

Nutrient Stress During Ontogeny Alters Patterns of Resource Allocation in two Species of Horned Beetles



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ABSTRACT

The elaboration of exaggerated, sexually selected weapons and ornaments often comes at a cost to other traits. For instance, by sustaining the growth of an exaggerated weapon during development, shared and limited resources such as morphogens, growth factors, and nutrients may become depleted and limit the size to which other structures can grow. Such interactions are characteristic of resource allocation trade-offs, which can constrain the production of phenotypic variation and bias evolutionary trajectories. Across many species of *Onthophagus* beetles, males produce extravagant horns that are used as weapons in male–male competition over mates. Previous studies have reported resource allocation trade-offs between horns and both proximally and distally developing structures. However, more recent studies have largely failed to recover these patterns, leading to the hypothesis that trade-offs may manifest only in certain species, populations, or environmental conditions. Here, we investigate (i) patterns of resource allocation into horns, eyes, and genitalia in *Onthophagus gazella* and *O. taurus*, and assess (ii) how these patterns of resource allocation are influenced by nutrient stress during larval development. We find that nutrient stress alters patterns of resource allocation within and among traits, but recover a trade-off only in the species that invests most heavily into horn production (*O. taurus*), and in individuals of that species that invested a disproportionately large or small amount of resources into horn growth. These results suggest that resource allocation trade-offs may not be as prevalent as previously described, and that their presence and magnitude may instead be highly context dependent. *J. Exp. Zool.* 325A:481–490, 2016. © 2016 Wiley Periodicals, Inc.

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INTRODUCTION

Investigations of the causes and consequences of resource allocation trade-offs, where the investment of limiting resources into one trait or process necessarily decreases investment elsewhere, are a common theme in the study of organismal physiology, development, and life history. For instance, resource allocation trade-offs have long been known to exist between life history traits such as reproduction and growth (Robinson and Doyle, '85; Van Rooij et al., '95; Roff, 2000), immunity (Gustafsson et al., '97; Nordling et al., '98), and survival (Jensen, '96; Gunderson, '97), as well as between propagule size and number (Fleming and Gross, '90; Stearns, '92; Venable, '92), but also during ontogeny when multiple morphological traits develop simultaneously (Klingenberg and Nijhout, '98; Simons and Emlen, 2006). Such developmental trade-offs, where

the elaboration of one morphological trait comes at the expense of another, may arise when developing traits are forced to compete for a shared pool of limited resources such as morphogens, growth factors (e.g., insulin-like peptides), nutrients

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(e.g., lipids, amino acids), or simply developmental space in order to enable their growth and differentiation (Moczek and Nijhout, 2004). As a consequence, resource allocation trade-offs can shape the phenotypic variation available to selection and possess the potential to bias both the magnitude and direction of trait evolution and diversification (Emlen, 2001; Simmons and Emlen, 2006).

Resource allocation trade-offs may be particularly pronounced in holometabolous insects such as butterflies, flies, and beetles: in these taxa, most of adult trait growth and differentiation occurs after the larva has stopped feeding, primarily in a short period before and during the quiescent pupal stage. For instance, experimental removal of the hindwing imaginal disc in the late larval stage of *Precis coenia* (Lepidoptera: Nymphalidae) results in disproportionate, compensatory growth in the neighboring forewings (Nijhout and Emlen, '98), and juvenile hormone has been shown to mediate the trade-off between both primary and secondary sexual traits in stalk-eyed flies (Fry, 2006).

Male beetles in the genus *Onthophagus* produce extravagant horns of various sizes and shapes that are used as weapons in competition for access to females. Previous studies have reported resource allocation trade-offs between these horns and both proximally (i.e., antennae, eyes, wings; Klingenberg and Nijhout, '98; Nijhout and Emlen, '98; Emlen, 2001; Pizzo et al., 2012) and distally located structures (i.e., genitalia; Moczek and Nijhout, 2004; Simmons and Emlen, 2006; Parzer and Moczek, 2008; Pizzo et al., 2012). Intriguingly, these trade-offs are responsive to artificial selection under laboratory conditions (Nijhout and Emlen, '98), and appear to shape the diversification of horn placement across the *Onthophagus* phylogeny so as to avoid limiting the elaboration of other functional traits (Emlen, 2001). Although resource allocation trade-offs may therefore influence morphological evolution, the degree to which the presence and strength of these trade-offs varies among populations, species, or environmental conditions is largely unclear.

In a recent study, we used *Onthophagus taurus*, a species of beetle that develops horns in proximity to the eyes, to assess the degree to which the well-characterized horn-eye trade-off (e.g., *O. acuminatus*: Nijhout and Emlen, '98; unnamed species: Emlen, 2001) can be influenced by genetic, developmental, and ecological factors (Schwab and Moczek, 2014). Specifically, we tested for the signature of trade-offs (i.e., negative correlation between residual trait values) among (i) natural populations of beetles that vary in their relative investment into horns, (ii) field-collected and laboratory-reared beetles, and (iii) male and female beetles in which horns were artificially reduced or induced to grow, respectively, via developmental-genetic manipulations. Contrary to expectations, we largely failed to recover evidence of a horn-eye trade-off under any set of comparisons. This result is, however, consistent with other recent studies that

question the consistency and evolutionary consequences of resource allocation trade-offs in this and similarly other well-characterized systems (Mole and Zera, '94; House and Simmons, 2012; McCullough et al., 2012; McCullough and Emlen, 2013). We therefore hypothesized that the nature of resource allocation trade-offs may be more complex than previously appreciated, and that resource allocation trade-offs may be manifest only in certain species, populations, or under particular (e.g., stressful) environmental conditions, as these factors are well known to influence the sign and magnitude of genetic correlations (Sgro and Hoffmann, 2004; Schwab and Moczek, 2014).

