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The Evolutionary Significance of Body Size in Burying Beetles

Ashlee Nichole Momcilovich
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The Evolutionary Significance of Body Size in Burying Beetles

Ashlee Nichole Momcilovich

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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ABSTRACT

The Evolutionary Significance of Body Size in Burying Beetles

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Doctor of Philosophy

Body size is one of the most commonly studied traits of an organism, which is largely due to its direct correlation with fitness, life history strategy, and physiology of the organism. Patterns of body size distribution are also often studied. The distribution of body size within species is looked at for suggestions of differential mating strategies or niche variation among ontogenetic development. Patterns are also examined among species to determine the effects of competition, environmental factors, and phylogenetic inertia. Finally, the distribution of body size across the geographic range of a species or group of closely related is looked at for indications of the effects of climate and resource availability on body size at different latitudes and altitudes. In this collection of research, I address the evolution and importance of body size in burying beetles (genus *Nicrophorus*). Body size is important to several aspects of burying beetle natural history, including competitive ability, fitness, parental care, climate tolerance, and locomotor activity. In Chapter 1, I use a large data set of body size measurements for seventy of the seventy-three *Nicrophorus* species to make inferences about the distribution of body size within the genus, across its geographic range, and the importance of body size in speciation. I found that the range of body sizes is not normally distributed, with an overrepresentation of small-sized species. I also found that expansion of the burying beetle range has been restricted by their inability to tolerate warm, dry climates, and therefore the majority of burying beetle diversity occurs in the temperature mid-latitudes of the northern hemisphere. Body size also seems to be important in speciation, as almost all sister taxa are significantly different in body size. In Chapter 2 I use common garden experiments to assess the importance of body size for males and females in competition, reproductive output, and starvation resistance. Body size is equally important for both sexes in starvation resistance, but it is more important for males in competitions for carcasses and for females in reproductive output. In Chapter 3 I test for fitness consequences of multigenerational effects of body size in offspring. I found that the larger offspring that are produced by larger mothers and on larger carcasses had higher fitness than small offspring. In Chapter 4 I test for the possibility of brood parasitism in two species of burying beetles, *N. guttula* and *N. marginatus*, which co-occur over part of their geographic ranges. I found that both species are able to detect and remove parasitic larvae. Finally, in Chapter 5 I compiled parent and offspring body sizes from seven species of burying beetles and use them to compare the heritability of body size among species using comparative techniques and a meta-analysis. I found that body size heritability is different between species, but is low for the genus as a whole. Together, these projects provide valuable information on the evolutionary significance of body size in *Nicrophorus*, and indicate compelling questions for future research into the evolution of body size in burying beetles.

Keywords: burying beetle, *Nicrophorus*, body size, evolution

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Chapter 1

Patterns of Body Size Evolution in the Genus *Nicrophorus*

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Abstract

Scientists are generally interested in body size because of its close relationship with a number of factors including fitness, life history strategy, and competitive ability. Body size is particularly important for burying beetles (genus *Nicrophorus*) as it can affect their ability to win access to resources and resource partitioning among species, as well as their reproductive output, thermal tolerance, survival, and their offspring's need for parental care. There is a large amount of variation in burying beetle body size, both between and among species, but we do not currently understand what drives these differences and how body size is distributed within the genus and across its geographic range. In this study, we used a large data set of body size measurements for species in the *Nicrophorus* genus to describe patterns of body size for the genus. We found that the body size distribution for burying beetles is skewed to the right, that body size is dispersed across the phylogeny for this species, that species richness and body size scale with resource availability, and that body size is important for speciation in this group. Thus, burying beetle body size and its distribution seems to be strongly affected by environmental conditions and resource availability.

Introduction

Body size is arguably one of the most important attributes of an organism from both an evolutionary and ecological perspective. Size has a predominant influence on an individual's physiological, life history, and fitness traits (Peters 1983; Reiss 1989; Roff 1992). Body size is also important in species interactions and community structure (Schoener 1974; Werner & Gillian 1984) as well as speciation (Nagel & Schluter 1998; Schluter 2001; Miraldo & Hanski 2014).

Several hypotheses have been proposed to explain how body sizes are distributed within taxonomic groups and across the geographic ranges of those groups. Hutchinson and MacArthur (1959) first noted that the frequency distribution of body sizes among species is highly right skewed, or that there are more small species than there are large species. They hypothesized that this occurs because smaller species are more specialized and therefore subdivide their environments and resources more narrowly than larger species. Other proposed explanations for the overrepresentation of small-sized taxa included a decrease in the number of niches with increasing body size (May 1978), a higher rate of speciation at small body sizes (Stanley 1973), and an energetically optimal small body size (Brown *et al.* 1996). Right-skewed size distributions have been demonstrated in a variety of taxa (e.g. Gardezi & da Silva 1999; Gaston & Blackburn 2000; Kozłowski & Gawelczyk 2002; Meiri 2008; Albert & Johnson 2012; Burbrink & Myers 2014; Gaston & Chown 2013; Feldman *et al.* 2015), and exceptions to the pattern are generally considered unusual.

Hutchinson also suggested that there is a relationship between body size and species interactions and niche partitioning. He proposed that for similar species to coexist in the same level of a food web, the ratio of the small to the large should be between 1.1 and 1.4, with the

mean being 1.3 (Hutchinson 1959). Although the validity of fixed body size ratios that allow coexistence has been questioned (Simberloff & Boeklin 1981), evidence from allopatric sister species indicates that species that are morphologically very similar cannot coexist because of significant niche overlap (Zink 2014).

The distribution of a trait, such as body size, among closely related taxa can also be assessed in a phylogenetic context. Trait variation between species reflects ecological influences, while similarities between species imply a phylogenetic signal (Blomberg *et al.* 2003). Therefore, if there is a high amount of variation in body size between closely related species, then environmental conditions have a strong influence on this trait as opposed to evolutionary history.

Several interspecific geographical patterns of body size have also been noted, and predict differences in mean body size with latitude, altitude, and environmental variation (reviewed in Gaston *et al.* 2008). Bergmann's rule predicts an increase in body size with increasing latitude (Bergmann 1847). Environmental variation may also affect body size through differences in competition, predation pressures, food availability, and temperature (Blanckenhorn 2000; Chown & Gaston 2010; McNab 2010), thus creating a complex set of variable that can influence body size.

Burying beetles (genus *Nicrophorus*) are well known in the field of evolutionary biology for their unique natural history, and are often used as a model system for investigations of parental care behaviors. All members of this genus use small vertebrate carcasses for feeding and reproduction (Scott 1998). The extent of parental care depends on the species and ranges from facultative to obligate biparental care (Capodeanu-Nägler *et al.* 2016; Jarrett *et al.* 2017). Parental care behaviors involve removing fur or feathers from the carcass, rolling it into a ball,

and burying it underground (Fetherston *et al.* 1990), followed by guarding the brood and regurgitating partially digested carrion to larvae after they hatch (Fetherston *et al.* 1990). One factor that is particularly important for burying beetle natural histories is body size, which affects burying beetles through physiological pathways such as thermoregulation (Merrick & Smith 2004), overwinter survival (Smith 2002), and starvation resistance (Trumbo & Xihani 2015; Smith & Belk 2018, Dissertation Chapter 2), fitness-related traits such as competitive ability (Bartlett & Ashworth 1988; Otronen 1988; Müller *et al.* 1990; Safryn & Scott 2000; Hopwood *et al.* 2013; Lee *et al.* 2014; Smith & Belk 2018, Dissertation Chapter 2), parental care behaviors (Steiger 2013; Smith *et al.* 2014; Pilakouta *et al.* 2015; Capodeanu-Nägler *et al.* 2016; Jarret *et al.* 2017), and reproductive output (Rauter *et al.* 2010; Hopwood *et al.* 2016; Smith & Belk 2018, Dissertation Chapter 2), and species interactions through resource partitioning (Wilson *et al.* 1984; Trumbo 1990; Scott 1998; Ikeda *et al.* 2006).

All burying beetle species are strikingly similar in their natural history, but there is a high level of variation in body size among species. There are 71 recognized *Nicrophorus* species (Sikes & Venables 2013; Sikes *et al.* 2016), most of which occur in the northern hemisphere (Sikes & Venables 2013) in a variety of habitat types (Peck & Anderson 1985; Peck & Kaulbars 1987; Scott 1998; Sikes & Peck 2000; Dekeirsschieter *et al.* 2011). The species in the *Nicrophorus* genus range in size from a mean pronotal width of 4.2mm in *N. montivagus* to 11.2mm in *N. concolor* (Sikes 2003; Sikes *et al.* 2006). Individual size is transmitted across generations through plastic effects generated by carcass size (Scott & Traniello 1990; Smith & Belk *in review*, Dissertation Chapter 2), maternal size (Steiger 2013; Smith & Belk 2018, Dissertation Chapter 2), maternal age (Lock *et al.* 2007), parental care strategy (Paquet *et al.* 2017), and environmental conditions such as temperature and altitude (Smith *et al.* 2000) and

population density (Creighton 2005). These plastic effects that affect offspring body size lead to differential lifetime fitness (Damron *et al. in prep*, Dissertation Chapter 3). Some species and populations of burying beetles exhibit low levels of heritability for body size, but no heritability of body size seems to be the case in most species (Smith *et al. in prep*, Dissertation Chapter 5). Thus, there are several pathways through which body size can affect life history strategy, fitness, species interactions, and speciation in burying beetles.

Although significant advancement has been made in determining how body size affects burying beetles at the microevolutionary scale, less attention has been paid to the macroevolutionary patterns of body size variation among *Nicrophorus* species and how body size is distributed across the geographic range of the genus. In this study we examine test four predictions regarding body size in the *Nicrophorus* genus: 1) The body size distribution for the genus is normally distributed, 2) Body size is dispersed rather than clustered in the *Nicrophorus* phylogeny, indicating a strong environmental influence on this trait, 3) Body size distributions are consistent with resource availability because burying beetles use rare and ephemeral resources for reproduction, and 4) Sympatric sister species are less similar in body size than allopatric or parapatric sister species due to competition and resource partitioning.

Methods

We test for the large-scale body size patterns predicted above for the *Nicrophorus* genus by assessing: 1) the distribution of mean body size within the genus, 2) the distribution of mean body size across the phylogeny, 3) the distribution of body sizes across the geographic range for the genus, and 4) the difference in body size between parapatric, sympatric, and allopatric sister species.

Body Size Range among Species

To explore the distribution of body size among species of the genus *Nicrophorus*, we used a data set compiled by D.S. Sikes that included 11,946 pronotum width measurements from museum specimens for 70 *Nicrophorus* species (See Sikes *et al.* 2016 for list of museums from which specimens were borrowed). Pronotal width is a standard measure of body size in beetles as it scales with body size in general, and it is important in determining the winners of competitions between burying beetles and resource partitioning in this group (Safryn & Scott 2000). We calculated the mean pronotum width for each species, and then we calculated the log of the mean pronotum width for each species. We used those values to create histograms, which we evaluated for normality and discontinuities. We then performed an Agostino test in the package ‘moments’ (Komsta & Novomestky 2015) in Program R (R Core Team 2013) to determine the normality of the distribution of body sizes in this genus.

To determine how body size is distributed across the *Nicrophorus* phylogeny, we created a phylogenetic heat map using the mean pronotum widths that were determined for each species. The chronogram from Sikes and Venable (2013) was imported and manipulated using the R package PHYLOCH (Heibl, 2008). Ancestral reconstructions of the continuous character trait pronotum width were estimated at the internal nodes by using a Maximum Likelihood (ML) function, fastANC in the R package phytools (Revell 2012) with the interpolation of the states along each edge using equation [2] of Felsenstein (1985). The reconstructions were then plotted using the contMap (Revell 2013) function in the R package phytools (Revell 2012).

Geographic Patterns

To determine how body size is distributed across the geographic range of *Nicrophorus*, we used the mean body sizes that were calculated for each species above. We used the cut function in Program R (R Core Team 2013) to divide the data into three categories. The data were divided as small = 4.14mm – 6.42mm, medium = 6.43mm – 8.7mm, and large = 8.8mm – 11.0mm. We then used locality records for each species recorded by DS Sikes and mapped the distribution of each size group in QGIS.

Patterns of Body Size Evolution

To determine whether there is a correlation between speciation and differences in body size, we used *t*-tests to compare the mean size of allopatric, parapatric, and sympatric sister species. Sister species were determined by examining the phylogenetic tree in Sikes and Venables (2013). We determined whether each pair of sister species occurred in sympatry, parapatry, or allopatry using locality data and information in published literature. To determine whether allopatric, sympatric, and parapatric sister species have significantly different body sizes, we calculated the mean difference in pronotum width and the factor difference between each sister species pair. We analyzed the data using ANOVAs, with the type of isolation (i.e. sympatry, allopatry, or parapatry) as the predictor variable and the mean difference or factor difference in pronotum width as the response variable. We also performed Tukey tests for comparisons between the three groups if the results of the ANOVA were significant. All statistical analyses were performed in Program R (R Core Team 2013).

Results

Body Size Range Among Species

The most apparent characteristic of the *Nicrophorus* species size distribution is that no species have a mean pronotum width between 8.0mm – 9.0mm, creating a gap in the distribution (Figure 1.1A). The break in the distribution occurs between what is categorized as medium and large in our Geographic Pattern analysis. Also, the distribution of the log mean body sizes for the species in the genus *Nicrophorus* is significantly right-skewed (two-sided Agostino test: skew = 1.1132, $z = 2.0906$, $p\text{-value} = 0.0366$) (Figure 1.1B).

The pronotum width heat map on the phylogenetic tree for the *Nicrophorus* genus shows that compared to the ancestral size, the mean pronotum width has increased and decreased several times in the evolutionary history of this group (Figure 1.2). Large body size has evolved three separate times, and large and small body sizes are not concentrated in any one part of the phylogeny.

Geographic Patterns

Our categorization placed 5 species (7% of the total number of species) in the large size category, 16 species in the medium size category (23% of the total number of species), and 49 species in the small size category (70% of the total number of species) (see Table 1.4 for a list of species in each category).

Visual analysis of the size distribution map (Figure 1.3) and the species richness map indicates four patterns of body size distribution across the geographic range of burying beetles.

(1) The largest diversity in body sizes and the highest species richness occurs in the mid-

latitudes. (2) Medium and large species mainly occur in the mid-latitudes, but small species are more likely to occur further north and south. (3) Large species of burying beetles occur across Europe and Asia, but not west of the Rocky Mountains in the United States. (4) The large *Nicrophorus* species occur in areas with high burying beetle biodiversity.

Patterns of Body Size Evolution

All sympatric sister species (Table 1.1) and parapatric sister species (Table 1.2) have significantly different mean pronotum widths. However, the mean pronotum widths of two allopatric sister species (29% of the group) are not significantly different from each other (Table 1.3). Out of the total seventeen *Nicrophorus* sister species pairs, 89% showed significant differences in body size between the pair (Tables 1.1-1.3).

Sympatric sister species differ from each other by a mean factor of 1.30 (range = 1.08 – 1.64) (Table 1.1). Parapatric sister species differ from each other by a mean factor of 1.10 (range = 1.05 – 1.18) (Table 1.2). Allopatric sister species differ from each other by a mean factor of 1.11 (range = 1.02 – 1.25) (Table 1.3).

There were moderately significant differences among the factor differences between sympatric, allopatric, and parapatric sister species (one-way ANOVA results: $df = 2$, $F = 3.43$, $p = 0.0578$). Results of a Tukey Test indicated that only the factor differences of sympatric and allopatric sister species were moderately different from each other ($p = 0.0827$), but the factor differences between sympatric and parapatric ($p = 0.1354$) and between allopatric and parapatric ($p = 0.9965$) sister species were not significantly different. Factor differences are on average 15% higher in sympatric compared to allopatric sister species.

There were no significant differences among the mean differences in body size between sympatric, parapatric, and allopatric sister species (one-way ANOVA results: $df = 2$, $F\text{-Value} = 2.18$, $p\text{-Value} = 0.1452$).

Discussion

The three ways that we assessed body size distribution in burying beetles produced interesting results, which indicate that the evolution of body size distributions in burying beetles is influenced by several factors. In particular, competition and resource partitioning and environmental conditions seem to have the greatest influence body size within the genus and within the geographic distribution of *Nicrophorus*. We discuss these influences in detail below.

Body Size Range Among Species

The mean pronotum width distribution for the *Nicrophorus* genus shows that there is a break between medium and large-sized species, but the distribution is continuous between small to medium-sized species (Figure 1.1A). The five species of burying beetles that we categorized as “large” also occur in the areas with the highest densities of burying beetles (Figure 1.3). The most well-studied large burying beetle species is *N. americanus*, which is the largest *Nicrophorus* species in North America (Schnell *et al.* 2008). This species competes intensely with *N. orbicollis* for carcasses because of a significant overlap in the geographic range, habitat preference, diel periodicity, and breeding season of the two species (Szalanski *et al.* 2000; Sikes & Raithel 2002), and this competition may have driven the evolution of large body size in *N. americanus*. *Nicrophorus americanus* breeds on carcasses that range from 30-500g, while *N.*

orbicollis breeds on carcasses that range from 7-150g (Trumbo & Bloch 2000). In competitions for carcasses, the largest competitors of each sex generally win access to the resource (Kozol *et al.* 1988; Bartlett & Ashworth 1988; Otronen 1988; Müller *et al.* 1990; Safryn & Scott 2000; Hopwood *et al.* 2013; Lee *et al.* 2014; Smith & Belk 2018, Dissertation Chapter 2), so competitions with other burying beetle species and resource partitioning could have driven the evolution of large body size in burying beetles. Although less is known about the natural history of the other large burying beetle species, reports of burying beetle community structures in Japan, where *N. concolor* occurs (i.e. Ikeda *et al.* 2006; Sugiura *et al.* 2013) and western Europe, where *N. germanicus* occurs (i.e. Aleksandrowicz & Komosiński 2005) indicate that intense competitions for carcasses probably also occur in the other areas where large burying beetle species are found.

