

Linear and threshold-dependent expression of secondary sexual traits in the same individual: insights from a horned beetle (Coleoptera: Scarabaeidae)

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Elaborate horns or horn-like structures in male scarab beetles commonly scale with body size either (a) in a linear fashion with horn size increasing relatively faster than body size or (b) in a threshold-dependent, sigmoid fashion; that is, males smaller than a certain critical body size develop no or only rudimentary horns, whereas males larger than the threshold size express fully developed horns. The development of linear vs. sigmoid scaling relationships is thought to require fundamentally different regulatory mechanisms. Here we show that such disparate regulatory mechanisms may co-occur in the same individual. Large males of the south-east Asian *Onthophagus* (*Proagoderus*) *watanabei* (Ochi & Kon) (Scarabaeidae, Onthophagini) develop a pair of long, curved head horns as well as a single thoracic horn. We show that unlike paired head horns in a large number of *Onthophagus* species, in *O. watanabei* the relationship between head horns and body size is best explained by a linear model. Large males develop disproportionately longer horns than small males, but the difference in relative horn sizes across the range of body sizes is small compared to other *Onthophagus* species. However, the scaling relationship between the thoracic horn and body size is best explained by a strongly sigmoid model. Only males above a certain body size threshold express a thoracic horn and males smaller than this threshold express no horn at all. We found a significant positive correlation between head and thoracic horn length residuals, contrary to what would be expected if a resource allocation tradeoff during larval development would influence the length of both horn types. Our results suggest that the scaling relationship between body size and horn length, and the developmental regulation underlying these scaling relationships, may be quite different for different horns, even though these horns may develop in the same individual. We discuss our results in the context of the developmental biology of secondary sexual traits in beetles. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, 83, 473–480.

ADDITIONAL KEYWORDS: allometry – developmental constraint – developmental tradeoff – *Onthophagus* – scaling.

INTRODUCTION

Several thousand species of beetles develop horns or horn-like structures (Arrow, 1951; Balthasar, 1963). Length of horns commonly scales with body size either

(a) in a linear fashion with horn length increasing relatively faster than body size or (b) in a sigmoid fashion; that is, males smaller than a certain critical body size develop small or no horns, whereas males larger than the threshold size express fully developed horns (Emlen & Nijhout, 2000). The latter pattern has been particularly well studied in dung beetles of the genus *Onthophagus* Latreille. Males in many *Onthophagus* species express elaborate horns on their head, thorax or both (Emlen, 2000). However, horn development

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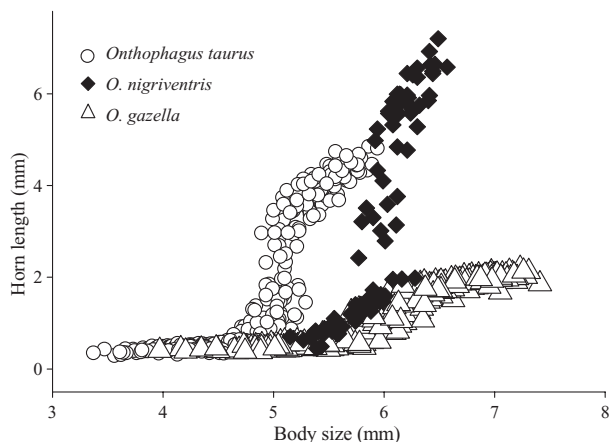


Figure 1. Typical scaling relationships between horn length and body size in the genus *Onthophagus*. Scaling relationships are S-shaped, but may vary in amplitude and the steepness of the transition from hornless to horned morphologies (data are from Moczek, in press).

commonly does not occur unless males exceed a certain critical species- or population-specific body size threshold (Moczek & Nijhout, 2002, 2003; Moczek, 2003). As a consequence of this threshold action natural populations are typically dimorphic for male shape, and the scaling relationship between body size and horn length appears S-shaped rather than linear (Fig. 1). The expression of linear vs. sigmoid scaling relationships is thought to require fundamentally different genetic and developmental control mechanisms (Emlen & Nijhout, 2000; Nijhout & Grunert, 2002). Here we explore whether the disparate developmental mechanisms necessary for the production of linear and sigmoid scaling relationships may co-occur in the same individual and differentially regulate the development of horns produced by different body parts.

