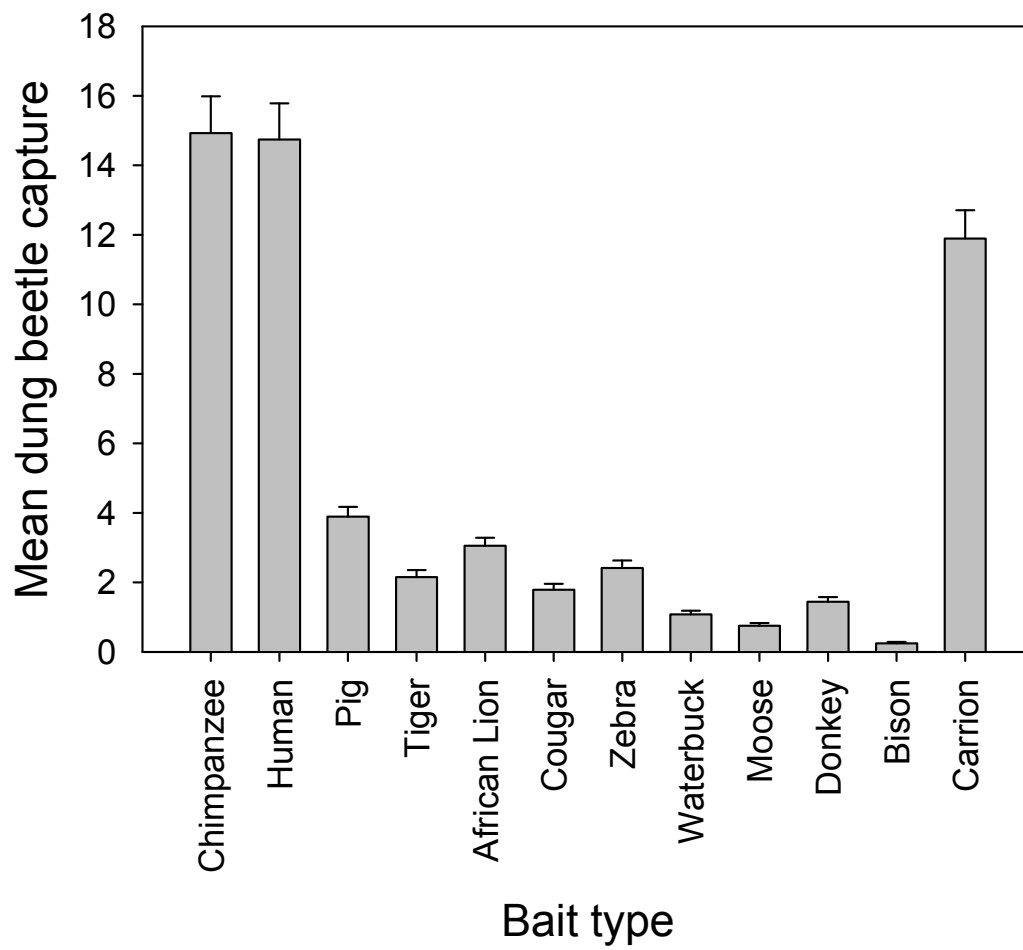
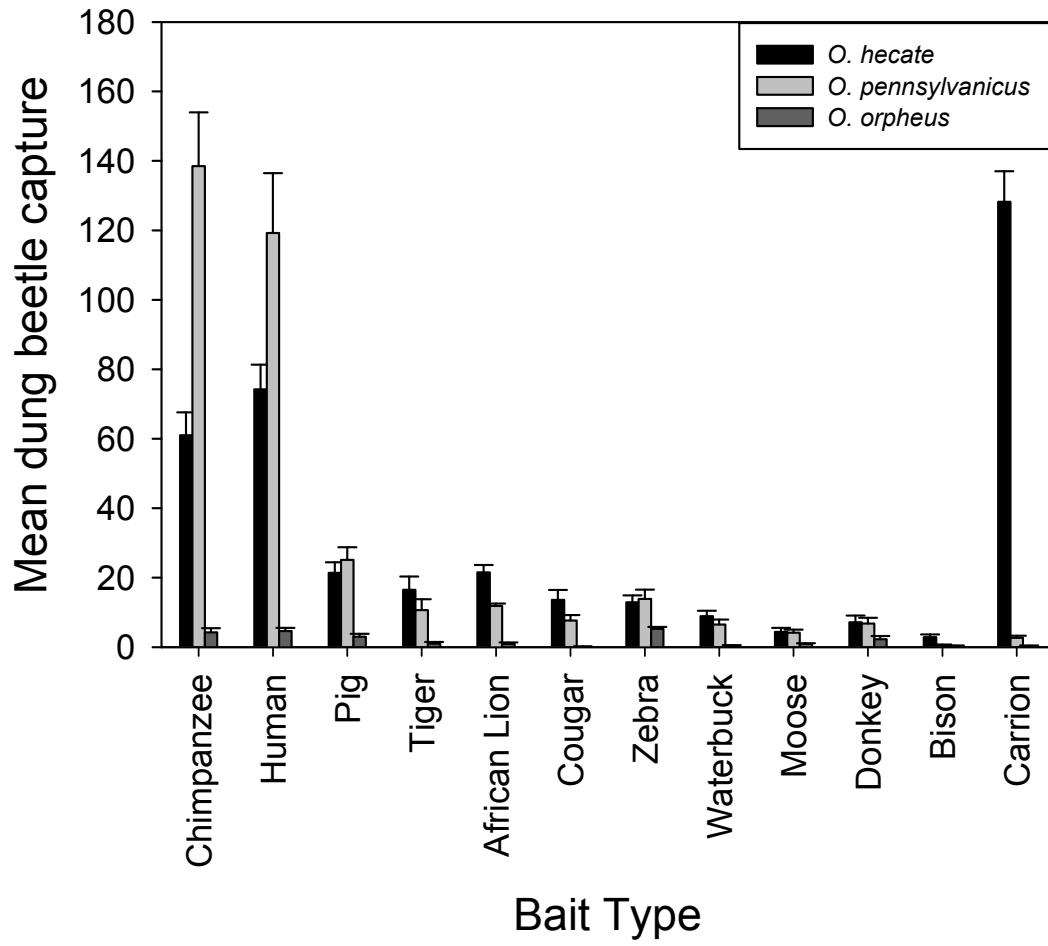


