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SHORT COMMUNICATION

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Utilizing geometric morphometrics to investigate gene function during organ growth: Insights through the study of beetle horn shape allometry

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Abstract

Static allometry is a major component of morphological variation. Much of the literature on the development of allometry investigates how functional perturbations of diverse pathways affect the relationship between trait size and body size. Often, this is done with the explicit objective to identify developmental mechanisms that enable the sensing of organ size and the regulation of relative growth. However, changes in relative trait size can also be brought about by a range of other distinctly different developmental processes, such as changes in patterning or tissue folding, yet standard univariate biometric approaches are usually unable to distinguish among alternative explanations. Here, we utilize geometric morphometrics to investigate the degree to which functional genetic manipulations known to affect the *size* of dung beetle horns also recapitulate the effect of horn *shape* allometry. We reasoned that the knockdown phenotypes of pathways governing relative growth should closely resemble shape variation induced by natural allometric variation. In contrast, we predicted that if genes primarily affect alternative developmental processes, knockdown effects should align poorly with shape allometry. We find that the knockdown effects of several genes (e.g., *doublesex, Foxo*) indeed closely aligned with shape allometry, indicating that their corresponding pathways may indeed function primarily in the regulation of relative trait growth. In contrast, other knockdown effects (e.g., *Distal-less*, *dachs*) failed to align with allometry, implicating these pathways in potentially scaling-independent processes. Our findings moderate the interpretation of studies focusing on trait length and highlight the usefulness of multivariate approaches to study allometry and phenotypic plasticity.

KEYWORDS
allometry, developmental plasticity, geometric morphometrics, *Onthophagus*, RNA interference, scaling

1 | INTRODUCTION

The scaling of relative trait size is integral to organismal function and its evolution represents a major contributor to morphological diversification (Gould, 1966; Schmidt-Nielsen, 1984). Consequently, the functional-genetic underpinnings of scaling—or static allometry—and its evolution have been heavily investigated, resulting in the identification of a wide diversity of genes and pathways thought to be involved in the regulation of proper scaling of trait size (e.g., genes involved in the insulin and TOR signaling pathways [reviewed in e.g.: Cobham & Mirth, 2020; Shingleton et al., 2007]). Collectively, this body of research posits that allometry is achieved through differences in trait growth as instructed by tissue-wide responses to systemic indicators of nutritional status or body size (e.g., insulin/insulin-like peptides [Emlen et al., 2012]). That is, a developmental signal that is tied to organismal size is thought to govern trait-specific changes in cell proliferation, cell shape, cell movement, and/or cell death to match trait size to body size. The evolution of the mechanisms believed to instruct these trait-specific responses is thought to represent a key contributor to the diversification in organismal form and function across species, morphs, and sexes.

Genes and pathways involved in the regulation of allometric growth are typically identified through functional genetic manipulations that cause a change in the relationship between trait size and body size. These approaches implicitly assume that changes in relative trait size—for instance, a reduction of appendage length—require changes in the mechanisms regulating allometry, ultimately causing a mismatch between body and organ size. However, in addition to processes related to allometry, relative trait size can also be modified through processes that are not per se related to scaling. Traits may, for instance, be shorter not just because of abberant scaling, but also because part of a structure failed to be patterned correctly or because tissue folding is perturbed. For instance, expression knockdown of *dachshund* leads to a drastic reduction in *Tribolium* leg length (e.g., Yang et al., 2009), yet these effects are due to the elimination of entire leg compartments rather than a reduction in the size of the leg as a whole. Likewise, homeotic transformations of appendages frequently result in odd-sized outcomes (e.g., haltere-to-forewing transformation in *Drosophila* [Lewis, 1978], or genitalia-to-leg transformation in fireflies [Stansbury & Moczek, 2014]) yet again it is obvious in such cases that radical changes in patterning rather than size sensing during organ growth underpin this outcome. In yet other instances such distinctions are, however, much harder to make. For example, the transcription factor *twist* facilitates the transition from epithelial to mesenchymal identity by instructing a switch from cadherin expression (which bind neighboring cells within an epithelium) to integrins (which allow cells to bind to an extracellular matrix and thus leave an epithelium) during gastrulation and other instances of tissue folding (e.g., Franco et al., 2010; Nam et al., 2015; Qin et al., 2012). Disruption of *twist* function impedes this process, thereby reducing in/outgrowth formation, including the size of mouse limbs (Firulli et al., 2007). Yet without knowledge of the molecular underpinnings and cellular context of *twist* function we might falsely infer a function in size/nutrition sensing, in particular if all that is available are linear measurements of trait size. Consequently, it is not clear a priori whether developmental manipulations that affect linear measures of trait size do so because they are directly involved in the regulation of allometric growth, or via other processes. By focusing on allometric changes in trait shape, we here propose an alternative multivariate approach that may help quantify *how closely* gene knockdown effects resemble the effects of allometry (i.e., whether they are aligned in direction or orthogonal). We posit that doing so may permit a more nuanced analysis of gene function during trait growth and development.

