8-2022

The influence of traits on species responses to climate change: Does warming negatively impact native species more than invasive species?

Margaret Anne Mamantov
mmamanto@vols.utk.edu

Recommended Citation

Mamantov, Margaret Anne, "The influence of traits on species responses to climate change: Does warming negatively impact native species more than invasive species?. " PhD diss., University of Tennessee, 2022.
https://trace.tennessee.edu/utk_graddiss/7271

To the Graduate Council:

I am submitting herewith a dissertation written by Margaret Anne Mamantov entitled "The influence of traits on species responses to climate change: Does warming negatively impact native species more than invasive species?." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Kimberly S. Sheldon, Major Professor

We have read this dissertation and recommend its acceptance:

Daniel S. Simberloff, James A. Fordyce, Todd M. Freeberg

Accepted for the Council:

Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

The influence of traits on species responses to climate change: Does warming negatively impact native species more than invasive species?

A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Margaret Anne Mamantov
August 2022

DEDICATION

For my grandmother
who helped pave the way for me
and so many other women

and

for Jackie Brown
whose ants sent me on this journey

ACKNOWLEDGEMENTS

First, thank you to my advisor, Dr. Kimberly Sheldon. Your advice, support, compassion, and expertise made this dissertation possible. Thank you to my dissertation committee, Dr. Jim Fordyce, Dr. Todd Freeberg, and Dr. Dan Simberloff for your helpful feedback, expert opinions, and wealth of knowledge which greatly improved this research. To my coauthors on these projects, your help was crucial in doing and communicating the science. Thanks to all those who helped me in the field and the lab; Kylie Hannahs, Matt McGhee, Morgan Fleming, Anchal Padukone, Dr. Amanda Wilson Carter, Will Kirkpatrick, and Dr. Nigel Andrews, your help was essential in finding beetles, scooping dung, and wrangling cattle. To the current and past members of the Sheldon Lab community, Dr. Kimberly Sheldon, Dr. Amanda Wilson Carter, Dr. Ethan Linck, Dr. Nigel Andrews, Claire Winfrey, Anchal Padukone, and Morgan Fleming, this research was greatly improved from your feedback and ideas.

I appreciate all those who funded this research or my education: the University of Tennessee Chancellor’s Fellowship, Shipley-Swann Fellowship, and Student/Faculty Grant; UTK Department of Ecology and Evolutionary Biology Graduate Research Fund; National Park Service Appalachian Highlands Research and Learning Center Research Grant; Sigma Xi Grants-in-Aid of Research; Audobon Society Apacheria Fellowship. Thanks to all of the farms and managed lands that allowed access to collect dung beetles and dung necessary for establishing this study system and for the projects described here. Thanks to private land owners Dr. Susan Reichert, Kevin Null, Proffitt Family Farms, and Cruze Dairy Farm. Thanks to the agricultural farms at the University of Michigan, Michigan State University, West Virginia University, and the University of Florida. And finally, thank you to Seven Islands State Birding Park and the Great Smoky Mountains National Park.

A special thanks to those who helped guide my development as an educator throughout my doctorate. Without your help, I would never have discovered the passion for teaching which now shapes my future. Thanks to Dr. Kimberly Sheldon, Dr. Beth Schussler, Dr. Randy Brewton, Dr. Randy Small, Dr. Caroline Weinhold, Dr. Jeremy

Chandler, Dr. Ben Keck, and Dr. Stephanie Madison whose encouragement and support helped develop my teaching and whose dedication to student success is inspirational.

Thanks to the graduate student community at the University of Tennessee whose support has been crucial throughout this journey. And most importantly, thanks to my family who provided me the resources and guidance to pursue my education and curiosity without limits. I would not have succeeded without you. I also never had to write a word or practice a talk without a few furry companions by my side.

Lastly, thank you to the dung beetles, whose behavior and charisma have inspired me throughout these six years.

ABSTRACT

Current climate change is increasing global temperatures so that many organisms are now experiencing temperatures outside of their thermal tolerance, which threatens their survival. Organisms respond to physiologically stressful temperatures to reduce this threat. Organisms respond to warming through three main mechanisms: range shifts, adjustments via phenotypic plasticity, and evolutionary adaptation. Organisms vary in their ability to utilize these three mechanisms, leading to differences in the magnitude and success of their adjustments to temperature change. Here, I examine how organismal traits influence variation in species response to climate change. Chapter one addresses how physiological tolerance may influence the rate of range shifts across elevation using a meta-analysis of twenty published data sets. Next, in chapter two, I address how invasive versus native species may respond differently to climate change because of predictable differences in traits, specifically phenotypic plasticity. Since plasticity often plays an important role in invasion success, invasive species may have higher plasticity than their native cogeners. Therefore, climate change may be more detrimental to native species than invasive ones, exacerbating the negative effects of invasive species on native biodiversity. To address this possibility, I examine differences in plasticity between an introduced and a native *Onthophagus* dung beetle species living in the Southeastern United States. In Chapter three, I investigate native dung beetle communities of the Great Smoky Mountain National Park to better understand possible impacts of introduced beetles. Then, I address variation in physiological plasticity (chapter four) and behavioral plasticity (chapter five) between the native species, *O. hecate*, and the introduced species, *O. taurus*. Taken together, these studies indicate that species traits, including physiological tolerance, acclimation ability, and reproductive behavior influence species responses to warming. The introduced beetle, *O. taurus*, is more capable of withstanding warming than the native *O. hecate* due to differences in these traits, indicating that climate change may increase the impacts of *O. taurus* on native dung beetle communities.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Current climate change is increasing mean environmental temperatures and increasing the frequency of extreme temperature events (Diffenbaugh & Field 2013; Allen *et al.* 2018). Climate change is thus causing some organisms to experience temperatures outside of their evolved thermal tolerance, threatening the persistence of many species across the globe (Seebacher *et al.* 2014; Gunderson & Stillman 2015). Organisms respond to physiologically stressful temperatures through three main mechanisms: range shifts, adjustments via phenotypic plasticity, and evolutionary adaptation (Bellard *et al.* 2012). If species are unable to use these mechanisms to successfully adjust to stressful temperatures, they are then threatened with extinction (Thomas *et al.* 2004; Bellard *et al.* 2012; Cahill *et al.* 2013; Urban *et al.* 2012; Urban 2015). While climate change has been the primary cause of extinction for only a few species (Cahill *et al.* 2013), the synergistic effects of climate change with habitat loss and biological invasions as well as the indirect effects of climate change on biotic interactions has led to dangerous threats to global biodiversity (Bellard *et al.* 2012; Alexander *et al.* 2015; Guo *et al.* 2018; Spence & Tingley 2020). It is thus a conservation priority to better understand and predict how species will respond to climate change. Every organism will use a different combination of range shifts, phenotypic plasticity, and evolutionary adaption in order to respond to warming. The degree to which organisms use each mechanism is directly linked to their specific traits, such as thermal physiology, behavior, diet, body size, and life history. Assessing how differences in traits influence species responses to warming may help explain the observed variation in organismal response to climate change.

Range shifts to higher latitude and elevation have been widely observed in response to warming (Parmesan 2006; Chen *et al.* 2011; Pecl *et al.* 2017). As organisms move to cooler latitude and elevations, they look to escape temperatures outside of their physiological limits. The rate at which individual species shift their range varies; some species have moved rapidly, some have stayed in place, and others have moved to warmer latitudes and elevations against predictions (Lenoir *et al.* 2010; Chen *et al.* 2011; Harris *et al.* 2012). Some variation in the rate of range shifts can be directly linked to species traits. For example, dispersal ability is a driver of variation in range shifts (Krause & Cobb 2015; Liang *et al.* 2017; Årevall *et al.* 2018; Williams & Blois 2018). Species physiological tolerance may also influence range shifts. Species with broader thermal tolerance are more able to handle temperature change and may not need to move as far or fast to track their preferred thermal niche (Rumpf *et al.* 2018; Mamantov *et al.* 2021a). Whether species are generalists or specialists could also influence rate of range shift; specialist are more closely tied to other species in their community, relying on specific biotic interactions for survival. Specialist range shifts may thus be more idiosyncratic than their generalist counterparts, tracking other species movement rather than thermal conditions.

Phenotypic plasticity may allow organisms to rapidly respond to climate change within an organismal lifespan through adjustments in phenology, behavior, and physiology. Phenotypic plasticity describes the influence of environment on phenotype (West-Eberhard 1989; Chevin *et al.* 2010). Plasticity is the ability of a single genotype to express different phenotypes over the course of an individual lifespan in response to environmental factors and is thus an important mechanism through which organisms can adjust their phenotype in order to survive warming (Snell-Rood 2013). One widely documented response to climate change is a shift in phenology where organisms adjust the timing of life history events such as emergence, flowering, migration, and hibernation to happen earlier in the year when it is cooler (Cleland *et al.* 2012; Socolar *et al.* 2017; Iler *et al.* 2021). Whether such phenological shifts are due to plasticity or genetic change via selection is often unclear; and in many cases, these mechanisms appear to act in concert (Gienapp *et al.* 2007; Anderson *et al.* 2012; Lane *et al.* 2018). Many species demonstrate behavioral plasticity in response to temperature stress. By adjusting thermoregulatory behaviors, individuals are able to avoid exposure to extreme heat by changing diel activity or increasing time spent in thermal refuges (Gross *et al.* 2010; Busch *et al.* 2012). Some species also protect offspring from overheating by burrowing eggs deeper in soil or water to buffer warming (Telemeco *et al.* 2016; Mamantov *et al.* 2021b). Organisms can also respond to warming via plasticity of thermal tolerance, where an organism adjusts its physiology in response to changes in environment, enabling increased survival at more extreme temperatures (Seebacher *et al.* 2014; Gunderson & Stillman 2015). Phenotypic plasticity is an evolved trait (Gotthard & Nylin 1995); the degree of plasticity varies within and among populations and among species. High degrees of environmental variation are thought to select for increased phenotypic plasticity (Snell-Rood 2013). Therefore, organisms will vary in their ability to use plasticity to respond to climate change.

Different populations have varying rates of evolutionary change in response to climate warming; this variation can be linked to generation time, genetic variation, and selection pressure. Some species may be capable of rapid evolution, demonstrating genetic changes in response to rapidly changing climate (Bradshaw & Holzapfel 2006; Hoffman & Sgró 2011). In some cases of phenological shifts, populations are undergoing selection for changes to life history, either alongside or in the absence of plasticity (Bradshaw & Holzapfel 2006; Anderson *et al.* 2012; Lane *et al.* 2018). It also possible for organismal thermal tolerance to evolve toward a higher critical thermal maximum (Seebacher *et al.* 2014; Gunderson & Stillman 2015), but in many populations, such adaptation lag behind warming (Wooliver *et al.* 2020), such that physiological evolution is not fast enough to buffer species from climate change (Gunderson & Stillman 2015). The ability for a population to show sufficient adaptation via evolution in response to warming depends on organismal traits, with fast-breeding, highly diverse organisms more likely to respond via evolution.

Taken together, understanding how species traits underlie organismal ability to respond to climate change via range shift, phenotypic plasticity, and/or evolutionary adaptation provides a framework through which to assess vulnerability to extinction. Being able to mechanistically explain idiosyncratic species responses to climate change could increase our ability to predict changes in community structure. If members of a community respond differently to climate change, then biotic interactions may be altered, affecting ecosystem services and leading to widespread indirect effects of climate change (Pecl *et al.* 2017). Here, I examine how organismal traits influence responses to climate change. Chapter one addresses how physiological tolerance may influence rate of range shifts across elevation using a meta-analysis of twenty published data set. Then, in chapter two, I examine how invasive versus native species may respond differently to climate change. Traits that improve invasion success are often also linked to organismal ability to respond to climate change, leading to the prediction that climate change may be more detrimental to native species than invasive ones. To address this possibility, I use *Onthophagus* dung beetles living in the Southeastern United States as a study system; chapter two also provides background information on this study system. In Chapter three, I address the distribution of dung beetles in the Great Smoky Mountains National Park in order to examine the range and seasonality of my study species, *O. taurus* (introduced) and *O. hecate* (native), and inventory the native dung beetle community in forested lands to complement previous censuses on pasture. I then address variation in acclimation ability between *O. taurus* and *O. hecate* in chapter four. Chapter five examines how differences in behavioral plasticity and behavioral traits between these species affects offspring survival. Taken together, these studies indicate that species traits, including physiological tolerance, acclimation ability, and reproductive behavior can influence species responses to climate. The introduced *O. taurus* is more capable of withstanding warming than the native *O. hecate* due to variation in these traits, indicating that climate warming may exacerbate the effects of this introduced dung beetle on native dung beetle communities.

CHAPTER I

CLIMATE DRIVEN RANGE SHIFTS OF MONTANE SPECIES VARY WITH ELEVATION

A version of this chapter is published by *Global Ecology and Biogeography* and was coauthored by Margaret A. Mamantov, Daniel K. Gibson-Reinemer, Ethan B. Linck, and Kimberly S. Sheldon:

Mamantov, M.A., Gibson-Reinemer, D.K., Linck, E.B., & Sheldon, K.S. 2021. Climate-driven range shifts of montane species vary with elevation. *Global Ecology and Biogeography* 30: 784-794. https://doi.org/10.1111/geb.13246.

Kimberly Sheldon and Daniel Gibson-Reinemer conceived the original idea. Margaret Mamantov and Kimberly Sheldon designed the study. Margaret Mamantov and Daniel Gibson-Reinemer gathered data. Margaret Mamantov performed analyses with contributions from Ethan Linck. Margaret Mamantov and Kimberly Sheldon wrote the manuscript. All authors discussed the results and commented on the manuscript.

Abstract

In response to warming, species are shifting their ranges toward higher elevations. These elevational range shifts have been documented in a variety of taxonomic groups across latitude. Yet the rate and direction of species range shifts in response to warming vary, potentially as a consequence of variation in species traits across elevation. Specifically, diurnal and seasonal climates are often more variable at higher elevations, which results in high elevation species that have broader thermal physiologies relative to low elevation species. High elevation species that are thermal generalists may not need to move as far to track their thermal niche as low elevation thermal specialists. We investigated whether rates of range shifts of 987 species of plants and animals varied systematically with increasing elevation across sixteen montane regions. We gathered published data on elevational range shifts from 20 transect studies comparing historical and recent distributions and examined how rates of range shifts changed across elevation. Specifically, we performed a meta-analysis to calculate the pooled effect of elevation on species range shifts. We found that rates of range shifts show a negative relationship

with elevation such that low elevation species have moved upslope farther than high elevation species on the same transect. This finding was primarily a result of shifts in the upper range limits. We also found that 28% of species shifted downslope against predictions, but elevation did not show a relationship with downslope range shifts.

Introduction

The climate of montane regions is projected to warm three times faster than the global average (Nogués-Bravo *et al.* 2007). In response to this warming, species are expected to shift their ranges toward higher elevations (Chen *et al.* 2011), and these elevational range shifts have already been documented in a wide variety of taxonomic groups across latitude (Konvicka *et al.* 2003; Parmesan & Yohe 2003; Hickling *et al.* 2006; Parmesan 2006; Lenoir *et al.* 2008; Lenoir & Svenning 2015; Pecl *et al*. 2017). However, the rate at which individual species have shifted their elevational ranges in response to warming varies; some species have moved upslope by tens of meters each year, other species have remained in place, and some species have moved downslope, counter to predictions (Lenoir *et al.* 2010; Harris *et al.* 2012; Brusca *et al.* 2013). Montane regions harbor some of the greatest biodiversity on Earth (Grenyer *et al*. 2006), and idiosyncratic range shifts will significantly alter these ecological communities, affecting ecosystem services and threatening human health (Körner 2004; Pecl *et al.* 2017). Understanding the drivers that explain fine scale variation in species range shifts is thus of great importance.

Observed range shifts of terrestrial species in response to climate warming have been greater in areas with a larger magnitude of temperature change (Chen *et al*. 2011). Evidence indicates that the rate of climate warming has generally been greater at high compared to low elevations (Beniston *et al.* 1997; Diaz & Bradley 1997; Pepin *et al.* 2015). This suggests that high elevation species may have shifted their ranges further upslope in response to contemporary warming compared to low elevation species over the same period.

However, in addition to the magnitude of temperature change, range shifts are likely driven by species' ability to handle temperature change, which may be greater at higher elevations. Specifically, the climatic variability hypothesis posits that the thermal tolerance of an organism should match the climatic variability it experiences (Dobzhansky 1950; Bozinovic *et al.* 2011). Diurnal and seasonal temperatures tend to be more variable at higher elevations (Sømme & Block 1991; Gaston & Chown 1999), and thus high elevation species should have broader thermal tolerance (i.e. able to tolerate a wider range of temperatures) compared to their low elevation counterparts (Janzen 1967; Sheldon *et al.* 2018). Indeed, a pattern of increasing breadth of thermal tolerance with increasing elevation has been observed in both terrestrial (Brattstrom 1968; Gaston & Chown 1999; Lüddecke & Sánchez 2002; Navas 2006; Sheldon *et al.* 2015) and aquatic ectotherms (Shah *et al.* 2017), though exceptions exist (Shah *et al.* 2017). In plants, heat tolerance declines with elevation, but less than would be expected by adiabatic lapse rates (Feeley *et al.* 2020), and more freeze tolerant genotypes are found at higher elevations (Körner 2003; Vitasse *et al.* 2014). High elevation plants may therefore have a broader thermal tolerance than low elevation species, though tests of both heat and cold tolerance in plants across elevation are generally lacking. In birds, but not mammals, species tend to have broader thermal tolerance in areas with greater climatic variability (Khaliq *et al.* 2014). Thus, for many taxa, high elevation species may be thermal generalists that do not need to move as far to track their thermal niche. Consequently, as climate has warmed, low elevation thermal specialists may have shifted their ranges more than high elevation thermal generalists to keep up with their narrower thermal niches (Rumpf *et al.* 2018).

Our goal was to determine where upslope movements have been greatest—higher or lower elevations—in order to better understand fine-scale variation in range shifts in montane regions. Because species in environments with reduced temperature variation have been shown to more closely track their thermal niche along elevational gradients in response to warming (Freeman & Freeman 2014), we made the *a priori* prediction that upslope range shifts have been greatest at lower elevations where temperature variation is reduced and species tend to be thermal specialists (Gaston & Chown 1999).

Methods

To examine species range shifts along montane gradients in response to warming, we collected data from the literature on elevational range limits from historic and recent surveys. Specifically, we did a Web of Science search in February 2020 using the terms "climate change, mountain*, range shifts, and elevation*" to find papers that published elevational range data from both an historic and a more recent survey. We also used recent meta-analyses (Gibson-Reinemer *et al.* 2015; Freeman *et al.* 2018; Rumpf *et al.* 2019) to identify additional papers containing relevant data.

To be included, published papers had to provide data on local temperature trends—specifically, the amount of warming between the historic and recent surveys—and changes in both the upper and lower limits of species distributions at two different time points. We limited our analyses to studies that were conducted in montane areas without significant anthropogenic habitat disturbance to minimize the confounding effect of habitat loss on range shifts (Guo *et al.* 2018; Lenoir *et al.* 2020). We removed species that were not present in both the historic and recent surveys (< 1% were present in one study but not the other) since we would be unable to calculate range shifts for these species. In total, data from 987 species of plants, insects, birds, mammals, amphibians, and reptiles located on 20 montane transects met these criteria (Table 1-1, Fig. 1-1; all tables and figures are included in the appendix; Raxworthy *et al.* 2008; Moritz *et al.* 2008; Chen 2011; Forero-Medina *et al.* 2011; Tingley 2011; Felde *et al.* 2012; Forero-Medina 2012; Tingley *et al.* 2012; Angelo & Daehler 2013; Brusca *et al.* 2013; Telwala *et al.*; Freeman & Freeman 2014; Menéndez *et al.* 2014; Ploquin *et al.* 2014; Rowe *et al.* 2015; Koide *et al.* 2017).

We quantified a species' elevational range as the distance (in meters) between the species' lower and upper elevational range limits and used this to determine the elevational midpoint of the range (hereafter range midpoint) for each species during both historic and recent surveys. We calculated range shift (in m/°C) for each species using both the change in range midpoint and the change in temperature between the historic and recent surveys reported in each study. We used m/°C rather than magnitude of range shift in order to standardize across studies. Because each transect study is independent, species ranges and warming were not always measured in the same way. This does not affect our results because the analyses we performed (see below) compare range shifts *within* mountain transects, not *among* mountain transects. Thus, as long as factors are controlled across elevations in the same study, our results are valid. We used range midpoint from the historic survey as a proxy for the elevation where the species occurred, and we predicted that rates of upslope range shifts of the range midpoint should be negatively correlated with midpoint elevation of the species historic range (i.e. lower elevation species have moved farther upslope).

To test our prediction, we examined range shifts along elevational gradients by subsetting the unaltered collection of aggregated data with three distinct sets of filtering criteria, each of which excluded certain species. These alternate datasets allowed us to determine if our results were robust to biases related to our filtering of species. For our ‘primary’ filtered dataset, we excluded any species with upper distributions that reached the maximum elevation of the transect during the historic survey, as we would therefore be unable to calculate the full extent of their upslope range shifts. Following the same logic, we also excluded species with lower distributions that reached the minimum elevation of the transect during the historic survey. After these exclusions, 698 species remained (Table 1). In our second filtered dataset, we excluded any species with upper or lower distributions that reached the maximum or minimum of the transect, respectively during either the historic or modern survey. In this dataset (610 species), species range shifts were entirely within the boundaries of their respective transects. While this data could lead to the underestimation of upslope range shifts for higher elevation species, it allowed us to measure the full extent of range shifts for species on a transect. Lastly, our third filtered dataset retained all species regardless of their maximum or minimum elevations relative to the transect during both the historic and modern surveys (987 species). This dataset was intended to measure whether we continued to see the same patterns in range shifts when including all species regardless of whether or not their range intersected the limits of a transect.

To determine the magnitude and direction of species range shifts across elevation, we used a meta-analysis approach that takes into account variation among transects and then pools individual effect sizes for all transects to get an overall effect size. To do this, we first plotted change in range midpoints (in m/°C) as a function of elevational midpoint of a species' range during the historic survey for all species on a transect (20 transects total). We then fit a linear regression to calculate the slope (β) and the standard error of the slope for each transect independently with the 'lm()' function in base R (version 3.6.3, R Development Core Team 2020). Next, we performed a meta-analysis using a random effects model to calculate pooled effect size, or the overall effect of elevation of historical range midpoint on shifts in range midpoint across all transects. This model assumes that the true effect size varies across transects, which is important since the studies varied in geographic location, taxa, and number of years between historic and recent surveys (Borenstein *et al.* 2009). To calculate pooled effect size from the value of β across all transects, we used the 'metagen()' function in the R package 'meta' and applied the Sidik-Jonkman method to estimate heterogeneity (R version 3.6.3, R Development Core Team 2020). In this calculation, the contribution of a given study to pooled effect size is weighted by sample size and the degree of variation in the study's data, such that a transect with many species and little variation in range shifts across species has a stronger influence on the value of the statistic than a transect with few species and a high level of range shift variation.

Because we found a negative relationship between elevation of historical range midpoint and shifts in range midpoint for all three datasets (see Results), we performed additional analyses to test whether this was due to greater upslope range shifts at lower elevations (as we predicted *a priori*) or three alternate hypotheses: 1) more frequent downslope range shifts at high elevations; 2) systematic contractions or expansions across elevation; or 3) asynchronous shifts in species' upper or lower range limits across elevation. To test alternate hypothesis 1), we further subset our primary dataset into only those species with range midpoints that shifted upslope or only those species with range midpoints that shifted downslope, and examined the effect of elevation of historical range midpoint on upslope and downslope movements independently. To test alternate hypothesis 2), we used our primary dataset and examined the relationship between elevation of historical range midpoint and change in range size. To test alternate hypothesis 3), we used our primary dataset and examined the relationship between elevation of historical range midpoint and shifts in both lower and upper range limits independently. For all three hypotheses, we again used linear regression and random effects meta-analyses to calculate the pooled effect size of elevation on response variables.

Finally, to test whether results from our primary filtered dataset could be an artifact of our filtering procedure, we ran a simulation that randomly assigned elevational range shifts drawn from the data to species along the transect under a null model of no relationship between elevational range and range shift direction or magnitude (see Appendix for model details). For the simulation, we used a non-parametric approach to randomly resample range shifts from empirically observed range shift values. We processed the randomly generated ranges identically to our primary dataset (i.e., by removing simulated species with original upper or lower range limits that reached the maximum or minimum transect elevation, respectively, during the historic survey). For most mountain transects, we found that the relationship between elevation and range shift was zero after randomly assigning range shifts to species. Three mountain transects demonstrated slightly negative relationships between species elevational ranges and range shifts. However, these negative relationships from randomly generated range shifts are smaller than those calculated empirically, and they do not change the interpretation of the data. Together, this, indicates that the empirical findings for the primary dataset were robust to analytical choices (see Appendix).

Results

In our primary dataset (where we excluded species with ranges that reached the top or bottom of each of the 20 transects during the historic survey), 61% of the 698 species have moved upslope (measured as change in range midpoint), 28% have moved downslope, and the remaining 11% have not shifted their ranges (Fig. 1-2). We found that for 7 of the 20 transects, range shifts showed a significant negative relationship with elevation (Fig. 1-2: r^2 = 0.05-0.56). Represented among these transects were two bird studies and one study each of plants, dung beetles, moths, reptiles, and mammals (Fig. 1-2). Another 9 transects had negative but non-significant relationships with elevation (Fig. 1-2: r^2 = 0-0.27). The remaining 4 transects had positive but non-significant relationships with elevation (Fig. 1-2: r^2 = 0.0002-0.0043). In the dataset where we excluded any species with range limits that reached the top or bottom of the transect during either the historic or recent survey, 9 transects showed a significant negative relationship between range shifts and elevation, 8 transects had negative but non-significant relationships with elevation, and 3 transects had positive but non-significant relationships with elevation (Fig. 1-3). Lastly, in the dataset where we did not exclude any species, 11 transects showed a significant negative relationship between range shifts and elevation, 8 transects had negative but non-significant relationships with elevation, and 1 transect had a positive but non-significant relationship with elevation (Fig. 1-4).

Analyses from all three datasets indicate that range shifts show a negative relationship with elevation (i.e. low elevation species have moved upslope farther than high elevation species on the same transect). In the primary dataset (698 species), the pooled effect size of elevation on range shift (m/°C) was -0.1776 (Fig. 1-5: 95% CI: -0.2680; -0.0871, $p = 0.0001$). This suggests that with 1°C of warming, a 100 m increase in elevation of the historical range midpoint of a species leads to a 17.76 m decrease in the upslope shift of the range midpoint. The pooled effect size was similar across the remaining two datasets, indicating that regardless of species exclusion criteria, the effect of elevation on range shift was negative and statistically significant. For the dataset where we excluded species whose ranges reached the top or bottom of the mountain during either the historic or recent survey (610 species), the pooled effect size was -0.2799 (Fig. 1-6; 95% CI: -0.4126; -0.1473, $p < 0.0001$). For the dataset where we kept all species regardless of their maximum or minimum elevations relative to the transect (987 species), the pooled effect size was -0.2100 (Fig. 1-7; 95% CI: -0.2991; -0.1209, p < 0.0001).

To test alternate hypotheses for the negative relationship between elevation and shift in the range midpoint of species, we examined range dynamics using different subsets of our primary dataset to examine different response variables. First, we looked at the relationships between elevation and all species that moved upslope or all species that moved downslope (alternate hypothesis 1). We found a significant decrease in upslope range shifts with increasing elevation and no significant change in the frequency of downslope range shifts across elevation. Among only those species with upslope range shifts (425 species), the pooled effect size of elevation on upslope movement was -0.1797 (Fig. 1-8; 95% CI: -0.2606; -0.0987, p < 0.0001), indicating that with 1℃ of warming, a 100 m increase in elevation leads to a 17.97 m decrease in the upslope shift of the range midpoint of species. Among only those species with downslope range shifts (196 species), the pooled effect size of elevation on downslope range shift was -0.0052 (Fig. 1-9; 95% CI: -0.0921; 0.0817, p = 0.91). Together, these results match our *a priori* prediction that the negative relationship between elevation and shift in range midpoint is due to changes in upslope range shifts with elevation, not downslope range shifts.

It was also possible that systematic changes in range sizes (i.e. range contractions or expansions) with elevation (alternate hypothesis 2) could be driving the negative relationship between elevation and range shifts. From our primary data set, 43% percent of species showed range contractions, 44% showed range expansions, and 13% showed no change in range size. When we examined changes in range sizes across elevation from the historic to recent survey, we found that range sizes changed significantly less with increasing elevation (Fig. 1-10; pooled effect size of elevation on changes in range size was -0.1825; 95% CI: -0.3502; -0.0149, p = 0.03). This pattern is explained by asynchrony in upper and lower range limits (alternate hypothesis 3); we found that shifts of upper range limits showed significant negative relationships with elevation, while shifts in lower range limits showed no relationship with elevation. The pooled effect size of elevation on upper range limits was -0.2441 (95% CI: -0.3590; -0.1291, p < 0.0001,

Fig. 1-11), and the pooled effect size of elevation on lower range limits was -0.1097 (95% CI: -0.2427; 0.0246, p = 0.11, Fig. 1-12). Thus, shifts in upper range limits in species moving upslope are driving the observed pattern in range shifts with elevation.

Discussion

Our analysis of 987 species range shifts across 20 montane transects indicates that low elevation species have moved upslope farther than high elevation species. Our results were robust to different assumptions, suggesting that this observation is not a product of which species were included in the analyses. The negative relationship between elevation and range shifts was primarily a result of variation in the upper range limits of species moving upslope. Our findings are consistent with a recent study (Rumpf *et al.* 2018) that found plant species in the Alps shifted upslope faster the lower in elevation they were situated historically on the mountain. However, contrary to our results, a meta-analysis (Rumpf *et al.* 2019) found upper and lower range limits of montane plant species are shifting at similar rates in response to climate change.

The relationship between elevation and range shifts (Fig. 1-2) may be driven in part by variation in the thermal physiology of species along montane gradients. Theory and empirical work suggest that high elevation species have evolved broader thermal tolerances in response to greater diurnal and seasonal temperature fluctuation (Stevens 1992; Gaston & Chown 1999; Janzen 1967; Ghalambor *et al.* 2006; Sheldon *et al*. 2018). This could allow high elevation, thermal generalist species to tolerate more warming relative to low elevation, thermal specialist counterparts, and allow high elevation species to stay in place longer. This mechanism could be responsible for the negative relationship we found between elevation and species range shifts in response to warming.

However, in addition to thermal physiology, variation in fecundity could also explain the patterns we observed. More fecund species have been shown to shift their ranges faster than less fecund species (Moritz *et al.* 2008). Because the length of the reproductive season influences the number of generations (Roff 1983; Stearns 1992; Altermatt 2010) and reproductive attempts per year (Badyaev 1997; Bears *et al.* 2009), low elevation species may have more opportunities to reproduce and disperse compared to high elevation species. This mechanism could allow low elevation species to shift their ranges farther over the same period compared to high elevation species (Crozier & Dwyer 2006). We cannot tease apart the roles of thermal physiology and fecundity in the observed patterns, and it’s possible both mechanisms play a role in the variation in range shifts of species along elevational gradients.

As elevation increases, geographic area and thus habitat space is thought to decrease (Colwell *et al.* 2008; Sekercioglu *et al.* 2008). If this is the case, high elevation species may be limited in their ability to shift to cooler habitats, which could result in reduced range shifts with increasing elevation. However, decrease in geographic area with elevation rests on the assumption that mountains are pyramid shaped. Through an analysis of 182 mountain ranges, Elsen & Tingley (2015) demonstrated the majority of mountains do not conform to this pattern, and in many cases geographic area was unaffected or actually increased with elevation. Therefore, it is unlikely that lack of area at higher elevations is leading to the observed pattern of reduced range shifts at higher elevations.

Previous research has also indicated that microhabitat complexity, which could buffer species from climate warming, increases with elevation (Scherrer & Körner 2010; Scherrer & Körner 2011; Elsen & Tingley 2015; Byrne *et al.* 2017; Rumpf *et al.* 2018). This suggests that species living at high elevation may not need to move as far to reach newly suitable habitats. The reduced distance to suitable habitats at high elevations may be contributing to our observation that range shifts decrease with elevation but is unlikely to be the primary factor. Increase in microhabitat complexity is especially pronounced in alpine and nival elevational zones (Scherrer & Körner 2010; Elsen & Tingley 2015). However, for many of the studies we analyzed, the transects do not reach these habitat zones and species on those transects still showed a negative relationship between elevation and range shifts (Fig. 1-2).

Our results are unlikely to be driven by elevational patterns in either habitat modification or warming. Previous work demonstrates that habitat modification at lower elevations can accelerate species' upslope movement (Guo *et al.* 2018). However, this is an unlikely driver of our results because we used data from studies that reported minimal habitat modification or that were conducted in protected areas to reduce the confounding effect of habitat loss on range shifts. Similarly, variation in climate warming along montane gradients is unlikely to drive the patterns we observed. Climate warming has tended to be greater at higher rather than lower elevations (Barry 2008; Ohmura 2012; Pepin *et al.* 2015), suggesting high elevation species should be moving upslope faster. However, our results show the opposite pattern.

Though our meta-analysis indicates a negative relationship between elevation and range shifts, different transects and different species within transects show heterogeneity in both the direction and magnitude of range shifts (Fig. 1-2). The idiosyncratic nature of range shifts in response to climate change is common (e.g. Lenoir *et al.* 2010; Chen *et al.* 2011; Crimmins *et al.* 2011; Gibson-Reinemer & Rahel 2015) and is likely the result of multiple factors, including abiotic changes that may or may not be related to climate change, species-specific traits, and biotic interactions (Gibson-Reinemer & Rahel 2015). As examples, natural changes in edaphic conditions are known to restrict species upslope movements in response to warming (Frei *et al.* 2010; Brown & Vellend 2014). Additionally, loss of snow cover or changes in precipitation associated with climate change at high elevations have led to declines in population sizes, occasionally resulting in local extirpations and thus downslope movement (Lenoir *et al.* 2010; Crimmins *et al.* 2011). Variation in species range shifts may also be affected by differences in species traits. For instance, greater ecological specialization can lead to range declines (Mattila *et al.* 2011; Angert *et al*. 2011). In addition, because oxygen availability decreases with elevation, species with higher oxygen demands may show counterintuitive range shifts with climate warming (Jacobsen 2020). Biotic interactions, including competition (Urban *et al.* 2012; Alexander *et al.* 2018) and facilitation (HilleRisLambers *et al.* 2013; Ettinger & HilleRisLambers 2017) can greatly influence distributions and alter range shifts in response to climate change. Finally, changes in community composition and local extinctions may result in novel biotic interactions that affect species' distributions

(Lenoir *et al.* 2010; Davis *et al.* 1998; Urban *et al.* 2013; Alexander *et al.* 2015). These factors could affect the direction or magnitude of range shifts and thus may be responsible for the idiosyncratic responses observed among species on the same mountain. However, these factors do not show a relationship with elevation and are therefore unlikely to be driving the relationship between range shifts and elevation that we observed.