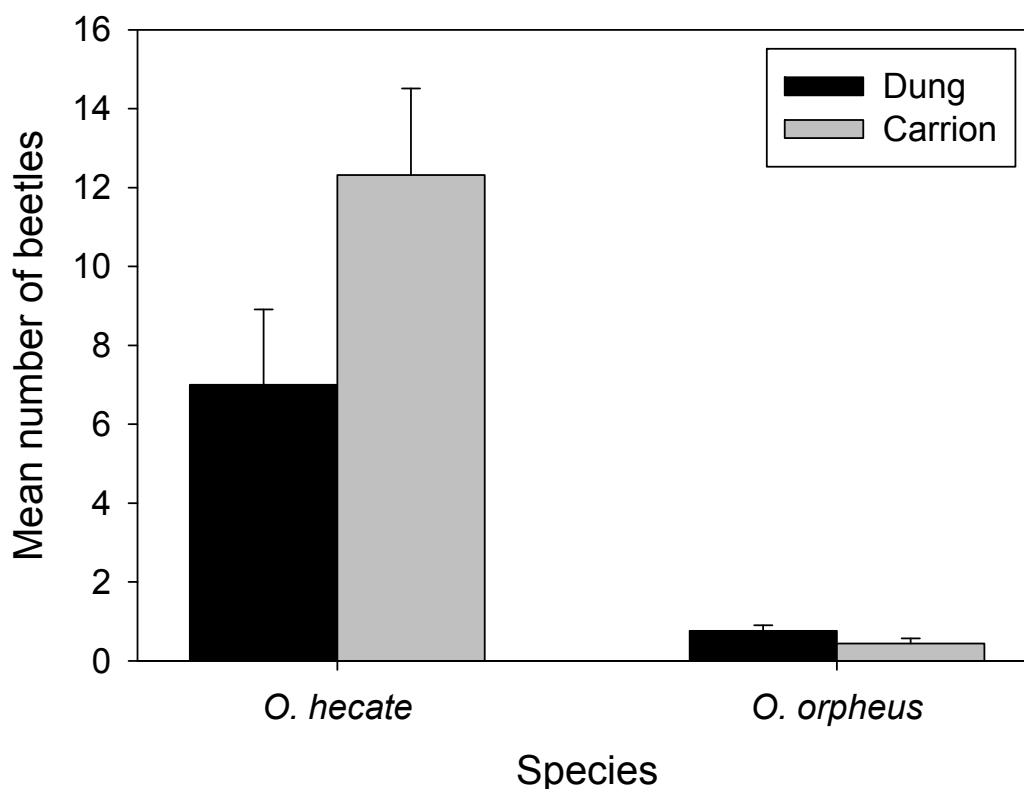


Fig. 3.2.



## Appendix A

Preliminary data was collected in the summer of 2009 by trapping a 32 km stretch of U.S. Highway 92 in western Nebraska. Ten 19 liter buckets were spaced three kilometers apart and baited with human feces or carrion (rotten rat), alternating bait between each trap. Traps were monitored daily for 10 consecutive days, switching bait (human feces, rotten rat) after five days. The two dung beetle species captured with frequency were *Onthophagus hecate* (Panzer) and *Onthophagus orpheus pseudorpheus* (Howden and Cartwright). There were 350 *O. hecate* captured on dung while 616 were captured on carrion. In the case of *O. orpheus pseudorpheus*, 38 were captured on dung compared to 22 on carrion. Significantly more (Mann-Whitney,  $p = 0.002$ ) *Onthophagus hecate* (Panzer) were captured at traps baited with carrion than traps baited with feces with a mean ( $\pm 1$  S.E.) of  $12.3 \pm 2.2$  compared to  $7.0 \pm 1.9$ . However, significantly more (Mann-Whitney,  $P = 0.021$ ) of the congener *Onthophagus orpheus pseudorpheus* (Howden and Cartwright) ( $0.76 \pm 0.14$ ) were collected with dung bait compared to carrion ( $0.44 \pm 0.13$ ).



## Appendix B

### Donkey

Winter: 1 c. ADF 16 (split between them)

### Pig

1.5 cups (.9 lbs.) Mazuri Mini Pig Elder BID  
 2 cups chopped assorted produce (winter only\*\*)  
 Alfalfa Hay a.m. and p.m.

### Chimpanzee

3 apples  
 3 cooked (soft) yams  
 3 bananas  
 3 oranges  
 1.5 onions (halved)  
 1.5 heads of romaine lettuce  
 6 carrots  
 2 scoops (2.5 cups) Mazuri leaf eater  
 1 scoop per chimp. Primate Basix PM

### Waterbuck

Winter: 1 scoop total AM ADF 16 grain / 2 scoops total PM ADF 16 grain  
 Summer: 1/2 scoop total AM ADF 16 grain/ 1 scoop total pm ADF 16 grain  
 Free choice grass hay

### Zebra

Summer: 3/4 scoop ADF 16 grain  
 Winter: 1 scoop ADF 16 grain  
 Free choice grass hay

<u>Tigers</u>	Mon.	Tues.	Wed.	Thurs.	Fri.	Sat.	Sun.
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(Beef)	12 lbs.	Fast	12	12	12	Fast	12
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### Cougars

(Beef)	3 lbs.	Fast	3	3	3	Fast	3
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**African lions**

(Beef)      12 lbs.    Fast    12      12      12      Fast    12

**Bison**

Free choice alfalfa hay  
1 scoop Mazuri ADF 16 each SID p.m.

**\*\*Winter feeding period is: 15 Sept – 31 Mar**

**Chapter 4:****Hypoxia Tolerance in Dung Beetles (Coleoptera: Scarabaeidae) Differs Among Species but Not Behavioral Groups**

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### Abstract

Dung beetles (Coleoptera: Scarabaeidae) are exposed to hypoxic conditions throughout their life cycle, including exposure to periods of severe hypoxia within dung pats and closed burrows as adults. Through exposure to hypoxic water (dissolved oxygen concentrations below 0.3 ppm), this study examined the hypoxia tolerance of five species of adult dung beetle (*Aphodius haemorrhoidalis* (L.), *Canthon pilularius* (L.), *Melanocanthon nigricornis* (Say), *Onthophagus hecate* (Panzer), and *Phanaeus vindex* MacLeay representing three behavioral groups (rollers, tunnelers, dwellers). Based upon nonoverlapping 95% confidence intervals, I found no differences in time to 50% mortality (LT<sub>50</sub>) among behavioral groups. Mean survival times ranged from 7-37 hrs with *P. vindex* having the shortest LT<sub>50</sub> of 7.87 hrs and *A. haemorrhoidalis* the longest at 37.04 hrs. *M. nigricornis* had an LT<sub>50</sub> of 19.06 hrs, which was significantly shorter than *A. haemorrhoidalis*, as well as *C. pilularius* and *O. hecate*, which had LT<sub>50</sub> values of 36.53 and 34.14 hrs, respectively. These results suggest that hypoxia tolerance in dung beetles is governed by more than life history, and is likely a consequence of numerous ecological and physiological factors.

Key Words: Anoxia, behavioral group, immersion, LT<sub>50</sub>, survival time

## Introduction

Dung beetles (Coleoptera: Scarabaeidae) are divided into three behavioral groups: Rollers, tunnelers, and dwellers (Hanski and Cambefort 1991). Rollers are characterized by the female fashioning a brood ball which the male and female roll away from the dung pat. A single egg is deposited in the brood ball where the larva develops through its instars. Tunnelers bury dung into the soil directly beneath the dung pat to lay their eggs while dwellers deposit their eggs into the main dung pat where they will develop into adults (Hanski and Cambefort 1991).