Trait shape commonly varies with trait size among individuals of the same life stage, that is, larger trait variants typically differ in shape from homologous yet smaller variants. Such static allometry (*sensu lato*) is commonly the most important source of shape variation (e.g., Klingenberg, 2016; Rohner, 2020). Because allometry affects many aspects of shape and can be readily quantified using geometric morphometrics, it can provide a multivariate null expectation against which the effects of functional genetic manipulations can be compared to (note that our geometric morphometric approach falls within the Gould–Mosimann school of thought [Klingenberg, 2016, 2022]. This approach treats size and shape as two separate properties and defines allometry as covariation between the two. This diverges from the Huxley–Jolicoeur approach which characterizes allometry as the covariation among traits in response to changes in overall size). If shape changes induced by functional genetic manipulation closely resemble the effects of shape allometry observed in a control group, the manipulated pathways function most likely in the transduction of size information to the developing trait primordia (i.e., the regulation of allometric growth including size and shape components of the entire structure). If, however, these effects align poorly, target genes may more likely be involved in aspects of development not primarily related to

allometric scaling (see Figure 1 for a hypothetical example). Using a multivariate approach, we investigate the extent to which functional genetic manipulations known to affect the size of dung beetle horns recapitulate the effect of horn shape allometry.

The head horns of the bull-headed dung beetle *Onthophagus taurus* have been widely used to study the role of development in shaping plasticity, robustness, and genetic differentiation (e.g., Moczek, 2009). In this species, males develop a pair of exaggerated and strongly nutrition-sensitive head horns (see Figure 2). Horn length exhibits a sigmoidal scaling relationship with a steep threshold that separates small "minor" males that only develop minute horns from large "major" males that develop a pair of elongated head horns used in male−male combat (Moczek & Emlen, 2000; see Figure 2). The switch between minor and major morphologies represents a nutritional polyphenism primarily determined by the nutritional environment larvae experience (Emlen, 1997), although population differentiation and other environmental factors can modify the body size threshold at which males switch between morphs to a minor degree (e.g., Rohner & Moczek, 2023). A large body of research documents the developmental processes and pathways involved in the allometric scaling and evolution of beetle horn length, including the doublesex, hedgehog, insulin, and serotonin signaling pathways. However, with few exceptions (Crabtree et al., 2020; Emlen et al., 2005; Rohner et al., 2022, 2021), prior work has focused nearly exclusively on horn length (reviewed in Casasa et al., 2017; Casasa & Moczek, 2019). Consequently, it is unclear whether changes in the relative size of horns are driven by a change in allometric scaling or alternative developmental processes.