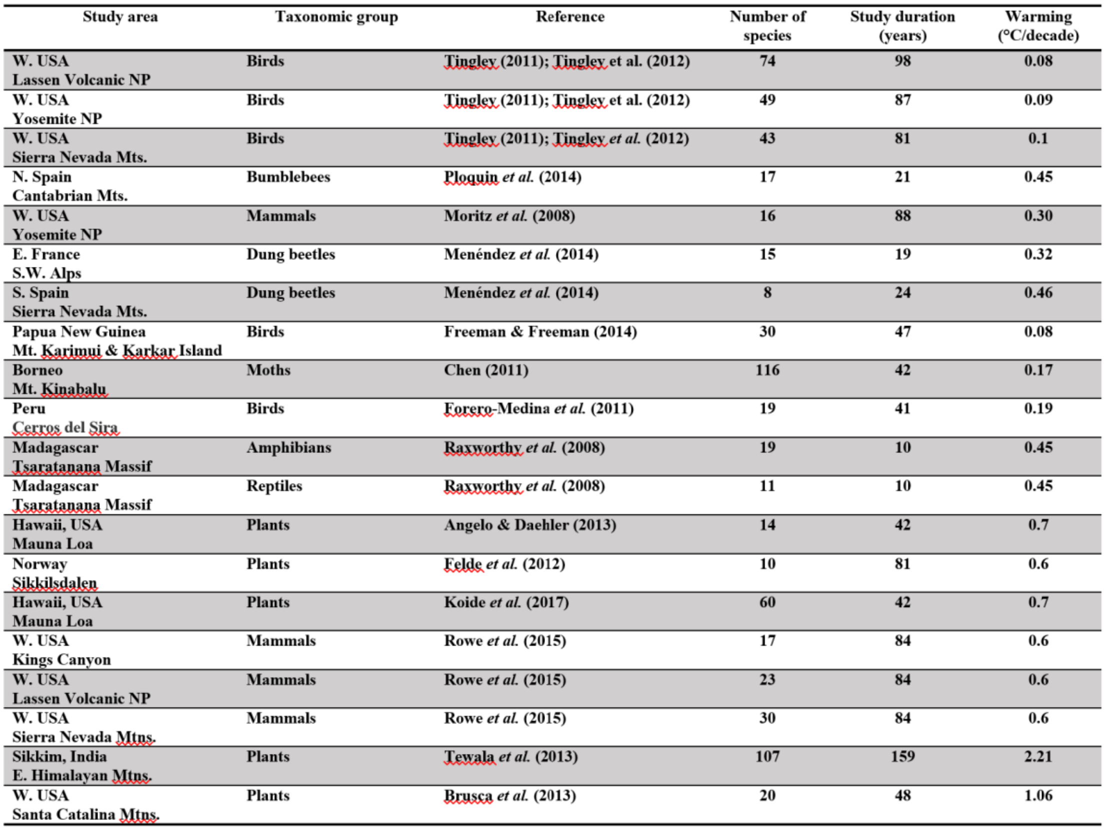
The majority of species ranges have shifted toward higher elevations likely in response to climate warming, and these upslope shifts consistently show greater movement of species that were historically present at lower elevations (Figs. 1-2, 1-5) (this meta-analysis; Rumpf *et al*. 2018). Asynchronous range shifts along montane gradients alter ecological communities (Gibson-Reinemer *et al.* 2015; Pecl *et al.* 2017). Because ranges of low elevation species have shifted more in response to climate warming compared to high elevation species, ecological communities at low elevations have likely experienced more biotic reshuffling and greater community disassembly. This is particularly troubling because low elevations tend to have greater species diversity compared to high elevations. Understanding species’ range shifts, which vary along elevational gradients in montane regions, improves our ability to formulate predictions of future distributions of global biodiversity and changes to community composition.

Acknowledgements

We are grateful to the many authors who completed historical and recent surveys and who made their data available for use. We thank Jim Fordyce for helpful discussions on analyses. This research was supported by the US National Science Foundation (IOS-1930829 to K.S.S.) and the University of Tennessee.

Appendix

Table 1-1. Studies used in primary meta-analysis (Fig. 1-3) of species range shifts along elevational gradients.



Study area	Taxonomic group	Reference	Number of species	Study duration (years)	Warming (°C/decade)
W. USA Lassen Volcanic NP	Birds	Tingley (2011); Tingley et al. (2012)	74	98	0.08
W. USA Yosemite NP	Birds	Tingley (2011); Tingley et al. (2012)	49	87	0.09
W. USA Sierra Nevada Mts.	Birds	Tingley (2011); Tingley *et al.* (2012)	43	81	0.1
N. Spain Cantabrian Mts.	Bumblebees	Ploquin *et al.* (2014)	17	21	0.45
W. USA Yosemite NP	Mammals	Moritz *et al.* (2008)	16	88	0.30
E. France S.W. Alps	Dung beetles	Menéndez *et al.* (2014)	15	19	0.32
S. Spain Sierra Nevada Mts.	Dung beetles	Menéndez *et al.* (2014)	8	24	0.46
Papua New Guinea Mt. Karimui & Karkar Island	Birds	Freeman & Freeman (2014)	30	47	0.08
Borneo Mt. Kinabalu	Moths	Chen (2011)	116	42	0.17
Peru Cerros del Sira	Birds	Forero-Medina *et al.* (2011)	19	41	0.19
Madagascar Tsaratanana Massif	Amphibians	Raxworthy *et al.* (2008)	19	10	0.45
Madagascar Tsaratanana Massif	Reptiles	Raxworthy *et al.* (2008)	11	10	0.45
Hawaii, USA Mauna Loa	Plants	Angelo & Daehler (2013)	14	42	0.7
Norway Sikkilsdalen	Plants	Felde *et al.* (2012)	10	81	0.6
Hawaii, USA Mauna Loa	Plants	Koide *et al.* (2017)	60	42	0.7
W. USA Kings Canyon	Mammals	Rowe *et al.* (2015)	17	84	0.6
W. USA Lassen Volcanic NP	Mammals	Rowe *et al.* (2015)	23	84	0.6
W. USA Sierra Nevada Mtns.	Mammals	Rowe *et al.* (2015)	30	84	0.6
Sikkim, India E. Himalayan Mtns.	Plants	Tewala *et al.* (2013)	107	159	2.21
W. USA Santa Catalina Mtns.	Plants	Brusca *et al.* (2013)	20	48	1.06

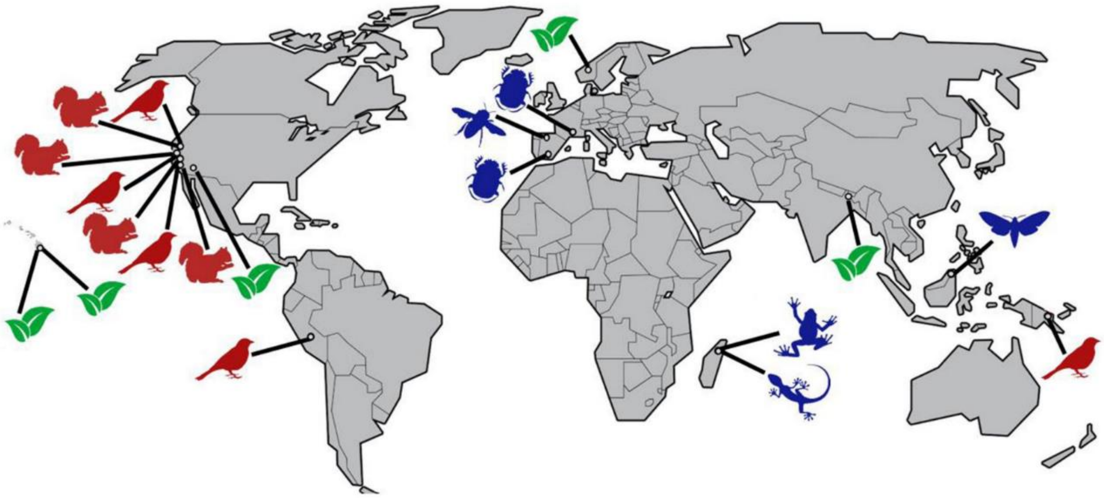


Figure 1-1. Map showing the study locations and taxa used in analyses. Symbols represent ectothermic (blue) and endothermic (red) animal taxa and plant taxa (green) sampled in transect studies, including dung beetles, bumblebees, moths, amphibians, reptiles, birds, mammals, and plants. References for studies used in analyses are listed in Table 1-1.

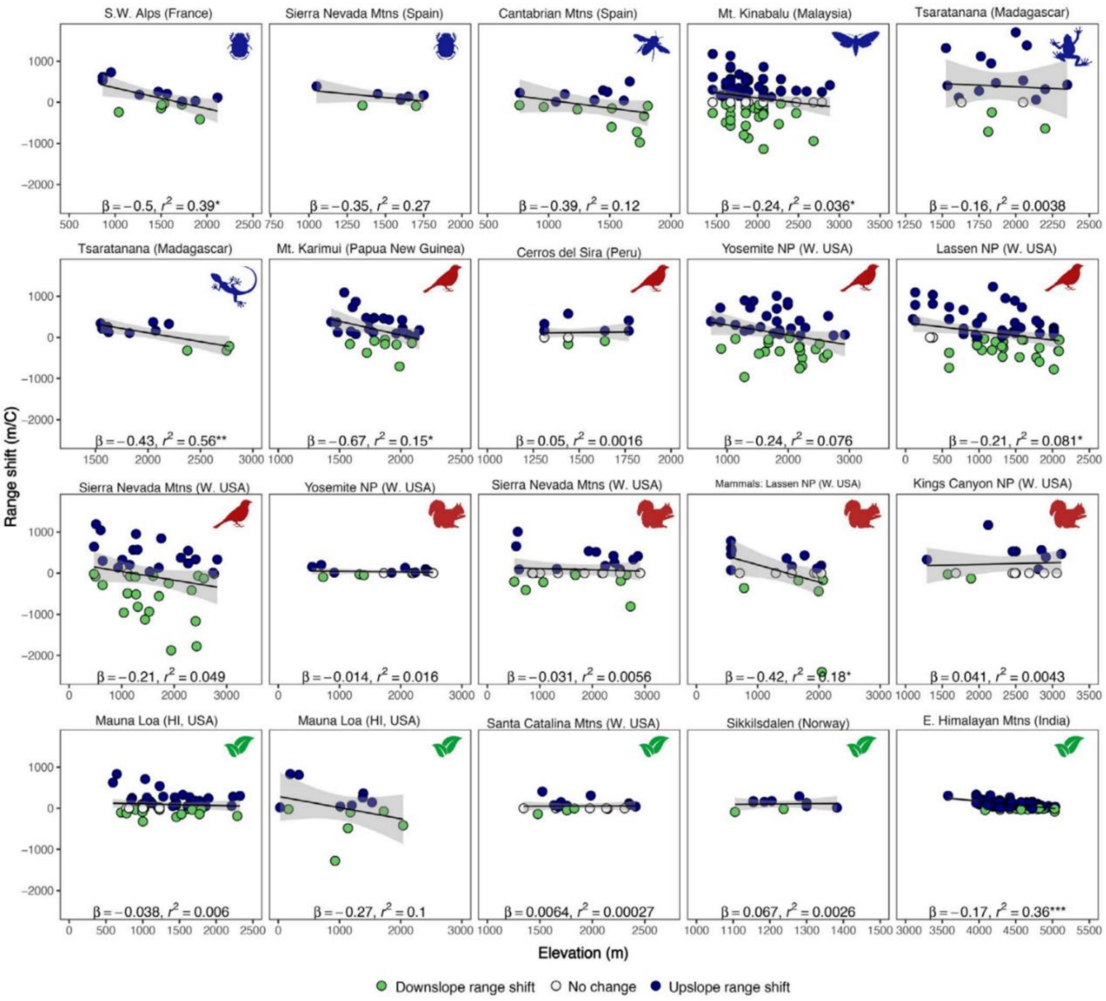


Figure 1-2. Rates of species range shifts decrease with increasing elevation**.** Range shifts (m/°C) were calculated as the total shift in midpoint of the elevational range of a species divided by the total change in temperature observed between historic and recent surveys. Elevation (m) is the midpoint of the species range from the historic survey. For each survey, elevation on the x-axis range from minimum to maximum elevation of the survey transect while range of range shift values on the y-axis are the same for each panel. Midpoint of species ranges have moved upslope (dark blue), downslope (green), or stayed the same (white). Results are presented by taxonomic group and study location. Symbols represent ectothermic (blue) and endothermic (red) animal taxa and plant taxa (green) sampled in transect studies, including dung beetles, bumblebees, moths, amphibians, reptiles, birds, mammals, and plants. Significant relationships between range shifts and elevation are marked with a * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.001$).

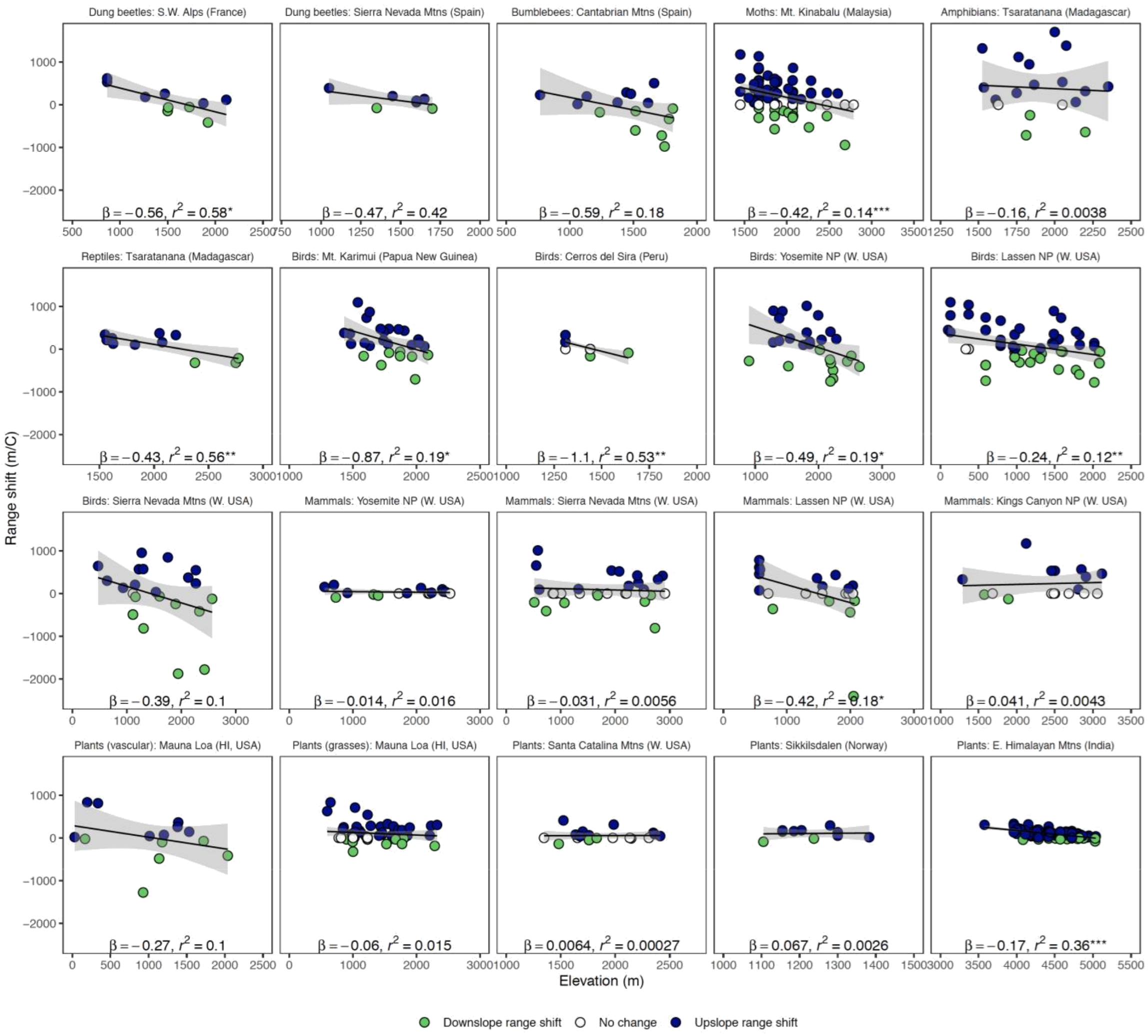


Figure 1-3. When we excluded species with ranges that reached the transect minimum or maximum during either the historic or recent survey, the rates of species range shifts decreased with increasing elevation. Range shifts (m/°C) were calculated as the total shift in midpoint of the elevational range of a species divided by the total change in temperature observed between historic and recent surveys. Elevation (m) is the midpoint of the species range from the historic survey. For each survey, elevation on the x-axis range from minimum to maximum elevation of the survey transect while range of range shift values on the y-axis are the same for each panel. Midpoint of species ranges have moved upslope (dark blue), downslope (green), or stayed the same (white). Results are presented by taxonomic group and study location. Symbols represent endothermic (red) and ectothermic (blue) animal taxa and plant taxa (green) sampled in transect studies, including birds, mammals, amphibians, reptiles, moths, bumblebees, and dung beetles. Significant relationships between range shifts and elevation are marked with a * ($p <$ 0.05), ** ($p < 0.01$), or *** ($p < 0.001$).

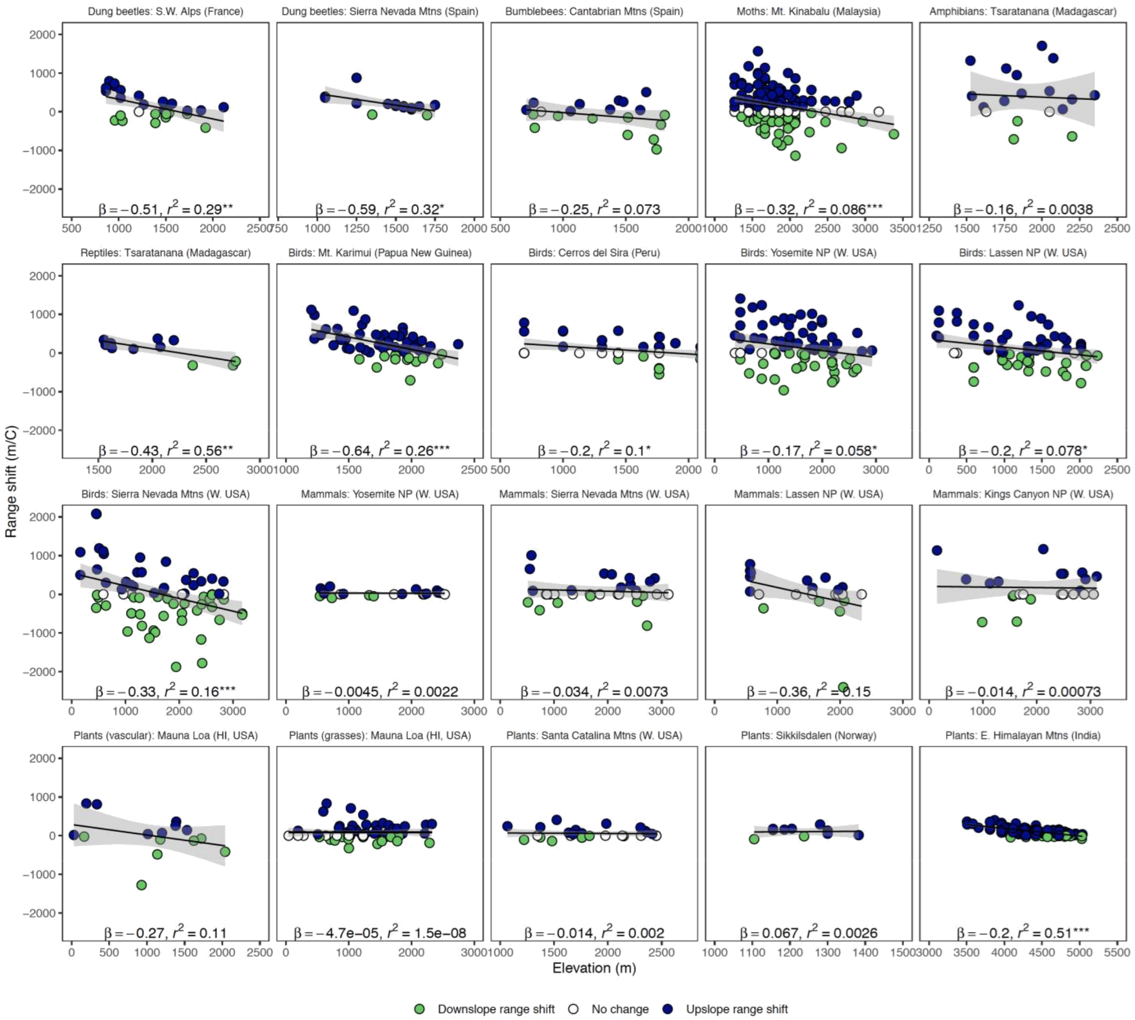
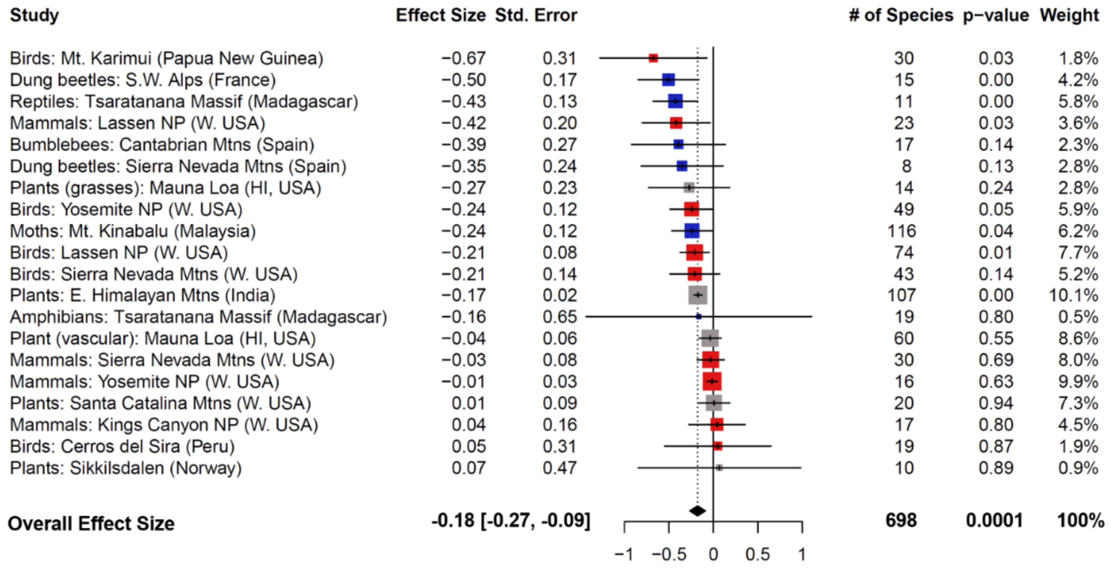
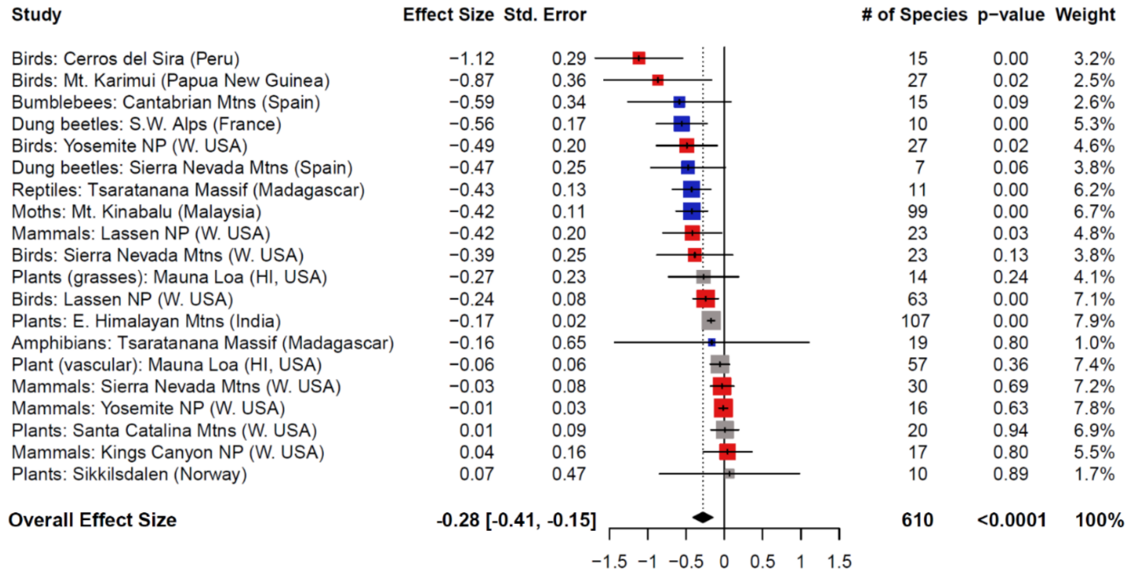


Figure 1-4. When we included all species regardless of elevation relative to the transect, the rates of species range shifts decreased with increasing elevation. Range shifts (m/°C) were calculated as the total shift in midpoint of the elevational range of a species divided by the total change in temperature observed between historic and recent surveys. Elevation (m) is the midpoint of the species range from the historic survey. For each survey, elevation on the x-axis range from minimum to maximum elevation of the survey transect while range of range shift values on the y-axis are the same for each panel. Midpoint of species ranges have moved upslope (dark blue), downslope (green), or stayed the same (white). Results are presented by taxonomic group and study location. Symbols represent endothermic (red) and ectothermic (blue) animal taxa and plant taxa (green) sampled in transect studies, including birds, mammals, amphibians, reptiles, moths, bumblebees, and dung beetles. Significant relationships between range shifts and elevation are marked with a * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.001$).



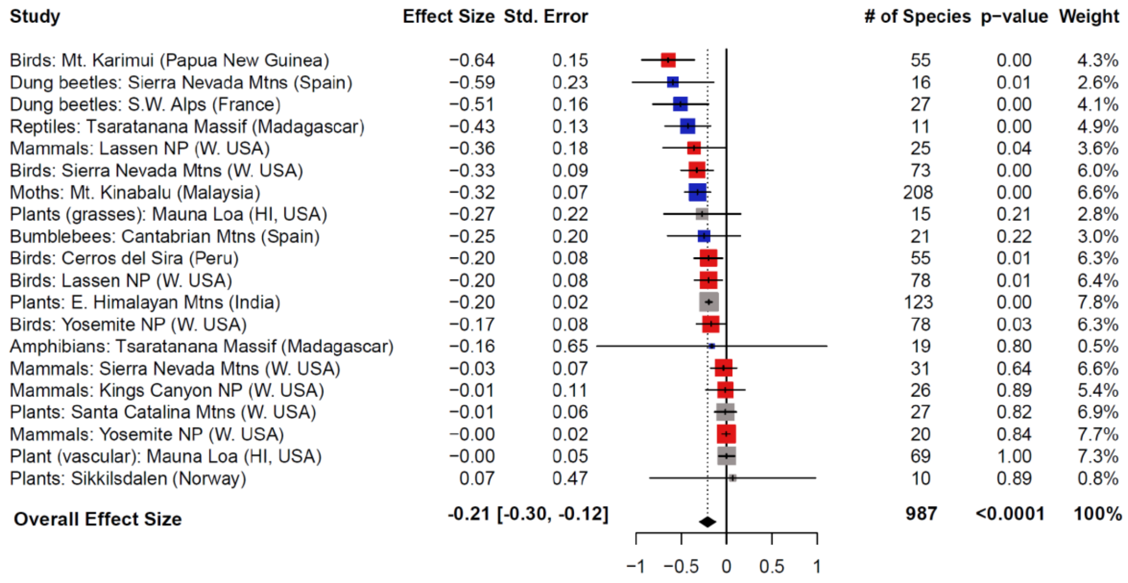
Study	Effect Size	Std. Error	# of Species	p−value	Weight
Birds: Mt. Karimui (Papua New Guinea)	−0.67	0.31	30	0.03	1.8%
Dung beetles: S.W. Alps (France)	−0.50	0.17	15	0.00	4.2%
Reptiles: Tsaratanana Massif (Madagascar)	−0.43	0.13	11	0.00	5.8%
Mammals: Lassen NP (W. USA)	−0.42	0.20	23	0.03	3.6%
Bumblebees: Cantabrian Mtns (Spain)	−0.39	0.27	17	0.14	2.3%
Dung beetles: Sierra Nevada Mtns (Spain)	−0.35	0.24	8	0.13	2.8%
Plants (grasses): Mauna Loa (HI, USA)	−0.27	0.23	14	0.24	2.8%
Birds: Yosemite NP (W. USA)	−0.24	0.12	49	0.05	5.9%
Moths: Mt. Kinabalu (Malaysia)	−0.24	0.12	116	0.04	6.2%
Birds: Lassen NP (W. USA)	−0.21	0.08	74	0.01	7.7%
Birds: Sierra Nevada Mtns (W. USA)	−0.21	0.14	43	0.14	5.2%
Plants: E. Himalayan Mtns (India)	−0.17	0.02	107	0.00	10.1%
Amphibians: Tsaratanana Massif (Madagascar)	−0.16	0.65	19	0.80	0.5%
Plant (vascular): Mauna Loa (HI, USA)	−0.04	0.06	60	0.55	8.6%
Mammals: Sierra Nevada Mtns (W. USA)	−0.03	0.08	30	0.69	8.0%
Mammals: Yosemite NP (W. USA)	−0.01	0.03	16	0.63	9.9%
Plants: Santa Catalina Mtns (W. USA)	0.01	0.09	20	0.94	7.3%
Mammals: Kings Canyon NP (W. USA)	0.04	0.16	17	0.80	4.5%
Birds: Cerros del Sira (Peru)	0.05	0.31	19	0.87	1.9%
Plants: Sikkilsdalen (Norway)	0.07	0.47	10	0.89	0.9%
Overall Effect Size	**-0.18 [-0.27, -0.09]**		**698**	**0.0001**	**100%**

Figure 1-5. Meta-analysis indicates a negative overall effect size of elevation on range shift. Species range shifts (m/°C) thus decrease with increasing elevation. The effect size of each study was calculated as the slope of the regression line (β) between midpoint elevation of the species range during the historical survey (m) and shift in midpoint of the species range by change in temperature (m/°C). The p- value for each study indicate the significance of this relationship. For each study, the center of the box represents the effect size, while the black bar shows the standard error of the effect size. Box color represent transect studies of endotherms (red), ectotherms (blue), and plants (green). Box size represents the study weight in the meta-analysis, which is based on the number of species and the standard error of the effect size of the transect study. Overall effect size and 95% confidence interval are listed in bold and represented by the diamond. The p-value of the overall effect size is also in bold as well as the total number of species included in the meta-analysis (right of plot).



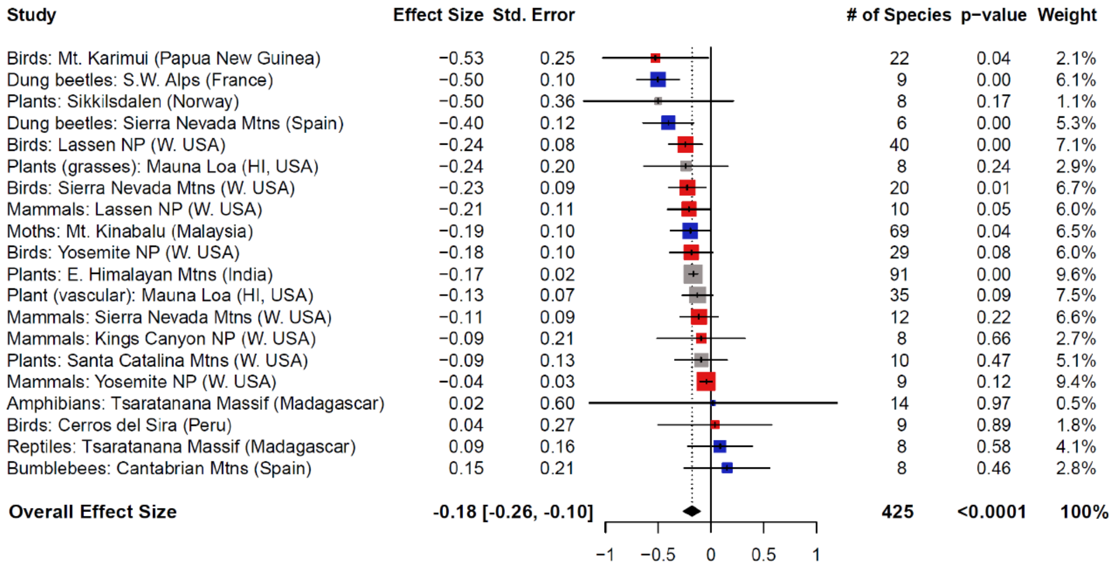
Study	Effect Size	Std. Error	# of Species	p−value	Weight
Birds: Cerros del Sira (Peru)	−1.12	0.29	15	0.00	3.2%
Birds: Mt. Karimui (Papua New Guinea)	−0.87	0.36	27	0.02	2.5%
Bumblebees: Cantabrian Mtns (Spain)	−0.59	0.34	15	0.09	2.6%
Dung beetles: S.W. Alps (France)	−0.56	0.17	10	0.00	5.3%
Birds: Yosemite NP (W. USA)	−0.49	0.20	27	0.02	4.6%
Dung beetles: Sierra Nevada Mtns (Spain)	−0.47	0.25	7	0.06	3.8%
Reptiles: Tsaratanana Massif (Madagascar)	−0.43	0.13	11	0.00	6.2%
Moths: Mt. Kinabalu (Malaysia)	−0.42	0.11	99	0.00	6.7%
Mammals: Lassen NP (W. USA)	−0.42	0.20	23	0.03	4.8%
Birds: Sierra Nevada Mtns (W. USA)	−0.39	0.25	23	0.13	3.8%
Plants (grasses): Mauna Loa (HI, USA)	−0.27	0.23	14	0.24	4.1%
Birds: Lassen NP (W. USA)	−0.24	0.08	63	0.00	7.1%
Plants: E. Himalayan Mtns (India)	−0.17	0.02	107	0.00	7.9%
Amphibians: Tsaratanana Massif (Madagascar)	−0.16	0.65	19	0.80	1.0%
Plant (vascular): Mauna Loa (HI, USA)	−0.06	0.06	57	0.36	7.4%
Mammals: Sierra Nevada Mtns (W. USA)	−0.03	0.08	30	0.69	7.2%
Mammals: Yosemite NP (W. USA)	−0.01	0.03	16	0.63	7.8%
Plants: Santa Catalina Mtns (W. USA)	0.01	0.09	20	0.94	6.9%
Mammals: Kings Canyon NP (W. USA)	0.04	0.16	17	0.80	5.5%
Plants: Sikkilsdalen (Norway)	0.07	0.47	10	0.89	1.7%
Overall Effect Size	**-0.28 [-0.41, -0.15]**		**610**	**<0.0001**	**100%**

Figure 1-6. When we excluded species with ranges that reached the transect minimum or maximum during either the historic or recent survey, our meta-analysis indicates a negative overall effect size of elevation on range shift. Species range shifts (m/°C) thus decrease with increasing elevation. The effect size of each study was calculated as the slope of the regression line (β) between midpoint elevation of the species range during the historical survey (m) and shift in midpoint of the species range by change in temperature (m/°C). The p-value for each study indicate the significance of this relationship. For each study, the center of the box represents the effect size, while the black bar shows the standard error of the effect size. Box color represent transect studies of endotherms (red), ectotherms (blue), and plants (grey). Box size represents the study weight in the meta-analysis, which is based on the number of species and the standard error of the effect size of the transect study. Overall effect size and 95% confidence interval are listed in bold and represented by the diamond. The p-value of the overall effect size is also in bold as well as the total number of species included in the meta-analysis (right of plot).



