Plastic responses to diet quality and thermal gradients in dung beetles

PLASTIC RESPONSES TO DIET QUALITY AND THERMAL GRADIENTS IN DUNG BEETLES

A Thesis by

Max Ryu Proctor

Bachelor of Arts, Claremont Mckenna College, 2018

Submitted to the Department of Biological Sciences
and the faculty of the Graduate School of
Wichita State University
in partial fulfillment of
the requirements for the degree of
Master of Science

May 2024

PLASTIC RESPONSES TO DIET QUALITY AND THERMAL GRADIENTS IN DUNG BEETLES

The following faculty members have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirement for the degree of Master of Science with a major in Biological Sciences.

____
Mary Liz Jameson, Committee Chair

____
Thomas Luhring, Committee Member

____
Moriah Beck, Committee Member

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Mary Liz Jameson, for the support and guidance these past two years. Initially I came to Wichita to work on a defined field-based project. However, I found myself drawn to exploring elements of sexual selection and animal weaponry. Over time my work transformed to a lab-based project on entirely different concepts—of course still about dung beetles! I was initially scared to incorporate these concepts into my work but Dr. Jameson encouraged me to pursue my interests. Her continued support has made me feel seen as a young scientist, and I am forever grateful. I have learned that being an academic is not only conducting experiments, analysis or writing. Being a scientist is also about showing up, collaborating, and being engaged with the people around you. Dr. Jameson has allowed me to grow and take on my own ideas, while providing guidance when it is most needed. And of course, she has shown me that it is possible to be productive and have fun, too!

Although Dr. Thomas Luhring is not officially my co-advisor, he has played such a critical role in my research that he might as well be. Dr. Luhring taught me about thermal ecology, allowed me to use his environmental chambers and generally has helped me think critically about experimental design and analyses. I stopped by his office unannounced countless times while tinkering with my models and was never turned away. I am also forever grateful that he welcomed me into his lab community and allowed me to tag along on herpetological excursions.

When I first met with my research committee, my project was a Frankenstein of vaguely related concepts that were strung together. As part of my committee, Dr. Moriah Beck helped me whittle down my project, so I had a clearer objective. Dr. Beck has helped me think of the role of nutrition, and she has brought a very helpful outside perspective as a chemist.

Outside of my instructors at WSU, there are countless people who have helped me with my research. I would like to thank Dr. Armin Moczek at the University of Indiana and Dr. Sofia Casasa at Boston University for their guidance on breeding dung beetles. They welcomed me into the fascinating world of dung beetle plasticity and gave me crucial tips that allowed me to successfully breed hundreds of beetles.

I thank Dr. Puni Jeyasingh at Oklahoma State University for collaborating on ionomic analyses of dung and beetle samples. I also thank Yetkin Ipek, Dr. Jeyasingh's PhD student, for working on ionomic analyses. Prior to this project I was always thinking at the organism and ecosystem level, but Dr. Jeyasingh's ideas about integrating ionomics into the project opened my eyes to the power of looking at smaller scale processes ("atoms first").

My experiment could not have been possible without the help of many people. I extend my gratitude to Bryanna Woodard, Stone Horton, Monica Cook, David Bohorquez and Megan Pappan for helping breed and monitor beetles. You guys are all a part of the beetle family. Thank you to Fawn Beckman for teaching me how to use the autoclave. I extend my sincere gratitude to Ralph Larovere (Clay County, Texas) for allowing me to collect beetles on his property. He generously took me around his ranch so I could pick dung beetles out of cow pats for two days. I am truly appreciative of his hospitality.

To everyone at the biology department, thank you for taking me in and showing me what community means. Not enough positive things can be said of Marcia Norton and Maria Martino. Marcia is a superhero and knew what to do in *every* sticky situation I found myself in. Maria has the grad students' best interests and has made me feel supported during my MS. I always walk out of the office with a big smile on my face, and I am forever grateful.

To my wonderful fellow grad students who not only welcomed me to Wichita but also kept me sane and grounded with your delightful banter: Sydney Falcon, Shania Burkhead-Harris, Barrett Houchen, Adrian Bethel, Sam Skerlec, and Steph Bristow, I extend my heartfelt gratitude. Thank you for always indulging my middle school sense of humor. You all have been an essential part of my support network, and I cherish the time we've shared. Thank you Bioshox!

Finally, I thank my family and girlfriend. Thank you to my parents who have always supported my interests in beetles. They never questioned me when I decided that I would spend the rest of my life looking at beetles. Without their love, I most definitely would not be doing what I do today. Thank you to my lovely girlfriend, Lexy Long. Whenever I don't believe in myself, she "believes in me for the both of us". Without her support, I wouldn't have been able to keep myself grounded in the day to day, and the long-term goals I set for my MS would have been too overwhelming. She makes me realize that it's okay to take a break from work, especially if there is ice cream involved. Thank you, Lexy, I am so excited for the next chapter in our lives.

Financial support for this research was provided by the Rachel Snyder Memorial Scholarship from the Grassland Heritage Foundation, as well as the Jayne and Glenn Milburn Travel Award and the Sheats Environmental Science Research Fellowship from Wichita State University.

ABSTRACT

In a dynamic world, organisms must swiftly adapt to environmental changes. Phenotypic plasticity enables organisms to alter their traits within their lifetime, providing a rapid response to shifting environments. Dung beetle horns display extreme plasticity, with males exhibiting varying horn sizes (horned "major" to hornless "minor" morphs) based on larval diet (dung type). Horns are utilized in male-male contests for mating, and body and horn size strongly influence success. Though plastic responses to dung quality are well studied, the interplay of temperature and nutritional conditions on these traits remains unexplored. We reared the dung beetle *Digitonthophagus gazella* in bison (*Bison bison)* and cattle (*Bos taurus*) dung, and across seven temperatures (22-34°C) to determine how these environmental factors interact to shape plastic responses in body size and horn length in dung beetles. Almost all (99%) of reared male beetles were major males in both cattle and bison dung treatments, indicating that both dung types are high quality resources for developing dung beetle larvae. The interaction between dung quality and temperature revealed distinct responses: beetles from bison dung show no temperature-related changes in body size and horns, while those from cattle dung exhibit larger sizes at intermediate (26-30°C) temperatures compared to those at colder (22-24°C) or warmer (32-34°C) temperatures. This suggests that cattle dung may be a higher quality resource for *D. gazella*, allowing beetles to utilize a thermal optimum. In addition, development rates increased with temperature, with a more pronounced increase in the bison dung treatment. The interactive effects of diet quality and temperature on morphological and physiological plasticity in dung beetles demonstrate that plastic responses to one factor are not always fixed and can vary with changes in another. To accurately predict how organisms will respond to changing climates, it is essential to evaluate how thermal responses are altered by other factors such as diet quality.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Ecosystems globally are rapidly changing. While evolution serves as a powerful mechanism for organisms to adapt to new environments, change occurs between generations. That is to say, adaptation to changing environments occurs slowly. In contrast, phenotypic plasticity allows organisms to adjust their phenotypes within their lifetimes, allowing them to increase their fitness in response to rapid environmental changes (Snell-Rood et al., 2018; Fox et al., 2019). Plastic responses have been shown to account for phenotypic changes in response to climate change, while evidence of evolutionary adaptation to rapid climate change is limited (Gienapp et al., 2008; Merilä and Hendry, 2014). Further, plastic responses in response to short term changes have been observed across levels of organization including the individual, population and species level (Matesanz et al., 2010). As a result, plasticity plays an important role in allowing organisms to persist in the face of major disturbances (Price et al., 2003; Casasa and Moczek, 2018; Snell-Rood et al., 2018). Most studies on plasticity examine plastic responses to one environmental factor (Westneat et al., 2019). However, ecosystems are complex and many factors are variable. For example, ecosystems can vary in both temperature and photoperiod (Gienapp et al., 2010), predator and competitor densities (Relyea, 2004), and elevated temperature and water availability (Rahman et al., 2021). Do plastic responses to changes in one environmental variable constrain or shape responses to another? In this time of rapid ecosystem change, an understanding of how organisms adapt to multiple environmental factors simultaneously and the role of phenotypic plasticity is essential.

Some of the most variable plastic traits can be found in the sexually selected horns used by male dung beetles of the genus *Onthophagus* to compete for mates (Emlen et al., 2005).

Horns are exceptionally variable across species and also variable within species (Emlen, 2000). The horns of males are highly responsive to larval nutritional conditions (animal dung) and are a model system for nutritional plasticity (Moczek, 2007). Horn size and body size are a major determinant of fight success over potential mates and, as a result, reproductive success (Emlen, 1997; Moczek and Emlen, 2000; Emlen, et al., 2005; Moczek, 2009; Knell, 2011). In females, body size is important for fitness because larger females have increased fecundity and offspring fitness (Hunt and Simmons, 2000; Simmons and Emlen, 2008).

Males in many species have two discrete horns morphs. Large 'major' males express fully developed horns, while small 'minor' males have only rudimentary horns or none at all (Kijimoto et al., 2013). Horn morphs are separated by a body size threshold – only beetles that reach a critical body size during larval development express horns (Snell-Rood et al. 2013, Moczek and Kijimoto, 2014). Body size is a major determinant of contest outcome, and the threshold regulation of horn morphs evolved such that beetles could adjust their phenotype to maximize their reproductive fitness (Hunt and Simmons, 2001). Females dig tunnels under dung to feed, mate and lay eggs (Emlen, 1997). Major male morphs defend females in tunnels from other major males, whereas minor morphs avoid fighting by digging side tunnels and sneak into tunnels to mate with the female (Moczek and Emlen, 2000). The quality of dung available to developing beetles is a major determinant of body size and, as a result, male horn phenotypes are also largely determined by the quality of dung that is provisioned by the female for larval development (Kijimoto et al., 2013). Developing larvae that feed on high quality dung are larger and are more likely to emerge as major morphs (Moczek, 1998; Tomkins and Hazel, 2011). In addition, both wild and experimental populations that feed on different kinds of dung display different threshold body sizes (Emlen, 1997; Moczek, 2002). As a result, changes in the quality of dung available to developing beetles, such as a shift in animal fauna in an ecosystem, can have important fitness consequences for dung beetles.

Body size is not only important for male reproductive fitness in dung beetles but also carries significant consequences for females (Macagno et al., 2018; Fleming et al., 2021). Larger females are more fecund and the provision larger brood balls to offspring (Hunt and Simmons, 2000; Hunt and Simmons, 2002). Offspring from smaller mothers are, on average, smaller and have reduced survival (Macagno et al., 2018). The body size of females can impact subsequent beetle size and survival even after two generations (Macagno et al., 2018). Female body size is also influenced by dung quality (Moczek, 1998). Thus, plasticity in growth and development in response dung quality significantly impacts fitness for both sexes.

Although dung beetle body size and horn length are highly responsive to dung quality, whether plastic responses to diet are consistent across temperatures is not understood. Temperature and food quality influence animal body size (Parker and Johnston, 2006), yet how these factors interact to shape plastic traits in dung beetles has yet to be studied. Dung beetles are ectotherms, and their growth and development are tied to temperature (Floate et al., 2014; Carter and Sheldon, 2020; Fleming et al., 2021). Previous studies have generally found that body size and temperature are inversely related in dung beetles, a trend found in most ectotherms known as the temperature size rule (TSR) (Atkinson, 1994; Macagno et al., 2018; Fleming et al., 2021; Rohner and Moczek, 2023). In other insects, diet quality has been shown to alter plastic responses to temperature. Hornworm caterpillars *Manduca sexta* (Diamond et al., 2010) and seed beetles *Callosobruchus maculatus* (Stillwell et al., 2007), however, have been studied for their response to both temperature and nutrition. In these insects, nutrients altered thermal responses in body size, even reversing the TSR, indicating that plastic responses to nutritional conditions are temperature-dependent (Stillwell et al., 2007; Diamond et al., 2010). Might dung beetles be the same?

By examining both growth and development rates across temperatures and nutritional conditions we can better determine how shifts in development affects plastic traits like body size or horn length. One mechanism by which the TSR is believed to function is by increasing development rate, thereby limiting the amount of time ectotherms can grow (Kingsolver et al., 2006). Dung beetles emerge faster at higher temperatures (Floate et al., 2017), but determining how growth rates respond to variation in both temperature and dung quality is crucial for elucidating the factors that contribute to variation in the expression of plastic traits. Nutrition has been identified as another factor influencing development rates (Rohner and Moczek, 2021). Moreover, faster development rates (early emergence) are linked to higher survival and increased female fecundity, suggesting that plastic responses in development can carry significant implications for dung beetle fitness, independent of changes in body size and horn size (Tyndale-Biscoe and Walker, 1992; Hunt and Simmons, 1997).

Temperature has a nonlinear effect on fitness-related traits because physiological processes typically increase with temperature up to a thermal maximum or optimum (CTmax) (Kingsolver and Umbanhowar, 2018). Thermal reaction norms that capture nonlinear trait responses across temperatures have been used to predict organismal responses to changing climates (Sinclair et al., 2016; Luhring et al., 2019). In dung beetles, thermal response curves have been used to predict the likelihood of establishment in non-native ranges (Floate et al., 2017). However, if nutritional conditions shape plastic responses to temperature, dung beetles may respond differently to temperature variation when the quality of dung varies in environments. In other insects, diet has been demonstrated to have an unequal effect of growth at different temperatures (Kingsolver et al., 2006), causing a change in the shape of thermal reaction norms (Luhring et al., 2019). Most studies that have investigated the relationship between dung beetle body size and temperature have compared growth under a limited range of temperatures (two or three temperature conditions) (Macagno et al., 2018; Rohner and Moczek, 2020; Fleming et al., 2021). Analyzing plastic responses across thermal gradients with many temperatures can detect incremental changes, revealing nonlinear patterns that can be missed if only comparing a limited number of temperatures (Luhring et al., 2019). One study that examined development rates in dung beetles across a thermal gradient (six temperatures) found nonlinear increases in development rates but did not examine changes in body size or horn length (Floate et al., 2017). Whether body size and horn length display nonlinear responses to broad thermal gradients has yet to be studied.