Large body size has evolved three separate times in burying beetles (Figure 1.2). With the exception of *N. germanicus* and *N. morio*, which are sister species, none of the other large burying beetle species are closely related (Figure 1.2). If, as proposed above, large body size evolves in response to competitive pressures, then the same competitive pressures likely drove the evolution of large body size in all five large burying beetle species. In support of this theory, none of the large *Nicrophorus* species co-occur. *Nicrophorus germanicus* and *N. morio* are parapatric in the middle of their ranges (Růžička *et al.* 2002; Sikes *et al.* 2002), but do not directly compete because they prefer different habitat types (Scott 1998; Růžička *et al.* 2002; Dekeirsschieter *et al.* 2011). Therefore, competition for carcasses may drive the evolution of large body size, but prevent the evolution of more than one large burying beetle species in any one region.

One of the most prevalent characteristics of many body size distributions is a disproportionate representation of small-sized taxa (Gaston & Blackburn 2000; Kozłowski & Gawelczyk 2002). Although this pattern is well documented in vertebrates (reviewed in Kozłowski & Gawelczyk 2002), less evidence has been found in invertebrates (Rainford *et al.* 2016). However, our results indicate that body size is significantly skewed to the right in the *Nicrophorus* genus both with raw values and when they are analyzed on the log scale (Figures 1.1A and 1.1B). Several hypotheses have been presented to explain right-skewed data (reviewed in Kozłowski & Gawelczyk 2002). One possible explanation for long-tailed body size distributions is a higher rate of speciation in small species, but a higher rate of extinction in large species (Gould 1988). This seems possible for burying beetles because the most recent speciation events have resulted in small species (Figure 1.2), and the largest species are at risk of extinction (Anderson 1982). Another possible explanation is that burying beetle body sizes are related to the size distribution of small vertebrates. Burying beetles partition carcasses according to body size, with smaller species utilizing smaller carcasses and larger species utilizing larger carcasses (Wilson *et al.* 1984; Trumbo 1990; Scott 1998; Ikeda *et al.* 2006). Mammal body size distributions also show an overrepresentation of small species (Blackburn & Gaston 1994), so smaller body size in burying beetles might be correlated with the availability of resources.

Geographic Patterns

Nicrophorus species occur predominantly in the temperate northern hemisphere, with only a few species spreading into South America and the Malay Archipelago (Figure 1.2). The genus is hypothesized to have originated in Asia (Hatch 1927; Peck & Anderson 1985; Sikes & Venables 2013), and then colonized the New World four separate times (Sikes & Venables

2013). Climatic sensitivity in burying beetles may constrain their ability to spread into areas outside of the northern hemisphere.

Species richness generally increases with decreasing latitude (Rozenzweig 1995; Gaston 1996a; Brown & Lomolino 1998). However, patterns of biodiversity in insects do not always follow this trend (Gaston 1996b; Kouki 1999; Skillen *et al.* 2000). One possible explanation for contradictions to the general pattern is known as the mid-domain effect, which predicts that species richness will be higher in the middle of a geographical domain. This occurs because species' ranges have boundaries caused by temperature, land formations, bodies of water, etc., and there is an increase in overlap in their ranges toward the center of the domain, causing greater species diversity in that area (Colwell & Lees 2000). This explanation seems to be likely for burying beetles because their environmental tolerance is narrow (Merrick & Smith 2004). Activity levels generally occur at moderate temperatures (Merrick & Smith 2004; Jacques *et al.* 2009; Laidlaw 2015), which may be due to an inability to function at low temperatures and risk of desiccation at high temperatures (Bedick *et al.* 2006) and a higher cost of reproduction at higher temperatures where bacteria and fungus are able to colonize and grow more rapidly (Jacques *et al.* 2009). In support of this hypothesis, *Nicrophorus* species in tropical areas are generally found at higher elevations (Sikes & Venables 2013) and do not occur in deserts (Sikes 2008). Therefore, the distribution of burying beetles is likely constrained by colder temperatures to the north and humid temperatures and competition with bacteria and fungi to the south.

The distribution of burying beetles also likely corresponds to areas where there are more resources (i.e. vertebrate carcasses) to inter for food and reproduction. In Europe, the mean body size of mammals is smallest in the west (Rodríguez *et al.* 2006), and in North America the mean body size of mammals is smallest in the east (Rodríguez *et al.* 2008). Burying beetle species

richness is also highest in Western Europe and Eastern North America (Figure 1.3), so the available resources may also explain the distribution of species. Additionally, the amount of resources available might explain the distribution of large burying beetle species. The “resource rule” predicts that larger species will occur in areas where there are more resources (McNab 2010). The largest species of burying beetles in North America and Europe occur in the areas where the mean size of small mammals most closely matches the size that burying beetles can use for reproduction, which may be because of the availability of small mammals.

Environmental conditions might also explain the distribution of large burying beetle species, which occur across Europe and Asia, but not west of the Rocky Mountains in the United States. *Nicrophorus americanus* is known to prefer deep soil of either grasslands or developed forests (Lomolino & Creighton 1996; Bedick *et al.* 1999), and thus the relatively shallow, rocky soils in the western United States may not be their preferred habitat type. Also, the lack of soil moisture might not allow large species such as *N. americanus* to survive west of the Rocky Mountains (Bedick *et al.* 2006), as large burying beetles seem to desiccate more quickly than small species. These same niche requirements might also explain why large burying beetle species only occur in the middle of the geographic range of burying beetles, and why burying beetles do not follow Bergmann’s rule.

Patterns of Body Size Evolution

Competition is an important driver of speciation (Schluter 2000a,b; Moen & Wiens 2009), and has been linked to speciation in other invertebrates such as dung beetles and amphipods (Miraldo & Hanski 2014; Jeffrey *et al.* 2017). Additionally, Wilson (1975) indicated that body size was a major factor in niche differentiation among closely related species. Burying

beetles engage in intense inter- and intraspecific competition for access to carcasses, and the largest individuals generally win access to the resource (Bartlett & Ashworth 1988; Otronen 1988; Müller *et al.* 1990; Safryn & Scott 2000; Hopwood *et al.* 2013; Lee *et al.* 2014). Therefore, competition should influence diversification in this group through resource partitioning according to body size. We tested for differences in size between sister species in two ways: 1) by determining whether mean pronotum widths of sister species were equivalent with Hutchinson ratios and 2) by testing whether the size differences between sister species were different between sympatric, parapatric, and allopatric sister species. The mean pronotum widths of all sister species were significantly different from each other, except the difference between two allopatric pairs (Tables 1.1-1.3). According to Hutchinson ratios, co-occurring species should be different by a factor of 1.1 – 1.4 (Hutchinson 1959). On average, the factor difference in mean pronotum width for sympatric, parapatric, and allopatric sister species were consistent with Hutchinson ratios (Hutchinson 1959; Tables 1.1-1.3), indicating that body size is an important factor in speciation in burying beetles.

We also found that body size differences were greater in sympatric sister species than in allopatric sister species. Traits such as body size have been proposed as the possible drivers of diversification because changes in these traits may have a significant impact on ecological opportunity and allow shifts in niche availability (Losos 2010), and the rate of body size evolution is correlated with diversification on a macroevolutionary scale (Ricklefs 2004; Rabosky *et al.* 2013). Rapid shifts in size have been noted alongside increased rates of diversification in several adaptive radiations (Schluter 1993, Harmon *et al.* 2010). Douglas (1987) showed that greater dissimilarities in body size within a clade resulted in lower competition coefficients, which may also reduce the level of competition among burying beetle

species. Sympatric sister species are in direct competition with each other, so they likely need to have greater differences in body size to be able to continue to co-occur.

It should be noted that all of the extant burying beetle species are not included in the current phylogeny by Sikes and Venables (2013). Therefore, some of the sister species that are included in our analysis might not actually be sister species. For example, in the current phylogeny, *N. vespilloides* and *N. defodiens* are sister species (Sikes & Venables 2013; Table 1.1; Figure 1.2), but recent molecular data has identified *N. hebes* as a valid species and the actual sister species to *N. vespilloides* (Sikes *et al.* 2016). Therefore, as more species are added to the phylogeny for this genus, some of the sister species reported here will be identified as incorrect. However, the pattern of significant differences between sizes of sister species will likely remain as body size is an important part of burying beetle community structure (Wilson *et al.* 1984; Trumbo 1990; Scott 1998; Ikeda *et al.* 2006), and possibly of speciation within the group.

Conclusions

In burying beetles, body size seems to be strongly related to environmental factors. The results of our analyses of body size in *Nicrophorus* genus indicate that mean body size is likely affected by the amount and size of resources available and the amount of competition for resources. The greatest burying beetle species richness occurs in areas where small vertebrates are most likely to be dense, and the number of species competing for access to carcasses seems to driven the evolution of large body size in this genus. Body size is also important in speciation, as most sister species are significantly different in body size, regardless of whether they occur in sympatry, parapatry, or allopatry. Future work addressing the relatedness of co-occurring species

and resource partitioning would provide interesting additional insight into the role of body size in speciation and burying beetle community assembly.

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Table 1.1 ANOVA (Analysis of Variance) results for the t-tests on the mean pronotal widths for each pair of sympatric sister species. The factor difference between the mean pronotal widths for each pair is also included.

Sympatric Sister Species	Sources	df	t-Value	p-Value	Factor Difference
<i>N. smefarka</i> and <i>N. przewalski</i>	Růžička <i>et al.</i> 2002; Sikes <i>et al.</i> 2002	39	13.88	<.0001	1.64
<i>N. americanus</i> and <i>N. orbicollis</i>	Anderson 1981; Peck & Kaulbars 1987; Kozol <i>et al.</i> 1988; Lomolino & Creighton 1996; Bedick <i>et al.</i> 1999; Trumbo & Bloch 2000; Walker & Hoback 2007	502	33.83	<.0001	1.64
<i>N. vespillo</i> and <i>N. nigricornis</i>	Růžička <i>et al.</i> 2002; Dekeirsschieter <i>et al.</i> 2011; Ghahari & Háva 2015	712	5.68	<.0001	1.09
<i>N. maculifrons</i> and <i>N. montivagus</i>	Ikeda <i>et al.</i> 2006; Sikes <i>et al.</i> 2006	443	23.53	<.0001	1.35
<i>N. vespilloides</i> and <i>N. defodiens</i>	Peck & Kaulbars 1987; Beninger & Peck 1992; Trumbo & Thomas 1998; Trumbo & Bloch 2000	794	6.09	<.0001	1.08
<i>N. obscurus</i> and <i>N. guttula</i>	Peck & Kaulbars 1987; Walker & Hoback 2007	187	7.81	<.0001	1.25
<i>N. apo</i> and <i>N. nepalensis</i>	Růžička <i>et al.</i> 2002; Sikes <i>et al.</i> 2006	611	3.98	<.0001	1.08
<i>N. hybridus</i> and <i>N. tomentosus</i>	Peck & Kaulbars 1987; Sikes <i>et al.</i> 2008	298	15.31	<.0001	1.28

Table 1.2. ANOVA (Analysis of Variance) results for the *t*-tests on the mean pronotal widths for each pair of parapatric sister species. The factor difference between the mean pronotal widths for each pair is also included.

Parapatric Sister Species	Sources	df	t-Value	p-Value	Factor Difference
<i>N. germanicus</i> and <i>N. morio</i>	Růžička <i>et al.</i> 2002; Sikes <i>et al.</i> 2002; Dekeirsschieter <i>et al.</i> 2011	631	7.87	<.0001	1.09
<i>N. mexicanus</i> and <i>N. nigrita</i>	Peck & Anderson 1985; Peck & Kaulbars 1987; Marquez <i>et al.</i> 2015	321	3.71	0.0002	1.05
<i>N. didymus</i> and <i>N. scrutator</i>	Peck & Anderson 1985; Sikes & Chaboo 2015	168	8.31	<.0001	1.18
<i>N. olidus</i> and <i>N. quadrimaculatus</i>	Peck & Anderson 1985; Marquez <i>et al.</i> 2015	120	2.77	0.0066	1.07

Table 1.3 ANOVA (Analysis of Variance) results for the t-tests on the mean pronotal widths for each pair of allopatric sister species. The factor difference between the mean pronotal widths for each pair is also included.

Allopatric Sister Species	Sources	df	t-Value	p-Value	Factor Difference
<i>N. dauricus</i> and <i>N. antennatus</i>	Tezcan & Háva 2001; Guéorguiev & Růžička 2002; Růžička <i>et al.</i> 2002	579	4.38	<.0001	1.04
<i>N. quadripunctatus</i> and <i>N. melissae</i>	Sikes <i>et al.</i> 2006; Růžička <i>et al.</i> 2011	457	1.25	0.2107	1.02
<i>N. insularis</i> and <i>N. kieticus</i>	Peck 2001; Sikes <i>et al.</i> 2006; Sikes & Mousseau 2013	158	2.99	0.0032	1.05
<i>N. oberthuri</i> and <i>N. lunatus</i>	Růžička <i>et al.</i> 2002; Sikes <i>et al.</i> 2002	118	8.35	<.0001	1.25
<i>N. pustulatus</i> and <i>N. hispaniola</i>	Peck & Kaulbars 1987; Sikes & Peck 2000	90	1.89	0.0622	1.09
<i>N. encaustus</i> and <i>N. investigator</i>	Peck & Kaulbars 1987; Sikes <i>et al.</i> 2008; Růžička <i>et al.</i> 2011	955	3.99	<.0001	1.16
<i>N. argutor</i> and <i>N. sepultor</i>	Růžička <i>et al.</i> 2002; Sikes <i>et al.</i> 2008; Dekeirsschieter <i>et al.</i> 2011	661	11.38	<.0001	1.13

Table 1.4 Mean pronotal width, range of pronotal widths in the sample, number of specimens measured, and size category (small, medium, or large) for all 70 burying beetle species measured for this study

Species	Mean Pronotum Width (mm)	Range (mm)	Sample Size	Size Category
<i>N. concolor</i>	10.97	7.95 – 13.64	332	Large
<i>N. americanus</i>	10.56	8.02 – 12.51	50	Large
<i>N. satanus</i>	10.46	8.49 – 12.11	37	Large
<i>N. germanicus</i>	9.83	5.99 – 13.19	366	Large
<i>N. morio</i>	9.03	5.94 – 11.34	266	Large
<i>N. carolinus</i>	7.88	5.23 – 10.47	136	Medium
<i>N. japonicus</i>	7.24	5.09 – 10.05	183	Medium
<i>N. obscurus</i>	7.23	4.38 – 9.52	29	Medium
<i>N. distinctus</i>	7.14	5.17 – 8.77	38	Medium
<i>N. przewalskii</i>	7.13	6.01 – 7.90	13	Medium
<i>N. validus</i>	7.06	5.75 – 8.64	9	Medium
<i>N. scrutator</i>	7.06	4.96 – 9.22	61	Medium
<i>N. humator</i>	7.00	4.01 – 9.32	673	Medium
<i>N. pustulatus</i>	6.88	4.63 – 8.83	81	Medium
<i>N. lunatus</i>	6.81	4.78 – 8.71	84	Medium
<i>N. hybridus</i>	6.77	4.80 – 8.95	58	Medium
<i>N. argutor</i>	6.73	4.29 – 8.97	381	Medium
<i>N. marginatus</i>	6.59	4.29 – 9.11	216	Medium
<i>N. ussuriensis</i>	6.56	5.61 – 7.30	8	Medium
<i>N. lethaeus</i>	6.47	6.31 – 6.79	3	Medium
<i>N. orbicollis</i>	6.43	4.49 – 8.68	454	Medium
<i>N. hispaniola</i>	6.34	5.50 – 7.55	11	Small
<i>N. nigrata</i>	6.33	3.88 – 7.69	181	Small
<i>N. nigricornis</i>	6.30	4.31 – 8.02	82	Small
<i>N. vestigator</i>	6.23	3.26 – 8.38	294	Small
<i>N. dauricus</i>	6.09	4.08 – 7.87	285	Small
<i>N. mexicanus</i>	6.01	3.86 – 7.96	142	Small
<i>N. reticulatus</i>	6.00	4.87 – 6.60	4	Small
<i>N. mongolicus</i>	5.97	4.22 – 7.23	109	Small
<i>N. sepultor</i>	5.96	3.98 – 7.78	282	Small
<i>N. semenowi</i>	5.96	4.96 – 6.88	29	Small
<i>N. didymus</i>	5.96	4.00 – 7.48	109	Small
<i>N. podagricus</i>	5.92	4.35 – 7.35	112	Small
<i>N. tenuipes</i>	5.91	3.64 – 7.21	201	Small
<i>N. investigator</i>	5.87	3.85 – 7.89	946	Small
<i>N. sayi</i>	5.84	4.02 – 7.70	24	Small
<i>N. antennatus</i>	5.82	3.84 – 7.58	296	Small
<i>N. guttula</i>	5.76	3.35 – 8.17	160	Small

<i>N. vespillo</i>	5.74	3.41 – 7.96	632	Small
<i>N. basalis</i>	5.72	3.78 – 7.41	166	Small
<i>N. sepulchralis</i>	5.70	4.22 – 7.17	73	Small
<i>N. efferens</i>	5.70	5.02 – 6.42	6	Small
<i>N. Charon</i>	5.65	4.70 – 7.00	18	Small
<i>N. maculifrons</i>	5.64	4.00 – 7.39	312	Small
<i>N. heurni</i>	5.58	4.24 – 6.87	143	Small
<i>N. trumboi</i>	5.58	4.56 – 6.35	17	Small
<i>N. interruptus</i>	5.53	2.93 – 7.59	651	Small
<i>N. herscheli</i>	5.48	4.66 – 6.54	9	Small
<i>N. oberthuri</i>	5.46	4.47 – 7.02	36	Small
<i>N. chilensis</i>	5.44	4.22 – 6.30	14	Small
<i>N. defodiens</i>	5.39	3.74 – 7.05	83	Small
<i>N. insularis</i>	5.38	4.23 – 6.45	91	Small
<i>N. hebes</i>	5.35	3.50 – 6.77	420	Small
<i>N. tomentosus</i>	5.27	3.61 – 6.93	242	Small
<i>N. nepalensis</i>	5.27	3.59 – 7.07	555	Small
<i>N. insignis</i>	5.25	4.31 – 6.42	18	Small
<i>N. sausai</i>	5.20	-	1	Small
<i>N. quadraticollis</i>	5.19	4.06 – 6.57	23	Small
<i>N. melissae</i>	5.18	3.66 – 6.26	47	Small
<i>N. schawalleri</i>	5.16	4.15 – 6.49	77	Small
<i>N. kieticus</i>	5.09	3.40 – 6.43	69	Small
<i>N. quadripunctatus</i>	5.06	3.40 – 6.77	412	Small
<i>N. quadrimaculatus</i>	5.06	4.41 – 5.64	20	Small
<i>N. encaustus</i>	5.05	4.36 – 5.93	11	Small
<i>N. vespilloides</i>	4.97	3.09 – 6.38	713	Small
<i>N. apo</i>	4.90	3.57 – 6.35	58	Small
<i>N. reichardti</i>	4.74	3.91 – 5.61	9	Small
<i>N. olidus</i>	4.71	3.38 – 6.18	102	Small
<i>N. smefarka</i>	4.34	2.80 – 5.50	28	Small
<i>N. montivagus</i>	4.15	2.96 – 5.21	133	Small