We examined patterns of allometric variation in the recently described Bornean dung beetle *Onthophagus watanabei* (Ochi & Kon, 2002). Male and female *O. watanabei* develop a pair of long, curved head horns, and large males also develop a single thoracic horn. We used a morphometric approach to quantify the scaling relationship between body size and head and thorax horns, and to distinguish between linear and sigmoid scaling relationships. We also used our results to investigate whether head horn development may influence the development of thoracic horns and vice versa. Recent studies have implicated resource allocation tradeoffs during larval development as possibly important mechanisms shaping patterns of morphological variation in insects (Kawano, 1995, 1997; Klingenberg & Nijhout, 1998; Nijhout & Emlen, 1998). Secondary sexual traits in beetles, in particular, have provided important first insights into how the

development of one structure, such as a horn, could influence the development of other structures that develop nearby (Emlen, 2001) or at the same time (Moczek & Nijhout, 2004). Because male *O. watanabei* develop horns on both head and thorax this species provides an interesting opportunity to investigate whether the relative expression of one horn type might be affected by the development of another horn type in another location of the same individual. We discuss the implications of our results for our understanding of the developmental biology of secondary sexual traits in insects.

MATERIAL AND METHODS

FIELD COLLECTING

Beetles were collected in Sabah, Borneo at the Deramakot Forest Reserve (c. 5°24'N, 117°28'E; 16.xii.1997–1.xi.1998; 10.iv.–28.iv.1998), Danum Valley Conservation Area (4°58'N, 117°48'E; 10.xii.1997–3.iv.1998, 4.–10.x.2000), Kabili-Sepilok Forest Reserve (5°52'N, 117°57'E; 14.–21.x.2000) and in an oil palm plantation at Segialud-Lokan (5°40'N, 117°32'E; 23.–25.x.2000); for a description of the areas see Chung (1999) and Brühl (2001). C. Brühl found *O. watanabei* searching through elephant dung on the dirt roads leading through secondary forest. No beetles were found in dry dung pads or dung pads older than three days. F.-T. Krell collected the beetles with pitfall traps baited with human dung. Beetles were deposited in The Natural History Museum London and in the Forest Research Centre, Sepilok.

MORPHOMETRIC MEASUREMENTS

All individuals were measured to the nearest 0.01 mm using a digital calliper (Chicago Brand Industrial Inc.) under a 5× illuminated magnifying glass. We used thorax width as an estimate for body size (for justification see Emlen, 1994; Moczek & Emlen, 1999). Individuals with broken or severely worn horns were excluded from the analysis.

STATISTICAL ANALYSES

Scaling relationships between head horn length and body size and thorax horn length and body size were analysed in two ways. First we fitted simple linear regressions to the data, which were expected to yield relatively high r^2 values if scaling relationships were linear. Secondly, we fitted a four-parameter non-linear, sigmoid regression model of the form

$$\text{horn length} = y_0 + \frac{a(\text{body size})^b}{c^b + (\text{body size})^b}$$

to the same data. In this model y_0 specifies minimum horn length, a describes the range of horn lengths in the sample, b specifies a slope coefficient, and c represents the body size at the point of inflection of the sigmoid curve. We obtained parameter values using Sigma Plot curve fitting procedures. This second analysis thus fitted an S-shaped regression model to the data, which would be appropriate if scaling relationships were sigmoid, as is generally widespread in the genus *Onthophagus* (Balthasar, 1963). To distinguish which model is the most appropriate when describing a particular scaling relationship we first compared r^2 values generated by the two regression types. A minor increase in fit when using the non-linear regression model is expected in most cases simply due to the larger number of parameters available and does not need to indicate an underlying sigmoid scaling relationship. However, a substantial increase in fit would indicate that a non-linear, sigmoid regression model would be a better descriptor of the underlying scaling relationship. We employed an Extra Sums of Squares F -test (Ramsey & Schafer, 2001) to quantify whether both models differed significantly in how well they fit the data. Results from F -tests are presented as $F_{\text{df numerator, df denominator}} = \text{test statistic}$.