We utilized a novel comparison of dung resources, cattle (*Bos taurus*) and bison (*Bison bison*) dung, reflecting a historical diet shift experienced by dung beetles native to North America (Tiberg and Floate, 2011). Previous research suggests that dung beetles native to North America were able to persist after the extinction of bison by utilizing dung from cattle imported from Europe (Tiberg and Floate, 2011). Whether this resource shift led to plastic changes in traits that are important for fitness has not been investigated. Despite being closely related, cattle and bison exhibit differences in their diets. Cattle mainly consume C4 grasses and consume more forbs than bison, whereas bison primarily consume C3 grasses (Plumb and Dodd, 1993). These dietary differences may influence the quality of dung for dung beetles. Bison dung has been previously documented to have a higher nitrogen content, which is associated with higher dung quality for dung beetles (Moore and Bower, 2001; Tiberg and Floate, 2011; Snell-Rood et al., 2013). Dung beetles are found in pastures grazed by both cattle and bison, and differences in these dung types may have developmental implications for dung beetles (Tiberg and Floate, 2011; Trible, 2017). Recent work found that dung beetles in grasslands foraged by bison and cattle display distinct seasonal shifts in body size and horn size (Allgire and Welti, in prep.). Across the planet, mean temperatures have increased in North American grasslands (Polley et al., 2013). Given that multiple environmental factors influence the development of life history traits important for fitness, understanding how species respond to changing conditions in complex environments can inform whether species can persist (Macagno et al., 2018).

In this study, we manipulated nutritional and temperature conditions for larvae of the male 6ncrease6c dung beetle *Digitonthophagus gazella* (previously *Onthophagus gazella)* to explore the individual and interactive effects of each environmental factor on the growth of dung beetles and the development of their sexually selected weapons. We allowed larvae to develop in either bison dung or cattle dung at seven different temperatures (22, 24, 26, 28, 30, 32 and 34°C) and compared thermal reaction norms for body size, male horn length, and underlying growth and development rates for beetles in each dung treatment. We hypothesized that, independent of temperature, beetles will exhibit significant differences in the scaling relationship between body size and male horn length (allometric plasticity) dependent upon their nutritional resource (bison or cattle dung). We also hypothesized that dung quality has a temperature-dependent effect on plastic traits in dung beetles, specifically body size and horn size.

CHAPTER 2

METHODS

2.1 Species Selection

Digitonthophagus gazella, commonly referred to as the gazelle scarab, is a dung beetle originally from Africa, Madagascar, and southern Asia (Pokhrel et al., 2020). It was deliberately introduced to Texas in the 1970s to aid in the decomposition of cattle dung (Fincher, 1986). Since then, has expanded its range in the US across the southern US (Fincher et al., 1983; Pokhrel et al., 2020). In Kansas, the species is documented in grasslands grazed by both cattle and bison (Trible, 2017; Proctor, in press). The species has been introduced in many regions and is one of the most widespread non-native dung beetles globally (Pokhrel et al., 2020). Many of the same characteristics that enabled *D. gazella* to establish around the world also make them useful in breeding studies.

Digitonthophagus gazella has high fecundity and short development time and, as a result, have been bred for many studies (Casasa et al., 2020; Pokhrel et al., 2020). Males of *D.* gazella have paired horns above the eyes and exhibit a moderately sigmoidal relationship between body and horn length (Casasa et al., 2020). The scaling of body size and horn length (allometry) is plastic in response to nutrition (Schwab and Moczek, 2016; Casasa et al., 2020). Further, development rate, which influences body size and horn length, has also been shown to increase with temperature (Floate et al., 2014). *Digitonthophagus gazella* is early diverging in the *Onthophagus* phylogeny, and horn morphology and allometry are considered the ancestral state within the group (Emlen, et al., 2005; Kijimoto et al., 2012; Casasa et al., 2020). Consequently, studying plastic responses in *D. gazella* may shed light on how these traits respond to environmental factors in other species. Variation among individuals and populations in *D. gazella* may have facilitated the species' adaptation to novel environments (Pokhrel et al., 2020), underscoring the importance of investigating plastic responses to complex environments.

2.2 Species Collection

Adult *D. gazella* were collected from a privately owned cattle ranch in Clay County, Texas. Beetles were collected on 06/16/2023 to 06/17/2023 under cow dung. Males (n=154) and females (n=168) were stored separately in containers (15.14 L) with a sand/soil mixture and a moistened sponge.

2.3 Dung Collection

Fresh dung from cattle (*Bos taurus*) and American bison (*Bison bison*) was collected from Konza Prairie Biological Field Station (Kansas State University and The Nature Conservancy; research permit 721 to ML Jameson and E Welti). The site is in the Flint Hills region of Kansas and is actively managed as tallgrass prairie. Herds of bison and cattle are managed in separate areas. Fresh dung (18.9 L) from both herbivore species was collected on 6-V-23. Dung was determined to be fresh by the visible moisture on the surface and minimal presence of other coprophagous invertebrates. To account for variation in the nutritional content of dung across individual cattle and bison, dung of the same herbivore species was homogenized using a drill equipped with a paint mixer. Homogenized dung was placed in freezer bags and frozen the same day.

To minimize fungal growth in breeding containers and to reduce moisture differences between cattle and bison dung treatments, excess water was removed from dung. After thawing, dung was placed in a nylon paint strainer, and water was squeezed out by hand until no further moisture was evident. 750 ml portions of dung were placed in individual freezer bags and frozen for use in breeding.

2.4 Experimental Design

We randomly assigned beetles to one of 18 breeding groups, each consisting of six females and three males. Breeding groups were randomly assigned to mitigate potential biases in body sizes across breeding groups because body size and reproductive behaviors of parents can influence the quantity of dung provisioned to developing beetles (Hunt and Simmons, 2001). Breeding groups were housed in 15.14 L square plastic containers (24.1 cm L x 21.1 cm W x 33.5 cm H), herein referred to as breeding containers. Breeding containers were filled with an autoclaved substrate consisting of a 2:1 sand:soil mixture and 1200 mL of water. To prevent collapse of tunnels that beetles constructed during the breeding period, the substrate was tightly packed using another 15.14 L plastic container filled with substrate. After packing, the substrate in all breeding containers was a uniform depth of 20 cm. Breeding groups were randomly assigned to either cattle or bison dung. Half (nine) of the breeding containers was provided 0.75 L of thawed cattle dung; the other half (nine) was provided 0.75 L of thawed bison dung. Before placing adults in breeding containers, beetles were inspected under the microscope for mites. Any mites were carefully removed using forceps under running water. Once mite free, breeding groups were introduced into breeding containers and covered with mesh paint strainers to prevent escape. Tarps with slits were secured over the top of breeding containers to prevent moisture loss and allow for gas exchange.

Breeding containers were placed in Percival Scientific environmental growth chambers (Model I-36VL) at 28° C, the ideal breeding temperature for *D. gazella* (Moczek pers. comm. 20 Jan. 2023), 60% relative humidity (RH), and constant darkness. After mating, females form and bury ovoid masses of dung referred to as a “brood balls” and lay a single egg in each brood ball (Floate et al., 2014). Each developing beetle gets all nutrition from its individual brood ball. Breeding groups remained in the environmental chambers for 7 days, after which brood balls were removed from breeding containers. Brood balls were weighed using a digital scale (Ohaus model NV222) to the nearest 0.01 g. Females will pack masses of dung that do not contain eggs, but serve as food caches (Mamantov and Sheldon, 2021). Ten dung masses under 2.00 grams, which are smaller than brood balls previously reported for *D. gazella* (Schwab et al., 2017), were opened and inspected under a microscope for the presence of eggs. None of the brood balls had signs of eggs, and dung masses balls under 2.00 g were removed from the experiment. All brood balls above 2.00 g were placed into individual 59 ml plastic cups with autoclaved, moistened substrate (referred to as rearing chambers). Lids were secured onto cups and punctured with toothpicks for improved air circulation.

To manipulate temperature conditions for developing larvae, brood balls from all breeding groups were randomly distributed among seven temperature treatments (22, 24, 28, 30, 32, 34°C), 60% RH and constant darkness. We selected seven temperatures extending the known thermal tolerance of *D. gazella* (Floate et al., 2014). Previous studies indicate that adult emergence for D. *gazella* was reduced below 26°C and above 32°C, and that 28°C is optimum (Floate et al., 2014; Moczek pers. comm. 20 Jan. 2023). Temperatures were selected to capture potential effects of temperature stress on body size and horn development. Although 34°C is hypothesized to be the thermal maximum for *D. gazella,* it has not been tested (Schwab et al., 2017). Brood balls were placed in environmental chambers before starting the temperature programs, allowing developing beetles to acclimate to the temperature treatments. Daily, all rearing chambers were opened and inspected for emerged adults. Emergence dates were recorded, and adults were euthanized by freezing. Development rate was calculated as the inverse of the number of days to adult emergence after a brood ball was placed in an environmental chamber.

Brood balls that did not emerge after two weeks post-emergence in the same temperature treatment were inspected (Floate et al., 2014). Brood balls were opened by hand and inspected under the microscope. Brood balls that had no emerging adult were considered mortalities if an egg chamber (small cavity) was present inside or if remnants of an egg, larvae (head capsule, molts), dead pupae, or dead adults were found inside the brood ball. Brood balls lacking signs of developing beetles may have been food caches or a result of disturbance during the breeding period (Floate et al., 2014; Mamantov and Sheldon, 2021); these were excluded from analyses of survival.

2.5 Morphological Measurements

Morphological measurements were taken using a Leica M165 C microscope connected to Leica Application Suite X (LAS X v. 3.7.3.23245). Measurements were taken using the segment line measurement tool in Leica Application Suite X. Body size in onthophagine dung beetles is commonly measured as the width of the widest point of the thorax (Moczek, 2006). Thorax width measurements for males were collected twice, and when the difference between the two measurements was less than 0.05 mm, the original measurement was used. When the difference was greater than 0.05 mm, a third measurement was taken and used as the final measurement.

Male *D. gazella* have horns resembling cones when short (minor morph) and curved cylinders when long (major morph). Horn length was measured as the nonlinear distance along the outer edge of the horn from the top of the eye to the tip of the horn (Fig. 1) [1]. We used the

[1] All figures can be found in Appendix B.

nonlinear measurement to account for investment into non-vertical growth (width and curvature of a horn) (Moczek, 2006). Growth rate for body size was calculated as the thoracic width in mm divided by the number of days to adult emergence. Growth rate for horn length was calculated as the horn length in mm divided by the number of days to adult emergence. Prior to GAMM analysis, we plotted horn length and body size and compared horn allometries between males from both dung treatments.

2.6 Statistical Analyses

All analyses were performed in Rstudio version 2023.12.1 (R Core Team, 2023).

2.6.1 Generalized Additive Mixed Models

Generalized Additive Mixed Models (GAMMs) were employed to capture nonlinear responses to temperature gradients often observed in plastic traits (Luhring et al., 2019). GAMMs were created with cubic regression splines using the 'mgcv' package (Wood, 2023). All GAMMs incorporated temperature terms with smoothers to capture potential nonlinear responses in traits across temperature. We incorporated the effect of sex into models for metrics related to body size (growth rate and development rate) because male *D. gazella* generally have slower development rates and reach larger body size than females (Rohner, 2021). We included an interaction term for sex and dung treatment with a temperature smoother to explore potential variation in temperature-dependence across treatment combinations (sex and dung treatment). For GAMMs related to horn length, traits expressed exclusively in males, the model features a dung treatment term with a temperature smoother instead of the interaction between dung treatment and sex. Dung treatment was included as a parametric effect in all GAMMs, whereas sex was included only as a parametric effect in the GAMM for body size. To separate the effect of the quality of dung from the quantity of dung provided and to account for potential variations in dung provisioning among different breeding groups, we incorporated brood ball mass as a random effect.

2.6.2 Curve-fitting

Before assessing the significance of GAMMs using the ‘anova.gam’ function in the ‘mgcv’ package (Wood, 2023), models were checked using the ‘gam.check’ function and by plotting residuals. The basis dimensions (k) of smoother terms in all models were initially set to 3 knots, representing the simplest model (Luhring et al., 2019). If gam.check found that models adequately captured nonlinear trends in residuals, we determined that 3 knots were sufficient. If p-values from the gam.check output were low ($P<0.05$), suggesting that models failed to capture additional nonlinear components of the data, we increased k (Wood, 2023). However, if increases in the basis dimension did not improve model fit, we defaulted to the simplest model with 3 knots (Wood, 2023). Additionally, if model fit improved based on gam.check output statistics, but it did not change the interpretation of the model using ANOVA, we defaulted to the simplest model (3 knots). After analyzing the output statistics from gam.check, we evaluated the normality of the residuals, examined QQ plots, and compared GAMM plots with scatterplot data. This process provided confirmation that models that incorporated the chosen number of basis dimensions effectively captured patterns in the data. After fitting models, we employed the anova.gam function to assess the significance of smoother terms including temperature, dung treatment, and sex (for body size metrics only) along with their interactions on the response variables. Finally, we visualized GAMM curves using the ‘plot_smooth’ function from the ‘itsadug;’ package (Rij et al., 2022).