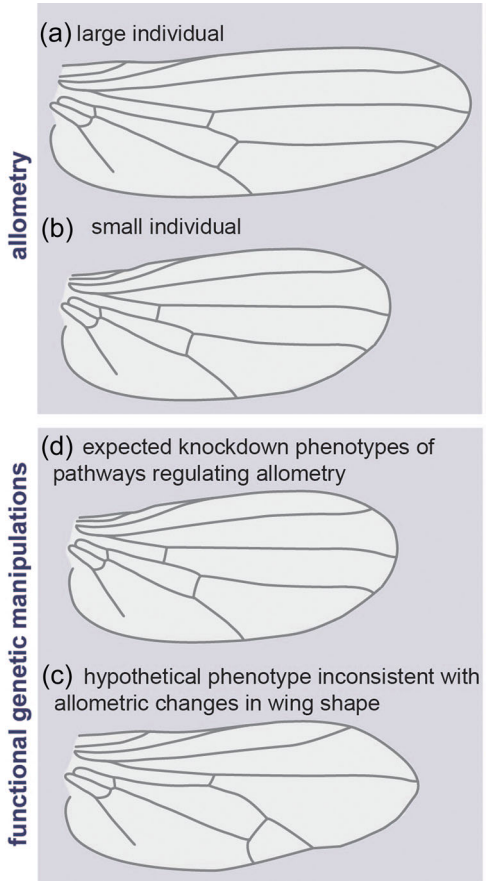

FIGURE 1 Trait shape often exhibits static allometry (a and b). In this hypothetical fly wing example, wings of large individuals (a) are overall more slender compared to the wings of smaller individuals (b). The associated changes in wing vein positioning can provide the null expectation against which the effects of functional genetic manipulations can be tested. If the knockdown phenotypes of a functional genetic manipulation closely resemble the effects of allometry, the manipulated gene/pathway is likely involved in the regulation of allometric shape changes. If, however, knockdown phenotypes are poorly aligned with shape allometry, a given target gene or pathway may function primarily in other aspects of trait formation, such as patterning or morphogenesis. In this hypothetical example, both knockdowns (c, d) reduce wing length (a linear measure of size) relative to a control (a) but only one of the two knockdown phenotypes closely resembles the effects of allometry. [Color figure can be viewed at wileyonlinelibrary.com]

Here, we use a multivariate approach to test how closely functional manipulations resemble the effects of allometric scaling. If the manipulated pathways function in the regulation of allometric scaling, we expect their phenotypic consequences to resemble those generated naturally by variation in trait size. That is, the induced shape changes are expected to resemble the effect of an increase or decrease in trait size in a control group (depending on whether the pathway of interest is a positive or negative regulator of allometric growth). If, however, knockdown phenotypes do not resemble allometric shape changes, the manipulated pathways may not constitute major regulators of allometry. We find that the effects of several genes indeed closely align with the effects of allometry, indicating that their corresponding pathways may function primarily in the sensing of size or nutritional status, or the transduction of this information. In contrast, other knockdown effects were largely independent of those associated with allometry, likely implicating these pathways in other developmental processes that may not primarily instruct scaling. Our findings moderate the interpretation of studies focusing on linear measurements of trait length and highlight the usefulness of multivariate approaches in the study of allometry.