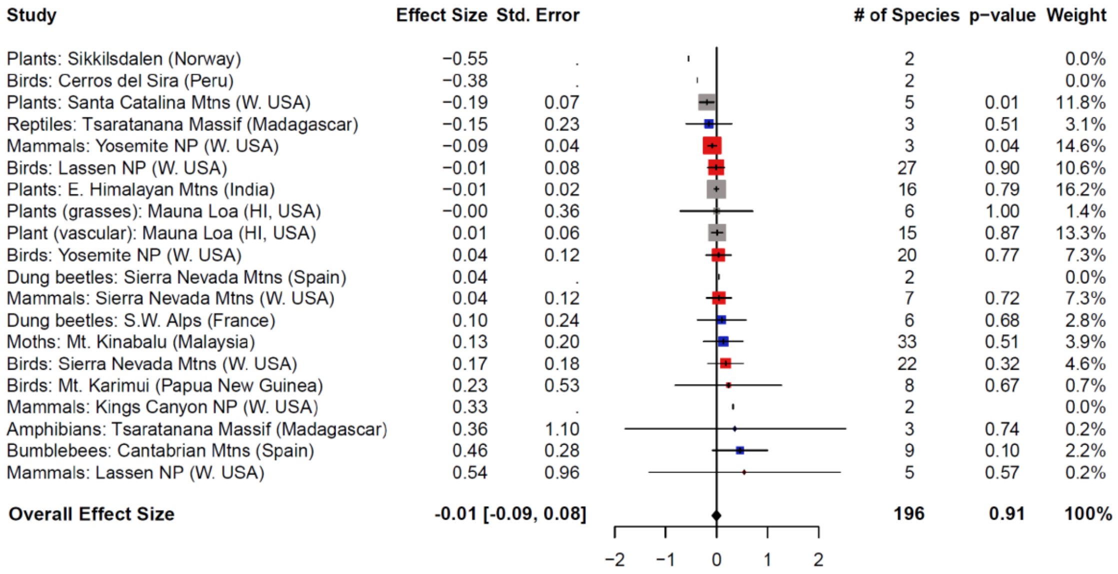
Study	Effect Size	Std. Error	# of Species	p−value	Weight
Birds: Mt. Karimui (Papua New Guinea)	−0.64	0.15	55	0.00	4.3%
Dung beetles: Sierra Nevada Mtns (Spain)	−0.59	0.23	16	0.01	2.6%
Dung beetles: S.W. Alps (France)	−0.51	0.16	27	0.00	4.1%
Reptiles: Tsaratanana Massif (Madagascar)	−0.43	0.13	11	0.00	4.9%
Mammals: Lassen NP (W. USA)	−0.36	0.18	25	0.04	3.6%
Birds: Sierra Nevada Mtns (W. USA)	−0.33	0.09	73	0.00	6.0%
Moths: Mt. Kinabalu (Malaysia)	−0.32	0.07	208	0.00	6.6%
Plants (grasses): Mauna Loa (HI, USA)	−0.27	0.22	15	0.21	2.8%
Bumblebees: Cantabrian Mtns (Spain)	−0.25	0.20	21	0.22	3.0%
Birds: Cerros del Sira (Peru)	−0.20	0.08	55	0.01	6.3%
Birds: Lassen NP (W. USA)	−0.20	0.08	78	0.01	6.4%
Plants: E. Himalayan Mtns (India)	−0.20	0.02	123	0.00	7.8%
Birds: Yosemite NP (W. USA)	−0.17	0.08	78	0.03	6.3%
Amphibians: Tsaratanana Massif (Madagascar)	−0.16	0.65	19	0.80	0.5%
Mammals: Sierra Nevada Mtns (W. USA)	−0.03	0.07	31	0.64	6.6%
Mammals: Kings Canyon NP (W. USA)	−0.01	0.11	26	0.89	5.4%
Plants: Santa Catalina Mtns (W. USA)	−0.01	0.06	27	0.82	6.9%
Mammals: Yosemite NP (W. USA)	−0.00	0.02	20	0.84	7.7%
Plant (vascular): Mauna Loa (HI, USA)	−0.00	0.05	69	1.00	7.3%
Plants: Sikkilsdalen (Norway)	0.07	0.47	10	0.89	0.8%
Overall Effect Size	**-0.21 [-0.30, -0.12]**		**987**	**<0.0001**	**100%**

Figure 1-7. When we included all species regardless of elevation relative to the transect, our meta-analysis indicates a negative overall effect size of elevation on range shift. Species range shifts (m/°C) thus decrease with increasing elevation. The effect size of each study was calculated as the slope of the regression line (β) between midpoint elevation of the species range during the historical survey (m) and shift in midpoint of the species range by change in temperature (m/°C). The p-value for each study indicate the significance of this relationship. For each study, the center of the box represents the effect size, while the black bar shows the standard error of the effect size. Box color represent transect studies of endotherms (red), ectotherms (blue), and plants (grey). Box size represents the study weight in the meta-analysis, which is based on the number of species and the standard error of the effect size of the transect study. Overall effect size and 95% confidence interval are listed in bold and represented by the diamond. The p-value of the overall effect size is also in bold as well as the total number of species included in the meta-analysis (right of plot).



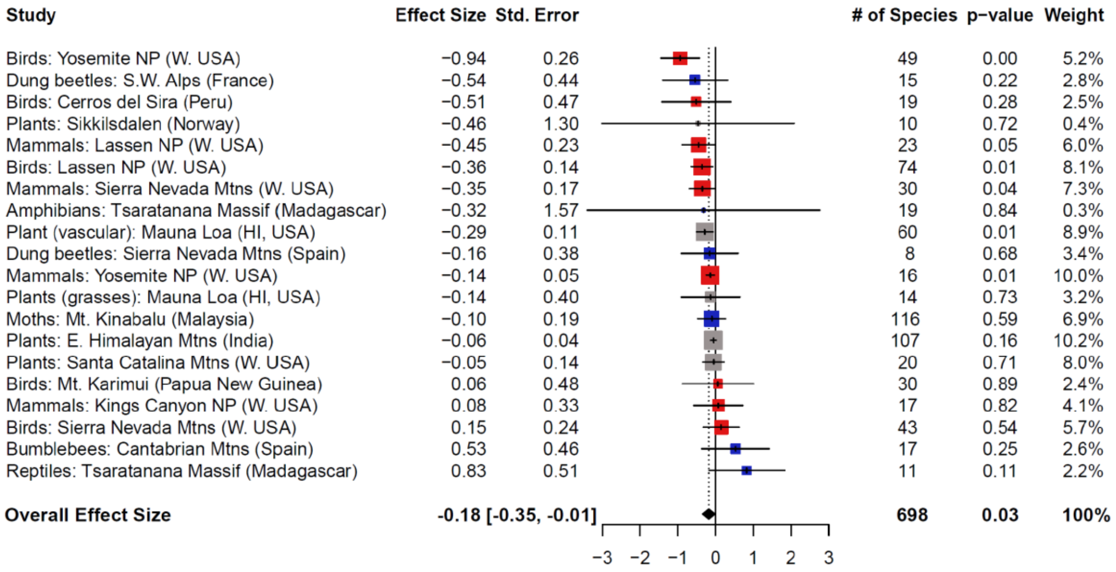
Study	Effect Size	Std. Error	# of Species	p−value	Weight
Birds: Mt. Karimui (Papua New Guinea)	−0.53	0.25	22	0.04	2.1%
Dung beetles: S.W. Alps (France)	−0.50	0.10	9	0.00	6.1%
Plants: Sikkilsdalen (Norway)	−0.50	0.36	8	0.17	1.1%
Dung beetles: Sierra Nevada Mtns (Spain)	−0.40	0.12	6	0.00	5.3%
Birds: Lassen NP (W. USA)	−0.24	0.08	40	0.00	7.1%
Plants (grasses): Mauna Loa (HI, USA)	−0.24	0.20	8	0.24	2.9%
Birds: Sierra Nevada Mtns (W. USA)	−0.23	0.09	20	0.01	6.7%
Mammals: Lassen NP (W. USA)	−0.21	0.11	10	0.05	6.0%
Moths: Mt. Kinabalu (Malaysia)	−0.19	0.10	69	0.04	6.5%
Birds: Yosemite NP (W. USA)	−0.18	0.10	29	0.08	6.0%
Plants: E. Himalayan Mtns (India)	−0.17	0.02	91	0.00	9.6%
Plant (vascular): Mauna Loa (HI, USA)	−0.13	0.07	35	0.09	7.5%
Mammals: Sierra Nevada Mtns (W. USA)	−0.11	0.09	12	0.22	6.6%
Mammals: Kings Canyon NP (W. USA)	−0.09	0.21	8	0.66	2.7%
Plants: Santa Catalina Mtns (W. USA)	−0.09	0.13	10	0.47	5.1%
Mammals: Yosemite NP (W. USA)	−0.04	0.03	9	0.12	9.4%
Amphibians: Tsaratanana Massif (Madagascar)	0.02	0.60	14	0.97	0.5%
Birds: Cerros del Sira (Peru)	0.04	0.27	9	0.89	1.8%
Reptiles: Tsaratanana Massif (Madagascar)	0.09	0.16	8	0.58	4.1%
Bumblebees: Cantabrian Mtns (Spain)	0.15	0.21	8	0.46	2.8%
Overall Effect Size	**-0.18 [-0.26, -0.10]**		**425**	**<0.0001**	**100%**

Figure 1-8. When we examined only those species whose range shifted upslope, our meta-analysis indicates a negative overall effect size of elevation on range shift. Species upslope range shifts (m/°C) thus decrease with increasing elevation. The effect size of each study was calculated as the slope of the regression line (β) between midpoint elevation of the species range during the historical survey (m) and shift in midpoint of the species range by change in temperature (m/°C). The p-value for each study indicate the significance of this relationship. For each study, the center of the box represents the effect size, while the black bar shows the standard error of the effect size. Box color represent transect studies of endotherms (red), ectotherms (blue), and plants (grey). Box size represents the study weight in the meta-analysis, which is based on the number of species and the standard error of the effect size of the transect study. Overall effect size and 95% confidence interval are listed in bold and represented by the diamond. The p-value of the overall effect size is also in bold as well as the total number of species included in the meta-analysis (right of plot).



Study	Effect Size	Std. Error	# of Species	p−value	Weight
Plants: Sikkilsdalen (Norway)	−0.55	.	2		0.0%
Birds: Cerros del Sira (Peru)	−0.38	.	2		0.0%
Plants: Santa Catalina Mtns (W. USA)	−0.19	0.07	5	0.01	11.8%
Reptiles: Tsaratanana Massif (Madagascar)	−0.15	0.23	3	0.51	3.1%
Mammals: Yosemite NP (W. USA)	−0.09	0.04	3	0.04	14.6%
Birds: Lassen NP (W. USA)	−0.01	0.08	27	0.90	10.6%
Plants: E. Himalayan Mtns (India)	−0.01	0.02	16	0.79	16.2%
Plants (grasses): Mauna Loa (HI, USA)	−0.00	0.36	6	1.00	1.4%
Plant (vascular): Mauna Loa (HI, USA)	0.01	0.06	15	0.87	13.3%
Birds: Yosemite NP (W. USA)	0.04	0.12	20	0.77	7.3%
Dung beetles: Sierra Nevada Mtns (Spain)	0.04	.	2		0.0%
Mammals: Sierra Nevada Mtns (W. USA)	0.04	0.12	7	0.72	7.3%
Dung beetles: S.W. Alps (France)	0.10	0.24	6	0.68	2.8%
Moths: Mt. Kinabalu (Malaysia)	0.13	0.20	33	0.51	3.9%
Birds: Sierra Nevada Mtns (W. USA)	0.17	0.18	22	0.32	4.6%
Birds: Mt. Karimui (Papua New Guinea)	0.23	0.53	8	0.67	0.7%
Mammals: Kings Canyon NP (W. USA)	0.33	.	2		0.0%
Amphibians: Tsaratanana Massif (Madagascar)	0.36	1.10	3	0.74	0.2%
Bumblebees: Cantabrian Mtns (Spain)	0.46	0.28	9	0.10	2.2%
Mammals: Lassen NP (W. USA)	0.54	0.96	5	0.57	0.2%
Overall Effect Size	**-0.01 [-0.09, 0.08]**		**196**	**0.91**	**100%**

Figure 1-9. When we examined only those species whose range shifted downslope, our meta-analysis indicates a non-significant effect size of elevation on range shift. Elevation does not affect species downslope range shifts (m/°C). The effect size of each study was calculated as the slope of the regression line (β) between midpoint elevation of the species range during the historical survey (m) and shift in midpoint of the species range by change in temperature (m/°C). The p-value for each study indicate the significance of this relationship. For each study, the center of the box represents the effect size, while the black bar shows the standard error of the effect size. Box color represent transect studies of endotherms (red), ectotherms (blue), and plants (grey). Box size represents the study weight in the meta-analysis, which is based on the number of species and the standard error of the effect size of the transect study. Overall effect size and 95% confidence interval are listed in bold and represented by the diamond. The p-value of the overall effect size is also in bold as well as the total number of species included in the meta-analysis (right of plot).



Study	Effect Size	Std. Error	# of Species	p−value	Weight
Birds: Yosemite NP (W. USA)	−0.94	0.26	49	0.00	5.2%
Dung beetles: S.W. Alps (France)	−0.54	0.44	15	0.22	2.8%
Birds: Cerros del Sira (Peru)	−0.51	0.47	19	0.28	2.5%
Plants: Sikkilsdalen (Norway)	−0.46	1.30	10	0.72	0.4%
Mammals: Lassen NP (W. USA)	−0.45	0.23	23	0.05	6.0%
Birds: Lassen NP (W. USA)	−0.36	0.14	74	0.01	8.1%
Mammals: Sierra Nevada Mtns (W. USA)	−0.35	0.17	30	0.04	7.3%
Amphibians: Tsaratanana Massif (Madagascar)	−0.32	1.57	19	0.84	0.3%
Plant (vascular): Mauna Loa (HI, USA)	−0.29	0.11	60	0.01	8.9%
Dung beetles: Sierra Nevada Mtns (Spain)	−0.16	0.38	8	0.68	3.4%
Mammals: Yosemite NP (W. USA)	−0.14	0.05	16	0.01	10.0%
Plants (grasses): Mauna Loa (HI, USA)	−0.14	0.40	14	0.73	3.2%
Moths: Mt. Kinabalu (Malaysia)	−0.10	0.19	116	0.59	6.9%
Plants: E. Himalayan Mtns (India)	−0.06	0.04	107	0.16	10.2%
Plants: Santa Catalina Mtns (W. USA)	−0.05	0.14	20	0.71	8.0%
Birds: Mt. Karimui (Papua New Guinea)	0.06	0.48	30	0.89	2.4%
Mammals: Kings Canyon NP (W. USA)	0.08	0.33	17	0.82	4.1%
Birds: Sierra Nevada Mtns (W. USA)	0.15	0.24	43	0.54	5.7%
Bumblebees: Cantabrian Mtns (Spain)	0.53	0.46	17	0.25	2.6%
Reptiles: Tsaratanana Massif (Madagascar)	0.83	0.51	11	0.11	2.2%
Overall Effect Size	**-0.18 [-0.35, -0.01]**		**698**	**0.03**	**100%**

Figure 1-10. When we used our primary data set to examine range dynamics across elevation, our meta-analysis indicates a negative overall effect size of elevation on change in range size between the historic and modern surveys. Species range size (m/°C) thus shows less change with increasing elevation. The effect size of each study was calculated as the slope of the regression line (β) between range size of species range during the historical survey (m) and change in range size of the species by change in temperature (m/°C). The p-value for each study indicate the significance of this relationship. For each study, the center of the box represents the effect size, while the black bar shows the standard error of the effect size. Box color represent transect studies of endotherms (red), ectotherms (blue), and plants (grey). Box size represents the study weight in the meta-analysis, which is based on the number of species and the standard error of the effect size of the transect study. Overall effect size and 95% confidence interval are listed in bold and represented by the diamond. The p-value of the overall effect size is also in bold as well as the total number of species included in the meta-analysis (right of plot).

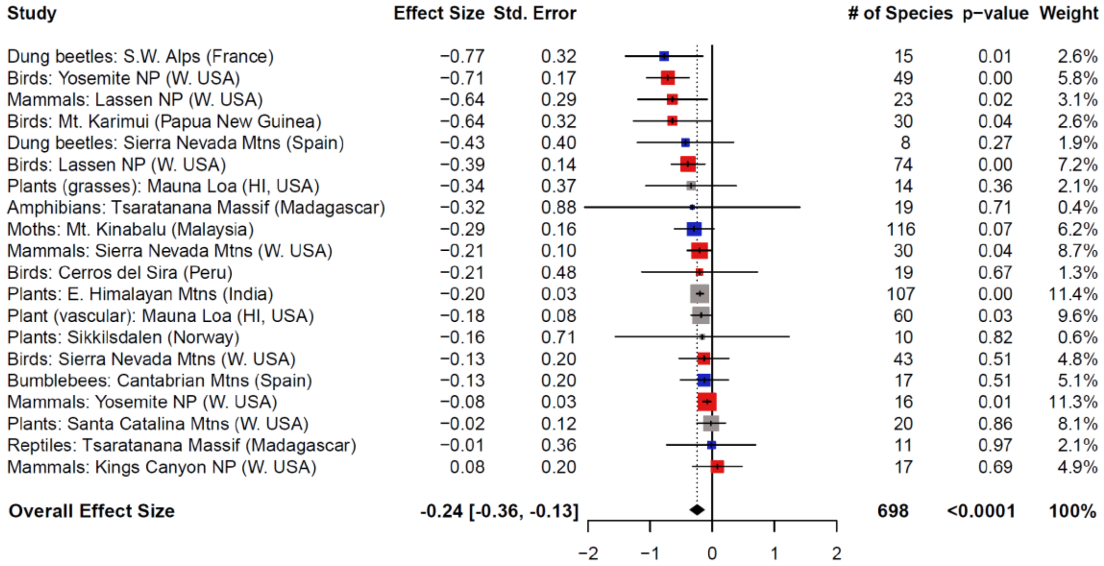


Figure 1-11. When we used our primary data set to examine range dynamics across elevation, our meta-analysis indicates a negative overall effect size of elevation on upper limit range shift. Species upper limit range shifts (m/°C) thus decrease with increasing elevation. The effect size of each study was calculated as the slope of the regression line (β) between midpoint elevation of the species range during the historical survey (m) and shift in upper limit of the species range by change in temperature (m/°C). The p-value for each study indicate the significance of this relationship. For each study, the center of the box represents the effect size, while the black bar shows the standard error of the effect size. Box color represent transect studies of endotherms (red), ectotherms (blue), and plants (grey). Box size represents the study weight in the meta-analysis, which is based on the number of species and the standard error of the effect size of the transect study. Overall effect size and 95% confidence interval are listed in bold and represented by the diamond. The p-value of the overall effect size is also in bold as well as the total number of species included in the meta-analysis (right of plot).

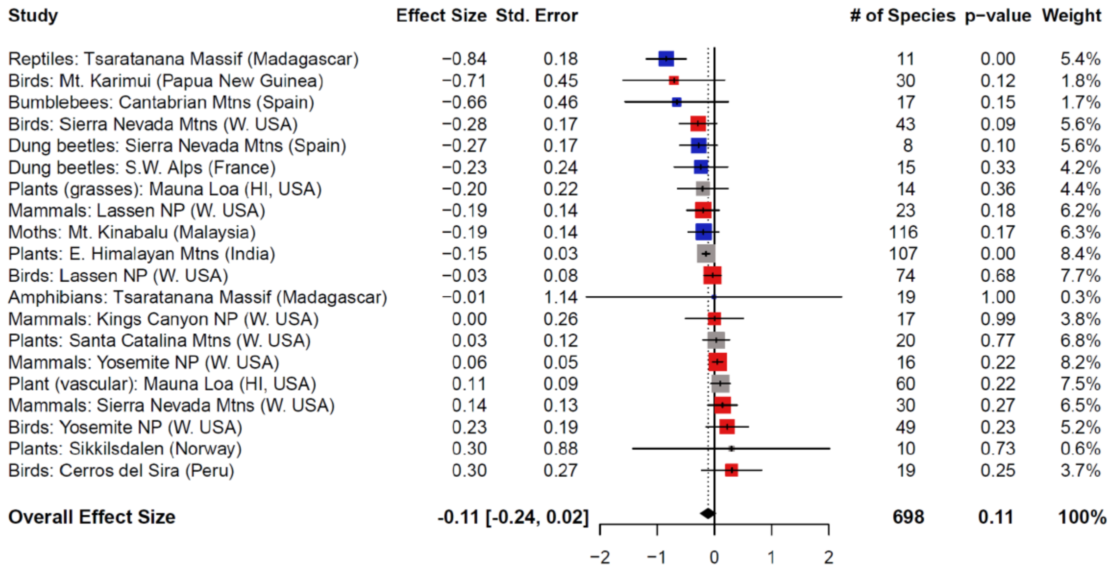


Figure 1-12. When we used our primary data set to examine range dynamics across elevation, our meta-analysis indicates a nonsignificant effect size of elevation on lower limit range shift. Species lower limit range shifts (m/°C) are not significantly affected by elevation. The effect size of each study was calculated as the slope of the regression line (β) between midpoint elevation of the species range during the historical survey (m) and shift in lower limit of the species range by change in temperature (m/°C). The p-value for each study indicate the significance of this relationship. For each study, the center of the box represents the effect size, while the black bar shows the standard error of the effect size. Box color represent transect studies of endotherms (red), ectotherms (blue), and plants (grey). Box size represents the study weight in the meta-analysis, which is based on the number of species and the standard error of the effect size of the transect study. Overall effect size and 95% confidence interval are listed in bold and represented by the diamond. The p-value of the overall effect size is also in bold as well as the total number of species included in the meta-analysis (right of plot).

Range Shift Simulation

To ensure that our filtering process was not leading to consistent underestimation of upslope shifts in high elevation species (i.e., created a statistical artifact responsible for our main findings), we used a simulation approach. For each mountain transect included in our analysis, we simulated random range shifts for the historical ("T1") elevational distribution of each species. Specifically, for each mountain transect, we used the empirical range shift values to calculate mean and standard deviation to generate a normal distribution. Random range shifts were then assigned to each species from within this normal distribution. We then applied the same filtering process used for our ‘primary’ dataset where we excluded any species with upper distributions that reached the maximum elevation of the transect during the historic survey, as we would therefore be unable to calculate the full extent of their upslope range shifts. Following the same logic, the simulation also excluded species with lower distributions that reached the minimum elevation of the transect during the historic survey. We ran the simulation 1000 times for each transect. Results from the simulation (below) demonstrate that the filtering procedure for the primary dataset does not lead to a null expectation of a negative relationship between midpoint of the elevational range and magnitude of upslope range shift. Thus, our filtering process does not lead to systematic underestimates of the magnitude of upslope shifts of high elevation species, and is not driving the reported pattern of decreasing magnitude of upslope range shift with increasing midpoint of the elevational range.

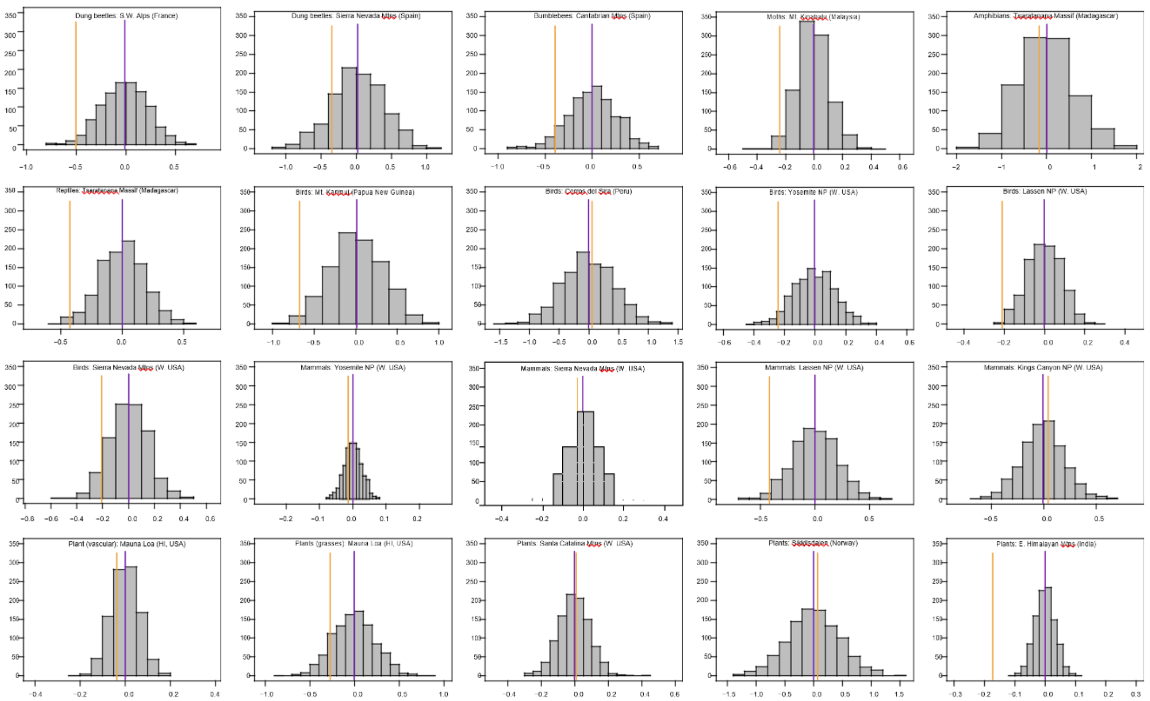


Figure 1-13. Histograms show the distribution of expected relationships (i.e., slopes) between elevational range midpoint and magnitude of upslope range shift for each mountain transect (n=20). The dashed purple line shows the average slope value following 100,000 simulations, and the solid orange line shows the empirical slope value calculated from our primary filtered data set. Because the simulated mean slopes are equal to 0 or are less than empirically calculated slopes for the transects, the simulation demonstrates that our filtering procedure in the primary data set does not lead to a null expectation of a negative slope value.

CHAPTER II
BACKGROUND INFORMATION & STUDY SYSTEM

Abstract

There are a number of traits that increase invasion success, allowing a species to establish and spread in a new environment. Many of these traits are also those that improve an organisms' ability to adjust to climate change. Therefore, it is likely that some invasive species will be more capable of surviving climate change than native species, resulting in climate change exacerbating the effects of some biological invasions. This synergism between species invasions and climate change is a threat to native diversity and deserves further study. Here I describe some potential ecological mechanisms behind this synergism. Next, I provide background information on the *Onthophagus* dung beetle system. This study system is then used in the following three chapters to investigate whether an introduced dung beetle outperforms a native congener under warming conditions due to differences in their phenotypic plasticity.

Climate change impacts biological invasions

Biological invasions often have devastating ecological and economic impacts. Invasive species modify habitats, outcompete and overconsume native species, and introduce novel diseases, resulting in biotic homogenization, loss of local diversity, and changes to ecosystem function (Elton 1958; Simberloff 2013). Invasive species cost the US economy an estimated $120 billion a year through damages to infrastructure, agricultural losses, declines in native species, and decreased ecosystem services (Pimentel 2005). For decades, biologists have sought predictive models of where and when invasions will occur (Elton 1958; Karieva 1996; Kolar & Lodge 2001). Some researchers have characterized environmental factors order to predict which ecosystems are most susceptible to new invasions. Others have focused on characterizing the traits of successful invaders (Simberloff 2013).

Many common traits of invasive species also increase their ability to adjust to climate change, such as broad thermal physiology, high dispersal capability, high phenotypic plasticity, and generalist feeding and habitat requirements. It is thus likely that invasive species will be more capable of surviving climate change than native species, and in some cases, may even spread and have increased impacts because of warming (Morrison *et al.* 2005; Bradley *et al.* 2010; Gilman *et al*. 2010; Simberloff 2013; Lin *et al.* 2018). This synergism between biological invasions and climate change is a threat to native diversity and deserves further study to elucidate how often invasive species outperform native species under warming, how competitive interactions between invasive and native species are impacted by climate change, and which traits of invasive species help them effectively adjust to warming.

Phenotypic plasticity is one such trait thought to increase organisms' ability to invade new environments and adjust to warming (Baldwin 1896; Agrawal 2001; Sol *et al.* 2002; Richards *et al.* 2006; Wright 2010; Davidson *et al.* 2011; Engel *et al.* 2011; Amiel *et al.* 2016). Phenotypic plasticity can be expressed in a number of traits including development, morphology, physiology, and behavior. For example, researchers have demonstrated that developmental plasticity of defensive armor may facilitate invasion by a water flea, *Daphnia lumholtzi* (Engel *et al.* 2011). Plasticity of foraging behavior, (Sol & Lefebvre 2000; Sol *et al.* 2011; Grey & Jackson 2012; Reisinger *et al.* 2017), anti-predator behavior (Hazlett *et al.* 2003; Reisinger *et al.* 2017), and habitat choice (Grey & Jackson 2012; Stroud *et al.* 2019) have also been linked to invasion success. High physiological plasticity has also been documented in invasive species. In fruit flies, plasticity of thermal limits was higher in a wide-spread invasive fruit fly compared to a narrowly distributed congener (Nyamukondiwa *et al.* 2010). Invasive cane toads' ability to invade high latitude sites has been attributed to physiological plasticity (Winwood-Smith *et al.* 2015). It is thus likely that many invasive species show elevated phenotypic plasticity in some traits.

Because phenotypic plasticity plays a role in biological invasions, it is likely that invasive species are more plastic than native species. Native species have a longer history of evolution in their environment and likely show local adaptation, which can replace phenotypic plasticity (Yeh & Price 2002; Price *et al.* 2003; Wright 2010) since plasticity can be costly to maintain (Dewitt *et al.* 1998). Few studies, though, have empirically measured differences in phenotypic plasticity between invasive and native species in response to temperature changes even though such differences may have important implications for the impacts of climate change (Davidson *et al.* 2011; Engel *et al.* 2015). Elevated plasticity in invasive species has been demonstrated in a few plant and marine species pairs (Smith *et al.* 2005; Funk *et al.* 2008; Engel *et al.* 2011), but a more thorough understanding of how plasticity varies been invasive and native species is an important step in evaluating whether climate change may exacerbate the spread and impact of damaging invasive species. In the following chapters, I investigate physiological plasticity (chapter four) and behavioral plasticity (chapter five) is higher in an introduced dung beetle species compared to a native species.

Dung beetle ecology & ecosystem services

There are 6,000 known species of dung beetles (Scarabaeinae) in 257 genera (Hunt *et al.* 2007; Simmons & Ridsdill-Smith 2011) that are distributed across all continents except Antarctica (Philips 2010). Dung beetles feed exclusively on dung and use it in their reproductive cycle. Dung beetles are organized into three main classes based on their reproductive behavior (Simmons & Ridsdill-Smith 2011, Fig. 2-1, figures are provided in the appendix). Paracoprids (tunnelers) dig tunnels underneath the dung pat, packing dung into brood masses at the end of the tunnel. They then lay a single egg within the brood mass, covering the egg with dung (Halffter & Edmonds 1982). Telecoprids (rollers) make a ball of dung at the dung pat and then roll the ball away from the dropping. After attracting a mate via pheromone emission, telecoprids bury the brood mass shallowly and lay a single egg (Halffter & Edmonds 1982). Finally, endocoprids (dwellers) create brood masses with eggs inside the dung pat.

Through their breeding behavior (Fig. 2-1), dung beetles provide crucial ecosystem services (Ridsdill-Smith & Edwards 2011). By burying brood masses underground (paracoprids and telecoprids), dung beetles release nutrients from the dung and return them to the soil at a much faster rate than decomposition alone (Anderson *et al.* 1984; Ridsdill-Smith & Edwards 2011). Dung beetles are particularly important in cycling P, K, and Mg (Bertone *et al.* 2005). Through digging tunnels (paracoprids), dung beetles improve soil percolation, aerate the soil, and introduce organic matter back to the soil (Bertone *et al.* 2005). Through changes to the soil structure and nutrient availability, dung beetles have been shown to affect vegetation structure and biomass. Finally, dung beetles compete with flies and other pests for dung resources. By creating brood balls, dung beetles also remove and damage pest eggs laid within the dung, decreasing the abundance of many pest species (Ridsdill-Smith & Edwards 2011). In the United Kingdom alone, Beynon *et al.* (2015) estimated that dung beetles provide approximately 450 million dollars in ecosystem services. Therefore, the ecosystem functions of dung beetles are ecologically and economically important.

***Onthophagus* dung beetles**

Onthophagus is a highly diverse and species-rich genus of dung beetle. *Onthophagus* dung beetles are paracoprids (tunnelers) with diverse and complex mating systems. For many species, males are dimorphic – major males have large horns while minor males have stunted horns and visually resemble females. Major males of *Onthophagus* show a variety of horn structures that are used to either flip or push opponents away from their tunnels, thereby minimizing access by other males to their female mate (Knell 2011). Minor males are considered “sneaker” males that move through tunnels under the soil in order to access females without engaging in physical bouts at the dung source (Moczek & Emlen 2002). Females are primarily responsible for digging tunnels and pulling dung under the soil to pack into brood masses (Hunt & Simmons 2002; Hunt & House 2011). Major males, though, will assist the females in these activities when they are not actively engaged in combat or other guarding behaviors; minor males do not assist females (Hunt & Simmons 2002; Hunt & House 2011). Eggs laid in the brood masses will fully develop into adult beetles within the brood mass; the size of the brood mass correlates with the size of the adult offspring because the provided dung makes up the sole source of nutrition for the developing larvae (Moczek 1998; Hunt & Simmons 2002).

Onthophagus dung beetles demonstrate plasticity of reproductive behaviors (Moczek 1998; Moczek 1999; Moczek 2003; Hunt & Simmons 2003). Females can vary the size, number, and burial depth of brood balls in response to environmental conditions (Hunt & Simmons 2003; Moczek & Emlen 2002; Hunt & House 2011; Macagno *et al.* 2016). Male behaviors, such as the time spent performing parental care through helping with brood mass creation, guarding, and in combat, are highly plastic and depend on the overall population density, the ratio of major to minor males, and abiotic factors (Moczek 1998; Moczek 2003; Hunt & Simmons 2003; Hunt & House 2011). Developmental plasticity of *Onthophagus* species has been widely studied and many phenotypic traits such as size and male morph type in *O. taurus* have been linked with different levels of nutrition during development (Moczek 1998; Moczek 1999; Moczek 2011). Therefore, *Onthophagus* beetles are an ideal study system for investigating questions of phenotypic plasticity.

Onthophagus taurus introduction

Onthophagus taurus has been introduced multiple times, both intentionally and accidently, into much of the United States throughout the 20th century (Fig. 2-2). *O. taurus* was first recorded in 1974 in the Southeastern United States on cattle pastures in northwestern Florida, central and southwestern Georgia, and southeastern Alabama (Fincher & Woodruff 1975). In this case, *O. taurus* is thought to have hitchhiked to the Florida panhandle in cattle dung brought overseas with transported cattle either by a farmer or perhaps the US Military (Hoebeke & Beuchke 1997). *O. taurus* established and began to expand northwards into much of the Southeastern United States. In the 1980s, *O. taurus* was intentionally introduced by the United States Department of Agriculture onto southern cattle pastures in California, Texas, and Georgia; at the same time, the New Jersey Department of Agriculture also released *O. taurus* into northern pastures (Floate *et al.* 2017; Pokhrel *et al.* 2021). Dung build-up results in pasture fouling which decreases grazing since livestock avoid feeding near dung pats (Anderson *et al.* 1984). The dung is also used as breeding resources for many agricultural pests, such as flies and intestinal parasites. Through their reproductive behavior, dung beetles damage pest eggs and larvae, decreasing populations of these pests (Ridsdill-Smith & Edwards 2011). Dung beetles also decrease methane emissions on cattle pastures through burying dung beneath the soil. Together, these ecosystem services make dung beetles an important player on livestock fields, encouraging local governments and private ranchers to introduce thousands of *O. taurus* and other non-native dung beetles into pastures. Managers believed that native dung beetles were unable to process the increased amount of dung produced by growing agricultural industries and thus turned to non-native beetles (Ridsdill-Smith & Edwards 2011).

By the late 1990s, *O. taurus* invasive range spread as far west as Texas and as far north as Maryland, with some established populations in California (Hoebeke & Beuchke 1997). Currently, *O. taurus* has spread throughout much of the eastern United States and southward into the Caribbean (Floate *et al.* 2017; Pokhrel *et al.* 2021). Environmental niche models indicate that *O. taurus* may continue to expand into most of the United States, Mexico, and the Caribbean (Floate *et al.* 2017).

After establishment and spread of non-native dung beetles in the United States, Hoebeke & Beuchke (1997) recorded high numbers of introduced dung beetles in pastures, to the extent that in some areas, these non-native beetles dominated communities. While this survey suggests that introduced dung beetles may be changing community structure, we do not have a good understanding of how introduced dung beetle species directly affect native species. In the United States and Canada, there were 37 species of *Onthophagus* dung beetles recorded prior to the introduction of *O. taurus* (Howden & Cartwright 1963). Little research, though, was performed to determine how the introduction of non-indigenous *Onthophagus* species would affect native communities of beetles, even though *O. taurus* and other introduced species are likely to compete with native tunneling beetles over dung resources and space under the dung pat. *O. taurus* may compete with native beetles through resource competition by removing and burying dung and/or through interference competition when male *O. taurus* physically exclude males from other species from tunnel space underneath the dung (Simmons & Ridsdill-Smith 2011).

