

# Pupal Remodeling and the Development and Evolution of Sexual Dimorphism in Horned Beetles

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**ABSTRACT:** Horns or hornlike structures in beetles have become an increasingly popular study system for exploring the evolution and development of secondary sexual trait diversity and sexual dimorphisms. The horns of adult beetles originate during a rapid growth phase during the prepupal stage of larval development, and differential activation of growth during this time is either implicitly or explicitly assumed to be the sole mechanism underlying intra- and interspecific differences in adult horn expression. Here I show that this assumption is not based on developmental reality. Instead, after their initial prepupal growth phase, beetle horns are extensively remodeled during the subsequent pupal stage via sex- and size-dependent resorption of horn tissue. I show that adult sexual dimorphism in four *Onthophagus* species is shaped partly or entirely by such pupal remodeling rather than by differential growth. Specifically, I show that after a sexually monomorphic growth phase, differential pupal horn resorption can generate both regular and reversed sexual dimorphism. Furthermore, I show that in cases in which initial growth is already dimorphic, pupal horn resorption can both magnify and reverse initial dimorphism resulting from differential growth. Finally, I show that complete resorption of pupal horns in both sexes can remove any trace of horn expression from all resulting adults. In such species, examination of adults only would result in the false conclusion that this species lacks the ability to develop a horn. Instead, such species appear to differ from those with sexually dimorphic adults merely in that they activate pupal horn resorption in both sexes rather than in just one. Combined, these results suggest that pupal remodeling of secondary trait expression is taxonomically widespread, at least among *Onthophagus* species, and is developmentally extensive and remarkably evolutionarily labile. These results have immediate implications for reconstructing the evolutionary history of horned beetles and the role of developmental processes in guiding evolutionary trajectories. I use

these results to revise current understanding of the evolutionary developmental biology of secondary sexual traits in horned beetles in particular and holometabolous insects in general. The results presented here seriously call into question whether descriptions of adult diversity patterns alone suffice for meaningful inferences toward understanding the developmental and evolutionary origin of these patterns. These results illustrate that a lasting integration of development into an evolutionary framework must integrate development as a process rather than define it solely by some of its products.

**Keywords:** allometry, evolvability, lability, *Onthophagus*, pupal remodeling, programmed cell death.

Sexual dimorphism is among the most striking and phylogenetically widespread patterns of phenotypic variation in nature, and the evolutionary origin and consequences of secondary sexual traits and sexual dimorphisms are classic themes in evolutionary biology (Andersson 1994; Shuster and Wade 2003). The evolutionary forces that shape sexual dimorphisms have been studied extensively in a wide range of organisms and on a variety of levels of biological organization (for excellent reviews on the subject, see the *American Naturalist* 2005 Vice Presidential Symposium, supplement to vol. 166; in particular, Delph et al. 2005; Fairbairn 2005; Ketterson et al. 2005). More recently, a developmental perspective has been added, aiming to better understand the evolution of sexual dimorphisms through understanding its ontogenetic underpinnings (Emlen 2000; Badyaev 2002; Emlen et al. 2005a, 2005b; Fry 2006).

Integrating ontogeny into an evolutionary understanding of the patterns of phenotypic diversity is important because a growing body of evidence suggests that the nature of developmental mechanisms has great potential to accelerate, limit, or bias phenotypic evolution (e.g., Klingenberg and Zimmermann 1992; Nijhout and Wheeler 1996; Klingenberg and Nijhout 1998; Yang 2001; Hughes and Kaufman 2002; Suzuki and Nijhout 2006). However, doing so is often challenging, since ontogeny is, by definition, a process, whereas phenotypic diversity, such as sexual dimorphism, represents one of many products of this process (Raff 1996; Minelli 2003). Studying the process

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of development is often far more cumbersome than trying to deduce ontogenetic properties on the basis of ontogenetic outputs. Even though the latter is a generally widespread approach (see below), it faces significant limitations. It overlooks the possibility that developmental processes may operate without leaving a signature of their existence in the phenotype and developmental stage under study. It also overlooks the possibility that different ontogenetic processes operating in different individuals may nonetheless give rise to the same phenotype or that the same ontogenetic process in two different individuals may give rise to different phenotypes under different circumstances. These shortcomings notwithstanding, attempts to deduce developmental processes from phenotypic products of development are commonplace in evolutionary biology. For example, comparative studies of scaling relationships between adult body parts continue to be used heavily to explore the ontogenetic basis of allometries and the evolution of shape, while actual attempts to understand the specification, formation, and growth of said parts during ontogeny are rare or absent (e.g., Simmons and Tomkins 1996; Knell et al. 2004; Tomkins et al. 2005a, 2005b). Similarly, adult phenotypes are mapped onto phylogenies to deduce the evolutionary history of a lineage's ability to develop a certain adult phenotype, while the actual developmental basis of this phenotype remains largely unstudied (e.g., swordfish [Meyer et al. 1994; Schluter et al. 1997], stick insects [Whiting et al. 2003], sepsid flies [Eberhard 2001], horned beetles [Emlen et al. 2005a, 2005b]). Here I explore the ontogenetic basis of sexual dimorphism in four closely related species of horn-dimorphic beetles and illustrate how relatively simple developmental studies can be sufficient to advance a deeper understanding of how ontogeny mediates phenotypic evolution and diversification.

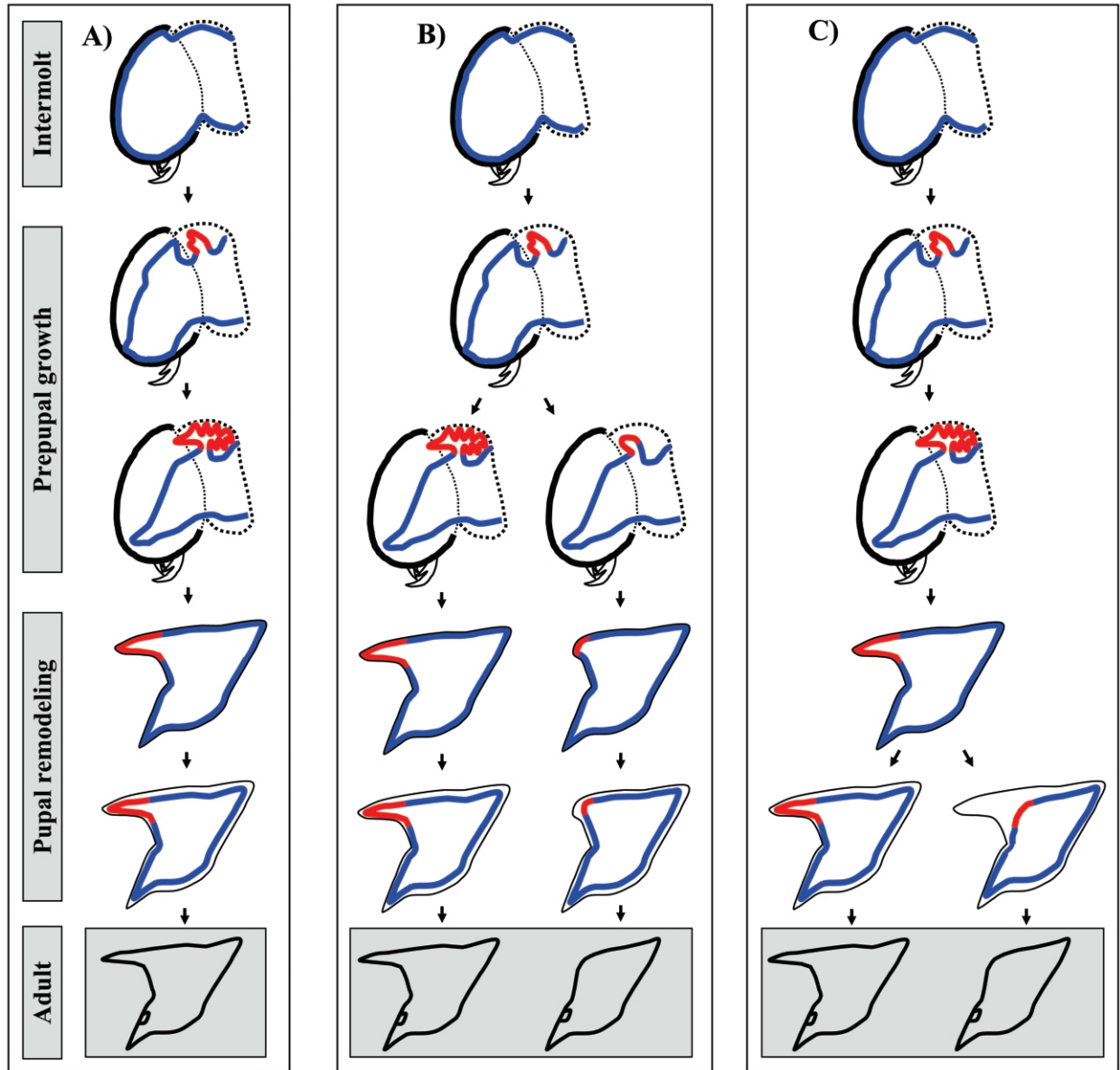
### Diversity of Horned Beetles and Beetle Horns

