

# Developmental regulation and evolution of scaling: novel insights through the study of *Onthophagus* beetles

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Scaling relationships play critical roles in defining biological shape, trait functionality, and species characteristics, yet the developmental basis of scaling and its evolution remain poorly resolved in most taxa. In the horned beetle genus *Onthophagus*, scaling relationships of most traits are largely comparable across many species, however, the morphology and scaling of horns, a recent evolutionary invention, has diversified dramatically, ranging from modestly to highly positively linear to more complex sigmoidal allometries. Through a series of transcriptomic screens and gene function assays, the *doublesex*, *hedgehog*, *insulin*, and *serotonin* signaling pathways have recently been implicated in the regulation of amplitude, slope, and threshold location of the highly sigmoidal horn allometry in *O. taurus*. These and other findings suggest that co-option of these pathways into the regulation of horn development may have been critical in the evolutionary transitions from isometric to positively allometric to sigmoidal allometries in *Onthophagus*, thereby contributing to the extraordinary diversification of one of the most species-rich genera in the animal kingdom.

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## Introduction

The study of scaling relationships marks a focal point at the intersection of several fundamental research programs in evolutionary developmental biology and allied fields. At the most basic level, scaling relationships, or allometries, are the product of differential growth of parts relative to each other, and as such the study of allometry is fundamental to our understanding of the biology of shape, how it is achieved and modified in development, and how it is

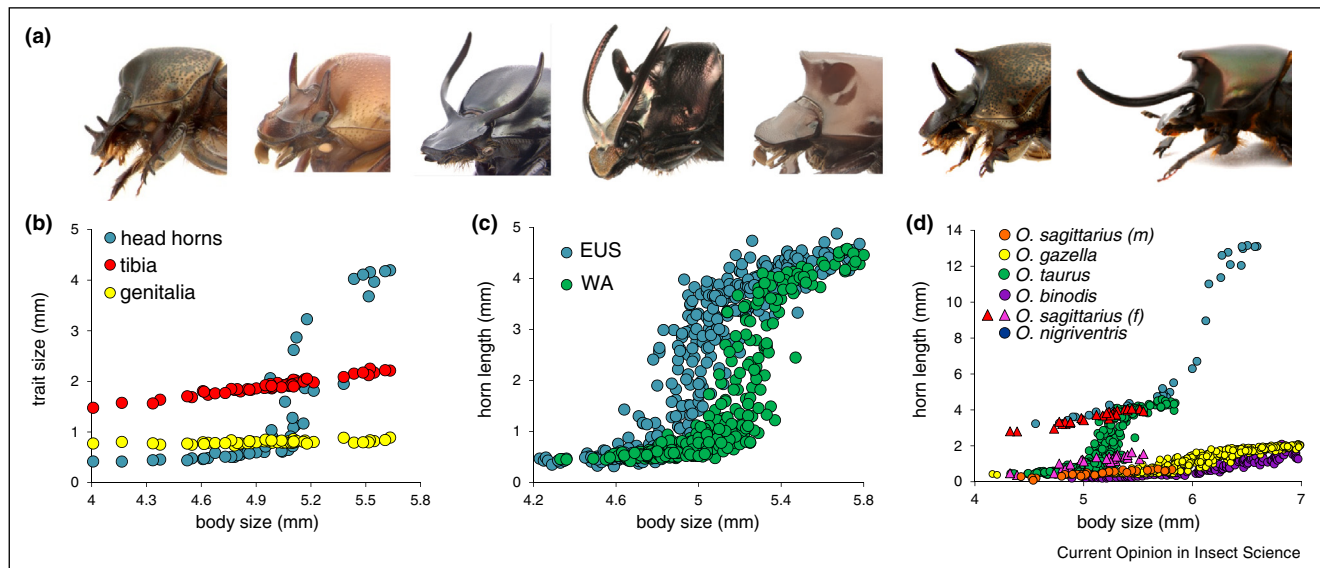
transformed in evolution [1]. At the same time, scaling relationships are highly dependent on biological context, and as such their study can provide a window into the biology of said contexts. For instance, in most sexually reproducing organisms sexual dimorphism is the leading source of intraspecific variation, brought about to a significant degree through sex-specific changes in the scaling of otherwise homologous body parts [2]. Similarly, environmental conditions such as nutrition, crowding, or season commonly impact how much developing organisms invest into specific structures, enabling adaptive changes in shape and scaling as a function of environmental conditions [3–5]. Investigating the developmental regulation of scaling relationships and its evolution can therefore inform multiple research programs at once, from the developmental evolution of biological shape in general to developmental origins and evolutionary diversification of sexual dimorphisms and developmental plasticity.

