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# Dominance status and sex influence nutritional state and immunity in burying beetles *Nicrophorus orbicollis*

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Intrasexual competition for mates or resources important for reproduction often leads to the establishment of dominance relationships that influence an individual's reproductive success. Although dominance can be an honest indicator of health or immunocompetence, the attainment and maintenance of dominance status can also influence an individual's ability to invest in immunity, making it difficult to disentangle cause and effect. Here we examine the relationship between intrasexual competition and the nutritional condition and immunity of burying beetles, *Nicrophorus orbicollis*, insects that reproduce on small vertebrate carcasses that serve as a larval food source. We staged intrasexual contests on carcasses in both sexes and compared the nutritional state and immunity of dominant and subordinate individuals with those of beetles reproducing on a carcass without competitors. The nutritional state and immunity of dominant beetles were not significantly different from beetles without competitors, but subordinates were characterized by a lower weight gain and reduced encapsulation response. In addition, we found a clear sex effect, with females gaining more weight than males and having superior immunity. We conclude that the subordinate's exclusion from the carcass plays an important role in mediating the difference in encapsulation. Our data suggest that this is not entirely a nutritional effect because better-fed subordinates did not exhibit higher immune responses. Rather, subordinates may have no need to invest in improved immunity because they do not participate in carcass maintenance and defense. *Key words*: burying beetles, dominance, immunity, intrasexual competition, nutritional condition, *Nicrophorus*. [Behav Ecol]

## INTRODUCTION

Intrasexual competition often leads to the establishment of dominance hierarchies in numerous species and can dramatically influence reproductive output (Andersson 1994). The ability to prevent rivals from gaining access to mates or resources important for reproduction can affect the fertilization success of males and the fecundity of females. In addition, females of many species are known to prefer dominant males as mates, and one explanation for this preference is that dominance reliably reflects certain aspects of male quality (Grafen 1990; Berglund et al. 1996; Qvarnström and Forsgren 1998). Specifically, dominance may serve as an honest indicator of health, as only immunocompetent males may have the ability to bear the cost of maintaining both a high dominance rank and effective immune defenses (e.g., Freeland 1981; Zuk and Johnsen 2000; Rantala and Kortet 2004). However, the interaction between dominance status and disease resistance can be complex, and the outcome of competitive interactions may have different consequences for the health of the dominant and subordinate individuals (Sapolsky 2005; Bartolomucci 2007). Immunocompetent individuals may be more likely to attain dominance, but dominance itself may have subsequent effects on immune function, making it difficult to disentangle cause and effect.

Here we examine the relationship between the outcome of competitive interactions and the competitors' nutritional state and immunity in burying beetles. Burying beetles are well suited to this task because the main determinant of dominance rank is body size (Pukowski 1933; Wilson and Fudge 1984; Bartlett and Ashworth 1988; Otronen 1988; Müller et al. 1990, 2007; Trumbo 1990; Scott 1997; Safryn and Scott 2000; Eggert et al. 2008). In *N. orbicollis*, size does not correlate with immune function (Steiger et al. 2011), and thus, it seems unlikely that immunocompetence has an important effect on the outcome of fights. Moreover, these insects provide the rare opportunity to examine simultaneously both male–male and female–female competition and to compare the relationship between immunity and dominance between the sexes. Burying beetles reproduce on small vertebrate carcasses that serve as the sole larval food source and also provide nutrition to the adults (Pukowski 1933; Eggert and Müller 1997; Scott 1998; Smiseth and Moore 2004; Trumbo and Robinson 2004; Eggert et al. 2008). When several individuals of the same sex locate the same small carcass, they engage in violent fights for its possession (Pukowski 1933; Bartlett and Ashworth 1988). In many species, beetles become more tolerant on larger size carcasses (Eggert and Müller 1992, 2000; Trumbo 1992; Trumbo and Wilson 1993;

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Scott 1994), but on small carcasses, aggressive behavior and exclusion of consexuals from the carcass occur in all species studied to date (Pukowski 1933; Bartlett 1988; Otronen 1988; Trumbo 1992; Eggert and Müller 1997). Fights can be so severe that they often lead to physical injuries, the loss of entire limbs, and even death.