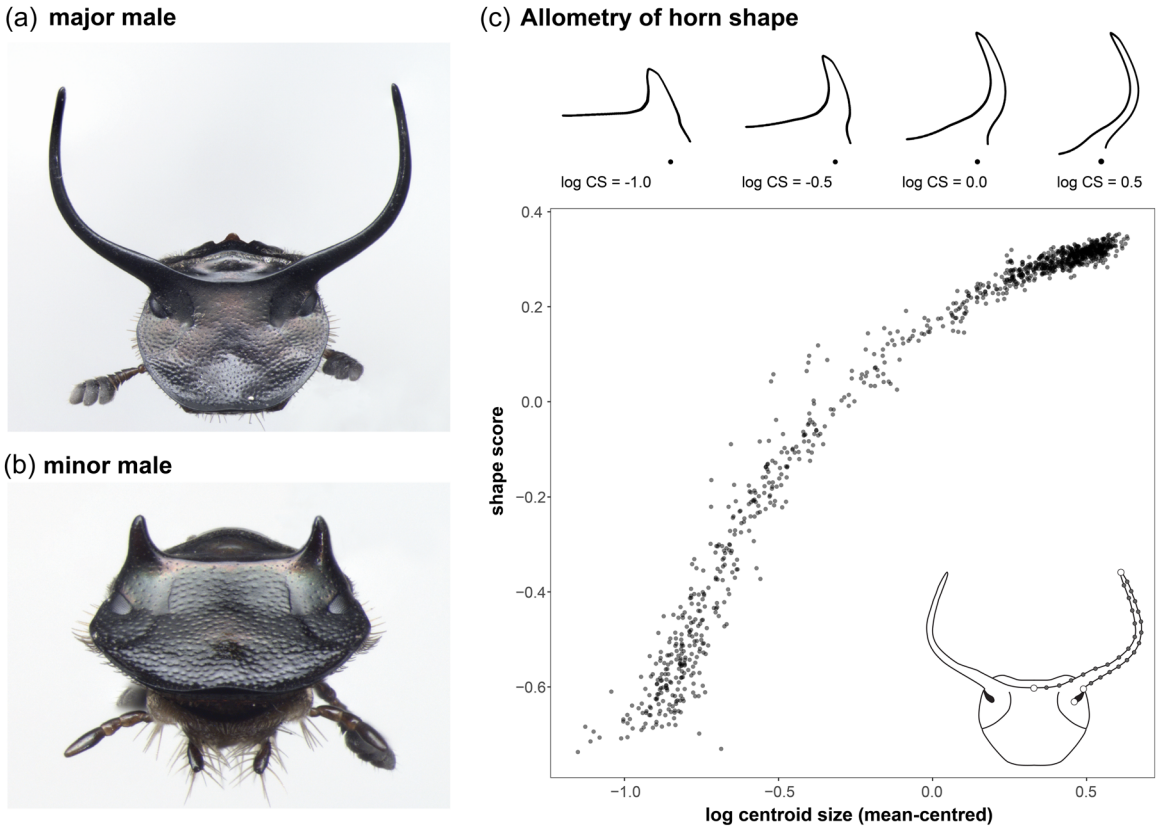

FIGURE 2 Horn morphology of major (a) and minor (b) male morphs of the dung beetle *Onthophagus taurus*. (c) Shows horn shape allometry. Shape scores were calculated by projecting shape data onto a vector in the direction of the common allometric relationship (following Drake & Klingenberg, 2008). The relationship between the shape score (*y*-axis) and size (*x*-axis) indicates the strength and shape of the overall relationship between log centroid size and horn shape. Small sketches on top depict the expected shape at a given log centroid size. Semilandmarks are indicated in gray in (c). [Color figure can be viewed at wileyonlinelibrary.com]

2 | MATERIALS AND METHODS

2.1 | Functional genetic manipulations

We here reanalyze horn shape data recently generated by Rohner et al. (2022). In that study, we revisited previous functional genetic experiments that investigated the roles of several major developmental pathways in the allometry of horn length. Manipulations investigated include: the inhibition of serotonin biosynthesis through the application of alpha-methyl-p-tyrosine (AMPT) (Schwab et al., 2020); RNA interference (RNAi) of the limb patterning gene *Distal-less* (*Dll*) (Moczek & Rose, 2009); RNAi of the somatic sex determination gene *doublesex* (*dsx*) (Kijimoto et al., 2012); RNAi of *Forkhead box, subgroup O* (*Foxo*), a key component of insulin signaling (Casasa & Moczek, 2018); RNAi-mediated knockdown of *dachs* (*d*); as well as RNAi targeting *histone deacetylase 3* (*HDAC3*) RNAi. In each experiment, beetle larvae were reared in artificial brood balls made from cow dung (Shafiei et al., 2001). All RNAi manipulations were conducted by injecting the corresponding dsRNA during the early third larval stage. The pharmacological manipulation of serotonin signaling was applied by mixing AMPT into cow dung, making it accessible to larvae by feeding. Cow dung is a heterogenous substrate that causes variation in larval nutrition, in turn yielding nutritionally plastic responses in body and horn size. We here take advantage of this nutritional variation in horn size to test whether functional manipulations affect horn shape allometry.

2.2 | Data collection

Horn shape was quantified using a two-dimensional geometric morphometric approach (see Rohner et al., 2022). Pictures of beetle head horns were taken using a digital camera (Scion) mounted on a Leica MZ-16 stereomicroscope. Four landmarks and 26 semilandmarks were digitized using TpsDig2 (Rohlf, 2009) following the approach developed by Crabtree et al. (2020) (see Figure 2). The landmark coordinates were subjected simultaneously to a Procrustes analysis in the R-package

geomorph (Adams et al., 2021). The position of semilandmarks was optimized by minimizing bending energy. Centroid size was extracted as an estimate of horn size.

2.3 | Statistical analysis

To test for the effects of size and functional genetic treatment on horn shape, we performed Procrustes ANOVAs (with randomized residual permutation procedure and type II sums of squares, as implemented in the R packages *geomorph* and *RRPP* [Collyer & Adams, 2019]). Because horn shape shows morph-specific shape allometry (Crabtree et al., 2020), we analyzed our data separately for major and minor males (pooling "intermediate" males with the latter). For each functional manipulation, we acquired Procrustes coordinates (as described above) for individuals subjected to RNAi or pharmacological treatments as well as the respective control injections. We then fitted horn shape as a function of treatment (T), log centroid size (Cs), and their interaction as:

$$Y \sim \beta_0 + \beta_1 \times Cs + \beta_2 \times T + \beta_3 \times Cs \times T + \varepsilon$$

