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Resource allocation during ontogeny is influenced by genetic, developmental and ecological factors in the horned beetle, *Onthophagus taurus*

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Resource allocation trade-offs arise when developing organs are in competition for a limited pool of resources to sustain growth and differentiation. Such competition may constrain the maximal size to which structures can grow and may force a situation in which the evolutionary elaboration of one structure may only be possible at the expense of another. However, recent studies have called into question both the consistency and evolutionary importance of resource allocation trade-offs. This study focuses on a well-described trade-off between the horns and eyes of *Onthophagus* beetles and assesses the degree to which it is influenced by genetic, developmental and ecological conditions. Contrary to expectations, we observed that trade-off signatures (i) were mostly absent within natural populations, (ii) mostly failed to match naturally evolved divergences in horn investment among populations, (iii) were subject to differential changes in F₁ populations derived from divergent field populations and (iv) remained largely unaffected by developmental genetic manipulations of horn investment. Collectively, our results demonstrate that populations subject to different ecological conditions exhibit different patterns of, and differential plasticity in, resource allocation. Further, variation in ecological conditions, rather than canalized developmental mechanisms, may determine whether and to what degree morphological structures engage in resource allocation trade-offs.

1. Introduction

Throughout ontogeny, resources such as nutrients, growth factors and morphogens are necessary to sustain growth and differentiation [1]. However, nutrition is a limiting resource during the development of a wide range of organisms, and morphogens and growth factors are commonly shared across structures and organs developing simultaneously in the same individual [2]. Under such conditions, resource allocation trade-offs may arise, whereby developing organs find themselves in competition for a limited pool of resources. Such competition may constrain the maximal size to which a structure can grow and may force a situation in which the evolutionary elaboration of one structure may only be possible at the expense of another. Resource allocation trade-offs thus have the potential to shape both patterns of phenotypic variation and lineage diversification [3].

Support for the existence and potential evolutionary significance of resource allocation trade-offs has come in part from studies on trait covariances in both hemi- and holometabolous insects. In hemimetabolous insects, many studies have focused on trade-offs arising from the costs of reproduction [4]. For instance, in many wing polymorphic taxa (e.g. crickets, planthoppers, aphids and grasshoppers), negative correlations exist between flight capability, and hence dispersal ability, and life-history traits such as fecundity and age at reproduction [5–9]. In holometabolous insects, much work has focused on taxa with elaborate secondary sexual traits, such as horned beetles [10–12]. For example, Parzer & Moczek [13] compared 10 species of horned beetles in the genus *Onthophagus*

and four rapidly diverging populations of *Onthophagus taurus* and demonstrated a significant negative correlation between the relative size of horns and that of the male copulatory organ [13]. Similar correlations were found by Emlen [10] in three horned *Onthophagus* species, documenting significant negative correlations between relative horn length and wing size, eye surface area or the length of antennae, respectively, consistent with localized resource allocation trade-offs [10].

Direct experimental manipulations of development as well as artificial selection experiments further support the existence and potential evolutionary significance of resource allocation trade-offs. For example, under equivalent nutritional conditions, artificially selected lines of the flightless, short-winged morph of the field cricket *Gryllus firmus* exhibited 100–400% greater ovarian growth coupled with a 30–40% decrease in flight fuel reserves (i.e. somatic triglycerides) when compared with flight-capable, long-winged individuals [9]. Similarly, experimental removal of the hindwing imaginal disc in the butterfly *Precis coenia* resulted in the disproportionate growth of the neighbouring forewings [11], ablation of the genital imaginal disc in the horned beetle *O. taurus* resulted in the production of larger horns [14], and artificial selection on relative head horn length in male *Onthophagus acuminatus* led to correlated evolution of relatively smaller eye size within only nine generations [11]. This latter result raised the possibility that horn–eye size allocation trade-offs may bias head horn-bearing species against a nocturnal ecology, a prediction supported by the results of an ecological survey of 161 neotropical *Onthophagus* [10]. Collectively, this evidence demonstrates that resource allocation trade-offs have the potential to determine not only the phenotypic products of development, but also to bias the evolutionary trajectory of species [10,15].

However, several studies have called into question both the consistency and evolutionary importance of resource allocation trade-offs. For example, the trade-off between ovarian and flight muscle mass can be eliminated by feeding a high-quality diet to long-winged, flight-capable *G. firmus* [16]. Similarly, despite the negative correlations between relative horn and aedeagus sizes in *Onthophagus* reported above [13], recent work on a single population of *O. taurus* detected zero genetic covariance between relative horn and aedeagus size [17], and studies of the rhinoceros beetle *Trypoxylus dichotomus* show that males that develop relatively longer head horns also develop relatively longer wings and greater wing area, flight muscle mass, eyes and copulatory organ length [18,19]. These results suggest that resource allocation trade-offs may not be as pervasive as previously suggested and raise the possibility that presence, absence or intensity of resource allocation trade-offs may depend on additional factors that contribute to the specific developmental and ecological conditions within which a given population or species may find itself.

Here, we use the horned beetle, *O. taurus* (Coleoptera: Scarabaeidae), to further investigate the proximate causes of, and population variation in, resource allocation trade-offs. Specifically, we focus on the developmental trade-off between head horns and adjacent eyes, which has been well documented through two earlier studies by Nijhout & Emlen [11] in *O. acuminatus* and Emlen [10] in a previously unclassified *Onthophagus* species. We focused on *O. taurus* because this species (i) develops horns of highly variable length at the back of the head (proximal to the eyes), making it an ideal

species for assessing horn–eye trade-offs, (ii) provides easy access to multiple populations, including populations that have diverged heritably in males' average investment into horn growth, and is amenable to both (iii) common garden rearing and (iv) developmental genetic manipulations of horn investment.

Onthophagus taurus was originally restricted to the European Mediterranean region [20], but became introduced accidentally to the Eastern US (first recorded in North Florida) and deliberately as part of a biocontrol programme to Western Australia (WA) in the early 1970s [21,22]. Since introduction, males in both exotic ranges have diverged dramatically, and heritably, in the average investment into horn development. Furthermore, since its initial introduction to the Eastern US in North Florida, *O. taurus* has continuously expanded its range and currently occurs as far north as New York and as far west as Indiana (IN).

