

Integrating Development with Evolution: A Case Study with Beetle Horns

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Rapid advances in development and evolution are providing unprecedented opportunities for synthesis of these fields. Increasingly, evolutionary biologists are using developmental mechanisms to explain large-scale evolutionary patterns (Alberch 1982, Bonner 1982, Oster et al. 1988, Hanken and Thorogood 1993, Akam et al. 1994, Raff 1996), including major transformations of complex phenotypes (e.g., Goldschmidt 1940, Carroll 1994, Halder et al. 1995, Raff 1996) and the origin of structures such as appendages (Coates 1994, Panganiban et al. 1997, Popadic et al. 1998) and eyes (Halder et al. 1995).

Developmental mechanisms are equally relevant to small-scale evolutionary processes because they may influence the response of a population to natural or sexual selection (e.g., Alberch 1982, Riska 1986, Pigliucci et al. 1996). For example, if some types of phenotypic variation are difficult or impossible to produce because of the nature of the underlying developmental processes, then these biases in the available phenotypic variation may constrain the direction of evolution in natural populations (Alberch 1982, Bonner 1982, Raff 1996). Similarly, if some phenotypes are especially easy to produce (e.g., if many different genetic mutations all result in the same phenotype), then independent evolutionary lineages may be unusually likely to converge on the same phenotype (Nijhout 1991). Studies of patterning mechanisms in butterfly wings (Nijhout 1991, 1994a) and avian plumage (Price and Pavelka 1996) reveal biases inherent in the types of color patterns that can be produced, so that some wing patterns are more easily generated than others. Similarly, studies of the development of tetrapod limbs reveal limits to the range of possible morphologies (Alberch and Gale 1985, Oster et al. 1988, Hinchliffe 1994). In each of these examples, studies of mechanism have illuminated boundaries to the range of likely phenotypic variants, and this developmental perspective helps explain patterns of natural variation within and among related species.

In principle, understanding how development affects the expression of morphological traits should help explain the evolution of those traits. However, empirical studies demonstrating an immediate relevance of development for understanding evolution in natural populations are rare because most population biologists do not study the

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developmental mechanisms regulating the expression of their traits of interest.

One trait in which to examine this question is the horns of beetles. The behavior associated with horns, the evolution of horns, and the development of horns have been explored for the same two species; consequently, it is now possible to integrate the results from these studies and to explore how knowledge regarding the mechanism of horn development influences our understanding of beetle horn evolution. In this article, I show how combining this information has enhanced our understanding of the evolutionary significance of beetle horns and illustrate how the integration of developmental studies with evolutionary studies may be used to identify unexpected avenues for future research.

Sexual selection and the evolution of beetle horns

Beetle horns are rigid extensions of the exoskeleton that are as dramatic as the antlers of elk or moose (although, unlike antlers, beetle horns are not shed; beetles must bear their horns for their entire adult life). Horns have arisen repeatedly during the evolution of beetles, and many thousands of extant species have horns (e.g., Arrow 1951, Enrodi 1985). This rich evolutionary history is reflected in the tremendous variety of horn morphologies: long cylindrical rods, curved flat blades, distended mandibles, and sundry spines, knobs, or tubercles. Even in closely related

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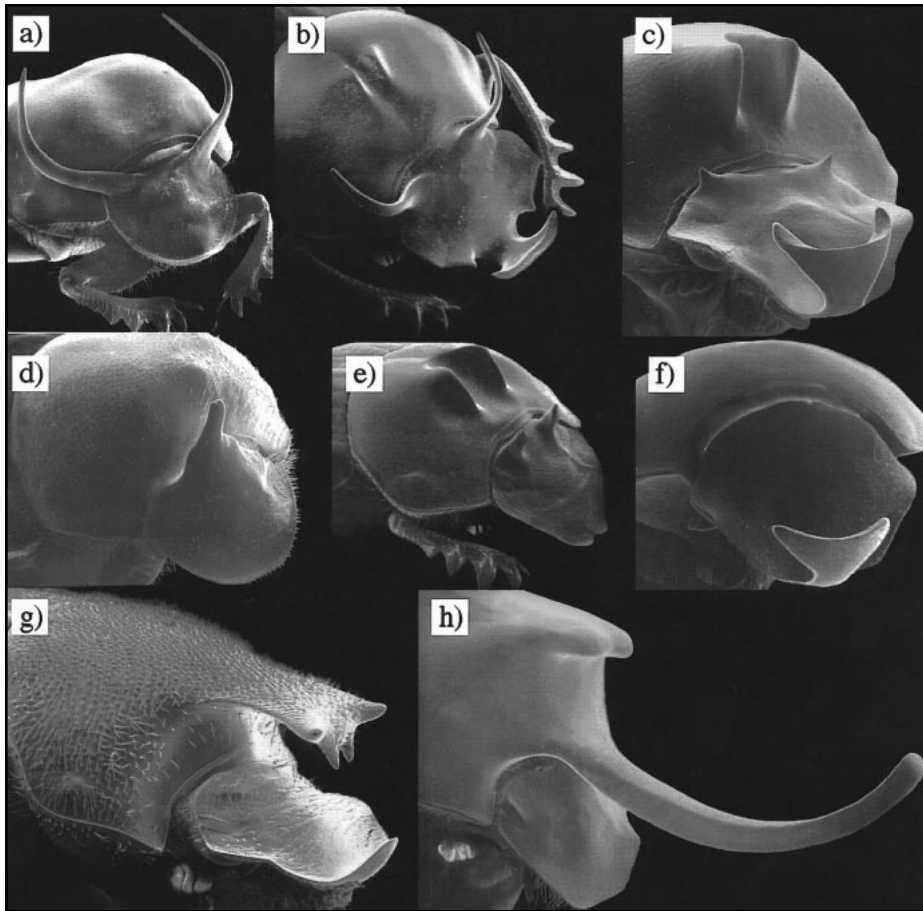


Figure 1. Myriad shapes, sizes, and locations of beetle horns in the dung beetle genus *Onthophagus* (Coleoptera: Scarabaeidae). Photographs show the head and thorax of each beetle. Depending on the species, horns can extend from the front of the head (clypeus), the back of the head, or the thorax. All photographs are of male beetles unless otherwise indicated. (a) *Onthophagus taurus*. (b) *Onthophagus clypeatus*. (c) *Onthophagus praecegens*. (d) *Onthophagus nuchicornis*. (e) *Onthophagus clypeatus* (female). (f) *Onthophagus sharpi*. (g) *Onthophagus hecate*. (h) *Onthophagus nigriventris*.

beetle taxa, horn sizes, shapes, and locations can vary extensively.