Depending upon behavioral group, adult dung beetles will be exposed to differential degrees and duration of hypoxia (Duncan and Byrne 2000, Scholtz et al. 2009). Oxygen concentrations can be as low as 1-2% within a dung pat (Holter 1991), and some adult dung beetles are able to continue movement and sustain respiration rate at these concentrations (Holter and Spangenberg 1997). Additionally, CO<sub>2</sub> and methane increase the hypoxic conditions within the dung (Holter 1994, Holter and Spangenberg 1997, Scholtz et al. 2009). Female dung beetles may spend several months in hypoxic conditions while brooding within closed burrows (Duncan and Byrne 2000, Scholtz et al. 2009). Although dung beetles spend a great deal of time in hypoxic conditions, few direct tests of hypoxia tolerance have been conducted (Scholtz et al. 2009).

Closely related species often differ in their ability to survive hypoxic conditions, which appears to be associated with ecology (Hoback 2011). Based upon frequency of exposure to flooding, individual larvae and adult *Cicindela hirticollis* Say tiger beetles from two populations differ in their ability to withstand hypoxia by 40% and 400%, respectively (Brust et al. 2005, Brust and Hoback 2009). Brust et al. (2007) examined

hypoxia tolerance of nymphal and adult grasshoppers and found survival to range from 3 to 21 hours. Many terrestrial insects display behavioral changes when exposed to hypoxia, and eventually fall quiescent, exhibit metabolic depression, and utilize anaerobic metabolism (Hochachka et al. 1993, Wegener, 1993, Hoback et al. 2000). This has not been examined in dung beetles, and there has been little evidence to suggest they undergo anaerobic metabolism (Holter and Spangenberg 1997).

Behavioral adaptations by dung beetles in order to avoid the intense inter and intra-specific competition for food and space (Hanski and Cambefort 1991, Scholtz et al. 2009) would exert variable selection pressures to cope with hypoxia, water loss, and metabolic constraints (Holter 1991, Holter and Spangenberg 1997, Duncan and Byrne 2000). Ball-making by rollers may expose the adult beetles to hypoxic condition for several minutes (Tribe 1976), or even hours (Osberg 1988). Tunnelers, and especially dwellers, would spend a larger amount of time immersed in dung, although the degree of hypoxia and hypercapnia will vary depending upon location within the dung pat (Holter 1991, Holter and Spangenberg 1997, Scholtz et al. 2009).

In this study, I tested the hypoxia tolerance of adult dung beetles from all three behavioral groups. I subjected the beetles to immersion in hypoxic water (Hoback et al. 1988, Brust and Hoback 2009) to test the null hypothesis that there is no difference in hypoxia survival time among dung beetle behavioral groups.

### **Materials and Methods**

Species chosen for study were the dweller *Aphodius haemorrhoidalis* (L.), the rollers *Canthon pilularius* (L.) and *Melanocanthon nigricornis* (Say), and the tunnelers



*Onthophagus hecate* (Panzer), and *Phanaeus vindex* MacLeay (Ratcliffe and Paulsen 2008). Adult dung beetles were collected using 18.9 liter buckets as pitfall traps baited with carrion (rotten rat) or human feces. Collection locations and number of individuals tested are specified in Table 4.1. Specimens were kept in damp sandy loam substrate for no more than 72 hrs prior to experimentation.

Beetles were exposed to severely hypoxic water following the methods of Hoback et al. (1998). Nitrogen gas was run through an air stone and bubbled in spring water for approximately 5 minutes per liter of water. This process renders the water extremely hypoxic, with dissolved oxygen concentrations below 0.3 ppm (Brust and Hoback 2009). Individual adults were placed in 20-ml screw cap glass vial to which the deoxygenated water was added. To prevent individuals from floating to the top, small pieces of tissue paper were added. In addition, glass vials were tapped lightly to release any air adhering to the glass vial or the beetles. Vials were then placed in a Percival® environmental chamber (Percival Scientific, Inc., Perry, IA) at 20°C in constant darkness.

Specimens were removed from hypoxic water in subsets of 5, 10, or 20 individuals (Table 4.1) at 6, 12, 18, 24, 30, 36, 42, 48, and 56 hrs. Controls consisted of individuals of each species (N=5) in 20-ml screw cap vials with a moist tissue, and were checked at each time interval that subsets of submerged individuals were removed. Specimens were placed in plastic containers and returned to the environmental chamber. Individuals were given 24 hrs to recover. A full recovery was defined by walking upright within the allotted recovery time.

Data were analyzed using Toxstat 3.4 software (Western Ecosystems Technology, Inc., Cheyenne, WY), which utilizes probit analysis to calculate the time to 50%

mortality (LT<sub>50</sub>) as well as 95% confidence intervals. Nonoverlapping 95% confidence intervals served to determine significant differences between survival times (Hoback et al. 1998, Brust and Hoback 2009).

## Results

Hypoxia tolerance of adult dung beetles varied between the species tested, and had mean survival times between 7 and 37 hrs (Figure 4.1). *A. haemorrhoidalis* survived the longest with an LT<sub>50</sub> of 37.04 hrs. Based on nonoverlapping confidence intervals, there was no significant difference between the survival times of *A. haemorrhoidalis* and *C. pilularius* or *O. hecate*, which had LT<sub>50</sub> values of 36.53 and 34.14 hrs, respectively. *M. nigricornis* had an LT<sub>50</sub> of 19.06 hrs, which was significantly shorter than *A. haemorrhoidalis*, *C. pilularius*, and *O. hecate*. The shortest survival time was observed for *P. vindex* with an LT<sub>50</sub> of 7.87 hrs, which was significantly lower survivorship than all other species. No control specimens perished during experimentation.

## Discussion

Although dung beetles differ in the duration and degree of exposure to hypoxic conditions (Holter 1991, Duncan and Byrne 200, Scholtz et al. 2009), mean survival times (LT<sub>50</sub>) do not correlate directly with behavioral group (Figure 4.1). Although the dweller *A. haemorrhoidalis* survived the longest, there was no significant difference between this species and the roller *C. pilularius*, or the tunneler *O. hecate*. Additionally, LT<sub>50</sub> was significantly different between the two rollers (*C. pilularius* and *M. nigricornis*) as well as the two tunnelers tested (*O. hecate* and *P. vindex*) (Figure 4.1).

Larger species are typically able to survive longer periods of hypoxia, potentially as a result of the tracheae being able to hold more air (Brust et al. 2007, Brust and Hoback 2009), although results have been mixed (Hoback et al. 2000). This is not the case in the dung beetle species tested, as the smallest species (*A. haemorrhoidalis*) had the longest survival time while the largest species (*P. vindex*) had the shortest survival (Figure 4.1). Increasing levels of atmospheric oxygen have been positively correlated with body size in both mealworms (*Tenebrio molitor* L.) and fruitflies (*Drosophila melanogaster* Meigen) (Schmitz and Harrison 2004). Larger insects invest a greater proportion of metabolic and gas exchange rates relative to their size (Harrison et al. 2010). Graham et al. (1995) speaks on the challenges larger insect may have with oxygen diffusion. Due to the extended length of their tracheae, oxygen delivery may prove to be difficult. When reared in hypoxic conditions, insects (including members from Tenebrionidae and Scarabaeidae) compensate for the lack of oxygen by expanding tracheal diameter and the amount of tracheoles (Harrison et al. 2010). In addition to the difficulties prompted by diffusion, there is a higher demand for metabolic energy due to the larger size of the insect. In compensation there is a greater regulation of metabolic enzymes responsible for basic maintenance (Harrison et al. 2010).