2.6.3 Relative Impacts of Development and Growth Rates

After modelling how trait sizes, growth rates and development rates independently responded to temperature, we sought to determine the relative influence of growth rates and development rates in shaping trait sizes across the temperature gradient. To achieve this, we plotted the average development and growth rates across temperatures for each treatment. We created separate plots for the growth rate of body size and horn length. We also included isoclines that represent combinations of growth and development rates leading to constant body sizes and horn lengths. By comparing how the points vary across isoclines, we were able to visualize the relative effects of development rate and growth rates on the resulting average body sizes and horn lengths.

2.6.4 Survival

We initially constructed a GAMM with binomial error distribution and a logistic link function with the same parametric and smoother terms as GAMMs for metrics of horn length. Due to the absence of specimens from brood balls where no beetles emerged, we were unable to account for the effect of sex on mortality. Initial GAMMs indicated that there were no support for a nonlinear relationship between temperature and log-odds of survival. As a result, we created a logistic regression using the 'glm' function for survival. The model included dung treatment, temperature, and brood ball mass as independent factors as well as the interaction of dung treatment and temperature.

CHAPTER 3

RESULTS

3.1 General Results

Independent of temperature, horn allometry did not differ by dung treatment in *D. gazella* (Fig. 2), but body sizes were larger in the cattle dung treatment (Fig. 3). When accounting for the effects of temperature on body size and horn length, the type of dung significantly influenced whether these traits displayed nonlinear responses to temperature changes or remained consistent across temperatures. In the cattle dung treatments, we observed a unimodal response to temperature for male and female body size as well as male horn length (Figs. 4-5). In contrast, in the bison dung treatment, body size and horn lengths did not change across the temperature gradient (Figs. 4-5). As a result, at intermediate temperatures (26-30°C), beetles from cattle dung treatments had larger body sizes and horns than those from bison dung treatments. Growth rates for body size and horn length increased with temperature for both sexes in each dung treatment (Figs. 6-7). Beetles from the bison dung treatments, however, had higher growth rates particularly at the highest temperatures (Figs. 6-7). Development rates similarly increased with temperature but were higher in the bison dung treatment, so beetles emerged earlier at the highest temperatures (Fig. 8). The increased body sizes and horn lengths at intermediate temperatures in the cattle dung treatments were due to slower development rates compared to bison dung treatments (Figs. 9-10).

3.2 Horn Allometry

Almost all males were larger in body size and had longer horns compared with previously reported laboratory and wild populations (Schwab and Moczek, 2016; Casasa et al., 2017; Casasa et al., 2020). Males did not span the full variation in body size and horn length for this species (Fig. 2). As a result, we focused our analyses on plastic trait responses in major males. In wild populations, 6 mm represents the threshold body size that separates male morphs (minor versus major) in wild populations (Schwab and Moczek, 2016). Four individuals (two males and two females) had thoracic widths below 6 mm which is more than 0.5 mm smaller than the next smallest females and 0.6 mm smaller than the next smallest males in this study. Removal of individuals below 6 mm improved model residuals for GAMM models of body size. To better model trait responses for large individuals, we excluded these individuals (1%) from analyses.

Several males exhibited misshapen horns with flattened apices and/or horns that bent in directions not found in natural populations (Fig. 11). The process of checking brood balls may have interfered with development, leading them to have horn forms that do not reflect typical investment in horn development. All male beetles were examined for misshapen horns and were categorically assigned as having developmental abnormalities. Individuals with abnormalities were excluded from analyses. In total, 10.4% of emerged males (n=22) were removed because of misshapen horns. Beetles with horn abnormalities were evenly distributed among temperature treatments (χ^2 = 7.909, df = 6, P=0.245, chi-square) and between dung treatments (χ^2 = 0.727, df = 2, P=0.394, chi-square). Following the removal of individuals with horn abnormalities (Fig. 11) or thoracic widths less than 6 mm, remaining males were included in GAMM analyses.

Horn length increased linearly with body size (thoracic width) among all males from both dung treatments (Fig. 3). Due to the lack of smaller males (typically found in wild populations), we could not analyze static allometries using conventional fitting of sigmoidal curves (Moczek 2002; Schwab and Moczek 2016). Linear regression analysis indicated that dung treatment did not affect horn allometry among the males in this study (Table 1[2]). When body size and horn

[2] All tables can be found in Appendix A.

length were analyzed separately, body size was significantly larger in the cattle dung treatment ($t = -2.00$, $df = 325$, $P = 0.047$), but there was no significant difference in horn lengths between males from bison and cattle dung treatments ($t = -1.13$, $df = 174$, $P = 0.261$).

3.3 Thermal Reaction Norms for Body Size and Horn Length

Body size showed distinct responses to temperature by dung treatment. For beetles developing in cattle dung, body size showed a unimodal response to temperature with a peak at intermediate temperatures (26-30°C) (Fig. 4). In contrast, for beetles from bison dung treatments, body size remained relatively constant across the temperature gradient (Fig. 4). The increase in body sizes at intermediate temperatures resulted in beetles from the cattle dung treatment reaching larger body sizes than those from the bison dung treatment. Although males were generally larger than females (Table 2), the effect of dung treatment on body size was consistent between the sexes (Fig. 4). Among the four combinations of sex and dung treatment, significant temperature-dependent responses only were observed for males from cattle dung treatments (Table 2).

As with body size, horn length displayed distinct responses to temperature due to dung treatment. Horn length showed a unimodal response to temperature for males from the cattle dung treatment (Table 3) but not in males from the bison dung treatment (Fig. 5). The fitted GAMM curve line for the bison dung treatment showed a weak increase in body size across temperature (Fig. 5), but the confidence intervals suggest the increase was non-significant. At intermediate temperatures, horns were larger in males from the cattle dung treatment than they were in the bison dung treatment (Fig. 5). Horn length did not differ by dung treatment alone (Table 3), indicating that the effects of dung treatments are only apparent when accounting for temperature.

3.4 Thermal Reaction Norms for Growth and Development Rates

Growth rate for body size increased with temperatures in all combinations of sex and dung treatments, but beetles from bison dung treatments had higher growth rates, particularly at higher temperatures (Fig. 6). All treatment combinations had significant smoother terms indicating distinct temperature-dependent responses in growth rate (Table 4). At the lowest temperatures, growth rates were similar across dung types, but at higher temperatures, beetles from bison dung treatments exhibited increased growth rates than those from cattle dung treatments (Table 4). Across all temperatures males have slightly higher growth rates than females, however the effect of sex was non-significant (Table 4).

The growth rate for horn length increased with temperature in both bison dung and cattle dung treatments (Fig. 7). Similar to the growth rate for body size, at lower temperatures growth rates for horn length were similar between dung treatments but diverged as the temperature increased (Table 5). Males from the bison dung treatment grew horns at higher rates than those in the cattle dung treatments at high temperatures (30-34°C).

Development rate increased with temperature for both sexes in cattle dung and bison dung treatments (Fig. 8). All smoother terms for each dung and sex treatment combinations were significant, indicating that all have unique nonlinear responses to temperature (Table 6). Like growth rates, development rates are faster in beetles from bison dung treatments compared to those from cattle dung treatments (Fig. 8). Development rates were faster in bison dung treatments for both sexes. Unlike the growth rate for body size however, females had faster development rates, particularly at higher temperatures.

3.5 Impacts of Growth and Development Rates on Trait Size

In general, growth rates and development rates increased simultaneously until reaching a peak at 32°C, after which both rates decreased. Even though body size remained constant across the temperature gradient for both males and females from bison dung treatments, growth and development rates were not constant and increased with temperature (Fig. 9). At intermediate temperatures (26-30°C), beetles from the cattle dung treatment experienced proportionally larger increases in growth rate compared to development rate, resulting in increased body sizes. Conversely, in the bison dung treatments, development rate saw relatively greater increases than growth rate, and body sizes did not increase. Notably, only males in bison dung treatments maintained a relatively constant growth rate at the two highest temperatures (32 and 34°C).

Similar to results for body size, slower development rates in cattle dung treatments allowed males to reach larger horn lengths at intermediate temperatures (Fig. 10). Despite faster or equal growth rates for horns in the bison dung treatment compared to those in the cattle dung treatment, faster development rates in bison dung treatments lead to smaller body sizes in bison dung treatments. At the two highest temperatures (32°C and 34°C), growth and development rates responded differently to the dung treatments. While both rates decreased in the cattle dung treatment, they remained constant in the bison dung treatment.

3.6 Parental Provisioning, Adult Emergence, and Survival

Breeding groups with bison dung produced 183 brood balls, whereas breeding groups with cattle dung produced 277 brood balls (total=460 brood balls). The mean mass of brood balls from the bison dung treatment (6.51 g) did not differ from the cattle dung treatment (6.44 g) (two sample t-test, $t = 0.421$, $df = 390$, $P = 0.674$). In total, 407 beetles emerged as adults, representing an 88.5% survival rate for all brood balls (bison dung = 183, cattle dung = 277). There was a relatively equal emergence by sex for both dung treatments, but a larger number of beetles emerged from cattle dung (Table 7). More beetles emerged from cattle dung across all temperature treatments (Table 8). Survival to emergence from brood balls was unaffected by temperature, dung type or brood ball mass (Table 9).

CHAPTER 4

DISCUSSION

4.1 General Discussion

Many dung beetles in the genus *Onthophagus* (closely related to *Digitonthophagus*) display morphological and developmental plasticity to maximize their fitness in response to variable nutritional conditions (Hunt and Simmons, 2001; Moczek, 2011). Although dung beetles that historically utilized bison dung in North America are believed to have persisted after the replacement of bison with cattle by utilizing cattle dung (Tiberg and Floate, 2011), the developmental consequences of this resource shift are not understood. We first sought to compare the horn allometry of *D. gazella* feeding on cattle and bison dung to determine how this resource shift affected plastic traits important for fitness in dung beetles. When we designed this project, the available literature indicated that bison dung has higher nitrogen content than cattle dung (Moore and Bower, 2001; Tiberg and Floate, 2011). The nitrogen content of dung is associated with increased resource quality for dung beetles (Dadour and Cook, 1996; Holter and Scholtz, 2007; Snell-Rood et al., 2013; Kaur et al., 2021; Thotagamuwa et al., 2023), and we hypothesized beetles would have larger body sizes and horn lengths in the bison dung treatment. However, we found that males were major morphs in both dung treatments indicating that both dung types are high-quality nutritional resources for *D. gazella.* Out of the 212 males that emerged in this study, only two (1%) had body sizes below 6 mm—the threshold that separates major and minor male morphs in *D. gazella* (Schwab and Moczek, 2016). As a result, males from our experiment were almost entirely major male morphs. In species where males are polymorphic, large males with fully developed horns arise from high-quality larval nutritional conditions while minor males arise from low-quality nutritional conditions (Emlen, 1997; Tomkins and Hazel, 2011; Snell-Rood et al., 2013; Moczek and Kijimoto, 2014). Beetles from the cattle dung treatment emerged at slightly larger body sizes but were large in both dung treatments, and there were no differences in the scaling relationship between male horn length and body size. This suggests that while cattle dung may offer slightly better nutrition, both dung types provided favorable nutritional conditions for *D. gazella*.

We hypothesized that bison dung would be a higher quality resource because it is reported to have higher nitrogen content than cattle dung (Moore and Bower, 2001; Tiberg and Floate, 2011). In contrast to predictions, male and female body size and male horn allometry did not differ between beetles that fed on bison and cattle dung, suggesting both are high quality resources for *D. gazella.* However, recent analyses comparing the nitrogen content of dung from cattle and bison at Konza Biological Station (where we collected dung samples) found that cattle and bison dung have similar nitrogen content, thus supporting our findings that cattle and bison are similar quality resources for dung beetles (Anguiano, 2024). Previous studies comparing the nitrogen content in bison dung to that of cattle dung involved animals given different supplemented diets (Tiberg and Floate, 2011). This variation in diet could potentially explain the reported differences in nitrogen content between the two types of dung. Dung samples utilized in our study were collected from cattle and bison grazing on pasture (Table 10). Grass-fed cattle dung is known to be a higher quality resource and produce larger beetles than hay and grain-fed cattle dung (Dadour and Cook, 1996; Casasa et al., 2020; Rohner and Moczek, 2021). Our results indicate that both bison and cattle dung may provide high quality resources when grazers are allowed to forage in natural grasslands, thus allowing dung beetles to emerge with large body sizes. Cattle were provided with mineral supplementation which could account for slightly larger body sizes in beetles from the cattle dung treatment. Although our analyses suggest that both cattle and bison dung provide high quality nutritional resources for dung beetles, we note that the dung quality of both ungulates can vary seasonally and by ecological and management contexts.