Here, we test this hypothesis by investigating the effect of developmental environment on the presence and strength of resource allocation trade-offs among horns, eyes, and genitalia. Specifically, we assess how resource allocation is influenced by an ecologically relevant stressor, starvation (i.e., nutrient stress (NuS)), during late larval development in two species of beetles that differ markedly in their average degree of investment into horns: *O. gazella* (low investor) and *O. taurus* (high investor; Fig. 1). We predicted that (i) NuS will result in smaller absolute trait sizes for both eyes and genitalia, and will shift the threshold for horn induction to smaller body sizes (as seen in Emlen, '97; Moczek, '98), (ii) NuS will alter the strength and direction of among-trait correlations, resulting in resource allocation trade-offs, and (iii) these trade-offs should be most pronounced in the species that invests most heavily into horns (*O. taurus*).

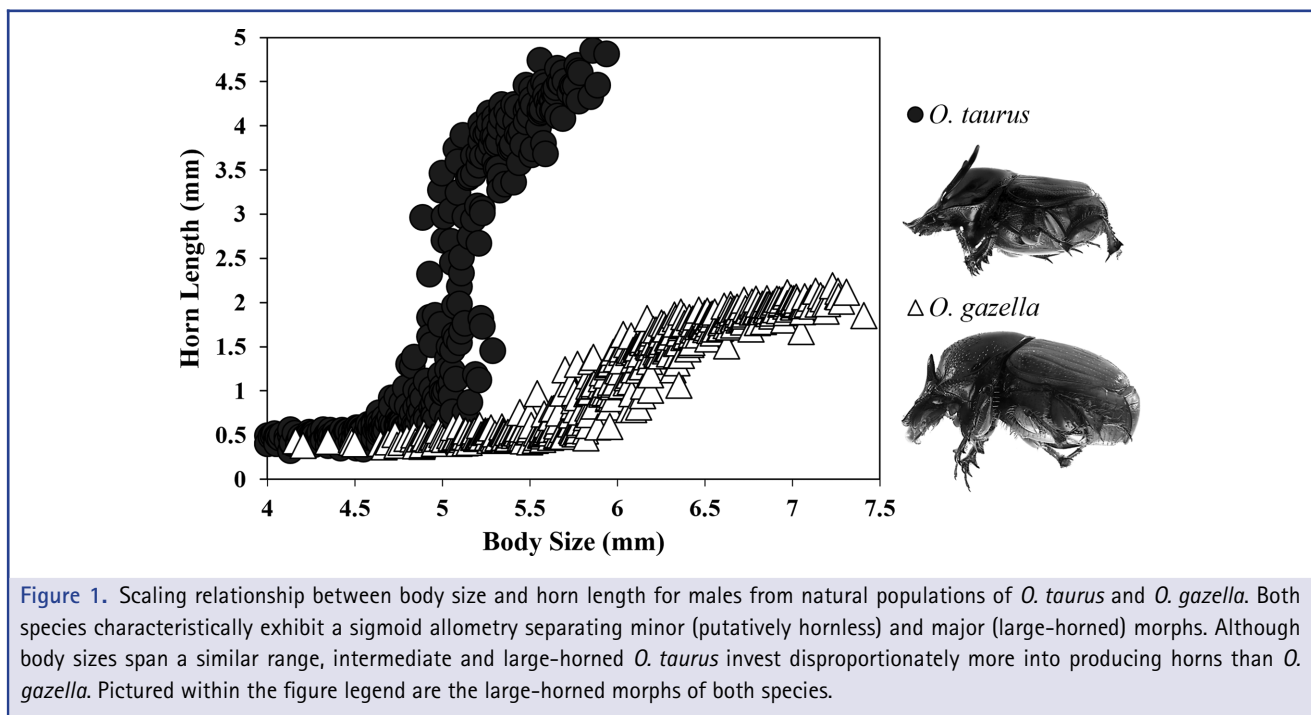
MATERIALS AND METHODS

Beetle Collection and Colony Maintenance

In June 2014, adult *O. taurus* and *O. gazella* were collected from cow dung pads at Marble Hill Farm in Bloomington, IN (39° 3' 8" N, 86° 36' 12" W) and Kualoa Ranch in Kaneohe, HI (21° 31' 15" N, 157° 50' 14" W), respectively. Beetles were maintained simultaneously within laboratory colonies in a moist sand-soil mixture at either 24° C (*O. taurus*) or 28° C (*O. gazella*) at 16 L: 8 D, and were fed cow dung ad libitum as described previously (Moczek et al., 2002).