Here, we assess the presence and strength of resource allocation trade-offs in *O. taurus* using three independent, but complementary, approaches. We first investigated whether exotic populations from WA and two US locations, North Carolina (NC) and IN, exhibit trait correlations that are consistent with a resource allocation trade-off, and whether these correlations were similar or different in direction and intensity across populations. Second, we reared offspring derived from two of these populations, WA and NC, under common garden conditions and explore whether environmental factors alter trade-off direction and intensity within and between populations. Finally, we use RNA interference (RNAi) to perturb the expression of *doublesex* (*dsx*), a somatic sex-determination gene that regulates both sex- and morph-specific elaboration of head horns in *O. taurus* [23]. By drastically reducing horn growth in males and inducing it in female beetles, we investigate the degree and nature of potential compensatory changes in eye investment.

2. Material and methods

(a) Field collections

Onthophagus taurus were collected from pastures in three geographically distinct locations. Beetles from WA were collected from cow dung pads at Narrikup (34.7667° S, 117.7333° E) in January 2001 and Perth (31.9554° S, 115.8585° E) in January 1997. In the United States, beetles were collected from pastures in Durham, NC (35.9939° N, 78.8989° W) from 1995 to 2001, as well as near Bloomington, IN (39.1653° N, 86.5264° W) in July 2012.

(b) Colony maintenance and common garden rearing

Beetles collected in 1997 in both NC and WA were maintained simultaneously and reared in the laboratory as described in detail in Moczek *et al.* [24]. In brief, beetles were maintained in a moist sand–soil mix at 26°C and a 16 L : 8 D cycle, and fed cow manure ad libitum. All colonies were kept separate but in otherwise identical conditions. Beetles were allowed to breed in plastic containers (25 cm tall, 20 cm in diameter) filled 3 : 4 with a moist sand : soil mixture. Five pairs of beetles were added to each container (eight containers per colony and week) and provided with approximately 0.5 l of homogenized dung. Six days later, beetles were recaptured and brood balls were collected and placed in separate containers until emergence. To minimize inbreeding, individual adult beetles were allowed to produce brood balls only once before being removed from the colony. Different generations were kept in separate containers. Over

1000 individuals were reared each generation for each strain. Great care was taken to provide both laboratory colonies with the exact same treatment and breeding set-up. Shelf positions of both colony and breeding containers were switched every week.

(c) Generation of *dsxRNAi* animals

Recent work has shown that the somatic sex-determination gene *dsx* regulates both sex- and morph-specific elaboration of horns in *O. taurus* [23]. Specifically, RNAi-mediated transcript depletion of the male-specific *dsx* isoform results in a dramatic reduction of horns in large males, whereas RNAi-mediated downregulation of the female-specific isoform induces ectopic horns in otherwise hornless females. We used animals generated by Kijimoto *et al.* [23] to contrast relative investment into eye development in genetically manipulated (*dsxRNAi*) and control injected individuals (double stranded DNA (dsRNA) derived from a vector sequence and buffer). Details on cloning, the generation of *dsxRNAi* and control constructs, injection procedures and knock-down validation can be found in Kijimoto *et al.* [23]. In brief, the following steps were carried out.

(d) Double stranded RNA generation and injection

dsRNA was generated following Moczek & Rose [25]. Select *dsx* fragments were cloned using StrataClone PCR Cloning Kit, followed by BigDye sequencing. The vector containing the fragment was purified using QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA, USA). The vector was then subjected to PCR by using M13 forward and reverse primers and the PCR product was used as a template for *in vitro* transcription. Forward and reverse RNA strands were produced using MEGascript T7 and T3 kit (Life Technologies, Carlsbad, CA, USA) as specified in the instructions and mixed at a 1:1 ratio by concentration. This mixture was incubated in a water bath at 80°C until 37°C was reached. The concentration of the annealed RNA was measured, confirmed by gel electrophoresis and stored at -80°C until injection. dsRNA (0.5 µg) was injected into larvae during the first 5 days of the final, third instar.

(e) Control injections

To execute control injections, animals were reared under the same conditions as RNAi-injected animals but were injected instead with dsRNA from a 167 bp PCR product derived from a pBluescript SK vector. The transcription reaction, DNaseI treatment and annealing of transcripts were performed as described above. One microgram of dsRNA was injected into larvae during the first 5 days of the final, third instar.

(f) Validation of *dsx* knock-down

First-day pupae were used for validation of *dsx* knock-down by qRT-PCR. Total RNA was extracted from whole bodies of six large *dsx* dsRNA injected males and females as well as three large wild-type males and females, respectively, using TRI reagent (Life Technologies). We followed the standard protocol provided by the manufacturer. qRT-PCR was performed separately on each independent set of samples. Each reaction was duplicated during the PCR step. One hundred nanograms of total RNA was treated and analysed as described above. Knock-down efficiency was assessed relative to *dsx* expression levels observed in wild-type individuals. The experiment was repeated three times with different RNA samples.

(g) Morphometric measurements

We used a standard two-dimensional morphometric set-up and IMAGEJ 1.44p software for all measurements. Horn length was

measured from the outer margin of the eye to the tip of the horn as described by Moczek [26]. To measure eye size, we obtained lateral images of the eye (following Emlen [10]), traced the outer edge of the eye and calculated the corresponding area using IMAGEJ v. 1.44p. Pronotum width was used as a proxy for body size and measured as described by Emlen [27]. All measurements were to the nearest 0.01 mm and were collected by the same person (D.B.S.).

(h) Analysis

Following approaches established by previous studies [10,11,13], we quantified relative investment into horns and eyes using a residual-based analysis. Briefly, the relative investment into horns and eyes for each beetle was standardized using a measure of pronotum width as a proxy for body size. Given the highly sigmoidal relationship between body size and horn length in *O. taurus* (figure 1), we fit a four-parameter Hill equation model to these data in order to calculate horn investment as

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

where *a* represents the range of horn lengths, *b* represents the maximum slope of horn length increase and *c* represents the inflection point of the sigmoidal curve (use justified in [14]). Using this analysis, we obtained means and standard errors for each parameter for each of our three focal populations. We used Welch's *t*-test to contrast inflection points (parameter *c*) across all three populations to determine whether among-population divergences in threshold sizes reported by earlier studies were also detectable in our samples.