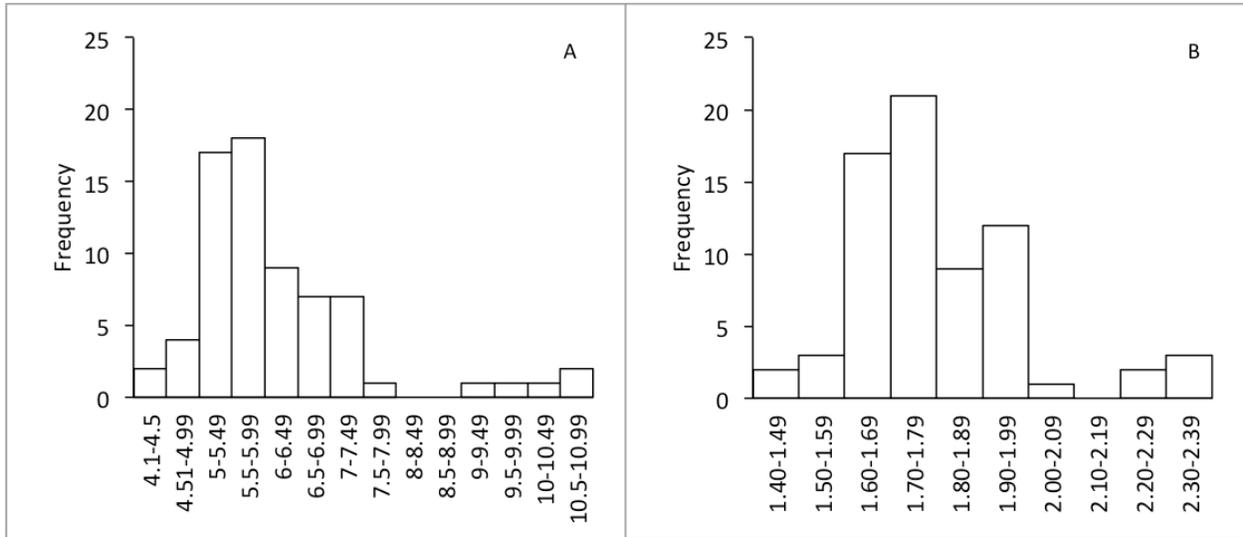


Figure 1.1 (A) Mean *Nicrophorus* species pronotal width (mm) distribution. (B) Log mean *Nicrophorus* species pronotal width distribution.

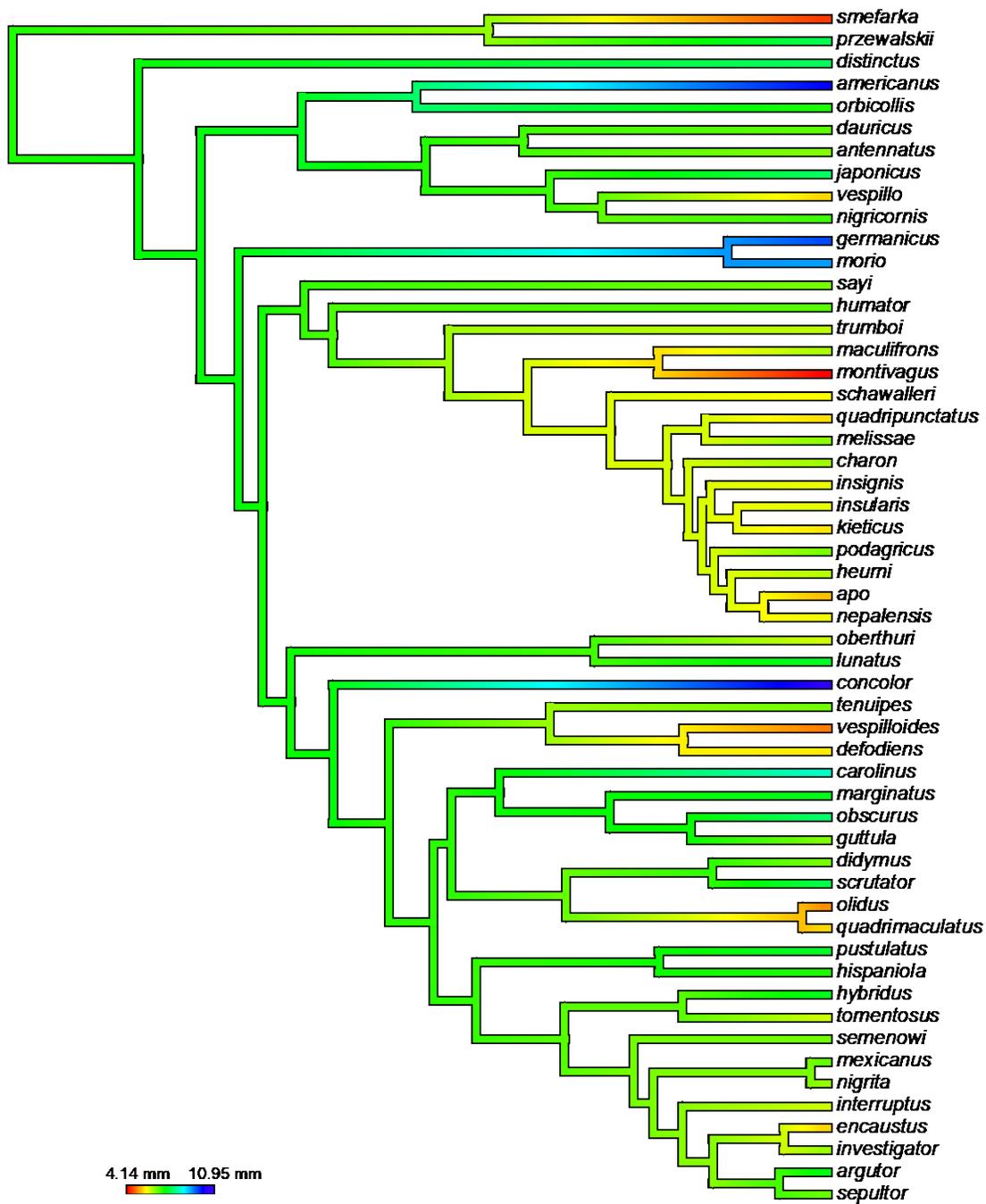


Figure 1.2 Mean species pronotal width mapped onto the existing molecular phylogeny by Sikes & Venables (2013).

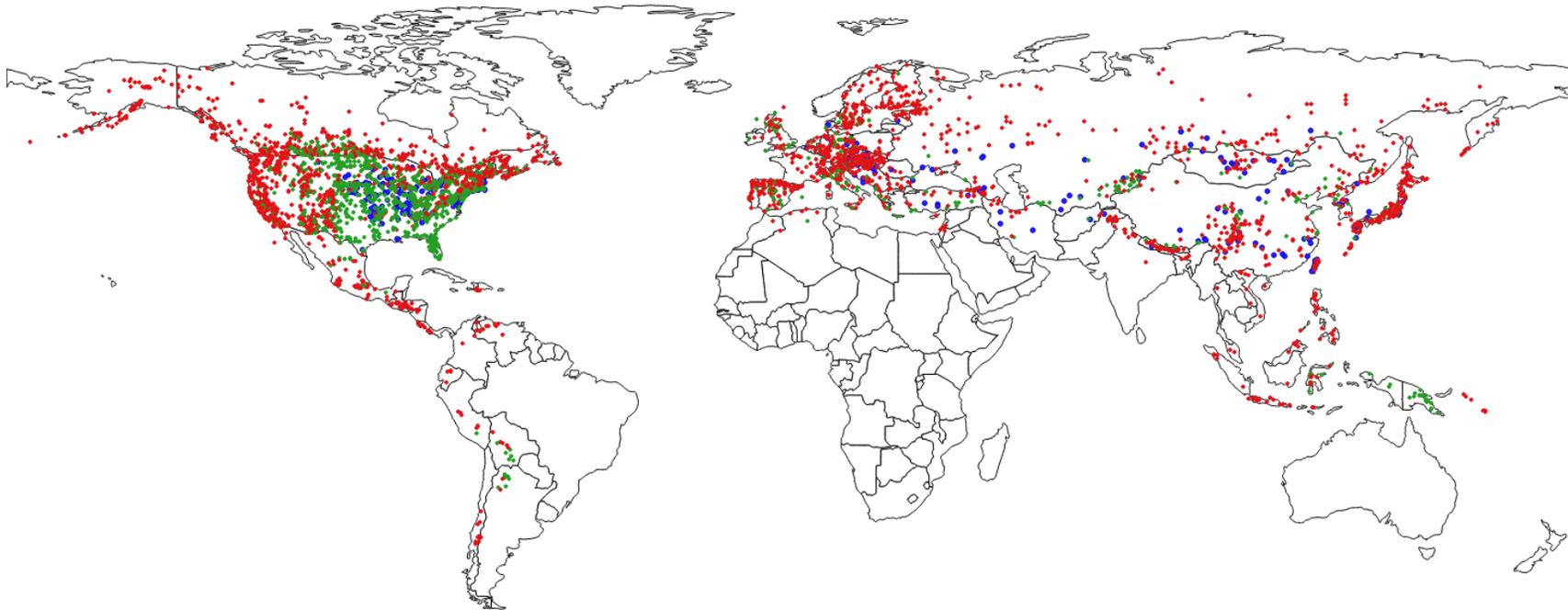


Figure 1.3 Geographical distribution of small (red, 4.14mm – 6.42mm pronotum width), medium (green, 6.43mm – 8.7mm pronotum width), and large (blue, 8.8mm – 11.0mm pronotum width) *Nicrophorus* species.

Chapter 2

Does Body Size Affect Fitness the Same Way in Males and Females?

A Test of Multiple Fitness Components

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Abstract

Body size generally has an important relationship with fitness, where larger body size leads to an increase in fitness through competition, reproductive output, and survivorship. However, the traits through which body size increases fitness often differ between the sexes. We tested for the effects of body size on fitness in both sexes using three separate experiments on competitive ability, reproductive output, and starvation resistance in the burying beetle *Nicrophorus marginatus*. Results varied between sexes as follows. 1) Larger body size increased competitive ability differentially between sexes. 2) Female body size, but not male body size, significantly affected reproductive output. Small females produced larger broods of smaller offspring, while large females produced smaller broods of larger offspring. 3) Large body size was positively associated with starvation resistance, but there was no differential response by sex. This study indicates that there is a complex relationship between body size, sex, and fitness, and that multiple fitness parameters need to be tested in both sexes to understand the evolution and importance of body size in a species.

Keywords: body size, burying beetle, competition, evolution, *Nicrophorus marginatus*, reproductive output, starvation resistance

Introduction

Body size of organisms is one of the most widely studied physical traits because of its influence on many behavioral, physiological, and life history traits (Roff, 1992). However, the diversity of pathways through which body size can affect fitness has been of particular interest. Fitness tends to increase with body size through sexual selection, an increase in reproductive success, increased viability, predator avoidance, and starvation resistance (Clutton-Brock, 1988; Blanckenhorn, 2000). Most studies have only addressed one pathway through which body size affects fitness (but see Amarillo-Suárez, Stillwell, & Fox, 2011 and Hsu & Soong, 2017 for exceptions), but single pathway studies cannot address the likely multivariate effect of body size on fitness.

Although there is a well-documented relationship between large body size and fitness, the degree and direction of this relationship often differs between males and females (Fairbairn *et al.*, 2007). Body size generally affects male fitness through male-male competition and sexual selection because females are usually the limiting sex, and therefore males must compete for access to females (Emlen & Oring, 1977; Clutton-Brock, 1983). However, reproductive output is more likely to vary with female body size through increased fecundity with larger body size (Emlen & Oring, 1977). Tests of sex-specific differences in survival have been mixed (e.g. Badyeav *et al.*, 2002; Råberg, Stjernman, & Nilsson, 2005; Husby *et al.*, 2006; Altwegg, Schaub, & Roulin, 2007; Cleasby *et al.*, 2010). Comprehensive tests on the importance of body size for multiple factors in both sexes are needed to understand the complex interactions that lead to the evolution of body size in males and females (Blanckenhorn, 2005).

In this study, we use the burying beetle *Nicrophorus marginatus* to test for the effect of body size on fitness in both sexes. Body size is closely linked to fitness through several aspects

of burying beetle natural history including: 1) Flight to locate carcasses for reproduction – larger beetles are able to fly at a broader range of temperatures (Merrick & Smith, 2004). 2) Competitions for carcasses for reproduction – larger beetles are more likely to win competitions for carcasses in both intra- and interspecific contests (Otronen, 1988; Eggert & Sakaluk, 2000). 3) Parental care - after winning access to a carcass, body size can affect the duration and extent of parental care (Smith, Belk, & Creighton, 2014; Steiger, 2013; Pilakouta *et al.*, 2015). 4) Reproductive output – body size has been linked to variation in brood size in both sexes (Hopwood *et al.*, 2016; Rauter *et al.*, 2010). 5) Survival - body size has been shown to affect overwinter survival and starvation resistance (Smith, 2002; Trumbo & Xhahani, 2015). Thus, there are several different ways that body size can affect fitness in burying beetles, but some of these factors might act differently in males and females.

The goal of this study was to determine whether body size is equally important for multiple aspects of fitness in both sexes in *N. marginatus*. Body size is likely to affect both males and females through competitions for carcasses, reproductive output, and starvation resistance in this species. We tested for these effects through three separate experiments that used 1) competitive trials between beetles of varying sizes, 2) tests of reproductive output of large and small beetles on large and small carcasses, and 3) tests of time to death under starvation conditions for a range of body sizes. For each of these traits, the sexes could be affected equally, differently, or not at all. We predicted that body size would be equally important for males and females for all three of these traits due to their similar natural histories and their engagement in biparental care. However, our results show a range of responses to these factors, indicating a complex relationship between body size and fitness between sexes in this species.

Methods

Source of Burying Beetles

Adult *N. marginatus* used to generate the laboratory population were captured at the Utah Wetland Preserve near Goshen, Utah in August 2014, July 2015, and July 2016 using pitfall traps baited with aged chicken. Wild-caught pairs were placed on 40g mouse carcasses and allowed to breed to generate the laboratory population. We created 41, 55, and 33 independent genetic lines from wild-caught pairs in 2014, 2015, and 2016, respectively. The date of eclosion was designated as the first day of adult life for all laboratory-bred beetles. Individuals were placed in small plastic containers (15.6 x 11.6 x 6.7 cm), fed *ad libitum* raw chicken liver twice weekly, and maintained on a 14:10 h light:dark cycle at 21°C until they were used in experiments.

Experimental Design

Competition Experiment

The purpose of this experiment was to determine the effect of body size (for both sexes) on the outcome of competitions for carcasses. We began each trial by randomly choosing two beetles of the same sex that were not genetically related. We then determined the difference in their body sizes by measuring their pronotum widths (replicated three times) using digital calipers. (We used pronotum width rather than mass because it is a better predictor of the outcome of fights in *Nicrophorus* [Safryn & Scott, 2000]) Each pair was then assigned to a size difference category as follows: 1 = 0mm – 0.1mm difference; 2 = 0.11mm – 0.2mm difference; 3 = 0.21mm – 0.3mm difference; 4 = 0.31mm – 0.4mm difference; and 5 = 0.41mm – 0.5mm difference. We randomly chose one beetle from the competing pair to be marked with a small dot

of white paper corrector fluid on one of its elytra for identification. (Marking a beetle did not negatively affect the outcome of the competition. In males, the marked beetle won 56% of competitions, and in females the marked beetle won 47% of competitions.) The pair was then placed in a large plastic container (88.5 x 42 x 15.5 cm) with a mouse carcass weighing 27g-33g. The two beetles were initially placed in opposite corners of the container. We then observed the pair for thirty minutes and noted any interactions between the beetles to determine whether the more aggressive beetle was the ultimate winner of the contest. The more aggressive beetle only won access to the carcass about 50% of the time, so we did not analyze the aggressive interactions further. After thirty minutes of observation, the lid was placed on the container and the pair was left overnight. The following day, we checked the container and determined the winner of the competition as the individual in possession of the carcass. We completed 100 competition trials for each sex, 20 in each of the 5 size categories for both sexes, which gave us a total of 200 trials. In two trials, one in size category 1 and one in size category 4, both females were found on the carcass after 24 hours, so those trials were excluded from the analyses.

Reproduction Experiment

The purpose of this experiment was to determine how body size and carcass size affect reproductive output. We measured three response variables: final brood size, mean individual offspring mass, and total brood mass. We addressed this question by allowing large and small beetles to reproduce on large or small carcasses. We used the pronotum widths of beetles collected in the field in 2014 to determine mean size for each sex, and sizes that were between 1 and 2 standard deviations above and below the mean were considered large and small, respectively. The mean pronotum width of females was 6.67mm, with a standard deviation of

0.78mm (N = 50). The mean pronotum width of males was 6.87mm, with a standard deviation of 0.72mm (N = 50). Therefore, the size range of large and small female beetles used in this experiment was 7.44mm – 8.22mm and 5.11mm – 5.89mm, respectively. The size range of large and small male beetles was 7.60mm – 8.32mm and 5.42mm – 6.15mm, respectively. This experiment represented a fully crossed factorial design. There were four size treatments - large male with large female, large male with small female, small male with large female, and small male with small female. Each size treatment was crossed with both small (20g) and large (40g) carcasses, for a total of eight treatment combinations. Twelve replicates were completed for each of the eight treatments. One replicate (large male, small female, 20g carcass) yielded no offspring, so it was excluded from the statistical analyses.