Competition of native tunnelers in North America with *O. taurus* has not been investigated either in the field or through laboratory experiments, so the ecological effects of *O. taurus* on North American dung beetle communities are not well understood. Studies in other systems, though, indicate that *O. taurus* will likely have an impact on native North American beetles. When competing with *O. taurus*, *O. illyricus*, a European native that has a sympatric range with *O. taurus*, buries brood balls significantly shallower than when kept in intraspecific colonies (Macagno *et al.* 2016). While this experiment investigated the effect of competition of *O. taurus* in its native range, Macagno *et al.* (2016) demonstrated that competition between dung beetle species can affect breeding behavior. Such effects of competition may also be occurring in North American habitats between *O. taurus* and native beetles. Ridsdill-Smith (1993) demonstrated that rate of dung processing varies between two *Onthophagus* species in Australia, which allowed *O. ferox* to outcompete *O. binodis* because *O. ferox* buried dung at a faster rate. If *O. taurus* processes dung faster than native species, *O. taurus* may outcompete native species in the United States. Research on *O. gazella*, a non-indiginous species introduced to the United States from Africa, demonstrates that *O. gazella* removes and buries dung at faster rate than two native tunnelers (Young 2007), suggesting that *O. gazella* may outcompete native species based on more efficient reproductive behavior. No such studies exist for *O. taurus*, but from my observations in lab colonies, *O. taurus* processes dung faster than native species. Finally, *O. taurus* was intentionally introduced to Australia in the 1950s and then established and spread. Now, *O. taurus* makes up about 81% of dung beetles caught on pastures (Ridsdill-Smith & Edwards 2011). *O. taurus* thus dominates many pasture communities in Australia and has the potential to drastically change community make-up in its invasive range. To better understand the effects of the *O. taurus* introduction, we need many more studies investigating interactions between *O. taurus* and native species across the United States, including detailed observations of native beetle communities in both pasture and wild lands. As a starting point, I performed a bioinventory of native dung beetle communities in the Great Smoky Mountain National Park (chapter three).

***Comparing traits of introduced* O. taurus *to the native species* O. hecate**

Onthophagus hecate is a small tunneling dung beetle native North America and is widely distributed across most of the United States, except the Pacific Coast (Fig. 2-2). *O. hecate* is most commonly found in open pastures, but it also occurs in forested areas. *O. hecate* is one of the most common North American dung beetle species due to its wide geographic range, broad habitat preference, and high abundance (Howden & Cartwright 1963; Nemes & Price 2015). *O. hecate* has a diel and geographic overlap with *O. taurus* and both species prefer to breed on large patties of mammalian dung. *O. hecate* and *O. taurus* are of similar size and therefore likely compete for access space under the dung for tunnels as well as dung to produce brood balls (Simmons & Ridsdill-Smith 2011). I therefore chose to analyze differences in traits, especially phenotypic plasticity, between *O. taurus* and *O. hecate* in order to investigate whether the introduced dung beetle would be better able to adjust to climate warming than the native species.

Appendix

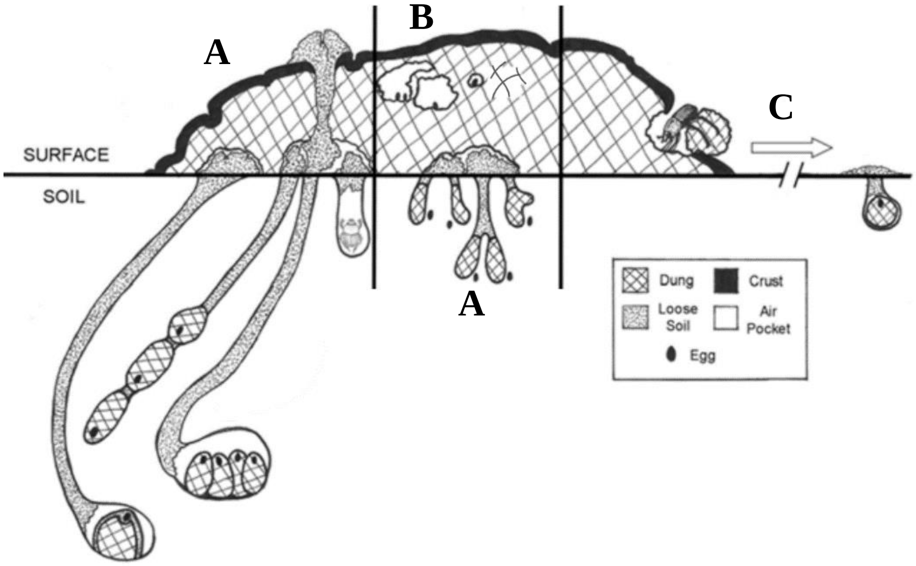


Figure 2-1. Three classes of dung beetles based on reproductive behavior (Halffter & Edmonds 1982). Paracoprids (tunnelers – A) bury brood masses with a single egg at the end of tunnels beneath the dung bat. Endocoprids (dwellers – B) created brood masses with eggs inside the dung pat. Telecoprids (rollers – C) roll brood masses away for burial a distance from the dung pat. Figure minimally adapted from Bertone *et al.* (2005).

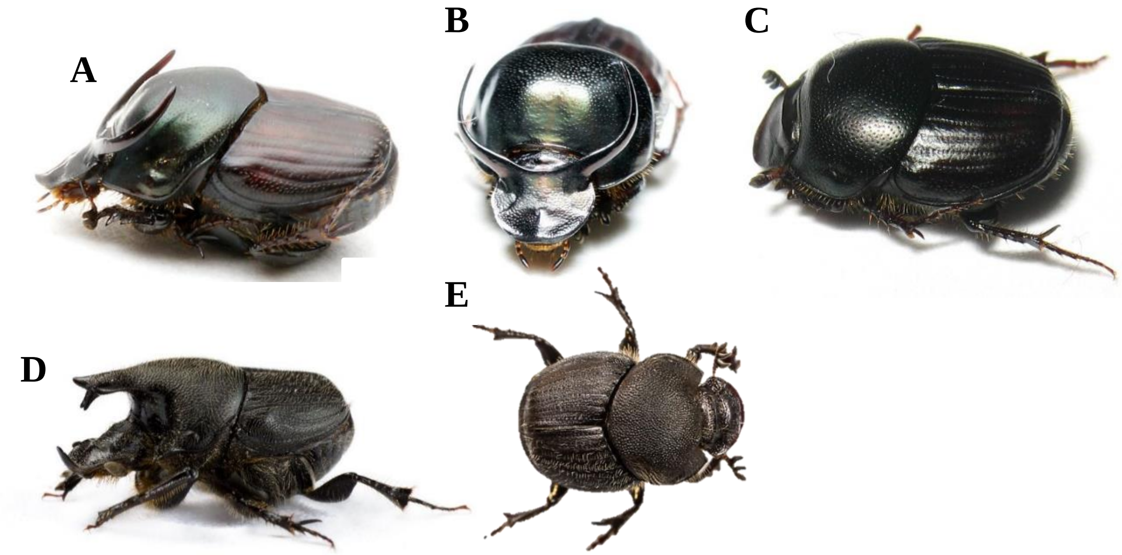


Figure 2-2. Study species. A – *O. taurus* male, from side. B – *O. taurus* male, from front. C – *O. taurus* female, from above. D – *O. hecate* male, from side. E – *O. hecate* female, from above. Photo credits: A-C – Jansuk Kim, D – Kevin Stohlgren, E – John Ros

CHAPTER III

SEASONALITY, DISTRIBUTION, AND DIVERSITY OF DUNG BEETLES IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK

A version of this chapter is in press at *The Coleopterists Bulletin* and was coauthored by Margaret A. Mamantov and Kimberly S. Sheldon:

Mamantov, M.A. & Sheldon, K.S. Seasonality, distribution, and diversity of dung beetles (Coleoptera: Scarabaeinae, Aphodiinae, and Geotrupinae) in the Great Smoky Mountains National Park. *The Coleopterists Bulletin. In Press*.

Margaret Mamantov and Kimberly Sheldon designed the study. Margaret Mamantov gathered data and performed analyses. Margaret Mamantov wrote the manuscript. All authors discussed the results and commented on the manuscript.

Abstract

Dung beetle communities provide crucial ecosystem services in a diverse range of habitats. As part of their breeding activities, dung beetles remove portions of a dung source and bury them under the soil. This behavior adds nutrients to the soil, aerates the soil, and disperses seeds. Dung beetle species are numerous in forest, prairie, savanna, and pasture ecosystems across the globe, but dung beetle communities vary across elevational gradients and habitat types. A variety of dung beetle species are native to the southeast region of the U.S., yet we have limited knowledge of the life history and community assemblage of these species. Previous research on Southeastern dung beetles has focused primarily on censusing the species inhabiting agricultural pasture land; bioinventories of dung beetle communities in the Appalachian mountain regions are thus incomplete. To fill this knowledge gap, we performed a census of dung beetles in the Great Smoky Mountains National Park, quantifying differences in abundance and distribution across season, habitat type, and elevation. Using pitfall traps baited with cattle dung for 24-hour periods, we identified dung beetles in six plots biweekly from April to September 2017. We found that communities of dung beetles varied both temporally and geographically. Low elevation communities were more diverse than high

elevation communities, and high elevation communities were dominated by non-native species. Population abundance peaked in late summer at both low and high elevations.

Introduction

Dung beetle communities provide crucial ecosystem services in a diverse range of habitats. As part of their breeding activities, dung beetles remove portions of a dung source and bury it under the soil for oviposition, and this dung is the sole food source of larvae during development (Halffter and Edmonds 1983). This behavior increases the amount of dung buried underground, which adds nutrients to the soil, aerates the soil, disperses seeds, and decreases survival of vertebrate pests (Nichols *et al.* 2008). Dung beetle species are numerous in forest, prairie, savanna, and pasture ecosystems across the globe, but species distributions change across space, and thus the composition of dung beetle communities varies across elevation and latitude (Andresen 2005; Hanski and Cambefort 1991; Jay-Robert *et al.* 2008; Simmons and Ridsdill-Smith 2011; Verdú *et al.* 2007). A variety of dung beetle species are native to the southeast region of the United States. Yet, we currently have limited knowledge of the life history and community assemblage of these species because previous research has focused primarily on agricultural pasture land (Bertone *et al.* 2005; Kaufman and Wood 2012). Thus, bioinventories of dung beetles in the Appalachian Mountains are rare and incomplete.

Documenting community assemblage of dung beetles is important for understanding the ecosystem services provided by beetles (Dangles *et al.* 2012). Dung beetles are split into three guilds based on breeding behavior. Rolling dung beetles (telecoprids) remove and roll dung away from the dung pat to a suitable site before laying a single egg within the dung mass and burying it in a shallow hole below ground (Halffter and Edmonds 1983). Tunneling dung beetles (paracoprids) dig a tunnel beneath the dung source, pack dung into a brood mass in the tunnels, lay a single egg within the brood mass, and then backfill the tunnel (Halffter and Edmonds 1983). Dwelling dung beetles (endocoprids) lay eggs in a brood mass that they shape within the dung pat, and thus dwellers do not place dung beneath the soil surface (Halffter and Edmonds 1983). The composition of the different functional guilds within a community significantly affects the rate of dung burial (Dangles *et al.* 2012). Furthermore, the dung burial rate and depth depend on the dung beetle's size (Dangles *et al.* 2012; Gregory *et al.* 2015; Mamantov and Sheldon 2021b). Determining the make-up of dung beetle communities across the Appalachian Mountains can thus provide insight into ecosystem services across this region's different elevations and habitat types.

Dung beetle communities in the Appalachians include native species as well as non-native species that were introduced to the region throughout the 20th century. During the 1960s-1970s, non-native dung beetles were intentionally introduced on agricultural land across the United States to increase the rate of dung removal (Fincher and Woodruff 1975; Floate *et al.* 2017; Hoebeke and Beucke 1997; Pokhrel *et al.* 2021). Since introduction, these species have spread to unintended areas and are likely competing with native species for access to dung resources and breeding space (Howden and Howden 2001; Howden and Scholtz 1986; Ridsdill-Smith 1993; Young 2007). These types of biological invasions are recognized as one of the major threats to biodiversity across the globe (Elton 1958; Simberloff 2013). Because we are lacking dung beetle bioinventories in the Appalachian Mountains, we know little about how these introduced dung beetle species affect native dung beetle species and community assemblage.

We performed a census of dung beetle communities to provide a biological inventory of the location, timing of activity, and abundance of native and introduced species in the temperate forests of the Appalachian Mountains within the Great Smoky Mountains National Park (GSMNP). Specifically, we wanted to know: (1) How do dung beetle communities in the GSMNP vary across habitat and elevation?; (2) How do dung beetle populations in the GSMNP vary seasonally?; and (3) How does the abundance of introduced dung beetle species vary across habitat and elevation? To address these questions, we performed a biweekly census from April–October 2017 at six sites in GSMNP varying in elevation and habitat type.

Methods

Study Sites

We cataloged dung beetles at six All Taxa Biodiversity Inventory (ATBI) plots in the GSMNP (Permit #GRSM-2017-SCI-2004) (Fig. 3-1, all figures and tables included in appendix). The ATBI plots are a project organized by Discover Life in America in conjunction with the National Park Service that works to inventory species and maintain species databases for the Smoky Mountains (https://dlia.org/; Nichols and Langdon 2007). The 1-hectare ATBI plots were established in 1998 and have been monitored for various taxa since their conception (Nichols and Langdon 2007). We chose six ATBI plots spanning different elevations and habitat types (Jenkins 2007), including (1) Catalouchee ATBI—high elevation old growth forest (1382 m); (2) Purchase Knob ATBI—high elevation forest edge (1524 m); (3) Indian Gap ATBI—high elevation beech gap forest (1672 m); (4) Cades Cove ATBI—low elevation meadow (522 m); (5) Tremont ATBI—low elevation early successional forest (549 m); (6) Twin Creeks ATBI—low elevation early successional forest (594 m) (Fig. 3-1, Table 3-1).

Trapping

We trapped dung beetles biweekly, starting on April 14, 2017, and finishing on September 27, 2017, to census throughout the entirety of the beetles' active period. Due to bear activity that impacted baited traps and site access, we had some variation in trapping periods among sites (Table 3-1). Within each plot, we set five pitfall traps within a 100 m radius circle. Traps were at least 25 m apart. Pitfall traps consisted of a buried 900 g plastic container with a funnel entrance filled with approximately three centimeters of field soil. All traps were baited with cattle dung that was wrapped in cotton fabric and hung from a metal frame. We used cattle dung that had been sterilized by autoclaving to prevent transfer of microorganisms into the park. The traps were covered with a white Styrofoam plate, which served as a rain cover. Traps were left open for 24 ± 4 hours in order to collect both diurnal and nocturnal beetles. We then sorted, identified, and released the insects. Voucher specimens were collected for each species and deposited at the Twin Creeks Science and Education Center, part of the National Park System.

Data Analysis

Using the VEGAN package in R v3.6.3 (Oksanen *et al.* 2016; R Core Team 2020), we examined how dung beetle communities vary across habitat and elevation. We calculated Shannon's diversity index (H) and species evenness (E) for each ATBI plot, comparing habitats and making comparisons between high elevation and low elevation sites. We then calculated abundance across the active season to compare seasonality among species and between high and low elevation sites. Finally, we examined abundance of introduced versus native species at high and low elevation sites.

Results

We trapped a total of 403 dung beetles from nine species in six genera, including one dwelling species, one rolling species, and seven tunneling species (Table 3-2). Seven of the nine species are native to the Appalachian region, and two species are introduced from Eurasia (Table 3-2).

Abundance, location, and seasonal activity of dung beetle species

Canthon chalcites (Haldeman, 1843)

We caught a total of 25 individuals of *C. chalcites* (Scarabaeinae: Deltochilini), which is a large (13–21 mm) rolling dung beetle that occurs throughout the eastern United States. The species has been collected from dung, rotting fruit, and road kill (Nemes and Price 2015). *Canthon chalcites* is either black or copper in color (Nemes and Price 2015), but we noted that all individuals in our survey were copper in color. In the GSMNP, *C. chalcites* was only found in lower elevation sites, including hardwood forests and grasslands (Table 3-2). Seasonally, we found that *C. chalcites* was active from June to late September and most abundant in mid-July (Fig. 3-2).

Aphodius fimetarius (Linnaeus, 1758)

We collected 223 individuals of *A. fimetarius* (Aphodiinae: Aphodiini), a small (5–9 mm) dwelling dung beetle with a distinctive bicolored orange and black pattern. Originally from Eurasia, *A. fimetarius* is now widely distributed across Asia, Europe, Northern Africa, Australia, and North America and can be found throughout the continental United States (Miraldo *et al.* 2014). Though it is thought to prefer cattle dung and open pastures (Gordon 1983), we collected individuals from forested and forest edge habitats. In the GSMNP, we only collected *A. fimetarius* at high elevation sites. Individuals were active from April to August, with a peak in early August (Table 3-2, Fig. 3-2). It is important to note that the classification of *A. fimetarius* has been under scrutiny in recent years and is now thought to be a species complex of *A. fimetarius* and *A. pedellus*, which can be genetically distinguished (Miraldo *et al.* 2014). Based on the rounded head shape of our specimens (Miraldo *et al.* 2014), we believe the beetles sampled in our survey are, in fact, *A. fimetarius*, but genetic tests would be necessary to confirm our identification.

Geotrupes splendidus (Fabricius, 1775)

Geotrupes splendidus (Geotrupinae: Geotrupini) is a mid-sized (13–15 mm), lustrous, earth-boring scarab beetle distributed across eastern North America. Earth-boring beetles feed on dung and decaying matter and thus are attracted to fungi, dung, and carrion, though they prefer fungi as a food resource (Fincher *et al.* 1970; Howden 1955; Simons *et al.* 2018). *Geotrupes* beetles, unlike true dung beetles, do not provision offspring with dung. Instead, *Geotrupes* beetles construct burrows underground and provision offspring with plant litter. The developing larvae feed on the decaying litter (Scholtz *et al.* 2009). In our survey, we collected 14 individuals of *G. splendidus* in forested plots at low elevations (Table 3-2). *Geotrupes splendidus* are usually metallic green in color but have also been observed in hues ranging from black to copper and even purple (Guarnieri and Harpootlian 2013). We collected beetles across this color spectrum, from lustrous dark brown to a brighter green color to a purplish hue. *Geotrupes splendidus* was active from

June through September, with a peak in mid-July (Fig. 3-2). Because they were still abundant during our last survey in late September, their activity likely extends into the fall.

Geotrupes blackburnii (Fabricius, 1781)

Geotrupes blackburnii (Geotrupinae: Geotrupini) is a mid-sized (10–13 mm), black, earth-boring scarab beetle that is abundant across much of eastern North America (Guarnieri and Harpootlian 2013). Like *G. splendidus*, *G. blackburnii* feeds on decaying plant litter, fungi, dung, and carrion. In the GSMNP, we collected 39 individuals of *G. blackburnii* in forested plots at both low and high elevations (Table 3-2), though the species was more abundant at high elevation. *Geotrupes blackburnii* was active from June through August, with a peak abundance in late July (Fig. 3-2).

Phanaeus vindex (Macleay, 1819)

Phanaeus vindex (Scarabaeinae: Phanaeini) is a mid-sized (11–22 mm) tunneling dung beetle distributed across much of the southern United States. *P. vindex* has a distinctive rainbow coloring with a coppery, red pronotum, and shiny green elytra. Major males have a large horn protruding from the center of the head, minor males have a short horn, and females are hornless. Due to their larger size, *P. vindex* tends to bury brood balls deeper than other co-occurring species (Gregory *et al.* 2015; Hanski and Cambefort 1991). The species prefers open fields and large mammal dung and carrion. In the GSMNP, we collected eight individuals of *P. vindex* from one habitat, a pasture ecosystem. The species was active from June through late September, with a peak in late August (Table 3-2, Fig. 3-2).

Copris fricator (Fabricius, 1787)

Copris fricator (Scarabaeinae: Coprini) is a mid-sized (10–18 mm), black tunneling dung beetle found in the eastern United States into Canada. Major males have a large, single horn on the center of their head and minor males have either a small horn or no horn present. Females have a rounded tubercle in the center of their head (Nemes and Price 2015). *Copris fricator* beetles show biparental care and bury brood balls below the dung pat (Scholtz *et al.* 2009). In the GSMNP, we collected 31 individuals of *C. fricator* from forested and pasture ecosystems at low elevations (Table 3-2). The species was active from late July to September and showed peak abundance in early August (Fig. 3-2).

Onthophagus hecate (Panzer, 1794)

Onthophagus hecate (Scarabaeinae: Onthophagini) is a small (5–9 mm) tunneling dung beetle widely distributed across most of the United States, except the Pacific Coast. *O. hecate* is most commonly found in open pastures, but it also occurs in forested areas. *O. hecate* is one of the most common North American dung beetle species due to its wide geographic range, broad habitat preference, and high abundance (Howden and Cartwright 1963; Nemes and Price 2015). Individuals of the species are matte black and have major males with a forked horn projecting forward from the pronotum. Minor males have a reduced pronotal horn or pronotal ridge, and females have a pronotal ridge. *O. hecate* prefers dung but also feeds on fungi, carrion, and decaying plant matter (Nemes and Price 2015). *O. hecate* bury oblong brood balls (approximately 1.0–2.5 g) around 3-10 cm below the dung pat (Mamantov and Sheldon 2021b). In the GSMNP, we trapped 10 individuals of *O. hecate* from the Cades Cove meadow (Table 3-2) from May through August, with peaks in abundance in mid-May and early July (Fig. 3-2).

Onthophagus orpheus (Panzer, 1794)

Onthophagus orpheus (Scarabaeinae: Onthophagini) is a small (5–9 mm) tunneling dung beetle distributed across the eastern United States with a preference for old growth forested habitats (Price 2004). *Onthophagus orpheus* individuals can be metallic green in color, purplish brown, reddish, or copper. Similar to *O. hecate*, major males have a forked horn projecting forward from the pronotum, minor males have a reduced pronotal horn or pronotal ridge, and females have a pronotal ridge. The species has been found feeding on mammalian dung and carrion (Howden and Cartwright 1963; Nemes and

Price 2015). In the GSMNP, we collected 19 individuals of *O. orpheus*, and all of the beetles had copper coloring. We trapped beetles from the low elevation, forested sites (Table 3-2), where they were present from mid-June until early August with no clear peak in abundance (Fig. 3-2).

Onthophagus taurus (Schreber, 1759)

Onthophagus taurus (Scarabaeinae: Onthophagini) is a small (8–11 mm), non-native tunneling dung beetle that is widely distributed across most of the United States, Central America, and Australia (Floate *et al.* 2017). The species is native to the Mediterranean region, but during the 20th century, *O. taurus* was introduced multiple times into much of the USA. *Onthophagus taurus* was first recorded in 1974 on cattle pastures in northwestern Florida, central and southwestern Georgia, and southeastern Alabama (Fincher and Woodruff 1975). In the 1980s, the species was intentionally introduced by the US Department of Agriculture onto cattle pastures in California, Texas, and Georgia to decrease dung build-up; at the same time, the New Jersey Department of Agriculture also released *O. taurus* beetles into pastures in the northern United States. *O. taurus* prefers open habitats and feeds primarily on cow and horse dung (Howden and Cartwright 1963; Nemes and Price 2015). The species has lustrous dark brown to black coloring. Major males have two long, curved horns projecting outwards from the center of the head, minor males have short, often straight horns, and females have a ridge along the head. *Onthophagus taurus* bury oblong brood balls (approximately 2.0–4.5 g) in clumps around 5-18 cm below the dung pat (Mamantov and Sheldon 2021b). In the GSMNP, we trapped 34 individuals of *O. taurus* in forested and meadow habitats at both high and low elevation, but beetles were more abundant at low elevation sites (Table 3-2). *Onthophagus taurus* was active throughout the entire sampling period from May to September, with a peak in early to mid-August (Fig. 3-2).

Dung beetle community structure at different elevations

Community structure varied between low and high elevation sites (Fig. 3-4). We trapped nine species at low elevation sites but only three at high elevation sites. At low elevation sites, we recorded rolling and tunneling Scarabaeinae and two *Geotrupes* species. Shannon's diversity index was 1.99 for low elevation communities (Tremont: S=6, H=1.40; Twin Creeks: S=5, H=1.38; Cades Cove: S=5, H=1.57). Low elevation sites had an even species distribution (E=0.96), and individuals of introduced species made up 18% of total dung beetle abundance. In contrast, the ecological community at high elevation sites consisted of a dwelling Aphodiinae, a tunneling Scarabaeinae, and one *Geotrupes* species. For high elevation communities, Shannon's diversity index was only 0.34 (Catalouchee: S=3, H=0.43; Indian Gap: S=2, H=0.22; Purchase Knob: S=2, H=0.08). At the high elevation sites, dung beetle communities were dominated by the non-native dweller *A. fimetarius*, which meant the sites showed low evenness (E=0.31), and 95% of the recovered dung beetles were individuals of introduced species.

Discussion

Through our biological inventory of dung beetles in the Great Smoky Mountains National Park, we collected 403 dung beetles from six species of Scarabaeinae, one species of Aphodiinae and two species of Geotrupinae attracted to cow dung. We recovered fewer species from our sample sites in the GSMNP than are typically recovered from cattle pastures in the Southeast United States; at least forty-one species of dung beetles have been collected from pastures in North Carolina and Tennessee (Benzanson and Floate 2019; Bertone *et al.* 2005). Furthermore, on pastures with grazing livestock, thousands of dung beetles can be collected in a single season (Bertone *et al.* 2005; Fiene *et al.* 2011; and Fincher *et al.* 1986), while we only collected four hundred. The lower abundance and species richness recovered in the GSMNP is likely due to dung availability. In the park, large herbivore dung is limited to deer and elk, both which produce pelleted dung unlike the wet, large dung mounds that these species prefer for breeding. We have recovered beetles from bear dung in the GSMNP, but this resource is much less abundant than dung mounds on pasture land because bear density varies across the landscape and over the course of the beetle breeding season. Furthermore, bears prefer habitat in mid elevations (600–1,000 m), while our sites were either below 600 m or above 1,000 m (van Manen 1994). There are a number of cattle, horse, and bison farms just outside the GSMNP in both Tennessee and North Carolina, including pasture land within eight kilometers from the park borders; therefore, collection sites near the border of the park may attract beetles who primarily breed on dung on pasture land, rather than dung found within the park itself. We also used cattle dung that had been autoclaved in order to prevent the spread of microorganisms into the park, but these bacteria are responsible for producing many of the volatile chemicals dung beetles use to locate food sources (Tribe and Burger 2011). The bait provided in our study may not have attracted as many beetles as non-sterilized dung and if this bioinventory was completed with other dung types or unsterilized cattle dung, more beetles and perhaps different species may be recovered.

We found that low elevation communities were more diverse than high elevation communities, which were dominated by the non-native dweller *A. fimetarius*. Low elevation communities varied by habitat type as some species were only collected on open meadow land in Cades Cove (Table 3-2). Species at high elevation sites experience cooler, more variable temperatures than species at low elevation sites, meaning these beetles should have a broader thermal tolerance at higher elevations (Gaston and Chown 1999; Janzen 1967). This could limit the elevational range of many species collected at low elevations (Sheldon and Tewksbury 2014; Verdú *et al.* 2007). Furthermore, resource availability may change across elevation since mammalian density varies density across the park.

While most species peaked in activity in late summer, seasonality varied among species (Fig. 3-2) and between the high and low elevation sites (Fig. 3-3). At high elevation sites, we observed two peaks in dung beetle activity, one in April and the other in late July, which was driven by the seasonality of *A. fimetarius*. At low elevation sites, we instead observed a single peak in activity in late July. Species varied in the length of their active period, with both introduced species (*O. taurus* and *A. fimetarius*) active for longer periods than the native species (Fig. 3-2), which may be due to broader thermal tolerances often observed in invasive species (Simberloff 2013; Kelley 2014; Zerebecki and Sorte 2011). This longer active period could also be due to differences in life history. *A. fimetarius* overwinters in the adult life stage, leading to an early spring peak in adults, which then breed, producing a late summer peak of the new generation of adults (Floate and Gill 1998; Gordon and Skelley 2007). Many of the native species instead overwinter as pupae, emerging later in the season, leading to the single peak in activity that we observed (Floate and Gill 1998; Gordon and Skelley 2007).

The Twin Creeks ATBI site was impacted by the Gatlinburg wildfires of 2016. These wildfires burned approximately 11,000 acres in the northern part of the GSMNP (Miller *et al.* 2017), which occurred approximately six months before we started our dung beetle survey. The site experienced moderate burning and had several downed and charred trees. Fire can impact dung beetle communities by changing plant community structure (Louzada *et al.* 2010). More specifically, by reducing forest canopy, fire creates open habitats preferred by some dung beetle species. Open habitat also allows the odor from baits to disperse more widely. Fire affects plant resources available to the mammalian herbivores and omnivores whose dung is preferred by many dung beetle species. Our study design does not provide a large enough sample to compare burned and unburned forests. However, it is interesting to note that the dung beetle communities of Tremont and Twin Creeks were similar in species richness and evenness, but one species, *C. fricator*, was found only in the unburned site. Furthermore, the abundance of *O. taurus* was highest in the burned site, suggesting that the fire disturbance may have provided a habitat change that promoted the spread of this non-native species. Habitat disturbance is thought to promote biological invasion (Buckley *et al.* 2007; Simberloff 2013), which could explain this pattern, but further research investigating burned and unburned sites with increased replication is crucial for understanding the impact of fire on dung beetle communities of the GSMNP.

Through our survey, we wanted to catalog the spread of introduced beetles from pasture land into natural systems. Because dung beetles provide numerous ecosystem services (Beynon *et al.* 2015), dung beetles have been introduced into U.S. pasture land over the past century, both accidently and through intentional introduction programs (Fincher and Woodruff 1975; Floate *et al.* 2017; Hoebeke and Beuchke 1997; Pokhrel 2021). A number of introduced species have been recorded on pasture land in the southeastern US, including the tunnelers *O. taurus, Digitonthophagus gazella* (Fabricius, 1787), and *Euonticellus intermedius* (Reiche, 1849), and the dwellers *Colobopterus erraticus* (formally *Aphodius erraticus;* Linnaeus, 1758), *A. fimetarius, Chilothorax distinctus* (formally *A. distinctus,* Müller, 1776), *Calamosternus granarius* (formally *A. granarius;* Linnaeus, 1767), and *Labarrus pseudolividus* (formally *A. pseudolividus*; Balthasar, 1941) (Benzanson and Floate 2019). Of these species, we found that two (*O. taurus* and *A. fimetarius*) have expanded their introduced range beyond managed pastures and into high and low elevation forested habitats in the GSMNP even though these species are thought to prefer open grassland habitats. The impact of non-native dung beetles on native communities, particularly in forested or non-pasture habitats, is unknown, but it is likely that these species compete for access to dung resources with native species. In high elevation sites, the non-native *A. fimetarius* dominates communities, which is likely due to the lack of large mammalian dung necessary to support other guilds of dung beetles. On pasture lands in the Southeast, *O. taurus* often inundate dung beetle communities. For example, Bertone *et al.* (2005) found that on North Carolina cattle pastures *O. taurus* made up approximately 45–85% of the dung beetle community. In our forested sites, we collected just 34 *O. taurus* across the breeding season (Table 3-2), suggesting that its effect on native communities in wooded sites may be less than on pastures. At Twin Creeks, though, where *O. taurus* was recovered in the greatest numbers, these beetles make up approximately 35% of the community, suggesting they may have a detrimental effect on the native community and should be monitored.

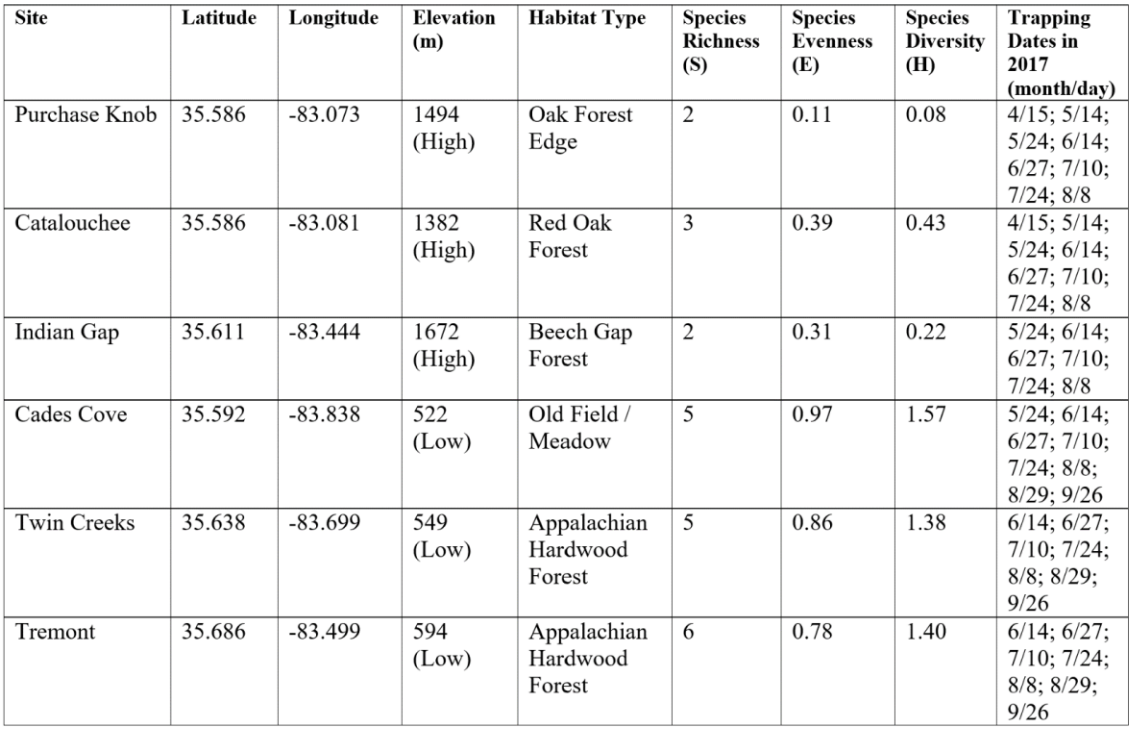
Dung beetles provide ecosystem services in the U.S. that exceed several hundred million dollars annually (Beynon *et al.* 2015; Fincher 19821; Losey and Vaughan 2006; Nichols *et al.* 2008). They are crucial members of ecological communities across a variety of habitat types, however, the services they provide vary in part due to community structure. Large-bodied beetles process more dung and bury dung deeper than their smaller-bodied counterparts, which influences nutrient cycling and seed dispersal (Dangles *et al.* 2012; Gregory *et al.* 2015; Simmons and Ridsdill-Smith 2011). However, small bodied beetles often arrive and occupy pats in greater numbers than large-bodied beetles, which can increase the rate of dung removal (Simmons and Ridsdill-Smith 2011). Furthermore, tunnelers and rollers are more effective at providing services than dwellers because they move dung away from the dung pat and bury it. In contrast, dwellers simply manipulate dung within the pat. Understanding the make-up of dung beetle communities is thus of great importance, and this survey suggests that in the GSMNP, dung beetle communities, and the ecosystem services provided, vary across habitat type and elevation.

Acknowledgments

We thank Matt McGee for assistance with field work in the GSMNP. We are grateful for the work done by Discover Life in America in maintaining and providing access to the ATBI sites as well as the National Park Service for permission to conduct this census (Permit #GRSM-2017-SCI-2004). We are especially thankful to Will Kuhn and Todd Witcher with Discover Life in America, as well as Paul Super with the National Park Service for their guidance and support while conducting field work. This project was generously funded by the Appalachian Highlands Science and Learning Center Research Grant, a program run by the GSMNP, the University of Tennessee, and the US National Science Foundation (grant no. IOS-1930829 to KSS).