Insects have featured prominently in the study of allometry by combining a diversity of transformational modes with overall easily measured external morphology, experimental manipulability, and ecological relevance. In particular, early studies focused on the hormonal regulation of metamorphosis provided many critical insights into the developmental mechanisms that impact growth and relative size, and the evolutionary biases these mechanisms may exert [6]. The advent of affordable next generation sequencing methods, coupled with the increasing availability of gene function assays such as RNA *interference*, is now allowing the field to move beyond these more traditional foci and explore pathways, processes, or developmental interactions that until recently have not been studied in the context of scaling and allometry evolution. In this article we briefly review current efforts to employ such approaches in the horned beetle genus *Onthophagus* to further advance our understanding of the developmental basis of scaling relationships and their evolution across important biological contexts.

## Biology and allometry in *Onthophagus*

The genus *Onthophagus* is among the most speciose genera of animals, encompassing over 2000 described species [7]. All *Onthophagus* are dung beetles in the narrow sense, i.e. rely on the dung of mostly herbivorous mammals as a food source for both larvae and adults. Natural populations of adult *Onthophagus* beetles typically consist of

Figure 1



Allometric diversity in the horned beetle genus *Onthophagus*. **(a)** Portraits of the head and thorax of six males and one female illustrating some of the diversity in head and thoracic horns present within the genus. From left to right: *O. sagittarius*, *O. gazella*, *O. taurus*, *O. watanabei*, *O. binodis*, *O. sagittarius* (female), and *O. nigriventris*. **(b)** Scaling relationships between nutritionally determined adult body size and head horn length, fore tibia length, and aedeagus (genitalia) length in male *O. taurus*, illustrating some of the diversity in scaling relationships and nutritional responsiveness different traits may exhibit despite being exposed to the same nutritional variation. **(c)** Horn length - body size allometries in two exotic *O. taurus* populations exemplifying a case of rapid allometric divergence in thresholds in populations subject to divergent ecological conditions. **(d)** Diversity in horn length - body size allometries among five *Onthophagus* species. Separate allometries are shown for head and thoracic horns of female *O. sagittarius*.

individuals spanning a wide range of body sizes, reflecting the diversity of resource environments experienced by larvae and providing easy opportunities to study the interplay between nutritional variation and scaling relationships across traits, sexes, populations, and species [8]. Moreover, *Onthophagus* possess a diversity of traits that make them especially attractive models for the study of scaling, most notably horns, single or paired structures projecting from the head and/or thorax and used primarily in male combat over access to females (Figure 1a). *Onthophagus* horns constitute an evolutionary novelty even by the strictest definition [9], and commonly display pronounced and sometimes extreme nutrition-dependent plasticity, in contrast to the more isometric growth typical of mouthparts, wings and legs, or the hypo-allometric growth of genitalia (Figure 1b). Further, while scaling relationships of non-horn traits are largely comparable across species, those of horns have diversified dramatically among *Onthophagus* populations (Figure 1c) and species (Figure 1d), ranging from modestly to highly positively allometric to polyphenic, where male morphologies are separated into a minor nearly hornless sneaker morph and a major fully horned fighter morph by a sharp body size threshold [8,10]. Lastly, at least one increasingly well-studied species is available in which males have secondarily lost nutrition-responsive horn growth while females have independently gained both conspicuous head- and

thoracic horns, resulting in a rare reversed sexual dimorphism [11]. Collectively, this striking intra- and interspecific diversity in scaling relationships over a range of phylogenetic distances offers interesting opportunities to explore the developmental regulation of relative growth, and to begin integrating the micro- and macro-evolution of scaling.