In order to calculate eye investment, a linear model was fit to all data points:

$$\text{eye size} = \text{minimal eye size} + a \text{ body size},$$

where *a* represents the slope of the linear regression. For each individual, we calculated the expected horn length and eye size given their body size. We then calculated the difference between the observed and expected values to obtain residual horn length and eye sizes, respectively. Model functions for both residual horn length and eye size were calculated in two different ways: (i) separately for each population and (ii) from a pooled sample of all three populations. We regressed residual horn length and eye size values against one another in order to examine the relationship between relative investment into horns versus eyes. A trade-off between investment into horns and eyes should be manifest in a significant negative correlation between horn length and eye size residuals. Because horn expression among male *O. taurus* is polyphenic and includes a sizeable fraction of *minor* males that only express rudimentary horns, we replicated our analyses separately for large, major males, small, minor males and males of intermediate body sizes (± 0.15 mm from the threshold body size of each population). We conducted one- and two-way ANCOVAs following Nijhout & Emlen [11] for all comparisons. We corrected for multiple comparisons using the Bonferroni-Holm correction [28] where appropriate. All analyses were executed in JMP v. 10.

3. Results

(a) Population variation in relative investment into horn length and eye size

We investigated natural variation in resource allocation to horn and eye development among two US (NC and IN) and one WA population of *O. taurus*. Previous studies have found that NC *O. taurus* develop horns at relatively small

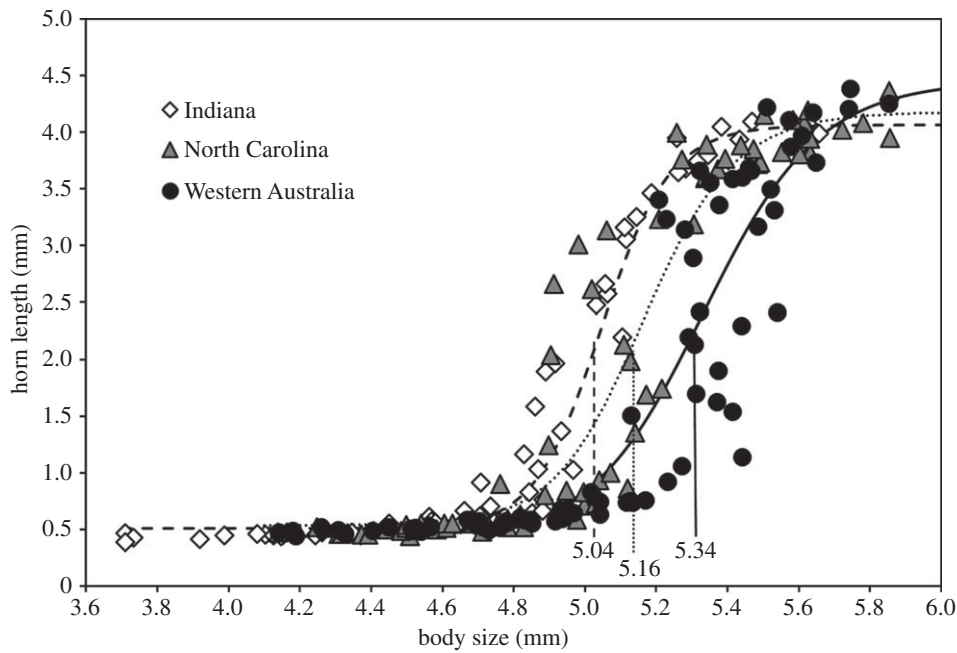


Figure 1. Scaling relationship between body size (x -axis) and horn length (y -axis) in males of three natural populations of *O. taurus*. All three populations exhibit a sigmoid allometry but differ significantly in the point of inflection, or threshold body size, separating minor and major morphs. Threshold body sizes and corresponding sigmoidal curves are indicated by dashed (IN), dotted (NC) and solid (WA) black lines, respectively.

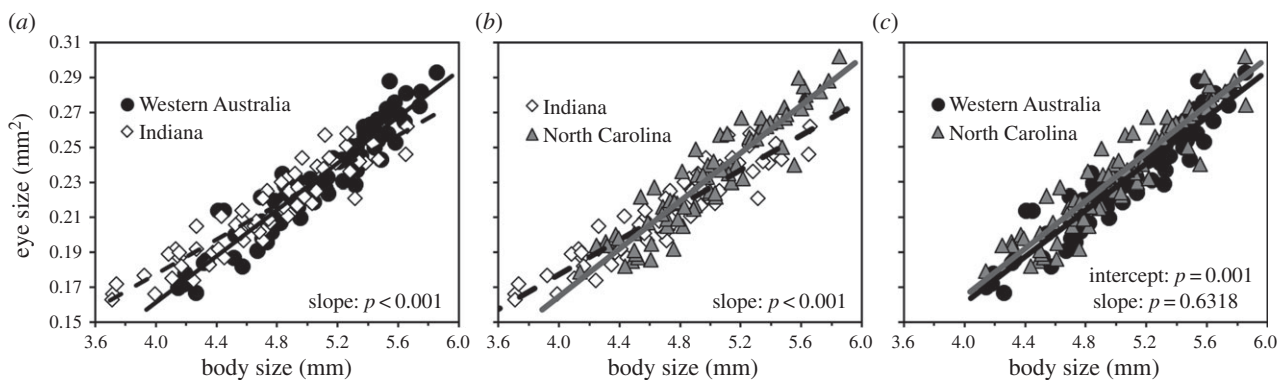


Figure 2. The relationship between body size and eye size (measured as area) among three populations of *O. taurus*. Resource allocation trade-offs predict that populations that invest relatively more into horns (IN > NC > WA) should invest relatively less into eyes. At large body sizes, IN beetles invest less into eyes than WA (a) and NC (b) beetles, consistent with a resource allocation trade-off. (c) By contrast, NC beetles invest more into eyes than WA beetles, despite also investing relatively more into horns. Line of best fit is indicated by dashed (IN), grey (NC) and black (WA) lines, respectively.

body sizes, thus investing relatively more resources into horn development when compared with same-sized individuals from WA [24]. We recover the same result among our field-collected samples of *O. taurus*. Specifically, the inflection point of male beetles collected from NC ($n = 75$) was significantly smaller ($5.16 \text{ mm} \pm 0.03$) than that observed for WA males ($5.34 \text{ mm} \pm 0.05$; $t = 0.002$; $p < 0.001$; $n = 75$), which closely matches results of earlier studies (figure 1) [24]. In addition, we found that the average body size threshold of IN beetles ($5.05 \text{ mm} \pm 0.01$; $n = 64$) was significantly smaller compared with both WA and NC populations ($p < 0.001$ for both comparisons). Therefore, *O. taurus* collected in IN exhibit the greatest relative investment into horns, followed by males collected in NC, and then WA.