To explore developmental correlations between head and thoracic horns we calculated the expected horn length for both head and thoracic horns given an individual's body size, using the regression models that best described the scaling relationship between horn length and body size obtained earlier. We then calculated the difference between observed and expected values, or horn length residuals, for each horn type, and tested for possible correlations between them using a standard linear regression model. We executed this analysis for the full data set as well as for a reduced data set, which excluded males that only developed head horns but no thoracic horn. Resource allocation tradeoffs during development should be manifest in a negative correlation between horn length residuals; that is males that develop relatively large head horns given their size should develop relatively small thoracic horns and vice versa (Emlen, 2001).

RESULTS

MALE HEAD HORN ALLOMETRY

All males expressed paired head horns ($N = 71$). Of those one male had to be excluded due to heavily worn horns. Visual inspections indicated that large males developed relatively larger horns than small males, but the difference in relative horn lengths was only moderate (Fig. 2). Gross head horn morphology did not allow the sorting of males into discrete morphs. This

was confirmed by our regression analyses (Fig. 3A). A simple linear regression model explained over 94% of the variation in horn lengths ($y = -19.13 + 2.59x$; $r^2 = 0.943$), whereas a sigmoid regression model generated an only slightly better fit ($y = 1.30 + 9.38x^{14.97}/(9.67^{14.97} + x^{14.97})$; $r^2 = 0.965$). However, even though this increase in fit only accounted for an additional 2% of the variation in horn lengths it was nonetheless significant ($F_{2,67} = 22.89$, $P < 0.05$; Fig. 3A). These results suggest that male head horns in this species scale largely linearly with body size, but that adding two additional parameters to the regression model marginally increases its descriptive power.

MALE THORAX HORN ALLOMETRY

Only 55 of 71 males examined expressed a thoracic horn. Of those one male had to be excluded due to a broken horn. The other 16 males expressed no thoracic horn at all and instead exhibited an indentation in the thorax (Fig. 2). Males transitioned from hornless to fully horned over a very narrow body-size range (~ 10 mm thorax width; Fig. 3B). A non-linear sigmoid regression of horn length on body size resulted in a substantially better fit ($y = -0.15 + 4.74 x^{32.58}/(10.03^{32.58} + x^{32.58})$; $r^2 = 0.932$) compared to a linear regression ($y = 15.97 + 1.83x$; $r^2 = 0.853$; Fig. 3B). The difference in fit between both models was highly significant ($F_{2,66} = 38.01$, $P < 0.001$). Combined, these results suggest that thorax horn length scales with body size in a sigmoid, threshold-dependent fashion, and that a non-linear sigmoid regression is the more appropriate descriptor of the scaling relationship between thorax horn length and body size in this species.

FEMALE HEAD HORN ALLOMETRY

All females developed a pair of relatively small head horns ($N = 110$). In contrast to male head horns both regression types generated nearly identical fit when applied to the scaling relationship between head horns and body size in female *O. watanabei* (linear regression: $y = -3.61 + 0.67x$; $r^2 = 0.772$; non-linear regression: $y = 1.86 + 2.33x^{12.82}/(9.88^{12.82} + x^{12.82})$; $r^2 = 0.775$; $N = 110$; $F_{2,106} = 0.70$, $P > 0.1$). Female head horn length, however, increased more slowly with increasing body size than did head horn length in males (Fig. 4).

RESOURCE ALLOCATION TRADE-OFF BETWEEN MALE HORNS

A resource allocation tradeoff during the development of head and thoracic horns should be manifest in a negative correlation between horn length residuals;