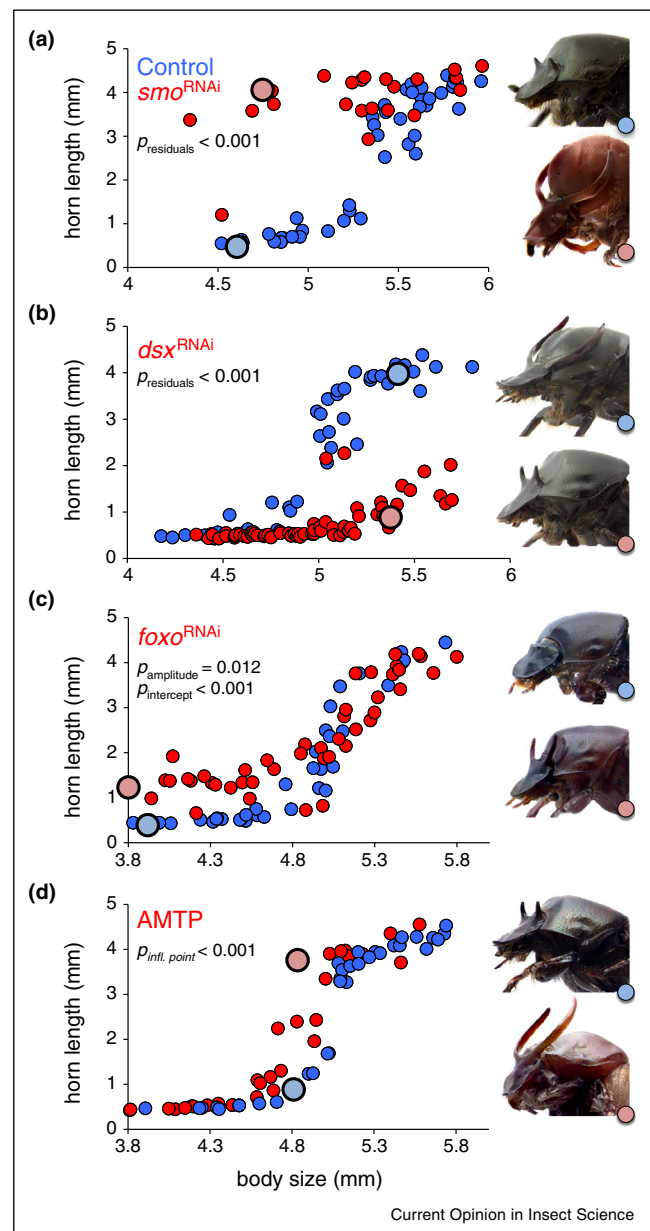
### The developmental evolution of thresholds: lessons from the sex determination and Hedgehog pathways

Many *Onthophagus* species are polyphenic, characterized by alternate male morphs so strikingly different that several were originally described as separate species [12]. Horn polyphenisms rely on a sharp body size threshold separating male larvae along one of two alternate, discrete developmental pathways. However, the developmental means by which such thresholds are specified and executed remain largely unclear, with early studies providing at best partial, and highly debated, insights [13,14]. Starting in 2009, a series of transcriptomic screens in *O. taurus* [15,16] began to document consistent and surprising differences in the expression levels of a transcription factor—Doublesex (*Dsx*)—across male body regions as well as nutritional states, suggesting that fold changes in *dsx* expression may somehow be related to the

relative degree of nutrition-dependent trait exaggeration. Up to this point *dsx* was well studied as a key member of the insect sex determination pathway, in the context of which it is alternatively spliced to encode male- and female-specific splice variants, which in turn are thought to regulate the sex-biased expression of target genes underlying sexually dimorphic traits [17,18]. While originally assumed to be expressed in all cells, seminal work in *Drosophila* showed that flies, and likely insects in general, are in fact mosaic for *dsx* expression: only a subset of cells acquire a *dsx*-mediated male or female identity during development, while the majority do not, suggesting that the spatial regulation of *dsx* is required for the elaboration of sex-specific structures [19–21]. Expression data in *O. taurus*, however, began to suggest that *dsx* may also contribute to variation *within* sexes, possibly by modulating growth responses to variation in nutrition. Subsequent work in *Onthophagus* and other beetle systems has since confirmed this suspicion: for example, in *O. taurus* down-regulation of the male Dsx isoform largely eliminates nutrition-responsive horn growth in males, while down-regulation of the female isoform *induces* moderate nutrition-responsive growth of horns in females (Figure 2b) [22]. Similarly, sex-specific Dsx isoforms promote and inhibit the formation of head horns in males and females of the rhinoceros beetle *Trypoxylus dichotomus*, respectively [23]. Unlike *O. taurus* males, male *T. dichotomus* are not polyphenic, but horn formation is similarly developmentally plastic, and cued primarily by larval nutrition [23]. Lastly, similar findings emerged from parallel studies on stag beetles (Lucanidae) famous for their nutrition-dependent elaboration of mandible length in males [24\*]. Taken together, these results suggest that the evolution of nutrition-dependent exaggeration of secondary sexual traits and the corresponding evolution of highly positive, hyper-allometric scaling relationships was made possible through the repeated and independent co-option of *dsx*-mediated growth regulation. At the same time it became clear, however, that *dsx* function in *Onthophagus* was insufficient to fully explain the development of a body size threshold and corresponding sigmoidal allometry: while *dsx* may account for the regulation of nutrition-responsive *exaggeration*, some other mechanism was needed to simultaneously inhibit trait induction in below-threshold individuals. Transcriptomic screens again began to point in a promising, albeit highly unexpected direction — the Hedgehog signaling pathway.