We began each trial by choosing a genetically unrelated pair of beetles that fit into one of the size treatments. We recorded the initial mass and pronotum width of each beetle. The pair was placed in a small brood container (16.5 x 15 x 9cm) filled with 6cm of moist soil and given either a 20g (± 1.0 g) or a 40g (± 2.0 g) mouse carcass. The containers were kept in an environmental chamber at 21°C on a 14:10 h light:dark cycle. The brood containers were checked daily, and after larvae arrived on the carcass, the lid of the small brood container was removed and the container was placed in an abandonment chamber (37.5x25.5x14.5cm; see Smith *et al.*, 2014 for details). We used the abandonment chambers to create a more natural experimental design that allowed the parents to remain with the brood or abandon it as they normally would, but we did not analyze any of the parental abandonment data.

Abandonment chambers were checked daily and the number of larvae was recorded every day, but we only used the number of eclosed offspring in our analysis. We counted the larvae by carefully removing each larva from the carcass with forceps. The larvae were returned to the

carcass immediately after they were counted. The cups in each corner of the abandonment chambers were also checked daily to see if an adult had abandoned the brood. If a parent was found in a cup, its mass and the date were recorded, and it was placed back in the small container with the brood. If a parent abandoned the brood for a second time, it was removed. When the larvae dispersed into the soil, the remaining parent(s) were removed and weighed. The larvae from each brood reached eclosion 4-5 weeks after dispersal. Number of newly-eclosed adult offspring was used to determine total number of offspring. Each newly-eclosed adult offspring was weighed, and we used these data to calculate the mean individual offspring mass and total offspring mass produced.

Starvation Resistance Experiment

The purpose of this experiment was to determine the effect of body size on starvation resistance. We began this experiment by placing 20 sexually mature *N. marginatus* pairs on 40g (± 2.0 g) carcasses and allowing them to reproduce. When larvae dispersed, each individual larva's mass was recorded and three larvae from each brood (the heaviest, the lightest, and one from the middle of the range) were placed individually in small containers with moist soil. The larvae were allowed to complete development, and their mass, sex, pronotum width, and date of eclosion were recorded. The eclosed offspring were not given any food, and were checked daily to determine when they died. Four beetles, one small, one medium, and two large, did not develop and died in the pupal stage, which gave us a total of 56 replicates (24 females and 32 males) for this experiment.

Statistical Analyses

Competition Experiment

To test for the probability of winning a carcass, we used a generalized linear model with a logit link function and assumed a binomial distribution of the response variable (Proc GENMOD in SAS; SAS 9.3 SAS Institute, Cary, North Carolina, USA). The data did not exhibit overdispersion (Harrison, 2015). We modeled the probability that the smaller beetle would win a competitive interaction. The response variable was either success (coded as 1) or failure (coded as 0) of the smaller beetle to win the competitive bout. Predictor variables were size difference (5 levels) and sex (2 levels), and we included the interaction between size and sex. The model also included pronotum width of the smaller beetle as a covariate to determine if absolute size influenced the competitive outcome.

Reproduction Experiment

To determine effects of body size on reproductive output, we used three response variables: final brood size (the number of eclosed adult offspring), total offspring mass, and individual offspring mass. In the competition experiment and in the starvation resistance experiment we used pronotum width as our measure of body size. In this experiment, we used pronotum width as our measure of body size for the parents, but we used mass of offspring as our measure of the response. We felt mass of offspring better reflected the energetic investment in offspring, and there is a strong correlation between body mass and pronotum width ($R^2 = 0.92$), so they are interchangeable as response variables for this analysis. For brood size we used a generalized linear model with a log link function and assumed a Poisson distribution of the

response variable (the data did not exhibit overdispersion; Harrison, 2014; Proc GENMOD in SAS; SAS 9.3 SAS Institute, Cary, North Carolina, USA). For total offspring mass and mean offspring size, we used a general linear model to determine the effect of parental size and carcass size on reproductive output (Proc GLM in SAS; SAS 9.3 SAS Institute, Cary, North Carolina, USA). For analysis of each response variable we used the same set of predictor variables: female parent body size (2 levels), male parent body size (2 levels), and carcass size (2 levels). The model also included all two-way and three-way interactions. We evaluated total offspring mass and individual offspring mass for normality of residuals and equal variances across treatment combinations by inspecting the distribution of residuals and studentized residuals versus predicted values. Untransformed data met these assumptions for these two response variables. For brood size, the log-link function satisfied the assumptions of normality of residuals and equal variances across treatment combinations. For each of the response variables, we tested the full model that included all interactions. Interactions were not significant for any of the three models. We removed all interaction terms and compared the AIC score from the model with only main effects to the full model. The reduced model exhibited better fit in all three cases, so we report results from the reduced model.

Starvation Resistance Experiment

We used a generalized linear model and assumed a Poisson distribution of the response variable (the data exhibited no overdispersion; Harrison, 2014). The response variable was number of days to starvation, and the predictor variables were pronotum width, sex, and their interaction. We also included the parental pair number as a random effect to control for family effects. We used the *glmer* function and the *blmeco* package (Korner-Nievergelt *et al.*, 2015) in

Program R 3.3.3 (R Core Team, 2013) for this analysis.

Results

Competition Experiment

Sex and the size difference between beetles significantly affected the probability of the smaller beetle winning a competitive interaction, but the pronotum width of the smaller competitor and the interaction between sex and size difference did not have significant effects on the outcome of competitions (Table 2.1). In males, the probability of the smaller beetle winning the competition decreased from 39% (not statistically different from 50%) in the smallest size difference category to 0% in the largest size difference category (Fig. 2.1). However in females, the probability of the smaller beetle winning the competition decreased from 42% (not statistically different from 50%) in the smallest size difference category to only 20% in the largest size difference category (Fig. 2.1). Overall, smaller males are 17% less likely to win in competitions than are smaller females across the entire range of size differences tested.

Reproduction Experiment

Parents produced significantly more offspring on 40g carcasses than on 20g carcasses and female size had a moderately significant effect on final brood size, but male size did not significantly affect final brood size (Table 2.2). Females produced about 42% more offspring (about 5 individuals) on 40g carcasses compared to 20g carcasses. Small females produced about 14% more offspring (2 individuals) in a brood than large females across both carcass sizes (Fig. 2.2A).

There were significant differences in mean individual offspring mass between carcass sizes, and the mass of the female parent had a significant effect, but male size did not (Table 2.2). Parents produced individual offspring that were about 19% heavier on 40g carcasses compared to 20g carcasses. Large females produced individual offspring that were about 8% heavier than those produced by small females (Fig. 2.2B).

There were significant differences in total offspring mass of a brood between carcass sizes, but male size, and female size did not have a significant effect (Table 2.2). Parents produced broods that were about 43% heavier on 40g carcasses compared to 20g carcasses (Fig. 2.2C).

Starvation Resistance Experiment

There were significant differences in starvation resistance according to pronotum width, but sex and the interaction between pronotum width and sex were not significant (Table 2.3). On average, the largest beetles of both sexes lived about 13 days without food, while the smallest beetles of both sexes lived about 2 days without food (Fig. 2.3).

Discussion

The results of the experiments in this study indicate that the pathways through which body size affects fitness differs between the sexes. In *N. marginatus*, large body size is important for fitness through competitions for carcasses in both sexes, but body size seems to be more important for males than for females. In contrast, female body size is related to the individual mass of offspring and number of offspring produced, whereas male body size is not related to

reproductive output. Finally, large body size affects starvation resistance in both sexes. These results are consistent with previous research that has shown that male body size is important in intrasexual competitions (Emlen & Oring, 1977; Clutton-Brock, 1983), but female body size affects reproductive output (Emlen & Oring, 1977). The unique natural history of burying beetles likely influences the similarities and differences through which body size affects fitness in each sex, which we discuss below.

Larger individuals of both sexes have more success at winning access to carcasses (Fig. 2.1). However in competitions between males, the larger individual is more likely to win compared to competitions between females. Therefore, body size seems to be more important for competitions in males than in females. This is consistent with the results of Scott & Traniello (1990), which showed that male *N. orbicollis* that successfully won a carcass were larger than the rest of the population, but there was no difference in body size of females that won a carcass compared to those that did not. Body size may be more important for males than for females in competing for carcasses because in burying beetles, females mate multiply (Eggert, 1992) and use sperm from previous matings to fertilize some of their eggs (Müller & Eggert, 1989), which results in approximately 15% of the offspring being sired by non-resident males (Müller *et al.*, 2007). Therefore, winning competitive interactions may be more important for males because they can achieve a much greater fitness if they are the dominant male on the carcass. Competitions may be less important for female fitness because they are able to produce offspring alone on a carcass with stored sperm, by finding a male on a carcass and producing offspring biparentally (Eggert & Müller, 1997), or by parasitizing another female's brood (Trumbo, 1994).

Nicrophorus marginatus is a facultatively biparental species, and therefore we would expect that the body size of both parents would affect offspring size. However, we found that

female, but not male, body size had a significant effect on this trait. The lack of an effect of male body size on both the number of offspring produced and the size of offspring might be due to their relatively minor role in parental care. Research on other burying beetle species has shown that females are more involved in direct care of offspring (Smiseth *et al.*, 2005; Parker *et al.*, 2015), and suggests that males remain with the brood to provide care if the female dies before the larvae are independent (Parker *et al.*, 2015). In support of this, handicapping and mate removal experiments have shown that males increase their effort when their partner is handicapped or removed, but females generally do not respond (Rauter & Moore, 2004; Smiseth *et al.*, 2005; Suzuki & Nagano, 2009; Creighton *et al.*, 2015). Additionally, males are less effective parents when they care for offspring uniparentally (Smith *et al.*, 2015). Therefore, male body size might not affect reproductive output because males are less actively involved in caring for offspring than females.

Both large and small females had larger broods on 40g carcasses compared to 20g carcasses, but large females produced significantly larger and slightly fewer offspring in their broods. Large female insects commonly produce larger eggs that then hatch into larger offspring (e.g. Steiger, 2013; Kojima 2015). Stegier (2013) found that *N. vespilloides* offspring tend to be similar in size to their mothers through transgenerational effects, which could also affect offspring size in *N. marginatus*. However, the largest effect on offspring size is carcass size, so it is possible that larger offspring are simply the result of more food resources from the carcass. However, it is unclear from our results whether the increase in offspring size is due to transgenerational effects of maternal size, additional resources from the larger carcass, or an interaction between those two factors.

Although we found that both large and small female *N. marginatus* produce more and larger offspring on larger carcasses, Hopwood *et al.* (2016) found that in *N. vespilloides*, large beetles had a reproductive advantage over small beetles on large carcasses, but small beetles had a reproductive advantage over large beetles on small carcasses. The opposing results of the two studies may be due to differences in the body sizes of these species compared to their competitors. In comparison with other burying beetle species that share the same habitat, *N. vespilloides* are small and *N. marginatus* are large (Scott, 1998). Different species of burying beetles are assumed to be able to coexist through resource partitioning according to body size (Scott, 1998; Ikeda *et al.*, 2006), but there is overlap in the sizes of carcasses that different species will use (Scott, 1998; Myers, 2014). Because large beetles typically win in competitions for carcasses (Otronen, 1988; Safryn & Scott, 2000; Lee *et al.*, 2014), they could exclude small beetles from the upper end of their carcass range, making small beetles more specialized in their carcass size use. Therefore, a broad range of carcass sizes may be in the natural history profile of *N. marginatus*, but not *N. vespilloides*, which in turn could cause the differences in the reproductive output between these two species seen on large and small carcasses.

In this study, larger beetles of both sexes lived longer after eclosion without food than small beetles. This is consistent with the results of a similar experiment done on *N. orbicollis* (Trumbo & Xhiani, 2015). Larger beetles may be able to survive without food because they have higher fat reserves. In other species of insects, fat content is positively correlated with body size (e.g. Strohm, 2000; O'Neill, Delphia, & O'Neill, 2014), which may allow large individuals to survive without food longer than small individuals. Although burying beetles can feed on a number of different types of carrion, carcasses are nutrient rich but ephemeral resources (Eggert & Müller, 1997), so the frequency at which burying beetles might encounter them is likely to be

unpredictable. Directly after eclosion larger beetles are able to withstand starvation for longer periods of time before finding a carcass, which makes them more likely to survive when environmental conditions are unfavorable.

For both males and females the advantage of body size in competitions, reproductive output, and starvation resistance might be context-dependent (Eggert & Müller, 1997) because population sizes and carcass availabilities are likely to fluctuate both within and between seasons (Scott, 1998; Smith *et al.*, 2000). Insect population sizes tend to fluctuate with their available resources (Dempster & Pollard, 1981), and burying beetles are no exception. Population densities of *N. investigator* fluctuate yearly (Smith *et al.*, 2000), and changes correspond with small rodent biomass in the previous year (Smith & Merrick, 2001). A broad range of body sizes exists in natural populations of burying beetles (e.g. Creighton 2005), and the context-dependent importance of body size could maintain these body size ranges. In dense populations, *N. vespilloides* that won access to carcasses were larger on average than individuals that were captured in pitfall traps (Otronen, 1988), but in low-density populations of *N. orbicollis*, there were no differences in body size between breeding and non-breeding beetles (Trumbo, 1990). Additionally, *N. orbicollis* parents adjust brood size according to population density, and raise fewer, larger offspring when competition is high (Creighton, 2005). However, when population density is low, parents produce more small offspring (Creighton, 2005), which is assumed to be because body size is less important when competition for carcasses is low. Larger offspring produced by larger females might only have an advantage when competition for carcasses is high.

The results of these experiments show a range of responses for the effect of body size on fitness. Body size is more important for males in competitions for carcasses, more important for

females in reproductive output, and equally important for males and females in starvation resistance. Therefore, looking at the importance of body size in only one sex or in relation to only one fitness parameter might not be sufficient to understand the complex interactions that lead to the evolution of this trait.

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Table 2.1 Competition experiment analysis of Variance (ANOVA) table for the effects of size difference, sex, the interaction between sex and size difference, and the pronotum width of the smaller competitor on the outcome of a competitive interaction. Significant values are shown in bold.

Response Variable	Source	Num df/ Den df	Chi-Square	p-Value
Small beetle wins (1), or loses (2)	size difference	4/177	4.38	0.002
	sex	1/177	8.96	0.003
	sex*size difference	4/177	1.26	0.29
	small beetle pronotum width	1/177	0.53	0.47

Table 2.2 Reproduction experiment analysis table for final brood size, total offspring mass, and mean offspring mass. Significant values are shown in bold.

Response Variable	Source	Num df/ Den df	F Value	<i>p</i>-Value
Final Brood Size	carcass size	1/91	21.41	< .0001
	male size	1/91	0.33	0.56
	female size	1/91	3.52	0.06
Mean Offspring Mass (g)	carcass size	1/91	29.86	< .0001
	male size	1/91	0.95	0.33
	female size	1/91	4.14	0.045
Total Offspring Mass (g)	carcass size	1/91	58.24	< .0001
	male size	1/91	0.02	0.89
	female size	1/91	1.5	0.22

Table 2.3 Starvation resistance analysis of Variance (ANOVA) table for days to death.

Significant values are shown in bold.

Response Variable	Source	Num df/ Den df	z Value	p-Value
Days to Death	pronotum width	1/50	1.29	<0.0001
	sex	1/50	5.06	0.1967
	pronotumwidth*sex	2/50	1.37	0.1700

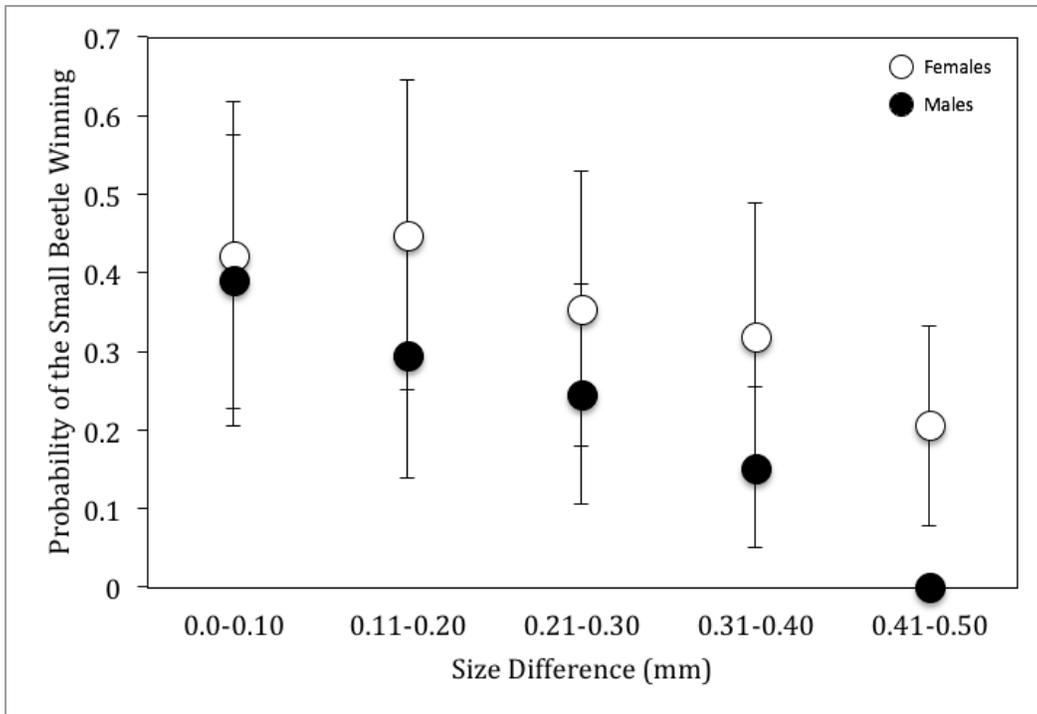


Figure 2.1 LSMeans (+/- 95% confidence interval) for the probability of the smaller beetle winning a competition according to size difference category and sex.