Rearing and Application of Nutrient Stress

All individuals used in this study were the offspring of field-collected (F_0) *O. taurus* and *O. gazella*. Briefly, F_0 beetles of both species were bred in plastic containers (25 cm tall, 20 cm diameter) filled 75% of the way with a moist sand-soil mixture. Three male and six female beetles were added to each container and provisioned with ~0.5 L of cow dung. Following 6 days of breeding, adult beetles were recaptured and brood balls, containing a single larva each, were collected and placed into separate plastic containers. Larvae were maintained within their natal brood balls for approximately 10 days, at which time they were



transferred to individual artificial brood balls (ABBs) within 12-well tissue culture plates containing cow dung ad libitum and reared within stand-alone incubators at either a constant 24° C (*O. taurus*) or 28° C (*O. gazella*). Immature *Onthophagus* complete all developmental transitions from egg to larval, pupa, and adult stages under these conditions, similar to larvae reared within the brood ball (Shafiei et al., 2001). Plate position was shifted every day to counteract the effects of potential microclimatic variation within incubators.

To investigate the effect of ecological stressors on the presence and severity of resource allocation trade-offs, we visually identified larvae which were beginning to show early signs of fat body accumulation, indicating that they had acquired competence to eventually reach pupation. This usually occurred early during the last (third) larval instar, which constitutes the main feeding and weight-gaining phase of larval development (Moczek and Nijhout, 2004). We then randomly assigned these larvae to one of two treatments: (i) permissive conditions, under which beetles were maintained in their individual well that contained dung ad libitum for the remainder of larval development (AL) or (ii) stressful conditions, under which beetles were exposed to NuS. Under the NuS treatment, larvae were removed from their ABB for a short (<1 min) duration, during which time all dung was removed and refilled to 50% capacity with moist vermiculite. Larvae were quickly transferred back into individual wells and left undisturbed for the remainder of larval and pupal development. During this time, larvae fed readily on the vermiculite,

which provides a source of hydration in the absence of nutrition and thereby exposes larvae to starvation conditions while avoiding dehydration artefacts. To control for potential effects of handling, AL larvae were removed from their ABB for an approximately similar amount of time and then transferred back into their original ABBs and left undisturbed for the remainder of development.

Morphometric Measurements

To assess the phenotypic outcomes of both treatments, we reared approximately 100 adult male individuals from *O. taurus* ($N_{AL} = 50$; $N_{NS} = 50$) and *O. gazella* ($N_{AL} = 52$; $N_{NS} = 50$). Head horn length, eye size, aedeagus length, and body width of all individuals were measured via a standard two-dimensional morphometric setup, including a Leica MZ16 stereomicroscope and ImageJ v. 1.44p software. We measured the left side of all symmetric structures (i.e., head horns and eyes) for residual analyses, and additionally measured horns on the right side for the analysis of fluctuating asymmetry (FA, see below). Head horns were measured from the outer margin of the eye to the tip of the horn, as described previously (Moczek, 2006). To obtain a measure of eye size, we took a lateral image of each eye, traced along its outer margins, and calculated the inner area following Emlen (2001). Aedeagus size was measured as the combined dorsal length of the paramere and phallobase, similar to Parzer and Moczek (2008). Pronotum width was used as a proxy for body size and

measured as in previous studies (e.g., Emlen, '94). All measurements were to the nearest 0.01 mm and collected by D.B.S.

Analysis

Following previous studies (Emlen, 2001; Parzer and Moczek, 2008; Schwab and Moczek, 2014), differences in the relative investment into head horns, eyes, and genitalia among treatments were examined via a residual-based analysis using a pooled sample of all AL and NuS beetles within each species. Relative investment into these traits was standardized for each beetle using pronotum width as a proxy for body size. Given the sigmoidal relationship between body size and horn length for both species of *Onthophagus* (Fig. 1), we fit a Hill four-parameter regression model to these data:

$$\text{horn length} = \text{minimal horn length} + \frac{a(\text{body size}^b)}{c^b + (\text{body size}^b)}$$

where a represents the range of observed horn lengths, b represents the maximum slope of horn increase, and c represents the inflection point of the sigmoidal curve (use justified in Moczek and Nijhout, 2004). This analysis generated means and standard errors for each parameter in both species and treatment groups, which were then compared among treatment groups using Welch's t -test. These parameters, such as the range of horn lengths (parameter a) and the inflection point (parameter c), have been reported to respond to variation in stressors such as local competition for dung and larval food quality, respectively, in *O. taurus* (Moczek, 2002, 2003; Buzatto et al., 2012).