The Hedgehog (Hh) signaling pathway is a highly conserved cellular transduction pathway best understood for its role in patterning anterior/posterior (A/P) polarity in body segments and appendages [25,26]. Key members of this pathway include the Hedgehog protein (Hh) — a diffusible morphogen required for activating Hh signaling, Patched (ptc) — the cell membrane bound Hh receptor that inhibits Hh signaling unless bound to Hh protein, and Smoothed (smo) — a membrane protein

Figure 2



Effect of *smo*<sup>RNAi</sup>, *dsx*<sup>RNAi</sup>, *foxo*<sup>RNAi</sup>, and AMTP application on the body size-horn length allometry in male *O. taurus*. **(a)** *smo*<sup>RNAi</sup> induces large horns in low-nutrition males which normally remain hornless. **(b)** *dsx*<sup>RNAi</sup> inhibits horn growth in high-nutrition males which normally develop a full set of horns. **(c)** *foxo*<sup>RNAi</sup> linearizes the horn length-body size allometry by significantly increasing horn growth in small, low-nutrition males while modestly decreasing it in large, high-nutrition males. **(d)** AMTP treatment decreases 5-HT and shifts the body size threshold for horn production to lower body sizes. Control and knock-down allometries are represented by blue and red circles, respectively. Enlarged circles correspond to the representative knock-down and control animals depicted on the right.

that, in the absence of Hh protein, is constitutively inhibited by *ptc*, but disinhibited once *ptc* binds to Hh, thereby activating the intracellular components of the pathway. Several transcriptomic screens in *O. taurus* identified Hh pathway members as differentially expressed not just in different appendage types, but also — unexpectedly — in response to sex and different levels of nutrition [27\*,28]. Subsequent functional analyses confirmed that Hh signaling indeed plays a critical role in the nutrition- and sex-dependent regulation of scaling relationships of a subset of traits, yet does so in a highly unusual and unexpected manner [29\*\*]. Specifically, RNAi-mediated down-regulation of *smo* and thus inactivation of the pathway resulted in the development of full-sized horns in small, low-nutrition *O. taurus* males, thereby converting the strongly sigmoidal scaling relationship between body size and horn length typical of wildtype individuals into a linear allometry (Figure 2a). High-nutrition males, in contrast, were unaffected. Similar, though less extreme phenotypes were obtained via knock-down of *hh* itself, which is also predicted to inactivate the pathway. Conversely, *ptc*<sup>RNAi</sup> — which is predicted to constitutively activate the pathway even in the absence of *hh*, rendered both low and high-nutrition males essentially hornless [29\*\*].

Corresponding results were obtained for pupal thoracic (pronotal) horns. Like all *Onthophagus* species studied so far, *O. taurus* pupae develop a horn on the pronotum, which aids in the shedding of the larval head capsule during the larval to pupal molt [30]. However, in a subset of species, including *O. taurus*, the pupal pronotal horn is fully resorbed prior to the adult molt via programmed cell death [31]. Unlike head horns, however, pupal thoracic horns scale linearly with body size and can be found in both sexes. Following *smo*<sup>RNAi</sup>, animals developed significantly longer thoracic horns relative to their body size compared to control-injected individuals, whereas *ptc*<sup>RNAi</sup> massively reduced or even eliminated thoracic horns in both sexes. At the same time, RNAi phenotypes observed elsewhere along the body axis were consistent with a conservation of pathway function in the context of patterning A/P polarity of segments and appendages, and double knock-downs confirmed conservation of the genetic relationships among pathway members [29\*\*]. Collectively, these data thus suggest that just like the sex-determination pathway, Hh signaling has been co-opted into the regulation of horn development, has acquired nutrition sensitivity in the process, but exerts its function in a manner opposite yet complimentary to that of *dsx*: by actively inhibiting horn formation in low-nutrition individuals.