Horned beetles, and in particular the genus *Onthophagus*, have become a popular study system for exploring how development and evolution interact in the genesis of phenotypic diversity (reviewed in Emlen 2000; Moczek 2005, 2006a, 2006b). Horn expression in *Onthophagus* beetles varies on at least four levels, and environmental and genetic contributions to phenotypic variation differ across these levels. Interspecific genetic differences account for the often dramatic differences in horn shape, number of horns grown, and exact location of horn expression observed between species (Arrow 1951; Balthasar 1963; Matthews 1972). Genetic differences also account for widespread sexual dimorphism within the majority of species (Emlen et al. 2005b). Typically, only males express fully developed horns, while females express no or greatly re-

duced horns (Balthasar 1963). Intraspecific variation is similarly extreme within the male sex, although here, environmental factors are responsible for most of the phenotypic diversity observed. Only males that as larvae had access to optimal feeding conditions eclose above a critical adult size threshold and develop a full set of horns, while male larvae with access to suboptimal feeding conditions eclose to a smaller adult size and remain female-like and largely hornless (Emlen 1994; Hunt and Simmons 1997; Moczek 1998; Moczek and Emlen 1999). Finally, the exact scaling relationship, or allometry, between male horn length and body size is affected subtly by environmental conditions (Emlen 1997; Moczek 2002) but can also diverge genetically between conspecific populations, sometimes generating heritable allometric divergences between conspecific populations that are similar in kind and magnitude to those observed between species (Moczek and Nijhout 2002, 2003; Moczek 2003). This study focuses on the development and evolution of thoracic horns, sexual dimorphisms in thoracic horn expression, and interspecific differences in degree and kind of sexual dimorphism.

### Developmental Basis of Beetle Horns

Beetle horns develop as epidermal outgrowths late in larval development during the prepupal stage (summarized in fig. 1; Emlen and Nijhout 2001; Moczek and Nagy 2005; Moczek 2006b). At the onset of the prepupal stage, the larval epidermis, which has so far tightly lined the larval cuticle, detaches or apolyses and generates a space between itself and the cuticle (Moczek and Nagy 2005). Selected epidermal regions then undergo rapid cell proliferation, and the resulting tissue becomes compacted and often folded underneath the larval cuticle. The old cuticle is then shed during ecdysis via internal hemolymph pressure, which is also used to inflate folded horn primordia into their final, fully expanded, state, followed by hardening of the pupal cuticle over the next several hours. Prepupal horn growth is a highly dynamic process, and in some species, 48 h is sufficient for the transition from initial prepupal apolysis to pupal ecdysis (Moczek and Nagy 2005). As remarkable as this process may seem, however, the development of beetle horns is in fact similar to that of traditional appendages, such as legs, mouthparts, or wings, in most holometabolous insects (Fristrom and Fristrom 1993; Nagy and Williams 2001; Moczek and Nagy 2005), with the only exception occurring in the higher flies, the wings of butterflies, and some beetles (reviewed in Kojima 2004; Angelini and Kaufman 2005). Here, adult appendage primordia are specified during embryonic development as epidermal invaginations or imaginal disks. Basic developmental patterning also takes place during the late embryo and early larva stages, and the majority of



**Figure 1:** Development of (A) thoracic horns and (B, C) thoracic horn dimorphisms in *Onthophagus* beetles. A, Between molts (intermolt), the larval epidermis (blue) fully lines the larval cuticle (black). At the onset of the prepupal growth phase, the larval epidermis apolyses from the cuticle, and selected regions (red) undergo rapid cell proliferation. The resulting extra tissue folds up underneath the larval cuticle. The epidermis subsequently secretes the future pupal cuticle, which upon the molt to the pupal stage forms the outermost layer of the pupa, lined once again by a layer of epidermal cells underneath. During this pupal molt, horn primordia are able to expand and unfold and are now visible externally. During the pupal remodeling stage, epidermal cells apolyse once more. This time, however, no significant growth of horn tissue follows apolysis. Instead, the epidermis undergoes more or less pronounced retraction and sculpting before secreting one last cuticle. Finally, the pupa molts to the final adult stage. B, Development of sexual horn dimorphisms through differential proliferation of prepupal horn tissue during the prepupal growth phase. During the prepupal stage, presumptive horn tissue proliferates more strongly in some individuals than in others, resulting in a sexual dimorphism among pupae and the adults eclosing from these pupae. C, Development of sexual horn dimorphisms through differential resorption of pupal horn tissue during the pupal remodeling phase. Here, differential proliferation of prepupal horn tissue is not necessary, and pupae may be sexually monomorphic. During the pupal remodeling phase, however, pupal horn epidermis is then resorbed more strongly in some individuals than in others, enabling sexually monomorphic pupae to eclose to sexually dimorphic adults (shaded box). This study aims to quantify the relative contributions of differential prepupal growth (B) and differential pupal resorption (C) to sexual dimorphism among adults of four *Onthophagus* species.

growth occurs while the disk is invaginated. Beetle horns therefore do not develop from imaginal disks but instead are the product of epidermal outbuddings lacking early embryonic patterning and an invaginated growth phase (Moczek and Nagy 2005). A series of recent reviews explores the significance of such differences for the evolutionary diversification of patterning mechanisms and appendage types (e.g., Prpic et al. 2003; Jockusch et al. 2004; Kojima 2004; Angelini and Kaufman 2005).

With the exception of imaginal-disk-derived appendages, however, beetle horns develop in a manner similar to that of traditional appendages such as legs and mouthparts (Kojima 2004), and differences in degree of adult horn expression between or within species have been taken implicitly or explicitly as evidence of differential growth of these structures (e.g., Cook 1987, 1990; Nijhout and Emlen 1998; Stern and Emlen 1999; Emlen 2000, 2001; Emlen and Nijhout 2000; Emlen and Allen 2004; Knell et al. 2004; Emlen et al. 2005a, 2005b; Tomkins et al. 2005a, 2005b). Recent studies have begun to contradict this notion and have suggested that explosive growth in the prepupa may at least in some species be followed by extensive remodeling of morphology during the pupal stage, including in some cases the complete loss of horns and the metamorphosis of a fully horned pupa into an entirely hornless adult (fig. 1C; Moczek 2005, 2006a, 2006b; Moczek and Nagy 2005; Moczek et al. 2006a, 2006b). Here I investigate pupal remodeling of horn expression in four sexually dimorphic congeneric species of horned beetles and quantify the contribution of pupal remodeling relative to that of differential prepupal growth in the development and evolution of intra- and interspecific diversity.

## Material and Methods

### *Species Choice and Husbandry*

The relative contribution of pupal remodeling in the development and evolution of sexual dimorphisms was studied by quantifying ontogenetic changes in the allometric scaling between body size and thoracic horn length from the pupal to the adult stage and the degree to which ontogenetic changes in allometries differed between sexes, both within and between species. In particular, I examined the ontogeny of thoracic horn allometries in four *Onthophagus* species. *Onthophagus nigriventris* and *Onthophagus binodis* exhibit a typical sexual dimorphism for thoracic horns, with horns present in males but only rudimentary in females. *Onthophagus sagittarius* exhibits a rare reversed sexual dimorphism, with females expressing a large thoracic horn, while their male counterparts are hornless. Finally, *Onthophagus taurus* lacks thoracic horns in both sexes; however, earlier studies indicated that both

sexes develop transient thoracic horns during the pupal stage that resemble those of species that maintain their pupal horns into adulthood, and this species was therefore included in this study (Moczek and Nagy 2005). In addition to their possible transient thoracic horns, *O. taurus* males also express two head horns in a conspicuous polyphenic manner: only males above a critical size express these horns, while smaller males remain female-like and hornless. The role of pupal remodeling in the expression of head horns, and in particular in the development of alternative male phenotypes, is examined in detail elsewhere (A. P. Moczek, unpublished data).

Field populations of all four species were used to establish laboratory colonies: *O. taurus* was collected from pastures around Bloomington, Indiana, *O. nigriventris* and *O. binodis* were collected from pastures near Waimea, Hawaii, and *O. sagittarius* was collected from pastures near Sunset, Hawaii. All species were maintained and reared as described by Moczek and Nijhout (2003) except for rearing temperature; *O. nigriventris*, *O. taurus*, and *O. binodis* were maintained at 23°C, whereas *O. sagittarius* was maintained at 27°C. Early third-instar larvae of each species were transferred from their natural brood ball into six-well plates to monitor larval development as described by Shafiei et al. (2001). First- to second-day pupae were measured, weighed, and then returned to their artificial brood ball until adult eclosion. At this stage, the pupal epidermis still fully lined the pupal cuticle (A. P. Moczek, unpublished data), and pupal size measurements are therefore a direct reflection of prior prepupal growth. Pupal remodeling occurs primarily in the second half of the pupal stage. However, repeated measurements of the same pupae throughout the pupal stage indicate that pupal size measurements actually do not change until just before eclosion (A. P. Moczek, unpublished data), primarily because remodeling of the pupal epidermis occurs underneath, and without altering, the shape of the pupal cuticle (fig. 1). Eclosing adults were retained in brood balls for an additional 3–4 days to allow full hardening of the adult cuticle and were then weighed, killed, stored in ethanol, and remeasured as described below.