Previous work on dung beetles in hypoxic environments has shown discontinuous gas exchange (DCG) to be exhibited (Holter 1991, Chown and Holter 2000, Scholtz et al. 2009). Chown and Holter (2000) found that under increasingly hypoxic conditions, *Aphodius fossor* (L.) switched from discontinuous gas exchange to a mode of continuous diffusion below 2.84% oxygen concentration. There has been little evidence to support that dung beetles display anaerobic metabolism under hypoxic conditions (Holter and

Spangenberg 1997). It may be possible that *P. vindex* is unable to achieve metabolic depression to the degree of other species tested. The ability to survive hypoxic conditions is strongly correlated with the ability to depress metabolism (Hand 1998, Hoback et al. 2000, Brust et al. 2006). Hoback et al. (2000) found that differences in survival of the tiger beetles *Cicindela togata* LaFerte and *Amblycheila cylindriformis* Say were related to the capacity for metabolic depression and quiescence. Under anoxic conditions, *A. cylindriformis* displayed simultaneous behavioral quiescence and reduction of ATP levels, while maintaining a higher metabolic rate than *C. togata* (Hoback et al. 2000). This same trend is seen in the grasshopper species *Schistocerca gregaria* (Forsk.) (Hochachka et al. 1993), as well as *Locusta migratoria* (L.) (Wegener 1993), which exhibit anaerobic metabolism under anoxic conditions. Neither species of grasshopper were able to survive longer than 6-8 hours, which is similar to the  $LT_{50}$  observed in *P. vindex*.

One possible explanation for the observed differences in hypoxia tolerance among dung beetles is burial strategy and location. The tunneler *P. vindex* burrows at the edge of dung pats (Fincher 1972, Ratcliffe and Paulsen 2008), which would be less hypoxic than other areas in the center of the dung pat (Holter 1991). The other tunneler tested in this study, *O. hecate*, digs vertical burrows directly beneath the dung pat (Ritcher 1966, Ratcliffe and Paulsen 2008), and may require more tolerance to hypoxic conditions. Depth of burial may also be a factor, as Anderson and Ultsch (1987) showed that oxygen levels near the soil surface are similar to atmospheric conditions. Additionally, *P. vindex* overwinters as either larvae or adults (Ratcliffe and Paulsen 2008), which may influence hypoxia tolerance as a result of time spent in low oxygen environments while in the

developmental stages. However, Brust and Hoback (2009) found no difference in hypoxia tolerance of adult tiger beetles (Coleoptera: Carabidae) based upon timing of development or life stage in which they overwinter.

The biology of *M. nigricornis* is not well known; however, it is found readily in sandy areas of the Nebraska Sandhills (Ratcliffe and Paulsen 2008). *P. vindex* is also common in sandy areas (Ratcliffe and Paulsen 2008, Price and May 2009). Among most plausible terrestrial habitats, subterranean soil habitats offer the greatest potential for hypoxic conditions (Schmitz and Harrison 2004). Oxygen and CO<sub>2</sub> diffuse more easily through sand than other soil types (Scholtz et al. 2009). *M. nigricornis* and *P. vindex* exhibited the lowest hypoxia survival times (Figure 4.1), which may indicate that species common to sandy areas are not exposed to severe hypoxia while in burrows and therefore do not require hypoxia tolerance to the degree of other species tested. However, all species tested in this study are habitat generalists, and can be found over a broad geographic range and diverse areas within the United States (Ratcliffe and Paulsen 2008).

Feeding preference may have a great deal to do with hypoxia tolerance in dung beetles. A dung resource is often defined as “patchy” (Scholtz et al. 2009), and generalist dung beetles must quickly secure a food source to avoid competition, desiccation, and colonization by flies (Scholtz et al. 2009). Although the dung beetles tested in this study are generalist feeders, it is well documented that beetles in the genus *Phanaeus* readily feed upon vertebrate carrion (Price and May 2009), which is a feeding environment that may prevent the *P. vindex* from being exposed to prolonged periods of hypoxia. Additionally, *M. nigricornis* is known to roll the fecal pellets of rabbits (Ratcliffe and Paulsen 2008), as well as fungi and vertebrate carrion (Williams and Kriska 2001), which

would also limit their exposure to the hypoxic conditions common within a dung pat (Holter 1991). Adult dung beetles may be able to sense oxygen availability in order to avoid mortality (Hoback 2011).

As suggested by Brust and Hoback (2009), the propensity for prolonged hypoxia survival may be a conserved ancestral trait that provides a selective advantage to insects exposed to variable degrees and durations of hypoxia. The results of this study indicate differences in hypoxia survival time that do not appear to be correlated with behavioral group. More research is needed across genera and over a broad geographic range (Hoback 2011) to explore the correlation between dung beetle ecology, physiology, life history, and hypoxia tolerance. This may be of particular interest in the regions of Nebraska, which are divided by geology, soil type, climate, and vegetation communities (Omernik 1987, 1995), largely as a result of differences in rainfall (Johnsguard 2001).

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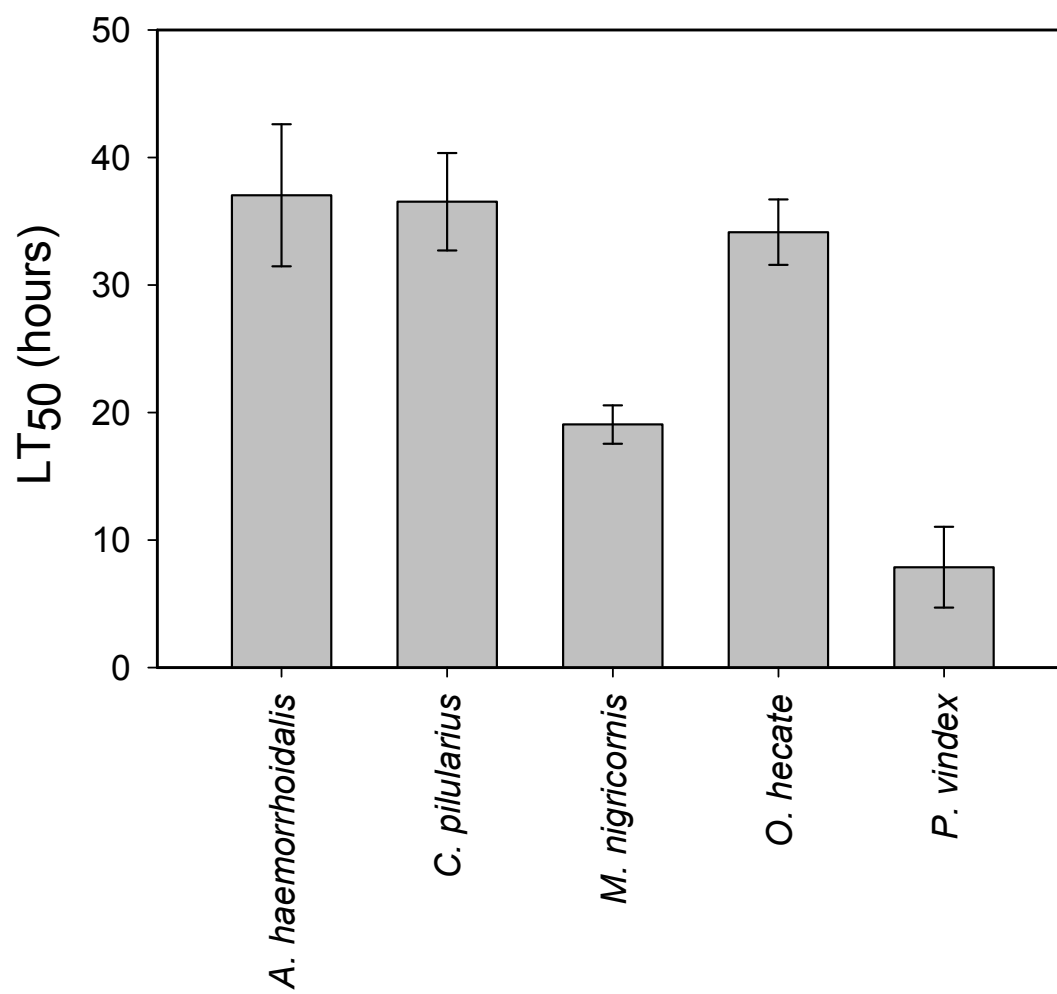
**Table 4.1.** Collection location, behavioral group, and number of each species of adult dung beetle tested for hypoxia tolerance.