One of the best studied species of burying beetle is *Nicrophorus vespilloides*, and for this species, several studies have documented that dominance is established after a few fights but that neither male nor female subordinates abandon the carcass immediately. Subordinate males continue to seek surreptitious copulations with the resident female or females (Bartlett 1988; Eggert 1992; Müller et al. 2007), whereas subordinate females remain near the carcass long enough to produce egg clutches of their own, which are typically slightly smaller than those of dominant females (Müller et al. 1990; Eggert et al. 2008; Eggert and Müller 2011). Despite the fact that subordinate males ("satellite males") and subordinate females ("intraspecific brood parasites") do not contribute to carcass burial, preparation, or larval feeding, they often succeed in producing a few offspring (Bartlett 1988; Müller et al. 1990, 2007; Eggert 1992; Eggert and Müller 1997; Scott 1997).

When female *N. vespilloides* compete for a small carcass, the dominant repels her subordinate rival from the carcass whenever possible, thus curtailing access to the carcass and preventing food intake by the subordinate female (Müller et al. 1990). A supplementation experiment documented that this reduced access to food causes subordinate females to have lower fecundity than dominants (Eggert et al. 2008), which contributes to the reproductive skew observed in this situation.

In the present study, we assessed the relationship between dominance status and sex on nutritional condition and immunity in burying beetles, *Nicrophorus orbicollis*. We predicted that the differential access to the carcass that arises as a result of dominance interactions might influence not only the nutritional condition, but also the immunity of the competitors. Fights per se might also have different effects on the immune function of dominant and subordinate individuals. Although female *N. orbicollis* are known to have a stronger individual immunity response than males (Steiger et al. 2011), it is not known whether the intensity of competition among males differs from that observed among females.

Our general approach was to experimentally create situations in which 2 males, or 2 females, compete for access to a carcass and to compare those with individuals reproducing in the absence of an intrasexual competitor. We compared the nutritional condition and immune response of dominant and subordinate individuals and that of beetles reproducing on a carcass without a competitor. If dominance is affected by immunocompetence, dominant individuals should exhibit the highest immunocompetence, subordinates the lowest, and the immune response of beetles reproducing without competitors should be intermediate between the 2. If dominance, or access to the carcass, causes improved immunocompetence, we would expect the immunocompetence of beetles without competitors to resemble that of dominant beetles. As it is conceivable that fights might be more severe in cases where the competitors are of similar size, or larger beetles pitted against very small competitors might be better able to monopolize the carcass, we also examined the relationship between size asymmetry (the size difference between the 2 competitors) and nutritional condition and immunity. If nutritional condition mediates immunity, we should see a stronger immune response in individuals with greater weight gain.

## MATERIALS AND METHODS

### Experimental beetles

Experimental *N. orbicollis* were the second-generation offspring of beetles collected from carrion-baited pitfall traps established in the Merwin Nature Preserve, a tract of secondary deciduous forest bordering the Mackinaw River in McLean County, IL, USA (40°40′N, 88°50′W). From their eclosion to the start of the experiment, beetles were kept in small plastic containers (480 mL) two-thirds filled with moist peat at 20 °C under a 16 h L:8 D light regime and fed small pieces of ground beef twice a week. One week before the start of the experiment, beetles were kept singly and fed ground beef ad libitum to ensure that all individuals were well fed and in about the same nutritional state. Beetles assigned to the different treatments were on average the same age (50 days).