Given the linear relationship between body size and both eye and aedeagus size, a linear regression model was fit to all data points:

$$\text{eye (or aedeagus) size} = \text{minimal eye (or aedeagus) size} + a \text{ body size}$$

where a represents the slope of the linear regression. For all individuals, we calculated the expected horn, eye, and aedeagus size given body size using the parameter estimates for each trait, and then calculated the difference between this and the observed sizes to obtain residual trait values. We regressed residual values for horns, eyes, and aedeagi against one another in order to examine the relationship between relative investment into each pair of traits. We evaluated differences in the slope, which can indicate a difference in the rate and/or sign of change in the size of trait Y relative to trait X, and the intercept, which can indicate a consistent increase or decrease in the size of trait Y relative to trait X, across the full body size range using ANCOVA following Nijhout and Emlen ('98) for all comparisons. A trade-off in investment was defined by a significant negative correlation in residual values. Regression models were fit and residuals calculated using SigmaPlot. We used Welch's t -test to assess differences in parameter estimates of the body size-

horn length sigmoid allometry, and ANCOVAs were conducted for all other allometric contrasts. FA of horns, a measure of developmental stress, was calculated by taking the absolute difference of left and right horn lengths and dividing by the mean. Differences in FA as well as adult body size were assessed using t -tests or Wilcoxon rank sum tests, and differences in mortality were assessed using the Chi-squared test. All analyses were conducted using SPSS statistical software v.22.

One common feature of sigmoid or threshold allometries, such as those that characterize horn polyphenism, is that residual values tend to be minimal in the smallest and largest size classes, and maximal at intermediate body sizes. In *O. taurus*, for instance, horn growth reaches its minimum in males below 4.8 mm and maximum in males above 5.25 mm, resulting in a large number of residual horn lengths near 0. Conversely, intermediate body sizes tend to be correlated with large residual horn lengths (e.g., see Schwab and Moczek, 2014). Indeed, after calculating residual values for all traits in our dataset from a pooled sample of individuals from the NuS and AL treatments, we found that a large number of residual horn sizes were clustered around zero for both *Onthophagus* species. Because trade-off signatures may be most apparent in individuals with relatively large negative or positive residual horn lengths, yet masked in our analyses by the numerous residual horn lengths near 0, we repeated our analyses following the removal of individuals from the second and third quartile of pooled residual horn lengths for both *O. taurus* ($N_{\text{AL}} = 22$; $N_{\text{NuS}} = 28$) and *O. gazella* ($N_{\text{AL}} = 29$; $N_{\text{NuS}} = 22$) in order to maximize our ability to detect treatment effects.

RESULTS

Effect of Nutrient Stress on Adult Body Size, Mortality, and FA

In order to confirm the efficacy of our treatment in stressing developing beetles, we first assessed the effect of NuS on body size, mortality, and horn FA. In *O. gazella*, nutrient stressed (NuS) beetles had smaller body sizes than ad libitum fed (AL) beetles at adulthood ($\bar{x}_{\text{AL}} = 6.386$ mm, $\bar{x}_{\text{NuS}} = 5.648$ mm; t -test: $t = 6.335$, $P < 0.001$), and experienced significantly higher mortality than AL beetles (Survival_{NuS} = 61%, Survival_{AL} = 80%; χ^2 test: $\chi^2 = 11.46$, $P < 0.001$). However, we found no treatment effect on horn FA, a common measure of developmental stress (Median_{AL} = 0.0230, Med_{NuS} = 0.0379; Wilcoxon Rank Sum test: $W = 1043$, $P = 0.156$). Conversely, in *O. taurus* we detected a significant effect of NuS on mortality (Survival_{NuS} = 69%, Survival_{AL} = 94%; χ^2 test: $\chi^2 = 19.47$, $P < 0.001$) as well as significantly elevated levels of FA (Med_{AL} = 0.0161, Med_{NuS} = 0.0299; Wilcoxon Rank Sum test: $W = 943$, $P = 0.035$), but failed to detect a significant effect on body size at adulthood (Med_{AL} = 4.894 mm, Med_{NuS} = 4.749 mm; Wilcoxon Rank Sum test: $W = 1436$, $P = 0.201$). Our treatment thus generated clear signatures of appreciable developmental stress in both species, while at the same time retaining a relatively wide and