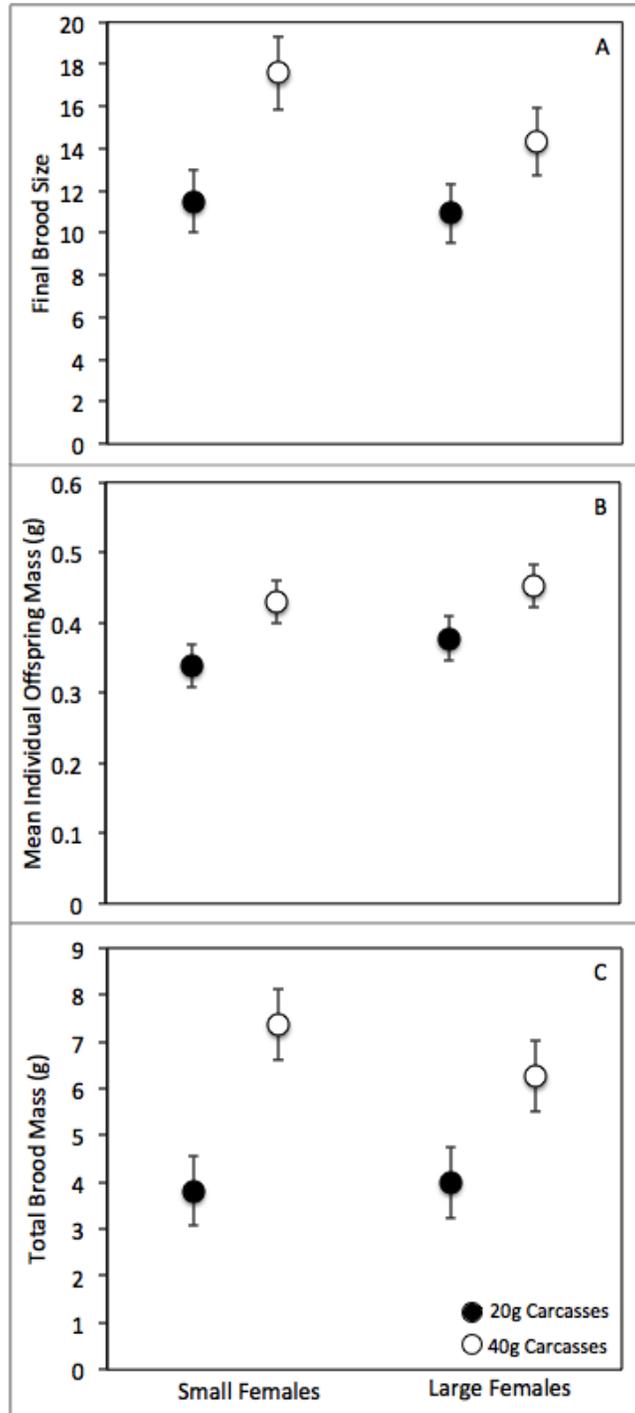


Figure 2.2 Mean (+/- 95% confidence interval) final brood size (A), individual offspring mass (B), and total brood mass (C) size produced by small and large females on both carcass sizes.

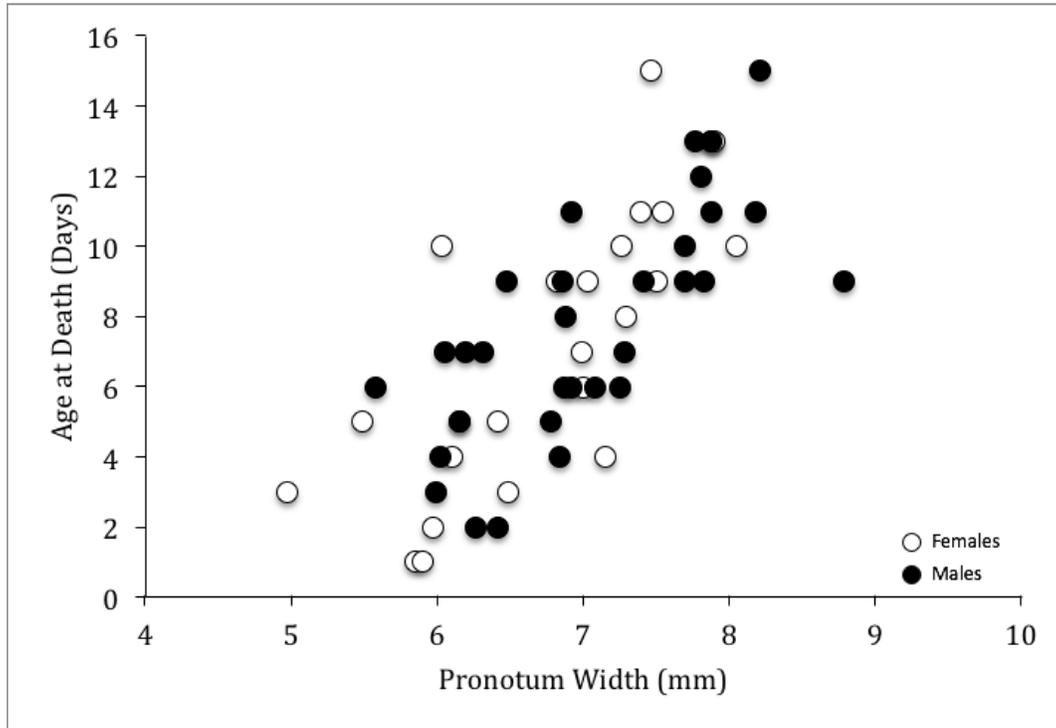


Figure 2.3 Age at death in days according to pronotum width for both sexes.

Chapter 3

Fitness Consequences of Multigenerational Effects in Burying Beetles

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Abstract

Multigenerational effects are changes in the behavior or physiology of offspring that are the result of parental state or environmental conditions. These effects are widespread in nature, and are often studied because of their important effects on offspring traits. Although multigenerational effects are commonly reported, few researchers have addressed whether they affect offspring fitness, or if they just result in benign variation in the next generation. In this study, we used the burying beetle *Nicrophorus marginatus* to test for differential offspring fitness as a result of multigenerational effects on offspring size. In a previous experiment, we showed that parental size and carcass size affect offspring body size, with larger females producing larger offspring and larger offspring being produced on larger carcasses. In this study, we measured the lifetime fitness of those offspring to determine whether the larger offspring also had increased lifetime fitness, as measured by their total number of offspring produced. We found that larger offspring produced in the previous experiment had an increased lifetime fitness compared to small offspring. Therefore, the multigenerational effects of body size in burying beetles cause differential offspring fitness.

Introduction

Multigenerational effects are components of the offspring phenotype that are the result of the parental phenotype and the parental environment as opposed to direct genetic effects (Rutledge *et al.* 1972; Kirkpatrick & Lande 1989; Rossiter 1996; Marshall & Uller 2007). These effects are widespread in nature and have been extensively studied in invertebrates (Bernardo 1996; Rossiter 1996; Mousseau & Fox 1998). Multigenerational effects have been reported in response to such factors as nutrients, egg size, the presence of predators, maternal age, maternal body size, and disease resistance (Fox *et al.* 1997; Mousseau & Fox 1998; Boots & Roberts 2012; Steiger 2013; Qazi *et al.* 2017). The phenotypic changes that occur in offspring as a result of multigenerational effects include, but are not limited to, changes in body size, age at maturity, developmental rate, dispersal, survival, lifespan, and diapause (Vinogradova & Reznik 2002; Prasad *et al.* 2003; Opit & Throne 2007; Singh 2009; Smallegange 2011; Ducatez *et al.* 2012; Mestre & Bonte 2012; Vargas *et al.* 2012).

One of the most frequently reported multigenerational effects is variation in body size. Body size is well studied because of its strong, positive relationship with fitness (Clutton-Brock 1988; Reiss 1989), and because an individual's size is typically easy to measure. Parental body size has been shown to cause multigenerational effects in offspring where larger females lay larger eggs (Berger 1989; Kim 1997; Fox & Savalli 1998; Wainhouse *et al.* 2001; Fischer *et al.* 2002; Vargas *et al.* 2013; Steiger 2013; Kojima 2015), which then hatch into larger offspring. Natal environment can also cause multigenerational effects of body size. On poor quality food resources, females tend to lay fewer, but larger eggs (Scott & Traniello 1990; Fox *et al.* 1997). There is also a positive correlation between resource availability and offspring size (Bartlett & Ashworth 1988). In crowded environments with more competition for resources, females tend to

lay fewer, larger eggs, which theoretically gives offspring a size advantage when competing for resources (Kawecki 1995; Visser 1996; Creighton 2005). Although there is ample evidence for multigenerational effects that affect offspring body size, it is unclear whether those effects translate into increases in offspring fitness.

The overall fitness of an individual can be measured as the total number of offspring produced over a lifetime (McGregor *et al.* 1981; MacColl & Hatchwell 2004). Estimating fitness in natural populations is difficult (Lewontin 1974; Allendorf & Leary 1986; Endler 1986; Wang *et al.* 2002), and many researchers use discrete traits such as body size, ejaculate size, and egg load as predictors of lifetime fitness (Bartlett & Ashworth 1988; Jervis & Ferns 2004; South & Lewis 2011; Kant *et al.* 2012). However, the link between these parameters and lifetime fitness is generally assumed but not empirically tested (ie, Langley *et al.* 1978; Gustafsson *et al.* 2005; Paquet & Smiseth 2017). Recent research has shown that increased offspring body size due to multigenerational effects does not necessarily lead to increase in fitness of offspring (Coakley *et al.* 2017), suggesting that the relationship between multigenerational effects and resulting fitness should be examined empirically.

Burying beetles (genus *Nicrophorus*) are ideal model organisms for studies of multigenerational effects of body size. These species use small vertebrate carcasses for food and reproduction, and the carcass serves as the sole food resource for both parents and offspring during reproduction (Scott & Traniello 1990). The parents provide their offspring extensive parental care in the form of regurgitating predigested carrion and defending the larvae from intruders (Fetherston *et al.* 1990; Rauter & Moore 1999). Adult body size is determined by the amount of carrion that an individual consumes as a larva, and parents cull the brood through filial cannibalism so that the brood size matches the size of the carcass (Scott & Traniello 1990;

Trumbo 2006), resulting in a positive correlation between offspring number and carcass size (Scott & Traniello 1990; Creighton 2005). Parents also cull the brood according to their competitive environment so that they raise fewer, larger offspring when competition for carcasses is high, and more, smaller offspring when competition is low (Creighton 2005). Body size is important for competitive interactions because inter- and intraspecific competitions for carcasses can be intense (Otronen 1988; Scott 1990, 1994; Eggert & Sakaluk 2000), and body size generally determines the winners of competitions for both males and females (Bartlett & Ashworth 1988; Otronen 1988; Müller *et al.* 1990; Safryn & Scott 2000; Hopwood *et al.* 2013; Lee *et al.* 2014; Smith & Belk 2018; Dissertation Chapter 2).

Previous studies in burying beetles have demonstrated multigenerational effects such as larger offspring being produced on larger carcasses (Scott & Traniello 1990; Smith & Belk 2018; Dissertation Chapter 2), larger mothers laying larger eggs, which hatch into larger offspring (Steiger 2013), young mothers producing smaller offspring (Lock *et al.* 2007), and mothers producing smaller offspring when the male parent is present (Paquet & Smiseth 2017). Body size and carcass size therefore seem to be the main modes through which multigenerational effects are transmitted. Larger females are more likely to win access to carcasses, and they are therefore more likely to win access to large, high quality carcasses. Large body size therefore allows females to attain more resources for their offspring. The amount of resources that a female burying beetle provides her offspring increases the size of her offspring (Scott & Traniello 1990; Smith & Belk 2018; Dissertation Chapter 2), so multigenerational effects on offspring size can be transmitted through both maternal size and carcass size.

In this study we tested whether multigenerational effects of parental size and natal carcass size that increase offspring size also cause an increase in lifetime offspring fitness in the

burying beetle *Nicrophorus marginatus*. We found that the multigenerational effects of parental body size and natal carcass size on offspring body size do cause differential fitness in offspring, as indicated by the total number of offspring that they produced over their lifetime.

Methods

Experimental Design

The purpose of this experiment was to determine whether the multigenerational effects of parent body size on offspring body size found by Smith and Belk (*In Review; Dissertation Chapter 2*) affect offspring fitness. We used the offspring of the pairs from the previous experiment to address this question (see Smith & Belk *In Review* for a detailed description of the experimental design used for the parental generation). The parental reproductive treatments in Smith and Belk (*In Review; Dissertation Chapter 2*) were designed as fully crossed treatments combinations where pairs of large or small males and females were allowed to reproduce once on either a 20g or 40g carcass for a total of eight treatment combinations. For the current experiment, two female offspring were randomly chosen from each brood from the parental experiment, and were randomly assigned to reproduce on either 20g or 40g carcasses. Therefore, in this experiment there were 16 treatments – female offspring from each of the 8 parental treatments reproduced on one of two carcass sizes. We completed six replicates for each treatment. However, we were not able to use the data from five of the replicates (two on 20g carcasses and three on 40g carcasses) because the females in those replicates were mistakenly frozen and killed instead of the males before their natural deaths. Therefore, we did not have complete lifetime data for those females. This left us with a total of 91 replicates for this experiment.

We began each trial by choosing a genetically unrelated male for each female and measuring both beetles' pronotum width. The pair was placed in a brood container (14 x 13 x 17cm) filled with 10cm of moist soil and given either a 20g (± 1.0 g) or a 40g (± 2.0 g) mouse carcass, depending on the treatment. The containers were kept in an environmental chamber at 21°C on a 14:10 h light:dark cycle and were checked daily. The male was removed within 24 hours of larvae arriving on the carcass, and the female parent was left to raise the brood alone. When the larvae dispersed into the soil, the female was removed and weighed, then was set up to breed with a new male two days later. Each female reproduced in this way for the duration of her lifetime. The larvae from each brood reached eclosion 5-6 weeks after dispersal. Number of newly-eclosed adult offspring produced throughout each female's life was used as our measure of fitness and to test whether the multigenerational effects of parental body size on offspring body size found by Smith and Belk (*In Review*; Dissertation Chapter 2) translate into differential offspring fitness.

Statistical Analyses

We used lifetime number of offspring to determine whether multigenerational effects of parental body size and natal carcass size affect offspring fitness. There was one main effect in this analysis, reproductive carcass mass (2 levels), and one covariate, the female's pronotum width. We included brood ID as a random effect to account for non-independence of nest mates in the second generation. We also included the interaction between pronotum width and carcass mass. We used a generalized linear model with a log link function and assumed a Poisson distribution of the response variable for these analyses (Proc GLIMMIX in SAS)(SAS 9.3 SAS Institute, Cary, North Carolina, USA).

Results

Lifetime number of offspring differs significantly by reproductive carcass mass and female pronotum width, and the interaction between pronotum width and carcass mass is also significant (Table 3.1). Large females produced 35% more offspring than small females on 20g carcasses, and 6% more offspring than small females on 40g carcasses (Figure 3.1). Small females produced 34% more offspring on 40g carcasses than on 20g carcasses, but there was no difference in the number of offspring produced by large females on the two carcass sizes (Figure 3.1).

Discussion

In this study, we found that the multigenerational effects on body size that parental size and carcass size have on *N. marginatus* offspring size (Smith & Belk 2018; Dissertation Chapter 2) cause differential offspring fitness. Large females produced more offspring on small carcasses than did small females over their lifetimes, but small and large females had equal fitness on large carcasses (Figure 3.1). Multigenerational effects of resource quality and parental size on offspring size are common in invertebrates (e.g Boersma *et al.* 2000; Ekbom and Popov 2004; Amarillo-Suárez & Fox 2006; Kosal & Niedzlek-Feaver 2007; Steiger 2013). However, most studies do not test for changes in offspring fitness that result from multigenerational effects on offspring size (but see Yanagi & Miyatake 2002 and Pieters & Liess 2006 for exceptions). Our results show that in *N. marginatus*, multigenerational effects on offspring body size influence the progeny's lifetime fitness on different carcass sizes.

Small females may have lower lifetime fitness on small carcasses due to an increased cost of reproduction compared to large females. Reproduction is costly for burying beetles (Creighton *et al.* 2009), and it is possible that the energetic expenditure required for carcass maintenance and larval provisioning is more costly for small individuals. *Nicrophorus marginatus* parents produce fewer offspring on small carcasses (Smith & Belk 2018; Dissertation Chapter 2), so if they have fewer reproductive attempts due to higher reproductive costs, this could lead to lower fitness on small carcasses. However, this hypothesis is currently untested.

In the previous experiment (Smith & Belk 2018; Dissertation Chapter 2), body size did not have a significant effect on brood size, but in the current experiment, we found that body size had a significant effect on lifetime number of offspring produced (Table 3.1). This implies that a single reproductive bout may not be indicative of potential lifetime fitness. Similar discrepancies between tests of the costs and benefits of biparental care that use data from a single reproductive bout compared to lifetime reproduction in *N. orbicollis* have also been found (i.e. Benowitz & Moore 2016 and Smith *et al.* 2017). Thus, the influence of female body size on fitness may not be apparent until the costs of reproduction have accumulated over a lifetime.

The results of this experiment indicate that the multigenerational effect that influences offspring body size in *N. marginatus* cause differential lifetime fitness in offspring. Based on the results of our study, it seems likely that multigenerational effects that affect offspring size (Scott & Traniello 1990; Lock *et al.* 2007; Steiger 2013; Paquet & Smiseth 2017) would also cause differential changes in offspring fitness. These multigenerational effects are also likely to occur in natural populations of burying beetles through the likelihood of a large female winning access to a large carcass and thus producing larger offspring.

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Table 3.1 Analysis of variance (ANOVA) table for lifetime number of offspring. Significant effects are bolded.