Where Y represents a matrix of shape coordinates; β_0 is the vector of intercepts; β_1, β_2, and β_3 are the vectors of regression coefficients for the effects of centroid size (i.e., allometry), treatment, and their interaction; and ε corresponds to the residual term. Significant treatment-by-size interactions were found for the effect of *HDAC3*$^{\text{RNAi}}$ in major males, and *dachs*$^{\text{RNAi}}$ and *Foxo*$^{\text{RNAi}}$ in minor males (see Supporting Information S1: Table 1). These interactions indicate that treatments affected the magnitude and/or the direction of allometry. Because we were primarily interested in differences in vector direction, we calculated vector correlations (also referred to as cosine similarity) and angles between the allometry vectors in the control and treatment groups as:

$$r_\beta = \cos(\theta) = \frac{\beta_{1,control} \cdot \beta_{1,treatment}}{\|\beta_{1,control}\| \times \|\beta_{1,treatment}\|}$$

where the numerator denotes the dot product of the partial coefficients of the allometry vectors (i.e., β_1) in the control and treatment groups, while the denominator represents their norms (Cheverud, 1982; Claude, 2008; Rohner et al., 2019). Bias-corrected and accelerated bootstrap (BCa) confidence intervals were calculated based on 9999 nonparametric bootstrap replicates. These correlations were strong for the *dachs* $^{\text{RNAi}}$ data sets ($r = .96$ [0.89−0.99] 95% BCa confidence interval; angle in degrees $\theta = 16.3°$ [8.1−27.1]) and the *HDAC3* manipulation ($r = .88$ [0.80−0.94]; $\theta = 28.4°$ [19.9−36.9]), indicating that overall, there was no strong indication that functional genetic manipulations had a major effect on the direction of horn shape allometry even when interactions were present. One potential exception was the insulin signaling manipulation that showed a strong interaction (Supporting Information S1: Table 1). However, closer inspection revealed that *Foxo*$^{\text{RNAi}}$ mainly reduced the magnitude of shape change from 1.06 [0.98−1.28] (95% BCa confidence interval) in the control group to 0.45 [0.36−0.66] in the treatment group, but had less of an effect on the direction of the shape change ($r = .75$ [0.56−0.89]; $\theta = 41.4°$ [27.1−55.9]).

To compare the treatment effects to the expected allometric shape changes, we next focused on models that only included main effects:

$$Y \sim \beta_0 + \beta_1 \times Cs + \beta_2 \times T + \varepsilon$$

We extracted vectors of coefficients for the partial effects of centroid size (β_1, i.e., allometry) and treatment (β_2) for each developmental manipulation and male morph. The alignment of significant treatment-mediated shape deformation vectors with allometric shape changes in the control group for each treatment j was quantified using pairwise vector correlations as:

$$r_{\beta_{1,j},\beta_{2,j}} = \frac{\beta_{1,j} \cdot \beta_{2,j}}{\|\beta_{1,j}\| \times \|\beta_{2,j}\|},$$

This vector correlation provides a quantitative assessment for the alignment between the developmental manipulation and shape changes expected due to allometry. Values close to ±1 indicate that treatment-mediated shape changes mirror those expected by allometric shape changes (i.e., the effect of the gene or pathway can be largely explained by changes in the way horns respond to an increase or decrease in overall size). If r is close to 0, morphological effects of developmental manipulations do not resemble the changes associated with allometry and, consequently, the functional genetic manipulation does not recapitulate the phenotypic effects expected by a disruption of tissue-wide responses of relative growth. Note that our approach of comparing vectors derived from main effect models assumes that treatments do not have strong effects on the direction of allometric shape change.

3 | RESULTS

The male head horns of *O. taurus* feature pronounced allometric shape changes. Horns not only become relatively longer and thinner, but also more curved as

their size increases (Figure 2, also see Crabtree et al. [2020]). These allometric shape changes superficially resemble knockdown effects of several genes that primarily affect horn curvature (Rohner et al., 2022; Figure 3). However, quantitative comparisons of the effects of functional genetic manipulations and shape allometry observed in an unmanipulated control group rendered heterogenous results. Functional manipulation of some pathways, such as *Foxo* indeed aligned very closely with the expected allometric pattern ($r = .89$ [0.83−0.95]; $\theta = 27.1°$ [18.2−33.9]). Put another way, changes in horn size as a result of $Foxo^{RNAi}$ were paralleled by changes in horn shape that one would normally observe in a control group. Similarly, we find alignments between the effects of dsx^{RNAi} and shape allometry ($r = -.91$ [−0.96 to −0.79]; $\theta = 155.5°$ [142.2−163.7]). These correlations were strong but negative, suggesting that that the effect of dsx^{RNAi} on horn shape is closely aligned with the expected allometric shape changes caused by a *decrease* in horn size. Likewise, the effect of serotonin synthesis inhibition on horn shape was also significantly correlated with the allometric shape changes, but this alignment was weaker than those observed for *Foxo* or *dsx* and clearly distinct from 1 ($r = .61$ [0.19−0.86]; $\theta = 52.4°$ [30.7−79.0]; Figure 4). This suggests that serotonin manipulations only