4.2 Interactive Effects of Diet Quality and Temperature

Understanding how nutrition shapes responses to temperature is key to predicting how ectotherms, like dung beetles, will respond to changing climates in different ecological contexts. We found that nutritional conditions altered the shape of thermal reaction norms for body size and horn size. Our research highlights that, while thermal reaction norms are useful to predict species responses to potential future climate, they may vary by nutritional context. Beetles that fed on cattle dung were larger and had longer horns than those that fed on bison dung, but only at intermediate temperatures. Increases in body size and horn length in beetles reared in cattle dung aligned with previously reported optimal growth temperatures (25–30°C) and optimal breeding temperature for *D. gazella* (28°C) (Pokhrel et al., 2020; Moczek pers. comm. 20. Jan. 2023), supporting the notion that these traits are responding to a thermal optimum. In contrast, neither of the traits appeared to change across temperature for beetles reared in bison dung. In other insects like *Drosophila*, body size is maximized at intermediate temperatures due to thermal stresses at high and low temperatures (Moreteau et al., 1997; Karan et al., 1998). Dung beetles feeding on cattle dung may respond similarly, that is they may be able to grow larger when freed from thermal stresses at intermediate temperatures. Bison dung may be a lower quality nutrient source in comparison, and beetles may not be able to grow to larger sizes even under optimal temperature conditions. Given that *D. gazella* is a generalist and can utilize a wide variety of dung types (Pokhrel et al., 2020), wild populations likely show varied responses to changes in temperature. Our findings highlight the need to account for nutritional conditions when determining how traits will scale with temperature.

Dung beetles from the cattle dung treatment are expected to have higher fitness than those from the bison dung treatment at optimal temperatures because body size and horn size are correlated with increased fecundity and reproductive success (Emlen, 1997; Hunt and Simmons, 2000). Larger females exhibit higher lifetime fecundity and provide more dung to their offspring, leading to increased offspring survival (Cook, 1988; Hunt and Simmons, 2000; Palestrini and Rolando, 2001; Simmons and Emlen, 2008). Moreover, studies have shown that body size largely determines fight outcome between major males (Emlen, 1997; Moczek and Emlen, 2000). For contests between equally sized males, horn size determines contest outcome (Moczek and Emlen, 2000). As a result, at optimal temperatures dung beetles that feed on cattle dung are expected be larger and have greater reproductive success than those that feed on bison dung.

Although body sizes and horn lengths were larger in the cattle dung treatment at intermediate temperatures, the resulting differences in trait size at intermediate temperatures remains relatively modest. The predicted differences in body sizes (~ 0.1mm) and horn lengths (~ 0.1mm) between dung treatments at intermediate temperatures represent 3% and 5% of the typical variation in body size and horn length (respectively) in *D. gazella* (Schwab and Moczek, 2016). Considering that reproductive success increases with trait size (Emlen, 1997), the slight differences in horn size and body size in the dung treatments under intermediate temperatures are unlikely to have large impacts on fitness. However, horn length variation less than 0.5 mm and body size variations of less than 1 mm have been demonstrated to significantly influence success in male contests among *Onthophagus* beetles (Emlen, 1997; Moczek and Emlen, 2000). The relatively small variation in horn size and body reflects that our analyses were restricted to large females and major males. The plastic traits analyzed, particularly horn size, are dimorphic in closely related *Onthophagus* dung beetles, and most of the variation is found between minor and major morphs (Emlen, 1994). However, even among large beetles in this study, we detected a consistent effect of dung treatment on the shape of thermal reaction norms for horn size and body size of both sexes, indicating that dung quality can modulate the effects of temperature on growth in a predictable manner.

4.3 Development and Growth Rates

We found that dung beetle growth and development rates increased with temperature, and both rates were higher in beetles feeding on bison dung than those feeding on cattle dung. Beetles in both dung treatments increased growth rates with temperature, and faster development rates led to a substantial reduction in body size. Faster development rates allowed beetles in the bison dung treatments to emerge earlier than beetles from the cattle treatment, particularly at the highest temperatures. Earlier emergence is associated with increased population persistence in dung beetles, so beetles feeding on bison dung may have an increased fitness at high temperatures (Rohner and Moczek, 2020). *Digitonthophagus gazella* can have multiple generations per year (Pokhrel et al., 2020), and shorter development times can increase fitness by allowing more generations per year (Tyndale-Biscoe and Walker, 1992; Kingsolver et al., 2012). Moreover, emerging earlier in the season can reduce the risk of pre-adult mortality due to predation by soil nematodes during development (Hunt and Simmons, 1997). As global climates warm, diet quality may play an increasing role in dung beetle fitness by altering development rates.

Although changes in both nutritional conditions and temperature altered dung beetle growth in our study, plasticity in parental behavioral could mitigate some of the effects of these environmental factors in natural systems. Mothers bury brood balls deeper where it is cooler when temperatures increase, which can mitigate negative effects of temperature on growth

(Macagno et al., 2018). In this study, brood balls were only insulated with a small amount of substrate inside of rearing chambers, and temperatures of brood balls in the soil are likely to be more insulated from surface temperatures. Though we did not observe differences in brood ball masses between beetles from bison and cattle dung treatments, mothers can also adjust the amount of dung provisioned in response to dung quality (Moczek, 1998). Mothers increase the amount of dung provisioned when the quality of quality is low (Moczek, 1998). Brood ball mass is also a major determinant of dung beetle body size and horn length (Moczek and Emlen, 1999), and as a result, plasticity in the amount of dung may also mitigate effects of variation in dung quality on thermal responses.

Our work highlights the need to account for nutritional conditions when predicting dung beetle responses to changing climates. The development rates observed in our study corroborate those reported for *D. gazella* under similar temperature gradients, and previous studies have demonstrated that nutritional conditions affect development time in *D. gazella* (Floate et al., 2014; Rohner and Moczek, 2021). However, our work shows that nutritional conditions can constrain plastic responses in development time to changes in temperature. Prior investigations of how development rates vary across temperatures served to inform whether dung beetles like *D. gazella* would establish populations in novel environments (Floate et al., 2014; Floate et al., 2017). An assumption of using thermal reaction norms to predict organismal responses to changing climate is that development time will scale consistently with temperature in different ecological contexts (Luhring et al., 2019). Our study reveals that thermal responses in development time are influenced by nutritional conditions, and that dung beetle populations feeding on higher quality dung will exhibit more pronounced plastic responses to changing temperatures.

4.4 Conclusions

The effects of nutritional conditions on body size, horn size, development rates, and growth rates varied with changing temperatures, highlighting the complex ways environmental variables interact to shape plastic traits. Beetles that provision their larvae with both cattle dung and bison dung were large, indicating both dung types provided favorable nutritional conditions.

Based on limited information about the nitrogen content of bison dung (Moore and Bower, 2001; Tiberg and Floate, 2011), we hypothesized that larvae feeding on bison dung would emerge at larger body size and longer horn lengths than beetles fed cattle dung. However, recent analyses by Anguiano (2024) on dung samples from the same cattle and bison herds we collected dung from revealed that both grazers have similar nitrogen content when foraging on pasture. Thus, both cattle and bison dung may be similar high-quality nutritional resources for dung beetles if both herbivores are grazing on natural grasslands and are not fed grain or hay diets. We note that cattle receiving mineral supplementation may explain the difference between the thermal reaction norms observed between dung beetles from bison dung and cattle dung treatments.

Comparing thermal response curves for body size and horn length between dung treatments revealed that nutritional conditions have temperature-dependent effect on plastic traits. Rather than causing a consistent change in trait size across temperatures, beetles from the cattle dung treatment were larger at intermediate temperatures. We observed significant differences in horn size and body size between dung type treatments at intermediate temperatures, but the differences in sizes were numerically small because all reared beetles were large (e.g., major male morphs) in both dung treatments (there was an overall lack of small individuals). While horn length and body size were similar between the dung treatments, underlying growth and development rates were higher in the bison treatment. As a result, plasticity in both growth and development rates may allow beetles to emerge earlier while having little consequence for trait size. Extensive research has shown that plastic traits in dung beetles are nutritionally cued, and our research highlights the need to examine the combined effects of nutrition and temperature on plastic responses in complex environments.

REFERENCES

REFERENCES

Anguiano NV. 2024. Bison and cattle grazing influences on soil microbial N cycling and ecosystem N pools in annually burned tallgrass prairies [Masters]. Manhattan Kansas: Kansas State University. 82 p.

Atkinson D. 1994. Temperature and organism size-A biological law for ectotherms? In: Begon M, editor. Advances in Ecological Research. Vol. 25. 1st ed. San Diego: Academic Press. p. 1–58.

Carter AW, Sheldon KS. 2020. Life stages differ in plasticity to temperature fluctuations and uniquely contribute to adult phenotype. J. Exp. Biol. 223(20):1–8. https://doi.org/10.1242/jeb.227884.

Casasa S, Moczek AP. 2018. The role of ancestral phenotypic plasticity in evolutionary diversification: population density effects in horned beetles. Anim. Behav. 137:53–61. https://doi.org/10.1016/j.anbehav.2018.01.004.

Casasa S, Schwab DB, Moczek AP. 2017. Developmental regulation and evolution of scaling: novel insights through the study of *Onthophagus* beetles. Curr. Opin. Insect Sci. 19:52–60. https://doi.org/10.1016/j.cois.2016.11.004.

Casasa S, Zattara EE, Moczek AP. 2020. Nutrition-responsive gene expression and the developmental evolution of insect polyphenism. Nat. Ecol. Evol. 4(7):970–978. https://doi.org/10.1038/s41559-020-1202-x.

Cook D. 1988. Sexual eelection in dung Beetles. Female fecundity as an estimate of male reproductive success in relation to horn size, and alternative behavioural strategies in *Onthophagus binodis* Thunberg (Scarabaeidae: Onthophagini). Aust. J. Zool. 36:521–532. https://doi.org/10.1071/ZO9880521.

Dadour IR, Cook DF. 1996. Survival and reproduction in the scarabaeine dung beetle *Onthophagus binodis* (Coleoptera: Scarabaeidae) on dung produced by cattle on grain diets in feedlots. Environ. Entomol. 25(5):1026–1031. https://doi.org/10.1093/ee/25.5.1026.

Diamond SE, Kingsolver JG, Heard AESB, et al. 2010. Environmental dependence of thermal reaction norms: Host plant quality can reverse the temperature-size rule. Am. Nat. 175(1):1–10. https://doi.org/10.1086/648602.

Emlen DJ. 1994. Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). Proc. R. Soc. Lond. B. 256(1346):131–136. https://doi.org/10.1098/rspb.1994.0060.

Emlen DJ. 1997. Diet alters male horn allometry in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). Proc. R. Soc. Lond. B. 264(1381):567–574. https://doi.org/10.1098/rspb.1997.0081.

Emlen DJ. 2000. Integrating development with evolution: A case study with beetle horns. Biosci. 50(5):403. https://doi.org/10.1641/0006-3568(2000)050[0403:IDWEAC]2.0.CO;2.

Emlen, DJ, Hunt J, Simmons LW. 2005. Evolution of sexual dimorphism and male dimorphism in the expression of beetle horns: Phylogenetic evidence for modularity, evolutionary lability, and constraint. Am. Nat. 166(S4):S42–S68. https://doi.org/10.1086/444599.

Emlen DJ, Marangelo J, Ball B, et al. 2005. Diversity in the weapons of sexual selection: Horn evolution in the beetle genus *Onthophagus* (Coleoptera: Scarabaeidae). Evol. 59(5):1060–1084. https://doi.org/10.1111/j.0014-3820.2005.tb01044.x.

Fincher GT. 1986. Mass rearing: Importation, colonization, and release of dung-burying scarabs. In: Biological Control of Muscoid Flies. College Park, MD: Entomological Society of America. p. 69. https://doi.org/10.4182/BVEE9801.61.69.

Fincher GT, Stewart TB, Hunter JS. 1983. The 1981 distribution of *Onthophagus gazella* Fabricius from releases in Texas and *Onthophagus taurus* Schreber from an unknown release in Florida (Coleoptera: Scarabaeidae). Coleop. Bull. 37(2):159–163.

Fleming JM, Carter AW, Sheldon KS. 2021. Dung beetles show metabolic plasticity as pupae and smaller adult body size in response to increased temperature mean and variance. J. Insect Physiol. 131:104215. https://doi.org/10.1016/j.jinsphys.2021.104215.

Floate KD, Watson DW, Coghlin P, et al. 2014. Degree-day models for development of the dung beetles *Onthophagus nuchicornis* , *O. taurus* , and *Digitonthophagus gazella* (Coleoptera: Scarabaeidae), and the likelihood of *O. taurus* establishment in southern Alberta, Canada. Can. Entomol. 147(5):617–627. https://doi.org/10.4039/tce.2014.70.

Floate KD, Watson DW, Weiss RM, et al. 2017. Bioclimatic analyses for the distributions of *Onthophagus nuchicornis* , *Onthophagus taurus* , and *Digitonthophagus gazella* (Coleoptera: Scarabaeidae) in North America. Can. Entomol. 149(4):504–524. https://doi.org/10.4039/tce.2017.20.

Fox RJ, Donelson JM, Schunter C, et al. 2019. Beyond buying time: The role of plasticity in phenotypic adaptation to rapid environmental change. Philos. Trans. R. Soc. B. 374(1768):20180174. https://doi.org/10.1098/rstb.2018.0174.

Gienapp P, Teplitsky C, Alho JS, et al. 2008. Climate change and evolution: disentangling environmental and genetic responses. Mol. Ecol. 17(1):167–178. https://doi.org/10.1111/j.1365-294X.2007.03413.x.

Gienapp P, Väisänen RA, Brommer JE. 2010. Latitudinal variation in breeding time reaction norms in a passerine bird. J. Anim. Ecol. 79(4):836–842. https://doi.org/10.1111/j.1365-2656.2010.01693.x.

Hunt J, Simmons LW. 1997. Patterns of fluctuating asymmetry in beetle horns: an experimental examination of the honest signalling hypothesis. Behav. Ecol. Sociobiol. 41(2):109–114. https://doi.org/10.1007/s002650050370.