Despite this diversity, all beetle horns are used for basically the same thing. Beetle horns generally are expressed only in males; in most species, females either have reduced horns or no horns. In all of the species studied so far, horns are used by males in combat with other males for access to resources used by females (e.g., Eberhard 1978, 1982, Palmer 1978, Siva-Jothy 1987, Conner 1988, Rasmussen 1994, Emlen 1997a). For example, stag beetles (*Dendrobias mandibularis*), harlequin beetles (*Acrocinus longimanus*), and some rhinoceros beetles (e.g., *Allomyrina dichotoma*) battle for small wounds or sap flows on trees that are visited by females (Goldsmith 1987, Siva-Jothy 1987, Zeh and Zeh 1992). Other rhinoceros beetles battle for emergent bamboo shoots or branches on shrubs that also are visited by females (e.g., *Golofa porteri*; Eber-

hard 1978) or for burrows carved into plant stems, in which females feed and lay eggs (e.g., *Podischnus agenor*; Eberhard 1982). Male dung beetles use their horns in battles for access to subterranean tunnels containing females (Palmer 1978, Otronen 1988, Cook 1990, Rasmussen 1994, Emlen 1997a).

Although the resources in question vary considerably from species to species, they share one key characteristic: they all tend to occur in discrete, readily defensible patches. Males with enlarged weapons are able to gain disproportionate access to these contested sites and, consequently, gain disproportionate access to females (Eberhard 1982, Goldsmith 1987, Siva-Jothy 1987, Zeh and Zeh 1992, Rasmussen 1994, Emlen 1997a, Moczek and Emlen 2000).

But beetle horns do not come without costs. Horns are likely to constitute a considerable investment by the bearer. Beetle horns comprising more than 10% of the animal's total body mass are not uncommon, and such large structures are energetically expensive to produce. Horn growth prolongs beetle development and can increase the risk of larval mortality (e.g., from soil nematodes that kill larvae; Hunt and Simmons 1997), and nutrients allocated to horn growth deplete resources that would have been available for

growth of other tissues (Nijhout and Emlen 1998). In addition, horns may impede the mobility or performance of individuals in ways that increase the risk of predation or starvation (as in Magnhagen 1991). For example, the increased wing loading associated with horns may make flight more energetically expensive, and horns may physically impede movement in some substrates. As a result, dispersal, foraging, and escape may be more difficult for beetles with well-developed horns.

Nevertheless, although beetle horns are likely to be expensive to generate and difficult to bear, they are useful for gaining reproductive access to females, and the reproductive benefits gained by having large horns presumably outweigh the costs of their production and maintenance. The intense sexual selection generated by contests over patchy, localized resources may have favored extravagant investment by males into the weapons that aid them in

securing access to females, leading to the evolution of some of the world's most dramatic sexually selected traits.

One genus of dung beetle that is characterized by prolific evolution of horns is the genus *Onthophagus*. This genus contains over 2000 described species, making it a contender for the largest genus of plant or animal in the world. In most of the species, males bear some type of horn, although the shapes and locations of these horns vary tremendously (e.g., Boucomont 1932, Howden and Cartwright 1963, Matthews 1972, Howden and Gill 1993; Figure 1). Onthophagine beetles occur on six continents and span most climate and habitat extremes, from tropical rain forests to temperate plains, African savanna, and even the Australian outback and the deserts of the southwestern United States. They feed on dung from antelope, buffalo, elephants, rabbits, kangaroos, emus, tapirs, deer, horses, monkeys, marmots, and even packrats.

Despite this wide array of habitat types and food resources, all of these beetles appear to behave in basically the same way: females dig tunnels in the soil beneath the dung and bury the dung below ground to use as larval provisioning, sometimes with the assistance of males (Halffter and Edmonds 1982, Cook 1990, Rasmussen 1994, Emlen 1997a, Hunt and Simmons 1998, Moczek 1998). Males fight with other males for possession of these tunnels, and males successful at guarding tunnels gain reproductive access to the females inside (Cook 1990, Rasmussen 1994, Emlen 1997a, Hunt and Simmons 1997, Simmons et al. 1999, Moczek and Emlen 2000).

In two species, *Onthophagus taurus* and *Onthophagus acuminatus*, the reproductive behavior occurring inside tunnels has been studied in detail. Males of both species produce a pair of horns that extend from the base of the head (Figures 1a and 2). The length of these horns varies considerably among individuals, and this variation scales with body size: large males produce long horns, whereas small males produce much shorter horns. The scaling relationship between horn length and body size is sigmoidal in both species (Figure 2; Emlen 1994, 1996, Hunt and Simmons 1997, 1998, Moczek and Emlen 1999). The shape of this scaling relationship means that the transition between minimal and complete horn expression occurs abruptly, at approximately the body size that is associated with the inflection of the sigmoid curve (3.35 mm; Figure 2). Males larger than this "threshold" size produce fully developed horns, whereas males smaller than this size produce only minimal horns or no horns at all. As a result, the distribution of horn sizes in both of these species is bimodal: populations contain large numbers of males with fully developed horns, large numbers of males with no or small horns, and relatively few males with horns of intermediate length (Figure 2, inset). Consequently, not only are these species sexually dimorphic with respect to horn expression (no females express horns), but a dimorphism exists within males. As it turns out, the two types of males use entirely different behavioral tactics to encounter

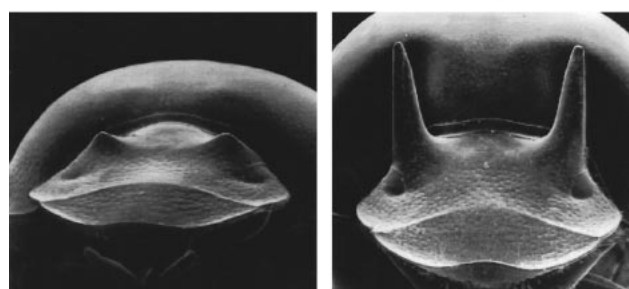
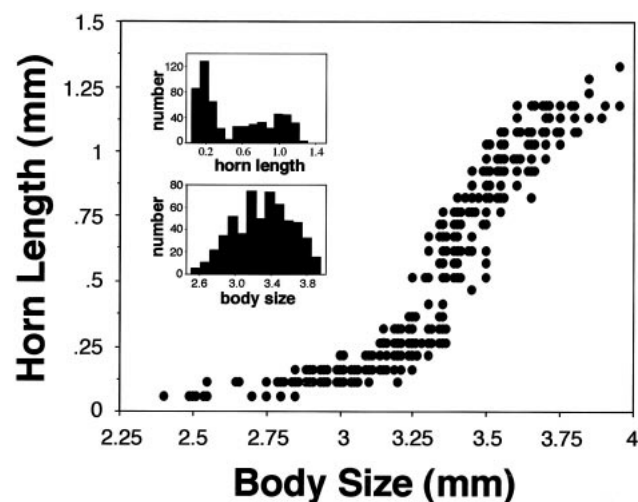


Figure 2. Scaling relationship between horn length and body size (prothorax width) for 500 male *Onthophagus acuminatus* (Coleoptera: Scarabaeidae) sampled from Barro Colorado Island, Panama. The sigmoidal relationship (top) is associated with a bimodal frequency distribution of horn lengths in natural populations (inset) and separates males into hornless (lower left) and horned (lower right) adult shapes. Reprinted from Emlen (1997b); data from *Onthophagus taurus* are similar.

and mate with females.