We then contrasted body size and eye size allometries among these populations. We predicted that if a resource allocation trade-off governs relative investment into eyes and horns, populations that invest relatively more into horns (IN, followed by NC) should exhibit relatively smaller eyes when

compared with same-sized individuals derived from a population that invests relatively less into horns (WA). Such differences should be manifest in significant differences in the y -intercept and/or slope of the body size–eye size allometry. We found partial support for this hypothesis. WA and IN populations show the most extreme divergence in horn investment (see figure 1), with the IN population investing significantly more into horns, and thus predicted to invest less into eyes. Consistent with this prediction, we found that IN males exhibited a significantly lower slope in their body size–eye size allometry, causing large males to develop smaller eyes than same-sized males from WA ($F = 20.835$, $p < 0.001$; figure 2a). We found a corresponding pattern when we contrasted both US populations: IN males invested more into horns than NC males (figure 1) and also exhibited a significantly lower slope in their body size–eye size allometry, causing large IN males to develop smaller eyes than size-matched males from NC ($F = 22.314$, $p < 0.001$; figure 2b). The remaining pairwise comparison failed to support the

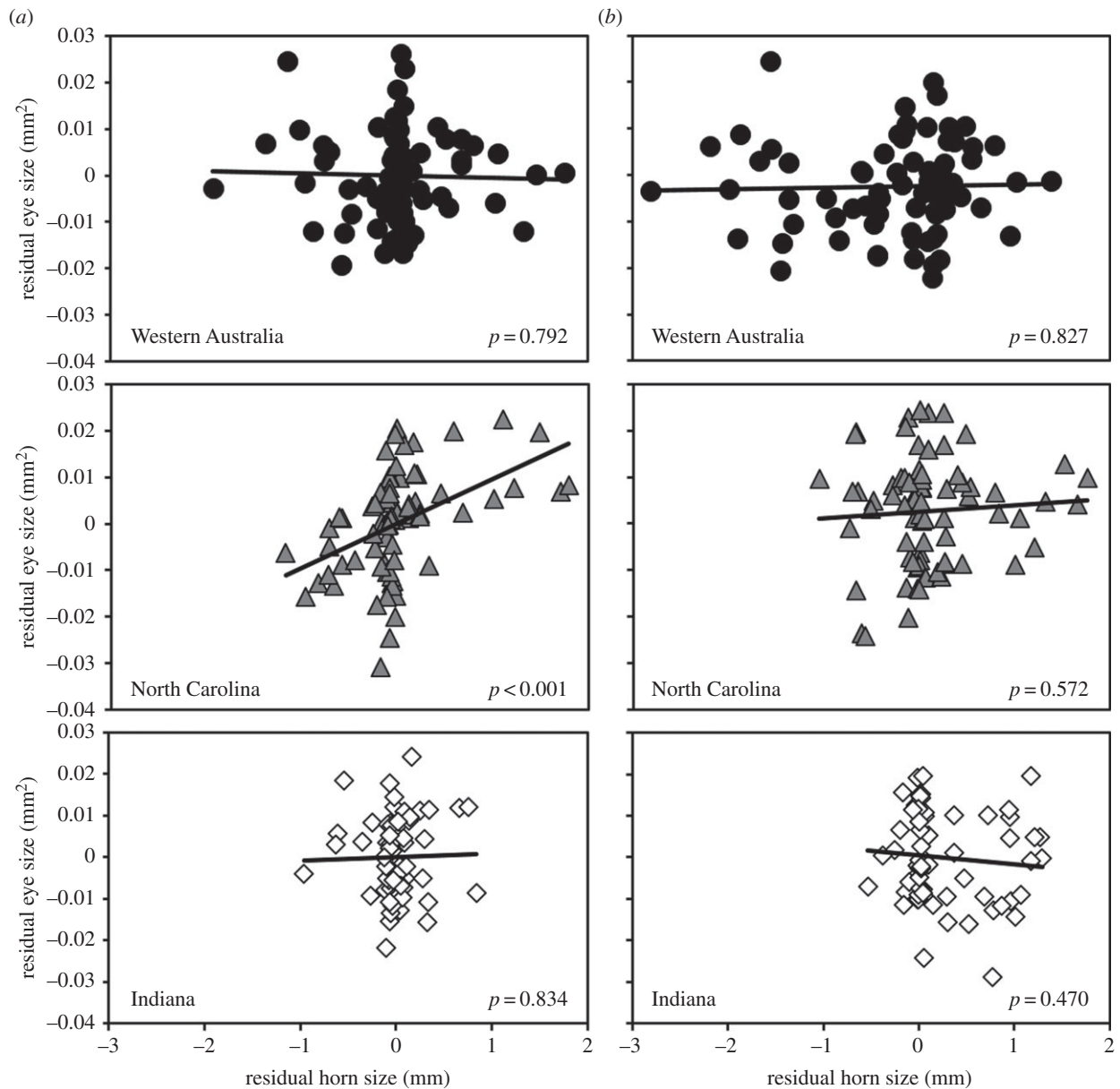


Figure 3. Bivariate plots of residual eye size versus residual horn length in three populations of *O. taurus*. Resource allocation trade-offs predict a negative correlation. (a) Residuals calculated based on a reference function generated separately for each population. None of the populations exhibits the predicted negative correlation. NC males exhibit a significant positive correlation ($p = 0.028$). (b) Residuals calculated based on a reference function generated from a pooled sample of all three populations. None of the populations exhibits the predicted negative correlation.

predictions associated with a resource allocation trade-off. NC males invest more into horns than WA males, but also develop significantly larger eyes over the entire body size range ($F = 10.841$, $p = 0.001$; figure 2c). These results suggest that population divergences in relative investment into eyes versus horns are only partly consistent with the predictions of a resource allocation trade-off between horn length and eye size.