Hunt J, Simmons LW. 2000. Maternal and paternal effects on offspring phenotype in the dung beetle *Onthophagus taurus*. Evol. 54(3):936–941. https://doi.org/10.1111/j.0014-3820.2000.tb00093.x.

Hunt J, Simmons LW. 2001. Status-dependent selection in the dimorphic beetle *Onthophagus taurus*. Proc. R. Soc. Lond. B. 268(1484):2409–2414. https://doi.org/10.1098/rspb.2001.1758.

Hunt J, Simmons LW. 2002. The genetics of maternal care: Direct and indirect genetic effects on phenotype in the dung beetle *Onthophagus taurus*. Proc. Natl. Acad. Sci. 99(10):6828–6832. https://doi.org/10.1073/pnas.092676199.

Karan D, Morin JP, Moreteau B, et al. 1998. Body size and developmental temperature in *Drosophila melanogaster* : Analysis of body weight reaction norm. J. Therm. Biol. 23(5):301–309. https://doi.org/10.1016/S0306-4565(98)00021-7.

Kijimoto T, Moczek AP, Andrews J. 2012. Diversification of doublesex function underlies morph-, sex-, and species-specific development of beetle horns. Proc. Natl. Acad. Sci. 109(50):20526–20531. https://doi.org/10.1073/pnas.1118589109.

Kijimoto T, Pespeni M, Beckers O, et al. 2013. Beetle horns and horned beetles: emerging models in developmental evolution and ecology: Developmental evolution of horned beetles. Wiley Interdiscip. Rev. Dev. Biol. 2(3):405–418. https://doi.org/10.1002/wdev.81.

Kingsolver JG, Diamond SE, Seiter SA, et al. 2012. Direct and indirect phenotypic selection on developmental trajectories in *Manduca sexta*. Funct. Ecol. 26(3):598–607. https://doi.org/10.1111/j.1365-2435.2012.01972.x.

Kingsolver JG, Shlichta JG, Ragland GJ, et al. 2006. Thermal reaction norms for caterpillar growth depend on diet. Evol. Ecol. Res. 8:703–715.

Kingsolver JG, Umbanhowar J. 2018. The analysis and interpretation of critical temperatures. J. Exp. Biol. 221(12):jeb167858. https://doi.org/10.1242/jeb.167858.

Knell R. 2011. Male contest competition and the evolution of weapons. In: Simmons LW, Ridsdill-Smith TJ, editors. Ecology and Evolution of Dung Beetles. 1st ed. Chichester, UK: John Wiley & Sons. p. 47–65. https://doi.org/10.1002/9781444342000.ch3.

Luhring TM, Vavra JM, Cressler CE, et al. 2019. Phenotypically plastic responses to predation risk are temperature dependent. Oecologia. 191(3):709–719. https://doi.org/10.1007/s00442-019-04523-9.

Macagno ALM, Zattara EE, Ezeakudo O, et al. 2018. Adaptive maternal behavioral plasticity and developmental programming mitigate the transgenerational effects of temperature in dung beetles. Oikos. 127(9):1319–1329. https://doi.org/10.1111/oik.05215.

Mamantov MA, Sheldon KS. 2021. Behavioural responses to warming differentially impact survival in introduced and native dung beetles. Behmer S, editor. J. Anim. Ecol. 90(1):273–281. https://doi.org/10.1111/1365-2656.13366.

Matesanz S, Gianoli E, Valladares F. 2010. Global change and the evolution of phenotypic plasticity in plants. Ann. N.Y. Acad. Sci. 1206(1):35–55. https://doi.org/10.1111/j.1749-6632.2010.05704.x.

Merilä J, Hendry AP. 2014. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. Evol. Appl. 7(1):1–14. https://doi.org/10.1111/eva.12137.

Moczek A. 1998. Horn polyphenism in the beetle *Onthophagus taurus*: larval diet quality and plasticity in parental investment determine adult body size and male horn morphology. Behav. Ecol. 9(6):636–641. https://doi.org/10.1093/beheco/9.6.636.

Moczek A. 2011. Evolution and development: *Onthophagus* beetles and the evolutionary developmental genetics of innovation, allometry and plasticity. In: Ecology and evolution of dung beetles. Chichester, UK: John Wiley & Sons Ltd. p. 126–151.

Moczek AP. 2002. Allometric plasticity in a polyphenic beetle. Ecol. Entomol. 27(1):58–67. https://doi.org/10.1046/j.0307-6946.2001.00385.x.

Moczek AP. 2006. A matter of measurements: Challenges and approaches in the comparative analysis of static allometries. Am. Nat. 167(4):606–611. https://doi.org/10.1086/501075.

Moczek AP. 2007. Pupal remodeling and the evolution and development of alternative male morphologies in horned beetles. BMC Evol. Biol. 7(1):151. https://doi.org/10.1186/1471-2148-7-151.

Moczek AP. 2009. The origin and diversification of complex traits through micro- and macroevolution of development: Insights from horned beetles. In: Current Topics in Developmental Biology. Vol. 86. Academic Press. p. 135–162. https://doi.org/10.1016/S0070-2153(09)01006-0.

Moczek AP, Emlen DJ. 2000. Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: Do alternative reproductive tactics favour alternative phenotypes? Anim. Behav. 59(2):459–466. https://doi.org/10.1006/anbe.1999.1342.

Moczek AP, Kijimoto T. 2014. Development and evolution of insect polyphenisms: novel insights through the study of sex determination mechanisms. Curr. Opin. Insect Sci. 1:52–58. https://doi.org/10.1016/j.cois.2014.05.004.

Moczek, Emlen. 1999. Proximate determination of male horn dimorphism in the beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). J. Evol. Biol. 12:27–37. https://doi.org/10.1046/j.1420-9101.1999.00004.x.

Moore AJ, Bower NW. 2001. Chemometric analysis of compositional variation in bison and cow pattie: A Biogeochemistry—environmental chemistry experiment. Chem. Educator. 6(2):86–90. https://doi.org/10.1007/s00897010459a.

Moreteau B, Morin JP, Gibert P, et al. 1997. Evolutionary changes of nonlinear reaction norms according to thermal adaptation: a comparison of two *Drosophila* species. C. R. Acad. Sci. 320(10):833–841. https://doi.org/10.1016/s0764-4469(97)85020-2.

Palestrini C, Rolando A. 2001. Body size and paternal investment in the genus *Onthophagus* (Coleoptera, Scarabaeoidea). J. Zool. 255(3):405–412. https://doi.org/10.1017/S0952836901001492.

Parker J, Johnston LA. 2006. The proximate determinants of insect size. J. Biol. 5:15. https://doi.org/10.1186/jbiol47.

Plumb GE, Dodd JL. 1993. Foraging eology of bison and cattle on a mixed prairie: Implications for natural area management. Ecol. Appl. 3(4):631–643. https://doi.org/10.2307/1942096.

Pokhrel MR, Cairns SC, Andrew NR. 2020. Dung beetle species introductions: when an ecosystem service provider transforms into an invasive species. PeerJ. 8:e9872. https://doi.org/10.7717/peerj.9872.

Polley HW, Briske DD, Morgan JA, et al. 2013. Climate change and North American rangelands: Trends, projections, and implications. Rangel. Ecol. Manage. 66(5):493–511. https://doi.org/10.2111/REM-D-12-00068.1.

Price TD, Qvarnström A, Irwin DE. 2003. The role of phenotypic plasticity in driving genetic evolution. Proc. R. Soc. Lond. B. 270(1523):1433–1440. https://doi.org/10.1098/rspb.2003.2372.

Proctor M. 2024. Westward expansion of *Onthophagus taurus* Schreber (Coleoptera: Scarabaeidae): Current distribution of a non-native species. Coleop. Bull. (in press).

Rahman A ur, Khan SM, Ahmad Z, et al. 2021. Impact of multiple environmental factors on species abundance in various forest layers using an integrative modeling approach. Global Ecol. Conserv. 29:e01712. https://doi.org/10.1016/j.gecco.2021.e01712.

Relyea RA. 2004. Fine-tuned phenotypes: Tadpole plasticity under 16 combinations of predators and competitors. Ecol. 85(1):172–179.

Rij J van, Wieling M, Baayen RH, et al. 2022. itsadug: Interpreting time series and autocorrelated data using GAMMs. Available from https://cran.r-project.org/web/packages/itsadug/index.html.

Rohner PT. 2021. A role for sex-determination genes in life history evolution? Doublesex mediates sexual size dimorphism in the gazelle dung beetle. J. Evol. Biol. 34(8):1326–1332. https://doi.org/10.1111/jeb.13877.

Rohner PT, Moczek AP. 2020. Rapid differentiation of plasticity in life history and morphology during invasive range expansion and concurrent local adaptation in the horned beetle *Onthophagus taurus*. Evol. 74(9):2059–2072. https://doi.org/10.1111/evo.14045.

Rohner PT, Moczek AP. 2021. Evolutionary and plastic variation in larval growth and digestion reveal the complex underpinnings of size and age at maturation in dung beetles. Ecol. Evol. 11(21):15098–15110. https://doi.org/10.1002/ece3.8192.

Rohner PT, Moczek AP. 2023. Allometric plasticity and the evolution of environment-by-environment (E×E) interactions during a rapid range expansion of a dung beetle. Evol. 77(3):682–689. https://doi.org/10.1093/evolut/qpac071.

Schwab DB, Casasa S, Moczek AP. 2017. Evidence of developmental niche construction in dung beetles: Effects on growth, scaling and reproductive success. Coulson T, editor. Ecol. Lett. 20(11):1353–1363. https://doi.org/10.1111/ele.12830.

Schwab DB, Moczek AP. 2016. Nutrient stress during ontogeny alters patterns of resource allocation in two species of horned beetles. J. Exp. Zool. 325(8):481–490. https://doi.org/10.1002/jez.2050.

Simmons LW, Emlen DJ. 2008. No fecundity cost of female secondary sexual trait expression in the horned beetle *Onthophagus sagittarius*. J. Evol. Biol. 21(5):1227–1235. https://doi.org/10.1111/j.1420-9101.2008.01575.x.

Sinclair BJ, Marshall KE, Sewell MA, et al. 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? Ecol. Lett. 19(11):1372–1385. https://doi.org/10.1111/ele.12686.

Snell-Rood EC, Kobiela ME, Sikkink KL, et al. 2018. Mechanisms of Plastic Rescue in Novel Environments. Annu. Rev. Ecol. Evol. Syst. 49(1):331–354. https://doi.org/10.1146/annurev-ecolsys-110617-062622.

Snell-Rood EC, Troth A, Moczek AP. 2013. DNA methylation as a mechanism of nutritional plasticity: Limited support from horned beetles. J. Exp. Zool. B. 320(1):22–34. https://doi.org/10.1002/jez.b.22479.

Stillwell RC, Wallin WG, Hitchcock LJ, et al. 2007. Phenotypic plasticity in a complex world: interactive effects of food and temperature on fitness components of a seed beetle. Oecologia. 153(2):309–321. https://doi.org/10.1007/s00442-007-0748-5.

Tiberg K, Floate KD. 2011. Where went the dung-breeding insects of the American bison? Can. Entomol. 143(5):470–478. https://doi.org/10.4039/n11-024.

Tomkins J, Hazel W. 2011. Explaining phenotypic diversity: The conditional strategy and threshold trait expression. In: Simmons LW, Ridsdill-Smith TJ, editors. Ecology and Evolution of Dung Beetles. Chichester, UK: John Wiley & Sons, Ltd. p. 107–125. https://doi.org/10.1002/9781444342000.ch6.

Trible ML. 2017. Effects of bison, cattle, and environmental factors on grassland dung beetle abundance and community structure [Masters]. Wichita KS: Wichita State University.

Tyndale-Biscoe M, Walker J. 1992. The phenology of the native dung beetle *Onthophagus australis* (Guerin) (Coleoptera, Scarabaeinae) in South-Eastern Australia. Aust. J. Zool. 40(3):303–311. https://doi.org/10.1071/zo9920303.

Westneat DF, Potts LJ, Sasser KL, et al. 2019. Causes and consequences of phenotypic plasticity in complex environments. Trends Ecol. Evol. 34(6):555–568.

Wood S. 2023. mgcv: Mixed GAM Computation Vehicle with Automatic Smoothness Estimation. Available from https://cran.r-project.org/web/packages/mgcv/index.html.

APPENDIXES

APPENDIX A

TABLES

Table 1. ANOVA table summarizing the linear regression analysis examining the relationship between thorax size and horn length, including the effect of dung treatment. Bold indicates significant effect of parameter.

	DF	Sum Sq	Mean Sq	F-Value	Pr(>F)
Thoracic width	1	331747	331746	27.067	**5.26e-7**
Dung	1	3814	1620	0.132	0.717
Thoracic Width * Dung	1	11634	837	0.068	0.791

Table 2. ANOVA Analysis of a Generalized Additive Mixed Model (GAMM) evaluating the effects of both parametric (left) and smoother terms (right) on body size (thoracic width). Temperature smoother effects were allowed to vary by the dung-sex interaction levels and, as a result, each interaction level has a separate p-value for nonlinear temperature-dependent effects. Bold indicates significant p-values.