Females of both species dig tunnels into the soil beneath pieces of dung. They reside within these tunnels, where they sequester pieces of dung for themselves and for larval provisioning. Females may spend several days inside a single tunnel, pulling pieces of dung down to the blind ends of tunnel branches and packing the pieces into dense oval masses (brood balls) that will eventually serve as food for developing larvae (Figure 3; Bornemissza 1970, Halffter and Edmonds 1982). In both *O. acuminatus* and *O. taurus*, females lay a single egg in a chamber at the top of each brood ball, and larvae complete their development in isolation within the confines of a single brood ball (Main 1922, Emlen 1994).

Females mate repeatedly with males during the days preceding and including egg laying. Because females are inside the tunnels during this period, entry into a tunnel is a prerequisite for mating. Male reproductive behavior revolves around methods of securing access to the females inside these tunnels; in both species, hornless males

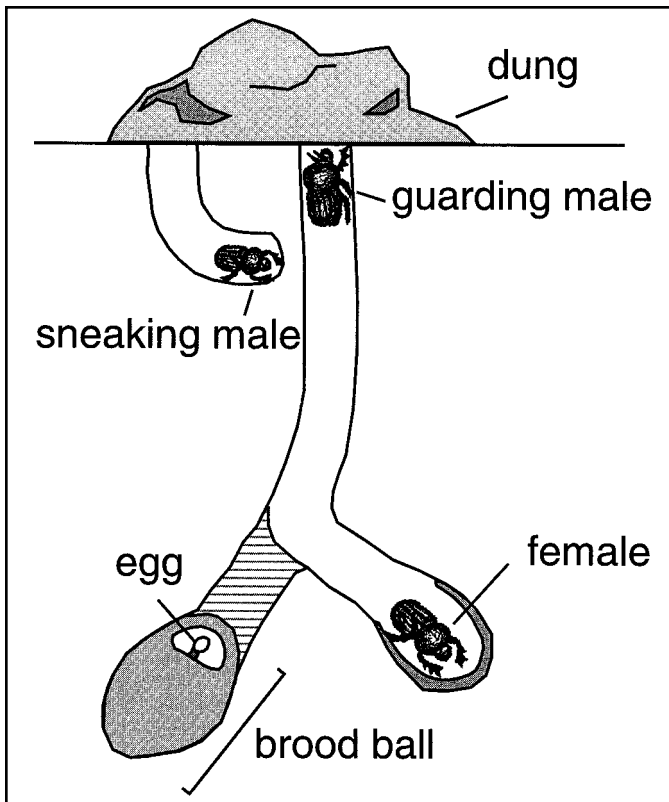


Figure 3. Alternative reproductive tactics used by male dung beetles (*Onthophagus acuminatus*) to gain reproductive access to females. Females dig tunnels into the soil beneath dung. They spend several days inside tunnels pulling fragments of dung below ground and using these to form oval masses (brood balls), which serve as food supplies for the larvae. Large, horned males guard the entrances to subterranean tunnels containing females, and successful males mate repeatedly with the resident female. Smaller, hornless males are ineffective at guarding tunnels; these males sneak into guarded tunnels either by slipping past the guarding male or by digging side tunnels that intercept the guarded tunnel below ground.

accomplish this task in a very different way from horned males (Figure 3).

Large, horned males guard entrances to tunnels that contain females, and these males fight with all other males that try to enter the tunnel. Large males win significantly more fights than small males; moreover, when the effects of body size are experimentally controlled for, males with longer horns win significantly more fights than same-sized rival males with shorter horns (Emlen 1997a, Moczek and Emlen 2000; see Rasmussen 1994 for similar results in *Phanaeus difformis*). Consequently, the largest horned males are the most likely to successfully defend access to tunnels containing females, generating strong sexual selection for long horns in males.

By contrast, small, hornless males generally are not effective at guarding entrances to tunnels, and these males

are evicted from tunnels whenever they encounter a larger, horned male. Hornless males therefore employ an alternative tactic to encounter and mate with females. These males attempt to enter guarded tunnels by circumventing the guarding male, either by slipping undetected past him (Emlen 1997a, Moczek and Emlen 2000) or by digging a side tunnel that intercepts the guarded tunnel below ground (Figure 3; Emlen 1997a). Occasionally, hornless males manage to sneak into guarded tunnels, mate with the female, and leave without being caught by the guarding male (Emlen 1997a, Moczek and Emlen 2000). If such “sneaker” males get caught by the resident male, not only do they get chased out of the tunnel, but the resident male returns to the female and mates with her immediately thereafter. This immediate remating may either dilute or displace sperm from a prior mating with the sneaker male (see Simmons et al. 1999 for discussion of sperm competition in *O. taurus*).

In principle, horns can influence the performance of males using either behavioral tactic, but the effect of the horns will differ dramatically depending on the behavioral tactic used. Long horns are clearly beneficial to males that guard. They improve male fighting ability significantly (Emlen 1997a, Moczek and Emlen 2000), probably because they provide leverage during contests and because they help block the tunnel entrance, making it more difficult for intruding males to push past the guarding male and into the tunnel. In contrast, horns appear detrimental to sneaker males, for the simple reason that they get in the way. Success at sneaking depends on rapid, undetected entry into tunnels. Horns impede movement below ground by scraping against tunnel walls as beetles run, which can slow males down (Moczek and Emlen 2000) and generate vibrations that alert resident males to the presence of an intruder (Douglas J. Emlen, unpublished observations). Consequently, the alternative methods for encountering females may have selected for opposing horn morphologies in large and small males. Males larger than most of their competitors can effectively guard tunnels, and these males guard tunnels more successfully if they have long horns. Small males enter guarded tunnels on the sly, and these males may enter and exit tunnels faster, and with less risk of detection, if they do not have horns.

The observed patterns of sexual selection on horns are consistent with the morphological variation present in natural populations, where large males produce the horns and smaller males do not (Figure 2). How is this adaptive association between horn expression and body size achieved? Experiments conducted on both of these species make it possible to begin to address this question.