### *Morphometric Measurements*

Pupae and adults of males and females of all four species were measured using a two-dimensional image analysis system including a digital video camera (Scion) mounted onto a dissecting scope (Leica MZ 16) and interfaced with a computer using Image J software. Pupal and adult thorax width were used as an estimate for body size (for justification, see Emlen 1994; Moczek and Emlen 1999). Pupal and thoracic horn length were measured as the linear distance from the anteriormost point of the prothorax, or

“tip” of the horn, to the posteriormost point of the prothorax bordering anteriorly on the scutellum of the second thoracic segment. Measuring thoracic horns in this fashion had the key advantage that homologous landmarks could be recognized unambiguously in both pupae and adults. A slightly modified measurement technique was used for horn length measurements in *O. nigriventris*. In this species, large males express some of the largest thoracic horns of the genus. This horn is drastically downcurved in the pupa, while in the adult it is curved upward. Therefore, in order to account for the drastic change in horn curvature, pupal and adult horn lengths were measured by following the outermost outline of the outgrowth. All measurements were taken to the nearest 0.01 mm.

### Statistical Analyses

All analyses were conducted using S-Plus 2000 (MathSoft). For each species, I first examined allometric scaling relationships between body size and horn length within developmental stages to determine whether pupae or adults exhibit significant sexual dimorphism, using a fixed-factor ANOVA model with horn length as the response variable and body size and sex as main effects. Sexual dimorphism in this scaling relationship would be evident as a significant effect of sex (equivalent to significant differences in  $y$ -intercepts of male and female allometries), a significant interaction between sex and body size (equivalent to significant differences in slopes), or both. This analysis was repeated for each stage and species. I used sequential Bonferroni procedures to correct for multiple comparisons within and between species where this was necessary (Sachs 1992; Sokal and Rohlf 1995). I then examined whether the relationship between male and female allometries changed significantly across developmental stages. To quantify the significance of such changes, I first combined the scaling relationship between body size and horn length of each individual into a single measure by computing the ratio of body size to horn length for each individual as a pupa and again as an adult. I then calculated the difference in ratios (adult ratio – pupal ratio) for each individual to obtain an individual measure of allometric change across stages. This was calculated separately for each sex, and sexes were compared using two-tailed  $t$ -tests. Significant differences would be expected if pupal remodeling altered body size–horn length allometries differently in different sexes. The same analysis was conducted separately for each species. To further characterize differential resorption of horn tissue during pupal development, I quantified relative resorption of pupal horn length (calculated as the percentage of pupal horn length not retained in the adult) and absolute resorption of pupal horn length (calculated as the absolute difference between pupal and

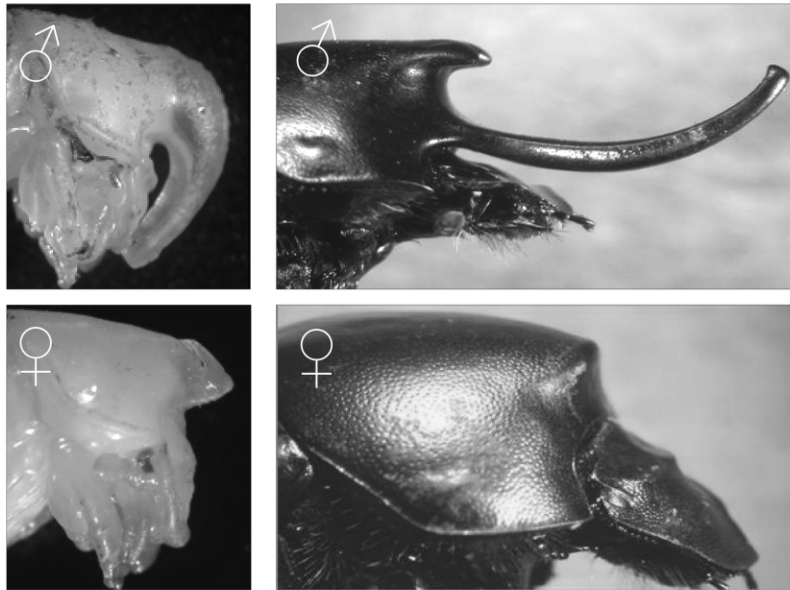
adult thoracic horn length) for each individual as a function of body size. Males and females within each species were then contrasted using a fixed-factor ANOVA model with horn resorption as the response variable and body size and sex as main effects. Sex-specific differential horn resorption would be evident in significant effects of sex, significant interactions between sex and body size, or both.

### Results

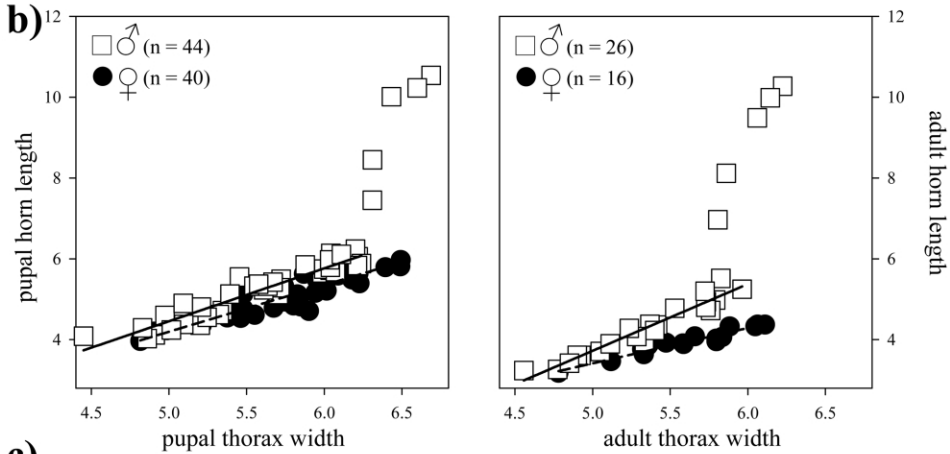
All four species examined exhibited highly significant sex-specific pupal remodeling of body size–horn length allometries, but which sex was affected, and in what manner, differed among all four species. *Onthophagus nigriventris* exhibited the mildest case of pupal remodeling of scaling relationships (fig. 2). In this species, sex-specific prepupal growth resulted in sexually dimorphic pupae; an ANOVA revealed a significant effect of sex ( $F = 15.82$ ,  $df = 1, 80$ ,  $P < .0002$ ) as well as a significant interaction effect between sex and size ( $F = 12.80$ ,  $df = 1, 80$ ,  $P < .001$ ; fig. 2a, 2b) on horn length. This sexual dimorphism was extreme only for the largest males and females and was modest, but still significant, among smaller individuals (fig. 2b). However, sexual dimorphism further increased significantly across developmental stages ( $t = 5.84$ ,  $df = 40$ ,  $P < .0001$ ) as a consequence of differential resorption of horn tissue in female pupae. Both relative resorption of pupal horn length (calculated as the percentage of pupal horn length not retained in the adult) and absolute resorption of pupal horn length (calculated as the absolute difference between pupal and adult thoracic horn length) were far greater in female *O. nigriventris* than in their male counterparts, with highly significant effects of sex as well as interaction effects between sex and size in both analyses (fig. 2c; relative resorption:  $F_{\text{sex}} = 70.66$ ,  $df = 1, 38$ ,  $P < .0001$ ;  $F_{\text{sex} \times \text{size}} = 16.6$ ,  $df = 1, 38$ ,  $P < .001$ ; absolute resorption:  $F_{\text{sex}} = 53.45$ ,  $df = 1, 38$ ,  $P < .0001$ ;  $F_{\text{sex} \times \text{size}} = 14.09$ ,  $df = 1, 38$ ,  $P = .0006$ ). Interestingly, relative horn resorption remained constant across female body sizes (as a consequence, absolute horn loss increased linearly with female size), but both relative and absolute horn resorption decreased dramatically in males (fig. 2c). This caused male *O. nigriventris* to resorb disproportionately less of their pupal thoracic horn the bigger their own body size and, thus, horn length, while in females, pupal horn resorption was roughly proportional to female body size and horn growth. In *O. nigriventris*, adult sexual dimorphism was therefore the product of two discrete developmental processes; differential prepupal growth generated sexually dimorphic pupae, and subsequent greater resorption of pupal horns in females compared to males further magnified this dimorphism into the adult stage.