### Design of competition experiment and measurements of nutritional state

To examine the effects of competition and dominance rank on nutritional state and immunity of beetles, we established 3 different treatment groups. In the "male–male competition" group, 2 males, together with 1 female, were placed on the same carcass ($n = 24$). In the "female–female competition" group, 2 females, together with 1 male, were placed on the same carcass ($n = 25$). Finally, in the "no competition" group, a pair consisting of 1 male and 1 female was provided with a carcass ($n = 23$). The beetles of each treatment group were left together for 36 h. This provided the beetles with sufficient time for aggressive interactions to occur and for the establishment of a dominance hierarchy. Each beetle was inspected thoroughly for any injuries before and after the experimental period. All treatment groups were matched for age to preclude the possibility that age might affect the outcome of fights and immunity. The beetles were selected nonrandomly, so that each treatment group was composed of beetles exhibiting a large variation in body size as measured by pronotum width ("male–male competition" group: 4.90–7.56 mm, "female–female competition" group: 4.97–7.24 mm, "no competition" group: 4.52–8.00 mm). In the competition groups, the 2 contestants were selected in such a way that the size asymmetry between them covered a wide range, from beetles that differed in their pronotum width by only 0.04 mm to beetles that showed a difference of 2.16 mm. We weighed each beetle thrice: ($t_0$) shortly before we provided them with a carcass, ($t_1$) 24 h later, before inserting an implant for measurement of immunity, and again ($t_2$) 14 h later, shortly before freezing the beetles. To ensure that differences in weight gain were not simply caused by initial size differences, with larger beetles gaining more weight, nutritional state was determined as the relative weight gain, that is, as a percentage of change in mass relative to the initial body mass ($[t_1 - t_0]/t_0$). Only the values determined after 24 h were used because some of the females started to lay eggs in the subsequent 14 h.

During the experimental period, beetles were kept in large plastic containers (20 × 30 × 15 cm) that were half filled with moist peat and contained a freshly defrosted mouse (~20 ± 3 g). Each mouse was secured with string to one corner of the box. This was done to create a situation in which the position of the carcass would be constant as it would be in the field, thus allowing subordinates the opportunity to hide from dominant beetles at some distance from the carcass. The boxes were kept in the dark and observations were made under red light. Twenty-four hours and 38 h after the start of the experiment, the position of the beetles with respect to the carcass was determined. In addition, the beetles' behavior was observed for 20 min. Beetles that were consistently observed

on the carcass were classified as dominant, whereas beetles that were observed off the carcass on both occasions were classified as subordinate.

## Measurements of immunity

Insects possess innate immunity, comprised humoral and cellular components (Gillespie et al. 1997; Söderhall and Cerenius 1998; Lawniczak et al. 2007). We examined both humoral and cellular responses in our study with methods commonly used to assess insect immunity (see references in Gershman et al. 2010). First, we measured the magnitude of the encapsulation response to a nylon monofilament. Insects utilize the encapsulation response to combat parasites that enter the hemocoel. This response invokes both cellular and humoral defenses that lead to the formation of a capsule consisting of layers of dead melanized hemocytes that isolate, cover, and kill invaders (Gillespie et al. 1997; Ryder and Siva-Jothy 2000). Second, we analyzed phenoloxidase (PO) activity, a key enzyme in the biochemical cascade leading to the production of melanin, which is the key component in the encapsulation response (Söderhall and Cerenius 1998). Finally, we estimated the amount of a hemolymph-bound enzyme lysozyme, which recognizes and attacks cell walls of gram-positive bacteria (Adamo 2004; Rantala and Roff 2005).

### Encapsulation rate

To measure the degree to which beetles were able to melanize a foreign body, experimental individuals were implanted with a 3-mm long, sandpaper-roughened segment of 0.255-mm diameter nylon monofilament fishing line 24h after the start of the experiment. A small hole was made between the fourth and fifth abdominal sternites with a 27-gauge syringe needle, and the implant was inserted until it was completely contained within the beetle's abdominal cavity. Prior to use, the needle and implants were sterilized in 70% ethanol. After implantation, beetles were returned to their respective containers and allowed to resume their activity. Exactly 14h after receiving an implant, beetles were freeze-killed and stored in a −80 °C freezer. We dissected the implants from the frozen beetles, removing any clumps of tissue, and photographed each one using a Nikon digital camera mounted on a stereomicroscope (Wild Heerbrugg Ltd, Heerbrugg, Switzerland). We photographed each implant thrice from different angles next to a control (nonimplanted) filament to account for variation in lighting. We measured the degree of implant melanization using ImageJ image-analysis software freely available from the National Institutes of Health (http://rsbweb.nih.gov). We outlined each implant and control using the polygon tool, which produced an average grey-scale value from all the pixels within each image. Darkness scores range from 0 (completely white) to 256 (completely black). The darkness score for each individual was calculated as the average difference between implant and control scores in the 3 images analyzed.