The development of beetle horns

Male horn expression in beetles is facultative. Despite the importance of horns to sexual selection and male reproductive success, horn production shows insignificant levels of heritable genetic variation in natural populations

(e.g., $h^2 = 0.006$, $P = 0.98$ in *O. acuminatus*, Emlen 1994; $h^2 = -0.162$, $P = 0.59$ in *O. taurus*, Moczek and Emlen 1999). Instead, nutritional conditions encountered by each larva as it develops determine both the final adult body size of that animal and the length of its horns (Emlen 1994, 1996, 1997b, Hunt and Simmons 1997, in press, Moczek 1998, Moczek and Emlen 1999). Males who encounter favorable larval conditions (e.g., large amounts of food) grow large and produce horns, whereas related (even sibling) males who encounter poor larval conditions remain small and do not produce horns. The developmental switch between hornlessness and horn growth occurs abruptly around a threshold body size: Individual males whose body sizes are below this threshold do not produce horns, and those whose body sizes are larger do produce horns. But what exactly are beetle horns, when do they grow, and how is their phenotypic expression coupled with growth in body size?

Horns grow during the larval period. Onthophagine beetles develop in isolation inside balls of dung that have been buried below ground (Main 1922, Halfpeter and Edmonds 1982). After hatching, beetles pass through three larval stages, undergoing exponential growth for most of this period (Figure 4). At the end of the third larval stage, the animals stop feeding and begin to purge their gut of all contents in preparation for metamorphosis. During this prepupal period they synthesize most of their adult structures, including their horns. Cells in localized regions of the epidermis that have detached from the larval exoskeleton (called imaginal discs) divide prolifically to produce the adult eyes, legs, wings, genitalia, antennae, and mouthparts (for review, see Cohen 1993). Consequently, a late third-instar larva (i.e., a prepupa) already has many adult morphological traits. But these structures are not visible on the outside—the larva still looks like a larva. Instead, these structures are folded up and tucked away on the inside of the animal. They unfold to take their adult form during molting, when beetles emerge from their old larval exoskeleton and expand to unwrinkle and fill out their new pupal exoskeleton.

Beetle horns develop at the same time and in the same

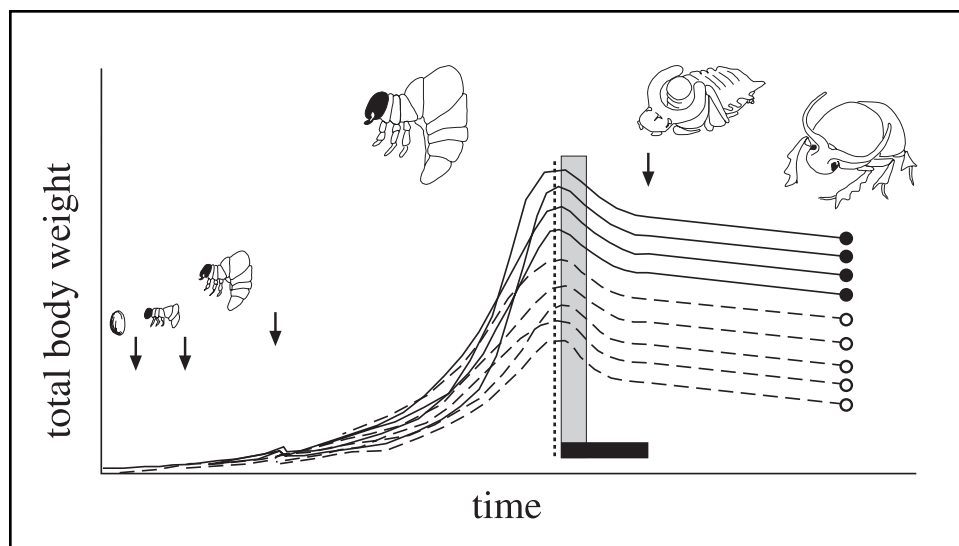


Figure 4. Life cycle of *Onthophagus taurus* showing developmental stages: egg, three larval instars, pupa, and adult. Molts between stages are indicated by arrows. Curved lines illustrate hypothetical growth trajectories of males. The vertical dotted line indicates the transition to the prepupal stage, when larvae stop feeding and begin to purge their gut. Morphological traits characteristic of adults (e.g., legs, wings, eyes, and horns) undergo most of their growth at the end of the third larval instar, during the prepupal period (indicated by the horizontal black bar), but they remain folded inside the larval cuticle. These structures expand rapidly to take their adult form when animals shed their larval cuticle and molt to the pupal stage. Male horn expression is regulated during a brief period occurring immediately after the gut purge (vertical gray bar). At this time, large male larvae produce horns (solid lines), whereas small male larvae do not (dashed lines).

manner as these other adult structures—that is, they begin as regional invaginations of the larval epidermis (Emlen and Nijhout 1999). In *O. taurus*, two small pockets of epidermis detach from the cuticle at the base of the head—one for each of the two horns (Figure 5a). These detached regions of epidermis undergo rapid cell divisions and dramatically increase in surface area, producing long, hollow tubes. However, because the outer larval cuticle is still in place, the horns cannot extend as they grow larger, so they remain trapped inside the larval exoskeleton (in *O. taurus*, the growing horns are actually trapped inside the rigid larval head capsule). Consequently, these tubes of growing cells invaginate repeatedly to produce a series of dense, concentric folds (Figures 5a and 5b). Externally, these animals still do not have visible horns, but large male larvae now have bulging pockets of folded epidermis trapped beneath the head cuticle (Figure 5c). When the animals shed their larval cuticle, the folded sacs expand rapidly to form the fully extended horns that are visible in pupae and adults (Figures 1a and 4). In small males (below the threshold size), this same region of larval epidermis grows minimally, and the males emerge with only rudimentary horns or with no horns (Emlen and Nijhout 1999).

With this knowledge of the details of horn development, we can refine the question of how horn growth is