Taken together, these findings now raise the possibility that a combination of Dsx-mediated promotion of horn growth under high-nutrition and Hh-mediated inhibition of horn growth under low nutrition may have played a

critical role in the evolutionary transition from exaggerated linear to sigmoidal scaling relationships by establishing a critical size threshold. Once in place, such a threshold could then have enabled the development of discrete, nutritionally cued horned and hornless morphs, and by recruiting additional genes and pathways further facilitated the evolution of morph-specific behaviors and physiologies, thereby enabling the development of alternate phenotypes from the same genome. Intriguingly, while the role of Hh signaling in plastic or polyphenic development has so far only been documented in the context of horn morphologies, recent findings suggest that the role of *dsx* may go well beyond the regulation of horns and also affect behavioral phenotypes, most notably aggression [Beckers and Moczek, unpublished data]. While these findings are beginning to hint at a master switch-like mechanism controlling and integrating suites of alternative morphological, behavioral and possibly physiological traits, clearly more work is needed in this area. A similarly exciting area for future study constitutes the nature of interactions between *dsx* and Hh pathways. A first window into possible interactions has been provided by a recent genome-wide analysis of *dsx* target genes in *O. taurus*, which found that several Hh pathway genes both change their expression following *dsx*<sup>RNAi</sup> and possess multiple high affinity Dsx binding sites in their promoters [21]. Perhaps most important, however, we need to learn where and how one or both pathways obtain information about the nutritional constitution of a given individual, a fundamental requirement for the establishment of a nutrition-sensitive threshold. Recent work on the insulin signaling pathway is beginning to provide some first insights.

### Insulin signaling as a possible mediator between nutritional status and growth regulation

The insulin/IGF signaling (IIS) pathway is a conserved physiological mechanism well known for its role in mediating nutrition-dependent growth across taxa, and is especially well studied in *Drosophila* [32–35]. These and other studies established that insulin-like peptides (ILPs) are produced by insulin producing cells (IPCs) in the brain in response to nutrient availability and then released directly into the haemolymph. Circulating ILPs then bind to the Insulin-like receptor (InR), which activates a phosphokinase signal transduction cascade that ultimately induces cell proliferation. Nutritional variation is thus transduced through development in part via the nutrition-dependent release of ILPs and the subsequent ILP-mediated regulation of organ- and body size [35,36].

The IIS pathway has also been implicated in mediating differential sensitivity to nutrition among different organs within an individual. For example, in *Drosophila*, growth of wings, palps and legs scales isometrically with body size in response to nutrition [37] while other body parts, such



as the central nervous system (CNS) and genitalia, are hypo-allometric [38–40]. Even though the mechanisms employed by the CNS and genitalia to render them insensitive to nutrition are different, they both ultimately rely on high levels of IIS activity, especially under low-nutrition conditions [40]. Specifically, the CNS achieves this by directly activating PI3-kinase (PI3 K), one of the components of the IIS pathway downstream of InR via the anaplastic lymphoma kinase, thereby ensuring high levels of IIS activity and cell proliferation regardless of nutritional status [38]. In contrast, genitalia exhibit low expression levels of the transcription factor Forkhead box, sub-group O (Foxo), a growth inhibitor downstream of InR and PI3K. This growth inhibitor is normally activated under nutrient stress, yet in genitalia constitutively low levels of *foxo* expression enable this organ to remain largely insensitive to nutritional status and to maintain a relatively constant size across a range of nutritional conditions and independent of final adult body size [39].