<b>Species</b>	<b>Behavioral Group</b>	<b>Location</b>	<b>Total no. tested</b>	<b>Controls</b>
<i>Aphodius haemorrhoidalis</i> (L.)	Dweller	Scott County, MO	45	5
<i>Canthon pilularius</i> (L.)	Roller	Cherry County, NE	45	5
<i>Melanocanthon nigricornis</i> (Say)	Roller	Holt County, NE	180	5
<i>Onthophagus hecate</i> (Panzer)	Tunneler	Dawson County, NE	180	5
<i>Phanaeus vindex</i> MacLeay	Tunneler	Cherry County, NE	45	5

### Figure Legend

**Figure 4.1.**  $LT_{50}$  and 95% confidence intervals for adult dung beetles exposed to hypoxic water (< 0.3 ppm) at 20° C. Confidence intervals are depicted by error bars.

Figure 4.1.



**Chapter 5:****Genetic Variability, Body Size, and Sex Ratios of *Digitonthophagus gazella* (F.)  
(Coleoptera: Scarabaeidae) from Puerto Rico and South Africa**

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### Abstract

*Digitonthophagus gazella* (F.) has been widely introduced to the New World and both natural dispersal and intentional releases continue. In this study, I compared a population of *D. gazella* from South Africa and from the island of Vieques, Puerto Rico using amplified fragment length polymorphisms (AFLP) analysis, body size measures, and sex ratios. Both Vieques, Puerto Rico and South African populations had a majority of females with sex ratios of 2.5:1 and 3.4:1, respectively. Between populations, mean female width was significantly narrower ( $p < 0.05$ ) in the Vieques population, suggesting possible differences in dung quality and availability. Genetic diversity was found to be high in both South Africa ( $H = 0.3623$ ) and Vieques ( $H = 0.3846$ ), providing no evidence of inbreeding depression on Vieques. Analysis of molecular variance (AMOVA) revealed that 69% of genetic diversity is within the populations and 31% of genetic diversity is between the populations, indicating that if interbreeding occurs between these populations, it is rare. The  $F_{st}$  value of 0.3143 also suggests that there is genetic isolation between populations in Africa and newly established populations in the New World. Because of its competitiveness and natural dispersal ability, additional comparisons of *D. gazella* populations, biology, and genetics are warranted.

Key Words: AFLP, dung beetles, gene flow, population genetics



## Introduction

The Afro-Asian dung beetle *Digitonthophagus gazella* (F.) was introduced into North America via Texas in 1972 (Peck 2011), with subsequent introductions in the 1970s to other states including Arkansas, California, Georgia, and Mississippi (Fincher 1981). Within 12 years the beetle had spread 700 km by natural means (Fincher et al. 1983, Kohlmann 1991). It currently ranges south into Mexico, and Central America (Hoebeke and Beucke 1997), with rapid expansion occurring in South America, the Caribbean, and West Indies (Fincher 1981, Fincher et al. 1983, Kohlmann 1991, Miranda et al. 2000, Ivie and Philips 2008, Matavelli and Louzada 2008, Peck 2009; 2011). *Digitonthophagus gazella* is highly mobile and has been known to disperse great distances, traveling as much as 29 km over open ocean (Scholtz et al. 2009).

Vieques, Puerto Rico is a 135 square km island located approximately 11 km east of the main island of Puerto Rico (Singer 2004). Although *D. gazella* was collected from Vieques in 2005 (W. W. Hoback, personal observation), it was not recorded by Peck (2009, 2011), and neither timing nor means of introduction is known. The source population of *D. gazella* on the island of Vieques (North America or South America) is also in question. With the dispersal capabilities and high reproductive output of *D. gazella* (Hanski and Cambefort 1991, Montes de Oca and Halffter 1995, Scholtz et al. 2009), the question of gene flow and genetic variability as a result of geographic and reproductive isolation is of interest.

With European settlement of Vieques in 1524 (Wetmore 1916), domestic livestock were introduced and are the only large mammalian herbivores present in Vieques. Livestock include Paso Fino horses introduced from Spain by Juan Ponce de

León (Singer 2004), and extensive cattle operations in the 1940s and 1950s after U.S. Navy expropriation (Casas and Fresneda 2006). Today, horses are still common, but few cattle operations remain on the island. Vieques is much different than *D. gazella*'s native range in Africa, which has the greatest diversity of both herbivorous mammals and dung beetles (Scholtz et al. 2009).

Although many dung beetles are generalist feeders, specialization is possible as a result of reduced dung availability (Halffter and Matthews 1966, Howden and Young 1981, Young 1981, Hanski 1989, Davis and Sutton 1997). Preliminary observations on the island of Vieques show *D. gazella* primarily feeds upon horse dung. Behavioral adaptation combined with high degrees of geographic and reproductive isolation may lead to large amounts of genetic variation between distant populations (Hedrick 2000). This could be especially important in Vieques considering *D. gazella* may outcompete the native dung beetle fauna of West Indian islands (Ivie and Philips 2008).

Amplified fragment length polymorphism (AFLP) is a molecular genetic technique that creates a large number of markers by using an organism's entire genome. Whole genomic DNA is first cleaved with restriction enzymes. Short segments of DNA are ligated to the sticky ends of the restriction fragments. The fragments are then amplified using primers that correspond to the sequences of the adapters. This technique was first developed by Vos et al. (1995) and has since been used in a wide variety of studies to analyze gene flow and genetic differentiation (Martinelli et al. 2007, Serikawa 2007). Here, I used AFLP analysis to compare the genetic variability of *D. gazella* on the island of Vieques, Puerto Rico to a population in the beetle's native range of South Africa.