Appendix

Table 3-1. All Taxa Biodiversity Inventory plots used as trapping sites in this study.



Site	**Latitude**	**Longitude**	**Elevation (m)**	**Habitat Type**	**Species Richness (S)**	**Species Evenness (E)**	**Species Diversity (H)**	**Trapping Dates in 2017 (month/day)**
Purchase Knob	35.586	-83.073	1494 (High)	Oak Forest Edge	2	0.11	0.08	4/15; 5/14; 5/24; 6/14; 6/27; 7/10; 7/24; 8/8
Catalouchee	35.586	-83.081	1382 (High)	Red Oak Forest	3	0.39	0.43	4/15; 5/14; 5/24; 6/14; 6/27; 7/10; 7/24; 8/8
Indian Gap	35.611	-83.444	1672 (High)	Beech Gap Forest	2	0.31	0.22	5/24; 6/14; 6/27; 7/10; 7/24; 8/8
Cades Cove	35.592	-83.838	522 (Low)	Old Field / Meadow	5	0.97	1.57	5/24; 6/14; 6/27; 7/10; 7/24; 8/8; 8/29; 9/26
Twin Creeks	35.638	-83.699	549 (Low)	Appalachian Hardwood Forest	5	0.86	1.38	6/14; 6/27; 7/10; 7/24; 8/8; 8/29; 9/26
Tremont	35.686	-83.499	594 (Low)	Appalachian Hardwood Forest	6	0.78	1.40	6/14; 6/27; 7/10; 7/24; 8/8; 8/29; 9/26

Table 3-2. Dung beetle species trapped in Great Smoky Mountains National Park from mid-April to late September 2017. A total of nine species (seven native, two non-native) were trapped at six study sites.

Species	Status	Guild	Study Site	Elevation	Number
Aphodius fimetarius	Non-Native	Dweller	Catalouchee	High	140
			Indian Gap	High	17
			Purchase Knob	High	66
Onthophagus taurus	Non-Native	Tunneler	Catalouchee	High	4
			Cades Cove	Low	6
			Purchase Knob	High	1
			Tremont	Low	1
			Twin Creeks	Low	22
Onthophagus hecate	Native	Tunneler	Cades Cove	Low	10
Onthophagus orpheus	Native	Tunneler	Tremont	Low	11
			Twin Creeks	Low	8
Phaneaus vindex	Native	Tunneler	Cades Cove	Low	8
Copris fricator	Native	Tunneler	Cades Cove	Low	5
			Tremont	Low	26
Canthon chalcites	Native	Roller	Cades Cove	Low	11
			Tremont	Low	11
			Twin Creeks	Low	3
Geotrupes blackburnii	Native	N/A	Catalouchee	High	15
			Indian Gap	High	1
			Twin Creeks	Low	22
			Tremont	Low	1
Geotrupes splendidus	Native	N/A	Tremont	Low	8
			Twin Creeks	Low	6

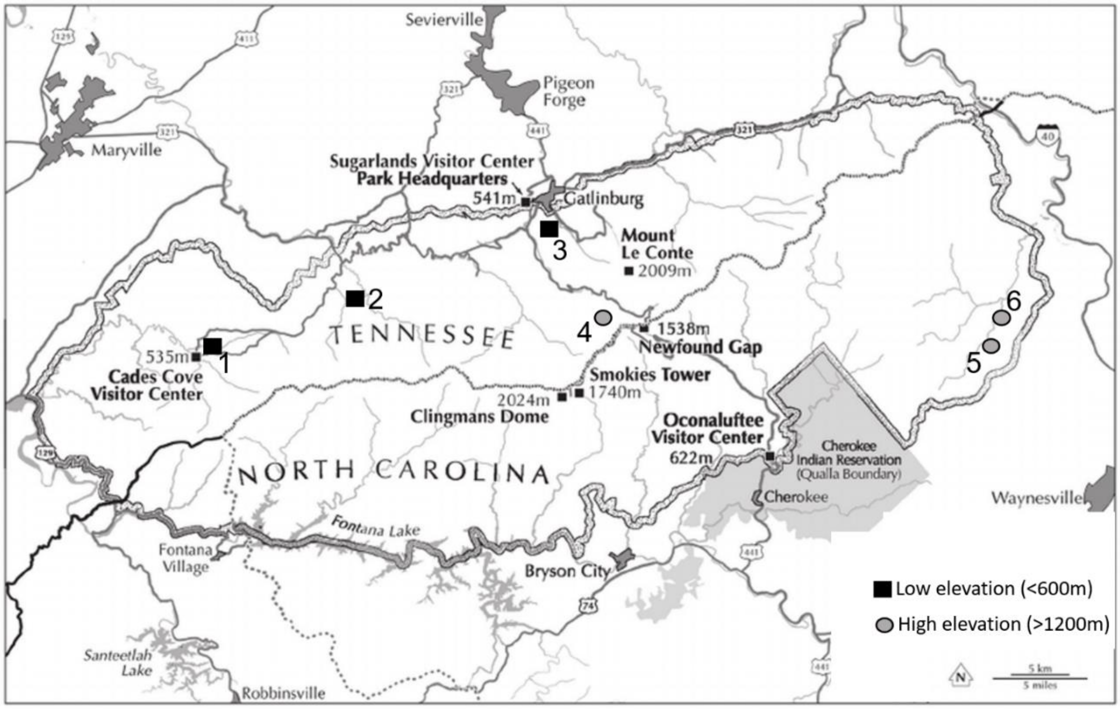


Figure 3-1. Location of the six All Taxa Biodiversity Inventory (ATBI) plots. High elevation (>1200m) plots are labeled with a grey circle and low elevation (<600m) are labeled with a black square. The numbers indicate the following plots: 1) Cades Cove, 2) Tremont, 3) Twin Creeks, 4) Indian Gap, 5) Catalouchee, and 6) Purchase Knob. Map modified from Miegrot *et al.* (2001).

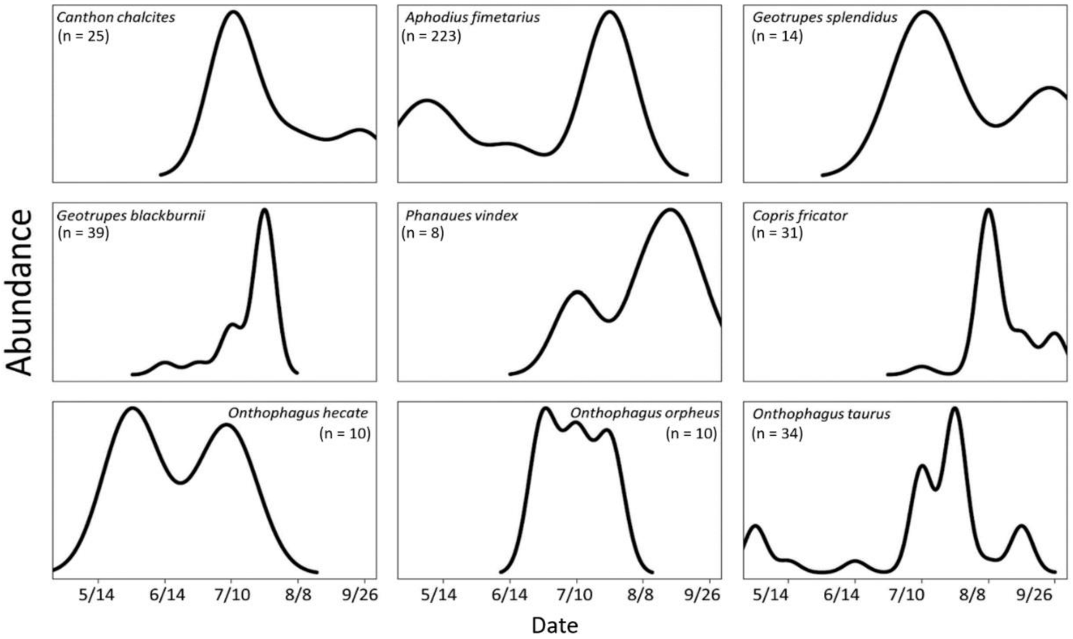


Figure 3-2. Seasonality and abundance of nine dung beetle species trapped in Great Smoky Mountains National Park from mid-April to late September 2017. Species varied in their seasonality and length of active period. Some species demonstrated unimodal peaks in abundance while others had bimodal peaks. The number of individuals trapped per species is included on each figure; for some species, very few individuals were recovered throughout the study so their observed seasonal distribution is likely to be less representative of the species true active period than the species with increased sample size.

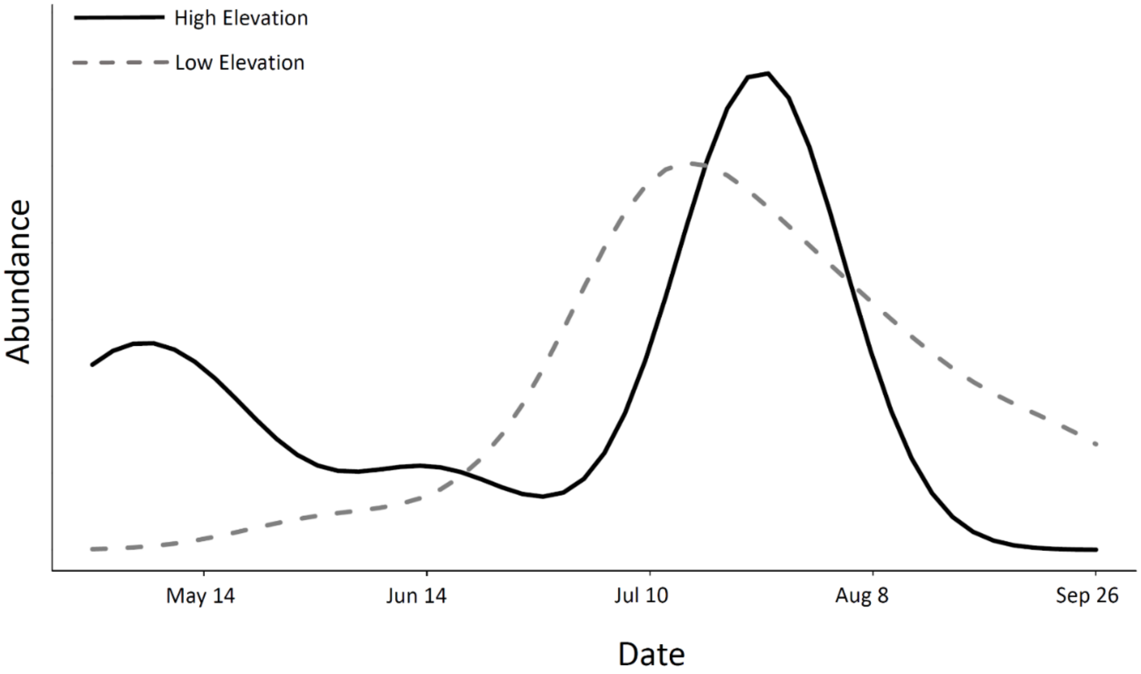


Figure 3-3. Seasonality and abundance of all dung beetles trapped at either high (>1200 m, black line) or low (<600 m, gray line) elevation sites in Great Smoky Mountains National Park in 2017. Note that the high elevation pattern is driven by one species, *Aphodius fimetarius.*

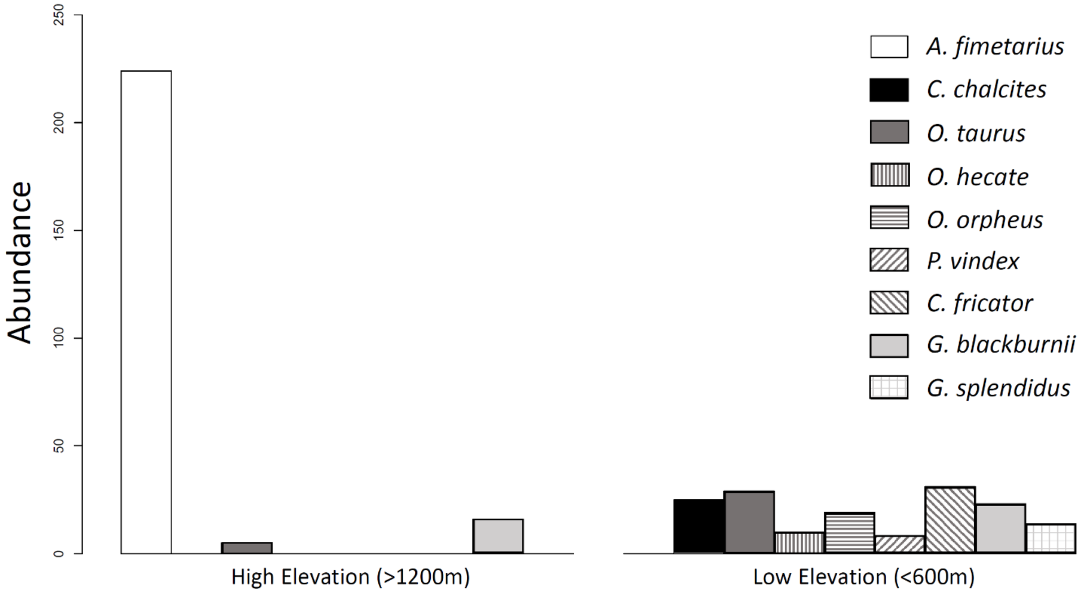


Figure 3-4. Abundance of dung beetles in high (>1200 m) versus low (<600 m) elevation communities in Great Smoky Mountains National Park in 2017. Bar height indicates the total number of individuals collected across the breeding season. Bar color indicates species guild: dwellers (white), rollers (black), tunnelers (dark gray), and earth-boring beetles (light grey). Bar shading varies by species.

CHAPTER IV

HIGHER ACCLIMATION ABILITY IN AN INTRODUCED VERSUS NATIVE DUNG BEETLE (*ONTHOPHAGUS* SPP.)

Abstract

Invasive species may be more capable of adjusting to climate warming via phenotypic plasticity than native species since plasticity is thought to increase invasion success. Physiological plasticity via acclimation is one way in which organisms can adjust their thermal tolerance in response to temperature change, but few studies have addressed whether invasive species have elevated acclimation ability compared to native congeners. Here we investigated whether acclimation ability varies between an *Onthophagus* dung beetle species pair, introduced *O. taurus* and native *O. hecate*, collected from Florida and Tennessee. We expected the introduced *O. taurus* to demonstrate higher acclimation ability than *O. hecate*; we also predicted that beetles from Florida would have reduced plasticity since their environment is less variable. To measure shifts in thermal tolerance after acclimation, we quantified time until loss of function, measured by leg mobility, in both hot and cold environments. We determined that *O. taurus* from Florida acclimate to warm temperatures, adjusting warm tolerance; unexpectedly, *O. taurus* from Tennessee showed no acclimation ability. *O. hecate* did not acclimate to warmer temperatures. Both species showed similar levels of cold acclimation. Taken together, our results suggest that the introduced species, *O. taurus* will be more capable of using physiological adjustments to respond to climate warming than the native species, *O. hecate.*

Introduction

Theory and empirical work suggest that invasive species have increased phenotypic plasticity (Baldwin 1896; Agrawal 2001; Sol *et al.* 2002; Nicolakakis *et al.* 2003; Gross *et al.* 2010; Wright 2010; Davidson *et al.* 2011; Engel *et al.* 2011; Amiel *et al.* 2016), which not only allows invasive species to establish in new environments but may also buffer them against environmental change. Native species, on the other hand, have a longer history of evolution in their environments and are more likely to demonstrate local adaptations rather than phenotypic plasticity, since plasticity is costly to maintain (Dewitt *et al.* 1998; Gotthard & Nylin 1999). Invasive species' higher capacity for phenotypic plasticity than native species may become especially concerning as climate change leads to increases in mean temperatures and the frequency of extreme thermal events (Allen *et al.* 2018). Physiological plasticity via acclimation allows organisms to adjust their physiology in response to temperature change (Diffenbaugh & Field 2013; Seebacher *et al.* 2014; Gunderson & Stillman 2015). Plasticity of thermal tolerance allows physiological traits such as critical thermal maxima and evaporative cooling to remain constant across a wider range of temperatures, increasing the organism's survival during overheating events (Stillman 2003; Seebacher *et al.* 2014). Because physiological plasticity could buffer the effects of climate change, invasive species may be more capable of surviving current climate warming than native species, exacerbating the effects of invasions (Stachowicz *et al.* 2002; Hellman *et al.* 2008; Walther *et al.* 2009; Mainka & Howard 2010; Huang *et al.* 2011).

Empirical tests of differences in plasticity between native and invasive species are sparse but generally conclude that invasives have greater behavioral and morphological plasticity than native species (Davidson *et al.* 2011; Engel *et al.* 2011), but whether the same is true of physiological plasticity is less understood. Previous research has demonstrated that invasive species have high levels of behavioral plasticity (Gross *et al.* 2010; Wright 2010; Engel *et al.* 2011; Beever *et al.* 2017), such as employing behavioral thermoregulation (Barker *et al.* 2018; Christensen *et al.* 2021) or shifts in reproductive behavior (Masson *et al.* 2016; Mamantov *et al.* 2021b) to adjust to new conditions. The limited evidence available on physiological plasticity suggests that it may drive invasion success of some organisms (Richards *et al.* 2006; Nyamukondiwa *et al.* 2010, McCann *et al.* 2018), but we lack studies quantifying whether physiological plasticity is greater in invasive species than native species. This gap in knowledge needs to be addressed since climate change and biological invasions could act synergistically in the decline of native species.

Thermal physiology is also thought to vary predictably by climate conditions. Temperature variation increases breadth of thermal tolerance (Janzen 1967; Navas 2006;

Sheldon & Tewksbury 2014), and organisms experiencing more seasonal and diurnal temperature variation have greater plasticity (Chown *et al.* 2004; Gunderson & Stillman 2015). Therefore, individuals living in more variable environments are expected to have broader thermal tolerance and increased physiological plasticity. This suggests that across the range of both invasive and native species, organisms may demonstrate varying levels of physiological plasticity and heat tolerance. The difference in plasticity and temperature tolerance between native and invasive species may thus vary by population, meaning that climate change may influence the impacts of invasions differently across species ranges.

Here we investigated whether physiological plasticity via acclimation varies between an introduced and a native *Onthophagus* dung beetle species and between populations separated by latitude. The introduced species, *O. taurus*, is native to Europe and was first recorded in the USA in 1974 on cattle pastures in northwestern Florida (Fincher & Woodruff 1975; Hoebeke & Beuchke 1997). The species established and began to expand northwards into much of the southeastern USA, helped by intentional introductions in the 1980s in California, Texas, New Jersey and Georgia to decrease dung build-up. Currently, *O. taurus* occurs throughout much of the eastern USA and southward into the Caribbean (Floate *et al.* 2017; Pokhrel *et al.* 2021). Environmental niche models indicate that *O. taurus* may continue to expand into most of the USA, Mexico, and the Caribbean (Floate *et al.* 2017). *Onthophagus taurus* overlaps in range with the native beetle *O. hecate*, which occurs from Florida to southern Canada. *Onthophagus taurus* and *O. hecate* have similar seasonal and diel activity patterns, dung use, and breeding behaviors and are often collected within the same dung pats. The two species are small-bodied dung beetles relative to the community as a whole, though *O. taurus* are larger than *O. hecate* (mean mass *O. taurus* = 0.07 g; *O. hecate* = 0.03 g).

We measured physiological plasticity in these species by quantifying shifts in ability to withstand extreme temperatures after acclimation to elevated or reduced temperatures. We collected beetles of both species from two populations across the introduced range of *O. taurus* - one population from northern Florida and the other from eastern Tennessee. We investigated the following questions: 1) does physiological plasticity vary between the introduced beetle, *O. taurus*, and the native beetle, *O. hecate*? and 2) does physiological plasticity vary between populations? We predicted that the invasive species *O. taurus*, regardless of source population, would demonstrate a higher acclimation ability than the native species *O. hecate*. We also expected that beetles from Tennessee, where temperatures are more seasonally variable, would show a higher acclimation ability than beetles from Florida, where temperatures are more stable across the dung beetle breeding season. Finally, we predicted that both species would show greater acclimation to cold temperatures versus warm temperatures since previous research has indicated that critical thermal minima are more responsive to acclimatization than critical thermal maxima (Gunderson & Stillman 2015).

Methods

Field collection

In May 2019, we collected adult *O. taurus* and *O. hecate* beetles from the University of Florida Sante Fe River Ranch Beef Unit in Alachua, FL (29° 55' 30.8"N, -82° 29' 26.9"W). We collected beetles via baited pit-fall traps and by manually searching through cow dung. Collection occurred over a three day period; beetles were held in groups of ~50 beetles in 2 L rectangular containers (13.5 x 10.2 x 28.2 cm) filled with a 4:1 mixture of topsoil:sand at constant room temperature (25°C). Colonies were fed ad libitum with field collected cow dung. These colonies were then transported back to the laboratory at University in Tennessee in a temperature controlled vehicle (24.2-25.3°C). Once in the laboratory, the colonies were held for an additional seven days at room temperature of 25°C and fed ad libitum with autoclaved cow dung.

In May 2021, we collected adult *O. hecate* beetles from Seven Islands State Birding Park (35° 57' 14.3"N, -83° 41' 22.1"W); in June 2021, we collected adult *O. taurus* beetles from a private cattle farm in Powell, TN (36° 03' 25.8' 'N, 84°04'19.8" W). We collected beetles via baited pit-fall traps and by manually searching through cow dung. We transported all field-caught beetles to the lab in plastic containers containing damp paper towels. Once in the laboratory, we kept beetles in colonies of 30-40 individuals within 2 L rectangular containers (13.5 x 10.2 x 28.2 cm) filled with a 4:1 mixture of topsoil:sand held at laboratory room temperature (24.4°C). We fed colonies autoclaved cow dung every four days, making sure to provide enough dung so that the food source was not fully depleted between feedings. We kept all beetles in these large colonies for 7 days prior to temperature trials.

Acclimation trials

To measure physiological plasticity, we quantified beetles' ability to acclimate to both cold and warm treatments. To measure shifts in thermal tolerance after acclimation, we randomly assigned dung beetles to the cold tolerance or heat tolerance trials. For beetles collected in Florida, we used both males and females to increase sample size, and each treatment had approximately the same ratio of male to female beetles. For Tennessee beetles, we only used female beetles for trials since physiological measurements can vary by sex, and we were able to collect high sample sizes using females exclusively. To measure shifts in cold tolerance, we held half of the beetles (FL: n=7 *O. hecate*, n=19 *O. taurus;* TN: n=20 *O. taurus*, n=20 *O. hecate*) in a cold treatment of 15 ± 0.8 °C for four days and the other half in a control temperature of 22 ± 0.9°C (FL) or 23 ± 1.1°C (TN). To measure shifts in heat tolerance trials, we held half of the beetles (FL: n=7 *O. hecate*, n=19 *O. taurus;* TN: n=20 *O. taurus*, n=20 *O. hecate*) in a warm treatment of 29 ± 0.9 (FL) or 30 ± 0.7 (TN) °C for four days and the other half in a control temperature of 22 ± 0.9°C (FL) or 23 ± 1.1°C (TN). We acclimated beetles at a control acclimation temperature of ~22.5°C since beetles breed readily at this temperature throughout the breeding season, suggesting this temperature does not induce thermal stress. The cold (~15°C) and hot (~29.5°C) acclimation temperatures reflect conditions that the beetles would experience in the field that are still well below lethal temperatures. For all acclimation treatments, beetles were housed in groups of 6-10 in 32oz plastic containers filled with soil and provided with 77.5 ± 2.5g of autoclaved cow dung. We acclimated beetles in Panasonic or Percival Incubators (50% RH, lights on).

After acclimation periods, we quantified shifts in thermal tolerance by comparing time until loss of function between beetles acclimated in control versus warm or cold treatments. To measure time until loss of function, we removed beetles from the plastic containers and recorded mass. Then, individuals were placed in clear, plastic chambers submerged into a water bath up to the container lip. The containers had air tight, clear plastic lids in order to maintain constant internal air temperature. The chambers had a layer of white sand on the bottom to increase traction for beetles. The water bath was held at 6°C for cold tolerance trials and 48°C for heat tolerance trials. We had one empty plastic chamber with a thermocouple during the entirety of the trials to monitor air temperatures inside the chambers, ensuring that air temperature matched the water bath temperature. Water bath temperatures were chosen to allow for variation in time until loss of function but also to ensure trials were not long enough to cause desiccation in beetles.

Individuals were monitored in the water bath for loss of function, in this case, the ability to move legs in coordinated movements. During trials, beetles usually attempted to walk or fly around the chamber; we monitored this movement and recorded when the beetles were no longer able to move limbs, which was shortly after forward motion and flight were impeded and shortly before death. If an individual did not attempt movement but instead tucked legs into the body, making it impossible to monitor leg movement, we used a paint brush to disturb the beetle, encouraging movement. If the beetle did not respond to this, the individual was removed from the water bath, and the trial ended (occurred in less than 2% of individuals). If an individual flipped upside down and was unable to right itself, we also used a paint brush to flip the individual upright. To confirm that this procedure did not change temperatures inside the submerged chamber, we also did mock flips in the empty chamber with a thermocouple, removing the lid, touching the chamber floor with the paintbrush, and replacing lids. This process confirmed that flipping beetles infrequently with a paintbrush did not alter interior air temperature. After trials, we recorded the mass of every individual again to monitor water loss over the course of the trial.

Statistical Analyses

To examine differences in physiological plasticity between populations and species, we used linear models for cold tolerance and heat tolerance trials in R Version 3.6.3 (R Core Team 2020) with the response variable of time until loss of function and the predictors of acclimation treatment (warm/cold or control), species (*O. hecate* or *O. taurus*), site (FL or TN), and beetle mass at the start of the trial (hereafter "starting mass"), and the interactions among species, treatment, and site. We included treatment in our model to test whether time until loss of function varied in response to acclimation. We included collection site in our model to test whether time until loss of function varied between TN and FL beetles, and we included species to test whether time until loss of function varied between *O. taurus* and *O. hecate*. To test for differences in acclimation ability between the two species living in the two populations, we included the interactions between species, site, and treatment. We included starting mass as a predictor because many physiological values vary by mass; larger organisms have a reduced surface to volume ratio, increasing heat and moisture retention. Therefore, larger beetles are expected to have increased cold tolerance but reduced heat tolerance and may be less able to respond to cooling by evaporative water loss. To determine best-fit models, we performed model selection using Akaike information criterion (AIC) scores.

Because time until loss of function at high temperature (48°C) could be influenced by acclimation ability or the ability to shed heat via water loss, we also examined if water loss varied between *O. taurus* and *O. hecate* during the heat tolerance (48°C) trials. To approximate water loss, we calculated the percent mass loss during the trial by dividing mass loss by total starting mass. We then examined whether percent mass loss varied by species, treatment, or site using a linear model with mass loss as the response variable and treatment, species, site, and the interaction between these variables as predictors. To determine the best-fit model, we performed model selection using Akaike information criterion (AIC) scores.

Results

Our goals were to test whether physiological plasticity via acclimation to warm and cold temperatures varies between the introduced beetle, *O. taurus*, and the native beetle, *O. hecate* and whether acclimation ability varies between beetles from the Tennessee versus the Florida populations.

Neither species collected in Tennessee demonstrated shifts in time to loss of function after acclimation to warmer temperatures. However, the introduced *O. taurus* collected in Florida showed a significant increase in time to loss of function after acclimation to warmer temperatures (Fig. 4-1). The best model for time to loss of function in warmer temperatures included the three way interaction between treatment, species, and site ($p<0.01$), indicating that *O. taurus* and *O. hecate* collected in different sites varied in their response to acclimation. To better understand this significant interaction among the predictors, we performed analyses on beetles from Florida and beetles from Tennessee individually. For beetles collected in Florida, *O. taurus* significantly increases time until loss of function after acclimation ($p < 0.001$), but *O. hecate* does not show significant adjustment ($p = 0.96$). For beetles collected in Tennessee, neither species significantly adjusted time until loss of function after acclimation. *O. hecate* showed an increase in time until loss of function, but this increase was only marginally significant ($p=0.06$) while *O. taurus* showed a non-significant decrease in time until loss of function after acclimation ($p=0.21$). The best model also included beetle starting mass ($p < 0.01$). In response to warming, larger *O. hecate* beetles had significantly longer time to loss of function in warm temperatures, but body size did not influence heat tolerance in *O. taurus* (Fig. 4-2).

Shifts in warm tolerance were not caused by increased water loss in warm-acclimated beetles (Fig 4-3). The best model of percent mass loss in beetles during warm acclimation trials only included site ($p < 0.0001$), not species ($p=0.39$) or treatment ($p=0.21$). Beetles from Tennessee, regardless of species, lost more mass during heat tolerance trials than Florida beetles, suggesting higher rates of evaporative cooling (Fig. 4-3).

In response to cooler treatments, both species from both populations demonstrated shifts in time to loss of function after acclimation at 15°C (Fig. 4-4). The best model for time to loss of function in cold acclimation trials included the interaction between site and treatment ($p < 0.01$) as well as the predictors of site, treatment, and species but not the interaction between species and treatment ($P=0.27$), suggesting that both species acclimate to cold temperatures but collection site affects this response. Therefore, while acclimation increases the time that beetles can tolerate cold temperatures, this plasticity does not vary by species (Fig. 4-4). The inclusion of starting mass did not significantly improve the statistical model ($p = 0.41$, Fig. 4-5). To better understand the interaction between site and treatment, we examined acclimation ability for beetles collected in Florida separately from those collected in Tennessee. For Florida beetles, *O. taurus* significantly increased time until loss of function after cold acclimation ($p < 0.001$). *O. hecate* also demonstrated increased time until loss of function after acclimation, but this response was only marginally significant ($p=0.07$). Therefore, in Florida, *O. taurus* has higher cold acclimation ability. In Tennessee, both beetle species showed significant acclimation ability to cold temperatures ($p < 0.0001$).

Discussion

To investigate whether increased physiological plasticity may favor invasive species over native species under climate warming, our goal was to determine acclimation ability of an introduced dung beetle, *O. taurus*, compared to a native congener, *O. hecate* from two different sites with varying climates. *O. taurus* collected in Florida showed the highest ability to acclimate to warmer temperatures (Fig. 4-1). We expected that the introduced *O. taurus* would have greater acclimation ability than the native *O. hecate*, but unexpectedly, we only observed this elevated plasticity in the Florida beetles, not in the Tennessee beetles, which experience more thermal variation. The native *O. hecate* beetles did not demonstrate significant ability adjustments in warm tolerance via acclimation regardless of geographic location. Even though plasticity of warm tolerance did not vary as predicted, the introduced *O. taurus* from Florida showed greater ability to withstand warmer temperatures compared to the native *O. hecate* or Tennessee *O. taurus* (Fig. 4-1). As predicted, cold tolerance was more plastic than warm tolerance, and all populations demonstrated the ability to acclimate to colder temperatures with the exception of *O. hecate* beetles from Florida. Beetles from both species collected from Tennessee had increased cold tolerance relative to beetles collected from Florida.

Acclimation to elevated temperatures was only seen in *O. taurus*, suggesting that physiological shifts may contribute to invasive species spread under climate warming. Previous research has indicated that invasive species can benefit from warming temperatures, allowing them to exploit new areas or become more competitive (Dukes & Mooney 1999; Morrison *et al.* 2005; Hellman *et al.* 2008; Walther *et al.* 2009; Bradley *et al.* 2010; Mainka & Howard 2010; Huang *et al.* 2011). In the case of *O. taurus*, increased ability to acclimate to warmer temperatures may favor the introduced beetle over its native congener, *O. hecate*, which showed no acclimation to warmer temperatures. Interestingly, we only observed this acclimation ability in *O. taurus* from Florida, not Tennessee, indicating that exposure to heat waves and higher average temperatures may select for maintenance of acclimation ability. Furthermore, this result indicates that plasticity may vary across an invasive species range.

We expected that beetles from Tennessee, where temperatures are more seasonally variable, would show a higher acclimation ability than beetles from Florida, where temperatures are warmer but more stable (Janzen 1967; Brattstrom 1968; Gaston & Chown 1999; Luddecke & Sanchez 2002; Chown *et al.* 2004; Navas 2006; Sheldon & Tewksbury 2014; Mamantov & Sheldon 2021a). In this case, though, exposure to greater seasonal variation only seems to have increased the ability to acclimate to cold temperatures. Previous research has indicated that critical thermal minima (CT_{min}) often shifts after acclimatization (Diffenbaugh & Field 2013; Seebacher *et al.* 2014; Gunderson & Stillman 2015); our results support this conclusion, indicating that cold tolerance is generally more plastic than warm tolerance in these beetle species. *O. hecate* from Florida had reduced acclimation ability to cold temperatures compared to the other populations (Fig. 4-4), which is not unexpected; rather than maintaining physiological plasticity, these populations may be locally adapted to the warm and stable environment of their the breeding season (Gotthard & Nylin 1999; Argarwal 2001).

Our results indicate that the spread of *O. taurus* into warmer regions may be facilitated by its ability to adjust to elevated temperatures through acclimation. Currently, *O. taurus* is spreading across Central America and has been collected in parts of northern South America (Floate *et al.* 2017; Pokhrel *et al.* 2020). In our study, *O. taurus* in Florida showed plasticity of warm tolerance and the highest tolerance of exposure to elevated temperature, indicating that some populations of *O. taurus* are capable of adjusting to elevated temperatures through acclimation, especially after exposure to warm climates. In previous work, we also found that *O. taurus* reproductive behaviors in response to warming make their offspring more likely to survive warming compared to *O. hecate*, (Mamantov & Sheldon 2021b). Taken together, *O. taurus* adults and offspring are less likely to be negatively influenced by climate change due to both physiological and behavioral adjustments, respectively, which may even allow the species to expand its range under warming conditions. In contrast, *O. hecate* will need to utilize other strategies to adjust to climate change or may experience population declines. In conclusion, our results suggest that variation in acclimation ability may be one mechanism through which climate change will exacerbate the spread and impact of invasive species.

Acknowledgements

We would like to thank Kevin Null, The University of Florida Sante Fe River Ranch, and Seven Islands Birding Park for allowing us to collect beetles from their properties. This research was funded by the US National Science Foundation under grant no. IOS-1930829 to KSS and funds from the University of Tennessee.

Appendix

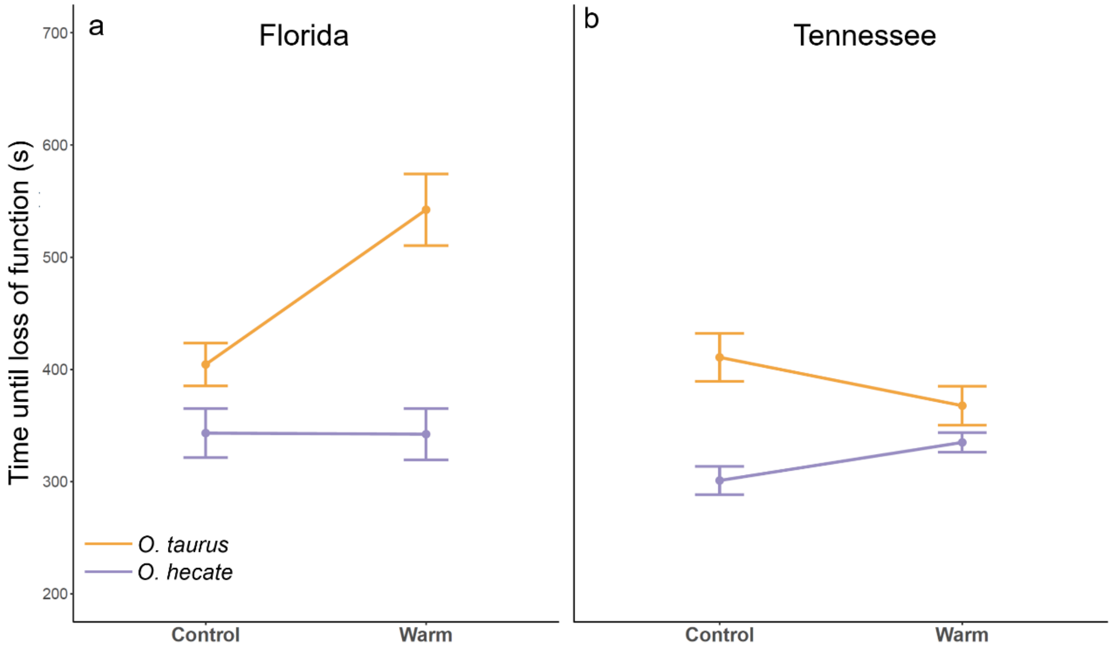


Figure 4-1. *O. taurus* from Florida demonstrate acclimation to warm temperatures, adjusting heat tolerance. *O. taurus* from both populations have greater heat tolerance, measured by time until loss of function while held at 48℃. Purple lines represent native *O. hecate* beetles; orange lines represent introduced *O. taurus* beetles. Plot 4-1a shows results for beetles collected in Florida; plot 4-1b shows results for beetles collected in Tennessee. The mean of treatment group is represented by the point on the graph, while the bars show standard error around the calculated mean.