Response Variable	Source	Num df/ Den df	F-Value	<i>p</i>-Value
Lifetime Number of Offspring	Pronotum Width	1/40	8.48	0.0058
	Carcass Mass	1/40	10.06	0.0029
	Pronotum Width*	1/40	7.14	0.0109
	Carcass Mass			

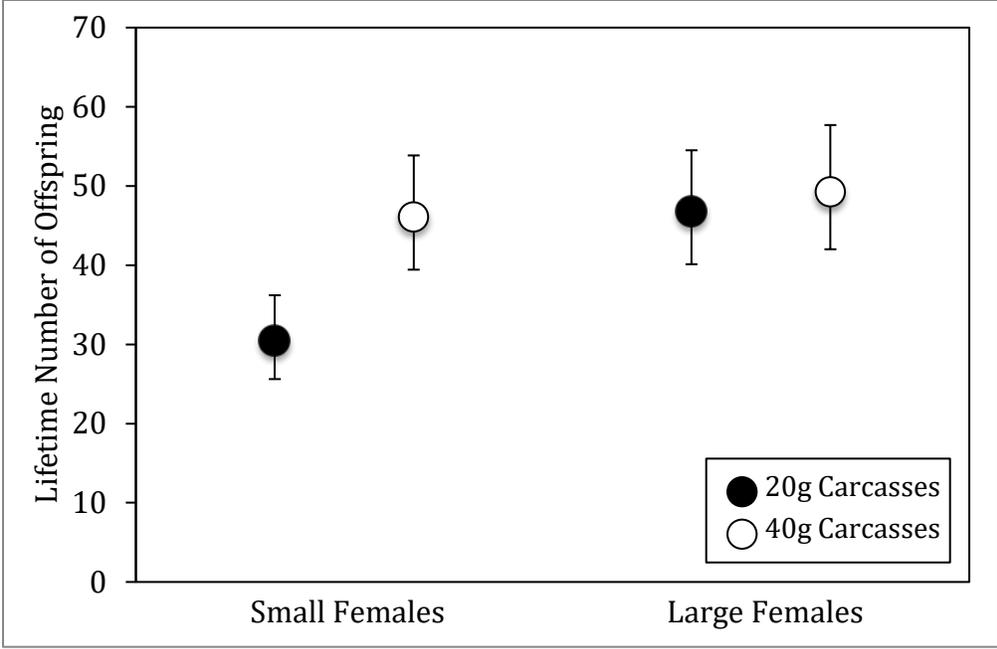


Figure 3.1 Mean (\pm 95% CI) Lifetime number of offspring produced by small and large females on 20g and 40g carcasses.

Chapter 4

Evidence for Interspecific Brood Parasite Detection and Removal in Burying Beetles

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Abstract

We tested whether brood parasitism could be successful between two co-occurring species of burying beetles, *Nicrophorus guttula* and *Nicrophorus marginatus*, and whether these species exhibit an adaptive response to brood parasitism by detecting and removing parasites. We cross-fostered larvae between broods of the two species and created mixed-species broods to simulate the addition of brood parasites. Brood parasites survived in both species' broods. *Nicrophorus marginatus* culled 86% of brood parasites compared to 56% of their own larvae; and *N. guttula* culled 50% of brood parasites compared to 22% of their own larvae. Additionally, *N. guttula* brood parasites were significantly smaller than *N. guttula* that were raised by *N. guttula* parents, but *N. marginatus* brood parasites were significantly larger than *N. marginatus* that were raised by *N. marginatus* parents. This paper provides the first evidence that burying beetles can discriminate between their own larvae and other species' larvae. We suggest that brood parasitism may be the selective force responsible for this ability.

Keywords: burying beetle, *Nicrophorus marginatus*, *Nicrophorus guttula*, interspecific brood parasitism

Introduction

Brood parasites directly affect the fitness of the host, typically by killing or competing with the host's offspring thus directing resources away from reproductive success of the host (González-Megías & Sánchez-Piñero 2003; Perry *et al.* 2003; Reader 2003; Garófalo *et al.* 2011; Manna & Hauber 2016). In response, hosts evolve mechanisms to avoid or ameliorate effects of brood parasites, and parasites evolve mechanisms to avoid detection (Kilner & Langmore 2011; Lorenzi *et al.* 2017). The result can be a co-evolutionary arms race between parasite and host where both species exhibit finely tuned reproductive behaviors (e.g. Marchetti 2000; Langmore *et al.* 2003; Soler 2009; Tizo-Pedroso & Del-Claro 2013; Mokkonen & Lindstedt 2016). Any species that provides protection or care to its young is susceptible to brood parasitism, but conditions that drive the evolution of brood parasitism seem to be narrow (Petrie & Møller 1991; Krüger & Davies 2002; Feeney *et al.* 2014; Zink & Lyon 2016). Brood parasitism may be a 'making-the-best-of-a-bad-job' strategy that females use when they do not have a nest site of their own or when the costs of rearing their own offspring are higher than the available resources (Petrie & Møller 1991; Goodell 2003; Reader 2003). For example, solitary bees are more likely to suffer from brood parasitism by cleptoparasitic wasps when resource levels are low (Goodell 2003). Additionally, brood parasitism may occur as a conditional tactic for when the availability of potential hosts is high, such as in treehoppers (Zink 2003).

Burying beetles (genus *Nicrophorus*) are potentially a model system for the study of the conditions that drive the evolution of brood parasitism. These species reproduce on small vertebrate carcasses, which serve as the sole source of food for both parents and offspring for the duration of the reproductive bout, during which parents provide facultative biparental care (Scott 1998) and cull the brood through filial cannibalism (Bartlett 1987; Scott & Traniello 1990;

Trumbo 2006) to produce a positive correlation between carcass size and offspring number (Bartlett 1987; Scott & Traniello 1990; Creighton 2005; Creighton *et al.* 2009). In most areas, several burying beetle species co-occur (Peck & Kaulbars 1987; Scott 1998), increasing competition for resources and interactions between individuals. Resource partitioning according to body size (Wilson *et al.* 1984; Trumbo 1990; Scott 1998; Ikeda *et al.* 2006) and seasonal and temporal variation in reproductive activity (Wilson *et al.* 1984; Scott 1998) may allow multiple species of burying beetles to coexist in the same areas.

Burying beetles are thought to be easy targets for both intraspecific and interspecific brood parasites. In these species, all larvae look similar, except for a size difference as larvae reach their third instar (Anderson 1982). Previous studies have shown that subordinate *N. vespilloides* females parasitize dominant females' broods of the same species (Müller *et al.* 1990), and females will accept the parasitic offspring as their own as long as the larvae do not hatch more than 8 hours before their own larvae should hatch (Müller & Eggert 1990; Eggert & Müller 2000; Eggert & Müller 2011). Interspecific brood parasitism has also been documented in burying beetles. *Nicrophorus pustulatus* can successfully parasitize *N. orbicollis* broods in the lab (Trumbo 1994), and the closely-related *Ptomascopus morio* is a brood parasite of *N. concolor* (Trumbo *et al.* 2001). Although these studies suggest that brood parasitism can occur between burying beetle species, they do not address the degree of success of brood parasites. Both previous studies on interspecific brood parasitism in burying beetles allowed females to parasitize host broods instead of the researchers switching in a certain number of larvae, so it is unclear how many parasites survived from hatching to adult. In this study we used two species of burying beetles, *N. guttula* and *N. marginatus*, to quantitatively measure the success of brood parasites.

Nicrophorus guttula and *N. marginatus* are closely related species of burying beetles (Sikes & Venables 2013), and both species are found in grassland habitats (Peck & Anderson 1985; Peck & Kaulbars 1987) in western North America. These species coexist in fields and meadows in central Utah, USA, presumably due to resource partitioning according to body size, temporal activity, or seasonal activity (reviewed in Scott 1998). However, wild populations of these two species have similar body sizes (body length of *N. marginatus* = 15 – 22 mm; body length of *N. guttula* = 14 – 20mm) (Peck & Anderson 1985), and in the laboratory, both species are able to reproduce on carcasses that range in size from 5g – 50g, although there is less success at the low end of this range for *N. marginatus* and the high end of this range for *N. guttula* (Myers 2014). They are also active at the same times of year (Walker & Hoback 2007; Hooper & Larson 2013) and day (Peck & Kaulbars 1987). Therefore, it is likely that these species are in direct competition for carcasses in the wild. Size is an important factor in determining access to carcasses (Bartlett & Ashworth 1988; Otronen 1988; Müller *et al.* 1990; Safryn & Scott 2000; Hopwood *et al.* 2013; Lee *et al.* 2014), but the similarity in body size between these two species suggests that one species is not always dominant over the other. These observations led us to hypothesize that there may be reciprocal interspecific brood parasitism between *N. guttula* and *N. marginatus* when resources for reproduction are limited.

In this study, we used cross fostering between *N. marginatus* and *N. guttula* broods to determine whether brood parasitism could be successful between these species, and whether they exhibit an adaptive response to brood parasitism by detecting and removing parasites. If brood parasites of either species survive in host broods that would suggest that brood parasitism is a viable mechanism to ameliorate effects of competition for carcasses. If brood parasites are detected and removed during the culling phase at a higher rate than each species' own offspring,

that would suggest that there has been an evolutionary history of brood parasitism between these species, and an adaptive response has evolved. Specifically, the purpose of this study was to determine (1) whether brood parasites are successful in other species' broods as measured by survival and size of parasites, and (2) whether parents have evolved a mechanism to detect and remove brood parasites from their broods as measured by differential culling rates of parasites.

Methods

Source of Burying Beetles

To generate the beetles needed for the experiments, we captured adult *N. marginatus* and *N. guttula* at the Utah Wetland Preserve near Goshen, Utah in July 2015 using pitfall traps baited with raw chicken. We placed wild-caught pairs on 30g mouse carcasses and allowed them to breed to generate the laboratory population. We recorded the date of eclosion for all first-generation laboratory-bred beetles, and designated this as the first day of life. We placed newly eclosed individuals in small plastic containers (15.6 x 11.6 x 6.7 cm), fed them *ad libitum* raw chicken liver twice weekly, and maintained them on a 14:10 h light:dark cycle in a temperature-controlled environmental chamber at 21°C until they reached sexual maturity.

Experimental Procedure

We began each trial by randomly choosing an unrelated virgin male and female beetle of the same species. The pair was placed in a plastic container (14 x 13 x 17cm) filled with 10cm of moist soil and given a 30g (± 1.0 g) mouse carcass. The containers were kept in an environmental chamber at 21°C on a 14:10 h light:dark cycle. We checked for larvae daily, and after larvae

arrived on the carcass, we counted the number of first instar larvae on both the first and second days after larvae had arrived on the carcass to account for any asynchronous hatching.

If there were more than seven larvae in the brood, we used the brood in the experiment. We switched seven first instar larvae from each brood with seven first instar larvae from a different brood. Intraspecific switches were used as the control treatment, and interspecific switches were used as the experimental treatment. We randomly paired broods for switches, and we recorded the ID number of the broods that were used in each switch. We checked the broods daily for dispersed offspring. When all larvae dispersed into the soil, the parents were removed. Starting at four weeks after dispersal, we checked the broods daily until all of the offspring eclosed. The larvae from each brood reached eclosion 4-5 weeks after dispersal. Number of newly-eclosed adult offspring was used to determine the final brood size. Each newly-eclosed adult offspring was weighed, which was used to calculate the total offspring mass. We also determined the species of each eclosed offspring using the identification key created by Sikes and Peck (2000). In the experimental treatments, the number of offspring in the brood that were from the other species was used to determine the number of successful parasites in the brood. Hereafter, we refer to offspring of the same species as the parents as “conspecific” and offspring that are from the other species as “parasitic”. We completed 22 control trials of *N. guttula* larvae switched into *N. guttula* broods, 21 control trials of *N. marginatus* larvae switched into *N. marginatus* broods, 21 experimental trials of *N. guttula* larvae switched into *N. marginatus* broods, and 21 experimental trials of *N. marginatus* larvae switched into *N. guttula* broods.

Statistical Analyses

To determine whether brood parasites were successful in *N. guttula* or *N. marginatus* broods, we analyzed number of successful parasites, and mean individual offspring mass. For the number of successful parasites we included one fixed factor - parent species (2 levels). Final brood size was included as a covariate, and the interaction between parent species and final brood size was also included (Proc GENMOD in SAS; SAS 9.3 SAS Institute, Cary, North Carolina, USA). The procedure assumes a poisson distribution and incorporates a log link function. The log link function is similar to a log-transform, and it leads to a more normal distribution of the residuals as determined by inspection of the residual plots. The analysis of mean individual offspring mass had two fixed effects - parent species (2 levels) and treatment (3 levels; individuals in non-parasitized broods, nest mates of parasites, and parasites). We also included final brood size as a covariate and the interaction between parent species and treatment (Proc Mixed in SAS; SAS 9.3 SAS Institute, Cary, North Carolina, USA).

To determine whether parents of either species are able to differentially remove brood parasites we used two analyses. First, we tested to see if proportion of offspring culled differed between species and between parasitized and non-parasitized broods. Second, we tested the proportion of parasites culled compared to the proportion of conspecific brood culled between species and between parasitized and non-parasitized treatments. For both tests we used a logistic regression framework where the response variable was the number of culled individuals relative to the number in the initial brood. For the first test (i.e., proportion of offspring culled), there were two fixed factors - parent species (2 levels) and treatment (2 levels; parasitized and non-parasitized). The interaction between parent species and treatment was also included (Proc GENMOD in SAS; SAS 9.3 SAS Institute, Cary, North Carolina, USA). For the second test we

compared the proportion of parasites culled in the parasitized broods to the proportion of offspring culled in the non-parasitized broods. The response variable was the proportion culled of either parasites or conspecific brood. There were two fixed factors - parent species (2 levels) and treatment (2 levels; parasitized and non-parasitized). The interaction between parent species and treatment was also included (Proc GENMOD in SAS; SAS 9.3 SAS Institute, Cary, North Carolina, USA).

Results

There are significant differences in the number of successful parasites in the final brood between the two species and final brood size has a significant effect (Table 4.1). There were about 2.5 more successful brood parasites in *N. guttula* broods than in *N. marginatus* broods, which means that 71% more brood parasites were successful in *N. guttula* broods (Fig. 4.1). Large final broods had more brood parasites than small final broods.

There are significant differences in mean individual offspring mass between species and between treatments, and the interaction between parent species and treatment was significant. Mean individual offspring mass was also significantly affected by final brood size (Table 4.2). *Nicrophorus guttula* offspring that were parasites in *N. marginatus* broods were significantly smaller than *N. guttula* in broods that were not parasitized (Fig. 4.2). In contrast, *N. marginatus* offspring that were parasites in *N. guttula* broods were significantly larger than *N. marginatus* offspring that were in broods that were, and were not, parasitized (Fig. 4.2)

Proportion of offspring culled differed by species but not by treatment, and the species by treatment interaction was not significant (Table 4.1). *Nicrophorus guttula* culled about 30% of

offspring in both parasitized and non-parasitized broods; whereas, *N. marginatus* culled 47% of offspring in both parasitized and non-parasitized broods (Fig. 4.3).

Proportion of parasites culled compared to proportion of conspecific larvae culled differed by species and treatment, and the species by treatment interaction was marginally significant (Table 4.1). *Nicrophorus guttula* culled 46% of parasites compared to 30% of conspecific larvae; whereas, *N. marginatus* culled 86% of parasites compared to 44% of conspecific larvae. The marginally significant interaction effect arises because of the difference in culling rate of parasites relative to conspecific larvae between species: *N. guttula* cull 16% more parasites than conspecific larvae, and *N. marginatus* cull 42% more parasites than conspecific larvae (Fig. 4.4).

Discussion

Both *N. guttula* and *N. marginatus* were able to rear their own offspring under laboratory conditions, so it seems likely that brood parasitism is facultative, not obligate, when it occurs in these species. One suggested driver for brood parasitic behavior is a lack of nest sites or high costs of rearing offspring (reviewed in Petrie & Møller 1991; Reader 2003). Carrion is an ephemeral resource, and competition between burying beetles for access to it can be intense (Otronen 1988; Scott 1990; Eggert & Sakaluk 2000). Body size determines the winners of competitions for carcasses (Bartlett & Ashworth 1988; Otronen 1988; Müller *et al.* 1990; Safryn & Scott 2000; Hopwood *et al.* 2013; Lee *et al.* 2014), so smaller individuals should be excluded from being dominant on carcasses, and should develop alternative reproductive strategies, such as brood parasitic behaviors. Therefore, we propose that facultative brood parasitism has evolved in burying beetles as an alternative reproductive tactic for when competition for resources is

high. This alternative reproductive strategy could explain how multiple species are able to co-occur, even with significant niche overlap.

Our results suggest that *N. guttula* and *N. marginatus* are able to discriminate between their own larvae and larvae of another co-occurring species, which is contrary to the results of previous studies on brood parasitism in burying beetles. In laboratory experiments, it was previously shown that *N. orbicollis* were not able to discern between entire broods of their own offspring and entire broods of *N. defodiens* larvae when they were switched between the species (Trumbo *et al.* 2001). Additionally, *N. pustulatus* could successfully parasitize *N. orbicollis* broods about 31% of the time after losing a competition for a carcass (Trumbo 1994). Our experiment differs from that of Trumbo (1994) because we switched larvae between broods instead of allowing females to lay their eggs near the carcass and parasitizing them on their own. The previous experiment's design did not allow for a comparison between the number of brood parasites that were successful and the number of parasitic eggs that were laid. *Nicrophorus pustulatus* typically have very large broods (Trumbo 1992), so it is possible that a higher number of parasitic eggs were laid in each brood and the host *N. orbicollis* parents were able to cull some, but not all of the parasitic larvae. In the current experiment, parents did not cull all of the parasitic larvae. We currently do not know the mechanism that allows parents to detect brood parasites, and it might increase the chance of a brood parasite being removed from a brood instead of being an exact process.

It is possible that in our experiment parents only culled offspring because they were switched in from another brood. However, this seems unlikely. *Nicrophorus vespilloides* females use a time-dependent cue to determine which larvae to cannibalize, and if larvae from a subordinate female arrive on the carcass during the same time period as the dominant female's

larvae, she will accept those offspring and raise them (Eggert & Müller 2011). All of our parasitic larvae were switched into their host broods during the hatching period of the host's larvae, so there was no difference in timing that the female could have used to detect the parasitic offspring. Parents of broods in which conspecific larvae were added culled significantly fewer offspring than parents with mixed-species broods (Table 4.3), so just switching larvae from another brood doesn't seem to cause parents to cull larvae.