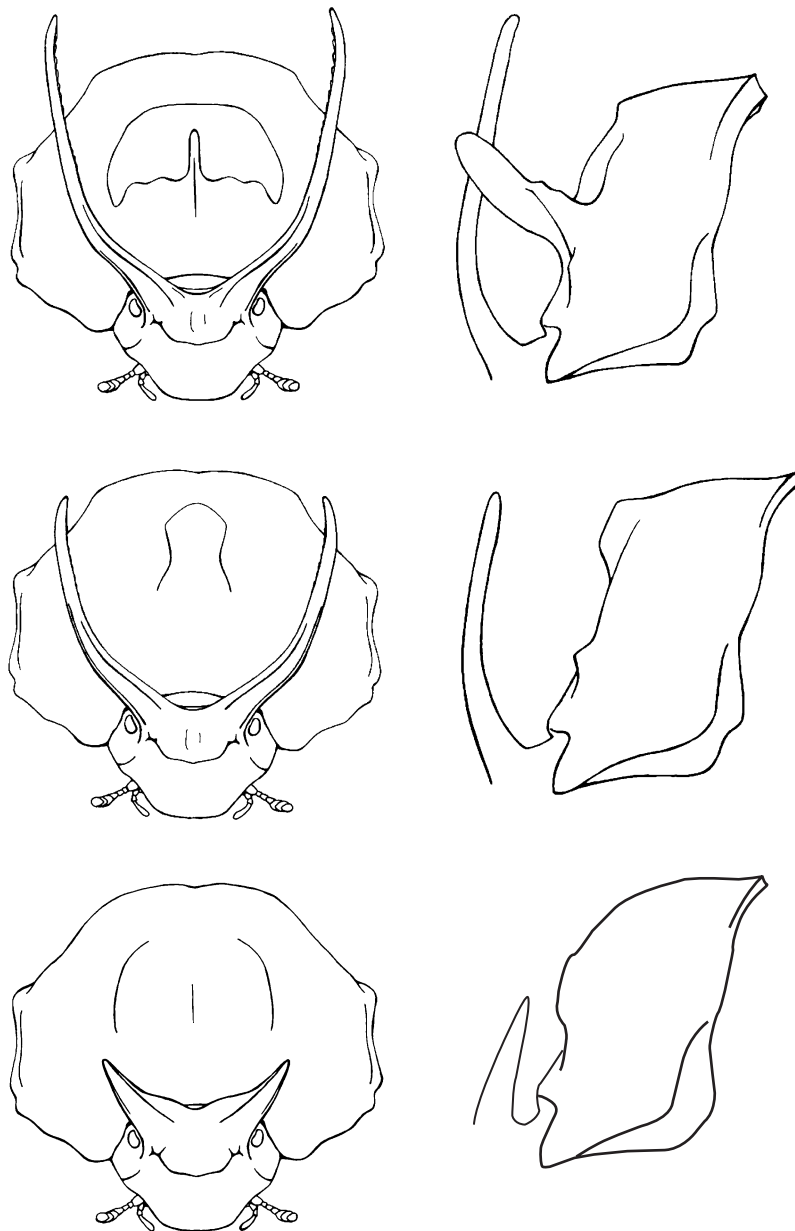


Figure 2. Head and thorax morphologies and horn sizes in *Onthophagus watanabei*. Top to bottom: large male, small male, large female. Left: frontal views; right: side views. For ease of comparison all drawings are adjusted to similar sizes. Note the relative sizes of head horns and the presence and absence of a central thorax horn in large and small males. Drawings by Beth Archie.

that is males that express relatively large head horns given their body size should grow relatively smaller thoracic horns and vice versa. Contrary to this expectation we found a moderate but significant positive correlation between head and thoracic horn length residuals ($N = 69$, $P = 0.028$). Removal of a single outlier substantially increased the significance of this correlation to $P = 0.0009$ (Fig. 5). The correlation persisted and remained significant if the reduced data

set was used for analysis ($N = 52$, $P = 0.0125$). Our results therefore do not support the hypothesis of a developmental tradeoff between horn types in this species.