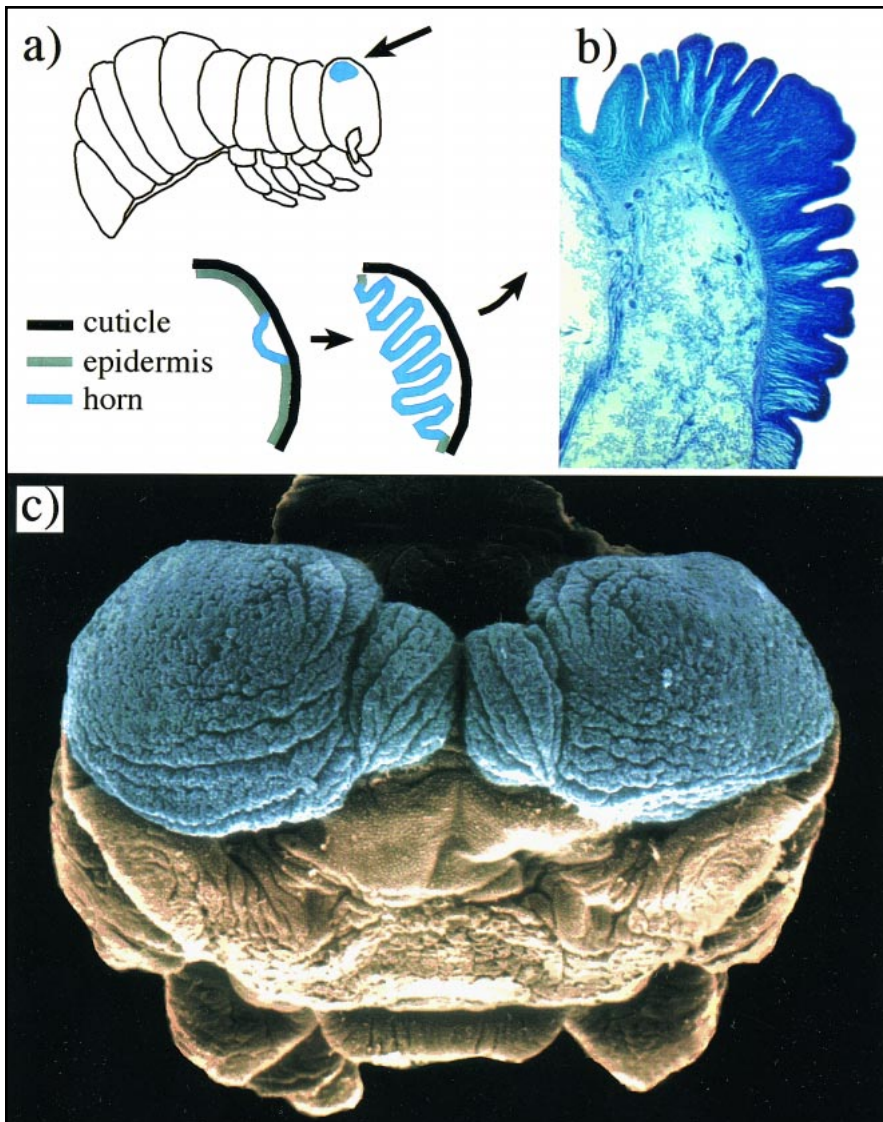


Figure 5. Development of horns in male *Onthophagus taurus*. (a) Horns start as a regional detachment of the epidermis from the larval cuticle (blue). This epidermis grows rapidly over a 24–48 hour period but is trapped beneath the larval cuticle as it grows and consequently invaginates into a dense series of telescoped concentric folds. (b) Cross-section of a developing horn, with larval cuticle removed (late third instar larva; animal is facing to the right, as in panel [a]). (c) By the end of the third larval period (prepupa), the two horns are visible beneath the head capsule as bulges of folded epidermis (front view, shown with head capsule removed; horns are indicated in blue).

regulated. Factors influencing the rate or amount of growth of these locally detached regions of larval epidermis ultimately determine whether a male produces horns. It is the localized growth of these pockets of larval epidermal cells that appears to be regulated by the body size threshold: males growing larger than a critical body size show pronounced cell proliferation in the regions where the horns will form, whereas males that have not attained this critical body size show minimal cell proliferation. Somehow, then, developing animals are able to incorporate information about their body size into the “decision” of whether or not to grow horns.

Horn growth is mediated by juvenile hormone.

This developmental decision appears to be mediated by levels of a circulating hormone in the blood of larvae (Emlen and Nijhout 1999). Hormones serve as messengers between tissues and may initiate changes in patterns of gene expression within cells expressing appropriate hormone receptors (e.g., Nijhout 1994b). In *O. taurus*, male

horn growth is mediated by juvenile hormone (Emlen and Nijhout 1999). At the end of the third larval stage, after larvae have completed all of their growth in body size and have begun to purge their guts of the remaining contents, the epidermal cells that will develop into horns become sensitive to the presence and level of juvenile hormone (JH). During a brief (30-hour) period, the hemolymph level of JH determines the amount of proliferation in these cells. High levels of JH appear to initiate rapid cell proliferation, resulting in future horned adults, whereas reduced levels of JH initiate

only minimal cell proliferation, resulting in future hornless adults (Emlen and Nijhout 1999). But what determines which individuals will have high levels of JH and which will not?

Levels of JH are affected by each individual's body size.

Hormone release is often triggered by the sensory system, and such release is one way in which insects regulate developmental events based on stimuli perceived from their environment (Nijhout 1994b, 1999). Sensory stimuli can include abiotic aspects of the physical environment, such as photoperiod or temperature—for example, hormonal changes in response to photoperiod initiate the onset of overwintering diapause (e.g., Tauber et al. 1986). Stimuli can also include aspects of the social or nutritional environment, as when hormonal changes initiate wing production by insects developing under crowded or deteriorated conditions (e.g., Pener 1985, Zera and Denno 1997). Finally, sensory stimuli can pertain to the growth or size of the animal itself. Indeed, a number of developmen-

tal events are initiated only after animals attain a certain critical body size (e.g., the onset of metamorphosis in tobacco hornworms is body size dependent; Nijhout and Williams 1974, Nijhout 1975, 1979, 1994b, Jones et al. 1981). In this final situation, conditions related to the body size of the animal (e.g., stretch in the softer regions of the larval cuticle; Beckel and Friend 1964, Anwyl 1972, Nijhout 1981, 1984, Chiang and Davey 1988) influence cellular events within the developing insect by affecting the levels of circulating hormones (Nijhout 1994b).

One hormone that may regulate body size-dependent expression of traits is JH (Wheeler and Nijhout 1984; for additional possibilities, see Stern and Emlen 1999). In a variety of insects, larval nutritional conditions (e.g., the amount or quality of food) and larval growth (e.g., attainment of a critical body size) directly affect circulating levels of JH (Johansson 1958, Asencot and Lensky 1976, Lenz 1976, Velthuis 1976, Dogra et al. 1977, de Wilde and Beetsma 1982, Rembold 1987, Rachinsky and Hartfelder 1990). For example, JH levels are thought to correlate with larval body size in the ant *Pheidole bicarinata*, and JH regulates the body size-dependent switch between worker and soldier development in that species (Wheeler and Nijhout 1983, 1984). Similar mechanisms may operate in *O. taurus*. Although the exact levels of JH in *O. taurus* larvae have not yet been measured, it appears that larger animals have higher concentrations of JH during this critical period than smaller individuals (see Emlen and Nijhout 1999). With this mechanism, cells initiating horn growth in response to high concentrations of JH also end up initiating horn growth only in the largest individuals.