This was not observed in *Onthophagus binodis*. In this

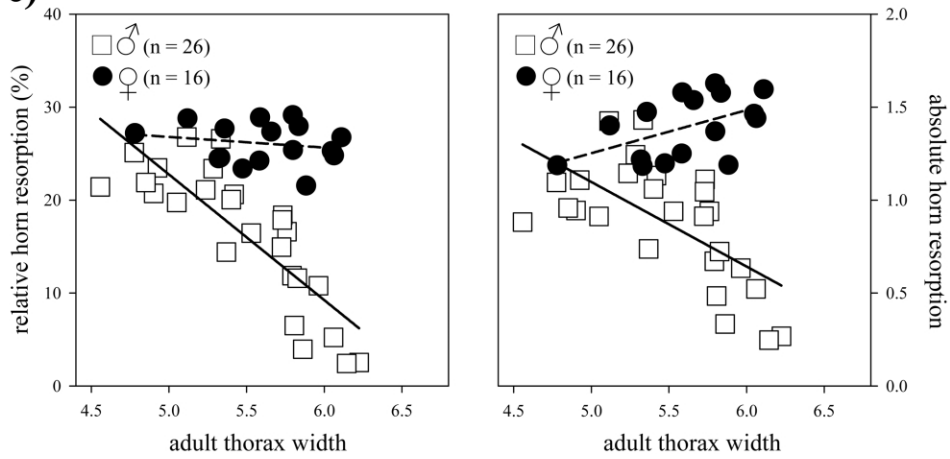
**a)**



**b)**



**c)**



species, pupae were not sexually dimorphic, and male and female pupae could not be distinguished by thoracic horn length (fig. 3a). Instead, pupal allometries were perfectly overlapping ( $F_{\text{sex}} = 0.70$ ;  $df = 1, 99$ ,  $P = .41$ ;  $F_{\text{sex} \times \text{size}} = 0.139$ ,  $df = 1, 99$ ,  $P = .71$ ). However, allometries changed highly significantly across developmental stages ( $t = 5.48$ ,  $df = 75$ ,  $P < .0001$ ), resulting in highly significant sexual dimorphism among adults (fig. 3b;  $F_{\text{sex}} = 91.88$ ,  $df = 1, 73$ ,  $P < .0001$ ;  $F_{\text{sex} \times \text{size}} = 25.83$ ,  $df = 1, 71$ ,  $P = .0001$ ) as a consequence of differential resorption of horn tissue in female pupae but not in males. As with *O. nigriventris* above, both relative and absolute resorption of pupal horn length were far greater in female *O. binodis* than in their male counterparts (fig. 3c; relative resorption:  $F_{\text{sex}} = 452.78$ ,  $df = 1, 65$ ,  $P < .0001$ ;  $F_{\text{sex} \times \text{size}} = 4.61$ ,  $df = 1, 65$ ,  $P = .036$ ; absolute resorption:  $F_{\text{sex}} = 43.84$ ,  $df = 1, 65$ ,  $P < .0001$ ;  $F_{\text{sex} \times \text{size}} = 0.775$ ,  $df = 1, 65$ ,  $P = \text{not significant}$ ). Surprisingly, relative horn resorption in females not only exceeded that in males but, in contrast to female *O. nigriventris*, also increased with female body size in this species, while it decreased dramatically with male body size. This caused females, but not males, to lose a disproportionate amount of their thoracic horn as their own body size increased. In *O. binodis*, monomorphic prepupal horn growth thus gave rise to sexually monomorphic pupae, which, followed by massive resorption of pupal horns, especially in large females, but retention of horns in all males, then generated the sexual dimorphism seen in the adult stage.

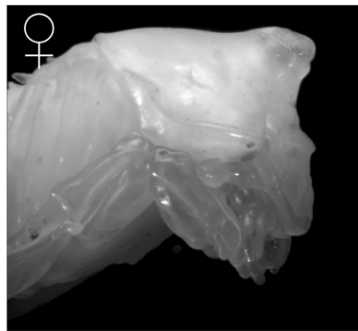
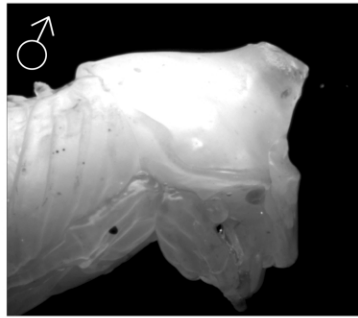
The third species, *Onthophagus sagittarius*, exhibited yet another behavior. In this species, pupae were already mildly sexually dimorphic, with males expressing slightly but significantly larger thoracic horns than females over the entire range of body sizes (fig. 4a, 4b;  $F_{\text{sex}} = 41.13$ ,  $df = 1, 81$ ,  $P < .0001$ ;  $F_{\text{sex} \times \text{size}} = 0.126$ ,  $df = 1, 81$ ,  $P = .27$ ). In adults, this initial sexual dimorphism then became reversed and far more extreme because of differential resorption of horn tissue in male pupae but retention of horns in females (fig. 3b;  $t = 23.58$ ,  $df = 61$ ,  $P < .0001$ ). This time, both relative and absolute resorption of pupal horn resorption in males far exceeded that of females (fig.

4c; relative resorption:  $F_{\text{sex}} = 1,081.91$ ,  $df = 1, 59$ ,  $P < .0001$ ;  $F_{\text{sex} \times \text{size}} = 35.48$ ,  $df = 1, 59$ ,  $P < .0001$ ; absolute resorption:  $F_{\text{sex}} = 957.29$ ,  $df = 1, 59$ ,  $P < .0001$ ;  $F_{\text{sex} \times \text{size}} = 60.41$ ,  $df = 1, 59$ ,  $P < .0001$ ). Relative horn resorption increased substantially with male body size, while it decreased slightly across female body sizes, causing males, but not females, to lose a disproportionately larger amount of their thoracic horn as their own body size increased. In this species, minor sexually dimorphic prepupal horn growth gave rise to moderately sexually dimorphic pupae; massive resorption of pupal horns in males but retention of horns in females then radically reversed and magnified sexual dimorphism and generated the reversed sexual dimorphism observed among adult *O. sagittarius*.

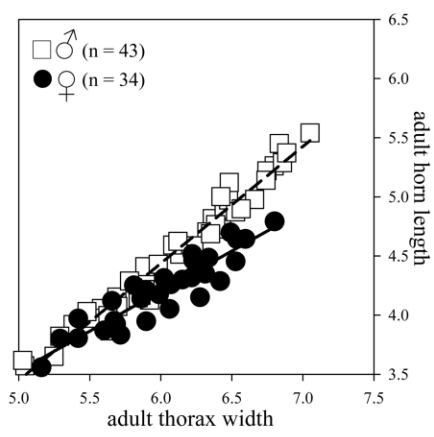
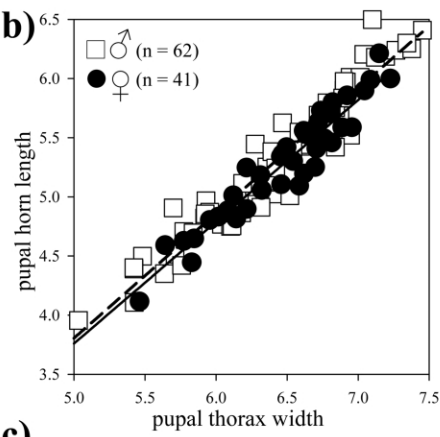
Patterns of horn resorption were once again different in the fourth species, *Onthophagus taurus*. In this species, differential prepupal growth gave rise to highly sexually dimorphic pupae, with male pupae expressing far larger thoracic horns than their female counterparts (fig. 5a, 5b;  $F_{\text{sex}} = 78.74$ ,  $df = 1, 110$ ,  $P < .0001$ ;  $F_{\text{sex} \times \text{size}} = 41.22$ ,  $df = 1, 110$ ,  $P < .0001$ ). As in other species, allometric differences between sexes changed highly significantly, this time resulting in adults that largely lacked a sexual dimorphism ( $t = 11.86$ ,  $df = 86$ ,  $P < .0001$ ). While male and female pupae had expressed large thoracic horns, male and female adults both lacked thoracic horns and differed merely in the degree of rounding of the prothorax, with the female prothorax dropping off more steeply than the male prothorax ( $F_{\text{sex}} = 335.52$ ,  $df = 1, 82$ ,  $P < .0001$ ;  $F_{\text{sex} \times \text{size}} = 35.99$ ,  $df = 1, 82$ ,  $P < .0001$ ). Both relative and absolute resorption of pupal horns were massive in both sexes, though significantly higher in males (fig. 5c; relative resorption:  $F_{\text{sex}} = 478.50$ ,  $df = 1, 82$ ,  $P < .0001$ ;  $F_{\text{sex} \times \text{size}} = 49.97$ ,  $df = 1, 82$ ,  $P < .0001$ ; absolute resorption:  $F_{\text{sex}} = 389.93$ ,  $df = 1, 82$ ,  $P < .0001$ ;  $F_{\text{sex} \times \text{size}} = 65.51$ ,  $df = 1, 82$ ,  $P < .0001$ ). Females resorbed approximately 25% of their prothorax length during the pupal stage regardless of their own body size, whereas relative horn resorption in males increased sharply with male size, reaching over 50% of prothorax length in the largest males, which represented the highest degree of pupal horn re-

**Figure 2:** Ontogenetic changes in allometric scaling between body size and thoracic horn length in male (squares) and female (circles) *Onthophagus nigriventris*. a, Photographs of male (top) and female (bottom) pupae (left) and the adults (right) that eclosed from them. b, Scaling relationship between body size and horn length in male and female pupae (left) and corresponding adults (right). The scaling relationship in both sexes was linear except in the very largest males, which grew disproportionately larger horns. Pupal *O. nigriventris* were already sexually dimorphic, which remained true even if the largest males and corresponding female were excluded from the analysis (as indicated by regression lines). However, sexual dimorphism increased significantly in adults as a consequence of differential resorption of horn tissue in female pupae illustrated in c. c, Relative resorption of pupal horn length (left; calculated as percentage of pupal horn length not retained in the adult) and absolute loss of pupal horn length (right; calculated as the absolute difference between pupal and adult thoracic horn length) as a function of adult body size in males and females. Horn resorption in female *O. nigriventris* far exceeded that of males. Interestingly, relative horn resorption remained constant across female body sizes but decreased dramatically in males. In this species, differential prepupal growth thus generated sexually dimorphic pupae, and greater resorption of pupal horns in females compared to males further magnified this dimorphism into the adult stage.