### Phenoloxidase and lytic activity

Three microliters of hemolymph were collected at the same time that the implant was removed. We mixed the hemolymph with 40 µL of phosphate-buffered saline (PBS) and stored the samples in a −80 °C freezer to halt enzymatic reactions and to induce cell lysis. To estimate PO activity, we added a known quantity of L-3,4-dihydroxyphenylanine (L-DOPA) to the hemolymph to replace the naturally occurring substrate. Because the amount of L-DOPA was constant across samples, any resulting differences in melanin production were due to individual differences in PO activity. We added 5 µL of thawed hemolymph solution and 7 µL of bovine pancreas a-chymotrypsin (Sigma-Aldrich, Germany) to each well of a microplate and allowed

them to react at room temperature for 20min. α-Chymotrypsin acts as a catalyst and converts all prophenoloxidase (PPO) present in the hemolymph into PO (Bailey and Zuk 2008). We then added 90 µL of a 15mM L-DOPA solution to each well and used a spectrophotometer (Power Wave 340; BioTek, Winooski, VT) to record the change in optical density (OD) at 490nm for 210min. OD readings were taken every 10min for 210min. This method estimates the total change in OD over the course of the reaction, ranging from an OD of 0 (transparent) to 4 (opaque). The PO activity rate was therefore calculated as the change in OD over time (OD∕time). We performed the same calculation on 12 control wells of each 96-sample plate containing only PBS and L-DOPA and then subtracted the average value of control samples from individual beetle values to obtain a final PO level. These protocols were adapted from Gershman et al. (2010).

We used 3mg of *Micrococcus lysodeikticus* (Sigma-Aldrich), a gram-positive bacterium, per 10L of PBS to determine the ability of a lysozyme-like enzyme to induce bacterial cell lysis. To estimate lytic activity, we added 10 µL of thawed hemolymph solution and 90 µL of PBS∕*M. lysodeikticus* solution to each well of a spectrophotometer microplate and recorded change in OD at 490nm for 150min. This method estimates the total change in OD from opaque to clear as lysozymes or lysozyme-like enzymes lyse the bacterial cells. OD readings were recorded every 5min. We performed the same calculation on 12 control wells of each 96-sample plate containing only PBS∕*M. lysodeikticus* solution and then subtracted the average value of control samples from individual beetle values to obtain a final level of lytic activity. Although OD decreases as more bacterial cells are lysed, lytic activity is given as a positive number for clarity. The experimental design described does not allow the characterization of the specific lysozyme responsible for cell lysis (Schneider 1985); the observed lytic activity is thus attributed to a lysozyme-like enzyme.

## Statistical analyses

Statistical analyses were performed using SPSS 19 (SPSS Inc., Chicago, Illinois, USA). A linear mixed effect REML model was used to assess the effect of sex, intrasexual competition, and dominance status on relative weight change and the different measures of immunity. Beetles placed on the same carcass were assigned the same carcass ID number. Carcass ID initially was included as a random effect in the model. However, Carcass ID had no effect on any of the immunity measurements (results not shown) and, therefore, was eliminated from the model in the analysis of immune responses. Instead, a multivariate analysis of variance (MANOVA) was employed in which the 3 immune measures were included as dependent variables, and sex, dominance status, level of intrasexual competition (present or absent), and size asymmetry were included as independent variables.