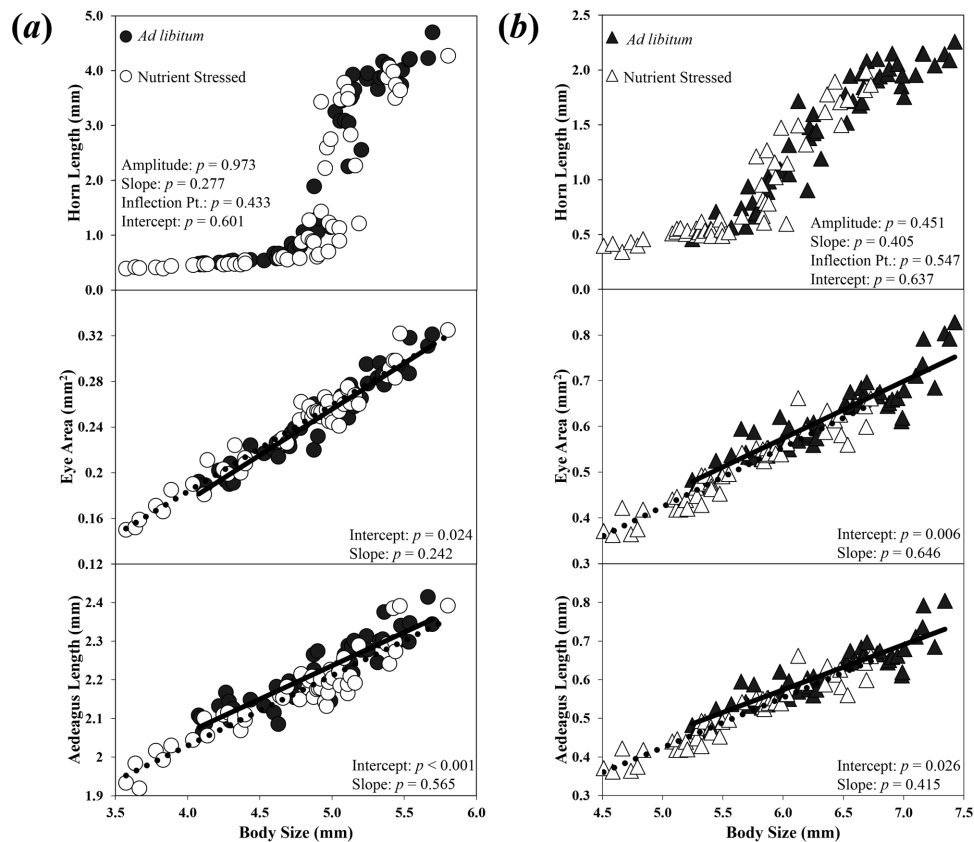


Figure 2. Scaling relationship between body size and horn length, eye area, and aedeagus length for males from *ad libitum* (AL) and nutrient stressed (NuS) treatments of *O. taurus* and *O. gazella*. NuS fails to influence the body size–horn size allometry in both (a) *O. taurus* and (b) *O. gazella*, but significantly alters overall investment into eyes and aedeagi, primarily by decreasing resource allocation across the entire body size range relative to control AL beetles (but see *O. taurus* body-size eye size allometry). Significant differences among treatments are denoted by the dotted (NuS beetles; open data points), and solid (AL beetles; closed data points) regression lines.

overlapping range of adult body sizes among treatment animals that is needed to assess the presence and severity of resource allocation tradeoffs in scaling relationships (AL_{*taurus*}: 4.078–5.695 mm, NuS_{*taurus*}: 3.574–5.802 mm; AL_{*gazella*}: 5.247–7.342 mm, NuS_{*gazella*}: 4.665–6.729 mm).

Effect of Nutrient Stress on Relative Investment into Horns, Eyes, and Aedeagus

We next contrasted body size and horn, eye, and aedeagus allometries between NuS and AL beetles to assess whether rearing conditions can alter patterns of resource allocation. We predicted that NuS should (i) decrease the size of the eyes and aedeagus in both species, and (ii) shift the threshold at which beetles develop horns to larger body sizes or decrease maximal horn growth in large horned individuals. We found partial support for these predictions in both species. In *O. taurus*, we found no effect of NuS

on any aspect of the body size–horn length allometry, including amplitude ($t_{98} = 0.03$, $P = 0.973$), slope ($t_{98} = 1.09$, $P = 0.277$), inflection point ($t_{98} = 0.79$, $P = 0.433$), and y-intercept ($t_{98} = 0.52$, $P = 0.601$; Fig. 2a). However, we found that beetles reared under NuS conditions developed significantly smaller aedeagi than AL beetles across the full body size range ($F_{1,97} = 14$, $P < 0.001$). Intriguingly, eyes showed an opposite response of similar magnitude and increased in relative size in NuS compared to AL beetles ($F_{1,97} = 5.24$, $P < 0.001$; Fig. 2a).

In *O. gazella*, as in *O. taurus*, we found no effect of NuS on horn allometry (amplitude: $t_{100} = 0.76$, $P = 0.451$; slope: $t_{100} = 0.84$, $P = 0.405$; inflection point: $t_{100} = 0.605$, $P = 0.547$; y-intercept $t_{100} = 0.473$, $P = 0.637$) and a significant negative effect on resource allocation to aedeagi across the entire body size range ($F_{1,97} = 5.11$, $P < 0.026$; Fig. 2b). However, contrary to results in *O. taurus*, and consistent with our hypothesis, we