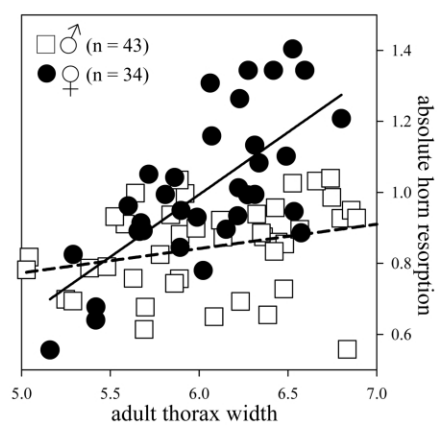
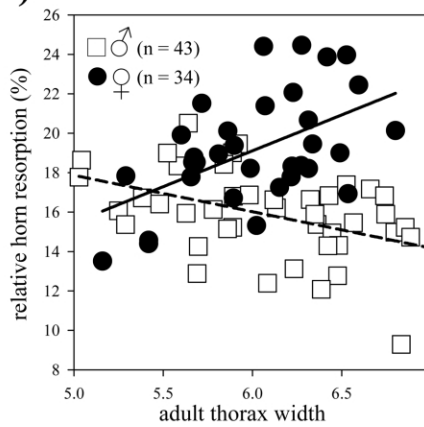
**a)**



**b)**



**c)**





sorption observed in any species. Combined, this caused males to resorb a disproportionately larger fraction of their prothorax the larger their own body size and molded the even slope of the final adult prothorax. Meanwhile, females resorbed a fraction of their pupal prothorax roughly proportional to their body size and retained a slight prothoracic drop-off. In *O. taurus*, highly sexually dimorphic prepupal horn growth therefore gave rise to a major sexual dimorphism among pupae. Massive resorption of pupal horns in both sexes then erased this dimorphism, causing horned male and female pupae to molt into hornless adults in both cases, distinguishable merely by slight but significant differences in prothoracic shape.

In contrast to the extreme lability of pupal horn resorption across species, changes in body size across developmental stages, measured as changes in thorax width from pupa to adult, were remarkably constant (fig. 6). In all cases, adult body size was slightly smaller than pupal body size. Average reductions in relative body size across stages were between 5% and 7%. In three of the four species, there was a small but significant effect of size on relative reduction in thorax width, with larger individuals experiencing slightly less reduction in thorax width (*O. nigriventris*:  $F = 4.33$ ,  $df = 1, 39$ ,  $P = .044$  [not significant if corrected for multiple comparisons]; *O. binodis*:  $F = 18.60$ ,  $df = 1, 73$ ,  $P < .01$ ; *O. taurus*:  $F = 16.53$ ,  $df = 1, 77$ ,  $P < .01$ ; *O. sagittarius*:  $F = 3.73$ ,  $df = 1, 59$ ,  $P = .58$ ). There were no significant effects of sex or significant interaction effects between sex and size on changes in body size in any of the four species studied.

### Discussion

Several insect taxa have become promising model systems for exploring the development and evolution of secondary sexual traits and sexual dimorphism (e.g., stalk-eyed flies [Wilkinson et al. 1998; Hurley et al. 2001], *Drosophila* [Kopp et al. 2000; Barmina et al. 2005], dung flies [Simmons and Ward 1991; Kraushaar and Blanckenhorn 2002; Blanckenhorn et al. 2003], mosquitoes [Hahn and Lanzaro 2005], waterstriders [Abouheif and Fairbairn 1997; Fairbairn 2005]). Horned beetles in general, and the highly

species-rich and morphologically diverse horned beetle genus *Onthophagus* in particular, have been especially attractive, as evidenced by at least 56 studies published since 1995 that investigate aspects of the evolutionary, ecological, or developmental underpinnings of secondary sexual trait expression in this genus (reviewed in Emlen 2000; Moczek 2005, 2006a, 2006b). The vast majority of these studies implicitly or explicitly assume that the expression of secondary sexual traits in adult beetles is the consequence of differential activation of growth of these structures during immature development (e.g., Cook 1987; Nijhout and Emlen 1998; Stern and Emlen 1999; Emlen 2000, 2001; Emlen and Allen 2004; Knell et al. 2004; Hartfelder and Emlen 2005; Tomkins et al. 2005a, 2005b). Magnitude and nature of differential expression among adults were then used to explore topics such as the costs and limits of secondary sexual trait expression (e.g., Hunt and Simmons 1997; Knell et al. 2004), developmental and evolutionary trade-offs between growing horns and growing other body parts (e.g., Emlen 2001; Tomkins et al. 2005a, 2005b), and the frequency of independent evolutionary events necessary to explain extant patterns of diversity (Emlen et al. 2005a, 2005b). Here I present results on the developmental origin of sexual dimorphism in four *Onthophagus* species that challenge this basic assumption.

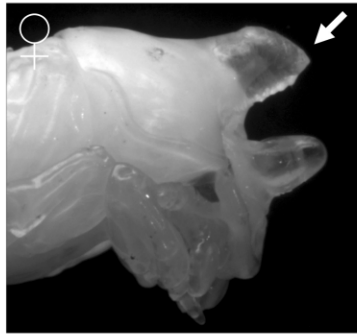
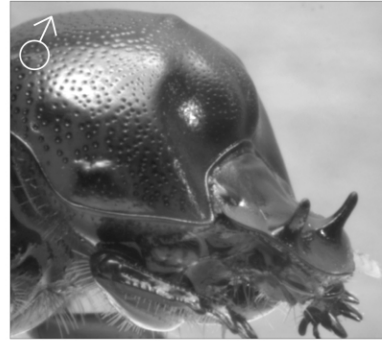
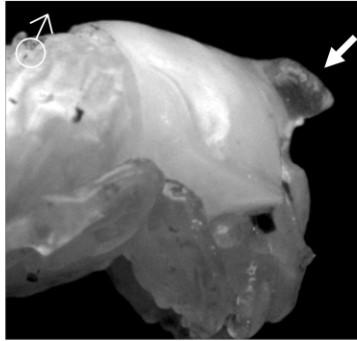
All four *Onthophagus* species studied here grew thoracic horns during the prepupal stage that were clearly visible and measurable in the resulting pupae. In all four species, however, pupal horns were not just intermediate stages toward a final adult structure but instead underwent moderate to severe remodeling via differential loss of horn primordial tissue. Kind and degree of pupal horn resorption varied dramatically both within and between species, suggesting a remarkable evolutionary lability of this mechanism.

For example, in *Onthophagus nigriventris*, dimorphic growth of horn primordia during the prepupal stage generated sexually dimorphic pupae, expressing a large down-curved horn in male pupae and only a moderate horn in female pupae. Differential resorption of female, but not male, pupal horns further amplified this dimorphism, resulting in a dramatic sexual dimorphism among adult *O.*

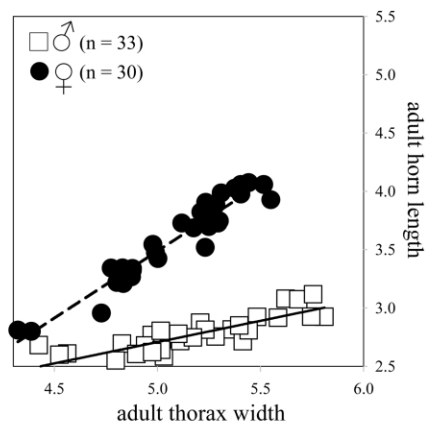
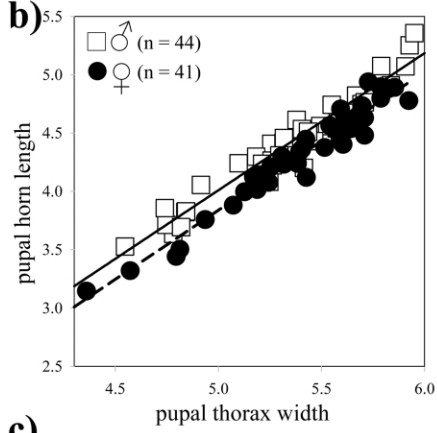
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**Figure 3:** Ontogenetic changes in allometric scaling between body size and thoracic horn length in male (squares) and female (circles) *Onthophagus binodis*. *a*, Photographs of male (top) and female (bottom) pupae (left) and the adults (right) that eclosed from them. *b*, Scaling relationship between body size and horn length in male and female pupae (left) and corresponding adults (right). The scaling relationship in both sexes was linear in both pupae and adults. Pupal *O. binodis* were not sexually dimorphic, and male and female pupae could not be distinguished by thoracic horn length. However, sexual dimorphism was highly significant in adults because of differential resorption of horn tissue in female pupae illustrated in *c*. *c*, Relative and absolute loss of pupal horn length as a function of adult body size in males and females. Horn resorption in female *O. binodis* far exceeded that of males. Interestingly, relative horn resorption in females not only exceeded that of males but also increased with female body size, while relative horn resorption decreased dramatically with male body size. In this species, monomorphic prepupal horn growth thus gave rise to sexually monomorphic pupae. Massive resorption of pupal horns, especially in large females, but retention of horns in males, then generated the sexual dimorphism observed in the adult stage.