Next, we examined whether, within and among each population, horn length and eye size residuals exhibited a negative correlation, as would be predicted if a trade-off drives resource allocation patterns within populations. After calculating residual values for horn length and eye size for each population separately, we found that a large number of residual horn sizes were clustered around zero, a result consistent with expectations for threshold allometries (e.g. *O. acuminatus* [29]). The regression of these two variables produced a significant positive, rather than the predicted negative, correlation between horn and eye size in NC ($F = 18.937$, $p < 0.001$) and no significant relationship for either IN ($F = 0.044$, $p = 0.834$) or WA

($F = 0.070$, $p = 0.791$) populations (figure 3a). No significant relationship for any of the populations emerged when residuals were calculated based on a pooled sample (figure 3b). Lastly, we recovered qualitatively identical results when we analysed horn length and eye size residuals separately for large, major males, small, minor males or males of intermediate body sizes ± 0.15 mm around a given population's threshold body size. We were thus unable to detect a trade-off signature for any of the three populations studied here, regardless of whether residuals were calculated individually for each population or relative to a combined sample of all three populations.

(b) Relative investment into horns and eyes in field and laboratory-reared individuals

We investigated the degree to which the relationship between horn and eye investment varies under different environmental conditions, drawing comparisons between wild-caught beetles from NC and WA and their laboratory-reared offspring. By

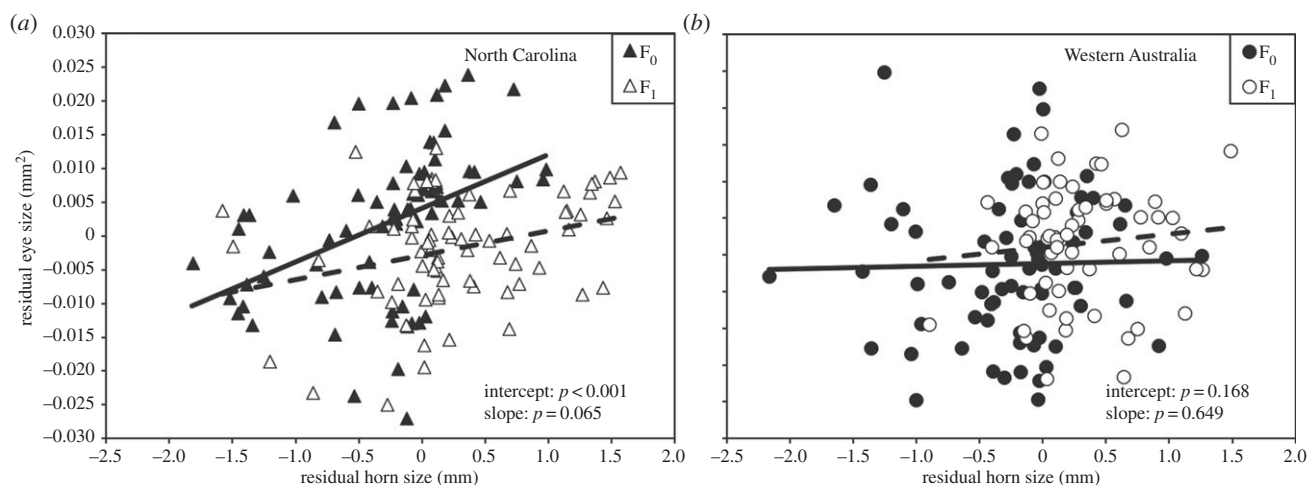


Figure 4. Residual horn versus eye size in field-collected F_0 and laboratory-reared F_1 generations of NC and WA beetles. NC F_1 invests significantly less into eyes for a given amount of horn investment than their field-collected counterparts ($p < 0.001$). There is no significant difference between the F_0 and F_1 generation in WA. Line of best fit is indicated by black (F_0) and dashed (F_1) lines, respectively.

allowing wild-caught beetles to breed under what we assume to be relatively permissive laboratory conditions, we were able to assess the extent to which patterns of resource allocation may be genetically fixed or are phenotypically plastic within and between populations. We found that laboratory rearing influences resource allocation, but does so unequally, among populations. In NC beetles, laboratory rearing resulted in a significant decrease of the y -intercept, but not slope, among F_1 individuals, causing F_1 ($n = 75$) males to invest relatively less into eyes for a given amount of investment into horns compared with their field-collected parental population ($F = 17.451$, $p < 0.001$; $n = 75$; figure 4). However, no differences were found between WA F_0 ($n = 75$) and F_1 ($n = 60$) populations ($F = 1.921$, $p = 0.168$).

(c) Developmental genetic basis of horn–eye trade-offs

Finally, we assessed the impact of *dsxRNAi* on horn–eye investment. Recall that RNAi-mediated transcript depletion of the male-specific *dsx* isoform results in a dramatic reduction of horns in large males, whereas targeting the female-specific isoform induces ectopic horns in females [23]. We reasoned that if horn and eye development trade-off with each other, *dsxRNAi* should result in relatively larger eyes in males but smaller eyes in females, compared with control injected individuals, respectively. We find that *dsxRNAi*-mediated horn induction in females indeed results in decreased eye size ($F = 20.116$, $p < 0.021$; $n_{dsxRNAi} = 27$, $n_{control} = 20$), consistent with our prediction. This effect increases with body size (significant body size \times eye size interaction; $F = 7.759$, $p = 0.008$; figure 5a), further paralleling the effect of *dsxRNAi* on horn induction, which is greatest in large females [23]. However, contrary to our expectations, *dsxRNAi*-induced reduction in horn length in males also results in a reduction in eye size ($F = 8.168$, $p = 0.006$; $n_{dsxRNAi} = 30$, $n_{control} = 20$; figure 5b). These results suggest either that only female, but not male, eyes engage in resource allocation trade-offs with horns, that *dsx* regulates eye development similarly in both sexes and independent of sex-specific horn elaboration and/or that resource allocation trade-offs do not exist or manifest under these conditions.

4. Discussion

Resource allocation trade-offs have the potential to shape patterns of phenotypic variation and lineage diversification. Here, we investigated the genetic, developmental and ecological factors that may influence allocation decisions during horn and eye development in the beetle *O. taurus*. We observed that trade-off signatures (i) were mostly absent within natural populations, (ii) mostly failed to match naturally evolved divergences in horn investment among populations, (iii) were subject to differential changes in F_1 populations derived from divergent field populations and (iv) remained largely unaffected by experimental developmental genetic manipulations of horn investment. Below, we discuss the most important implications of our results.