Nicrophorus marginatus brood parasites were significantly larger than offspring that were raised by *N. marginatus* parents (Fig. 4.2). The differences in body size of brood parasites relative to offspring that were raised by their own species might be due to differences in begging behavior between the two species' larvae. Other studies have shown that the larvae of some species of burying beetles such as *N. vespilloides* and *N. pustulatus* do not require regurgitation from parents for survival; whereas *N. orbicollis* larvae need parental care for growth and survival (Trumbo 1991; Trumbo 1992; Eggert *et al.* 1998; Rauter & Moore 2002; Smiseth *et al.* 2003; Capodeanu-Nägler *et al.* 2016; Jarret *et al.* 2017). *Nicrophorus guttula* may not need as much parental care as *N. marginatus*, and therefore may not beg for food as often. However, when *N. marginatus* were parasites in *N. guttula* broods, they may have begged significantly more than their nest mates and therefore received more regurgitations from their host parents. An alternative explanation for our results is that the parasitic offspring starved to death rather than being cannibalized by the host parents. This seems unlikely for *N. marginatus* because brood parasites of this species were significantly larger than offspring reared by their own species (Fig. 4.2). Conversely, parasitic *N. guttula* were significantly smaller than *N. guttula* in non-parasitized broods (Fig. 4.2), which might make their *N. marginatus* hosts perceive them as low quality. Filial cannibalism of low-quality offspring has been suggested as an adaptive parental

care strategy (Klug & Bonsall 2007), so *N. marginatus* parents could use offspring size as a cue for which offspring to remove from their broods. However, this hypothesis requires additional testing.

The two species of burying beetles that we used to test for brood parasitism in this study, *N. guttula* and *N. marginatus*, are similar in size (Peck & Anderson 1985) and co-occur with several other species of burying beetles throughout their ranges (Walker & Hoback 2007). Therefore, they have probably been subjected to selective pressures for the development of a mechanism to detect brood parasitism. Larger burying beetles typically win access to resources (Bartlett & Ashworth 1988; Otronen 1988; Müller *et al.* 1990; Safryn & Scott 2000; Hopwood *et al.* 2013; Lee *et al.* 2014), so large species should be targeted for brood parasitism more often than small species. It would be interesting to conduct a similar experiment using small and large co-occurring species of burying beetles, for example *N. defodiens* and *N. orbicollis*, to determine whether a mechanism for detecting interspecific brood parasitism exists in all species, or only those that are likely targets for brood parasites. It may be important to use species that naturally co-occur because a recent study using *N. vespilloides* and *N. orbicollis*, which are allopatric, showed that *N. orbicollis* could not distinguish between their own offspring and entire broods of *N. vespilloides* (Benowitz *et al.* 2015).

To our knowledge, this is the first evidence that burying beetles are able to discriminate between their own larvae and parasitic larvae of other species. The detection and removal of brood parasites indicates that brood parasitism between species is likely to occur under natural conditions for both *N. guttula* and *N. marginatus*, and previous studies have also indicated that brood parasitism is likely to occur in wild populations of burying beetles (Müller *et al.* 1990; Trumbo 1994; Trumbo *et al.* 2001; Suzuki & Nagano 2006). However, additional experiments

are required to determine the extent of brood parasitism in the wild and the mechanism through which hosts are able to detect brood parasites, as well as the ecological factors that drive this behavior.

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Table 4.1 Analysis of variance (ANOVA) table for number of successful parasites, proportion of offspring culled, and proportion of parasites compared to the number of conspecific larvae culled. Significant values are bolded.

Response Variable	Source	Num df/ Den df	Chi-Square Value	<i>p</i>-Value
Number of Successful Parasites	Parent Species	1/42	5.11	0.0238
	Final Brood Size	1/42	4.91	0.0267
Proportion of Offspring Culled	Species	1/85	65.63	<.0001
	Treatment	1/85	1.32	0.2509
	Species*Treatment	1/85	0.81	0.3682
Proportion of Parasites vs. Conspecific Larvae Culled	Species	1/85	81.09	<.0001
	Treatment	1/85	68.04	<.0001
	Species*Treatment	1/85	3.55	0.0595

Table 4.2 Analysis of Variance (ANOVA) table for the effects of the parent species, treatment, final brood size, and the interaction between parent species and treatment on the mean offspring mass of individual offspring. Significant values are bolded.

Source	Num df/ Den df	F-Value	<i>p</i>-Value
Parent Species	1/27	209.33	<.0001
Treatment	2/27	12.46	0.0001
Final Brood Size	1/27	37.46	<.0001
Parent Species* Treatment	2/27	27.60	<.0001

Table 4.3 Mean values (\pm SE) for number of 1st instar larvae, number of conspecific larvae culled, number of parasitic larvae culled, final brood size, and number of successful parasites in *N. guttula* and *N. marginatus* broods that were parasitized by conspecifics.

	Mean # of 1st Instar Larvae	Mean # of Conspecific Larvae Culled	Mean # of Parasitic Larvae Culled	Mean Final Brood Size	Mean # of Successful Parasites
<i>N. guttula</i> Broods					
Parasitized by Conspecifics	19.1 (\pm 0.93)	5.7 (\pm 0.80)	-	16.1 (\pm 0.96)	-
Parasitized by Heterospecifics	22.6 (\pm 1.04)	4.6 (\pm 0.68)	3.2 (\pm 0.37)	16.3 (\pm 0.90)	3.5 (\pm 0.43)
<i>N. marginatus</i> Broods					
Parasitized by Conspecifics	30.0 (\pm 1.19)	12.8 (\pm 1.54)	-	13.3 (\pm 0.79)	-
Parasitized by Heterospecifics	27.3 (\pm 1.14)	7.8 (\pm 1.56)	6.0 (\pm 0.30)	11.8 (\pm 0.73)	1.0 (\pm 0.22)

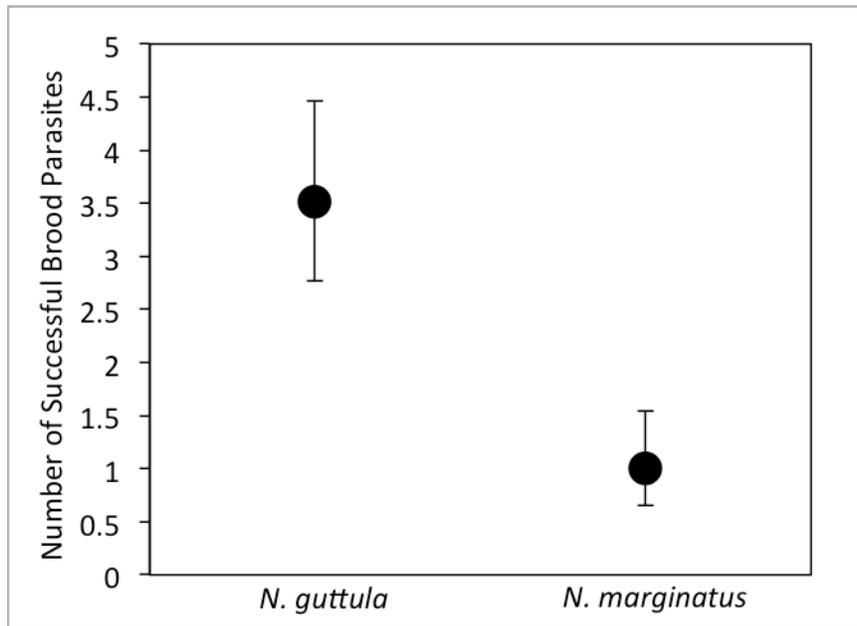


Figure 4.1 LSMeans (\pm 95% confidence intervals) for the number of successful parasites in *N. guttula* and *N. marginatus* broods.

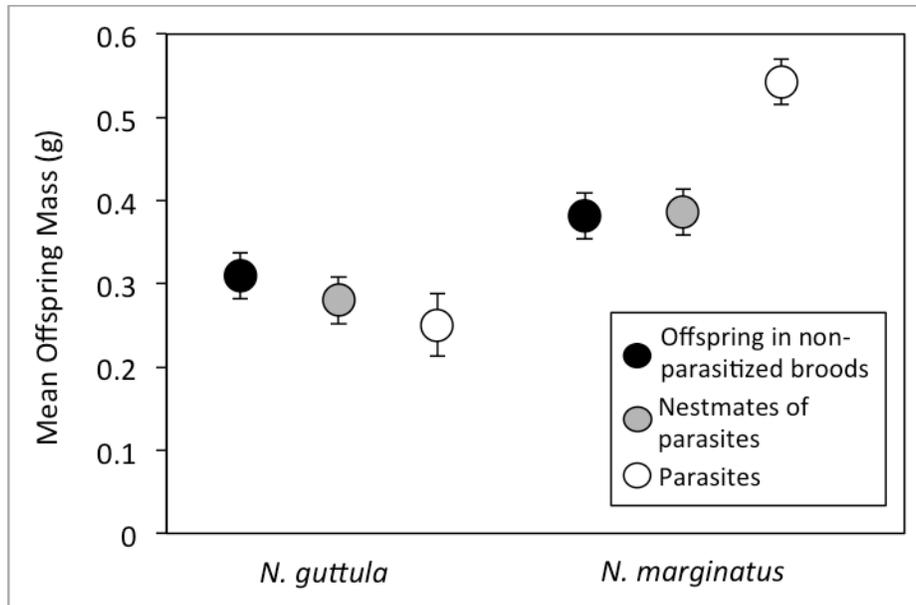


Figure 4.2 LSMeans (\pm 95% confidence intervals) for the mean mass of adult *N. guttula* and *N. marginatus* offspring that were in non-parasitized broods, that were nest mates of parasites, and that were parasites.

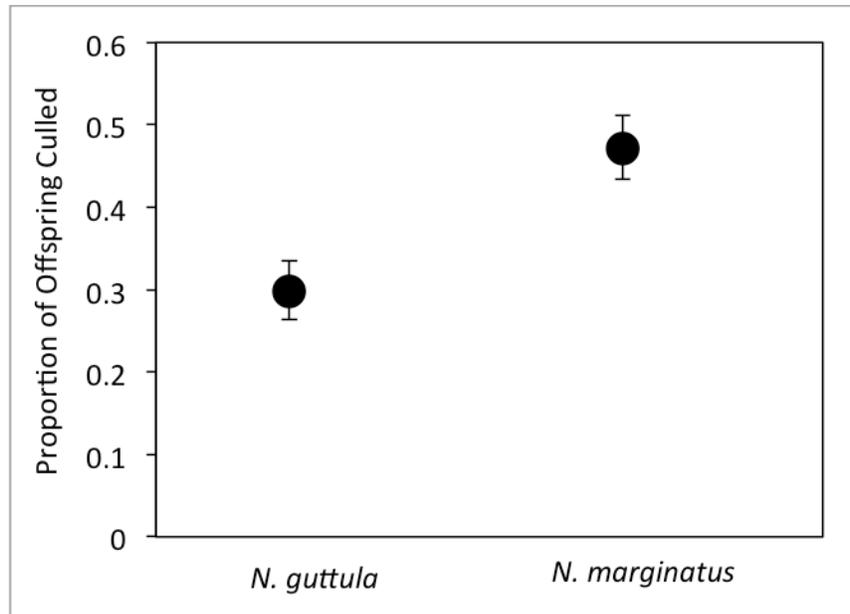


Figure 4.3 LSMeans (\pm 95% confidence intervals) for the mean proportion of offspring culled in *N. guttula* and *N. marginatus* broods.

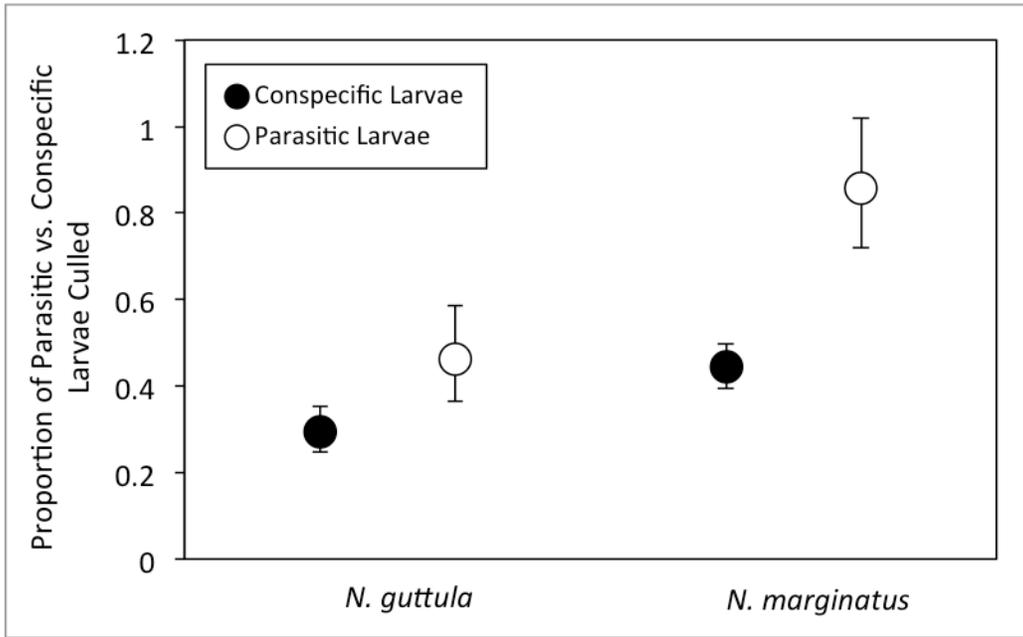


Figure 4.4 LSMeans (\pm 95% confidence intervals) for the mean proportion of conspecific and parasitic larvae culled in *N. guttula* and *N. marginatus* broods.

Chapter 5

Heritability of Body Size in Burying Beetles

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Abstract

Narrow-sense heritability (h^2) provides an estimate of the amount of phenotypic variation that is a result of direct genetic effects. The coefficient of genetic variation (CV_A) provides an estimate of the amount of genetic variation that exists in a population. Together, these two values allow inferences to be made about the evolvability of a trait. In this study, we calculated h^2 and CV_A for 17 population samples from 7 species of burying beetles, *Nicrophorus americanus*, *N. defodiens*, *N. guttula*, *N. marginatus*, *N. orbicollis*, *N. pustulatus*, and *N. vespilloides*. Some of our samples were from different populations for the same species, and some were from different years within the same population for a species. The different samples allowed us to make comparisons between species, and between populations of a species. We also performed a meta-analysis to determine whether there are consistent levels of heritability within the genus. We found that both h^2 and CV_A differed between species as well as between populations of a species. Our meta-analysis indicated that the overall heritability of body size is low ($h^2 = 0.14$), and may not be different from zero. However, there were significant differences between species, and body size heritability is higher in some species than in others. Our CV_A estimates indicated that the amount of genetic variation in each species is also very low (mean $CV_A = 6.25$). Taken together, these estimates indicate that the environment has a large effect on body size in this genus, and suggest that there has been strong past selection on this trait in the *Nicrophorus* genus. This brings into question how the large range of body sizes seen in this genus could have come about, as well as how body size is involved in speciation.

Keywords: *Nicrophorus*, body size, heritability, coefficient of genetic variation, meta-analysis

Introduction

Heritability determines how a population will respond to selection pressures in the present, but the degree of heritability for a population is shaped by past selection (Falconer & Mackay 1996). For a trait to be heritable, some additive genetic variance must be present in the population (Falconer & Mackay 1996). Narrow-sense heritability (h^2) is an estimate of the proportion of total phenotypic variation due to the additive effects of genes and is defined as

$$h^2 = \frac{V_A}{V_P}$$

where V_A is the additive genetic component of variation and V_P is the total phenotypic variation (Falconer & Mackay 1996). Generally, the heritability of traits that affect fitness, such as life history traits, tend to be lower than morphological traits (Mousseau & Roff 1987; Price & Schluter 1991), which is attributed to reduced genetic variation in these traits caused by directional selection (Fisher 1958; Mousseau & Roff 1987).

The evolvability of a trait, or its ability to respond to selection pressures, depends on the amount of additive genetic variation that exists for that trait (Houle 1992). The coefficient of genetic variation, or CV_A , is a measure of genetic variation, and is therefore a measure of evolvability (Garcia-Gonzalez *et al.* 2012). CV_A can be calculated as

$$CV_A = \frac{\sqrt{V_A}}{\bar{X}}$$

where the square root of the additive genetic variance is divided by the phenotypic mean of the trait. Heritability (h^2) estimates the proportion of phenotypic variation that is attributed to genetic factors. However, it does not provide an estimate of the amount of genetic variation that exists for the trait being measured (Iraqi *et al.* 2014). CV_A is a useful measure of additive genetic variation because it is standardized by the trait mean, and thus independent of other sources of

variance. For these reasons, it is also useful for comparison across species and studies (Garcia-Gonzalez *et al.* 2012).

Adult body size is a complex morphological trait with influences from both genetic (Mousseau & Roff 1989; Simmons & Ward 1991; Ryder & Siva-Jothy 2001; Pappers *et al.* 2002; Seko *et al.* 2006) and environmental factors (Nijhout 2003). The heritability of body size is often studied because of its indirect effect on fitness, which generally increases with body size in both sexes (Clutton-Brock 1988; Reiss 1989). In females, there is generally a positive correlation between body size and fecundity (Honěk 1993; Blanckenhorn 2000), and in males body size is important for both inter- and intrasexual selection (reviewed in Andersson 1994; Kingsolver & Huey 2008). Body size heritability has been shown to differ between populations of the same species (e.g. Coyne & Beecham 1987; Mousseau & Roff 1989; Bitner-Mathé & Klaczko 1999; Loh & Bitner-Mathé 2005), but comparisons among closely related species are lacking.