The most convincing evidence that JH links horn growth with body size in beetles comes from an experiment that perturbed JH levels during the 30-hour sensitive period for horn growth. By experimentally augmenting levels of JH, it was possible to induce tiny *O. taurus* males to produce horns (the morphology of large males was unaffected by this manipulation; Emlen and Nijhout 1999). This finding suggests that amplifying the levels of JH in small males caused the cells that grew to become horns to “misread” the body sizes of those individuals. This ability of extra JH to “trick” small males into producing morphological traits that typically are expressed only at large body sizes implicates JH as the link between larval body size and horn growth.

It is also noteworthy that horn length is not determined until very late in larval development. The cells that produce the horns respond to perturbations of JH only during a brief period that occurs after larvae have stopped feeding and have begun to purge their guts (Figure 4; Emlen and Nijhout 1999). It is at the end of the feeding stage that larvae can fully assess their nutritional state. At this time, all increases in body size have ceased, and the size of a larva should accurately predict the final body size of that animal as an adult (as in insects generally, beetles do not grow as adults). If horn lengths match the size of a

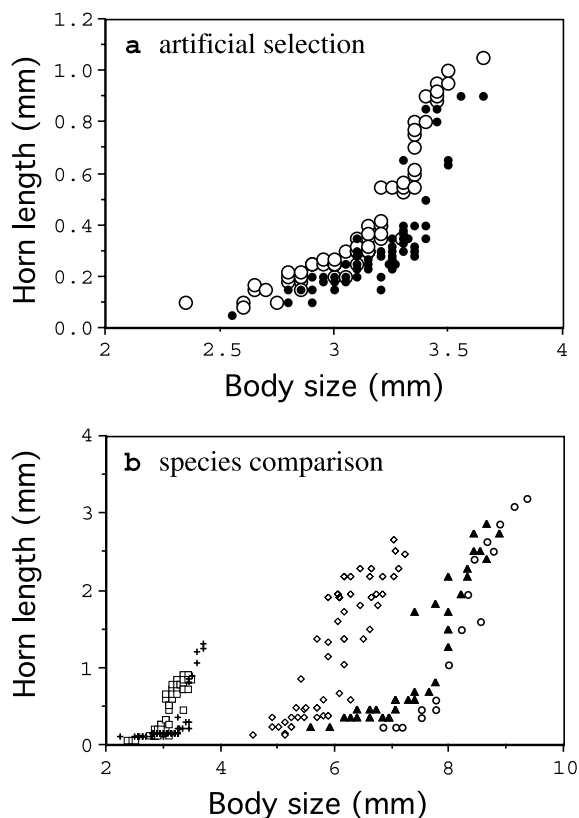


Figure 6. Evolution of a threshold. (a) Artificial selection for relatively long and relatively short horns in *Onthophagus acuminatus* shifted the threshold body size for horns. After seven generations of selection, males selected for relatively long horns (open circles) produced horns at a significantly smaller threshold body size than males selected for relatively short horns (solid circles). (b) Related species of *Onthophagus* also differ in the threshold body size for horns, suggesting that one major avenue for macroevolution in this genus involves changes in the threshold body size at which horn growth is initiated. Open squares, *Onthophagus acuminatus*; plus symbols, *Onthophagus marginicolis*; open diamonds, *Onthophagus striatulus*; solid triangles, *Onthophagus batesi*; open circles, *Onthophagus incensus*. All of these species produce a pair of horns that extends from the base of the head (as in Figure 1a). Modified from Emlen (1996).

larva during this prepupal period, they will also match the size of the adult that emerges.

Synthesis of horn development. The findings discussed in this section show that beetle horns result from localized regions of larval epidermis that undergo a very rapid period of growth immediately before pupation. The amount of proliferation in these localized regions of epi-

dermis ultimately determines the length of the adult horns. In *O. acuminatus* and *O. taurus*, horn growth is affected by how large each individual becomes, so that large male larvae show extensive growth in these epidermal regions and small male larvae show only minimal growth. The developmental switch between minimal and complete horn growth occurs abruptly and is associated with attaining a specific threshold body size.

Body size, in turn, is affected by the nutritional environment. Larvae encountering favorable conditions grow large, whereas larvae encountering poor conditions remain small. Consequently, effective matches between horn length and body size require a mechanism that translates the actual growth conditions encountered by each larva into levels of horn growth. In *O. taurus*, this translation is mediated by circulating levels of JH during a brief period just before pupation (and after all growth in body size has been completed). During this period, large males appear to have higher concentrations of JH than small males, and high levels of JH initiate growth of the cells that produce the horns. The threshold body size for producing horns thus appears to be a threshold of sensitivity to JH: cells encountering a sufficient concentration of JH initiate a period of exponential growth and produce long horns, whereas cells not encountering sufficient JH show only minimal horn growth. Because horn production takes place very late in the larval period, after all increases in body size have ceased, the amount of horn growth is matched with the actual individual variations in body size, even though body size is influenced by external environmental conditions encountered by larvae as they develop. The result is a facultative mechanism of horn expression, in which horns are produced consistently in only the largest males.

A developmental perspective adds to the study of beetle horn evolution

In addition to being interesting in its own right, an understanding of horn development has important implications for studies of beetle horn evolution. Information pertaining to this proximate regulatory mechanism enhances our perception and interpretation of sexual selection in beetles, illuminating how horns evolve, identifying constraints on horn evolution, and revealing hidden costs of horn production.

How horns evolve. Even a preliminary grasp of the mechanisms that regulate the expression of morphological traits can offer insights into how specific evolutionary changes arise. The low heritabilities for beetle horn length might be interpreted as indicating that these structures have limited potential for evolutionary modification. In fact, this conclusion is not true, as evidenced by the tremendous diversity of horn types present among even closely related taxa (e.g., Figure 1). By studying the mechanisms regulating horn growth, we can begin to appreciate

how horn evolution might have occurred.

Evolution of the threshold. For natural populations of *O. acuminatus*, there exists an average threshold body size at which the switch from minimal to complete horn growth occurs (3.35 mm; Figure 2). However, individuals within these populations vary slightly in the precise body size at which they initiate horn growth, and in *O. acuminatus* these differences are heritable (Emlen 1996). Evolutionary shifts in this threshold body size were generated through artificial selection experiments (Emlen 1996). After seven generations of selection, one population initiated horn growth at a significantly smaller body size than the starting population, and another population that had been selected in the opposite direction initiated horn growth at a significantly larger body size (Figure 6a).

Microevolutionary changes in the threshold size for horn production illustrate how genetic variation in components of a developmental regulatory mechanism can lead to evolutionary changes in the expression of morphological traits. Although shifts in the *O. acuminatus* threshold were generated in the laboratory, similar evolutionary changes have occurred in natural populations. Indeed, a comparison of several onthophagine species reveals that one major avenue of evolutionary change in this group has involved shifts in the threshold body size regulating horn growth (Figure 6b).