## Materials and Methods

*Digitonthophagus gazella* specimens were collected from the island of Vieques, Puerto Rico in July, 2008 and 2010, as well as from Borakalalo National Park within the North West Province of South Africa (S25.2758 E27.7776) in January, 2011. Collection was done by actively searching dung pats. Collected beetles were sexed and measured for length (tip of clypeus to apex of elytra) and width (across humeri) using digital calipers (Ratcliffe and Paulsen 2008). Beetles were then transferred into 95% ethanol. Upon reaching the laboratory samples were stored at -80°C. Length and width measurements within gender and between years and populations were analyzed utilizing a One-Way Analysis of Variance (ANOVA) with a Tukey test as a post-hoc (SigmaPlot 3.1).

### DNA Extraction

Before the DNA extraction process was initiated, specimens were first washed in 70% ethanol and then in nanopure water. DNA was extracted from the head and thorax. DNA was isolated from dung beetle specimens using a cetyl trimethylammonium bromide (CTAB) method modified from Doyle and Doyle (1987). Extracted DNA was suspended in 50 µl 1x TE buffer (10 mM Tris-HCL; 0.1 mM EDTA) and stored at -20°C. DNA concentration and purity was determined using the Nanodrop 2000 (Thermo Scientific, Wilmington, DE). The AFLP process was initiated using a template concentration of 100-300 ng/µl.

### Amplified Fragment Length Polymorphism

The AFLP procedure used was adapted from Vos et al. (1995) and Lindroth (2011) (Appendix C). The AFLP process consisted of four basic steps. DNA was first

digested with *EcoRI* and *MseI* restriction enzymes. Short oligonucleotides were then ligated onto the sticky ends of the resulting fragments of DNA. The resulting fragments were then amplified non-selectively using primers that match the adapter sequences. After pre-amplification, the DNA was selectively amplified using primers with a three basepair extension sequence in addition to the adapter sequence. The resulting PCR product was run on a 6.5% polyacrylamide gel and visualized via infrared laser scanner (LI-COR Biosciences, Lincoln, NE). The sequences of all adapters and primers are given in Table 5.1. A total of 29 beetles from Vieques, Puerto Rico (all from 2010 sampling period) and 28 beetles from South Africa were analyzed using 136 AFLP markers from primer pairs M-CAC + E-ACG (53-455 bp) and M-CAC + E-ACT (75-430 bp).

#### **Data Scoring and Analysis**

An IRD-700 labeled 50-700 bp size standard was used to calibrate the gels. Gels were scored using the program SAGA MX 3.2 (LI-COR Biosciences, Lincoln, NE). The data were converted to a Boolean vector for further analysis, with a “1” indicating band presence and a “0” indicating absence.

DBOOT v. 1.1 (Coelho 2001) was used to determine whether the number of loci used was sufficient to explain the genetic variation among *D. gazella* subpopulations. The population genetics software Popgene v. 1.32 (Yeh and Boyle 1997) was utilized to assess genetic diversity at the subpopulation level with assumed Hardy-Weinberg equilibrium. The percentage of polymorphic loci and Nei's Gene Diversity were calculated for each location. The Popgene software was also used to estimate  $G_{st}$  (Nei 1973).

The software package Arlequin v. 3.5 (Excoffier et al. 2005) was used to conduct the analysis of molecular variance (AMOVA) as well as for calculation of  $F_{st}$ , a measure of genetic differentiation. The AMOVA tested for genetic structure between and within subpopulation levels. Significance testing was accomplished by running 1,023 permutations of the data.

## Results

### Body Size Measurements and Sex Ratios

A total of 98 beetles (70 females, 28 males) were collected and measured from Vieques, Puerto Rico and 35 beetles (27 females, 8 males) from South Africa (Table 5.2). Mean female width was significantly different between beetles from Vieques in 2008 ( $5.72 \text{ mm} \pm 0.08$ ) and South Africa ( $6.11 \text{ mm} \pm 0.09$ ) ( $p < 0.05$ , One-Way ANOVA, Tukey test post-hoc) (Table 5.2). There were no statistical differences in female length, male length, or male width between *D. gazella* populations from Vieques and South Africa (Table 5.2). More females were collected in both populations, with an overall sex ratio of 2.5:1 in Vieques (2.9:1 in 2008, 1.8:1 in 2010) and 3.4:1 in South Africa (Table 5.2).

### AFLP Analysis

When the coefficient of variation was calculated (Coelho 2001), it was determined that the markers account for more than 93% of genetic variation within these beetle populations (Fig. 5.1). Nei's gene diversity and the number of polymorphic loci were high for both populations (Table 5.3). The South African population had a slightly higher level of polymorphic loci (99%) than the Vieques population (96%). However,

Nei's gene diversity was slightly higher in the Vieques population (0.3846) than in the South African population (0.3623).

Analysis of molecular variance revealed that the majority of variation is within populations (68.57% of variation within populations and 31.43% of variation between the two populations) (Table 5.4). As calculated by Arlequin (Excoffier et al. 2005),  $F_{st}$  was 0.3143.

### **Discussion**

Mean female body size was statistically different between beetles collected in Vieques compared to those from South Africa ( $p < 0.05$ ) (Table 5.2). However, there was no difference in male body measurements. Body size in dung beetles is highly variable and depends on the quantity and quality of dung available to the developing larvae (Scholtz et al. 2009). In *D. gazella*, larger body size has been shown to increase the rate of dung burial, size of brood balls, and ultimately the size of the offspring (Lee and Peng 1981). Additionally, offspring of *D. gazella* are significantly smaller when dung quality is low and adult density is high (Lee and Peng 1982). Because the current majority of vertebrate herbivore dung available in Vieques is that of Paso Fino horses which are common on the island, dung quality and availability may be higher with less competition (Scholtz et al. 2009). Differences observed in female body size measurements may also suggest variability in the partitioning and utilization of the dung resource between genders and populations. An adequate supply of dung is necessary for survival and reproduction, and body size effects are known to result from an insufficient dung resource (Halffter and Matthews 1966, Hanski and Cambefort 1991, Scholtz et al.

2009). However, sample size was relatively small in this study, and more data are needed to examine body size of *D. gazella* over a broad geographic range.

Sex ratios (Table 5.2) were highly skewed towards females and ranged from 2.9:1 in Vieques to 3.4:1 in the South African population. The presence of a greater number of female beetles has the possibility of numerous explanations and is highly speculative.

*Digitonthophagus gazella* is an *r*-selected species, and females of most *r*-selected dung beetle species do not exhibit bisexual cooperation in terms of dung burial and brood ball formation (Scholtz et al. 2009). Other possibilities include sex allocation theory or sex-specific mortality (House et al. 2011), male-male competition reducing the number of males on a dung pat, environmental variables, seasonality, and feeding rather than brood ball formation (Hanski and Cambefort 1991, Scholtz et al. 2009).