DISCUSSION

As with numerous species in the genus *Onthophagus*, male *O. watanabei* develop a pair of long curved horns

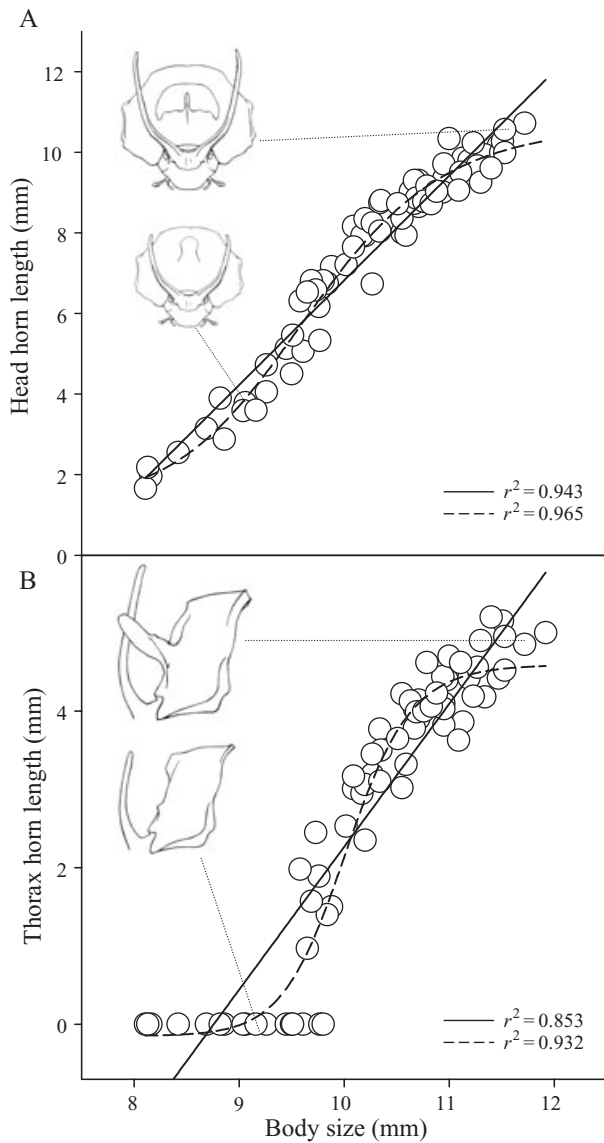


Figure 3. Scaling relationship between body size and A, head horn length and B, thorax horn length in male *Onthophagus watanabei*. Solid line: linear regression; dashed line: non-linear sigmoid regression. See text for further details. Inserts: typical morphologies of small and large male *O. watanabei*. Drawings by Beth Archie.

on their head. Unlike most species studied so far, however, head horn length in *O. watanabei* scales in a largely linear fashion with body size (Fig. 3A), rather than in a sigmoid fashion (Fig. 1, for other examples see also Emlen, 1996, 2000; Moczek *et al.*, 2002). The thoracic horn developed by some male *O. watanabei*, on the other hand, develops in a strongly body size threshold-dependent fashion, and clearly separates males into two alternative morphologies (Fig. 3B). Combined, these findings suggest that horns expressed by different body parts can exhibit consider-

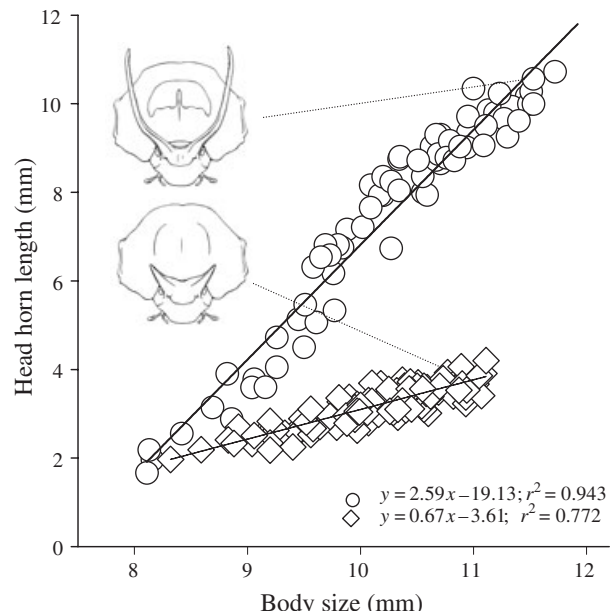


Figure 4. Scaling relationship between body size and head horn length in male (○) and female (◇) *Onthophagus watanabei*. Solid lines indicate best fit linear regressions. Inserts: typical morphologies of large male and female *O. watanabei*. Drawings by Beth Archie.