## RESULTS

### Outcome of fights

In 96% of the cases with 2 beetles of the same sex on a carcass (47/49), a clear hierarchy with a dominant and a subordinate was established, that is, 1 beetle was found consistently on the carcass 24 and 38h after the start of the experiment, the other off the carcass hiding in the peat. In 2 cases, both competitors were found on the carcass and no fights occurred between them despite multiple encounters. Because there was no obvious winner in these situations, they were excluded from further analyses. Body size was a good predictor of dominance: the larger beetle was dominant in 43 cases, the smaller one in only 4 ($\chi^2_1 = 32.36$, $P < 0.0001$).

Despite the large arena provided, 6 subordinate females and 2 subordinate males were found dead. All of them were missing limbs and had most likely been killed by the dominant individuals. None of the dominants died. Of the female survivors, 5 subordinates and 2 dominants had visible external injuries. Of the male survivors, only one subordinate was injured. Pooling across all individuals who were either killed or injured, there were significant effects of sex and dominance status. Injuries were more common among females than males ($\chi^2_1$ QUOTE = 6.25, $P = 0.02$), and subordinates received more injuries—lethal or not—than did dominants (QUOTE $\chi^2_1 = 7.12$, $P = 0.01$).

### Effects of intrasexual competition, dominance status, and size asymmetry on nutritional state and immunity

For all measurements taken, there was no difference between beetles that had only one partner of the opposite sex or 2 (competing) partners of the opposite sex. Therefore, results from these beetles were pooled and included in the "no competition" treatment for all subsequent analyses. In 1 competing pair of males and 1 competing pair of females, there was no clear winner. These individuals were excluded from the analysis.

*Nutritional state*
On average, beetles in all treatment groups gained weight (Figure 1). However, sex ($F_{1,179} = 34.96$, $P < 0.0001$) and status (mixed model, $F_{1,138} = 40.90$, $P < 0.0001$) had a significant effect on the weight gain (Figure 1). This was not simply an effect of larger individuals gaining more weight, as we looked at relative weight change, that is, weight gained relative to the individual's initial body mass. Females gained relatively more weight than males and dominants more than subordinates (follow-up contrasts, $P < 0.0001$). Having a competitor only had consequences for the nutritional status of subordinates: there was no difference in weight gain between dominants and beetles without competitors (follow-up contrast, $P = 0.91$), but subordinates gained less weight than beetles without competitors (follow-up contrast, $P < 0.0001$). The relative weight gain of dominants and subordinates was not dependent on the size asymmetry between the 2 competitors (dominants, males: $n = 24$, $r^2 = 0.06$, $P = 0.25$; females: $n = 23$, $r^2 = 0.029$, $P = 0.43$; subordinates, males: $n = 21$, $r^2 = 0.008$, $P = 0.68$; females: $n = 21$, $r^2 = 0.10$, $P = 0.14$), and the weight gain of subordinates did not depend on their absolute size ($n = 42$, $r^2 = 0.001$, $P = 0.87$).

*Immunity*
Sex had a significant effect on both implant darkness and PO activity, with females exhibiting higher values than males (Table 1). There was no significant difference between the sexes in lytic activity. Status also had a significant effect on immunity, but this was true only for implant darkness (Table 1; Figure 2): dominants had a higher encapsulation rate than subordinates (follow-up contrast, $P < 0.025$). The presence of an intrasexual rival only had consequences for the encapsulation rate of subordinates. Subordinates had lighter implants than beetles without competitors (follow-up contrast, $P < 0.047$; Figure 2), but there was no difference in implant darkness between dominants and beetles without competitors (follow-up contrast, $P = 1.00$). In dominant individuals, there was no significant relationship between implant darkness and size asymmetry (males, $n = 24$, $r^2 = 0.011$, $P = 0.63$; females, $n = 23$, $r^2 = 0.05$, $P = 0.30$). Because subordinates are typically smaller than the dominants, the status effect on immunity might simply be an effect of their smaller body size. However, there was no significant correlation between body size and implant darkness in control ("no