Response	Parametric Terms	df	F	P	Smoother Terms	Ref. df	F	P
Body Size	Dung	1	11.381	**0.0008**	Temperature	0.800	19.677	**8.77e-05**
	Sex	1	567.913	**<2.22e-16**	Temperature: Female Bison	0.800	0.678	0.462
					Temperature: Male Bison	1.341	0.420	0.722
					Temperature: Female Cattle	1.796	7.155	**0.001**
					Temperature: Male Cattle	1.792	4.980	**0.005**
					Random: Brood Ball Mass	1.000	18.04	**1.71e-05**

Table 3. ANOVA Analysis of a Generalized Additive Mixed Model (GAMM) evaluating the effects of both parametric (left) and smoother terms (right) on the horn length in males. Temperature smoother effects were allowed to vary by each dung treatment and, as a result, both dung treatments have a separate p-value for nonlinear temperature-dependent effects. Bold indicates significant p-values.

Response	Parametric Terms	*df*	F	*P*	Smoother Terms	*Ref. df*	F	*P*
Horn length	Dung	1	2.438	0.120	Temperature	0.667	7.040	**0.032**
					Temperature: Bison	0.667	2.311	0.216
					Temperature: Cattle	1.652	4.436	**0.017**
					Random: Brood Ball Mass	1.00	8.619	**0.002**

Table 4. ANOVA Analysis of a Generalized Additive Mixed Model (GAMM) evaluating the effects of both parametric (left) and smoother terms (right) on the horn length in males. Temperature smoother effects were allowed to vary by each dung treatment and, as a result, both dung treatments have a separate p-value for nonlinear temperature-dependent effects. Bold indicates significant p-values.

Response	Parametric Terms	df	F	P	Smoother Terms	Ref. df	F	P
Growth rate (Body size)	Dung	1	10.841	0.001	Temperature	1.800	1795.769	**<2.22e-16**
	Sex	1	3.2.846	0.092	Temperature: Female Bison	0.800	65.128	**<2.22e-16**
					Temperature: Male Bison	0.800	57.694	**<2.22e-16**
					Temperature: Female Cattle	0.800	42.040	**<2.22e-16**
					Temperature: Male Cattle	0.897	36.285	**<2.22e-16**
					Random: Brood Ball Mass	1.000	30.666	**<2.22e-16**

Table 5. ANOVA Analysis of a Generalized Additive Mixed Model (GAMM) evaluating the effects of both parametric (left) and smoother terms (right) on the growth rate of horn length in males. Temperature smoother effects were allowed to vary by each dung treatment and, as a result, both dung treatments have a separate p-value for nonlinear temperature-dependent effects. Bold indicates significant p-values.

Response	Parametric Terms	*df*	F	*P*	Smoother Terms	*Ref. df*	F	*P*
Growth rate (Horn length)	**Dung**	1	6.179	0.014	Temperature	1.639	662.813	**< 2.22e-16**
					Temperature: Bison	0.667	196.544	**< 2.22e-16**
					Temperature: Cattle	1.148	32.327	**< 2.22e-16**
					Random: Brood Ball Mass	1.000	21.080	**5.37e-06**

Table 6. ANOVA Analysis of a Generalized Additive Mixed Model (GAMM) evaluating the effects of both parametric (left) and smoother terms (right) on development rates. Temperature smoother effects were allowed to vary by each dung treatment and, as a result, both dung treatments have a separate p-value for nonlinear temperature-dependent effects. Bold indicates significant p-values.

Response	Parametric Terms	df	F	P	Smoother Terms	Ref. df	F	P
Development rate	Dung	1	7.384	**0.0001**	Temperature	1.800	1836.975	**< 2.22e-16**
	Sex	1	16.014	**6.07e-05**	Temperature: Female Bison	0.800	81.663	**< 2.22e-16**
					Temperature: Male Bison	0.800	48.273	**< 2.22e-16**
					Temperature: Female Cattle	0.800	58.676	**< 2.22e-16**
					Temperature: Male Cattle	0.800	32.484	**9.41e-07**
					Random: Brood Ball Mass	1.000	19.263	**1.29e-05**

Table 7. Contingency table for number of emerged adults by dung treatment and sex.

Dung Treatment	Female (n=195)	Male (n=212)
Bison dung (n=162)	77	85
Cattle dung (n=245)	118	127

Table A-8. Adult emergence of beetles across temperatures by dung treatment.

Temperature	22°C	24°C	26°C	28°C	30°C	32°C	34°C
Bison	29	21	27	25	24	19	17
Cattle	41	33	37	33	35	35	31

Table 9. Summary table of term statistics for logistic regression to analyze responses in log-odds of survival.

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
Dung Type	-0.753	2.17	-0.346	0.729
Temperature	-0.033	0.061	-0.535	0.593
Dung x Temp	0.026	0.077	0.349	0.727
Brood Ball Mass	0.015	0.092	0.164	0.869

APPENDIX A (continued)

Table 10. Management for Bison and Cattle Herds at Konza Prairie Research Station.

Herd	**Ivermectin Use**				**Supplemental Feeding**		**Herd Type**	**Livestock Grazing Regime**					**Notes:**	**Contact:**
	Last date administered	Active ingredient/ Chemical name	Dosage	Method	On-site (last date)	Off-site (last date)		Breed	Head/Acre and duration	Location of origin	Date On-site	Date Off-site		
C35A (cattle)	Early May	Cydectin (cows, heifers & calves <2 yrs; >2 yrs is not treated)	Not provided	pour on or oral	non-medicated mineral (during the growing season)	not provided	cow-calf pairs or heifers	Angus-Hereford cross	1 cow-calf pair per 8 acres	home-raised and originated in Riley County, KS	~1 May	~30 Sept		KC Olson <kcolson@ksu.edu>
C3A (cattle)	Early May	Cydectin (cows, heifers & calves <2 yrs; >2 yrs is not treated)	not provided	pour on or oral	non-medicated mineral (during the growing season)	not provided	cow-calf pairs or heifers	Angus-Hereford cross	1 cow-calf pair per 8 acres	home-raised and originated in Riley County, KS	~1 May	~30 Sept		KC Olson <kcolson@ksu.edu>
N20B (bison)	10/24/2019	Prohibit (Levamisole)	7.4ml/100lbs	oral drench	20% beef range cubes used for roundup baiting. Less than 1/2lb/head/day. Last date: 10/15/22	NA	mixed	bison (no mtDNA introgression)	1AU/12.3 acres (or 1head/10.3 acres) 12 months	NA	1992	NA	ivermectin use from 2000-2016; levamisole 2017-2019; nothing currently used	
N1B (bison)	10/24/2019	Prohibit (Levamisole)	7.4ml/100lbs	oral drench	20% beef range cubes used for roundup baiting. Less than 1/2lb/head/day. Last date: 10/15/22	NA	mixed	bison (no mtDNA introgression)	1AU/12.3 acres (or 1head/10.3 acres) 12 months	NA	1992	NA	ivermectin use from 2000-2016; levamisole 2017-2019; nothing currently used	Jeffrey Taylor <jht@ksu.edu>

APPENDIX B

FIGURES

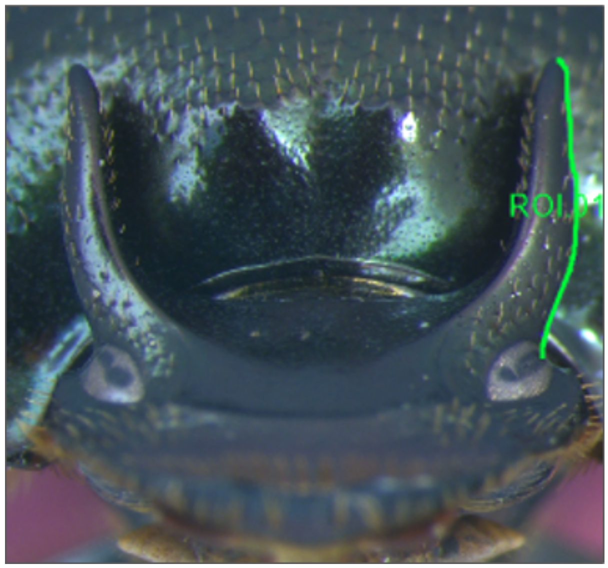

Figure 1. Frontal view of the head of *D. gazella*, major male morph, showing the method for nonlinear measurement of horn length (green line that follows the outer edge of right cephalic horn from the apex of the eye to the apex of the horn).

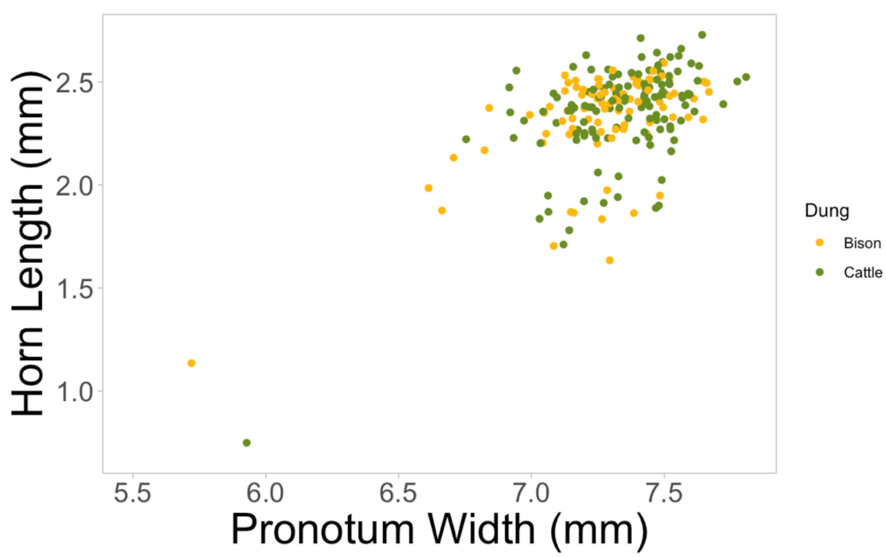

Figure 2. Scaling between body size and horn length for male *D. gazella*. All emerged males from the study are depicted including those with horn abnormalities and thoracic widths below 6 mm. Colors differentiate males from each dung treatment.

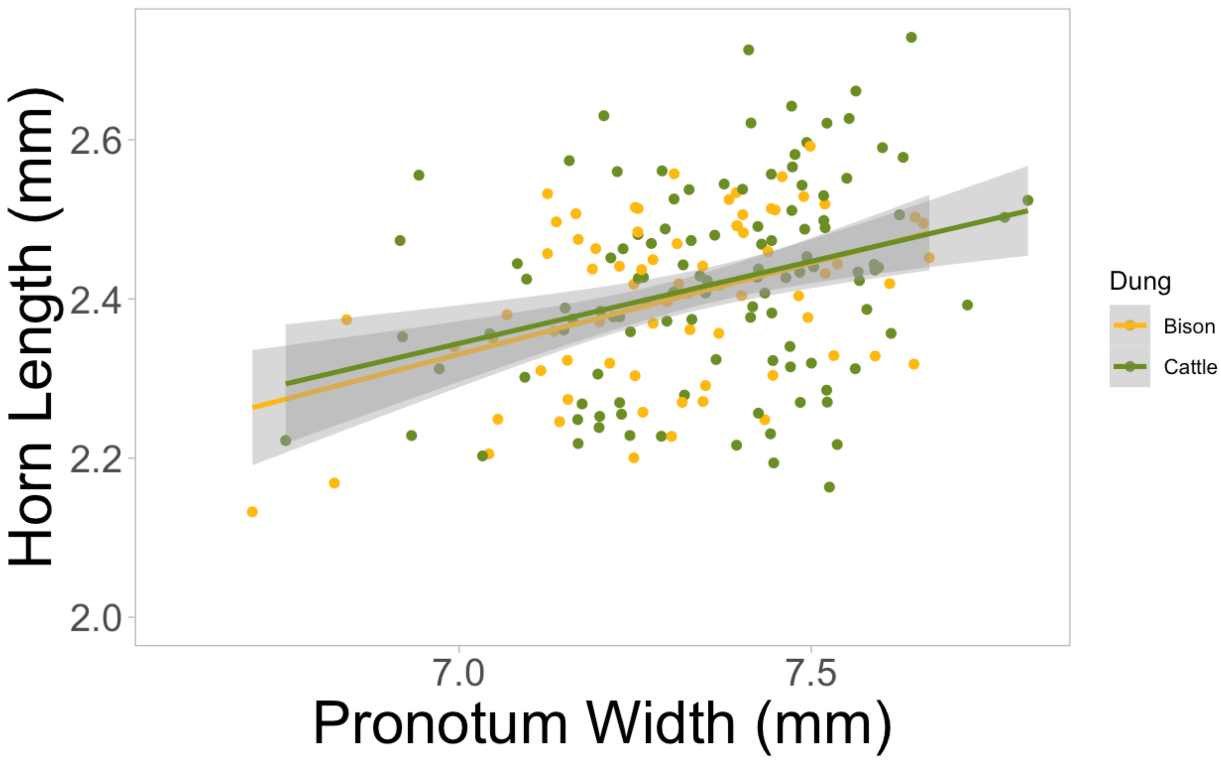

Figure 3. Scatterplot illustrating the relationship between pronotum width and horn length among male specimens by dung treatment. Lines fitted by linear regression with 95% confidence intervals are differentiated by dung treatment. Linear allometries do not differ between large males from bison dung and cattle dung treatments (P = 0.791).