(a) Trade-off signatures within and among populations

In contrast to previous studies in horned beetles [10–12], we find no signatures of resource allocation trade-offs between horns and eyes within any of our focal populations of *O. taurus*. Instead, residual horn length and eye size are either uncorrelated (IN, WA), or exhibit a positive correlation (NC) within each population (figure 3). By contrast, we detect interpopulation divergences in body size–eye size allometries that are only partly consistent with a resource allocation trade-off: for two of three pairwise comparisons, and for at least part of the body size range, males from populations that have evolved increased investment into horns show relatively reduced investment into eyes (figure 2). These results raise the possibility that resource allocation trade-offs between horns and eyes may not be nearly as prevalent as suggested by previous studies [10]. Therefore, we consider two alternative hypotheses that may help explain our results in the light of previous findings: (i) that resource allocation trade-offs are species-specific, and/or (ii) that trade-offs may be environmentally sensitive, with different populations exhibiting alternative trait correlations given prevailing environmental conditions.

Previous studies have suggested that the presence of trade-offs may reflect the ecology of a species and may therefore be species-specific [10]. For example, nocturnal or crepuscular

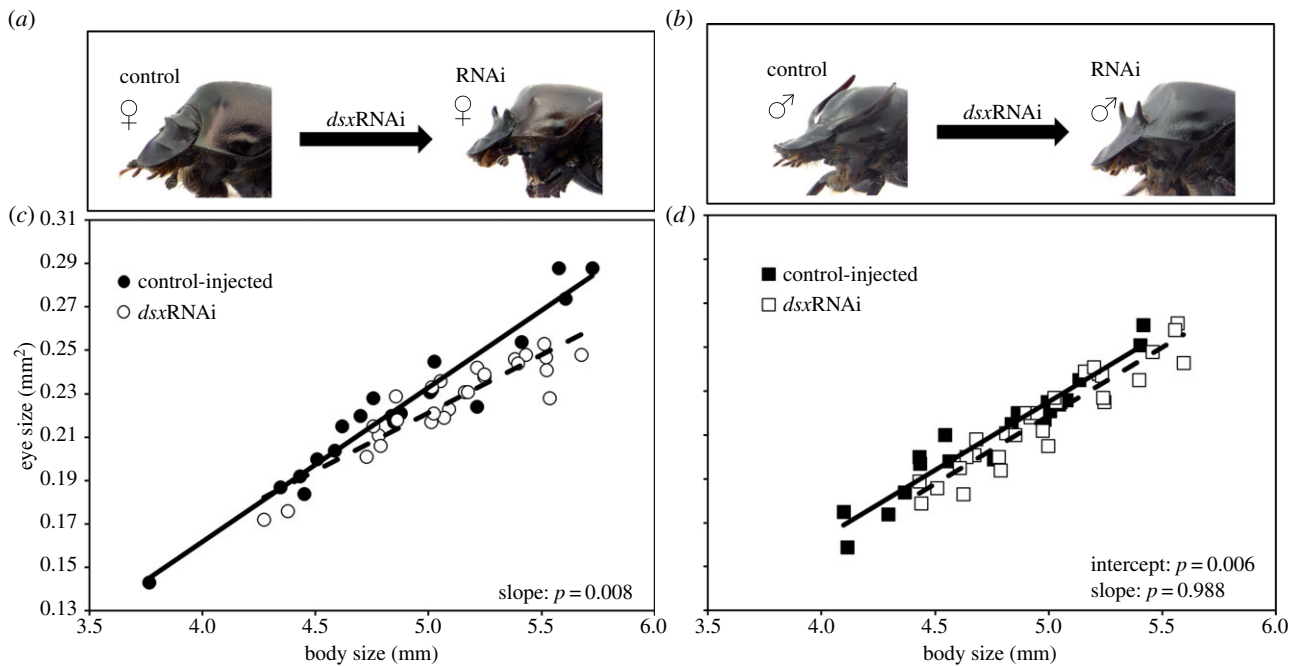


Figure 5. The relationship between body size and eye size in size-matched *dsxRNAi* and control injected beetles. Resource allocation trade-offs predict that the experimental induction of horns in females (a) should result in decreased eye investment, whereas the experimental suppression of horn growth in males (b) should result in increased eye investment. (c) Induction of horns in large females correlates with a significant reduction in eye size in *dsxRNAi* females, consistent with a resource allocation trade-off. (d) Contrary to predictions, *dsxRNAi* males also invest less into eyes than control injected individuals, despite experimental downregulation of horn development. Line of best fit is indicated by black (control injected) and dashed (*dsxRNAi*) lines, respectively. (Online version in colour.)

species, which require larger eyes in order to navigate limited light conditions, tend not to bear horns at the back of their heads, as these horns are believed to engage in resource allocation trade-offs with eyes [10]. It is possible that *O. taurus*, given its diurnal activity pattern, or due to other species-specific characteristics, simply does not engage in horn length–eye size resource allocation trade-offs. If correct, observed patterns of resource allocation to horn and eye formation in *O. taurus* may thus have little to do with growth competition between both traits and may instead be shaped by other factors, such as additional trait interactions (e.g. between horns and genitalia [13]) not addressed in this study, or by genetic effects (e.g. antagonistic pleiotropy) that regulate both horn and eye production [30]. Alternatively, presence, absence or degree of trade-off signatures may be influenced by the current environmental conditions in which a population finds itself. Nutrient-rich or otherwise benign environments may buffer populations against resource limitations and may instead result in the expression of zero or even positive trait correlations [17]. By contrast, challenging and stressful, or novel, environments could constrain developmental processes in such a way that trade-offs are more likely to manifest. Support for this hypothesis has been provided from experimental studies of the long- and short-winged morphs of *G. firmus*, for which trade-offs have been eliminated under permissive rearing conditions [9,16]. Indeed, studies from a diversity of taxa suggest that life-history trade-offs are most strongly expressed when nutrients are limited (e.g. amphibians [31], molluscs [32] and reptiles [33]). The results presented here provide at least some support for condition-dependency in resource allocation: in *O. taurus* collected from NC, one generation of laboratory rearing resulted in a significant reduction in relative eye size (figure 4), whereas laboratory rearing had no effect on WA *O. taurus*. These results suggest that changes in rearing conditions can impact resource allocation patterns,

and may do so differentially across populations. More generally, our results provide limited evidence for resource allocation trade-offs between horns and eyes and suggest that the direction and intensity of their correlation may vary by population and environment.