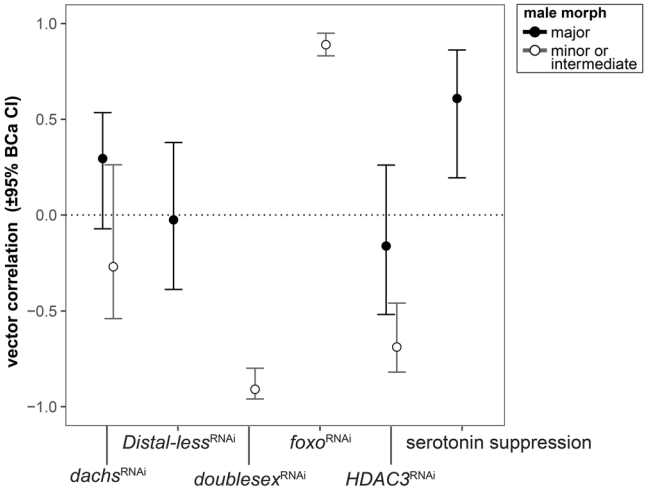

FIGURE 4 Correlations between the shape deformation vectors associated with a given treatment and shape allometry, shown separately for minor/intermediate and major males (with associated 95% bias-corrected and accelerated bootstrap confidence intervals). Correlations close to ±1 indicate that shape changes due to functional genetic manipulations closely resemble those expected by an increase or decrease in size and thus replicate the expected effects of shape allometry. Correlations close to zero indicate that the effects of functional genetic effects are not aligned with the expected allometric shape changes. Only significant shape change vectors are included. Dll^{RNAi} and dsx^{RNAi} could only be assessed in majors or minor males, respectively.

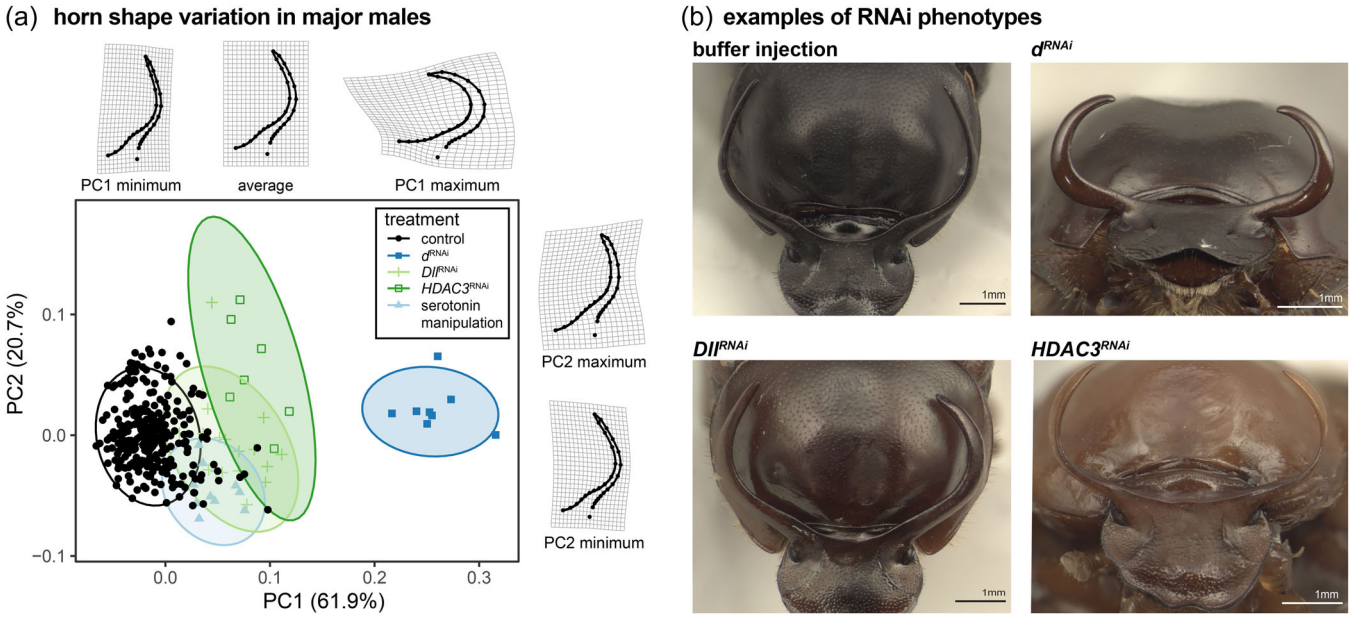

FIGURE 3 Effect of functional genetic manipulations on major male horn shape. (a) Shows how individuals cluster in a morphospace defined by the first two principal components calculated based on the covariance matrix of horn shape coordinates of major males only (together accounting for 82.6% of the total variation). (b) Illustrates the phenotypic effects of RNAi-mediated gene expression knockdown of *dachs* (*d*), *Distal-less* (*Dll*), and *Histone deacetylase 3* (*HDAC3*). [Color figure can be viewed at wileyonlinelibrary.com]

partially resemble the expected allometric shape changes.