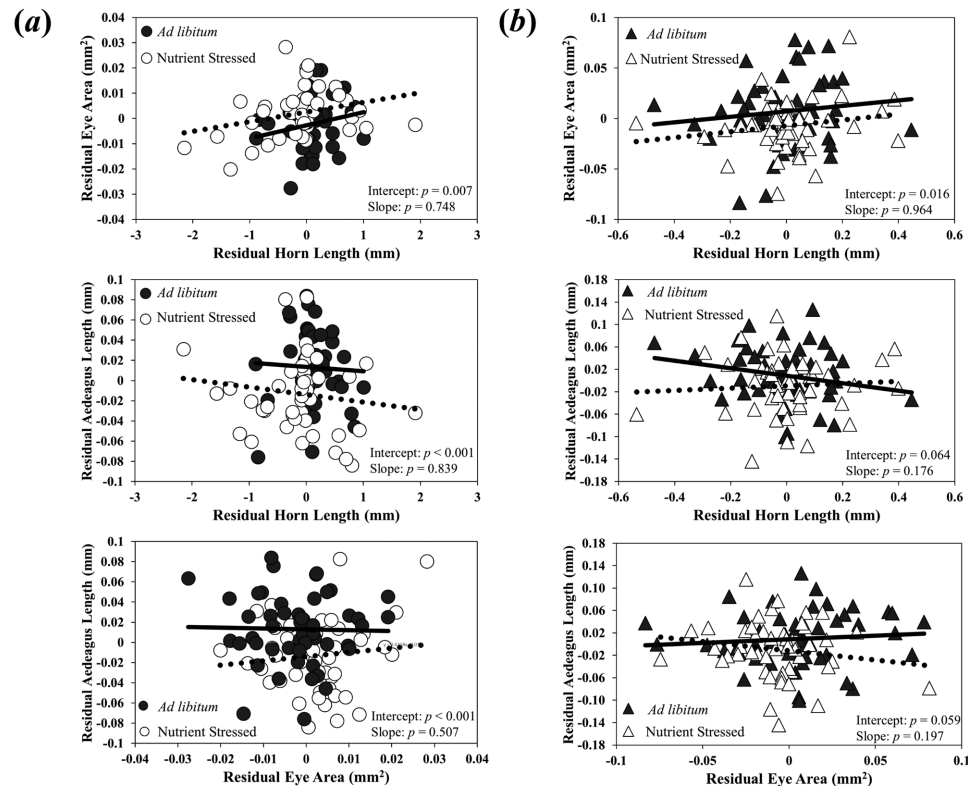


Figure 3. Bivariate plots of residual horn, eye, and aedeagus sizes in *O. taurus* and *O. gazella*. Resource allocation trade-offs predict a significant negative correlation, and are most likely to occur under stressful developmental conditions (i.e., NuS treatment) and in the species that invests disproportionately into horns (i.e., *O. taurus*). Both (a) *O. taurus* and (b) *O. gazella*, exhibit significant treatment effects but no significantly positive or negative correlations amongst any pair of traits. Significant differences among treatments are denoted by the dotted (NuS beetles; open data points), and solid (AL beetles; closed data points) regression lines.

found that *O. gazella* reared under NuS conditions developed significantly smaller eyes than AL beetles ($F_{1,99} = 8.03$, $P < 0.006$; Fig. 2b). Combined, these results demonstrate that NuS treatment is sufficient to alter patterns of resource allocation in both *O. taurus* and *O. gazella*, but suggest that the presence and nature of this effect may vary among traits and species.

We next sought to examine whether stressful rearing conditions alter correlations between horn length, eye, and aedeagus size residuals, and whether this responsiveness manifests in negative correlations indicative of potential resource allocation trade-offs among traits. We predicted that (i) beetles reared under NuS conditions will most strongly express altered trait correlations, maintaining investment into some structures while reducing investment into others, (ii) *O. taurus*, the species that shows the most extreme nutrition-responsive horn growth, will be most susceptible to alterations in trait correlations, and (iii) trade-offs are therefore most likely to arise in *O. taurus* reared

under NuS conditions. In *O. taurus*, rearing beetles under NuS conditions significantly altered residual trait correlations for all bivariate comparisons, but did so by significantly shifting the y-intercept without altering the slope of the respective scaling relationship in a manner that matched the treatment responses observed in the absolute body size trait size scaling relationships seen above (horns vs. eyes: $F_{1,97} = 7.59$, $P = 0.007$; horns vs. aedeagus: $F_{1,97} = 14.85$, $P < 0.001$; eyes vs. aedeagus: $F_{1,97} = 13.77$, $P < 0.001$). For example, the NuS treatment resulted in smaller aedeagi across the entire body size range (Fig. 2a), resulting in a corresponding reduction in aedeagi residuals regardless of body size (Fig. 3a). Results in *O. gazella* were qualitatively similar, with significant decreases in the y-intercept for NuS beetles across all bivariate comparisons (horns vs. eyes: $F_{1,99} = 6.06$, $P = 0.016$; horns vs. aedeagus: $F_{1,97} = 3.51$, $P = 0.064$; eyes vs. aedeagus: $F_{1,97} = 3.66$, $P = 0.059$; Fig. 3b). All correlations were nonsignificantly different from zero.