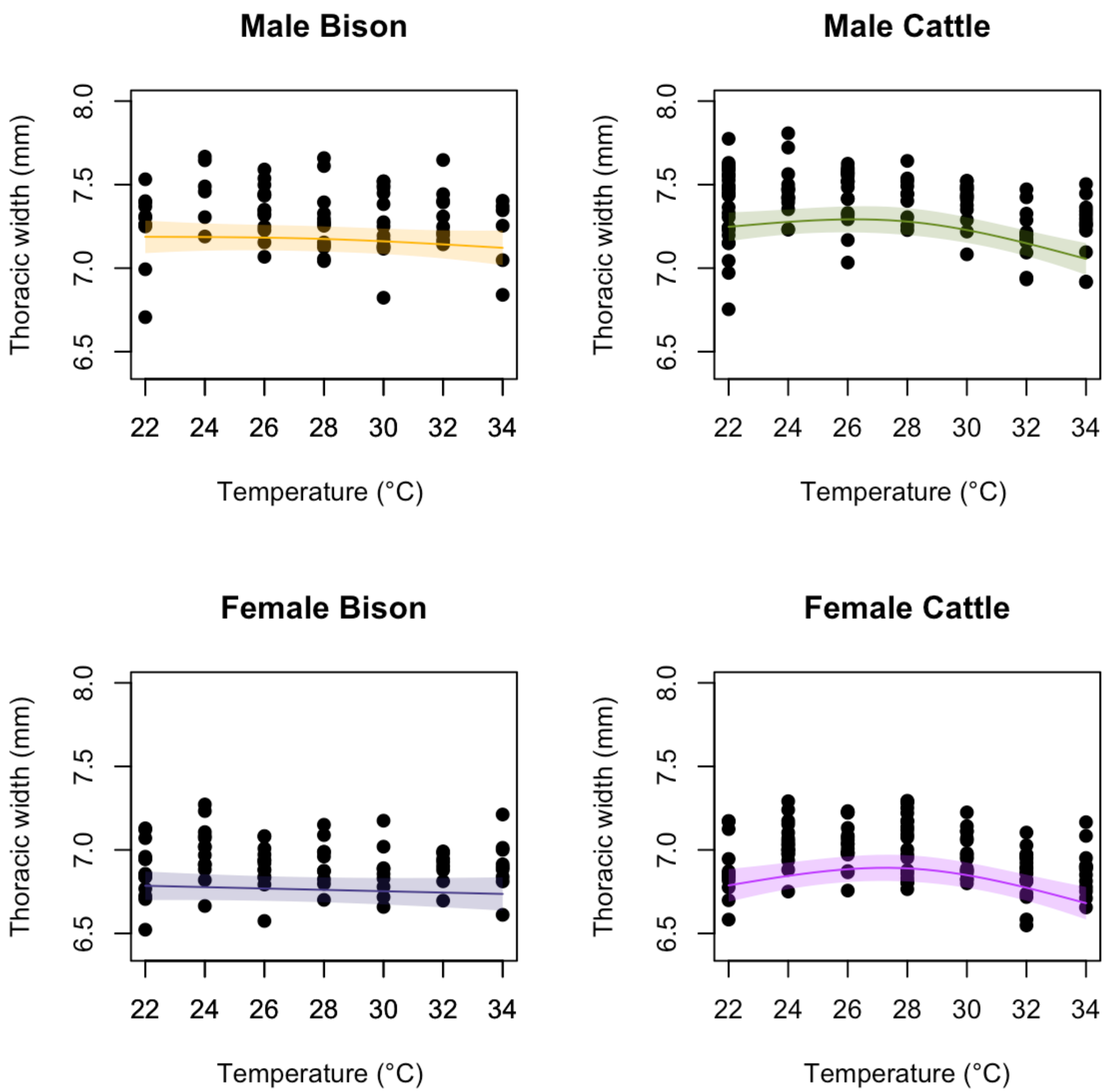

Figure 4**.** Fitted curves with 95% confidence intervals (CI) of the response of body size to the combined effects of temperature, dung type and sex derived from generalized additive mixed models (GAMMs). Males were roughly 0.4 mm larger than females across temperatures ($P < 2.22e{-16}$). For both sexes developing in cattle dung, body size scaled nonlinearly with temperature and peaked at intermediate temperatures (26-38°C) (male $P = 0.005$, female $P = 0.001$). Body size in both sexes that developed in bison dung did not change with temperature (male $P = 0.722$, female $P= 0.462$).

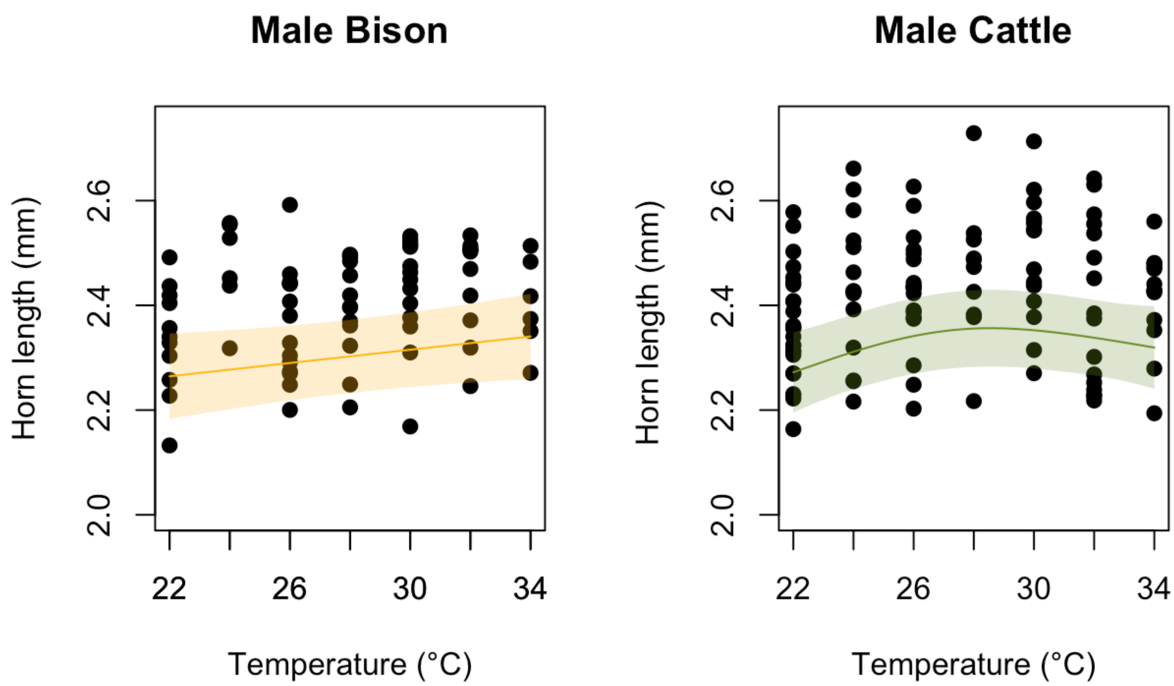

Figure 5. Fitted curves with 95% confidence intervals (CI) of the response of horn length to the combined effects of temperature and dung type derived from generalized additive mixed models (GAMMs). Horn lengths scaled unimodally with temperature in cattle dung treatments with greatest horn lengths at intermediate temperatures (26-30° C) ($P = 0.017$), but there were no nonlinear trends in horn length across temperature in the bison dung treatment ($P = 0.216$).

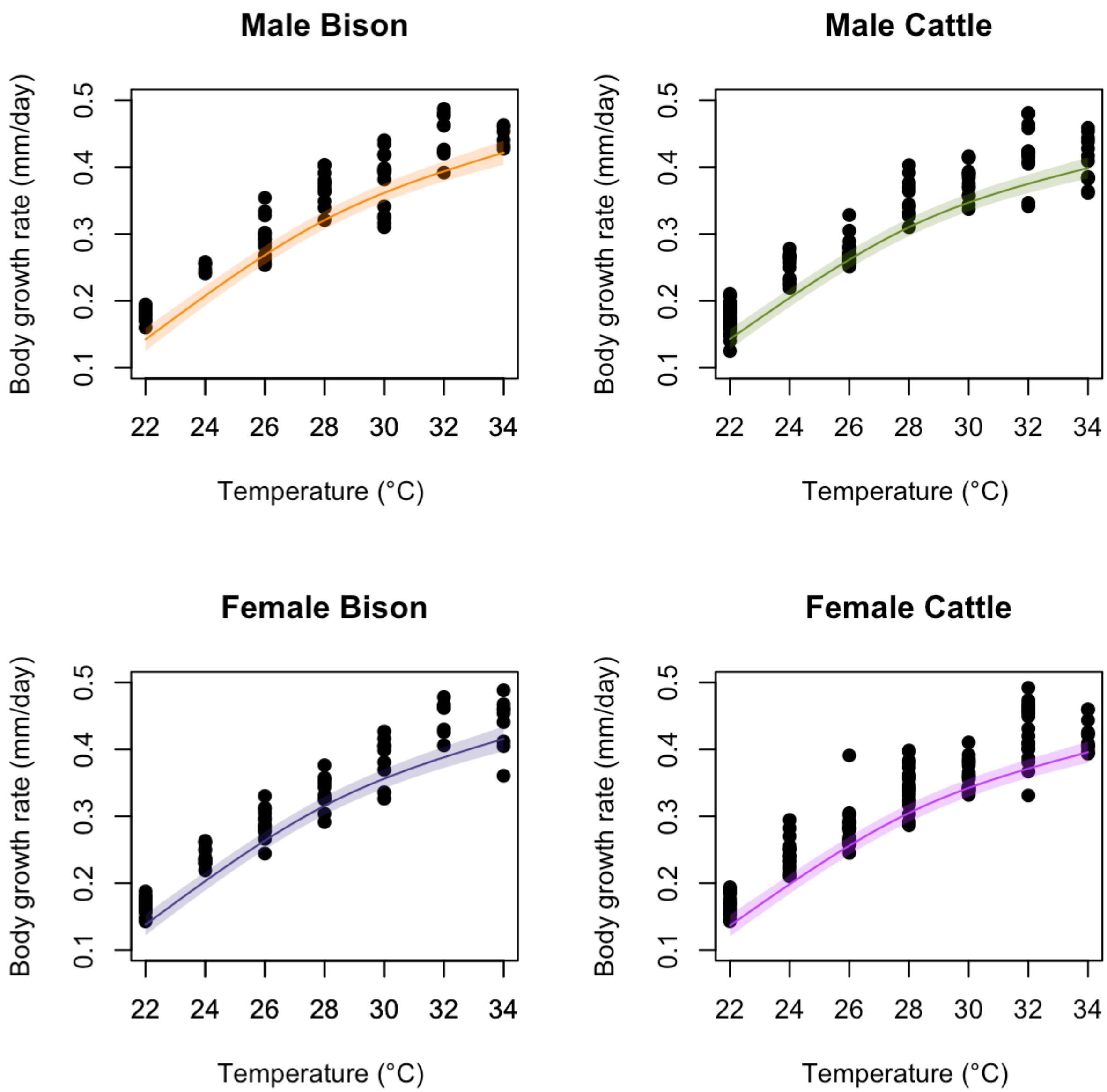

Figure 6. Fitted curves with 95% confidence intervals (CI) of the response of the growth rate of body size (mm/day) to the combined effects of temperature, dung type and sex derived from generalized additive mixed models (GAMMs). Growth rates of all sex and dung treatment combinations increased with temperature, but bison dung treatments had greater increases in growth rate than cattle dung treatments leading to higher growth rates at the highest temperatures ($P<2.22e-16$).

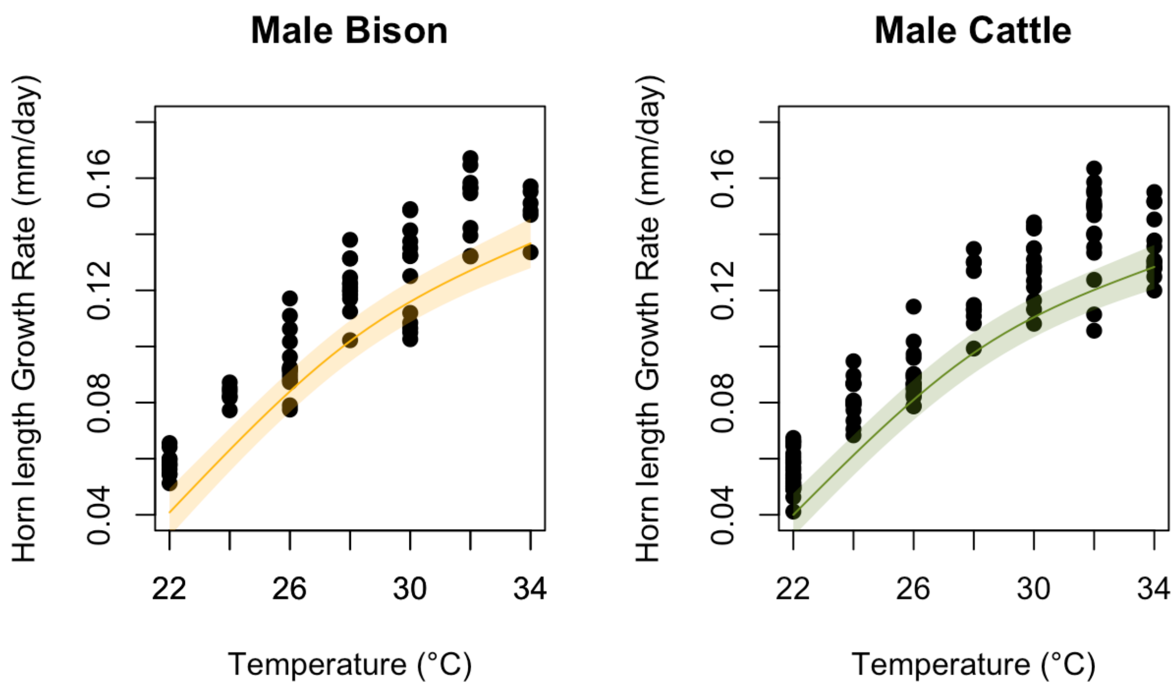

Figure 7**.** Fitted curves with 95% confidence intervals (CI) of the response of the growth rate of horns (mm/day) to the combined effects of temperature and dung type derived from generalized additive mixed models (GAMMs). The growth rate increases with temperature for both treatments (P<2.22e-16). Males from bison dung treatments had higher growth rates, highlighting a distinct nonlinear response between the dung treatments (Bison P< 2.22e-16; Cattle P< 2.22e-16).