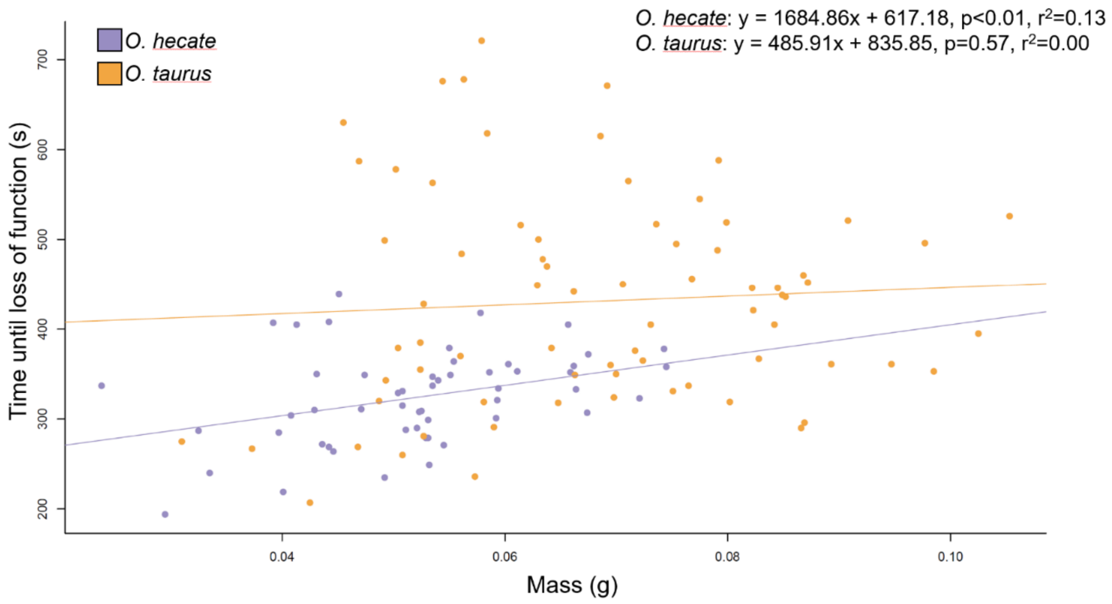


Figure 4-2. Larger bodied *O. hecate* have increased cold tolerance; mass does not affect cold tolerance in *O. taurus*. Purple points represent individual native *O. hecate* beetles; orange points represent individual introduced *O. taurus* beetles. The line of best fit was calculate via regression of time until loss of function with beetle mass (g). The calculated line of best fit and r^2 value is provided. Beetles from Florida and Tennessee were combined for this analysis.

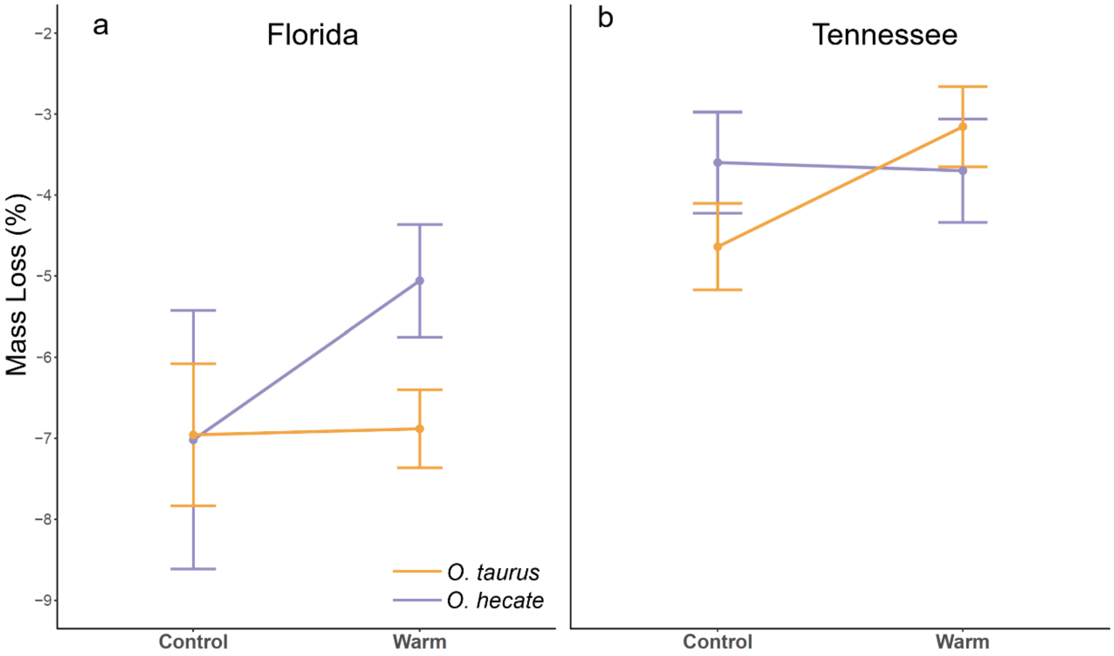


Figure 4-3. Percent mass loss varies by collection site. Purple lines represent native *O. hecate* beetles; orange lines represent introduced *O. taurus* beetles. Plot 4-3a shows results for beetles collected in Florida; plot 4-3b shows results for beetles collected in Tennessee. The mean of treatment group is represented by the point on the graph, while the bars show standard error around the calculated mean.

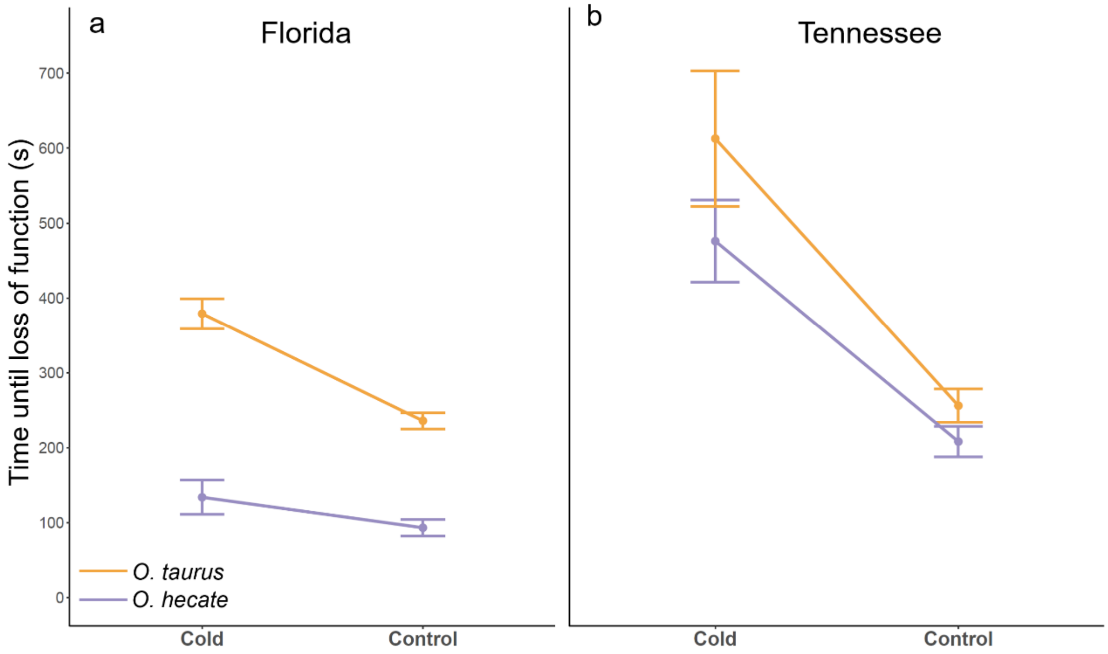


Figure 4-4. Both species acclimate to cold temperatures. Purple lines represent native *O. hecate* beetles; orange lines represent introduced *O. taurus* beetles. Plot 4-4a shows results for beetles collected in Florida; plot 4-4b shows results for beetles collected in Tennessee. The mean of treatment group is represented by the point on the graph, while the bars show standard error around the calculated mean.

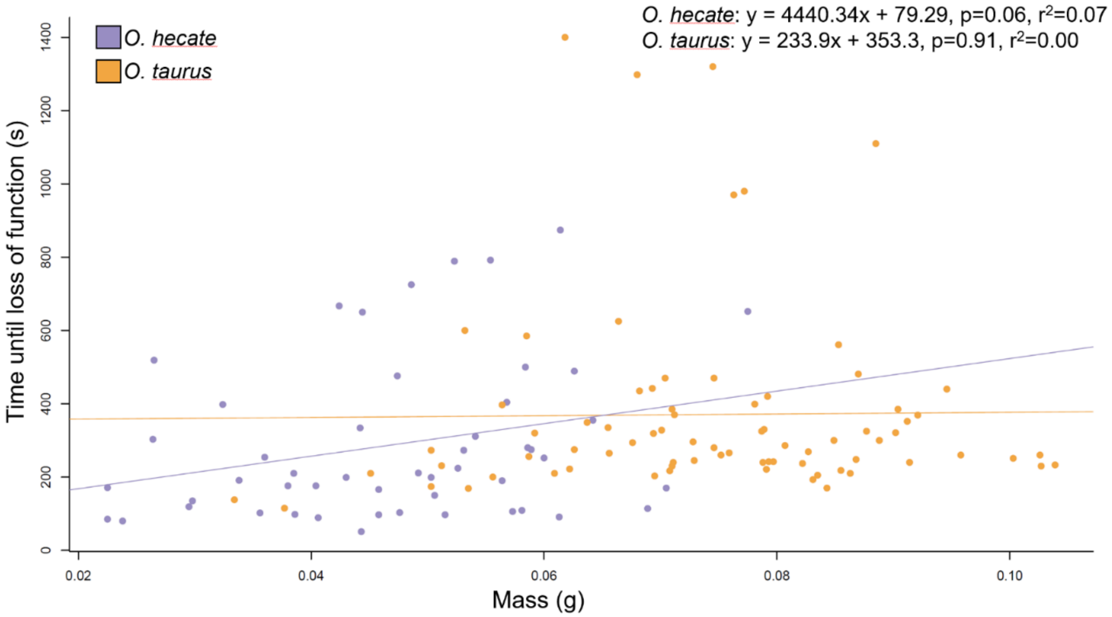


Figure 4-5. Larger bodied *O. hecate* have marginally significant increased heat tolerance; mass does not affect heat tolerance in *O. taurus.* Purple points represent individual native *O. hecate* beetles; orange points represent individual introduced *O. taurus* beetles. The line of best fit was calculate via regression of time until loss of function with beetle mass (g). The calculated line of best fit and r^2 value is provided. Beetles from Florida and Tennessee were combined for this analysis.

CHAPTER V

BEHAVIORAL RESPONSES TO WARMING DIFFERENTIALLY IMPACT SURVIVAL IN INTRODUCED AND NATIVE DUNG BEETLES

A version of this chapter is published by *Journal of Animal Ecology* and was coauthored by Margaret A. Mamantov and Kimberly S. Sheldon:

Mamantov, M.A. & Sheldon, K.S. 2021. Behavioural responses to warming differentially impact survival in introduced and native dung beetles. *Journal of Animal Ecology* 90: 273-291. https://doi.org/10.1111/1365-2656.13366.

Margaret Mamantov and Kimberly Sheldon conceived the ideas and designed methodology; Margaret Mamantov collected the data; Margaret Mamantov and Kimberly Sheldon analyzed the data. Margaret Mamantov led writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Abstract

Anthropogenic changes are often studied in isolation but may interact to affect biodiversity. For example, climate change could exacerbate the impacts of biological invasions if climate change differentially affects invasive and native species. Behavioral plasticity may mitigate some of the impacts of climate change, but species vary in their degree of behavioral plasticity. In particular, invasive species may have greater behavioral plasticity than native species since plasticity helps invasive species establish and spread in new environments. This plasticity could make invasives better able to cope with climate change. Here our goal was to examine whether reproductive behaviors and behavioral plasticity vary between an introduced and a native *Onthophagus* dung beetle species in response to warming temperatures and how differences in behavior influence offspring survival. Using a repeated measures design, we exposed small colonies of introduced *O. taurus* and native *O. hecate* to three temperature treatments, including a control, low warming, and high warming treatment, and then measured reproductive behaviors, including the number, size, and burial depth of brood balls. We reared offspring in their brood balls in developmental temperatures that matched those of the brood ball burial depth to quantify survival. We found that the introduced *O. taurus* produced more brood balls and larger brood balls, and buried brood balls deeper than the native *O. hecate* in all treatments. However, the two species did not vary in the degree of behavioral plasticity in response to warming. Differences in reproductive behaviors did affect survival, such that warming temperatures had a greater effect on survival of offspring of native *O. hecate* compared to introduced *O. taurus*. Overall, our results suggest that differences in behavior between native and introduced species is one mechanism through which climate change may exacerbate negative impacts of biological invasions.

Introduction

Organisms are facing human-induced environmental changes, such as climate change and biological invasions, that threaten native species and ecological communities. Most research to date has focused on either climate change or biological invasions in isolation even though they can interact to drive biodiversity declines (Engel *et al.* 2011; Oliver *et al.* 2016). For example, climate change can expand the range of some invasive species (Dukes & Mooney 1999; Morrison *et al.* 2005; Bradley *et al.* 2010) or worsen their impacts on their new environment (Oliver *et al.* 2016; Fahey *et al.* 2018). However, only a few studies have empirically tested the interactive effects of climate change and biological invasions. We thus have limited understanding of how climate change will modify the ecological effects of invasive species on native species and their new environment.

Due to differences in phenotypic plasticity, invasive and native species may respond to climate change in different ways or to different degrees. Research suggests introduced species can invade novel environments because they have high levels of phenotypic plasticity (Baldwin 1896; Agrawal 2001; Sol *et al.* 2002; Richards *et al.* 2006; Wright 2010; Davidson *et al.* 2011; Amiel *et al.* 2016), which helps them to establish and spread (Baldwin 1896; Price *et al.* 2003; Yeh & Price, 2004). One type of plasticity – behavioral plasticity – may be an especially important trait of invasive species since behavior is highly labile, allowing rapid but reversible responses to novel environmental pressures (Snell-Rood 2013). For example, plasticity of foraging behavior, which allows access to novel food sources in new environments, is a crucial driver of invasion success in species of crayfish and birds (Sol & Lefebvre 2000; Sol *et al.* 2002; Sol *et al.* 2005; Sol *et al.* 2011; Grey & Jackson 2012; Reisinger *et al.* 2017). Plasticity in anti-predator behavior (Hazlett *et al.* 2003; Reisinger *et al.* 2017) and habitat choice (Grey & Jackson 2012; Stroud *et al.* 2019) have also been linked to invasion success. Greater behavioral plasticity could also help invasive species adjust to other environmental changes, such as climate change, providing a competitive advantage to invasive species over native species (Sol *et al.* 2002; Wright 2010; Engel *et al.* 2011; Amiel *et al.* 2016; Beever *et al.* 2017). Few studies have empirically measured differences in behavioral plasticity among invasive and native animal species in response to temperature changes even though such differences may have important implications for the impacts of climate change (Engel *et al.* 2011).

Here we examined whether behavioral plasticity in response to warming varies between introduced and native dung beetles in the genus *Onthophagus* living in the eastern USA. Specifically, we measured changes in reproductive behaviors in response to increased temperatures in *O. taurus*, an introduced species, and *O. hecate*, a native species. To reproduce, *Onthophagus* spp. dig tunnels, bury dung, and use the dung to create a brood ball that contains a single egg (Halfter & Edmonds 1983). Offspring develop within the brood ball from egg to adulthood, feeding exclusively on dung from the brood ball.

Given their burrowing behavior, *Onthophagus* dung beetles are an interesting study system for investigating behavioral plasticity in response to climate change. Burrowing animals may be able to alter den or nest depth to select underground thermal microhabitats that are cooler and less variable than surface temperatures, and this plasticity in burrowing behavior could play a large role in buffering animals from climate change (Telemeco *et al.* 2009; Snell-Rood *et al.* 2016; Telemeco *et al.* 2016). Burrowing behavior is widespread across a variety of taxa, but measuring belowground processes is often difficult. However, dung beetles offer an opportunity to measure nest site selection and have been successfully used in previous research on responses to climate change (Snell Rood *et al.* 2016; Macagno *et al.* 2018).

Female *Onthophagus* vary the number, size, and burial depth of brood balls in response to environmental cues, which in turn, determine the developmental environment for their offspring (Hunt & Simmons 2003; Moczek & Emlen 2002; Macagno *et al.* 2016; Snell-Rood *et al.* 2016). Brood ball number and size reflect reproductive effort. Brood ball size influences body size of offspring upon eclosion, which can affect fitness since larger females have higher fecundity and larger males have a competitive advantage (Hunt & Simmons 2000). Brood ball burial depth determines developmental temperatures experienced by offspring (Snell-Rood *et al.* 2016). Offspring developing in warmer temperatures emerge faster, resulting in smaller body size and lower survival (Macagno *et al.* 2018; Pettersen *et al.* 2019). Plasticity of reproductive behaviors thus affects fitness proxies of dung beetles, such as offspring number and survival (Hunt & Simmons 2000; Hunt & Simmons 2002; Hunt & Simmons 2003).

During the 20th century, *O. taurus* was introduced multiple times into much of the USA. *O. taurus* was first recorded in 1974 on cattle pastures in northwestern Florida, central and southwestern Georgia, and southeastern Alabama (Fincher & Woodruff 1975). *O. taurus* is thought to have hitchhiked to the Florida panhandle in the dung of cattle transported overseas by farmers or the US Military (Hoebeke & Beuchke 1997). The species established and began to expand northwards into much of the southeastern USA. In the 1980s, *O. taurus* was intentionally introduced by the US Department of Agriculture onto cattle pastures in California, Texas, and Georgia to decrease dung build-up; at the same time, the New Jersey Department of Agriculture also released beetles into pastures in the northern United States. By the late 1990s, the range of *O. taurus* in the USA spread as far west as Texas and north to Maryland with some established populations in California (Hoebeke & Beuchke 1997). Currently, *O. taurus* occurs throughout much of the eastern USA and southward into the Caribbean (Floate *et al.* 2017). Environmental niche models indicate that *O. taurus* may continue to expand into most of the USA, Mexico, and the Caribbean (Floate *et al.* 2017).

Though the effects of the *O. taurus* introduction have not been directly studied, previous research suggests the species may impact communities of native dung beetles. In some areas, high numbers of introduced dung beetles have been recorded to the extent that these non-native beetles are dominating communities (Hoebeke & Beuchke 1997). *O. taurus* likely competes with native dung beetles by removing and burying dung and by physically excluding males of other species from dung or space below dung pats. A native species, *O. hecate*, ranges from Florida to southern Canada. *Onothophagus taurus* and *O. hecate* are similar in seasonal and diel activity patterns, dung use, and breeding behaviors and are often collected within the same dung pats. The two species are small-bodied dung beetles, though *O. taurus* are heavier than *O. hecate* (mean mass *O. taurus* = 0.07g; *O. hecate* = 0.03g). Comparing behavioral plasticity and responses to warming between these species is thus ecologically relevant because the two species overlap spatially and temporally and individuals likely interact within pasture habitats.

We exposed small breeding colonies of *O. taurus* and *O. hecate* to different warming treatments and recorded reproductive behaviors to address the following questions: (1) Do introduced *O. taurus* and native *O. hecate* species show differences in reproductive behavior? (2) Does the degree of behavioral plasticity in response to warming temperatures vary between the introduced species *O. taurus* and the native species *O. hecate*?, and (3) How do these behavioral responses of adults to warming affect fitness? We predicted that the introduced species *O. taurus* would have more successful reproductive phenotypes and demonstrate greater plasticity in reproductive behaviors than the native species *O. hecate*, resulting in greater survival of *O. taurus* offspring in comparison to *O. hecate* offspring.

Methods

Field Collection

From May-August 2018, we collected adult *O. taurus* and *O. hecate* beetles from two sites in their current range: Powell, TN (36°03'25.8"N, 84°04'19.8"W) and Kings Mountain, NC (35°15'53.7"N, 81°21'18.6"W) using baited pit-fall traps. After collection, we transported all field-caught beetles to Knoxville, TN in plastic containers containing damp paper towels. Once in the laboratory, we kept beetles in colonies of 50-75 individuals within 3.8 L jars held at laboratory room temperature (25°C). We fed colonies autoclaved cow dung every three days, making sure to provide enough dung so that the food source was not fully depleted between feedings. We kept all beetles in these large colonies for at least seven days prior to temperature trials. We collected cow dung from an organic dairy farm in Knoxville, TN between February – September 2018. We autoclaved and then froze the dung in clean plastic containers. We then removed and thawed the dung to room temperature prior to feeding colonies throughout the experiment.

Behavioral plasticity trials

To investigate behavioral plasticity in these species, we used a repeated measures design to quantify reproductive behaviors of single species colonies (n=18 colonies per species) in response to changes in their thermal environment. Each colony had five beetles (two males and three females). We used beetles in small colonies to measure behavioral plasticity rather than individual mated pairs (one male and one female) because *O. hecate* rarely reproduced when housed as mated pairs. We used only major males – those with fully developed horns – since reproductive behaviors vary between major and minor males (Hunt & Simmons, 2000). Before the start of the trials, we weighed each beetle to control for the effects of body size on brood ball size. We placed all experimental beetle colonies in plastic 2 L rectangular containers (13.5 x 10.2 x 28.2 cm) filled to a depth of 24 cm with a 4:1 mixture of topsoil:sand. We mixed the soil with water to create a standardized moisture level across colonies, and we covered the container with aluminum mesh to prevent escape of the beetles.

We used 43W halogen light bulbs to heat experimental colonies because the bulbs produce a gradient of warming in the soil, mimicking soil gradients produced by the sun. The distance of the bulb to the soil surface determined the degree of warming at the surface of the soil and the steepness of the thermal gradient. We had three different temperature treatments: control (25°C surface temperature, no temperature gradient), low warming (30°C surface temperature), and high warming (39°C surface temperature). While this set-up allowed us to produce soil gradients similar to field settings, we were unable to simultaneously measure the effect of increased temperature variation on reproductive behavior; the temperature gradients produced by our warming treatments were thus consistent throughout the trial and did not fluctuate. The control treatment temperature is slightly lower than field averages over the breeding season but has led to high reproductive output in laboratory conditions for these species. The low warming treatment mimics average ambient high temperatures in the field throughout the summer breeding season (29.5°C). The high warming treatment reflects temperatures commonly reached during heat waves at our collection sites, and such heat waves are predicted to become more common due to climate change (IPCC 2014). To record soil temperatures experienced by our colonies in all three treatments, we buried data loggers (Onset Hobo Pendant Temperature/Light Logger) at the surface, middle, and bottom of containers that were filled with soil but did not have beetles three times during the experimental period. To maintain warming conditions throughout the trial length, we kept bulbs on during the entire trial, such that all colonies experienced consistent light (no dark periods).

We held each experimental colony at each of the three temperature treatments in random order for ten days (30 days total/colony). We fed colonies 130 ± 5g of autoclaved cow dung on days one, three, and six of each ten-day trial. On day ten, we searched through the soil in three cm sections (0-3cm, 3-6 cm, 6-9cm, 9-12 cm, 12-15cm, 15-18cm, 18-21cm, 21-24cm) and removed any brood balls produced by the experimental colony. For each brood ball we recorded mass and soil section where it was buried (i.e. burial depth). Before starting the next temperature treatment, we replaced any dead beetles so that each colony was always composed of two major males and three females at the beginning of each treatment. We did not observe differences in adult mortality across treatments or between species. The mass of each beetle was recorded at the start of each new treatment. This repeated measures design, where each colony experienced all three temperature treatments in a randomized order, allowed us to quantify behavioral plasticity using reaction norms (Gotthard & Nylin 1995; West-Eberhard 2003; Dingemanse & Wolf 2013). Behavioral reaction norms describe each colonies' behavioral response to the different thermal environments; the slope of the line describes the level of behavioral plasticity, with a greater slope reflecting greater plasticity of the behavior (Dingemanse & Wolf 2013). We randomized the order in which colonies experienced the three temperature treatments because we are interested in mean-level differences of colonies among treatments, and pre-exposure to one treatment may influence the response to the next treatment (Dochtermann 2010; Bell 2013). For example, if beetles were trialed in a particular treatment order (e.g. control, low warming, then high warming treatment), it is possible the previous environment could prime the beetles to respond in a particular way. By randomizing the order of trials, we are able to examine differences among treatments regardless the environment the beetles experienced previously (Bell 2013).

Offspring survival

To determine the effect of behavioral plasticity on offspring size and survival, we reared all brood balls at the average temperature of the soil layer in which they were buried. To approximate this burial temperature, we binned the container into thirds based on depth, including the top (0-9cm deep), middle (9-15 cm), and bottom (15-24 cm) of the container. We used data from the data loggers to quantify temperatures for each treatment and depth. We then used incubators to rear offspring in temperatures that reflected the brood ball location and, thus, the soil temperature in the containers. For the control treatment, we reared offspring in brood balls found in all three sections of the container at 25°C, which reflects the lack of thermal gradient in these containers. For the low warming treatment, we reared offspring in brood balls found in the top third at 29°C, those in the middle third at 26°C, and those in the bottom third at 25°C. For the high warming treatment, we reared offspring in brood balls found in the top third at 33°C, those in the middle third at 27°C, and those in the bottom third at 25°C. For rearing offspring, we placed each brood ball in an individual, sealed 75ml plastic cup with holes punched in the lid. We placed each brood ball at the bottom of the plastic cup and packed soil around the brood ball up to the lip of the cup. Throughout development, we added water to the cups using a spray bottle to maintain soil moisture.

We checked brood balls for beetle emergence starting four weeks after the end of the experimental trial. If beetles had not emerged after six weeks, we determined if the brood ball had an egg chamber (hollow portion of the brood ball). If the brood ball did not have an egg chamber, we considered the brood ball empty and we removed it from data analysis since it could be a food cache that does not reflect parental investment or reproductive behavior. If the brood ball had an egg chamber, we categorized it as a mortality event during development.

Statistical analyses

To examine differences in behavioral plasticity between *O. taurus* and *O. hecate*, we used linear mixed-effects models in R Version 3.4.3 (R Core Team 2017) with the response variables of burial depth, number, or size of brood ball and the fixed effects of temperature treatment, species, and the interaction of the two variables. Including the fixed effect of temperature treatment tests whether beetles demonstrate behavioral plasticity, or a change in behavioral phenotype in different thermal environments. The fixed effect of species tests whether behavioral phenotype varies between species across treatments. The interaction term indicates whether the species respond differently to changes in temperature and thus tests for differences in behavioral plasticity between the two species. In all models, we included two random effects. First, the random effect of experiment start date controlled for blocking effects such as slight differences in time since field capture of beetles, soil moisture, and seasonality. The random effect of colony ID accounted for the non-independence of the colony responses at the three temperature treatments. To determine the best-fit models we used maximum likelihood ratio comparisons.

To determine the effect of temperature treatment on offspring survival, we used generalized linear mixed-effects models where offspring survival (binomial distribution) is predicted by the fixed effects of developmental temperature (incubator temperature), species, and the interaction of the two variables. The models included the same two random effects as described above: experiment start date and colony ID. We used maximum likelihood comparisons to determine which model best predicted survival and body size.

Results

Our goal was to determine whether the introduced species, *O. taurus*, and the native species, *O. hecate* vary in reproductive behavior and behavioral plasticity in response to experimental warming in the United States. We found significant differences in behavior, but not behavioral plasticity, between the species. At the end of our trials, we had 35 colonies (18 *O. taurus*, 17 *O. hecate*) for analyses. Twenty-nine colonies (17 *O. taurus*, 12 *O. hecate*) bred in all three temperature treatments. The remaining six colonies (5 *O. hecate*, 1 *O. taurus*) produced brood balls in two of the three temperature treatments. The 35 colonies produced a total of 1,217 brood balls (761 *O. taurus*, 456 *O. hecate*).

Both species showed plasticity in brood ball burial depth in response to temperature changes, but the degree of behavioral plasticity did not significantly vary between the species (i.e. the slopes of the reaction norms were similar between species) (Figure 5-1, all figures are included in the appendix). The linear mixed-effects model that best predicted brood ball burial depth included temperature treatment (F=5.74, df=2, $p = 0.008$) and species (F=35.86, df=1, $p < 0.0001$) as predictors, but not their interaction (F=0.48, df=2, p=0.61), suggesting the species did not respond differently to the warming treatments. Both species demonstrated behavioral plasticity by burying brood balls deeper in the high warming treatment (mean depths: *O. taurus* 11.1 cm, *O. hecate* 5.8 cm) than in the control (mean depths: *O. taurus* 9.6 cm, *O. hecate* 5.3 cm) or low warming treatments (mean depths: *O. taurus* 9.4 cm, *O. hecate* 5.2 cm) (Figure 5-1). Across all temperature treatments, *O. taurus* buried brood balls deeper in the soil than *O. hecate* (Figure 5-1).

For brood ball size, the best fit model included species (F=208.75, df =1, p<0.0001) as a predictor, but not treatment (F=0.94, df = 2, p=0.39) (Figure 5-2). Thus, neither species demonstrated plasticity of brood ball size in response to warming, which is indicated by the horizontal reaction norms for each species. Across all treatments, *O. taurus* made larger brood balls than *O. hecate* (mean brood ball size: *O. taurus* 3.0 g, *O. hecate* 1.6 g).

For brood ball number, the best fit model included the fixed effects of temperature treatment, species, and the interaction of the two variables (F=12.18, df=2, p = 0.047), suggesting that the species are adjusting the number of brood balls in response to warming in different ways (Figure 5-3). *Onthophagus taurus* decreased brood ball production in response to warming (negative slope of the reaction norm), while *O. hecate* increased the number of brood balls in response to warming (positive slope of the reaction norm) (Figure 5-3). Even with these contrasting responses to temperature, *O. taurus* produced more brood balls than *O. hecate* in all three warming treatments (Figure 5-3).

We assessed if differences in reproductive behavior or degree of behavioral plasticity in brood ball burial depth affects fitness by modifying the temperatures offspring experience during development. To do this, we examined how developmental temperatures at the depth that the adults buried brood balls influenced the survival of offspring. The best-fit model describing offspring survival included the fixed effects of species ($\chi 2=190.76$, df=4, p = 0.0003) and developmental temperature ($\chi 2=13.26$, df=1,p < 0.0001) but not their interaction ($\chi 2=6.62$, df=4, p=0.16), indicating that offspring survival of the two species did not respond differently to developmental temperatures (Figure 5-4). Both species survived at a significantly lower rate when brood balls were reared at 33°C (Figure 5-4). However, less than a quarter of *O. taurus* offspring (23%) were exposed to this temperature during development because adult *O. taurus* beetles experiencing the high warming treatment buried their brood balls and, thus, offspring, in either the middle (46%) or bottom (31%) third of the soil where developmental temperatures are cooler. In contrast, a high proportion of *O. hecate* offspring (77%) were reared at 33°C. Despite plasticity in burial depth, *O. hecate* adults did not bury their offspring far enough down to avoid the warmest rearing temperature: only 19% of brood balls were buried in the middle third of the bucket and only 3% were buried in the bottom third. Furthermore, a higher proportion of *O. taurus* offspring survived in each developmental temperatures compared with *O. hecate* offspring (Figure 5-4). Thus, we found that *O. taurus* had higher survival in all temperatures relative to *O. hecate*, and, because of burial depth of brood balls, fewer *O. taurus* offspring were exposed to the stressful developmental temperature of 33°C compared with *O. hecate* offspring.

Discussion

In order to investigate how climate change impacts biological invasions, our goal was to determine whether reproductive behavior and the degree of behavioral plasticity varied between an introduced and native species and how behavioral differences affect offspring survival. We found that the introduced *O. taurus* outperforms the native *O. hecate* based on reproductive traits because *O. taurus* produced more brood balls (Figure 5-3), larger brood balls (Figure 5-2), and buried brood balls deeper than *O. hecate* (Figure 5-1). A greater proportion of *O. taurus* offspring survived across all developmental temperatures compared to *O. hecate*.

The differences in brood ball mass and burial depth are likely driven by differences in body size; *O. taurus* adults are approximately double the size of *O. hecate* adults (mean mass *O. taurus* = 0.07g; *O. hecate* = 0.03g). Brood ball mass is proportional to body size across dung beetle species, and larger dung beetles have been shown to dig deeper tunnels (Gregory *et al.* 2015). The greater number of brood balls produced by the introduced *O. taurus* compared to the native *O. hecate* is likely due to faster dung removal from the pat. Previous research has suggested that rapid dung sequestration is an important trait for the establishment and competitive success of introduced dung beetles, which allows small tunneling beetles, like *O. taurus,* to outcompete native congeners as well as large rolling dung beetles (Ridsdill-Smith 1993; Young 2007). The differences in behavior between *O. taurus* and *O. hecate* that we observed are important because the greater number of brood balls and higher offspring survival are likely drivers in the successful establishment and spread of *O. taurus*.

Interestingly, the two species did not vary in the degree of plasticity of reproductive traits in response to warming; in terms of brood ball depth and number, both species demonstrated the same magnitude of plasticity in warmer conditions. Both species also showed no change in brood ball size in response to temperature changes. This result is consistent with previous research demonstrating that the size of *O. taurus* brood balls did not change in experimental warming chambers (Holley & Andrew 2020). Other dung beetle species show the same consistency in brood ball size despite temperature changes, including the tunneling dung beetle *Euoniticellus fulvus* (Holley & Andrew 2019b) and a rolling dung beetle species, *Sisyphus rubrus* (Holley & Andrew 2019a). Thus, it appears there may be strong selection for a particular brood ball size for each species despite environmental fluctuations.

Even though behavioral plasticity did not vary between species, *O. taurus* offspring survival was less impacted by warming than *O. hecate* offspring. This variation in survival is driven by differences in brood ball placement by parents that affect developmental temperatures experienced by offspring (Figure 5-1, Macagno *et al.* 2018). Digging tunnels is energetically costly, but brood balls buried deeper underground are better protected from extreme surface temperatures and environmental fluctuations. Across all temperature treatments, *O. taurus* buried their brood balls deeper than *O. hecate*, likely because *O. taurus* beetles are larger (Gregory *et al.* 2015). Though both species adjusted brood ball burial depth across temperature treatments, *O. hecate* did not bury brood balls deep enough in the soil to protect developing offspring from warm temperatures. Thus, in the high warming treatment, the majority of *O. hecate* brood balls were placed in the top third of the breeding container and thus developed at 33°C, leading to high mortality. In contrast, adjustments in burial depth by *O. taurus* were sufficient to move brood balls away from the surface so that more than three quarters of *O. taurus* brood balls were placed in the middle or bottom thirds in the high warming treatments and thus developed at temperatures at or below 27°C. Thus, even though the degree of behavioral plasticity did not vary between the species, differences in their reproductive behaviors had a significant impact on offspring survival.