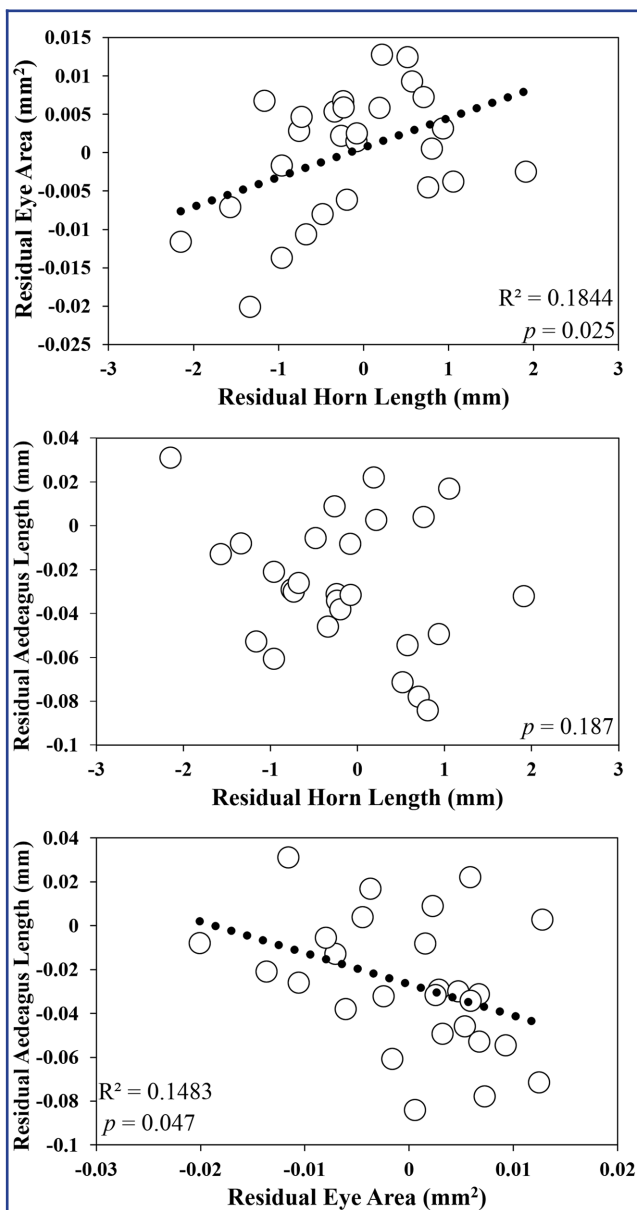


Figure 4. Bivariate plots of residual horn, eye, and aedeagus sizes in nutrient stressed (NuS) *O. taurus* following the removal of residual values near 0. *Onthophagus taurus* reared under NuS conditions exhibit a significant positive correlation between residual horn and eye sizes ($P = 0.025$), a nonsignificant negative correlation between residual horn and aedeagus sizes ($P = 0.187$), and a significant negative correlation between residual eye and aedeagus sizes ($P = 0.047$), which is consistent with the presence of a resource allocation trade-off. Dotted regression lines indicate a significant correlation. One outlier was removed from the dataset.

Given our failure to detect trade-offs using residual trait values from the entire dataset, we repeated our analysis following the removal of individuals from the second and third quartile of pooled residual horn lengths for both species of beetle. In *O. gazella*, we remained unable to recover a significant positive or negative correlation for any pair of traits in either treatment. In contrast, in *O. taurus*, the species that exhibits the most pronounced nutrition-responsive investment into horns, we found a significant positive correlation between horns and eyes ($SS = 0.0003$, $F = 5.65$, $r^2 = 0.18$, $P = 0.025$), a negative but nonsignificant correlation between horns and aedeagus ($SS = 0.0016$, $F = 1.84$, $r^2 = 0.07$, $P = 0.187$), and a significant negative correlation between eyes and aedeagus ($SS = 0.0035$, $F = 4.39$, $r^2 = 0.15$, $P = 0.047$) (Fig. 4). Therefore, the NuS treatment was sufficient to generate a trade-off, but was only able to do so in the individuals and species that invested the most into horn production.

DISCUSSION

Identifying the conditions under which resource allocation trade-offs manifest is key to understanding the structure of phenotypic variation within populations and its evolutionary consequences. In this study, we investigated the effect of a nutritionally stressful environment on the presence and strength of resource allocation trade-offs among horns, eyes, and genitalia in *O. gazella* and *O. taurus*. We observed that the removal of nutrition during the mid-third larval instar was sufficient to stress developing beetles by decreasing adult body size and increasing both mortality and FA. We then found that NuS altered patterns of resource allocation within and among traits, but that the presence of a trade-off was only detected among a subset of stressed individuals in *O. taurus*. Below, we discuss the most important implications of our results.

nutrient stressed Alters Absolute Sizes of Developing Traits

We found mixed support for our prediction that NuS would shift the threshold for horn induction to smaller body sizes and generate adult beetles with smaller absolute trait sizes for eyes and genitalia. Although the threshold at which horns are produced has been shown to shift to smaller body sizes in response to a low-quality diet in *O. acuminatus* (Emlen, '97) and *O. taurus* (Moczek, '98), we failed to find a similar response in our NuS individuals, for reasons that are presently unclear. In addition, we recovered divergent responses to NuS on eye growth among species, which grew to larger and smaller sizes in *O. taurus* and *O. gazella*, respectively, whereas genitalia were smaller across the full body size range in both species (Fig. 2). This latter result is particularly surprising, given that there is characteristically little variation in copulatory organ size relative to body size across arthropod taxa such as spiders and insects (Eberhard, '98). Further, the copulatory organs of many insect species are generally relatively unresponsive to variation in nutrition (e.g.,

Cayetano and Bonduriansky, 2015; House et al., 2015), as well as to developmental genetic manipulations of key growth regulatory pathways such as insulin signaling (e.g., Shingleton et al., 2005; Emlen et al., 2012; but see Snell-Rood et al., 2013), when compared to other traits. However, the size of the *Drosophila* copulatory organ demonstrates genotype-by-environment variation in response to factors such as nutrition (Shingleton et al., 2009), and previous studies of *Onthophagus* have demonstrated substantial among-population variation in copulatory organ size and shape (Parzer and Moczek, 2008, and in review). In combination with these observations, our findings suggest that environmentally induced size variation in copulatory organs may be greater than previously appreciated.