(b) *Dsx* and horn–eye trade-offs

We observed that both *dsxRNAi*-mediated induction of horns in female *O. taurus* as well as *dsxRNAi*-mediated reduction in male horn growth similarly resulted in smaller eyes. While the former result is consistent with a resource allocation trade-off, the latter is not. These results may be explained by two, non-mutually exclusive, hypotheses.

First, female, but not male, *O. taurus* may engage in resource allocation trade-offs between developing horns and eyes. If so, this raises the possibility that mechanisms governing sex-specific development may also influence whether or not resource allocation trade-offs manifest during development, similar to sex-biased trade-offs between immunological function and reproduction in *Drosophila melanogaster* [34]. Species in which females naturally express horns (either in addition to or instead of males) may provide interesting opportunities to further investigate this possibility. Alternatively, and perhaps more likely, our results raise the possibility that *dsx* may directly and positively affect the regulation of eye size during development. Unlike the role of *dsx* in horn development, which involves the promotion of horns in males but their inhibition in females, *dsx* may be promoting eye development in both sexes. This in turn would explain why *dsxRNAi* results in similarly reduced eyes in both sexes, despite opposing consequences on horn formation. However, it is worth noting that even though *dsx* has been implicated in the sex-specific development of genitalia [35], mesothoracic ganglia [36], sex combs [37] as

well as diverse abdominal traits [38], there is presently no evidence suggesting an additional role in eye elaboration.

5. Conclusion

Our results provide only limited support for the hypothesis that resource allocation trade-offs bias relative investment into horns and eyes among three naturally divergent populations of *O. taurus*, contradicting findings from previous studies [10–12]. Instead, our results suggest that relative investment into horns and eyes can be rather variable among populations, more commonly exhibits a positive or no correlation rather than negative correlation, and may be influenced by rearing environment in some populations (NC) but not others (WA). Collectively, our results suggest

that the role of trade-offs in biasing developmental outcomes as well as evolutionary trajectories may be highly complex and suggests that presence, absence and degree of resource allocation trade-offs may need to be evaluated empirically for each species, population and environmental condition.

Data accessibility. Our data are available as an online appendix on Dryad ([doi:10.5061/dryad.jg43t](https://doi.org/10.5061/dryad.jg43t)).