There are several possible reasons why we did not detect differences in behavioral plasticity between *O. taurus* and *O. hecate* as we had predicted. First, developmental temperatures likely exert strong selection on both species. Warmer temperatures can be stressful on offspring, leading to faster development, smaller body size, and lower survivorship (Figure 5-4) (Macagno *et al.* 2018). Behavioral adjustments by adult beetles can thus protect offspring from stressful developmental conditions. *O. taurus* and *O. hecate* responded to warming by altering brood ball burial depth in the same direction and magnitude. This suggests that both species experience selection to maintain plasticity of burial depth to keep offspring in optimal developmental conditions.

Second, phenotypic plasticity can be costly (Dewitt *et al.* 1998). There are energetic costs to maintaining the sensory and regulatory mechanisms of behavioral plasticity (Dewitt *et al.* 1998; Chevin *et al.* 2010). For some traits, we may thus see reduced selection for behavioral plasticity, especially if one reproductive strategy is successful across different environments. For brood ball size, it is likely advantageous for adult beetles to produce large brood balls across many environments since the dung contained in the brood ball is the offspring's sole source of nutrition.

Third, *O. taurus* may have experienced genetic assimilation following introduction. In such cases, plastic traits become genetically fixed, which is often followed by a loss of plasticity (Baldwin 1896; West-Eberhard 1989; Price *et al.* 2003). Specifically, as an invasive species adapts to its new environment, specialized traits via local adaptation may replace phenotypic plasticity (Yeh & Price 2002; Price *et al.* 2003; Wright 2010); the result would be that the invasive species would then have similar levels of plasticity as a locally adapted native. In most of the southeastern United States, *O. taurus* has been established since the 1980s (Hoebeke & Beuchke 1997), so it is possible that populations have local adaptations that led to a degree of plasticity that matches native species.

Overall, warming had a greater negative effect on offspring survival of the native species, *O. hecate*, than the introduced species, *O. taurus*. This result indicates that warming could lead to unchanged or even increased populations of *O. taurus* and concomitant decreases in *O. hecate* populations. Our results suggest that warming could increase the spread and impact of *O. taurus* because its behavior can better mitigate the negative impacts of climate change. Furthermore, our results indicate that *O. hecate* may need to respond to warming temperatures through mechanisms beyond behavioral shifts since adjustments in burial depth were not sufficient to protect offspring from warming. Unlike *O. taurus*, *O. hecate* are commonly found in forests as well as pasture and emerge from overwintering earlier than *O. taurus* in spring. This suggests selection may lead to habitat shifts of *O. hecate* into cooler, shaded areas from warming pastures, or adjustments to the timing of the breeding season that would reduce competition with *O. taurus*. Otherwise, there may be population declines in the native species.

O. taurus may have widespread effects on dung beetle communities beyond competition with *O. hecate.* In large numbers, *O. taurus* can outcompete even large-bodied dung beetles through rapid dung sequestration (Ridsdill-Smith & Edwards 2011), which could threaten slow-breeding tunneling dung beetles and rolling dung beetle, especially those also impacted by climate change (Holley & Andrew 2019). The loss of these large-bodied beetles negatively affects ecosystem functions provided by dung beetle communities, such as nutrient cycling, fly pest removal, and seed dispersal (Dangles *et al.* 2012). In conclusion, this study indicates that variation in behavior between invasive and native species, not necessarily plasticity, is one mechanism through which climate change may exacerbate negative impacts of biological invasions.

Acknowledgements

We thank M.J. Fleming, A. Wilson Carter, and A. Padukone for help with field work, and S. Riechert and S. Proffitt for allowing us to collect beetles on their property. Thanks to M.J. Fleming and K. Hannahs for help with beetle husbandry, behavioral plasticity trials, and brood ball monitoring. M.J. Fleming assisted with the artwork for the graphical abstract. This research was funded by a Sigma Xi Grant in Aid of Research to MAM, the US National Science Foundation under grant no. IOS-1930829 to KSS, and funds from the University of Tennessee.

Appendix

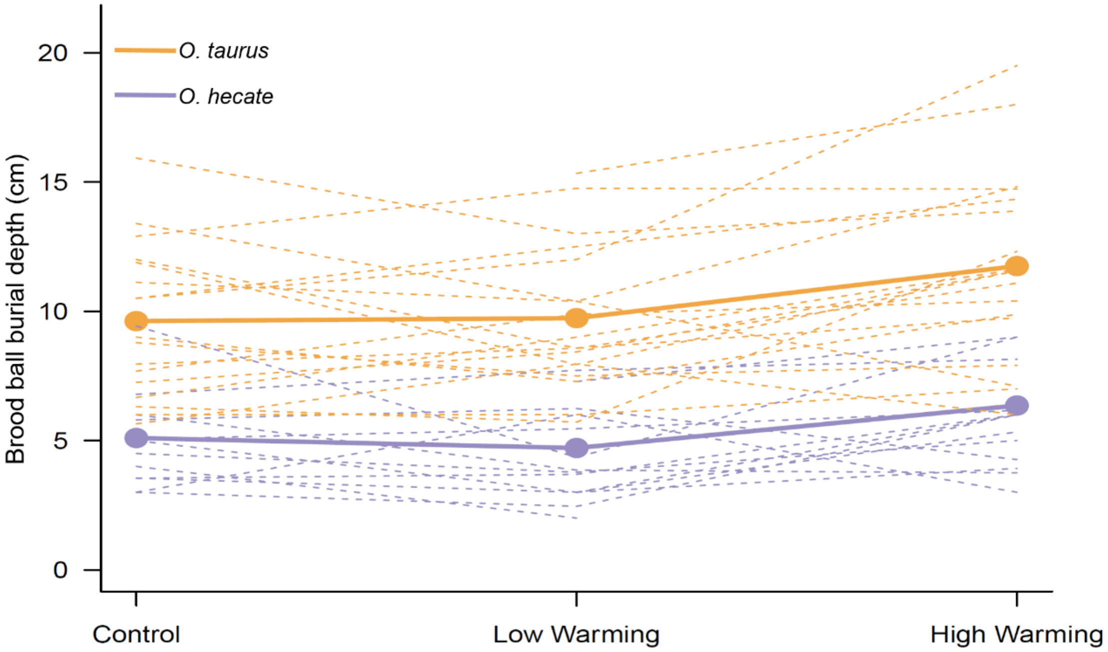


Figure 5-1. Species did not differ in the degree of behavioral plasticity of brood ball burial depth in response to warming. Each dashed line represents a single colony's reaction norm (n=35), and the slope of the reaction norm shows the degree of behavioral plasticity. *O. taurus* colonies (n=18) are indicated by orange lines (n=18) and *O. hecate* colonies (n=17) are indicated by purple lines. The points and solid lines represent mean colony responses of the two species. Reaction norms that do not span all three treatments are from colonies that only reproduced in two of the three treatments.

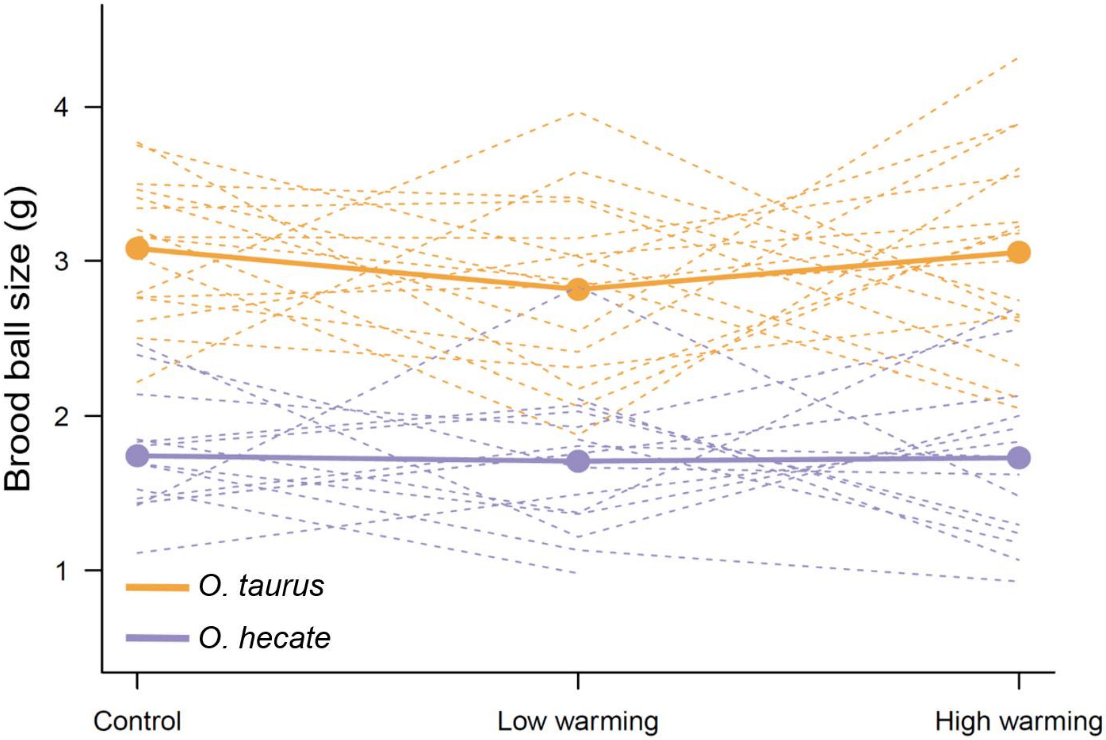


Figure 5-2. Neither species demonstrated behavioral plasticity of brood ball size in response to warming. Each dashed line represents a single colony's reaction norm (n=35), and the slope of the reaction norm shows the degree of behavioral plasticity. *O. taurus* colonies (n=18) are indicated by orange lines and *O. hecate* colonies (n=17) are indicated by purple lines. The points and solid lines represent mean colony responses of the two species. Reaction norms that do not span all three treatments are from colonies that only reproduced in two of the three treatments.

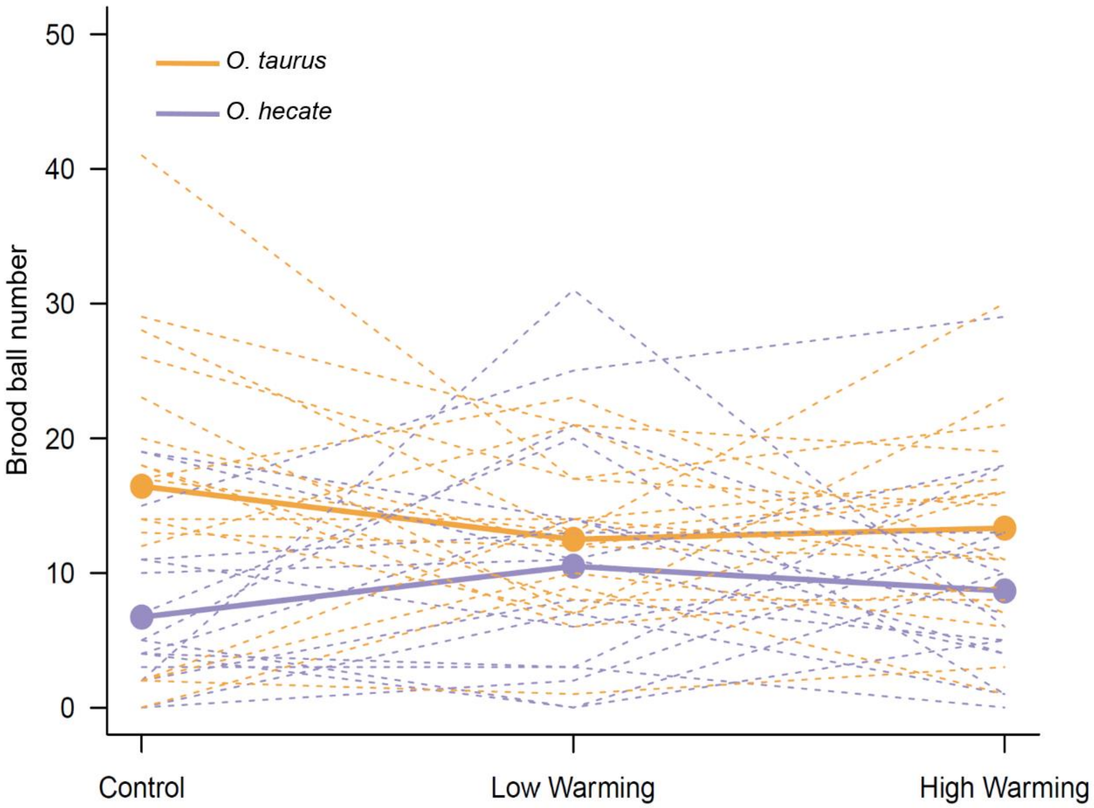


Figure 5-3. Species did not differ in the magnitude of behavioral plasticity of brood ball number in response to warming, but differed in the direction of behavioral response. Each dashed line represents a single colony's reaction norm (n=35), and the slope of the reaction norm shows the degree of behavioral plasticity. *O. taurus* colonies (n=18) are indicated by orange lines and *O. hecate* colonies (n=17) are indicated by purple lines. The points and solid lines represent mean colony response of the two species. Reaction norms that do not span all three treatments are from colonies that only reproduced in two of the three treatments.

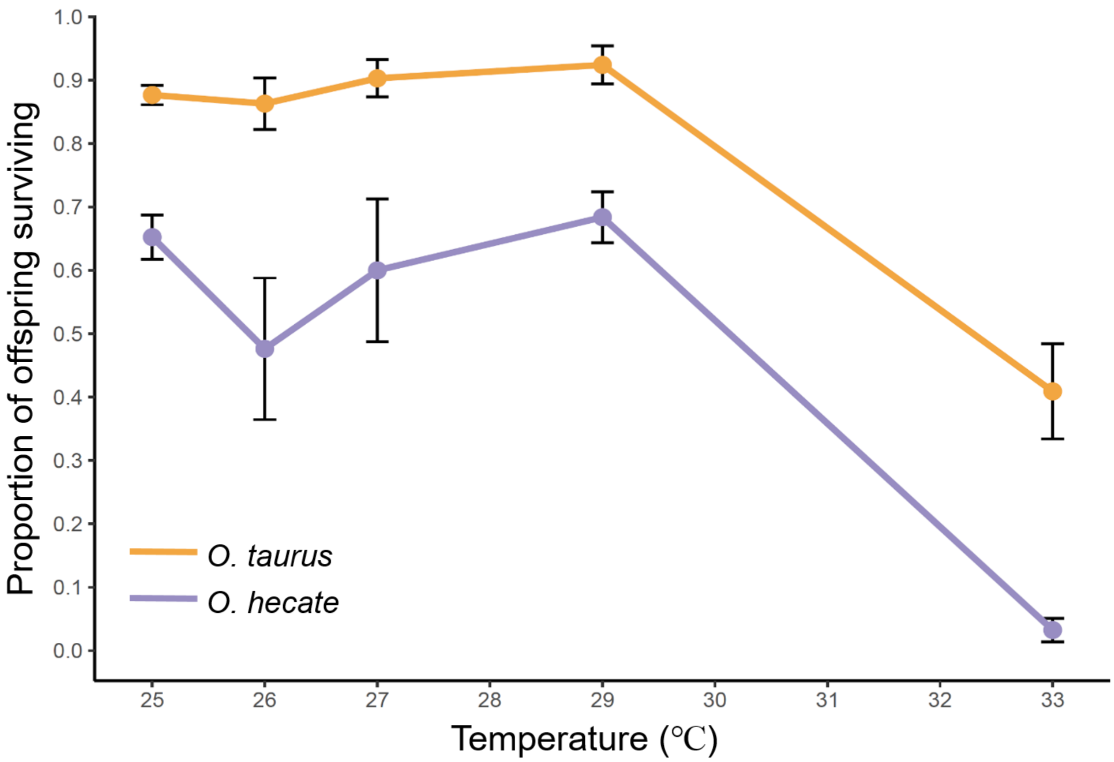


Figure 5-4. Proportion of offspring surviving was lower for the native *O. hecate* in every developmental temperature compared to the introduced *O. taurus*. *O. taurus* and *O. hecate* are shown in orange and purple lines, respectively. Points and error bars represents the mean and standard error of the proportion of offspring surviving per species at each developmental temperature.

CONCLUSION

Climate change is causing many organisms to experience temperatures outside of their evolved thermal breadth (Seebacher *et al.* 2014; Gunderson & Stillman 2015). Species vary in their response to climate change, with some organisms benefitting from warming and expanding their range while others have faced population declines and local extinctions (Bellard *et al.* 2012). Understanding why the effects of warming vary widely across species is crucial because these idiosyncratic responses drive changes in communities. Changes to community structure have important consequences, such as disrupting biotic interactions, affecting ecosystem services, and threatening human health (Körner 2004; Pecl *et al.* 2017). Traits, such as physiological tolerance, behavioral thermoregulation, dispersal ability, and feeding habits, directly influence how organisms respond to climate via range shifts, phenotypic plasticity, and/or evolutionary adaptation. With a clearer understanding of how traits influence magnitude and type of response to warming, we increase our ability to predict which species and which communities are most vulnerable to climate change.

My first chapter investigates the role of species thermal physiology in determining the rate and magnitude of range shifts in montane regions in order to explain some of the widespread variation in species range shifts (Lenoir et al. 2010; Harris et al. 2012; Brusca et al. 2013). We expected that species living at high elevations experience increased seasonal and diurnal temperature variation, which selects for broader thermal tolerance (Janzen 1967; Sheldon *et al.* 2018). We predicted that high elevation species are thus thermal generalist that do not need to shift their range as far in order to track their preferred thermal niche; therefore, we expected greatest upslope range shifts for species living in low elevations. To test this, we performed a meta-analysis of range shifts of 987 species living along twenty mountain transects across the globe. We found that, as predicted, low elevation species have moved upslope farther than high elevation species. This result suggests that thermal physiology is an important trait to consider when examining how species will respond to climate; species with broad thermal physiologies will likely show delayed and/or reduced responses to warming.

I then investigated how climate change may influence biological invasions because many common traits of invaders also promote persistence in the face of warming. This is crucial because most research to date has focused on either climate change or biological invasions in isolation even though they can interact to drive biodiversity declines (Engel *et al.* 2011; Oliver *et al.* 2016). Phenotypic plasticity is one such trait thought to increase organisms' ability to invade new environments and adjust to warming (Baldwin 1896; Agrawal 2001; Sol *et al.* 2002; Richards *et al.* 2006; Wright 2010; Davidson *et al.* 2011; Engel *et al.* 2011; Amiel *et al.* 2016). Because phenotypic plasticity plays a role in biological invasions, it is likely that invasive species are more plastic than native species, which are more likely to demonstrate local adaptation. Few studies, though, have empirically measured differences in phenotypic plasticity between invasive and native species in response to temperature changes (Davidson *et al.* 2011; Engel *et al.* 2011).

Working with *Onthophagus* dung beetles, I examined whether the introduced *O. taurus* shows elevated levels of phenotypic plasticity compared to the native *O. hecate.* Before beginning empirical experiments, I performed a census of dung beetles communities in the Great Smoky Mountains National Park (chapter three). Dung beetle communities have been inventoried on pasture lands in the Southeastern United States, documenting a number of introduced species at high densities, including *O. taurus* (Bertone *et al.* 2005; Kaufman & Wood 2012). My third chapter is one of the first to census forest habitat in the Appalachian Mountains. Through this survey, we determined that community makeup varied by elevation and habitat type, with the most diverse communities at low elevations. The abundance and species richness were lower in our study sites compared to pasture land likely due to the lack of large dung patties preferred for breeding. We also found that two introduced species had colonized sites in the Great Smoky Mountains National Park, *O. taurus* and *Aphodius fimetarius* indicating that these species can disperse from pastures into other habitats, which could threaten native diversity. We also collected *O. taurus* and *O. hecate* from the same site in the Great

Smoky Mountains National Park, indicating that these species do have niche overlap, both on agricultural and forested habitats.

After establishing the natural history of our study organisms and the wider dung beetle community, we then investigated whether phenotypic plasticity varied between the introduced *O. taurus* and *O. hecate*. In chapter four, we addressed whether *O. taurus* and *O. hecate* exhibit different degrees of physiological plasticity via acclimation than the native *O. hecate* and whether different populations separated by latitude showed variation in acclimation ability. We expected that *O. taurus* would show greater acclimation ability than *O. hecate.* We also expected beetles collected in Tennessee to have increased acclimation ability than beetles collected in Florida since they experience greater climate variation (Chown *et al.* 2004; Gunderson & Stillman 2015). Unexpectedly, we found that *O. taurus* collected in Florida were the only population able to acclimate to warm temperatures and adjust their tolerance to extreme heat. This result indicates that exposure to warm climates and heat waves may maintain acclimation ability and that some populations of *O. taurus* may be capable of range expansion or population growth in response to warming.

In chapter five, we investigated whether behavioral plasticity varies between introduced *O. taurus* and *O. hecate* and how behavioral traits influence offspring survival. We expected *O. taurus* to have greater behavioral plasticity than native *O. hecate*, resulting in increased survival of *O. taurus* offspring. We found that behavioral plasticity did not vary between the two species; both species adjusted their breeding behavior in response to warming. Behavioral traits did vary between species; *O. taurus* buried their brood balls deeper and made larger brood balls than *O. hecate* across all temperatures. These behavioral differences led to greater survival of *O. taurus* compared to *O. hecate* offspring, indicating that *O. taurus* is more suited for warming than *O. hecate*. Taken together, chapters four and five indicate that warming will negatively impact the native *O. hecate* more so than the introduced *O. taurus* due to lower acclimation ability and variation in behavioral traits. Therefore, as *O. taurus* continues to expand its range into South America and across the United States, the impact of these nonnative beetles is likely to increase, which could negatively impact the diversity and ecosystem services of native dung beetle communities. Future research needs to address whether the differences observed between native and introduced species in response to warming then influences interactions among members of communities; for example, little is known about the effect of *O. taurus* on native dung beetle communities even though any negative impacts will likely magnify under warming.

Overall, the research presented here demonstrates the value of mechanistically investigating the role of specific organismal traits when examining variation in how species respond to climate change. The results presented here highlight that traits influence both range shifts and plastic adjustments to warming; it is likely that similar patterns would be seen in evolutionary responses to warming. This research increases our understanding of the different traits that drive variation in climate change responses, which is a crucial step in protecting species diversity from declines due to warming.

REFERENCES

1. Agrawal, A.A. 2001. Phenotypic plasticity in the interactions and evolution of species. *Science* 294: 321–326.
2. Alexander, J.M., Chalmandrier, L., Lenoir, J., Burgess, T.I., Essl, F., Haider, C.K., … Pellissier, L. 2018. Lags in the response of mountain plant communities to climate change. *Global Change Biology* 24: 563-579.
3. Alexander, J.M., Diez, J.M., & Levine, J.M. 2015. Novel competitors shape species' responses to climate change. *Nature* 525: 515–518.
4. Allen, M.R., Dube, O.P. ,Solecki, W., Aragón-Durand, F., Cramer, W., Humphreys, S., Kainuma, M., Kala, J., Mahowald, N., Mulugetta, Y., Perez, R., Wairiu, M., & Zickfeld, K. 2018. Framing and Context. In: Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. *IPCC Special Report*.
5. Altermatt, F. 2010. Climatic warming increases voltinism in European butterflies and moths. *Proceedings of the Royal Society B Biological Sciences* 277: 1281-1287.
6. Amiel, J.J., Tingley, R., & Shine, R. 2011. Smart moves: effects of relative brain size on establishment success of invasive amphibians and reptiles. *PLoS One* 6: e18277.
7. Anderson, J.R., Merritt, R.W., & Loomis, E.C. 1984. The insect-free cattle dropping and its relationship to increased dung fouling of rangeland pastures. *Journal of Economic Entomology* 77: 133-141.
8. Anderson, J.T., Inouye, D.W., McKinney, A.M., Colautti, R.I. & Mitchell-Olds, T. 2012. Phenotypic plasticity and adaptive evolution contribute to advancing

flower phenology in response to climate change. *Proceedings of the Royal Society B* 279: 3843-3852.

9. Andresen, E. 2005. Effects of season and vegetation type on community organization of dung beetles in a tropical dry forest. *Biotropica* 37: 291–300.

10. Angelo, C.L. & Daehler, C.C. 2013. Upward expansion of fire-adapted grasses along a warming tropical elevation gradient. *Ecography* 36: 551-559.

11. Angert, A.L., Crozier, L.G., Rissler, L.J., Gilman, S.E., Tewksbury, J.J. & Chunco, A.J. 2011. Do species' traits predict recent shifts at expanding range edges? *Ecology Letters* 14: 677–689.

12. Årevall, J., Early, R., Estrada, A., Wennergren, U., & Eklöf, A.C. 2018. Conditions for successful range shifts under climate change: the role of species dispersal and landscape configuration. *Diversity and Distributions* 24: 1598-1611.

13. Badyaev, A.V. 1997. Avian life history variation along altitudinal gradients: an example with carduelin finches. *Oecologia* 111: 365-374.

14. Baldwin, J. M. 1896. A new factor in evolution. *American Naturalist* 30: 441–451: 536–553.

15. Barker, B.D., Horodysky, A.Z. & Kerstetter, D.W. 2018. Hot or not? Comparative behavioral thermoregulation, critical temperature regimes, and thermal tolerances of the invasive lionfish *Pterois* sp. versus native western North Atlantic reef fishes. *Biological Invasions* 20: 45-48.

16. Barry, R.G. 2008. *Mountain weather and climate, 3rd edn.* Cambridge University Press: New York, NY, USA.

17. Bears, H., Martin, K., & White, G. C. 2009. Breeding in high-elevation habitat results in shift to slower life-history strategy within a single species. *Journal of Animal Ecology* 78: 365-375.

18. Beever, E.A., Hall, L.E., Varner, J., Loosen, A.E., Dunham, J.B., Gahl, M.K., Smith, F.A., & Lawler, J.J. 2017. Behavioral flexibility as a mechanism for coping with climate change. *Frontiers in Ecology and the Environment* 15: 299-308.

19. Bell, A. 2013. Randomized or fixed order for studies of behavioral syndromes? *Behavioral Ecology* 24: 16-20.

20. Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., & Courchamp, F. 2012. Impacts of climate change on the future of biodiversity. *Ecology Letters* 15: 365-377.

21. Beniston, M., Diaz, H.F., & Bradley, R.S. 1997. Climatic change at high elevation sites: an overview. *Climatic Change* 36: 233–252.

22. Bertone, M., Green, J., Washburn, S., Poore, M., Sorenson, C., & Watson, D.W. 2005. Seasonal activity and species composition of dung beetles Coleoptera: Scarabaeidae and Geotrupidae inhabiting cattle pastures in North Carolina. *Annals of the Entomological Society of America* 98: 309–321.

23. Beynon, S.A., Wainwright, W.A., & Christie, M. 2015. The application of an ecosystem services framework to estimate the economic value of dung beetles to the U.K. cattle industry: Economic benefits of dung beetles. *Ecological Entomology* 40: 124–135.

24. Bezanson, G.A. & Floate, K.D. 2019. An updated checklist of the Coleoptera associated with livestock dung on pastures in America North of Mexico. *The Coleopterists Bulletin* 73: 655–683.

25. Borenstein, M., Hedges, J.P., Higgins, J.P.T, & Rothstein, H.R. 2009. *Introduction to Meta-Analysis*. John Wiley & Sons LTD: West Sussex, United Kingdom.

26. Bozinovic, F., Calosi, P., & Spicer, J.I. 2011. Physiological correlates of geographic range in animals. *Annual Review of Ecology, Evolution, and Systematics* 42: 155-179.

27. Bradley, B.A., Wilcove, D.S., & Oppenheimer, M. 2010. Climate change increases risk of plant invasion in the Eastern United States. *Biological Invasions* 12: 1855–1872.

28. Bradshaw, W.E. & Holzapfel, C.M. 2006. Evolutionary response to rapid climate change. *Science* 312: 1477-1478.

29. Brattstrom, B.H. 1968. Thermal acclimation in *Anuran* amphibians as a function of latitude and altitude. *Comparative Biochemistry and Physiology* 24: 93-111.

30. Brown, C.D. & Vellend, M. 2014. Non-climatic constraints on upper elevational plant range expansion under climate change. *Proceedings of the Royal Society B Biological Sciences* 281: 20141779.

31. Brusca, R.C., Wiens, J.F., Meyer, W.M., Eble, J., Franklin, K., Overpeck, J.T., & Moore, W. 2013. Dramatic response to climate change in the Southwest: Robert Whittaker's 1963 Arizona Mountain plant transect revisited. *Ecology and Evolution* 3: 3307–3319.

32. Buckley, Y.M., Bolker, B.M, & Rees, M. 2007. Disturbance, invasion and re-invasion: managing the weed-shaped hole in disturbed ecosystems. *Ecology Letters* 10: 809–817.

33. Busch, S., Kirillin, G. & Mehner, T. 2012. Plasticity in habitat use determines metabolic response of fish to global warming in stratified lakes. *Oecologia* 170: 275–287.

34. Byrne, M., Millar, M.A., Coates, D.J., Macdonald, B.M., McArthur, S.M., Zhou, M., & van Leeuwen, S. 2017. Refining expectations for environmental

characteristics of refugia: two ranges of differing elevation and topographical complexity are mesic refugia in an arid landscape. *Journal of Biogeography* 44: 2539-2550.

35. Cahill, A.E, Aiello-Lammens, M.E., Fisher-Reid, M.C., Hua, X., Karanewsky, C.J., Ryu, H.Y., Sbeglia, G.C., Spagnolo, F., Waldron, J.B., Warsi, O., and Wiens, J.J. 2013. How does climate change cause extinction? *Proceedings of the Royal Society B* 280: 20121890.

36. Chen, I.-C. 2011. Impacts of climate warming on range shifts with an emphasis on tropical mountains. [PhD Thesis], University of York, U.K.

37. Chen, I.-C., Hill, J.K., Ohlemüller, R., Roy, D.B., & Thomas, C.D. 2011. Rapid range shifts of species associated with high levels of climate warming. *Science* 333: 1024–1026.

38. Chevin, L.-M., Lande, R., & Mace, G. M. 2010. Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biology* 8: e1000357.

39. Chown, S.L., Gaston, K.J. & Robinson, D. 2004. Macrophysiology: large-scale patterns in physiological traits and their ecological implications. *Functional Ecology* 18: 159–167.

40. Christensen, E.A.F., Norin, T., Tabak, I., van Deurs, M., & Behrens, J.W. 2021. Effects of temperature on physiological performance and behavioral thermoregulation in an invasive fish, the round goby. *Journal of Experimental Biology* 224: jeb237669.

41. Cleland, E.E., Allen, J.M., Crimmins, T.M., Dunne, J.A., Pau, S., Travers, S.E., Zavaleta, E.S., and Wolkovich, E.M. 2012. Phenological tracking enables positive species responses to climate change. *Ecology* 93: 1765-1771.

42. Colwell, R.K., Brehm, G., Cardelus, C.L., Gilman, A.C., & Longino, J.T. 2008. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science* 322: 258-261.

43. Crimmins, S.M., Dobrowski, S.Z., Greenberg, J.A., Abatzoglou, J.T., & Mynsberge, A. R. 2011. Changes in climatic water balance drive downhill shifts in plant species' optimum elevations. *Science* 331: 324–327.

44. Crozier, L & Dwyer, G. 2006. Combining population-dynamic and ecophysiological models to predict climate-induced insect range shift. *The American Naturalist* 167: 853-866.

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

46. Davidson, A.M., Jennions, M., & Nicotra, A.B. 2011. Do invasive species show higher phenotypic plasticity than native species, and, if so, is it adaptive? A meta-analysis. *Ecology Letters* 14: 419-431.

47. Davis, A.J., Jenkinson, L.S., Lawton, J.H., Shorrocks, B., & Wood, S. 1998. Making mistakes when predicting shifts in species range in response to global warming. *Nature* 391: 783–786.

48. Dewitt, T.J., Sih, A., & Wilson, D.S. 1998. Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution* 13: 77–81.

49. Diaz, H.F. & Bradley, R.S. 1997. Temperature variations during the last century at high elevation sites. *Climatic Change* 36: 253–279.

50. Diffenbaugh, N.S. & Field, C.B. 2013 Changes in ecologically critical terrestrial climate conditions. *Science* 341: 486–492.

51. Dingemanse, N. J. & Wolf, M. 2013. Between-individual differences in behavioral plasticity within populations: causes and consequences. *Animal Behavior* 85: 1031-1039.

52. Dobzhansky, T. 1950. Evolution in the tropics. *American Scientist* 38: 208-221.

53. Doctermann, N.A. 2010. Behavioral syndromes: carryover effects, false discovery rates, and a priori hypotheses. *Behavioral Ecology* 21: 437-439.

54. Dukes, J. S., & Mooney, H.A. 1999. Does global change increase the success of biological invaders? *Trends in Ecology & Evolution* 14: 135–139.

55. Elsen, P.R. & Tingley, M.W. 2015. Global mountain topography and the fate of montane species under climate change. *Nature Climate Change* 5: 772-776.

56. Elton, C.S. 1954. *The Ecology of Invasions by Animals and Plants.* Methuen: London, UK.

57. Engel, K., Tollrian, R., & Jeschke, J.M. 2011. Integrating biological invasions, climate change and phenotypic plasticity. *Communicative & Integrative Biology* 4: 247–250.

58. Ettinger, A. & HilleRisLambers, J. 2017. Competition and facilitation may lead to asymmetric range shift dynamics with climate change. *Global Change Biology* 23: 3921-2933.

59. Fahey, C., Angelini, C., & Flory, S.L. 2018. Grass invasion and drought interact to alter the diversity and structure of native plant communities. *Ecology* 99: 2692-2702.

60. Feeley, K., Martinez-Villa, J., Perez, T., Silva Duque, A., Triviño Gonzalez, D., & Duque, A. 2020. The thermal tolerances, distributions, and performances of tropical montane tree species. *Frontiers in Forests and Global Change* 3: 10.3389.

61. Felde, V.A., Kapfer, J., & Grytnes, J.-A. 2012. Upward shift in elevational plant species ranges in Sikkilsdalen, central Norway. *Ecography* 35: 922-932.

62. Fiene, J.G., Connior, M.B., Androw, R., Baldwin, B., & McKay, T. 2011. Surveys of Arkansas dung beetles Coleoptera: Scarabaeidae and Geotrupidae: phenologies, mass occurrences, state and distributional records. *American Midland Naturalist* 165: 319–337.

63. Fincher, G.T. 1981. The potential value of dung beetles in pasture ecosystems. *Journal of the Georgia Entomological Society* 16: 316–333.

64. Fincher, G.T. & Woodruff, R.E. 1975. A European dung beetle, *Onthophagus taurus* Schreber, new to the U.S. Coleoptera: Scarabaeidae. *The Coleopterists Bulletin* 29: 349–350.

65. Fincher, G.T., Steward, T.B., & Davis, R. 1970. Attraction of coprophagous beetles to feces of various animals. *The Journal of Parasitology* 56: 378–383.

66. Fincher, G.T. & Woodruff, R. E. 1975. A European dung beetle, *Onthophagus taurus* Schreber, new to the U.S. Coleoptera: Scarabaeidae. *Coleopterist Bulletin* 29: 349–350.

67. Fincher, G.T., Blume, R.R., Hunter, J.S., and Beerwinkle, K.R. 1986. Seasonal distribution and diel flight activity of dung-feeding scarabs in open and wooded pasture in east-central Texas. *Southwestern Entomologist* 10: 1–35.

68. Floate, K.D. & Gill, B.D. 1998. Seasonal activity of dung beetles Coleoptera: Scarabaeidae associated with cattle dung in southern Alberta and their geographic distribution in Canada. *Canadian Entomologist* 130: 131–151.

69. Floate, K.D., Watson, D.W., Weiss, R.M., & Olfert, O. 2017. Bioclimatic analyses for the distributions of *Onthophagus nuchicornis*, *Onthophagus taurus*,

and *Digitonthophagus gazella* Coleoptera: Scarabaeidae in North America. *The Canadian Entomologist* 149: 504–524.

70. Forero-Medina, G. 2012. Elevational range shifts driven by climate change in tropical mountains: Assessment and conservation opportunities. [PhD Thesis], Duke University, USA.

71. Forero-Medina, G., Terborgh, J., Socolar, S.J., & Pimm, S.L. 2011. Elevational ranges of birds on a tropical montane gradient lag behind warming temperatures. *PloS One* 6: e28535.