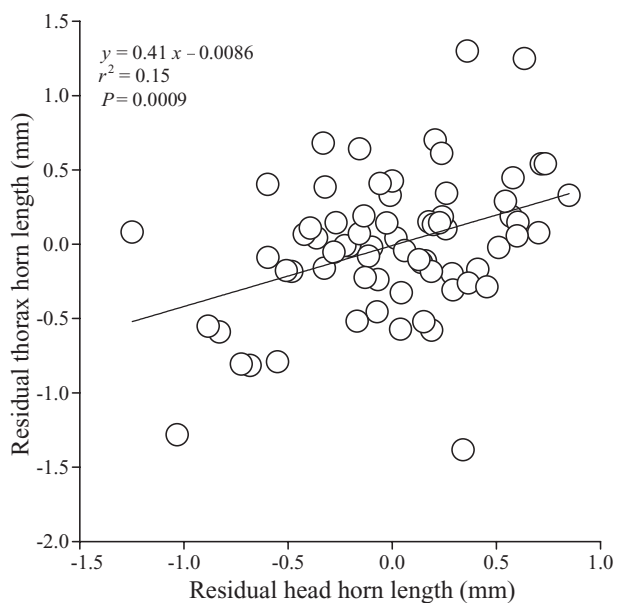


Figure 5. Positive correlation between horn length residuals of head horns (x-axis) and thoracic horns (y-axis) in male *Onthophagus watanabei* ($P = 0.0275$; after removal of a single outlier $P = 0.0009$, $N = 68$) Resource allocation tradeoffs predict a negative correlation.

able differences in scaling relationships even though these horns are produced by the same individual.

Several studies have shown that body size and horn length in *Onthophagus* beetles are largely determined by larval feeding conditions. Typically, larvae exposed to suboptimal feeding conditions eclose to small adult sizes and develop no or greatly reduced horns (Lee & Peng, 1982; Hunt & Simmons, 1997; Moczek, 1998; Moczek & Emlen, 1999). Horns develop from imaginal disk-like tissues in the epidermis of the head or thorax, which in large individuals undergo explosive growth during the prepupal stage of late larval development (Emlen & Nijhout, 1999). As such, horns develop in a manner similar to regular appendages in holometabolous insects. The sigmoid allometries so common in the genus *Onthophagus* are at least in part mediated by juvenile hormone (JH) titres, which are thought to correlate with the nutritional state of the animal (Emlen & Nijhout, 1999). Recent studies suggest that JH titres above a certain critical concentration during late larval development induce cell proliferation of epidermal cells that will eventually give rise to horns, whereas below threshold concentrations cause the same cells to remain dormant and to give rise to a hornless individual (Moczek & Nijhout, 2002). Epidermal cells only respond to JH during a very brief, sensitive period which, combined with the late, explosive growth of the horn tissue following activation, allows such species to generate the highly non-linear, S-shaped allometries so common in this genus (Emlen & Nijhout, 1999; Moczek & Nijhout, 2002).

Surprisingly little is known about the mechanisms necessary to allow imaginal disk growth to be proportional to somatic growth and to generate a linear scaling relationship (Nijhout & Grunert, 2002). In fact, until recently it remained a complete mystery how imaginal disk growth is regulated such that an individual develops wings, legs and mouthparts appropriate for its own body size. Several recent studies have now begun to shed some light on this issue. For instance, in the buckeye butterfly *Precis coenia*, as for butterflies in general, wing disks start to grow relatively earlier in larval development than do horns in *Onthophagus* beetles. During this time *P. coenia* wing disk growth requires an optimal concentration of ecdysone plus the insulin-like growth factor bombyxin, with the concentration of the latter fluctuating with the nutritional state of the larva (Nijhout & Grunert, 2002). Poor nutritional conditions cause wing disks to reduce or even halt growth within hours, which can be reversed by improving feeding conditions (Miner, Rosenberg, & Nijhout, 2000). Insulin-like peptides and other growth factors have also recently been implicated in the control of somatic and imaginal disk growth in *Drosophila melanogaster* (Kawamura *et al.*, 1999; Oldham *et al.*, 2000; Brogiolo *et al.*, 2001). Pro-