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References

- Gilbert SF. 2013 *Developmental biology*, 10th edn. Sunderland, MA: Sinauer Associates.
- Klingenberg CP, Nijhout HF. 1998 Competition among growing organs and developmental control of morphological asymmetry. *Proc. R. Soc. Lond. B* **265**, 1135–1139. ([doi:10.1098/rspb.1998.0409](https://doi.org/10.1098/rspb.1998.0409))
- Angilletta MJ, Wilson RS, Navas CA, James RS. 2003 Tradeoffs and the evolution of thermal reaction norms. *Trends Ecol. Evol.* **18**, 234–240. ([doi:10.1016/S0169-5347\(03\)00087-9](https://doi.org/10.1016/S0169-5347(03)00087-9))
- Harshman LG, Zera AJ. 2007 The cost of reproduction: the devil in the details. *Trends Ecol. Evol.* **22**, 80–86. ([doi:10.1016/j.tree.2006.10.008](https://doi.org/10.1016/j.tree.2006.10.008))
- Denno RF, Olmstead KL, McCloud ES. 1989 Reproductive cost of flight capability: a comparison of life history traits in wing dimorphic planthoppers. *Ecol. Entomol.* **14**, 31–44. ([doi:10.1111/j.1365-2311.1989.tb00751.x](https://doi.org/10.1111/j.1365-2311.1989.tb00751.x))
- Zera AJ, Denno RF. 1997 Physiology and ecology of dispersal polymorphism in insects. *Annu. Rev. Entomol.* **42**, 207–230. ([doi:10.1146/annurev.ento.42.1.207](https://doi.org/10.1146/annurev.ento.42.1.207))
- Zhang Y, Kongming WU, Wyckhuys KA, Heimpel GE. 2009 Trade-offs between flight and fecundity in the soybean aphid (Hemiptera: Aphididae). *J. Econ. Entomol.* **102**, 133–138. ([doi:10.1603/029.102.0119](https://doi.org/10.1603/029.102.0119))
- Steenman A, Lehmann AW, Lehmann GUC. In press. Life-history trade-off between macroptery and reproduction in the wing-dimorphic pygmy grasshopper *Tetrix subulata* (Orthoptera: Tetrigidae). *Ethol. Ecol. Evol.* ([doi:10.1080/03949370.2014.885466](https://doi.org/10.1080/03949370.2014.885466))
- Zera AJ, Harshman LG. 2001 The physiology of life history trade-offs in animals. *Annu. Rev. Ecol. Syst.* **32**, 95–126. ([doi:10.1146/annurev.ecolsys.32.081501.114006](https://doi.org/10.1146/annurev.ecolsys.32.081501.114006))
- Emlen DJ. 2001 Costs and the diversification of exaggerated animal structures. *Science* **291**, 1534–1536. ([doi:10.1126/science.1056607](https://doi.org/10.1126/science.1056607))
- Nijhout HF, Emlen DJ. 1998 Competition among body parts in the development and evolution of insect morphology. *Proc. Natl Acad. Sci. USA* **95**, 3685–3689. ([doi:10.1073/pnas.95.7.3685](https://doi.org/10.1073/pnas.95.7.3685))
- Pizzo A, Macagno AM, Dusini S, Palestini C. 2012 Trade-off between horns and other functional traits in two *Onthophagus* species (Scarabaeidae, Coleoptera). *Zoomorphology* **131**, 57–68. ([doi:10.1007/s00435-012-0148-1](https://doi.org/10.1007/s00435-012-0148-1))
- Parzer HF, Moczek AP. 2008 Rapid antagonistic coevolution between primary and secondary sexual characters in horned beetles. *Evolution* **62**, 2423–2428. ([doi:10.1111/j.1558-5646.2008.00448.x](https://doi.org/10.1111/j.1558-5646.2008.00448.x))
- Moczek AP, Nijhout HF. 2004 Tradeoffs during the development of primary and secondary sexual traits in a dimorphic beetle. *Am. Nat.* **163**, 184–191. ([doi:10.1086/381741](https://doi.org/10.1086/381741))
- Simmons LW, Emlen DJ. 2006 Evolutionary trade-off between weapons and testes. *Proc. Natl Acad. Sci. USA* **103**, 346–351. ([doi:10.1073/pnas.0603474103](https://doi.org/10.1073/pnas.0603474103))
- Mole S, Zera AJ. 1994 Differential resource consumption obviates a potential flight–fecundity trade-off in the sand cricket (*Gryllus firmus*). *Funct. Ecol.* **8**, 573–580. ([doi:10.2307/2389917](https://doi.org/10.2307/2389917))
- House CM, Simmons LW. 2012 The genetics of primary and secondary sexual character trade-offs in a horned beetle. *J. Evol. Biol.* **25**, 1711–1717. ([doi:10.1111/j.1420-9101.2012.02559.x](https://doi.org/10.1111/j.1420-9101.2012.02559.x))
- McCullough EL, Weingarden PR, Emlen DJ. 2012 Costs of elaborate weapons in a rhinoceros beetle: how difficult is it to fly with a big horn? *Behav. Ecol.* **23**, 1042–1048. ([doi:10.1093/beheco/ars069](https://doi.org/10.1093/beheco/ars069))
- McCullough EL, Emlen DJ. 2013 Evaluating the costs of a sexually selected weapon: big horns at a small price. *Anim. Behav.* **86**, 977–985. ([doi:10.1016/j.anbehav.2013.08.017](https://doi.org/10.1016/j.anbehav.2013.08.017))
- Cartwright OL. 1965 Monographie der Scarabaeidae und Aphodiidae. Der palaearktischen und orientalischen Region. Volumes 1, 2, and 3. Vladimir Balthasar. *Q. Rev. Biol.* **40**, 387–388. ([doi:10.1086/404776](https://doi.org/10.1086/404776))
- Fincher GT, Woodruff RE. 1975 A European dung beetle, *Onthophagus taurus* Schreber, new to the U.S. (Coleoptera: Scarabaeidae). *Coleop. Bull.* **29**, 349–350.
- Tyndale-Biscoe M. 1996 Australia's introduced dung beetles: original releases and redistributions, Technical Report No. 62. Canberra, Australian Capital Territory: CSIRO, Division of Entomology.
- Kijimoto T, Moczek AP, Andrews J. 2012 Diversification of *doublesex* function underlies morph-, sex-, and species-specific development of beetle horns. *Proc. Natl Acad. Sci. USA* **109**, 20 526–20 531. ([doi:10.1073/pnas.1118589109](https://doi.org/10.1073/pnas.1118589109))
- Moczek AP, Hunt J, Emlen DJ, Simmons LW. 2002 Evolution of a developmental threshold in exotic populations of a polyphenic beetle. *Evol. Ecol. Res.* **4**, 587–601. ([doi:10.1093/beheco/arg062](https://doi.org/10.1093/beheco/arg062))
- Moczek AP, Rose DJ. 2009 Differential recruitment of limb patterning genes during development and diversification of beetle horns. *Proc. Natl Acad. Sci. USA* **106**, 8992–8997. ([doi:10.1073/pnas.0809668106](https://doi.org/10.1073/pnas.0809668106))
- Moczek AP. 2006 A matter of measurements: challenges and approaches in the comparative analysis of static allometries. *Am. Nat.* **167**, 606–611. ([doi:10.1086/501075](https://doi.org/10.1086/501075))
- Emlen DJ. 1994 Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* Coleoptera, Scarabaeidae. *Proc. R. Soc. Lond. B* **256**, 131–136. ([doi:10.1098/rspb.1994.0060](https://doi.org/10.1098/rspb.1994.0060))
- Holm S. 1979 A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* **6**, 65–70. ([doi:10.2307/4615733](https://doi.org/10.2307/4615733))
- West-Eberhard MJ. 2003 *Developmental plasticity and evolution*. New York, NY: Oxford University Press.
- Glazier DS. 2009 Trade-offs. In *Resource allocation theory applied to farm animal production* (ed. WM Rauw), pp. 44–60. Wallingford, Oxfordshire, UK: CAB. ([doi:10.1079/9781845933944.0044](https://doi.org/10.1079/9781845933944.0044))
- Waelti MO, Reyer HU. 2007 Food supply modifies the trade-off between past and future reproduction in a sexual parasite–host system (*Rana esculenta*, *Rana lessonae*). *Oecologia* **152**, 415–424. ([doi:10.1007/s00442-007-0671-9](https://doi.org/10.1007/s00442-007-0671-9))

32. Jokela J, Mutikainen P. 1995 Phenotypic plasticity and priority rules for energy allocation in a freshwater clam: a field experiment. *Oecologia* **104**, 122–132. (doi:10.1007/bf00365570)
33. French SS, DeNardo DF, Moore MC. 2007 Trade-offs between the reproductive and immune systems: facultative responses to resources or obligate responses to reproduction? *Am. Nat.* **170**, 79–89. (doi:10.1086/518569)
34. McKean KA, Nunney L. 2005 Bateman's principle and immunity: phenotypically plastic reproductive strategies predict changes in immunological sex differences. *Evolution* **59**, 1510–1517. (doi:10.1111/j.0014-3820.2005.tb01800.x)
35. Hildreth PE. 1965 *Doublesex*, recessive gene that transforms both males and females of *Drosophila* into intersexes. *Genetics* **51**, 659–678.
36. Rideout EJ, Billeter JC, Goodwin SF. 2007 The sex-determination genes *fruitless* and *doublesex* specify a neural substrate required for courtship song. *Curr. Biol.* **17**, 1473–1478. (doi:10.1016/j.cub.2007.07.047)
37. Tanaka K, Barmina O, Sanders LE, Arbeitman MN, Kopp A. 2011 Evolution of sex-specific traits through changes in HOX-dependent *doublesex* expression. *PLoS Biol.* **9**, e1001131. (doi:10.1371/journal.pbio.1001131)
38. Wang W, Yoder JH. 2012 Hox-mediated regulation of *doublesex* sculpts sex-specific abdomen morphology in *Drosophila*. *Dev. Dyn.* **241**, 1076–1090. (doi:10.1002/dvdy.23791)