Nutrient Stress Alters Among-Trait Correlations and Generates a Trade-Off

The condition dependence of resource allocation trade-offs, particularly under food limitation, has been well established for a diversity of life history (Stearns, '92; French et al., 2007; Waelti and Reyer, 2007) and morphological traits. For instance, the well-characterized trade-off between flight muscle and ovarian mass in female sand crickets, *G. firmus*, is eliminated when long-winged individuals that generate substantial flight muscles are fed a high-quality diet (Mole and Zera, '94). Consistent with our predictions, we found that NuS altered among-trait correlations in comparison with ad libitum fed individuals (Fig. 2). Although NuS significantly shifted the y -intercept for all trait comparisons in both *O. gazella* and *O. taurus*, we failed to find any significant positive or negative (i.e., trade-off) correlations among traits. The failure to identify trade-offs is not uncommon in studies of resource allocation to life history and morphological traits (e.g., Reznick et al., 2000; House and Simmons, 2012), and this absence may be underlain by several factors, including genotypic variation in efficiency of resource use and resource allocation among traits, as well as variation in resource reserves prior to NuS (Glazier, 2009). Indeed, here we were able to detect significant correlations only in *O. taurus*, the species that shows the greater developmental response to nutrition, and only among the subset of beetles that exhibited the relatively largest positive or negative residual trait values. Among these, we found that NuS generated an unexpected positive correlation between horns and eyes and a trade-off (i.e., negative correlation) between eyes and genitalia. This suggests that trade-offs are most likely to be found when assessing variation in resource allocation among particular genotypes or populations within a species, and that the species selected for study should be those with the greatest residual investment into exaggerated traits.

The observation of a positive phenotypic correlation between horns and eyes is consistent with the positive correlation between these traits in the rhinoceros beetle, *Trypoxylus dichotomus* (a lineage that independently evolved horns;

McCullough et al., 2012; McCullough and Emlen, 2013), but inconsistent with other experimental studies of *Onthophagus* (Nijhout and Emlen, '98; Pizzo et al., 2012). Similarly, we failed to find a significant negative correlation between horns and genitalia, which is consistent with results from some studies (e.g., House and Simmons, 2012), but not others (Moczek and Nijhout, 2004; Parzer and Moczek, 2008). In combination, these observations call into question the proposed ubiquity and significance of proximity-based trade-offs (Emlen, 2001), and instead support the argument that the presence and severity of negative correlations among structures such as horns, eyes, and genitalia may be highly dependent on trait, species, and ecological context, with positive or neutral correlations just as, if not more, commonly found in nature (Schwab and Moczek, 2014).

To our knowledge, the trade-off that we observed between eyes and genitalia has not previously been described in *Onthophagus*, and it is presently unclear how the growth of these traits may become linked during development, though it is possible that this response is a byproduct of the positive correlation between horns and eyes (Fig. 4). Functional traits such as eyes, wings, and legs, which normally scale isometrically with body size, have been hypothesized to be developmentally canalized against engaging in trade-offs due to the substantial functional penalty associated with deviations from their optimal size (Parzer and Moczek, 2008). These functional consequences may underlie the strong association between the autoecology of *Onthophagus* species and their horn placement: in comparison with diurnal species of beetles, nocturnal species are significantly less likely to develop their horns near the eyes, where they may engage in trade-offs with horns (Emlen, 2001). Although early studies of resource allocation trade-offs favored a model in which proximity determined the degree to which traits competed for local pools of resources, and thereby their likelihood of engaging in trade-offs (Klingenberg and Nijhout, '98; Nijhout and Emlen, '98; Emlen, 2001), the results presented here favor a more complex model in which traits are equally capable of engaging in trade-offs with distantly proliferating traits (Moczek and Nijhout, 2004; Fry, 2006; Parzer and Moczek, 2008; Pizzo et al., 2012). While the physical basis of such trade-offs remains unclear, the local (e.g., morphogenic) and systemic (i.e., hormonal) signaling pathways that direct trait growth, as well as the degree of crosstalk among these pathways, is becoming increasingly well resolved. For instance, insulin-like peptides can coordinate growth among imaginal discs (Colombani et al., 2012; Garelli et al., 2012) and have been implicated in well-established trade-offs such as those between dispersal and reproduction (Lin et al., 2016). Further, these systemic signals may in part be integrated with within-disc growth via e.g. the Fat/Hippo signaling pathway among other growth regulatory pathways (Gotoh et al., 2015). Future studies using developmental genetic techniques (e.g., RNA interference) to manipulate these and other pathways may be necessary to further elucidate the

mechanistic basis of resource allocation patterns, as well as how these patterns come to vary across populations, species, and environmental conditions.

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