AFLP analysis has been used to determine genetic variability in a number of studies. For example, AFLP analysis allowed the New World screwworm, *Cochliomyia hominivorax* (Coquerel), to be distinguished from similar non-pest species (Skoda et al. 2002, Alamalakala et al. 2009). AFLP analysis has shown low levels of gene flow among subpopulations of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Clark et al. 2007). Krumm et al. (2008) used AFLP analysis to determine that gene flow is high among subpopulations of European corn borer, *Ostrinia nubilalis* (Hubner), indicating that resistance to control methods could easily spread between different regions. In this case, AFLP analysis suggests that *D. gazella* populations in South Africa and Vieques are genetically distinct. AMOVA results (Table 5.4) indicate that genetic isolation between these populations is likely, because 31% of genetic variation was found to be between populations, while 69% of genetic variation was found within populations. An  $F_{st}$  value

(Table 5.4) of 0.3143 indicates that recent gene flow between these two populations is unlikely. Gene flow ( $Nm$ ) may be estimated from  $F_{st}$  ( $F_{st} \sim 1/(4Nm+1)$ ) (Allendorf and Luikart 2007). An  $Nm$  value greater than one reflects enough migration to overcome genetic drift (Hedrick 2000). The  $F_{st}$  value for these two populations is 0.3143, giving an  $Nm$  value of approximately 0.55. These results suggest there is not enough interbreeding between these two populations to overcome genetic drift. While *D. gazella* is known to fly relatively long distances (Fincher et al. 1983, Hanski and Cambefort 1991, Kohlmann 1991, Scholtz et al. 2009), any genetic exchange between Old and New World subpopulations would most likely be mediated by human activity (intentional or accidental introduction).

When a small number of individuals establish a new population, a decrease in genetic variability, commonly referred to as a founder effect or bottleneck, is often observed (Hedrick 2000). Founder effects may be especially likely when a new population establishes on an island. However, *D. gazella* does not appear to have experienced a bottleneck when it became established on Vieques. While the number of polymorphic loci (Table 5.3) in the Vieques population (96%) is slightly lower than the number of polymorphic loci in the South African population (99%), the difference is minor. Additionally, Nei's gene diversity (Table 5.3) was slightly higher for Vieques (0.3846) than for South Africa (0.3623), suggesting slightly more heterozygosity in the Vieques population. These results indicate that there is no reduction of genetic diversity in the Vieques population.

The high genetic variability found within populations of *D. gazella* in this study contrast with the low genetic variability found within a single population from Uberaba,



Brazil. Martins and Contel (2001) used isozymes derived from esterases and found that of the 23 loci analyzed, only three loci were polymorphic, suggesting low genetic variability within the population. It is possible that Brazilian populations have significantly diverged from other populations of *D. gazella* or that inbreeding depression has taken place in Brazil. Further studies encompassing *D. gazella* from throughout their range could help resolve this question.

More research is needed to clarify the amount of gene flow between populations of *D. gazella*. Other island populations in the Caribbean and West Indian islands should be included in future research and compared to native populations in Africa and Asia. With more populations from other islands, it may be possible to determine how often interbreeding and dispersal occurs. *Digitonthophagus gazella* has been shown to be especially good at securing dung resources in comparison to other dung beetle species (Hanski and Cambefort 1991, Scholtz et al. 2009). Their role in dung removal and the biological control of pest fly species and internal parasites of livestock is well documented (Reinecke 1960, Bornemissza 1970; 1976, Bryan 1973; 1976, Fincher 1973, 1981). Because *D. gazella* is available for purchase by land owners and appears to be rapidly expanding, more research is needed to characterize their local population structure and adaptations to new habitats, including islands.

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**Table 5.1.** AFLP adapters and primers. Sequences from Vos *et al.* (1995).

Oligonucleotide	Purpose	Sequence (5'-3')
<i>Eco</i> RI-forward adapter	Adapter Ligation	CTCGTAGACTGCGTACC
<i>Eco</i> RI-reverse adapter	Adapter Ligation	AATTGGTACGCAGTCTAC
<i>Mse</i> I-forward adapter	Adapter Ligation	GACGATGAGTCCTGAG
<i>Mse</i> I-reverse adapter	Adapter Ligation	TACTCAGGACTCAT
<i>Eco</i> RI primer	Pre-Amplification	GACTGCGTACCAATTC
<i>Mse</i> I primer	Pre-Amplification	GATGAGTCCTGAGTAA
E-ACG	Selective Amplification	GACTGCGTACCAATTC + ACG
M-CAC	Selective Amplification	GATGAGTCCTGAGTAA + CAC
E-ACT	Selective Amplification	GACTGCGTACCAATTC + ACT

**Table 5.2.** Measurements ( $\pm$  SE) and sex ratio data for *D. gazella* collected in Vieques, Puerto Rico and South Africa. Length is measured from the tip of clypeus to the apex of the elytra and width is measured across humeri. Different letters indicate significance ( $p < 0.05$ , One-Way ANOVA, Tukey test post-hoc).

	<b>Vieques 2008</b>	<b>Vieques 2010</b>	<b>South Africa 2011</b>
<b>Number Female</b>	50	20	27
<b>Number Male</b>	17	11	8
<b>Sex ratio</b>	2.9:1	1.8:1	3.4:1
<b>Mean female length (mm)</b>	10.20 $\pm$ 0.14a	9.75 $\pm$ 0.19a	9.81 $\pm$ 0.13a
<b>Mean female width (mm)</b>	5.72 $\pm$ 0.08a	5.79 $\pm$ 0.10a	6.11 $\pm$ 0.09b
<b>Mean male length (mm)</b>	10.95 $\pm$ 0.17a	10.62 $\pm$ 0.17a	10.43 $\pm$ 0.22a
<b>Mean male width (mm)</b>	6.18 $\pm$ 0.09a	0.13 $\pm$ 6.30a	6.42 $\pm$ 0.13a

**Table 5.3.** Single subpopulation statistics. Nei's gene diversity ( $H$ , where  $J = \sum_k x_k^2$  and  $H = 1 - J$ ) and polymorphic loci.

	<b>Nei's Gene Diversity</b>	<b>Polymorphic Loci</b>
<b>South Africa</b>	0.3623	99%
<b>Vieques</b>	0.3846	96%
<b>Total</b>	0.4490	100%