Conversely, pronounced and drastic changes in organ growth in response to variation in nutrition, such as those seen in exaggerated secondary sexual traits, have also been shown to be regulated by the IIS pathway. A recent study in the rhinoceros beetle, *T. dichotomus* [41], provided the first hint that differential expression of *InR* may enable diverse body regions to differ in their sensitivities to nutrition, thereby enabling disparate growth responses to the same nutritional gradient. Specifically, larval RNAi-mediated transcript depletion of *T. dichotomus InR* affected the nutritional responsiveness of horns much more severely than that of wings, whereas genitalia were least affected. These findings raised the possibility that IIS activity may enable differential sensitivity to nutritional variation, thereby allowing linear responses to the same nutritional gradient to be more or less severe. However, if and how the IIS pathway also enables non-linear growth responses, such as those underlying polyphenic development, is presently unclear. A first hint suggesting that IIS signaling may also play a major role in the regulation of polyphenisms comes from the planthopper *Nilaparvata lugens*. A recent study found that a duplication of *InR*, followed by functional divergence, was involved in mediating the production of long- versus short-winged morphs [42]. Duplications of *InR* are widespread across insects, however, their potential role in regulating alternative morphologies, particularly in the context of nutrition-responsive growth, is just beginning to be elucidated.

Recent and ongoing work in *Onthophagus* also implicates the IIS pathway in the regulation of nutrition-responsive growth, although the exact details appear to differ considerably when compared to findings in other taxa, or even from one *Onthophagus* species to another. A recent study in *O. nigriventris*, a species with a nutritionally cued

male polyphenism involving a single and enormous thoracic horn, has shown that *foxo* plays only a modest role in regulating nutrition-responsive horn growth [43]. In contrast, nutrition-responsive development of paired head horns in *O. taurus* appears to be greatly influenced by *foxo*. Preliminary data in this species show that *foxo*<sup>RNAi</sup> results in a significant *increase* in horn length in small, low-nutrition males, but a modest *decrease* in horn length in larger, high-nutrition males, causing the resulting body size — horn length allometry to lose much of its sigmoidal nature (Figure 2c). These results raise the possibility that insulin signaling in *O. taurus*, though not *O. nigriventris*, may play an important role in regulating the steepness of the allometric transition between hornless and horned morphologies, i.e. the slope at the point of inflection of the sigmoidal allometry. Intriguingly, *foxo*<sup>RNAi</sup> phenotypes parallel at least in part those observed for *dsx*<sup>RNAi</sup> (under high nutrition) and *smo*<sup>RNAi</sup> (under low nutrition), raising the possibility that *dsx*, or *hh* signaling, or both may be downstream targets of IIS. However, if and how the IIS pathway interacts with these and other pathways is presently unknown, and represents exciting opportunities for future research. Moreover, recent work on yet another pathway, the serotonin signaling pathway, suggest additional opportunities for studying the developmental regulation and possibly evolutionary diversification of body size thresholds.

### Serotonin signaling as a determinant of threshold body size

The serotonin (5-HT) signaling pathway is an ancient and highly conserved feature of plant, fungal, and animal physiology [44]. Serotonin biosynthesis takes place within specialized (i.e. serotonergic) neurons located in the brain and ventral nerve cord of all insects and requires two steps, beginning with the hydroxylation of the essential amino acid tryptophan by tryptophan hydroxylase (TPH) to form 5-hydroxytryptophan (5-HTP), and followed by the decarboxylation of 5-HTP to 5-HT by tryptophan decarboxylase (TPD). Serotonin is released from serotonergic neurons into either the synaptic cleft as a neurotransmitter or neuromodulator, or secreted into general circulation as a neurohormone, before binding to one or more members of the three 5-HT receptor families (5-HTR<sub>1</sub>, 5-HTR<sub>2</sub>, and 5-HTR<sub>7</sub>) that are present in insects [45•].

Both in vertebrates and invertebrates, 5-HT has long been studied as a regulator of many key physiological processes and behaviors, including circadian rhythms, diuresis, learning and memory, as well as feeding behavior and social interactions [45•]. Yet, recent insights have begun to expand the role of 5-HT and other monoamines beyond these processes and into the regulation of growth, plasticity, and scaling relationships. For instance, the monoamine dopamine has been shown to regulate plasticity in the feeding arm length of sea urchin larvae in

response to food availability [46], and 5-HT mediates the shift from the solitary to the gregarious morph of phase polyphenic locusts in response to high conspecific density [47]. In addition, 5-HT signaling can interact with IIS by suppressing the release of ILPs from the brain of *Drosophila* to negatively regulate adult body size and developmental timing [48].