**Table 1**

**Follow-up analysis of variance from a MANOVA of the fixed effects of sex, status, and their interactions on measurements of individual immunity (implant darkness, PO, and lytic activity).**

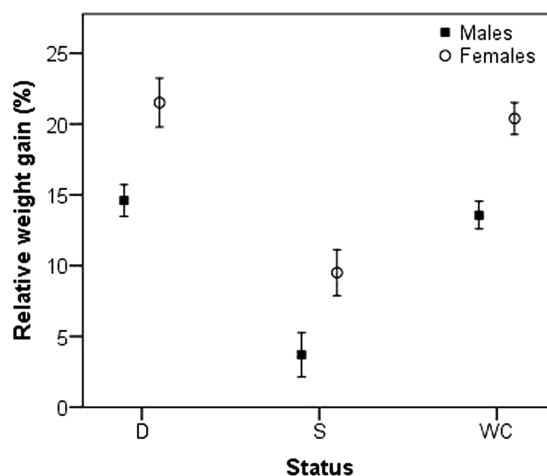| Factors | Implant darkness | PO activity | Lytic activity |
|---|---|---|---|
| Sex | $F_{1,155} = 4.06$ $P = 0.046$ | $F_{1,155} = 20.66$ $P < 0.001$ | $F_{1,155} < 0.001$ $P = 0.99$ |
| Status | $F_{2,155} = 3.94$ $P = 0.021$ | $F_{2,155} = 0.99$ $P = 0.38$ | $F_{2,155} = 0.06$ $P = 0.94$ |
| Sex x status | $F_{2,155} = 0.43$ $P = 0.65$ | $F_{2,155} = 0.19$ $P = 0.83$ | $F_{2,155} = 0.49$ $P = 0.62$ |

Bold values are statistically significant.



**Figure 1**
Relative weight gain (mean ± SE) of dominants (D), subordinates (S), and beetles without competitors (WC) during the first 24h on a carcass.
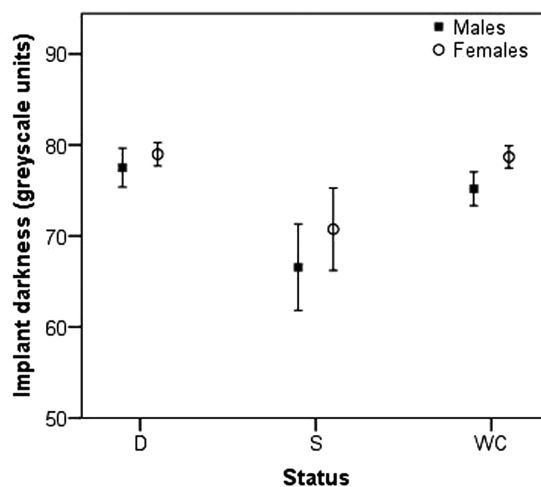


**Figure 2**
Implant darkness of dominants (D), subordinates (S), and beetles without competitors (WC).

competition") beetles ($n = 90$, $r^2 = 0.021$, $P = 0.177$), despite the wide range of body sizes established for this group. To further explore the lower immunity of subordinates, we analyzed implant darkness in relation to the size asymmetry between the dominant and the subordinate, the weight of a beetle at

the time of implant insertion, the relative weight gain, and the body size. None of the factors tested together (multiple regression, male: $n = 19$, $r^2 = 0.12$, $P = 0.77$; female: $n = 16$, $r^2 = 0.45$, $P = 0.14$) or separately (for all variables, $P > 0.05$) were good predictors of encapsulation rate of the subordinates. If nutrition affects the immune response, implant darkness should depend on the beetle's relative weight gain. However, the encapsulation response of subordinates did not depend on their relative ($n = 34$, $r^2 = 0.02$, $P = 0.49$) or absolute ($n = 34$, $r^2 = 0.03$, $P = 0.33$) weight gain.