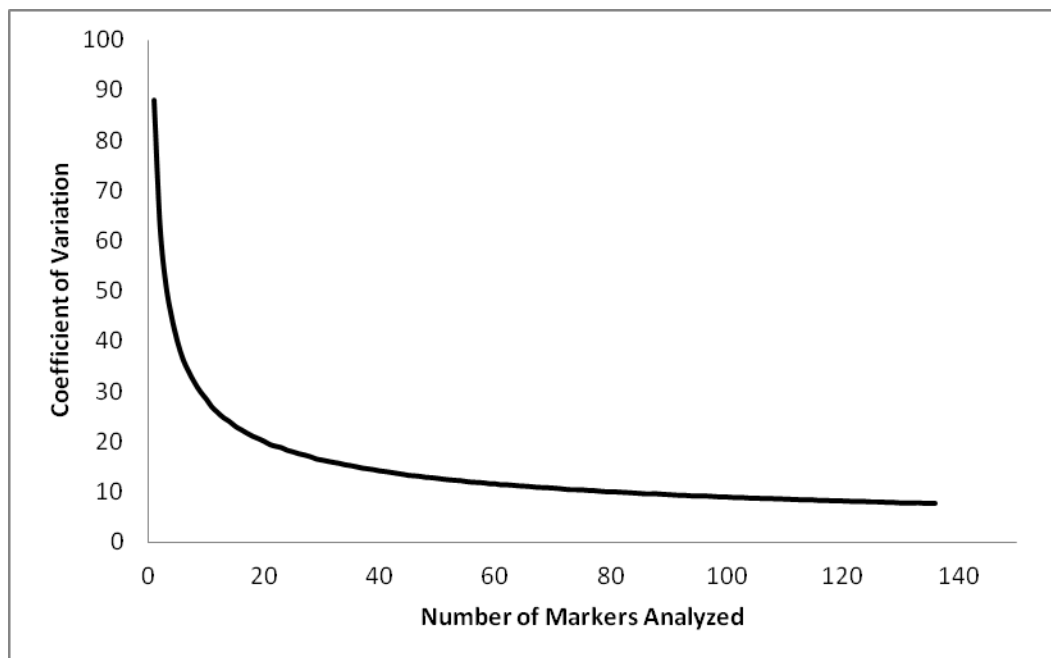
**Table 5.4.** Two-level AMOVA results and fixation indices.  $F_{st} = \sigma_a^2 + \sigma_b^2 / \sigma^2$ .

Significance testing accomplished with 1,023 permutations.

<b>Source of Variation</b>	<b>D.F.</b>	<b>Sum of Squares</b>	<b>Variance Components</b>	<b>Percentage of Variation</b>
<b>Among Populations</b>	1	319.573	10.419 Va	31.43
<b>Within Populations</b>	55	1249.936	22.726 Vb	68.57
<b>Total</b>	56	1569.509	33.145	
<b>Fixation Indices</b>	<b>Va and Fst</b>	0.3143	<b>P-value</b>	0.000 ± 0

### Figure Legend

**Fig. 5.1.** Coefficient of variation. The number of markers scored plotted against coefficient of variation values. A high number of markers decrease the coefficient of variation. > 93% of genetic variation is encompassed by the markers used.

**Fig. 5.1.**

## Appendix C

### AFLP Protocol

#### **Step 1**      **Restriction Digestion**

<b><u>Stock</u></b>	<b><u>Component</u></b>	<b><u>1 RxN</u></b>
10x	one phorall buffer	1.25 $\mu$ l
10 $\mu$ / $\mu$ l	MSEI enzyme	.125 $\mu$ l
20 $\mu$ / $\mu$ l	ECORI enzyme	.0625 $\mu$ l
10mg/ml	BSA	.125 $\mu$ l
	dH2O	3.94 $\mu$ l (bring up to 5.5 $\mu$ l total)

Dispense 5.5  $\mu$ l of above master mix and to each micro tube with 7 $\mu$ l ( or 150ng DNA/ $\mu$ l of template DNA and put it into PCR machine at 37 C, 2.5 hr ( 70 C 15 min), store at 4 C (program: resdig)

#### **Step 2**      **Adapter ligation**

<b><u>Component</u></b>	<b><u>1RxN</u></b>
ECORI prepared adapter	0.5 $\mu$ l
MSEI prepared adapter	0.5 $\mu$ l
T4 DNA kigase	0.15 $\mu$ l (in AFLP box)
T4DNA ligase buffer	0.5 $\mu$ l
dH2O	3.35 $\mu$ l
<b>Total</b>	<b>5.0 <math>\mu</math>l</b>

Dispense 5  $\mu$ l of ligation mix into tubes containing digestion product from step1. Incubate at 25 c for 8 hrs programme (ADAPLIGA)

#### **Step 3**      **diluting the ligation mixture**

- Add 135  $\mu$ l 1x TE buffer to the solution from step 2. Mix well and store at -20 c
- This is a 1:10 dilution

#### **Step 4**      **Preamplification**

<b><u>Component</u></b>	<b><u>1RxN</u></b>
Preamplification mix II	10 $\mu$ l
10x PCR buffer	1.25 $\mu$ l
15mM Mgcl2	0.75 $\mu$ l
Taq polymerase 5 $\mu$ l/ml	0.25 $\mu$ l
<b>Total</b>	<b>12.25 <math>\mu</math>l</b>

Add 12.25  $\mu$ l master mix and 1.25  $\mu$ l diluted DNA solution from step 3 to new micro tubes.

**PCR programme:**    94 C for 30 sec\sec

56 C for 1 min  
 72 C for 1 min  
 20 cycles  
 Soak/store 4 C

### **Step 5      Checking preamplified DNA**

- Run samples on .8% agarose gel  
 1  $\mu$ l sample /1  $\mu$ l dye  
 Run 15 min at 80V

### **Step 6      Dilution of pre-amp**

- 1:20 dilution----- 190  $\mu$ l dH<sub>2</sub>O and 10  $\mu$ l pre-amp mix from step4
- If too much, try 195  $\mu$ l dH<sub>2</sub>O

### **Step 7      Selective amplification**

<b><u>Component</u></b>	<b><u>1Rxn</u></b>
dH <sub>2</sub> O	4.1 $\mu$ l
10x PCR buffer-II	1.2 $\mu$ l
15mM MgCl <sub>2</sub>	0.72 $\mu$ l
Taq polymerase	.08 $\mu$ l
MSEI primer	2.0 $\mu$ l
ECORI primer	0.4 $\mu$ l
<b>Total</b>	<b>8.5 <math>\mu</math>l</b>

- Do this step in the dark, primers are light sensitive
- Quantity of ECORI primer dependent upon insect----- amount is 0.3  $\mu$ l
- Use 8.62  $\mu$ l of the solution and 2.0  $\mu$ l of DNA template from step 6. PCR cycles:  
 94 C for 30 s, 65 C for 30 s, 72 C for 1 min    (1 cycle)  
 94 C for 30 s, 72 C for 1 min                    (12 cycles)  
 94 c for 30 s, 56 for 30 C, 72 for 1 min        (23 cycles)

### **Step 8      Stop the reaction**

- Add 2.5  $\mu$ l of stop solution to each tube
- Do this step in the dark

### **Step 9      Denature**

**PCR program:** 94 C 1 min, keep at 4 C

### **Preparing AFLP gel**

1. Clean plates with 1 % vionex soap .rinse with 70 % isopropanol and let it dry
2. Prepare ammonium persulphate solution
3. Prepare comb buding solution
4. Wash plates with 100 5 isopropanol and dry



5. Apply comb budding solution to plates
6. Put mold together
7. Mix gel
8. Use large pipette to mix and pour gel into the mold, fast and even, no bubbles
9. Insert comb----pore more gel over comb
10. Let it dry for 1.5-2 hr
11. Pour nano pure water on comb and remove
12. Use razor blade to remove excess gel
13. Wash glass and dry with isopropanol
14. Running buffer- 1x TBE - fill machine
15. Stop gel and do a pre run
16. If unacceptable, check connections, and then redo the gel