portional somatic and imaginal disk growth therefore appears to rely on a tight linkage between nutritional status and growth factor titres, which in turn exert control over disk growth over extended periods of time. Our results suggest that even though the mechanisms involved in generating linear and sigmoid scaling relationships may thus be quite different, they are not mutually exclusive and may co-occur in the same animal to differentially regulate the development of horns in different body regions. Interestingly, fitting a sigmoid regression model to the scaling relationship between body size and male head horns in *O. watanabei* resulted in a slight yet nonetheless significant improvement in fit compared to the linear model. Combined with the subtle sigmoid appearance of this allometry (Fig. 3A) this raises the possibility that largely linear and strongly sigmoid allometries need not necessarily require two fundamentally different developmental mechanisms, but may in fact represent quantitatively different outcomes of the same developmental mechanism operating with different growth parameters. If this is correct an important implication of this would be that evolutionary changes in scaling relationships from sigmoid to linear and vice versa may only require relatively simple changes in growth parameters rather than fundamental changes in the underlying developmental machinery.

Recent studies on several insects have implicated resource allocation tradeoffs during larval development as possibly important mechanisms for shaping patterns of morphological variation (Nijhout & Emlen, 1998). Allocation tradeoffs during development may arise when two or more structures compete for a shared and limited pool of resources necessary to sustain their growth, such as a hormone or growth factor (Kawamura *et al.*, 1999; Gibson & Schubiger, 2000; Oldham *et al.*, 2000; Brogiolo *et al.*, 2001; Nijhout & Grunert, 2002). Such limiting resources may thus constrain the absolute sizes to which a structure can grow, and the presence or absence of a growing structure may therefore influence the size to which other structures are able to develop (Nijhout & Wheeler, 1996). Phenotypic correlations obtained from a variety of insect species support the existence of developmental tradeoffs, in particular between structures that grow in close proximity to one another, or grow during the same time (Kawano, 1995, 1997; Klingenberg & Nijhout, 1998; Nijhout & Emlen, 1998; Moczek & Nijhout, 2004). Here we did not find evidence supporting a possible tradeoff in the development of head and thoracic horns in *O. watanabei*. Instead, we observed a significant positive correlation between head and thorax horn length residuals; that is males that expressed relatively long head horns given their body size also expressed a relatively long thorax horn and vice versa.

The absence of a tradeoff may be expected as both horn types develop in very different regions of the animal, i.e. head and thorax. Earlier studies have argued that tradeoff intensity should decay with distance between growing structures (Emlen, 2001). A recent experimental study on *Onthophagus taurus* (Schreber), however, suggested that even distant structures such as head horns and copulatory organs can engage in a developmental tradeoff provided their growth periods overlap (Moczek & Nijhout, 2004), which raises the possibility that timing of growth, rather than exact location, might determine if and how severely two structures will affect each other's development. We do not know exactly when head vs. thoracic horn growth is initiated in *O. watanabei*, but related species exhibit differences in the timing of horn expression depending on the region in which horns develop. Large *Onthophagus nigriventris* d'Orbigny initiate the growth of their single thoracic horn at least 24 h earlier than *O. taurus* initiate the growth of their paired head horns (A. P. Moczek & L. Nagy, unpubl. data). It is conceivable that such differences in timing of growth are also present in *O. watanabei* and allow this species to avoid a possibly constraining tradeoff in the expression of different horn types. These hypotheses, however, only help to explain the absence of a negative correlation between relative head and thorax horn development. A positive correlation between both horn types, as detected here, suggests that (a) some aspect of the regulation of horn growth is shared between horn types and affects different horns in the same fashion, and (b) that males differ from each other with respect to this regulatory mechanism. One possible candidate mechanism could be the regulation of the exact onset of horn growth. As horns grow explosively over a relatively short period of time, even minor changes in the length of the growth period could produce measurable differences in horn length (Nijhout & Wheeler, 1996). If such differences in the exact onset of horn growth differ between individuals but are shared between horns within an individual this could produce the kind of positive correlation detected in the present study. Clearly, much more work remains to be done to elucidate the mechanisms of growth regulation and resource allocation during development to understand when and by what means growing structures might affect each other's development.

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