## DISCUSSION

Our experiments showed that dominance status was associated with differences in weight gain and encapsulation rate in *N. orbicollis*. Although intrasexual competition had no effect on dominant beetles, the subordinate of 2 competing males or females gained less weight and exhibited a weaker encapsulation response than both the dominant and beetles without competitors. In addition, we found a clear sex effect, with females gaining relatively more weight than males and having superior immunity (encapsulation rate and PO activity). The latter result confirms earlier findings (Steiger et al. 2011).

When 2 males or females competed for a carcass, the larger beetle was usually found on the carcass at both points in time, 24 and 38 h after carcass provisioning, and this beetle gained relatively more weight than his or her smaller competitor. Thus, it is likely that subordinates' access to the carcass was curtailed by the dominants, resulting in lower food intake in subordinates compared with dominant beetles. This effect has been demonstrated previously in female *N. vespilloides* competing for 15 g mice: dominant females were in significantly better nutritional condition than subordinates (Müller et al. 1990). We also found that male *N. orbicollis* gained less weight than females. Female burying beetles arrive on a carcass with ovaries that do not contain mature ova and undergo rapid ovarian growth during their first day on a carcass prior to initiating oviposition, and thus, they might have a greater need to feed on the carcass than males. In a recent study, Eggert et al. (2008) found that subordinate females lay fewer eggs than dominants. Fecundity differences disappeared when supplemental food was made available to the subordinates, indicating that access to the carcass and food intake is essential for producing high numbers of eggs (see also Steiger et al. 2007). The fact that subordinate females gained more weight than subordinate males also implies that females fed on the carcass more than males. They may have stayed on the carcass for longer periods or visited it more frequently, or they may have spent a greater portion of their time on the carcass feeding. Therefore, subordinate females may take greater risks than subordinate males to gain access to the carcass, as suggested by their higher rate of injuries. To be able to produce at least some offspring, it may be essential for females to gain prolonged access to the carcass, and therefore, the additional risks taken may be adaptive. By contrast, subordinate males do not have to feed on the carcass at all to sire offspring. They need only to gain access to the resident female to obtain copulations; indeed, female *N. vespilloides* are known to make occasional forays from the carcass into the soil, increasing the opportunity for subordinate matings (Pettinger et al. 2011). However, risk-taking strategies in burying beetles appear to be flexible, with adjustments based on dominance status and the context in which competitors are encountered. When carcass owners are confronted with intruders in the brood chamber, males take greater risks than females, with males appearing both more willing and able to guard the brood (Trumbo 2006). In

general, an animal's willingness to take risks and engage in escalated fights is thought to depend on various factors such as the value of the resource, the motivation of the opponent, and the competitors' intrinsic fighting abilities (Houston and McNamara 1988; Brown et al. 2006; Jonart et al. 2007). Burying beetles appear to assess their situation and modify their level of risk-taking accordingly.

Surprisingly, the size asymmetry between the 2 contestants in our study did not influence their nutritional condition, and thus, it appears that access to the carcass by subordinates was not influenced by the relative size of their dominant rivals. We had assumed that relatively larger beetles would be more effective at monopolizing the resource, but this does not appear to be the case. Possibly, very small individuals might be more capable of evading detection by dominants. It is not likely that very small beetles were motivated by danger of starvation to make greater efforts to access the carcass, as even the smallest of our experimental individuals were well fed before the start of the experiment.

The encapsulation responses of breeding pairs in this study were lower than those reported in a previous study (Steiger et al. 2011). This is likely due to age differences between the experimental beetles used. Beetles used in this study were on average 50 days old, whereas in the earlier study, we established 2 age classes of beetles 22 and 38 days old, respectively. In our previous study, age had a negative effect on the encapsulation response of breeding beetles (Steiger et al. 2011), and our current data support these age effects. As in the previous study, neither the PO nor the lysozyme assay differed significantly between our treatment groups. It appears that an increased encapsulation response is of greater importance during breeding. The ultimate reason for this may lie in the fact that burying beetles also visit larger carcasses that they cannot bury and utilize them as food sources. Such larger decomposing carcasses are heavily colonized by bacteria, but the beetles do not engage in fights there. Thus, high antibacterial activity may be required outside of breeding attempts, whereas injury repair may not.