Transcriptomic screens in *Onthophagus* are now providing the first hints of a possible role of 5-HT signaling in the regulation of horn growth and scaling. These efforts demonstrated not only that 5-HTR<sub>1</sub> is up-regulated exclusively in the horn tissue of major males [15], but also that this receptor, alongside other members of the 5-HT biosynthetic pathway (i.e. TPH and TPD), exhibits differential expression across developmental stages, sexes, and perhaps most importantly, across *O. taurus* populations that have diverged rapidly in the precise location of the body size threshold separating alternate male morphs [21,49, and Pespeni and Moczek, unpublished data]. In addition, recent genome-wide analysis of *dsx* target genes in *O. taurus* (see Section 3) found that 5-HTR<sub>1</sub> expression in male head horns is up-regulated by *dsx*, and that the 5-HTR<sub>1</sub> gene possesses several putative high affinity *dsx* binding sites in its promoter region [21]. Combined, these data implicate 5-HT signaling in the nutrition-responsive horn growth of *O. taurus*, and suggest a thus far undescribed interaction between *dsx* and 5-HT signaling.

Preliminary functional analyses are beginning to confirm a role for 5-HT in regulating the nutrition-responsive growth of head horns in *O. taurus*. Specifically, while pharmacologically *increasing* 5-HT biosynthesis via treatment with 5-HTP produces no effect on horn growth, pharmacologically *decreasing* 5-HT biosynthesis using  $\alpha$ -methyltryptophan (AMTP), a competitive antagonist of TPH, shifts the threshold at which horns are produced to a substantially lower body size, while all other aspects of the allometry remain unaffected (Figure 2d). Intriguingly, the magnitude of this threshold shift is congruent with those observed among *O. taurus* populations (Figure 1c) [49], implicating variation in 5-HT signaling as a potential mechanism underlying rapid threshold evolution in nature. In contrast, even though other morphological features such as the wings and aedeagus begin their elaboration much earlier than horns, the scaling relationships of these and other traits are unaffected by both AMTP and 5-HTP, providing experimental validation of expression data suggesting that the development of horns alone is sensitive to 5-HT signaling.

These manipulations have since been replicated in two additional species of *Onthophagus*: *O. sagittarius*, a species that has secondarily lost male horn polyphenism and now generate horns that scale linearly with body size in both sexes, and *O. gazella*, a primitively polyphenic species that is basal in the *Onthophagus* phylogeny. In *O. sagittarius*,

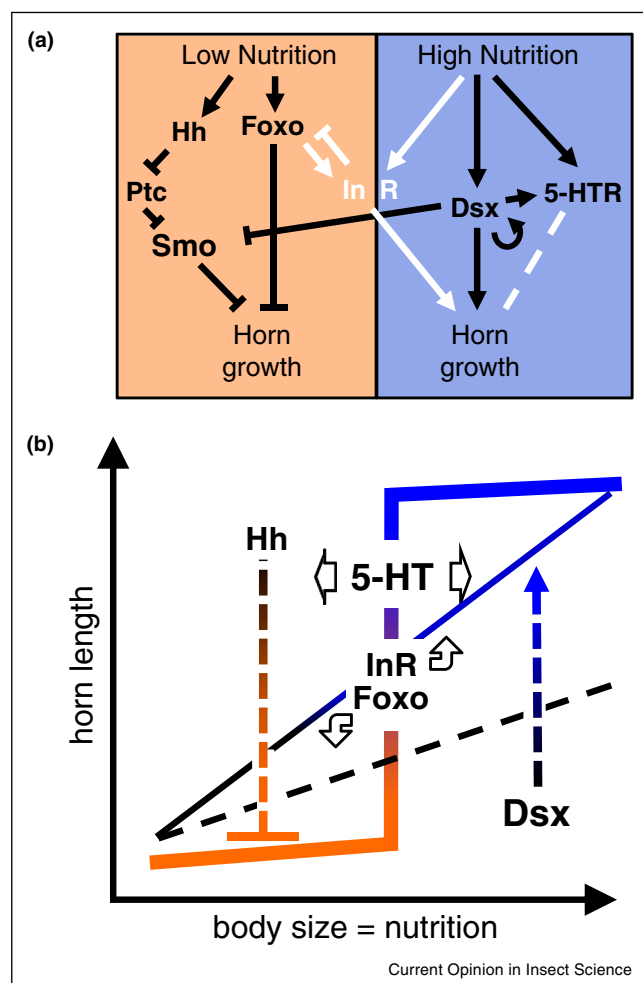
neither AMTP nor 5-HTP treatment has any effect on the horn allometry of males and females, whereas the effect of these treatments on *O. taurus* is largely replicated in *O. gazella*. These preliminary results suggest (i) that 5-HT signaling was co-opted early in *Onthophagus* evolution, (ii) that 5-HT function may be limited to setting the location of the body size threshold, and (iii) that, in the absence of a threshold to set, 5-HT has no effect on the nutrition-dependence of horn growth.