In contrast to the manipulation of *Foxo*, *dsx* and serotonin signaling, knockdown effects of other pathways were unrelated to shape allometry. Specifically, $dachs^{RNAi}$ yielded shape changes uncorrelated with allometric shape variation in both male morphs (d^{RNAi}: major males: $r = .29$ [−0.08 to 0.53], $\theta = 73.1°$ [58.0−94.6], minor males: $r = -.27$ [−0.55 to 0.23], $\theta = 105.7°$ [76.7−123.4]). Similarly, knockdown of *Distal-less* generated phenotypes that are not congruent with those expected by allometric shape changes (major males: $r = -.03$ [−0.39 to 0.37]; $\theta = 91.7°$ [68.3−113]). Interestingly, the alignment between the effects of an expression knockdown of *HDAC3*, a histone deacetylase, and shape allometry differed even between minor and major morphs ($r = -.69$ [−0.82 to −0.45], $\theta = 133.6°$ [116.7−145.1] and $r = -.16$ [−0.52, 0.24]; $\theta = 99.2°$ [76.1−121.3], respectively), indicating that the relationship between knockdown phenotypes and the expected allometric pattern may be context dependent.

4 | DISCUSSION

Recent years have seen a rapid accumulation of studies aimed at identifying the developmental genetic mechanisms that regulate trait size relative to body size. The most common approaches employ perturbation experiments such as RNAi-mediated transcript depletion (e.g., Emlen et al., 2012; Rohner et al., 2021; Tang et al., 2011) or topical hormone applications (e.g., Fry, 2006; Shelby et al., 2007) or a combination of approaches (e.g., Gotoh et al., 2014) whose effects on trait size are assessed by the degree to which typically simple, linear measurements of traits size relative to some proxy of body size deviate between treated and control individuals. However, such deviations may arise via a great diversity of developmental mechanisms which may or may not be related directly to nutrient sensing and the regulation of trait size. Here we sought to propose and put to the test a geometric morphometric approach aimed to distinguish developmental mechanisms primarily involved in the regulation of organ size relative to body size, from those that may be affecting trait size through other means. Such a distinction is biologically relevant because it informs our understanding of the degree to which the developmental regulation of relative organ size may be decoupled from other aspects of trait formation, and hence able to evolve independently, and to prioritize candidate pathways and genes for more detailed analyses. Specifically, we asked how closely the phenotypic effects of functional genetic manipulation of genes involved in the regulation of horn *length* resemble the effects of horn *shape* allometry. Our results indicate that while some knockdown phenotypes are consistent with the expected allometric changes in shape, others align only poorly. Below we discuss the potential implications of our results for our understanding of the developmental basis of trait size and the utility of this approach for future applications.

In agreement with previous interpretations, we found that RNAi-mediated knockdowns of some genes were strongly aligned with horn shape allometry (Figure 4). This was especially true for the RNAi-mediated knockdown of *Foxo*, a major regulator of insulin signaling. $Foxo^{RNAi}$ had previously been shown to increase horn length in minor males. Here, we show that $Foxo^{RNAi}$ also affects horn shape and that these shape deformations correlate strongly with the expected shape effects associated with an increase in horn size. $Foxo^{RNAi}$ phenotypes thus recapitulate allometric shape changes, which supports the conclusions of previous studies indicating that *Foxo* in particular, and insulin signaling more generally, specifies allometric growth throughout the entire horn tissue (e.g., Casasa & Moczek, 2018; Rohner et al., 2023). Similarly strong but negative alignments were found between horn shape allometry and the knockdown of *dsx* expression. Because dsx^{RNAi} *reduces* horn length, and the shape changes are strongly negatively correlated with the effects of nutritional plasticity, *DSX* is thus likely to instruct tissue-wide allometric growth. These findings are consistent with the hypothesis that *DSX* is a significant regulator of allometry, potentially in complex interaction with *FOXO* or other components of insulin signaling (see Casasa & Moczek, 2018).