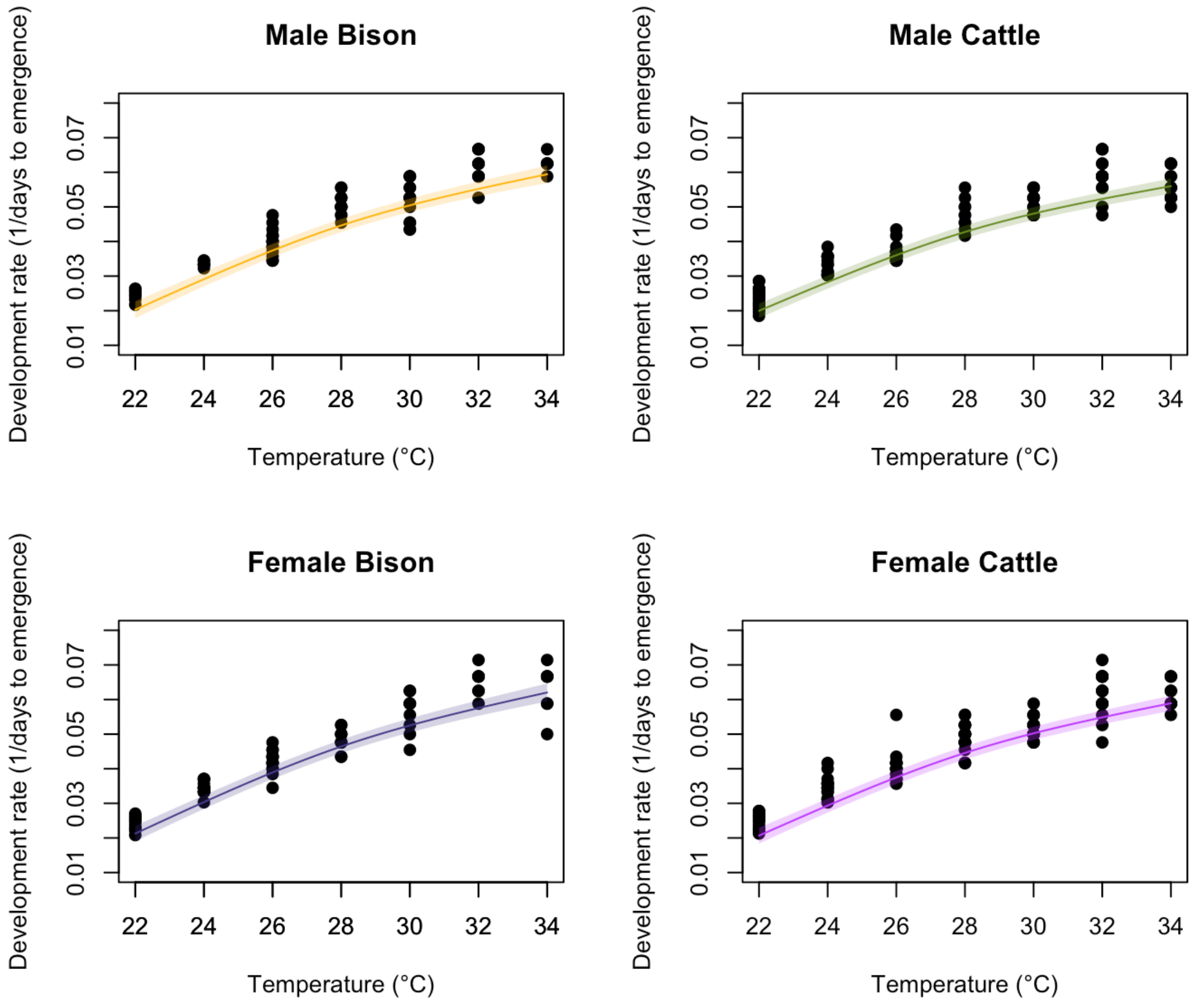

Figure 8. Fitted curves with 95% confidence intervals (CI) of the response of development rates (1/days to emergence) to the combined effects of temperature, dung type and sex derived from generalized additive mixed models (GAMMs). All treatment combinations display a nonlinear increase in growth rates with rising temperature, exhibiting a reduced slope at higher temperatures (30-34°C). With increasing temperatures, development rates diverge by treatment combination: bison dung treatments demonstrate faster development rates (male: $P < 2.22e-16$, female: $P < 2.22e-16$) than cattle dung treatments (male: $P= 9.41e-07$, female $P< 2.22e-16$). Females displayed faster development rates when compared to males in the same dung treatments.

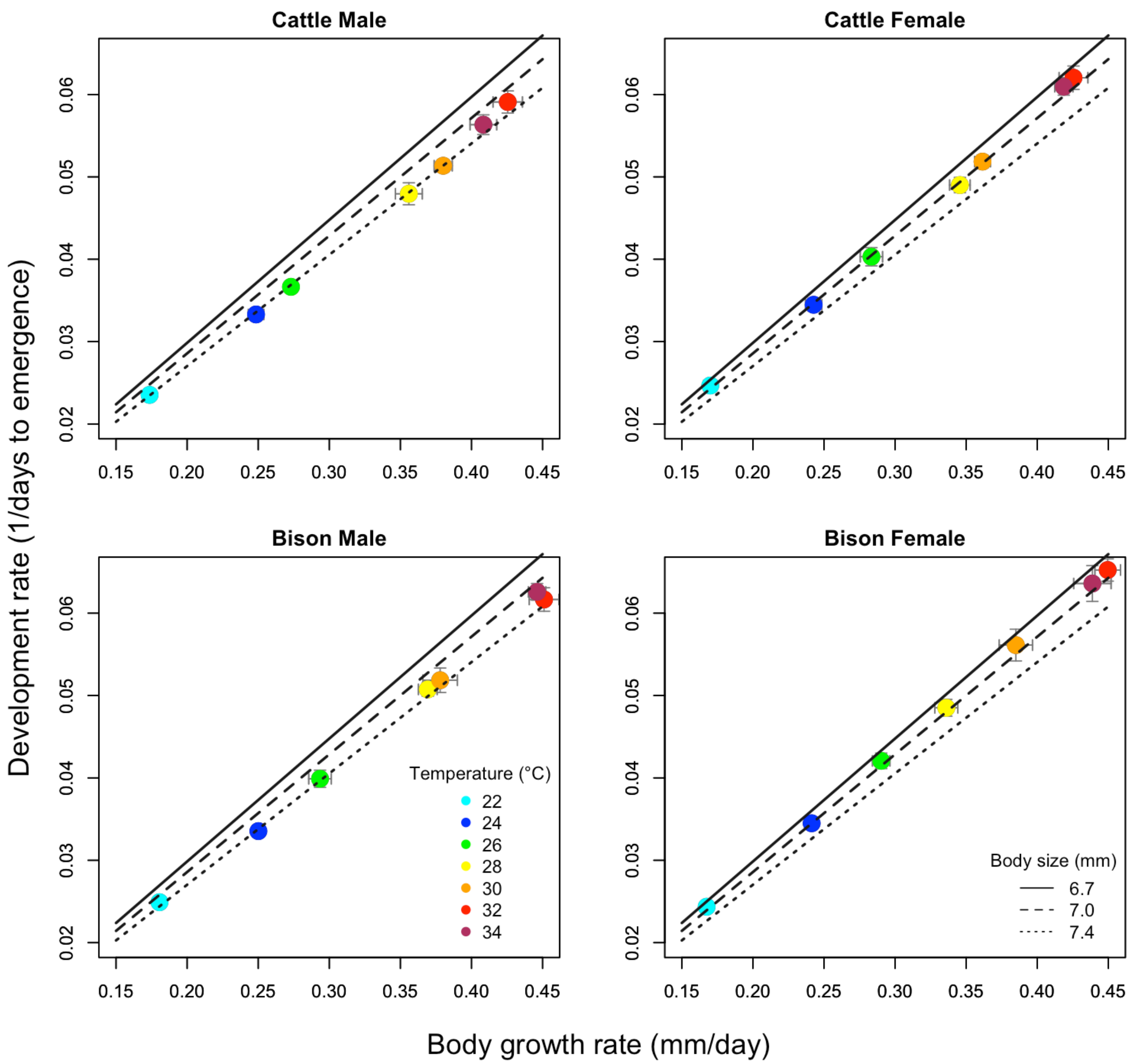

Figure 9. Average development rate (y=1/days to emergence) and body growth rate (x=thoracic width (mm)/days to emergence) of all beetles in each dung and sex treatment combination. The average male horn length at each temperature is represented by a dot with 95% error bars. Three isoclines correspond to combinations of growth and development rates where male horn length is constant.

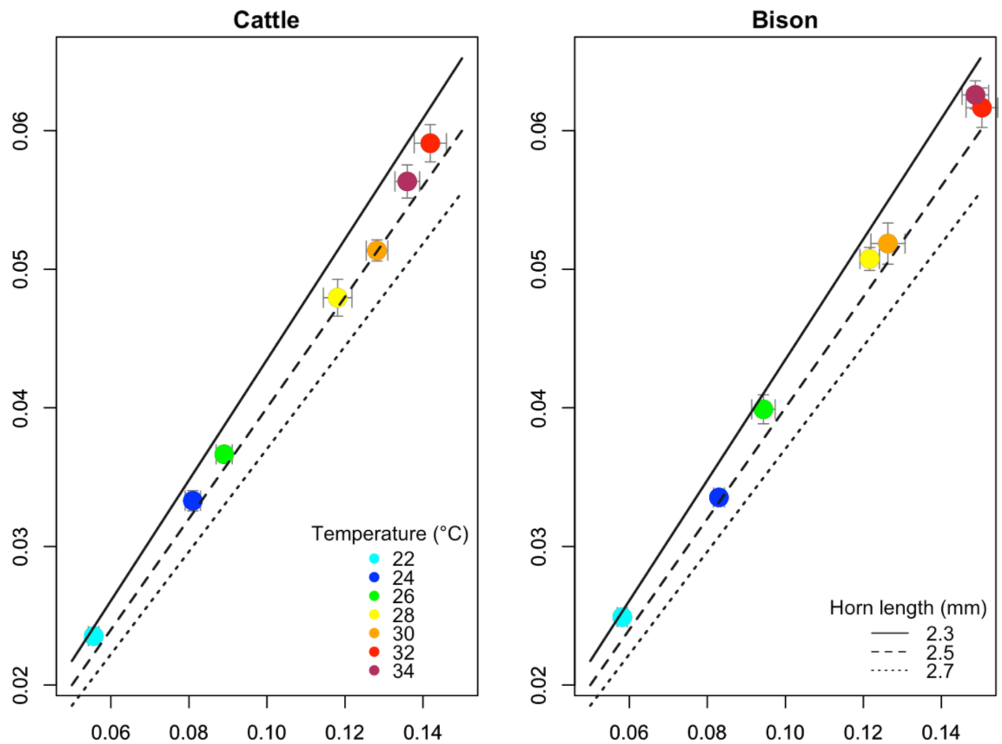

Figure 10. Average development rate (y=1/days to emergence) and horn growth rate (x=horn length (mm)/days to emergence) of male beetles in both dung treatments. The average horn length at each temperature is represented by a dot with 95% error bars. Three isoclines correspond to combinations of growth and development rates where horn length is constant.

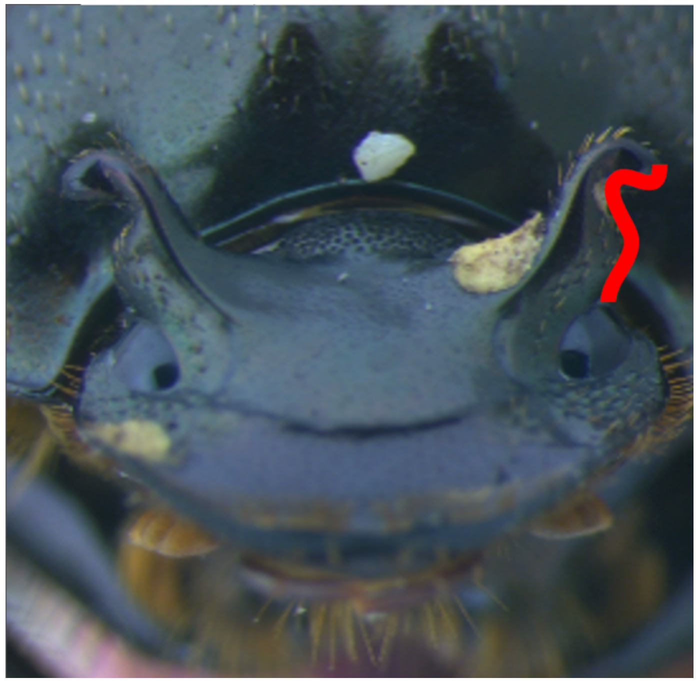

Figure 11**.** Example of a male with horn abnormalities (red line). Misshapen horns may have been due to human interference during development.