## Summary and future directions

*Onthophagus* horned beetles combine rich morphological diversity within and among species with experimental accessibility and a growing arsenal of transcriptomic and genomic resources. Applying this tool box to the study of allometry has begun to add critical new pathways as well as possible pathway interactions to our understanding of the mechanistic basis of scaling regulation and its evolution across a range of phylogenetic distances (Figure 3a). In particular, the discovery that *Dsx* and *Hh* signaling interact to enable the development of alternate male morphs separated by a sharp body size threshold constitutes a significant advancement in our understanding of the biological basis and evolutionary origins of complex allometries. Conversely, even though IIS has been a focal pathway for much research into the regulation of scaling over the past decade, research in *Onthophagus* is contributing to a better understanding of the surprising evolutionary lability of this pathway and its role in differentially regulating nutrition-responsive growth in different traits, sexes, and species. Lastly, serotonin signaling is emerging as an unexpected yet possibly critical regulator of size thresholds and their diversification (Figure 3b).

Taken together, these new discoveries open up critical new opportunities for future research. For example, exactly how and under what conditions *Dsx*, *Hh*, insulin, and serotonin signaling interact remains largely unknown for most traits and species, yet is likely critical for understanding how the same nutritional gradient can be transduced into disparate growth responses by different body parts of the same individual, and how these interactions - and the allometries they give rise to - might diversify across species. Similarly, we have barely begun to explore the roles played by these pathways in possibly co-regulating morphological and behavioral development, yet doing so may provide critical insights into how divergent social conditions, e.g. those encountered in newly colonized environments, may drive subsequent, rapid allometric divergences [50]. Such an effort would be especially opportune with respect to serotonin signaling given its well established role as a modulator of behavior on one side [45<sup>\*</sup>], and the critical interdependencies of alternative male morphologies and reproductive tactics in horned beetles on the other [51]. Given their allometric diversity and experimental accessibility, *Onthophagus*

Figure 3



Proposed models for the regulation and evolution of nutrition-dependent development of alternative horned and hornless male morphs in *Onthophagus taurus*. (a) Diverse pathways contribute to the nutrition-dependent expression of alternate male morphs. Results to date suggest that the Hh signaling pathway negatively regulates horn growth in low-nutrition males [29\*]. Similarly, preliminary data suggests that IIS negatively regulates horn growth under low nutrition via activation of the growth inhibitor Foxo, but may promote horn development under high nutrition conditions, via up-regulation of the Insulin-like receptor InR (Casasa and Moczek, unpublished data; [52]). Horn growth under high nutrition conditions is also promoted by the somatic sex determination gene *dsx* [22], which binding site analyses suggest positively regulates *smoothed* (a member of Hh signaling) as well as itself [21]. Lastly, preliminary data suggests that serotonin signaling also positively regulates horn development under high nutrition conditions, and one cardinal member of this pathway, the serotonin receptor, appears positively regulated by *dsx* ([21] and Schwab, unpublished data). Black arrows and bars indicate effects and interactions supported by functional (RNAi), co-expression (qPCR, RNAseq), pharmacological manipulations, or transcription factor binding site analyses, whereas white arrows indicate hypothesized interactions yet to be validated in *Onthophagus*. The dashed line indicates an interaction between aspects of serotonin signaling and horn growth, yet nature and direction of this interaction are presently unclear. (b) *dsx*-mediated promotion of horns in high-nutrition conditions converts ancestral isometric (dashed line) into exaggerated hyper-allometric scaling relationships (solid line), and when coupled

beetles are ideally positioned to help address these and related questions in coming years.

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with Hh-mediated inhibition of horns in low-nutrition conditions has the potential to further transform hyper-allometric into sigmoidal scaling relationships. IIS in turn may play a critical role in fine tuning the severity of the transition between alternate morphs (i.e. the steepness of the allometry at its point of inflection) whereas serotonin signaling appears to aid in establishing the precise nutritional state and body size at which this transition occurs.

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