72. Freeman, B.G. & Freeman, A.M.C. 2014. Rapid upslope shifts in New Guinean birds illustrate strong distributional responses of tropical montane species to global warming. *Proceedings of the National Academy of Sciences of the USA* 111: 4490–4494.

73. Freeman, B.G., Lee-Yaw, J.A., Sunday, J.M., & Hargreaves, A.L. 2018. Expanding, shifting and shrinking: The impact of global warming on species' elevational distributions. *Global Ecology and Biogeography* 27: 1268–1276.

74. Frei, E., Bodin, J., & Walther, J.-R. 2010. Plant species' range shifts in mountainous areas—all uphill from here? *Botanical Helvetica* 120: 117–128.

75. Funk, J. L. 2008. Differences in plasticity between invasive and native plants from a low resource environment. *Journal of Ecology* 96: 1162–1173.

76. Gaston, K.J. & Chown, S.L. 1999. Elevation and climatic tolerance: a test using dung beetles. *Oikos* 86: 585-590.

77. Ghalambor, C.K., Huey, R.B., Martin, P.R., Tewksbury, J.J., & Wang, G. 2006. Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integrative and Comparative Biology* 46: 5–17.

78. Gibson-Reinemer, D.K. & Rahel, F.J. 2015. Inconsistent range shifts within species highlight idiosyncratic responses to climate warming. *PloS One* 10: e0132103.

79. Gibson-Reinemer, D.K., Sheldon, K.S., & Rahel, F.J. 2015. Climate change creates rapid species turnover in montane communities. *Ecology and Evolution* 5: 2340–2347.

80. Gienapp, P., Teplitsky, C., Alho, J.S., Mills, J.A., & J. Merilä. 2008. Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology* 17: 167-178.

81. Gilman, S.E., Urban, M.C., Tewksbury, J., Gilchrist, G.W., & Holt, R.D. 2010. A framework for community interactions under climate change. *Trends in Ecology & Evolution* 25: 325–331.

82. Gordon, R.D. 1983. Studies on the genus *Aphodius* of the United States and Canada Coleoptera: Scarabaeidae. VII. Food and habitat; distribution; key to eastern species. *Proceedings of the Entomological Society of Washington* 85: 633–652.

83. Gordon, R.D. & Skelley, P.E. 2007. A Monograph of the *Aphodiini* Inhabiting the United States and Canada Coleoptera: Scarabaeidae: Aphodiinae. American Entomological Institute: Gainesville, FL, USA.

84. Gotthard, K. & Nylin, S. 1995. Adaptive plasticity and plasticity: a selective review of plasticity in animal morphology and life history. *Oikos* 74: 3-17.

85. Gregory, N., Gómez, A., Oliveira, T.M.F de S., & Nichols, E. 2015. Big dung beetles dig deeper: trait-based consequences for faecal parasite transmission. *International Journal for Parasitology* 45: 101–105.

86. Grenyer, R., Orme, C.D.L., Jackson, S.F., Thomas, G.H., Davies, R.G., Davies, T.J., … Owens, I.P.F. 2006. Global distribution and conservation of rare and threatened vertebrates. *Nature* 444: 93-96.

87. Grey, J. & Jackson, M.C. 2012. 'Leaves and eats shoots': direct terrestrial feeding can supplement invasive red swamp crayfish in times of need. *PLoS One*, 7: e42575.

88. Gross, K., Pasinelli, G., & Kunc, H. 2010 Behavioral plasticity allows short-term adjustment to a novel environment. *American Naturalist* 176: 456–464.

89. Guarnieri, F.G. & Harpootlian, P.J. 2013. An illustrated key to the genus *Geotrupes* Latreille Coleoptera: Geotrupidae of Maryland including notes on *G. ulkei* Blanchard, a rare southern species recently discovered in Morgan County, West Virginia. *The Maryland Entomologist* 6: 6–13.

90. Gunderson, A.R. & Stillman, J.H. 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences* 282: 20150401.

91. Guo, F., Lenoir, J., & Bonebrake, T.C. 2018. Land-use change interacts with climate to determine elevational species redistribution. *Nature Communications* 9: 1315.

92. Halffter, G., and W. D. Edmonds. 1982. The Nesting Behavior of Dung Beetles Scarabaeinae. An ecological and Evolutive Approach. Instituto de Ecologica: Mexico City, Mexico.

93. Hanski, I. & Cambefort, Y. 1991. Dung Beetle Ecology. Princeton University Press: Princeton, USA.

94. Harris, J.B.C., Yong, D.L., Sheldon, F.H., Boyce, A.J., Eaton, J.A., Bernard, H., … Wei, D. 2012. Using diverse data sources to detect elevational range changes

of birds on Mount Kinabalu, Malaysian Borneo. *Raffles Bulletin of Zoology* 25: 197–247.

95. Hazlett, B.A., Burba, A., Gherardi, F., & Acquistapace, P. 2003. Invasive species of crayfish use a broader range of predation-risk cues than native species. *Biological Invasions* 5: 223-228.

96. Hellmann, J.J., Byers, J.E., Bierwagen, B.G., & Dukes, J.S. 2008. Five potential consequences of climate change for invasive species. *Conservation Biology* 22: 534-543.

97. Hickling, R., Roy, D.B., Hill, J.K., Fox, R., & Thomas, C.D. 2006. The distributions of a wide range of taxonomic groups are expanding polewards. *Global Change Biology* 12: 450–455.

98. HilleRisLambers, J., Harsch, M.A., Ettinger, A.K., Ford, K.R., Theobald, E.J. 2013. How will biotic interactions influence climate change-induced range shifts? *Annals of the New York Academy of Sciences* 1297: 112-125.

99. Hoebeke, E.R. & Beucke, K. 1997. Adventive *Onthophagus* Coleoptera: Scarabaeidae in North America: geographic ranges, diagnoses, and new distributional records. *Entomological News* 108: 345-362.

100. Hoffmann, A.A. & Sgrò, C.M. 2011. Climate change and evolutionary adaptation. *Nature* 470: 479-485.

101. Holley, J. M. & Andrew, N. R. 2019a. Experimental warming disrupts reproduction and dung burial by a ball-rolling dung beetle. *Ecological Entomology* 44: 206-216.

102. Holley, J.M. & Andrew, N.R. 2019b. Experimental warming alters the relative survival and emigration of two dung beetle species from an Australian dung pat community. *Austral Ecology* 44: 800-811.

103. Holley, J. M. & Andrew, N.R. 2020. Warming effects on dung beetle ecosystem services: brood production and dung burial by a tunneling dung beetle, *Onthophaugs taurus* Coleoptera: Scarabaeidae is reduced by experimental warming. *Austral Ecology* 59: 353-367.

104. Howden, H. 1955. The biology and taxonomy of North American beetles of the subfamily Geotrupinae with revisions of the genera *Bolbocerosoma*, *Eucanthus*, *Geotrupes*, and *Peltotrupes* Scarabaeidae. *Proceedings of the United States National Museum* 104: 151–319.

105. Howden, H.F. & Scholtz, C.H. 1986. Changes in a Texas dung beetle community between 1975 and 1985 Coleoptera: Scarabaeidae, Scarabaeinae. *The Coleopterists Bulletin* 40: 313–316.

106. Howden, H.F. & Cartwright, O.L. 1963. Scarab beetles of the genus *Onthophagus* Latreille north of Mexico Coleoptera: Scarabaeidae. *Proceedings of the United States National Museum* 114: 1–143.

107. Howden, H. & Howden, A. 2001. Change through time: a third survey of the Scarabaeinae (Coleoptera: Scarabaeidae) at Welder Wildlife Refuge. *The Coleopterists Bulletin* 55: 356–362.

108. Huang, D., Haack, R.A., & Zhang, R. 2011. Does global warming increase establishment rates of invasive alien species? A centurial time series analysis. *PLoS ONE* 6: e24733.

109. Hunt, J. & House, C. 2011. The evolution of parental care in onthophagine dung beetles. In: *Ecology and Evolution of Dung Beetles*. Simmons, L.W. & Ridsdill-Smith, J.T, eds. Blackwell Publishing Ltd: Sussex, UK.

110. Hunt, J. & Simmons, L.W. 2000. Maternal and paternal effects on offspring phenotype in the dung beetle *Onthophagus taurus*. *Evolution* 54: 936–941.

111. Hunt, J. & Simmons, L.W. 2002. Behavioral dynamics of biparental care in the dung beetle *Onthophagus taurus*. *Animal Behavior* 64: 65–75.

112. Hunt, J. & Simmons, L.W. 2003. Optimal maternal investment in the dung beetle *Onthophagus taurus*? *Behavioral Ecology & Sociobiology* 55: 302–312.

113. Hunt, T., Bergsten, J., Levkanicova, Z, Papadopoulou, A., St. John, O., Wild, R., …Volger, A.P. 2007. A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. *Science* 318: 1913–1916.

114. Iler, A.M., CaraDonna, P.J., Forrest, J.R.K. & Post, E. 2021. Demographic consequences of phenological shifts in response to climate change. *Annual Review of Ecology, Evolution, and Systematics* 52: 221-245.

115. Jacobsen, D. 2020. The dilemma of altitudinal shifts: caught between high temperature and low oxygen. *Frontiers in Ecology and the Environment* 18: 211-218.

116. Janzen, D.H. 1967. Why mountain passes are higher in the tropics. *The American Naturalist* 101: 233–249.

117. Jay-Robert, P., Lumaret, J.-P., & Lebreton, J.-D. 2008. Spatial and temporal variation of mountain dung beetle assemblages and their relationships with environmental factors Aphodiinae: Geotrupinae: Scarabaeinae. *Ecology and Population Biology* 101: 58–69.

118. Jenkins, M.A. 2007. Vegetation communities of Great Smoky Mountains National Park. *Southeastern Naturalist* 6: 35–56.

119. Kareiva, P. Developing a predictive ecology for non-indigenous species and ecological invasions. *Ecology* 77: 1651–1652.

120. Kaufman, P.E. & Wood, L.A. 2012. Indigenous and exotic dung beetles Coleoptera: Scarabaeidae and Geotrupidae collected in Florida cattle pastures. *Annals of the Entomological Society of America* 105: 225–231.

121. Kelley, A.L. 2014. The role thermal physiology plays in species invasion. *Conservation Physiology* 2: 1–14.

122. Khaliq, I., Hof, C., Prinzinger, R., Böhning-Gaese, K., & Pfenninger, M. 2014. Global variation in thermal tolerances and vulnerability of endotherms to climate change. *Proceedings of the Royal Society B Biological* 281: 20141097.

123. Knell, R. 2011. Male contest competition and the evolution of weapons. *Ecology and Evolution of Dung Beetles*. Ed. Simmons, L.W. & Ridsdill-Smith, T.J. Oxford: Blackwell Publishing Ltd.

124. Koide, D., Yoshida, K., Daehler, C.C., & Mueller-Dombois, D. 2017. An upward elevation shift of native and non-native vascular plants over 40 years on the island of Hawai'i. *Journal of Vegetation Science* 28: 939-950.

125. Kolar, C.S. & Lodge, D.M. 2001. Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution* 16: 199–204.

126. Konvicka, M., Maradova, M., Benes, J., Fric, Z., & Kepka, P. 2003. Uphill shifts in distribution of butterflies in the Czech Republic: Effects of changing climate detected on a regional scale. *Global Ecology and Biogeography* 12: 403–410.

127. Körner, C. 2003. *Alpine plant life: Functional Plant Ecology of High Mountain Ecosystems*. Berlin: Springer.

128. Körner, C. 2004. Mountain biodiversity, its causes and function. *Ambio* 13: 11–17.

129. Krause, C.M., Cobb, N.S., & Pennington, D.D. 2015. Range shifts under future scenarios of climate change: dispersal ability matters for Colorado plateau and endemic plants. *Natural Areas Journal* 35: 428-438.

130. Lane, J.E., McAdam, A.G., McFarlane, S.E., Williams, C.T., Humphries, M.M., Coltman, D.W., Gorrell, J.C. & Boutin, S. 2018. Phenological shifts in North American red squirrels: disentangling the roles of phenotypic plasticity and microevolution. *Journal of Evolutionary Biology* 31: 810-821.

131. Lenoir, J. & Svenning, J.-C. 2015. Climate-related range shifts – a global multidimensional synthesis and new research directions. *Ecography* 38: 15–28.

132. Lenoir, J., Bertrand, R., Comte, L., Bourgeaud, L., Hattab, T., Murienne, J. & Grenouillet, G. 2020. Species better track climate warming in the oceans than on land. *Nature Ecology & Evolution* 4: 1044–1059.

133. Lenoir, J., Gégout, J.C., Marquet, P.A., de Ruffray, P., & Brisse, H. 2008. A significant upward shift in plant species optimum elevation during the 20th century. *Science* 320: 1768–1771.

134. Lenoir, J., Gegout, J.-C., Guisan, A., Vittoz, P., Wohlgemuth, T., Zimmermann, N. E., … Svenning, J.-C. 2010. Going against the flow: Potential mechanisms for unexpected downslope range shifts in a warming climate. *Ecography* 33: 295–303.

135. Liang, Y., Duveneck, M.J., Gustafson, E.J., Serra-Diaz, J.M., & Thompson, J.R. 2018. How disturbance, competition, and dispersal interact to prevent tree range boundaries from keeping pace with climate change. *Global Change Biology* 24: e335-e351.

136. Lin, Z.-H., Wu, C.-H. & Ho, C.-K. 2018. Warming neutralizes host-specific competitive advantages between a native and invasive herbivore. *Scientific Reports* 8: 11130.

137. Losey, J.E. & Vaughan, M. 2006. The economic value of ecological services provided by insects. *BioScience* 56: 311–323.

138. Louzada, J., Lima, A.P., Matavelli, R., Zambaldi, L., & Barlow, J. 2010. Community structure of dung beetles in Amazonian savannas: role of fire disturbance, vegetation and landscape structure. *Landscape Ecology* 25: 631–641.

139. Lüddecke, H. & Sánchez, O. R. 2002. Are tropical highland frog calls cold-adapted? The case of the Andean frog *Hyla labialis*. *Biotropica* 34: 281-288.

140. Macagno, A.L.M., Moczek, A.P., & Pizzo, A. 2016. Rapid divergence of nesting depth and digging appendages among tunneling dung beetle populations and species. *American Naturalist* 187: 143-151.

141. Macagno, A.L.M., Zattara, E.E., Ezeakudo, O., Moczek, A.P., & Ledón-Rettig, C.C. 2018. Adaptive maternal behavioral plasticity and developmental programming mitigate the transgenerational effects of temperature in dung beetles. *Oikos* 127: 1319–1329.

142. Mainka, S.A. & Howard, G.W. 2010. Climate change and invasive species: double jeopardy. *Integrative Zoology* 5: 102-111.

143. Mamantov, M.A. & Sheldon, K.S. 2021. Behavioural responses to warming differentially impact survival in introduced and native dung beetles. *Journal of Animal Ecology* 90: 273–281.

144. Masson, L., Brownscombe, J.W., & Fox, M.G. 2016. Fine scale spatio-temporal life history shifts in an invasive species at its expansion front. *Biological Invasions* 18: 775-792.

145. Mattila, N., Kaitala, V., Komonen, A., Päivinen, J., & Kotiaho, J. S. 2011. Ecological correlates of distribution change and range shift in butterflies. *Insect Conservation and Diversity* 4: 239–246.

146. McCann, S.M., Kosmala, G.K., Greenlees, M.J., & Shine, R. 2018. Physiological plasticity in a successful invader: rapid acclimation to cold occurs only in cool-climate populations of cane toads *Rhinella marina*. *Conservation Physiology* 6: cox072.

147. Menéndez, R., González-Megías, A., Jay-Robert, P., & Marquéz-Ferrando. R. 2014. Climate change and elevational range shifts: evidence from dung beetles in two European mountain ranges. *Global Ecology and Biogeography* 23: 646–657.

148. Miegroet, H., Creed, I., Nicholas, N., Tarboton, D., Webster, K., Shubzda, J., … Moore, S. 2001. Is there synchronicity in nitrogen input and output fluxes at the Noland Divide Watershed, a small N- saturated forested catchment in the Great Smoky Mountains National Park? *The Scientific World Journal* 1: 480–942.

149. Miller, A.N., Raudabough, D.B., Iturriaga, T., Matheny, P.B., Petersen, R.H., Hughes, K.W., Gube, M., Powers, R.A., James, T.Y., & O'Donnell, K. 2017. First report of the post-fire morel *Morchella exuberans* in eastern North America. *Mycologia* 109: 710–714.

150. Miraldo, A., Krell, K.-T., Smalén, M., Angus, R. B.., & Roslin, T. 2014. Making the cryptic visible – resolving the species complex of *Aphodius fimetarius* Linnaeus and *Aphodius pedellus* de Geer Coleoptera: Scarabaeidae by three complementary methods. *Systematic Entomology* 39: 531–547.

151. Moczek, A.P. 1998. Horn polyphenism in the beetle Onthophagus taurus: larval diet quality and plasticity in parental investment determine adult body size and male horn morphology. *Behavioral Ecology* 9: 636–641.

152. Moczek, A.P. 1999. Facultative paternal investment in the polyphenic beetle *Onthophagus taurus*: the role of male morphology and social context. *Behavioral Ecology* 10: 641–647.

153. Moczek, A.P. 2003. The behavioral ecology of threshold evolution in a polyphenic beetle. *Behavioral Ecology* 14: 841–854.

154. Moczek, A.P. 2011. Evolution and development: *Onthophagus* beetles and the evolutionary development genetics of innovation, allometry, and plasticity [pp 126-151]. In: *Ecology and Evolution of Dung Beetles*. Simmons, L.W. & Ridsdill-Smith, J.T, eds. Blackwell Publishing Ltd: Sussex, UK.

155. Moczek, A.P. & Emlen, D.J. 2000. Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: do alternative reproductive tactics favour alternative phenotypes? *Animal Behavior* 59: 459–466.

156. Moritz, C., Patton, J.L., Conroy, C.J., Parra, J.L., White, G.C., & Beissinger, S.R. 2008. Impact of a century of climate change on small-mammal communities in Yosemite National Park, USA. *Science* 322: 261.

157. Morrison, L.W., Korzukhin, M.D., & Porter, S.D. 2005. Predicted range expansion of the invasive fire ant, *Solenopsis invicta,* in the eastern United States based on the VEMAP global warming scenario: Range expansion of an invasive fire ant. *Diversity and Distributions,* 11: 199–204.

158. Navas, C.A. 2006. Patterns of distribution of anurans in high Andean tropical elevations: Insights from integrating biogeography and evolutionary physiology. *Integrative and Comparative Biology* 46: 82–91.

159. Nemes, S.N. & Price, D.L. 2015. Illustrated keys to the Scarabaeinae Coleoptera: Scarabaeidae of Maryland. *Northeastern Naturalist* 22: 318–344.

160. Nichols, B.J. & Langdon, K.R. 2007. The Smokies All Taxa Biodiversity Inventory: history and progress. *Southeastern Naturalist* 6: 27–34.

161. Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S., Favila, M.E., & The Scarabainae Research Network. 2008. Ecological functions and ecosystem

services provided by Scarabaeinae dung beetles. *Biological Conservation* 141: 1461–1474.

162. Nicolakakis, N., Sol, D., & Lefebvre, L. 2003. Behavioral flexibility predicts species richness in birds, but not extinction risk. *Animal Behavior* 65: 445-452.
163. Nogués-Bravo, D., Araújo, M.B., Errea, M.P., & Martínez-Rica, J.P. 2007. Exposure of global mountain systems to climate change. *Global Environment Change* 17: 420–428.
164. Nyamukondiwa, C., Kleynhans, E. & Terblanche, J.S. 2010. Phenotypic plasticity of thermal tolerance contributes to the invasion potential of Mediterranean fruit flies Ceratitis capitata. *Ecological Entomology* 35: 565–575.
165. Ohmura, A. 2012. Enhanced temperature variability in high-altitude climate change. *Theoretical and Applied Climatology* 110: 499–508.
166. Oksanen, J., Blanchet, F.G., Friendly, M., & Kindt, R. 2016. VEGAN: community ecology package. R package version 2.4-3. *Vienna: R Foundation for* Statistical Computing, Vienna, Austria.
167. Oliver, I., Dorrough, J., Doherty, H., & Andrew, N.R. 2016. Additive and synergistic effects of land cover, land use and climate on insect biodiversity. *Landscape Ecology* 31: 2415-2431.
168. Parmesan, C. & Yohe, G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37–42.
169. Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics* 37: 637–669.
170. Pecl, G.T., Araújo, M.B., Bell, J.D., Blanchard, J., Bonebrake, T.C., Chen, I.-C., … Williams, S.E. 2017. Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science* 355: eaai9214.

171. Pepin, N., Bradley, R.S., Diaz, H.F., Baraer, M., Caceres, E.B., Forsythe, N., … Yang, D. Q. 2015. Elevation-dependent warming in mountain regions of the world. *Nature Climate Change* 5: 424–430.

172. Pettersen, A.K., White, C.R., Bryson-Richardson, R.J., & Marshall, D.J. 2019. Linking life-history theory and metabolic theory explains the offspring size-temperature relationship. *Ecology Letters*, 22: 518–526.

173. Philips, K.T. 2011. The evolutionary history and diversification of dung beetles [pp 21-45]. In: *Ecology and Evolution of Dung Beetles*. Simmons, L.W. & Ridsdill-Smith, T.J., eds. Blackwell Publishing Ltd: Oxford, United Kingdom.

174. Pimentel, D., Zuniga, R. & Morrison, D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52: 273–288.

175. Ploquin, E.F., Herrera, J.M., & Obeso, J. R. 2014. Bumblebee community homogenization after uphill shifts in montane areas of northern Spain. *Oecologia* 173: 1649–1660.

176. Pokhrel, M.R., Cairns, S.C., Hemmings, Z., Floate, K.D., & Andrew, N.R. 2021. A review of dung beetle introductions in the Antipodes and North America: status, opportunities, and challenges. *Environmental Entomology* 50: 762–780.

177. Price, D. L. 2004. Species diversity and seasonal abundance of scarabaeoid dung beetles Coleoptera: Scarabaeidae, Geotrupidae and Trogidae attracted to cow dung in central New Jersey. *Entomologica Americana* 112: 334–347.

178. Price, T.D., Qvarnström, A., & Irwin, D.E. 2003. The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B: Biological Sciences* 270: 1433–1440.

179. R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

180. Raxworthy, C.J., Pearson, R., Rabibisoa, N., Rakotondrazafy, A.M., Ramanamanjato, J.-B., Raselimanana, A.P., … Stone, D.A. 2008. Extinction vulnerability of tropical montane endemism from warming and upslope displacement: A preliminary appraisal for the highest massif in Madagascar. *Global Change Biology* 14: 1703–1720.

181. Reisinger, L.S., Elgin, A.K., Towle, K.M., Chan, D.J., & Lodge, D.M. 2017. The influence of evolution and plasticity on the behavior of an invasive crayfish. *Biological Invasions* 19: 815-830

182. Richards, S.L, Bossdorf, O., Muth, N.Z., Gurevitch, J., & Pigliucci, M. 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters* 9: 981-993.

183. Ridsdill-Smith, T. J. 1993. Asymmetric competition in cattle dung between two species of *Onthophagus* dung beetle and the bush fly, *Musca vetustissima*. *Ecological Entomology* 18: 241–246.

184. Ridsdill-Smith, T. J., and P. B. Edwards. 2011. Biological control: ecosystem functions provided by dung beetles [pp. 245–266]. In: *Ecology and Evolution of Dung Beetles*. Simmons, W & Ridsdill-Smith, J.T., eds. Blackwell Publishing Ltd: Sussex, UK.

185. Roff, D. 1983. Phenological adaptation in a seasonal environment: a theoretical perspective [pp. 253-270] In: *Diapause and life cycle strategies in insects*. Brown, V.K. & Hodeck, I., eds. Kluwer: Junk, Netherlands.

186. Rowe, K.C., Rowe, K.M.C., Tingley, M.W., Koo, M.S., Patton, J.L., Conroy, C.J., … Moritz, C. 2015. Spatially heterogeneous impact of climate change on

small mammals of montane California. *Proceedings of the Royal Society B Biological Sciences* 282: 20141857.

187. Rumpf, S.B., Hülber, K., Klonner, G., Moser, D., Schütz, M., Wessely, J., … Dullinger, S. 2018. Range dynamics of mountain plants decrease with elevation. *Proceedings of the National Academy of Sciences of the USA* 115: 1848–1853.

188. Scherrer, D. & Körner, C. 2010. Infra-red thermometry of alpine landscapes challenges climatic warming projections. *Global Change Biology* 16: 2602-2613.

189. Scherrer, D. & Körner, C. 2011. Topographically controlled thermal-habitat differentiation buffers alpine plant diversity against climate warming. *Journal of Biogeography* 38: 406-416.

190. Scholtz, C.H., Davis, A.L.V, & Kryger, U. 2009. *Evolutionary Biology and Conservation of Dung Beetles*. Pensoft Publications: Sofia-Moscow, Russia.

191. Seebacher, F., White, C.R., & Franklin, C.E. 2014. Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change* 5: 61.

192. Sekercioglu, C.H., Schneider, S.H., Fay, J.P., & Loarie, S.R. 2008. Climate change, elevational range shifts, and bird extinctions. *Conservation Biology* 22: 140-150.

193. Shah, A.A., Gill, B.A., Encalada, A.C., Flecker, A.S., Funk, W.C., Guayasamin, J.M., … Ghalambor, C.K. 2017. Climate variability predicts thermal limits of aquatic insects across elevation and latitude. *Functional Ecology* 31: 2118-2127.

194. Sheldon, K.S. & Tewksbury, J.J. 2014. The impact of seasonality in temperature on thermal tolerance and elevational range. *Ecology* 95: 2134–2143.

195. Sheldon, K.S., Huey, R.B., Kaspari, M., & Sanders, N.J. 2018. Fifty years of Mountain Passes: A perspective on Dan Janzen's classic article. *The American Naturalist* 191: 553–565.

196. Sheldon, K.S., Leaché, A.D., & Cruz, F.B. 2015. The influence of seasonality in temperature on elevational range size across latitude: A test using Liolaemus lizards. *Global Ecology and Biogeography,* 24: 632–641.

197. Simberloff, D. 2013. Invasive species: what everyone needs to know. Oxford University Press, New York, USA.

198. Simmons, L.W. & Ridsdill-Smith, J.T. 2011. Reproductive competition and its impact on the evolution and ecology of dung beetles [pp. 1–20]. In: *Ecology and Evolution of Dung Beetles*. Simmons, L.W. & Ridsdill-Smith, J.T, eds. Blackwell Publishing Ltd: Sussex, UK.

199. Simons, P., Molina, M., Hagadorn, M.A., & Price, D.L. 2018. Monitoring of dung beetle Scarabaeidae and Geotrupidae activity along Maryland's Coastal Plain. *Northeastern Naturalist* 25: 87–100.

200. Smith, L.D. 2009. The role of phenotypic plasticity in marine biological invasions [pp 177-202]. In: *Biological invasions in marine ecosystems: ecological, management and geographic perspectives.* Rilov, G., Crooks, J.A., ed. *Ecological Studies Series*. Berlin: Springer.

201. Snell-Rood, E.C. 2013. An overview of the evolutionary causes and consequences of behavioral plasticity. *Animal Behavior* 8: 1004–1011.

202. Snell-Rood, E.C., Burger, M., Hutton, Q., Moczek, A.P. 2016. Effects of parental care on the accumulation and release of cryptic genetic variation: review of mechanisms and a case study of dung beetles. *Evolutionary Ecology* 30: 251-265.

203. Socolar, J.B., Epanchin, P.N, Beissinger, S.R., & Tingley, M.W. 2017. Phenological shifts conserve thermal niches in North American birds and reshape expectations for climate-driven range shifts. *Proceedings of the National Academy of Sciences* 114: 12976-12981.

204. Sol, D. & Lefebvre, L. 2000. Behavioral flexibility predicts invasion success in birds introduced to New Zealand. *Oikos,* 90: 599-605.

205. Sol, D., Duncan, R.P., Blackburn, T.M., Cassey, P., & Lefebvre, L. 2005. Big brains, enhanced cognition, and response of birds to novel environments. *PNAS* 102: 5460-5465.

206. Sol, D., Griffin, A.S., Bartomeus, I., Boyce, H. 2011. Exploring or avoiding novel food resources? The novelty conflict in an invasive bird. *PLoS One* 6: 319535.

207. Sol, D., Timmermans, S., & Lefebvre, L. 2002. Behavioral flexibility and invasion success in birds. *Animal Behavior* 63: 495–502

208. Sømme, L. & Block, W. 1991. Adaptations to alpine and polar environments in insects and other arthropods [318-359]. In: *Insects at low temperature.* Lee, R.E. & Denlinger D.L., eds. Chapman and Hall: London, United Kingdom.

209. Spence, A.R. & Tingley, M.W. 2020. The challenge of novel abiotic conditions for species undergoing climate-induced shifts. *Ecography* 43: 1571-1590.

210. Stachowicz, J.J., Terwin, J.R., Whitlatch, R.B., & Osman, R.W. 2002. Linking climate change and biological invasions: ocean warming facilitates nonindigenous species invasions. *PNAS* 99: 15497-15500.

211. Stearns, S.C. 1992. *The evolution of life histories.* Oxford University Press: Oxford, United Kingdom.

212. Stevens, G.C. 1992. The elevational gradient in altitudinal range: an extension of Rapoport’s latitudinal rule to altitude. *The American Naturalist* 140: 893–911.

213. Stillman, J.H. 2003. Acclimation capacity underlies susceptibility to climate change. *Science* 301: 65.

214. Stroud, J.T., Colom, M., Ferrer, P., Palermo, N., Vargas, V., Cavallini, M., Lopez, J., & Jones, I. 2019. Behavioral shifts with urbanization may facilitate biological invasion of a widespread lizard. *Urban Ecosystems* 22: 425-434.

215. Telemeco, R.S., Elphick, M.J., & Shine, R. 2009. Nesting lizards *Bassiana dupperreyi* compensate partly, but not completely, for climate change. *Ecology* 90: 17-22.

216. Telemeco, R.S., Fletcher, B., Levy, O., Riley, A., Rodriguez-Sanchez, Y., Smith, C., Teague, C., Waters, A., Angilletta, M.J. Jr., & Buckley, L.B. 2016. Lizards fail to plastically adjust nesting behavior or thermal tolerance as needed to buffer populations from climate warming. *Global Change Biology* 23: 1075-1084.

217. Telwala, Y., Brook, B.W., Manish, K., & Pandit, M.K. 2013. Climate-induced elevational range shifts and increase in plant species richness in a Himalayan biodiversity epicentre. *PloS One* 8: e57103.

218. Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., … Williams, S.E. 2004. Extinction risk from climate change. *Nature* 427: 145–148.

219. Tingley, M.W. 2011. Long-term trends in the avifauna of the Sierra Nevada: community dynamics and species occupancy over a century of climate change. [PhD Thesis], University of California, Berkeley, U.S.A.

220. Tingley, M.W., Koo, M.S., Moritz, C., Rush, A.C., & Beissinger, S.R. 2012. The push and pull of climate change causes heterogeneous shifts in avian elevational ranges. *Global Change Biology* 18: 3279–3290.

221. Tribe, G.D. & Burger, B.V. 2011. Olfactory ecology [pp. 87–106]. In: *Ecology and Evolution of Dung Beetles.* Simmons, L.W. & Ridsdill-Smith, J.T. eds. Blackwell Publishing Ltd: Sussex, UK.

222. Urban, M.C., Tewksbury, J.J., Sheldon, K.S. 2012. On a collision course: competition and dispersal differences create no-analogue communities and cause extinctions during climate change. *Proceedings of the Royal Society B Biological Sciences*, 279: 2072-2080.

223. Urban, M.C., Zarnetske, P.L., & Skelly, D.K. 2013. Moving forward: dispersal and species interactions determine biotic responses to climate change. *Annals of the New York Academy of Sciences* 1297: 44–60.

224. Urban, M.C. Accelerating extinction risk from climate change. *Science* 348: 571-573.

225. Van Manen, F.T. 1994. Black bear habitat use in the Great Smoky Mountains National Park. [PhD thesis], University of Tennessee, Knoxville, U.S.A.

226. Verdú, J.R., Arellano, L., Numa, C. & Micó, E. 2007. Roles of endothermy in niche differentiation for ball-rolling dung beetles Coleoptera: Scarabaeidae along an altitudinal gradient. *Ecological Entomology* 3: 544–551.

227. Vitasse, Y., Lenz, A., Kollas, C., Randin, C.F., Hoch, G., & Körner, C. 2014. Genetic vs. non-genetic responses of leaf morphology and growth to elevation in temperate tree species. *Functional Ecology* 28: 243-252.

228. Walther, G.R., Roques, A., Hulme, P.E., Sykes, M.T., Pysek, P., Kuhn, I., … & Settele, J. 2009. Alien species in a warmer world: risks and opportunities. *Trends in Ecology & Evolution,* 24: 686-693.

229. West-Eberhard, M.J. 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology, Evolution, and Systematics,* 20: 249–278.

230. West-Eberhard, M.J. 2003. *Developmental Plasticity and Evolution.* New York: Oxford University Press.

231. Williams, J.E. & Blois, J.L. 2018. Range shifts in response to past and future climate change: can climate velocities and species' dispersal capabilities explain variation in mammalian range shifts? *Journal of Biogeography* 45: 2175-2189.

232. Winwood-Smith, H.S., Alton, L.A., Franklin, C.E. & White, C. R. 2015. Does greater thermal plasticity facilitate range expansion of an invasive terrestrial anuran into higher latitudes? *Conservation Physiology* 3: cov010.

233. Wooliver, R., Tittes, S.B., & Sheth, S.N. 2020. A resurrection study reveals limited evolution of thermal performance in response to recent climate change across the geographic range of the scarlet monkeyflower. *Evolution* 74: 1699-1710.

234. Wright, T.F. 2010. Behavioral flexibility and species invasions: the adaptive flexibility hypothesis. *Ethology Ecology and Evolution*, 22: 393-404.

235. Yeh, P.J. & Price, T. D. 2004. Adaptive phenotypic plasticity and the successful colonization of a novel environment. *American Naturalist* 164: 531–542.

236. Young, O. P. 2007. Relationships between an introduced and two native dung beetle species Coleoptera: Scarabaeidae in Georgia. *Southeastern Naturalist* 6: 491-504.

237. Zerebecki R.A. & Sorte, C.J. 2011. Temperature tolerance and stress proteins as mechanisms of invasive species success. *PLoS ONE* 6: e14806

VITA

Margaret A. Mamantov was born in Knoxville, TN to Mark and Cathy Mamantov. She grew up in Knoxville with two sisters, Elizabeth Mamantov Goeddel and Ellen Mamantov, in a family that valued knowledge, curiosity, animals, sports, nature, and travel. Margaret graduated from Webb School of Knoxville, leaving childhood and Knoxville with a love of animals, science, history, reading, and soccer. She then attended Grinnell College in Grinnell, IA where numerous professors shared their expertise and passion. While working toward her Bachelor of Arts at Grinnell, Margaret cultivated a passion for scientific thinking while working with cooperative ants under the mentorship of Dr. Jonathan Brown. During her undergraduate education, she also spent a summer working with house sparrow nestlings with Dr. Dave Westneat at the University of Kentucky, gaining interest in animal behavior. Margaret graduated Grinnell College in December 2015 with a BA in both history and biology. Her honors thesis investigated the population structure of a cooperative ant species.

In fall 2016, Margaret started her graduate program in the Department of Ecology & Evolution at the University of Tennessee, advised by Kimberly S. Sheldon. Throughout her graduate career, she has spent many hours exploring cattle pastures and protected areas across the eastern United States, searching for dung beetles and collected thousands of pounds of cow dung. She also spent many hours searching though soil, investigating the reproductive behavior of beetles. She also developed a love of teaching and is happiest in the classroom. The results of this time are summarized in this dissertation. In August of 2022, she will graduate from the University of Tennessee and begin her teaching career as a lecturer in the Division of Biology at the University of Tennessee.