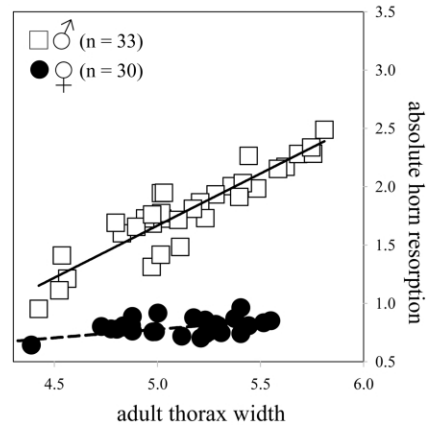
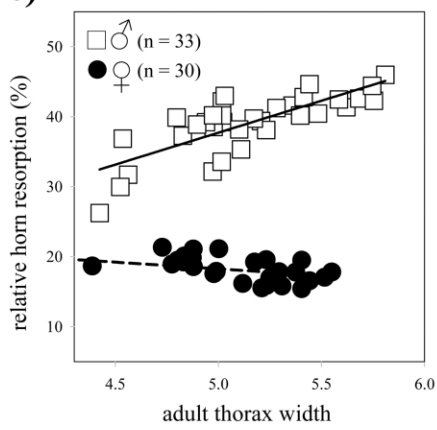
a)



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c)



*nigriventris*. In this species, pupal horn remodeling magnified an already existing sexual dimorphism stemming from the preceding prepupal growth period.

In contrast, in *Onthophagus binodis*, initial prepupal growth of horns was identical in both sexes and generated monomorphic pupae. In this species, subsequent differential resorption of horn tissue in females but not males was the sole mechanism responsible for generating sexually dimorphic adults.

The same situation was observed in a third species, *Onthophagus sagittarius*, except in a sex-reversed manner. While initial prepupal growth was nearly identical in both sexes, only female pupae retained their horns into adulthood, while male pupae resorbed their horns before the final molt. As in *O. binodis*, differential sex-specific resorption of horn tissue, rather than differential growth, was the sole mechanism responsible for generating sexually dimorphic adults in *O. sagittarius*.

Finally, both sexes in *Onthophagus taurus* also grew a thoracic horn during the prepupal stage. In this species, initial growth was dimorphic and generated dimorphic pupae similar to those of *O. nigriventris*; however, in this species, both sexes resorbed their thoracic horns during the pupal stage and removed any trace of its existence before the final adult molt. Interestingly, resorption of thoracic horn tissue was less severe in female than in male *O. taurus*, generating a minor thoracic ridge in female but a much rounder, even-sloped thorax in male adults.

On one side, pupal remodeling of horn expression therefore appears to be ubiquitous in the genus *Onthophagus*; on the other, it exhibits surprising lability, as even closely related species differ rather remarkably in which sex horn expression is remodeled and whether this mechanism is used to generate, magnify, erase, or reverse sexual dimorphism after the prepupal growth phase. Importantly, pupal remodeling of horns after prepupal growth was far more variable than prepupal growth itself, at least among the four species studied here. Together, these observations force a revision of current understanding of the developmental and evolutionary biology of beetle horns and horn dimorphisms, as discussed below.

### The Developmental Basis of Horn Resorption

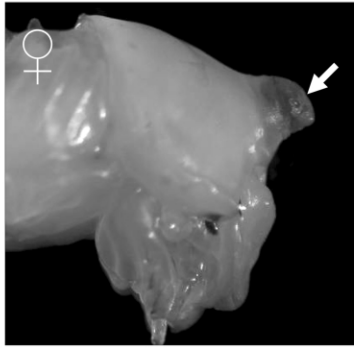
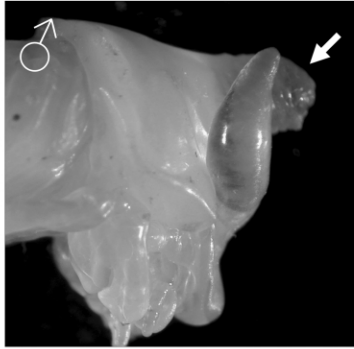
Which developmental and genetic mechanisms mediate size, sex, and species-specific resorption of pupal horn tissue? Recent advances in our understanding of the developmental basis of horn and horn dimorphisms are beginning to generate testable hypotheses to address this question. Beetle horns, even though they lack segments, joints, muscles, and nervous tissue, develop in a manner reminiscent of the development of more traditional insect appendages, such as legs, mouthparts, and antennae (Emlen and Nijhout 1999, 2001; Moczek and Nagy 2005; Moczek 2006b). Recent studies suggest that beetle horns and traditional appendages also share at least some of the same patterning mechanisms known to instruct appendage formation and differentiation during the ontogeny of a great diversity of arthropods, including the initiation of outgrowth formation via joint expression of the morphogens *wingless* and *decapentaplegic* (A. P. Moczek, unpublished data), establishment of the proximodistal axis via the transcription factors *Distal-less*, *aristaleless* (Moczek and Nagy 2005), *homothorax*, *extradenticle*, and *dachshund* (Moczek et al. 2006b), and possibly the establishment of region-specific identity via HOX genes such as *sex combs reduced* and *deformed* (A. P. Moczek and B. Kesselring, unpublished data).

However, the sometimes radical resorption of pupal horn tissue reported here is at odds with typical appendage development, at least when compared to that of other holometabolous insects. Like beetle horns, traditional appendages of most holometabolous insects undergo explosive growth during the prepupal stage but are then typically retained during the pupal stage and into the adult stage. Two of the main functions of the pupal stage are in fact to allow for the “wiring” and final “sculpting” of appendages (Snodgrass 1924; Leudeman and Levine 1996; Cullen and McCall 2004). Pupal appendages such as legs, mouthparts, and antennae are initially not much more than hollow tubes lacking most of the machinery necessary to carry out their adult function. Wiring is achieved during the pupal stage as muscle tissue invades the new appendage,

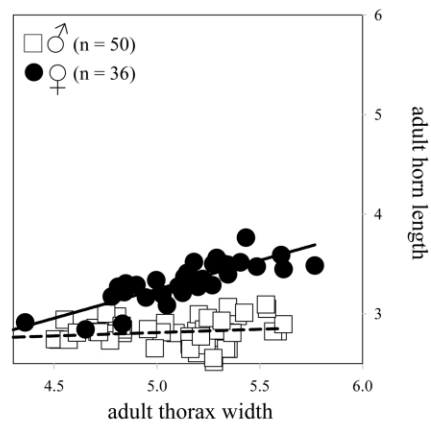
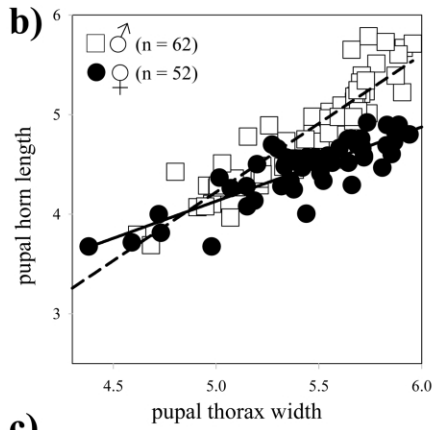
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**Figure 4:** Ontogenetic changes in allometric scaling between body size and thoracic horn length in male (squares) and female (circles) *Onthophagus sagittarius*. *a*, Photographs of male (top) and female (bottom) pupae (left) and corresponding adults (right). *b*, Scaling relationship between body size and horn length in male and female pupae (left) and corresponding adults (right). The scaling relationship in both sexes was linear in both pupae and adults. Pupal *O. sagittarius* were already mildly sexually dimorphic, with males expressing slightly but significantly larger thoracic horns than females. In adults, this initial sexual dimorphism then became reversed and far more extreme, as a consequence of differential resorption of horn tissue in male pupae (*c*). *c*, Relative and absolute resorption of pupal horn length as a function of adult body size in males and females. This time, horn resorption in males far exceeded that in females, and relative horn resorption increased with male body size, while it remained constant across female body sizes. In this species, moderately sexually dimorphic prepupal horn growth thus gave rise to modestly sexually dimorphic pupae. Massive resorption of pupal horns in males but retention of horns in females then radically reversed and magnified sexual dimorphism and generated the reversed sexual dimorphism observed among adult *O. sagittarius*.

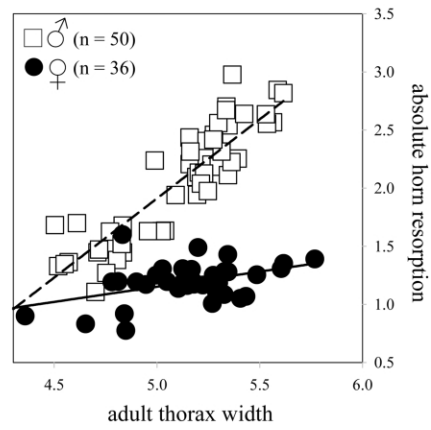
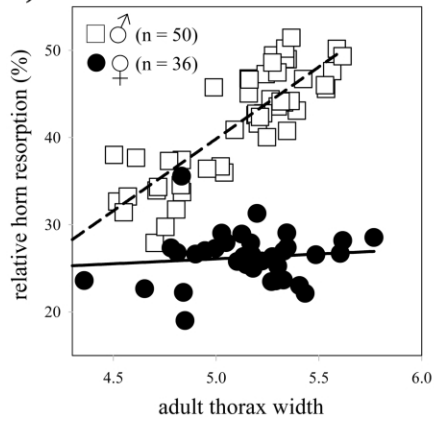
**a)**



**b)**



**c)**



attaches to appropriate locations, and becomes invaded by a corresponding tracheal and nervous support system (Leudeman and Levine 1996; Champlin et al. 1999; Hemming 2003). Sculpting, in turn, is typically achieved via subtle resorption of surplus pupal tissue via programmed cell death (Cullen and McCall 2004).