Our experiments revealed that subordinate beetles had a lower encapsulation response than dominant beetles. Cause and effect are somewhat difficult to disentangle for this association. At first glance, it seems that the effect could have resulted from weaker, immunodeficient individuals losing fights and becoming subordinates. If such had been the case, the immune response of beetles without competitors should have been intermediate between the response of dominant and subordinate individuals. However, beetles without competitors were as immunocompetent as dominant individuals (see Figure 2), which strongly suggests that losing fights, or reduced access to the carcass, causes individuals to exhibit reduced immune responses rather than vice versa. The proximate and ultimate causes of this effect are unclear at the present time. The lesser response of subordinates could result from constraints associated with their subordinate status that preclude them from mounting a strong response. Possible constraints could be nutritional limitations because of limited access to the carcass and its high-quality protein or greater energetic expenses required of smaller individuals in the physical challenge of fights. Although our experiments clearly show that fights were more costly to subordinates, this cost came in the form of greater injury risk. Currently, there is no evidence that fights are more energetically expensive to smaller individuals. If energy expended in fights reduces resources available for immunity, we would predict that all beetles competing for a carcass should exhibit a lower immune defense than beetles reproducing in the absence of competitors, but this was not the case. It thus seems more likely that the subordinate's

exclusion from the carcass, and possibly the concomitant reduction in food intake, plays an important role in mediating the difference in immune responses. Although none of our experimental individuals were nutritionally stressed going into the experiment, it is likely that subordinates were largely prevented from ingesting any of the high-quality protein the carcass presents. A positive relationship between nutritional condition and insect immune function has been shown in studies of other insect species (Povey et al. 2009; Srygley et al. 2009), and protein quality in particular can have significant effects on insect immune function (Lee et al. 2008). However, we have no information about the temporal course of weight gain and immune response in breeding beetles. We do not know whether beetles need to feed on the carcass before they mount an immune response, during the response, or both. The lack of a relationship between subordinates' weight gain and their immune response suggests that immunocompetence may not actually be mediated by nutritional condition.

Rather than resulting from nutritional constraints, however, the reduced immunity in subordinates could be an adaptive response to losing fights if subordinates have little need to mount strong encapsulation responses. Losing fights may be a cue to the individual that it will not likely spend much time directly on the carcass where it would be exposed to potential pathogens, or that it will not likely be defending the carcass against other beetles in the near future, which would carry an increased injury risk. The upregulation of immunity (i.e., encapsulation rate) that occurs in breeding *N. orbicollis* shortly after the discovery of a carcass (Steiger et al. 2011) is likely an adaptive response to protect against parasites and pathogens, fungi, and other micro-organisms, growing on the carcass. Because subordinates spend very little time on the carcass (e.g., in *N. vespilloides*, subordinates spend only 5% of the first 24 h on the carcass, whereas dominants spend 78%; Müller et al. 1990), it may not be necessary for them to maintain their immune system at an enhanced level of readiness. Subordinate individuals also abandon the carcass before the larvae hatch and, thus, will not engage in fights after this time, whereas dominants continue to defend the carcass once larvae are present.

Although the above-mentioned lack of a significant relationship between subordinate nutrition and immunity suggests that it is not nutrition that causes the differential immune response of dominants and subordinates, our current results do not allow us to definitively rule out a role for nutrition. A future study in which supplemental food is made available to subordinates away from the carcass could help more clearly resolve the issue. However, regardless of the underlying proximate mechanism, our results clearly show that a dominance hierarchy can influence the immunity of the contestants. In addition, our study suggests that a lower immunity does not necessarily imply that an individual is of lower physiological quality. Differences in social roles, spatial distribution, or residency may entail differences in the optimal level at which individuals should invest in the functioning and maintenance of their immune system.

## FUNDING

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