But what *is* a threshold trait, and how can it be modified by selection? By combining what is known about endocrine mechanisms that regulate the expression of threshold traits in other insects with results from experiments exploring horn development, I can now suggest a plausible scenario for how this threshold trait might evolve. Although several parts of this model are still untested (and several alternative possibilities exist), it allows us to begin to visualize how evolutionary changes in horn morphology may have arisen.

How might this threshold evolve? Growth in horn length is regulated by a hormone. Hormones regulate a diverse array of developmental processes in insects, yet insects have surprisingly few hormones (Nijhout 1994b). One of the ways a limited number of hormones can regulate so many processes is that not all cells are sensitive to a particular hormone at the same time. Often, specific tissues respond to hormones only during brief critical periods, or windows, of sensitivity (Nijhout 1994b). In this way, a single hormone may serve different functions at different times.

What determines when cells will respond to the presence of a hormone? Whether specific cells are sensitive to a hormone at any particular time depends on the receptors present in the cells: cells become sensitized to a hormone when they synthesize receptors for that hormone (e.g., Nijhout 1994b, 1999, Fujiwara et al. 1995, Jindra et al. 1996). The duration of time that receptors are present determines the length of the critical period when cells are sensitive, and the density of receptors presumably deter-

mines how sensitive those cells are to that hormone at that time. Cells expressing high densities of receptors should be more sensitive to the hormone (and should therefore respond to lower concentrations of the hormone) than similar cells expressing fewer receptors.

In *O. taurus*, the cells that will become the horns are sensitive to JH for a brief period at the end of larval development. Cells that are exposed to sufficiently large doses of JH during this period undergo rapid cell division and produce long horns. If these same cells do not encounter sufficient levels of JH, they do not produce horns. The threshold body size for horn production appears to correspond to a level of JH that is just high enough to bind to sufficient receptors to initiate horn growth. In this mechanism, whether horns are produced depends on two factors: first, how large each animal grows to be (which is reflected in the circulating levels of JH during the sensitive period) and, second, how sensitive that animal is to the level of JH (as determined by the concentration of JH receptors expressed during the sensitive period).

Levels of both JH and JH receptors can vary among individuals, and genetic variation affecting the levels of either could lead to an evolutionary shift in the threshold body size at which horn growth is initiated. Mutations affecting how much JH is secreted, when JH is secreted, and how quickly JH is removed or inactivated would all change the level of JH that is present during the sensitive period, and any of these mutations could result in a shift in the threshold body size for horn growth. However, mutations affecting circulating levels of a hormone are likely to affect the expression of other morphological traits as well. All other tissues that share the same sensitive period as horns would be affected by a change in hormone level (and most of the adult structures grow at the same time as horns; Figure 4). In contrast, the second possible avenue for horn evolution involves mutations whose effects are tissue specific. Mutations affecting the sensitivity of particular cells (e.g., the cells that produce the horns) to a circulating hormone will affect the expression of the structure produced by those cells but should not interfere with the endocrine regulation of other traits. For this reason, changes in the sensitivity of the target cells may be a more likely avenue for horn evolution than changes in levels of circulating hormone. Consequently, for the remainder of this article, I focus on the potential implications of evolutionary changes in the sensitivity of target cells.

If the cells destined to produce horns become sensitized to JH by synthesizing the appropriate receptors during the critical period, then it is possible that beetle genotypes vary in their sensitivity to JH by varying in the expression of JH receptors. Genotypes overexpressing receptors in horn imaginal cells would be more sensitive to JH and would initiate horn growth at a lower threshold concentration of hormone, and thus at a proportionately smaller body size. In this case, quantitative differences in the critical size for horn production could result from something

as simple as variation in the rate of receptor production, and it is tempting to speculate that the interspecific evolutionary changes in the relationship between male horn length and body size (Figure 6) were brought about in this way.

Evolution of horn location, horn size, and sexual dimorphism. Visualizing the mechanism regulating horn growth may inform us about other types of horn evolution as well. The genus *Onthophagus* is characterized by extensive variation in horn morphology. Species differ in the location of horns, the relative sizes of horns, and which sex expresses horns. For the first time, we can begin to envision how these evolutionary changes may have been brought about because each avenue of morphological diversification can now be interpreted in light of the basic regulatory mechanism described above.

Extant species of *Onthophagus* bear horns on several different parts of the body. Many species have horns that extend from the base of the head, as in *O. acuminatus* and *O. taurus* (Figure 1a; see also 1d). Other species have horns that extend from the front of the head, or clypeus (e.g., Figure 1f). Still others have horns that extend from the thorax (Figures 1g and 1h). In addition, some species exhibit multiple horns, with all possible combinations of horn locations (Figures 1b, 1c, and 1e), and it is clear from preliminary phylogenetic analyses that horn locations have changed multiple times within the history of the genus (Douglas J. Emlen, Clifford Cunningham, Leigh W. Simmons, Jen Marangelo, unpublished data).

If horn growth is initiated by localized clusters of epidermal cells expressing receptors to JH during the critical period, then changing which cells express the receptors may also change which regions of epidermis produce the horns (Figure 7a). In this case, evolutionary changes in the location of horns simply may require shifts in the specific clusters of epidermal cells that express receptors to JH during the horn-specific critical period. Consequently, it is possible to begin to visualize major (interspecific) morphological transformations that have occurred within this genus as having resulted from simple, graded alterations in JH receptor expression.

A second way that onthophagine horns differ is in their relative sizes. Horns range from enormous extensions that constitute more than 10% of total body mass in some species (e.g., Figure 1h) to tiny knobs in other species. Some species do not express horns at all. Again, we can now begin to understand how evolutionary changes in the relative sizes of horns may have been brought about. One possibility is that there have been changes in the number of cells that are sensitive to JH during the critical period for horn growth. Because these cells grow exponentially to produce the horns, changing the starting conditions (i.e., the size of the patch of epidermis that grows in response to JH) can lead to changes in the final horn size. Animals with many epidermal cells expressing JH receptors at the sensitive time for horn development would grow longer horns than animals with fewer dividing cells (i.e., fewer