Programmed cell death is a basic physiological process used by all metazoan organisms to eliminate harmful or superfluous cells and to sculpt organs during morphogenesis, and it relies on a tier of phylogenetically highly conserved genetic and developmental processes (White et al. 1994; Jacobson et al. 1997; Potten and Wilson 2004). Programmed cell death is executed via apoptosis or autophagy, two processes that differ primarily in the mechanisms used for degradation of the dying cell (Martin and Baehrecke 2004). Even though in holometabolous insects, sculpting of pupal body parts mediated by programmed cell death is essential for permitting the attainment of a given body part's final adult shape and function (Snodgrass 1924; Lohmann et al. 2002; Cullen and McCall 2004), it typically does not involve the wholesale loss of an entire structure, as happens with transient beetle horns. One exception occurs in *Pheidole* ants, in which apoptosis mediates the caste-specific degeneration of wing disks during the prepupal-pupal transition (Sameshima et al. 2004). A second exception occurs in some Lepidoptera in which winglessness in adult females is achieved developmentally via sex-specific apoptotic wing degeneration during pupal development (Nitsu 2001; Lobbia et al. 2003). In *Onthophagus* pupae, loss of entire thoracic horns is similarly severe and is likely to rely on many of the same developmental mechanisms. Companion studies on the developmental regulation of pupal remodeling have recently provided the first evidence that programmed cell death can be widespread during early pupal horn development, providing a likely mechanism for the sex- and size-dependent removal of horn primordial tissue (fig. 7; T. Kijimoto, A. P. Moczek, and J. Andrews, unpublished data).

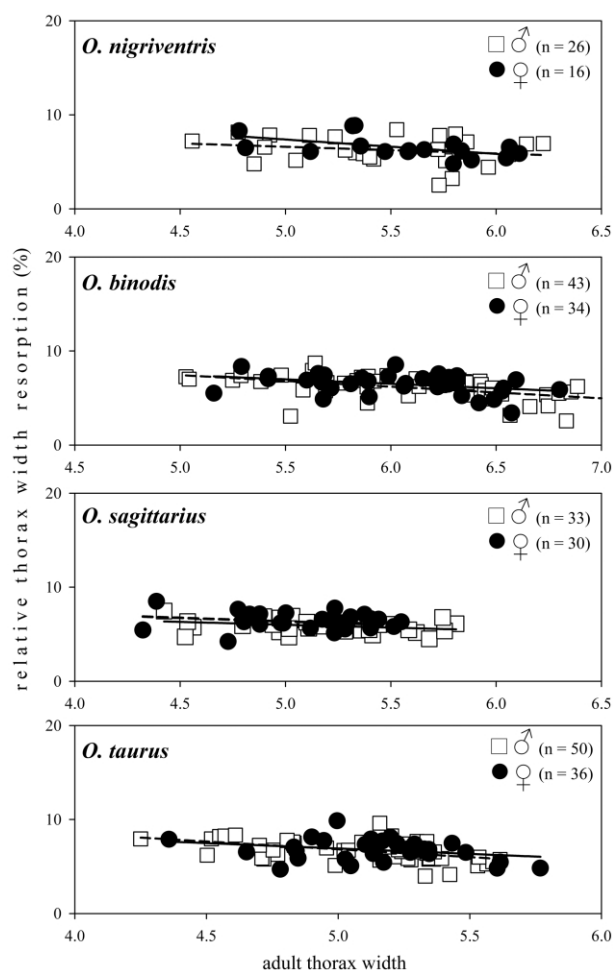
Interestingly, the developmental regulation of programmed cell death plays a central role in the biology of cancer, and the pathways involved in activating or inhibiting programmed cell death are focal areas of current medical research (reviewed in Potten and Wilson 2004). One such pathway is the epidermal growth factor receptor (EGFR) signaling pathway (Cao et al. 2000; Baker 2001; Kamer et al. 2004). This pathway is particularly interesting because it has also been demonstrated to be vital for the patterning of at least some insect appendages. For example, in *Drosophila*, EGFR ligands are expressed in the form of steep gradients from the future apical tip to the proximal regions of the developing appendage (Barolo and Posa-kony 2002; Campbell 2002; Galindo et al. 2002), and graded EGFR ligand expression regulates a suite of additional transcription factors such as *aristaleless*, *argos*, *BarH1/BarH2*, *bric-a-brac*, and *rotund* (Campbell 2002; Klein et al. 2004; Kojima 2004). One of these targets, the transcription factor *aristaleless*, has been shown to be functionally required for patterning tarsal segments of *Drosophila* legs (Campbell 2002) and the formation of the arista on the antenna (Schneitz et al. 1993), and its orthologue in crickets is expressed in the distal portions of developing legs, mouthparts, and antennae and cerci (Miyawaki et al. 2002). At the same time, EGFR signaling plays a prominent role in the regulation of apoptosis (Cao et al. 2000; Baker 2001; Kamer et al. 2004). For example, a second prominent EGFR signaling component, *argos*, is both an extracellular inhibitor of EGFR signaling (Klein et al. 2004) and an inducer of apoptotic cell death (Sawamoto et al. 1998). Interestingly, *Onthophagus* orthologues of *aristaleless* (Moczek and Nagy 2005) and *argos* (A. P. Moczek, unpublished data) are expressed in transient thoracic horns. Their exact roles, if any, in regulating pupal horn resorption are currently examined through functional analysis.

#### *The Biological Significance of Transient Thoracic Horns*

Recent studies on the developmental genetics of beetle horns show that persisting and transient horns develop at

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**Figure 5:** Ontogenetic changes in allometric scaling between body size and thoracic horn length in male (squares) and female (circles) *Onthophagus taurus*. *a*, Photographs of male (top) and female (bottom) pupae (left) and corresponding adults (right). *b*, Scaling relationship between body size and horn length in male and female pupae (left) and corresponding adults (right). The scaling relationship in both sexes was linear in both pupae and adults. Pupal *O. taurus* are highly sexually dimorphic, with male pupae expressing far larger thoracic horns than their female counterparts. This difference changed highly significantly across stages. In adults, the sexual dimorphism seen among pupae was absent. Instead, both male and female adults lacked a thoracic horn and differed merely in the degree of rounding of the prothorax, with the female prothorax dropping off more steeply than the male prothorax. *c*, Relative and absolute resorption of pupal horn length as a function of adult body size in males and females. In this species, both relative and absolute resorption of pupal horns were massive in both sexes but higher in males, which resorbed 30%–50% of their prothorax length. Relative horn resorption increased sharply and highly significantly with male body size, while it remained high but constant across female body sizes. This caused males to resorb a disproportionately larger fraction of their thoracic horn and prothorax the larger their own body size, while females resorbed their entire thoracic horn yet retained a slight prothoracic drop-off. In *O. taurus*, highly sexually dimorphic prepupal horn growth thus gave rise to a major sexual dimorphism among pupae. Massive resorption of pupal horns in both sexes then erased this dimorphism, causing horned male and female pupae to molt into thorax-hornless adults, in both cases distinguished merely by slight yet significant differences in prothoracic shape.



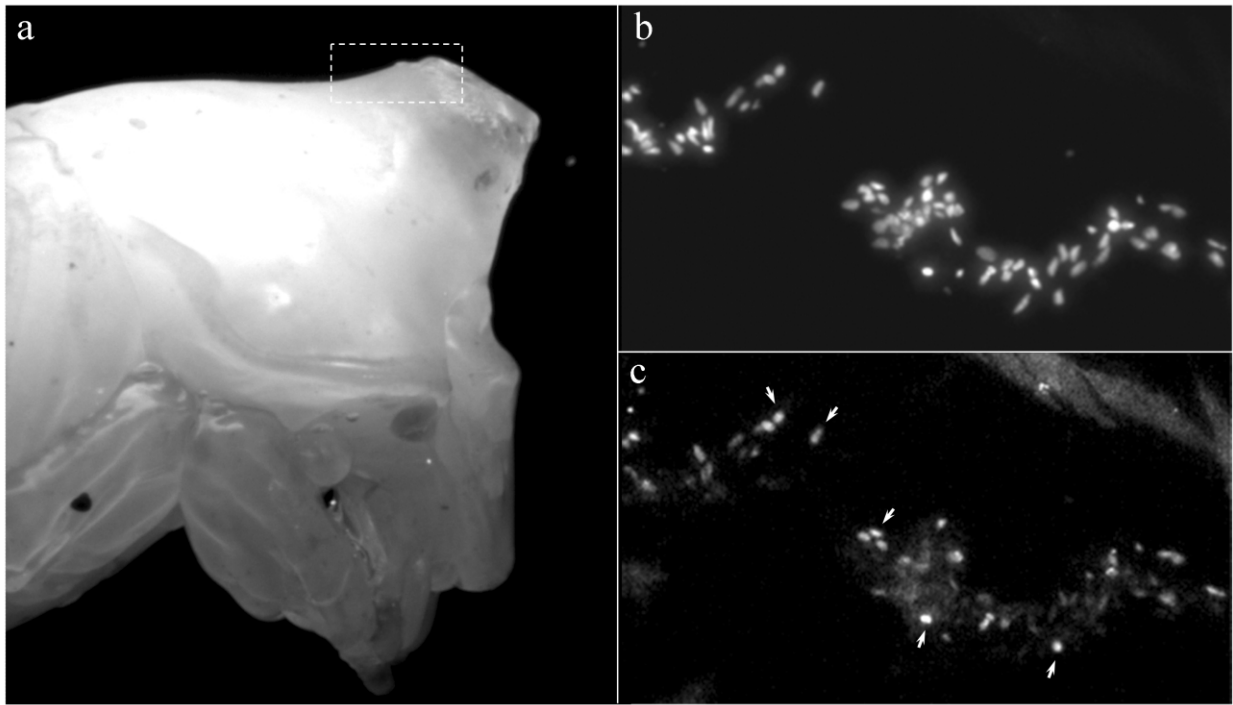
**Figure 6:** Relative changes in body size from pupal to adult stage (measured as reduction percentage in adult thorax width relative to pupal thorax width) in males (squares) and females (circles) in four *Onthophagus* species as a function of final adult body size. Adult body size in most individuals was 5%–7% smaller than pupal body size. All species except *Onthophagus sagittarius* exhibited a small but significant effect of size, causing larger individuals to lose a slightly smaller fraction of their pupal body size during pupal development. Effects of sex and sex  $\times$  size interactions were nonsignificant for each species. Body size thus undergoes a very moderate reduction as individuals transition from pupa to adult, and this reduction is remarkably constant across sexes and species.