Although some functional genetic manipulations aligned well with horn shape allometry, this was not the case for all pathways. For instance, although the manipulation of *dachs*, a gene involved in appendage formation in *Drosophila* (e.g., Mao et al., 2006), leads to a reduction in horn length, the effects on horn shape were poorly aligned with allometric shape changes. Similarly, knockdown of *Distal-less*, a gene involved in appendage patterning (Moczek & Rose, 2009), induced phenotypes that are not congruent with allometric shape changes. Although d^{RNAi} and Dll^{RNAi} both reduce horn length, these effects are not aligned with the expected allometric shape changes and instead raise the possibility that one or both genes may be executing functions related to patterning and/or morphogenesis. This contrasts with interpretations based on measures of horn length alone, indicating that a multivariate approach may reveal more about gene function than analyses focusing exclusively on univariate estimates of trait size.

Interestingly, our data suggest that the alignment between knockdown effects and shape allometry can be complex and may itself be context dependent. For instance, the effect of serotonin synthesis inhibition on horn shape was overall similar to horn shape allometry, but the correlation was much smaller than 1 (Figure 4). These partial alignments could be caused if a pathway regulates allometry only in part of the structure. As the pharmacological manipulation of serotonin signaling was administered through feeding, it may also be caused by increased variability in the strength of the treatment, which we were unable to control. The involvement of serotonin in regulating allometry thus requires further investigation. Furthermore, the alignment between the effects of expression knockdown of *HDAC3*, a histone deacetylase, and allometry differed even between minor and major morphs. This indicates that the degree to which gene function relates to allometry may depend on the precise developmental context.

It is worth emphasizing that the degree of alignment between knockdown effects and shape allometry may further depend on the exact timepoint at which developmental manipulations are administered. All functional manipulations reported on here were applied during the early third larval instar. While this allows us to investigate gene function during the later larval, prepupal, and pupal stages (i.e., the stage at which horn primordia undergo apolysis, cell proliferation, morphogenesis, and cuticular differentiation), we cannot exclude the possibility that our manipulations missed potential early effects during embryonic or early larval development. In cases where knockdown phenotypes do not recapitulate the effects of nutritional plasticity, more detailed, temporally fine-grained analyses may be required to characterize gene function unless clear predictions for the phenotypic effects of particular developmental processes are available.

Taken together, our findings indicate that the degree to which the effects of functional genetic manipulations resemble horn shape allometry is dependent on target gene and developmental context. Because beetle head horns are evolutionary novelties lacking any obvious homology to other structures or species (Emlen et al., 2007), it is unclear whether similar findings can be expected for other traits and developmental contexts. However, we expect at least some of the patterns documented here to be general. For instance, the insulin/TOR signaling pathway is canonically thought of as the main component of allometric scaling. Consequently, functional genetic manipulation of its components is expected to closely recapitulate the effects of allometry across traits and species. Similarly, *Dll* is a highly conserved appendage patterning gene. Its knockdown may thus a priori be expected to change the way trait size scales with body size, yet due to its deeply conserved role in patterning rather than allometry. The approach detailed here may therefore provide a useful analytical method to begin characterizing the roles of diverse genes in trait development where a detailed molecular or cellular understanding of gene function is lacking. Future research in other species and traits will be necessary to further investigate potential general patterns.

5 | CONCLUSIONS

Studying the effects of functional genetic manipulations on the development of beetle horn shape, we show that the knockdown phenotypes of a subset of genes and pathways investigated by past work indeed replicate the effects of horn shape allometry. However, other genes and pathways previously shown to affect the scaling of horn length induce shape changes inconsistent with allometric shape change (e.g., *Dll, d*). These genes or pathways are thus unlikely to be main instructors of allometric growth. Our findings illustrate the usefulness of geometric morphometrics in the investigation of gene function and the developmental regulation of allometric shape change. Similar approaches could be used to test the degree to which genes involved in sexual differentiation recapitulate shape differences between males and females, or whether the manipulation of genes acting as developmental switches during polyphenic development indeed recapitulate morph-specific shape differences (e.g., in polyphenic nematodes Sieriebriennikov et al., 2017). Geometric morphometrics may therefore benefit the study of gene function and its evolution and context-dependency.

AUTHOR CONTRIBUTIONS

Patrick T. Rohner and Armin P. Moczek conceived and designed the study. Yonggang Hu conducted previously unpublished functional manipulations. Patrick T. Rohner performed all analyses and drafted the initial version of the manuscript. All authors contributed to later versions of the manuscript.

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DATA AVAILABILITY STATEMENT

The data used in this study were derived from the following resources available in the public domain: Data from Rohner et al. (2022), https://doi.org/10.5061/dryad.ksn02v77n

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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