In this study, we tested for heritability and genetic variation of body size across seven species of burying beetles (genus *Nicrophorus*) to compare heritability estimates and the amount of genetic variation for body size both within and among species of the genus. Burying beetles provide extensive parental care to their offspring. All species in the *Nicrophorus* genus use small vertebrate carcasses for food and reproduction (Scott & Traniello 1990), which is a highly contested and ephemeral resource (Eggert & Müller 1997). Body size is strongly related to fitness in burying beetles through thermoregulatory capabilities (Merrick & Smith 2004), overwinter survival (Smith 2002), starvation resistance (Trumbo & Xihani 2015; Smith & Belk 2018; Dissertation Chapter 2), competitive ability (Bartlett & Ashworth 1988; Otronen 1988; Müller *et al.* 1990; Safryn & Scott 2000; Hopwood *et al.* 2013; Lee *et al.* 2014; Smith & Belk

2018; Dissertation Chapter 2), parental care behaviors (Steiger 2013; Smith *et al.* 2014; Pilakouta *et al.* 2015; Capodeanu-Nägler *et al.* 2016; Jarret *et al.* 2017), reproductive output (Rauter *et al.* 2010; Hopwood *et al.* 2016; Smith & Belk 2018; Dissertation Chapter 2), and species interactions through resource partitioning (Wilson *et al.* 1984; Trumbo 1990; Scott 1998; Ikeda *et al.* 2006). There is also significant variation in body size both within and among burying beetle species (Sikes 2003; Smith *et al.* *in prep*), and body size seems to be an important factor in speciation for the *Nicrophorus* genus (Smith *et al.* *in prep*). Because body size has had an important role in burying beetle evolutionary history, we wanted to determine the heritability of this trait and thus its potential role in speciation and community structure. We calculated the narrow-sense heritability (h^2) and the coefficient of genetic variation (CV_A) for body size for seventeen samples from seven species of burying beetles (*N. americanus*, *N. defodiens*, *N. guttula*, *N. marginatus*, *N. orbicollis*, *N. pustulatus*, and *N. vespilloides*). We also conducted a meta-analysis on the h^2 estimates from each sample to estimate the heritability of body size within the *Nicrophorus* genus.

Methods

Experimental Design

Each author collected burying beetles and performed experiments individually (see Table 1 for location and year for each sample), and most of these samples were part of larger experiments (e.g. Meyers 2014; Smith *et al.* 2015; Capodeanu-Nägler *et al.* 2016; Smith *et al.* 2017; Smith & Belk 2018). In each experiment, we measured the pronotum width of each parent before reproduction, and used the mean of their pronotum widths as our mid parent size measurement. After the offspring eclosed, we measured the pronotum width for each member of

the brood and used the mean pronotum width of all of the offspring as our mid offspring size measurement.

For two of our sample populations, one from *N. guttula* and one from *N. marginatus* (collected from 2011-2012 by PJM), we did not have data on eclosed offspring pronotum widths, but we did have data on larval mass at dispersal. There is a strong positive correlation between larval mass and pronotum width after eclosion (Lock *et al.* 2004; Trumbo & Xhahani 2015), so for the 2011-2012 samples of *N. guttula* and *N. marginatus*, we converted the larval masses to pronotum widths using the results of a linear regression of larval mass on adult pronotum width with data from ANS. The slope of the regression was significantly different from zero (df = 53, Estimate = 5.15, SE = 0.21, $t = 25.03$, $p < 0.0001$), indicating that there is a strong, positive relationship between larval mass and adult pronotum width (Adjusted $R^2 = 0.92$). Converting the larval masses to adult pronotum widths was necessary to test these two samples on the same scale as the other sample populations.

Statistical Analyses

We used a parent-offspring regression (Falconer & Mackay 1996) to determine the heritability of body size for each of our sample populations. We used mid parent size as the independent variable and mid offspring size as the dependent variable. We also included carcass mass (when different carcass sizes were used in the experiment) in the model to account for potential environmental impacts on offspring body size. We used the protocol outlined by Lynch and Walsh (1998) to calculate the coefficient of genetic variance for each sample. We then used an ANOVA to compare the h^2 and CV_A estimates between species. We used Program R 3.4.1 (R Core Team 2013) for these analyses.

We also applied a meta-analysis to our h^2 estimates as a measure of heritability across the genus. We used a weighted means method (see Becker & Wu 2007). The weighted average of the parent-offspring regression slopes was calculated using the inverse of the corresponding slope variances as weights, which are a function of sample size. We also used a method (Meier 1953) for calculating the variance of the weighted mean to obtain a 95% confidence interval (CI) for heritability within the genus. A second meta-analysis used only a subset (7) of the sample populations that had sample sizes greater than or equal to 50. The majority of the complement, or samples with less than 50 replicates, had standard errors that were larger by a factor of 3 to 4, and in one case by nearly an order of magnitude. These analyses were performed in Microsoft Excel.

Results

The h^2 estimates of body size ranged from -0.31 in a *N. guttula* sample to 0.38 in a *N. orbicollis* sample (Table 5.1). Heritability was significantly greater than zero in four of our seventeen tests, spread across four different species (*N. guttula*, *N. marginatus*, *N. orbicollis*, and *N. vespilloides*) (Table 5.1). Carcass mass had a significant effect on mid offspring size in 55% of the samples where it was included (Table 5.1). Heritability was relatively consistent between the two samples from *N. vespilloides* (range = 0.10), but varied more among the samples from *N. guttula* (range = 0.41), *N. marginatus* (range = 0.20), and *N. orbicollis* (range = 0.32).

The h^2 estimates differed significantly by species ($F = 4.642$, $p = 0.0188$). Our body size heritability estimates were about 140% higher in *N. orbicollis* and *N. vespilloides* than in *N. defodiens*, and about 69% higher in *N. vespilloides* than in *N. guttula*.

The CV_A estimates for our samples ranged from 2.31 in the *N. defodiens* sample to 9.62 in a *N. marginatus* sample (Table 5.1). The mean CV_A estimate across all 17 of our samples was 6.25, indicating that the amount of genetic variation across all of our sample estimates is consistently low.

The CV_A estimates differed significantly by species ($F = 4.96, p = 0.0153$). Our body size CV_A estimates were about 70% higher in *N. marginatus* than in *N. defodiens*, and about 55% higher than in *N. vespilloides*.

In the overall meta-analysis, the weighted mean slope (h^2) was 0.0212 (SE = 0.0376, 95% CI = -0.0557 – 0.0982). This suggests that the heritability for the genus is not significantly different from zero since the CI does not overlap zero. The second meta-analysis using large sample size populations shows a weighted mean slope of 0.1352 (SE = 0.1002, 95% CI = -0.1099 – 0.3804). The confidence interval for this subset also overlaps zero, suggesting that heritability for the genus is not significantly different from zero.

Discussion

We used data from seven species of burying beetles to test whether the heritability of body size is generalized across species and populations. Our sample included species with a wide range of body sizes (Sikes 2003), as well as species that are found across a broad geographic range (Table 5.1). We found evidence for body size heritability in at least one population sample of *N. guttula*, *N. marginatus*, *N. orbicollis*, and *N. vespilloides*, but not in *N. americanus*, *N. defodiens*, or *N. pustulatus* (Table 5.1). However, sample size likely contributed to the lack of a significant effect in several of our population samples. In species where we had multiple population samples, all samples had similar heritability estimates, but the samples with the

lowest sample sizes also had the highest standard errors. This indicates that some sample sizes were not large enough to detect a significant genetic effect. We only had one population sample for *N. americanus*, *N. defodiens*, and *N. pustulatus*, and those had low sample sizes, so it is difficult to determine from our data whether or not there is a significant direct genetic effect of parent size on offspring size in those species. However, our estimate of heritability for *N. pustulatus* is similar to that obtained by Rauter & Moore (2002), so it is likely that the heritability estimate we obtained for this species is valid.

Our meta-analysis of h^2 estimates indicates that little, if any, of the variation in body size for the *Nicrophorus* genus is attributable to additive genetic effects and our estimates of genetic variation within each sample are also low. These two factors should only occur together if total phenotypic variation is high and the amount of variation is mostly due to environmental factors. Natural populations of burying beetles often show high levels of variation in body size, and even individual broods have a wide range of offspring sizes within them (Smith, unpublished data). Additionally, offspring size is heavily dependent on resource size, with larger offspring generally produced on larger carcasses (Smith & Belk 2018; Dissertation Chapter 2). Two previous studies on heritability in burying beetles, one on *N. pustulatus* (Rauter & Moore 2002) and the other on *N. vespilloides* (Jarrett *et al.* 2017), also found that the heritability of body size was not significantly different from zero. Thus, burying beetle body size seems to be mostly influenced by environmental factors rather than genetic variation.

It is also possible that the low heritability estimates that we obtained for body size across the *Nicrophorus* genus are the result of strong past selective pressures that reduced the genetic variability of this trait. Traits that are closely related to fitness, such as life history traits, are expected to have lower heritability than morphological and behavioral traits (Mousseau & Roff

1987; Price & Schluter 1991; Visscher *et al.* 2008) because strong directional selection should reduce genetic variation in these traits (Fisher 1958; Mousseau & Roff 1987). As discussed above, body size has an important role in several aspects of burying beetle natural history (i.e. physiology, fitness components, and community structure), and therefore might be under stronger selection than typical morphological traits, thus reducing the amount of additive genetic variation for this trait.

Environmental variation that resulted from different methodologies used by each researcher who participated in this study may have caused some of the variation in our h^2 estimates between samples. The heritability of a trait is the proportion of the total phenotypic variance that can be attributed to additive genetic variance (Falconer & Mackay 1996). The magnitude of heritability partly depends on the contribution of nongenetic factors to the total variation, so traits that are influenced heavily by environmental factors will have low heritabilities (Barton & Turelli 1989; Price & Schluter 1991). Burying beetle body size is particularly sensitive to environmental variation. Biparental parents produce smaller broods than uniparental parents (Scott 1989; Smith *et al.* 2017), offspring mass is significantly higher when offspring receive parental care than when they do not (Eggert *et al.* 1998), and parents produce smaller broods at higher temperatures (Laidlaw 2015). Parents also adjust brood size according to the size of the carcass that they are given (Scott & Traniello 1990; Trumbo 2006), and reduce brood size to increase the size of individual offspring when competition is high (Creighton 2005). In the present study, we found that carcass size contributed significantly to environmental variation (Table 5.1). The duration and type of parental care (i.e. biparental or uniparental) in each sample probably also contributed to environmental variation, and therefore our heritability estimates.

Our results indicate that there are differences in body size heritability between populations of the same species, which is consistent with a recent comparative genetic study on *N. vespilloides* that showed that there is significant genetic structuring among populations (Pascoal & Kilner 2017). Our body size heritability estimates for *N. orbicollis* were from three separate population samples (collected in Wisconsin, Illinois, and Oklahoma) and for *N. vespilloides* were from two separate population samples (collected in Germany and the United Kingdom). Burying beetle community structure, and therefore the level of competition, differs across the range of these species, which could impact the importance of body size. For example, at the northern part of its range (Ontario, Canada), *N. orbicollis* co-occurs with five other burying beetle species (Robertson 1992), whereas in the middle of its range (Nebraska, USA) it co-occurs with seven other burying beetle species (Walker & Hoback 2007). Of the species that occur in Ontario, *N. orbicollis* is the largest burying beetle species, but in Nebraska *N. orbicollis* co-occurs with the larger *N. americanus*, so the importance of body size probably varies depending on competitive environment. Our results also showed population differences in genetic variation. In flour beetles, genetic variation increases variation of niche breadth (Agashe & Bolnick 2010), so competition could also affect the amount of genetic variation in each population. Our *N. orbicollis* sample from Oklahoma (collected by JCC) co-occurred with *N. americanus*, so the low h^2 estimate and low CV_A for that population could be due to competition with a larger species that limits the resources that can be used by the smaller *N. orbicollis*. Heritability of body size and the amount of genetic variation could be dependent on the community structure that burying beetles encounter in different parts of their geographic ranges, and therefore dependent on the recent evolutionary history of each population.

The lowest body size heritability estimates were found in *N. americanus*, *N. guttula*, and *N. marginatus*. Each of these three species is found in diverse habitat types (Shubeck 1983; Peck & Anderson 1985; Peck & Kaulbars 1987; Lomolino *et al.* 1995; Trumbo & Bloch 2000; Sikes & Raithel 2002; Walker & Hoback 2007; Hooper & Larson 2012), which may contribute to their low body size heritability estimates. Heritability is expected to be lower in variable environments (Simons & Roff 1994), so the habitat generalist strategy used by *N. americanus*, *N. guttula*, and *N. marginatus* might cause the low heritability of body size in these species because a single strategy might not be optimal under all conditions. Heritability may be lower in species that encounter variable conditions because the environmental variance component of heritability is higher under those conditions (Visscher *et al.* 2008). Although all of our tests of heritability were conducted under laboratory conditions, they should still be representative of heritability under natural conditions. Previous studies have shown that there is no difference between heritability measured under field and laboratory conditions (e.g. Weigensberg & Roff 1996; Blanckenhorn 2002), so our results are likely to be representative of heritability in natural populations.

Although the overall heritability of body size seems to be low for the *Nicrophorus* genus, *N. vespilloides* had a higher body size heritability than the other species that we tested. Recently, Hopwood *et al.* (2016) found that in *N. vespilloides* reproductive output varied with carcass size and parent body size. Large females produced larger broods on large carcasses than did small females, but small females produced larger broods on small carcasses than large females (Hopwood *et al.* 2016). However, in *N. marginatus* there is no association between reproductive output, body size, and carcass size (Smith & Belk 2018; Dissertation Chapter 2). Therefore, it is possible that there is a genetic component to carcass-related fitness that causes a genotype by environment interaction in *N. vespilloides* that does not exist in other species of burying beetles.

Both of the body size heritability estimates reported in the present study for *N. vespilloides* are significantly higher than that reported by Jarrett *et al.* (2017) for the same species, which may also be a result of differences in the environment and community structure between the samples.

The results of this comparison of body size heritability and genetic variation estimates in burying beetles indicate that these estimates vary among species and populations of the same species. It also suggests that heritability estimates cannot be generalized as the heritability of body size is higher in some species than in others, and that differences in the recent evolutionary history of each population likely have an important impact on their heritabilities. The overall heritability for the *Nicrophorus* genus and the amount of genetic variation in each population is low, indicating that most of the variation in body size is attributable to environmental factors, and that past selective pressures could have reduced the genetic variation for this trait. The size of the species in question also does not seem to affect the heritability of body size, as there were no significant differences between the heritability of size or the amount of genetic variation of small species such as *N. vespilloides* and *N. defodiens* and the largest species in our study, *N. americanus*. We have shown interesting patterns in the heritability of body size between populations and species that provide a framework for future comparative studies on the basis of these differences with regard to each species' unique natural history and the distinct selective pressures of each population. This study also indicates that heritability estimates should not be generalized across closely related species or even across populations of the same species, but additional heritability estimates for each species and population are needed to fully assess the heritability of body size in burying beetles. Our results also bring into question how such a large range of body sizes has evolved in this genus, as well as the role of body size in speciation.

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Table 5.1 Heritability (h^2) (\pm SE), ANOVA, and coefficient of genetic variation (CV_A) results for all species. Significant values are bolded. Sample source indicates which author collected the data and where the beetles were collected. 1 = Cherokee County, OK, USA, 2 = Goshen, UT, USA, 3 = Benjamin, UT, USA, 4 = Big Falls, WI, USA, 5 = Lexington, IL, USA, 6 = Ulm, Germany, 7 = Edinburgh, UK.

Species	Sample Source	Year	h^2	Independent Variable	df	t-value	p-value	CV_A	
<i>N. americanus</i>	JCC ¹	2016	0.06 (\pm 0.19)	Mid Parent Pronotum	2/22	0.35	0.7328	6.57	
				Carcass Mass	2/22	2.23	0.0366		
<i>N. defodiens</i>	JCC ⁴	2017	-0.25 (\pm 0.14)	Mid Parent Pronotum	1/10	1.78	0.1055	2.31	
<i>N. guttula</i>	ANS & MCB ²	2016	0.02 (\pm 0.15)	Mid Parent Pronotum	1/22	0.15	0.8842	5.25	
	ANS & MCB ²	2015	-0.31 (\pm 0.19)	Mid Parent Pronotum	1/31	1.62	0.1150	6.72	
	PJM & MCB ²	2011-2012	0.10 (\pm 0.05)	Mid Parent Pronotum	2/240	2.13	0.0343	6.38	
				Carcass Mass	2/240	10.21	<.0001		
<i>N. marginatus</i>	ANS & MCB ²	2017	0.11 (\pm 0.11)	Mid Parent Pronotum	2/57	1.05	0.2975	9.62	
				Carcass Mass	2/57	3.70	0.0005		
	ANS & MCB ²	2016	0.003 (\pm 0.15)	Mid Parent Pronotum	1/46	0.03	0.9791	6.31	
	ANS & MCB ²	2015	-0.06 (\pm 0.26)	Mid Parent Pronotum	2/28	0.25	0.8084	8.72	
	ANS & MCB ²	2014	0.14 (\pm 0.06)	Mid Parent Pronotum	2/92	2.24	0.0274	6.73	
					Carcass Mass	2/92	3.38	0.0011	
	ANS & MCB ³	2013	0.10 (\pm 0.07)	Mid Parent Pronotum	1/193	1.42	0.1588	6.53	
	PJM & MCB ²	2011-2012	0.05 (\pm 0.05)	Mid Parent Pronotum	2/340	0.94	0.3480	8.40	
				Carcass Mass	2/340	16.44	<.0001		
<i>N. orbicollis</i>	ACN & SS ⁵	2014	0.38 (\pm 0.39)	Mid Parent Pronotum	2/13	0.99	0.3431	8.82	
				Carcass Mass	2/13	0.72	0.4870		
	ANS & JCC ³	2009-2011	0.17 (\pm 0.07)	Mid Parent Pronotum	2/176	2.26	0.0251	7.56	
				Carcass Mass	2/176	3.51	0.0006		
	JCC ¹	1992	0.06 (\pm 0.13)	Mid Parent Pronotum	2/17	0.44	0.6681	4.73	
				Carcass Mass	2/17	0.26	0.7974		

<i>N. pustulatus</i>	ACN & SS ⁵	2014	-0.15 (± 0.13)	Mid Parent Pronotum	2/15	1.13	0.2753	4.21
				Carcass Mass	2/15	0.06	0.9532	
<i>N. vespilloides</i>	ACN & SS ⁶	2014	0.36 (± 0.18)	Mid Parent Pronotum	2/15	1.96	0.0694	2.91
				Carcass Mass	2/15	0.04	0.9668	
	PTS ⁷	2012	0.26 (± 0.09)	Mid Parent Pronotum	2/90	2.91	0.0046	4.06
				Carcass Mass	2/90	0.10	0.1643	