the same time and location, utilize the same tissue regions and types, show identical prepupal and pupal differentiation patterns, and share at least qualitatively, and often quantitatively, similar expression patterns of basic appendage-patterning genes (Moczek and Nagy 2005; Moczek 2006b; Moczek et al. 2006a). Combined, these observations leave little doubt that persisting and transient horns are homologous structures, with the only difference being that the former are retained into the adult stage, while the latter are not. This raises a number of questions,

including why females, and in some cases both sexes, in many species invest in the growth of these structures during prepupal development, if this growth is then followed by secondary loss through pupal resorption.

Three nonexclusive hypotheses are currently being evaluated. First, transient horns could be the result of genetic and developmental correlations between the sexes. According to this hypothesis, selection favoring expression of horns in one sex forces members of the other sex to also express portions of the underlying developmental pathways. Genetic and developmental correlations between sexes have been documented to play important roles in shaping sexual dimorphisms (Lande 1980; Slatkin 1984; Delph et al. 2004, 2005; McDaniel 2005) yet are probably insufficient to explain transient thoracic horns for at least two reasons. First, several species, such as *O. taurus*, grow transient thoracic horns in both sexes rather than just one. Second, other horn types, such as head horns, are readily grown in a sexually dimorphic manner in a wide range of *Onthophagus* species without any signs of correlated expression in the sex that lacks the adult horn (Emlen and Nijhout 2001; Moczek and Nagy 2005).

Apparently, the developmental machinery necessary to grow horns in a dimorphic manner right away during the prepupal growth phase is already available to *Onthophagus* beetles, yet for some reason it is not used in the process of generating thoracic horn dimorphisms. One such reason could be that pupal thoracic horns may actually have an adaptive significance separate from simply being an intermediary to adult horns, causing selection to maintain pupal thoracic horns regardless of whether they are retained into adulthood. Recent studies identified such a possible function by showing that thoracic horns are crucial for correct ecdysis of the larval head capsule during the larval-to-pupal molt (Moczek et al. 2006b). During this molt, the thoracic horn primordium expands anteriorly, inserts itself underneath the larval head capsule, and subsequently expands into the space between the head capsule and the underlying head epidermis. Further expansion then leads to the splitting of the head capsule along suture lines, which is then followed by complete ecdysis of the capsule. If the precursor cells that generate the prepupal horn primordium are ablated earlier in larval development, animals cannot grow a thoracic horn but will develop normally otherwise. Such animals pupate successfully and shed the abdominal and thoracic larval cuticle in the process but fail to eclose from their larval head capsules and die a few days later. Replication of this experiment inside and outside the genus *Onthophagus* suggests that this putative dual function of thoracic horns may be unique to *Onthophagus* beetles and absent in other scarabaeine genera (Moczek et al. 2006b). These results therefore suggest a plausible scenario as to why many *On-*



**Figure 7:** Apoptosis in thoracic horn tissue during early pupal development. *a*, Thorax and head of a female *Onthophagus binodis*. Dashed rectangle indicates approximate region from which sagittal sections were obtained. *b*, DAPI staining of pupal epidermis to highlight cell nuclei. *c*, TUNEL staining of the same section highlighting nuclei undergoing apoptotic DNA fragmentation (examples are indicated by arrows; data are from T. Kijimoto, A. P. Moczek, and J. Andrews, unpublished data).

*thophagus* species grow thoracic horns even though those outgrowths are not used to give rise to a functional structure in the adult.

Despite these promising results, however, it is difficult to envision how this hypothesis could explain the observations that different species grow transient horns of very different sizes (e.g., compare the pupae of female *O. binodis* [fig. 3*a*] with those of *O. sagittarius* [fig. 4*a*]) and that in some species, such as *O. taurus*, transient horn expression is sexually dimorphic (fig. 5*a*). This could be explained by a third hypothesis, which suggests that transient thoracic horns may be developmental fossils, or remnants, of horns that used to be present in adults. This hypothesis would not be contradicted by the observation that the relative sizes of transient horns differ markedly across species, as it could be argued that presumably the relative horn sizes of the ancestral adults exhibited the same behavior, which can also be readily observed among extant species (e.g., Balthasar 1963; Matthews 1972). The same would be true for the observation of sexually dimorphic transient horns, where sexual dimorphism could be interpreted as a developmental signature of an adult horn dimorphism that has now been secondarily lost alongside the dimorphic

traits. Interestingly, both the dual-function hypothesis and the developmental-remnant hypothesis would carry with them the interesting implication that the secondary loss of horns was achieved developmentally by adding a new developmental step (the resorption of a fully developed pupal horn) rather than by not growing the primordium in the first place. Further evaluation of these hypotheses is currently under way via a combination of developmental, functional, and phylogenetic studies.

#### *Have Thoracic Horns Evolved Multiple Times within the Genus Onthophagus?*

In a recent phylogenetic analysis of beetle horn evolution, Emlen et al. (2005*a*) mapped adult horn morphologies onto a molecular phylogeny of 48 *Onthophagus* species, which represents approximately 3% of extant species diversity within this extraordinarily species-rich genus. Based on this analysis, Emlen et al. (2005*a*) concluded that thoracic horns must have been gained independently nine times in males and seven times in females to explain present-day patterns of diversity. This analysis relied solely on horn expression in adults to infer whether a given

lineage possessed the ability to develop such horns and to determine the number of independent evolutionary origins of thoracic horns necessary to explain the current phylogenetic distribution of species with thoracic horns. This analysis included all four species examined in this article, yet Emlen et al.'s (2005a) scoring of the presence or absence of thoracic horns in these species stands in conflict with the ontogenetic data presented here. For example, *O. binodis* (fig. 3; misspelled as "*O. binodus*" in phylogenies but not text in Emlen et al. 2005a; see Thunberg 1818 for original description of this species) was scored by Emlen et al. (2005a) as expressing a thoracic horn in males only, and thoracic horn development was postulated to have originated in this species in a sex-specific manner.

Here I show that both sexes grow a similarly sized, shaped, and positioned horn. The major differences between the male and female *O. binodis* does not lie in their respective abilities to develop a horn, which are identical, but in the degree to which their investment in horns is maintained into the adult stage. At least one other species, *Onthophagus hecate*, was scored similarly by Emlen et al. (2005a), even though ontogenetic data indicate that females grow male-sized transient horns similar to those of *O. binodis* (Moczek et al. 2006b). While in these instances, the restriction to adult phenotypes mainly inflates the estimate of sex-specific origins of thoracic horns, the greatest challenge to Emlen et al.'s (2005a) analysis comes from species such as *O. taurus*, which was scored as lacking a thoracic horn in adults of both sexes. Results reported here, however, show that male and female *O. taurus* grow a large, sexually dimorphic thoracic horn during development, but unlike in the other three species, both sexes resorb it entirely before the final adult molt.

Current work is examining whether other species that lack thoracic horns in both adult sexes express similarly transient horn growth during development. Preliminary results are available so far for at least 14 more species (Moczek et al. 2006b) that all lack adult thoracic horns yet grow transient horns during ontogeny in both sexes. This suggests that transient horn growth may actually be taxonomically widespread within the genus. If these preliminary results are confirmed further, this would suggest that the ability to develop a thoracic horn may have evolved only a few times or possibly only once during *Onthophagus* evolution and that what actually diversified was a given lineage's ability to resorb or retain a pupal horn into the adult stage. In addition, this would provide a relatively simple developmental mechanism by which adult horns could be lost and regained without requiring de novo evolution of horn growth or patterning processes. More generally, these observations provide a good example illustrating how relying on descriptions of adult diversity

patterns alone may be insufficient at best, and seriously misleading at worst, for any attempt to reconstruct the evolutionary history of phenotypic diversity.

### Acknowledgments

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