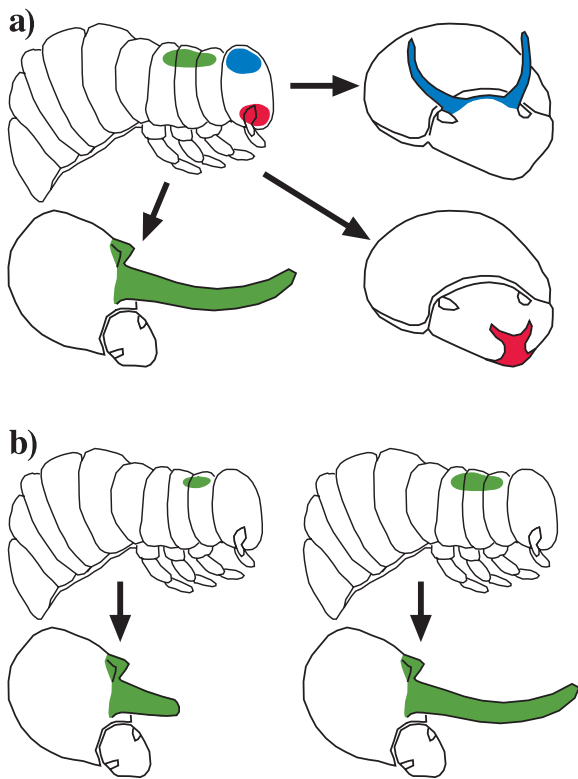


Figure 7. Proposed mechanisms for the evolution of horn location and relative horn size. (a) Epidermal cells sensitive to juvenile hormone (JH) during the critical period for horn determination (e.g., cells expressing receptors for JH during this period) proliferate rapidly to produce the horns. Genetic changes affecting which epidermal cells are sensitive to JH during the critical period should shift the body region exhibiting proliferation, altering the location of the resulting horns. (b) Genetic changes affecting how many epidermal cells are sensitive to JH at the start of this period of continuous proliferation should affect the rate of horn growth, altering the relative size of the resulting horn.

initial cells expressing receptors for JH; Figure 7b).

Finally, sex-specific expression of these same receptors may underlie the evolution of sexual dimorphism in horn production. Most species of *Onthophagus* are sexually dimorphic for horns. Female larvae encounter the same range of environmental conditions as do males, and female body size varies over the same range as males. Yet in virtually all species, females do not produce horns. The simplest way to achieve sexual dimorphism, given this regulatory mechanism, would be for females to not express JH receptors in the cells that would otherwise become horns. Females in these species would be insensitive to circulating JH (in terms of horn production), and consequently would not produce horns. This same mechanism could also account for the few exceptional species in which females do express horns (e.g., Figure 1e). By expressing

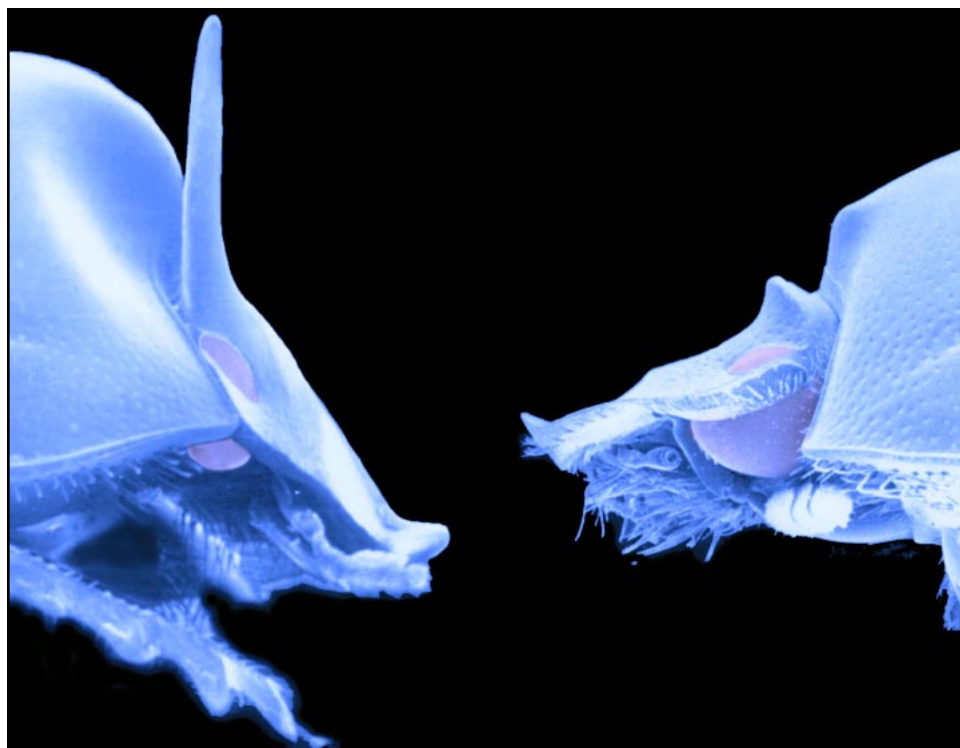
JH receptors, these females may be able to initiate patterns of growth typically expressed only in males.

All of these hypotheses about beetle horn evolution generate explicit predictions that should be testable in the near future. For example, the major differences between species are predicted to arise from changes in one or more of the following: which sex expresses receptors to JH at the appropriate time, which cells express receptors for JH at the appropriate time, how many cells express these receptors, and how many receptors are produced by each of these cells. One way to assess if, and how, any of these factors differ among species uses fluorescently labeled antibodies to characterize the cellular distribution of JH receptors. Once antibodies have been generated (JH receptors are only just being described; Wyatt and Davey 1996, Jones and Sharp 1997, Ashok et al. 1998), it should be feasible to rapidly assess where and when receptors are expressed (by assaying which regions of the larval epidermis bind the labeled antibody) and how many receptors are expressed (by quantifying how much binding occurs) in related species that differ in specific aspects of their horn morphology.

Although much of this analysis is preliminary and many alternative possibilities exist to explain the evolution of beetle horns (e.g., horn evolution could have resulted from changes in hormone levels or from evolution of downstream patterning and growth genes that respond to hormone signals, instead of from changes in the expression of hormone receptors), this approach illustrates how even a rudimentary understanding of developmental mechanisms can help fill in the “black box” connecting genotype with phenotype. By learning how horn expression is regulated, we can begin to think about how horn expression might be modified. Specifically, we can explore how perturbations to the mechanism of horn expression would affect the resulting phenotype, and, using these results, we can begin to think about what types of modifications are likely to have occurred in the past to generate the diversity of forms that exists today.

Constraints on horn evolution. Developmental processes translate genotype to phenotype, and modifications to developmental mechanisms generate morphological variation within populations. Once generated, variant individuals act and interact within their social and physical environments, and those individuals that are most successful at surviving and reproducing contribute disproportionately to subsequent generations. When specific traits or combinations of traits contribute to the relative success of individuals within populations, and when variation in the expression of these traits is heritable, then populations may evolve toward increased expression of the relevant traits. However, natural and sexual selection can act only on available phenotypic variation. Consequently, evolutionary responses to selection may be limited by the variation that the developmental mechanisms are capable of

Figure 8. Tradeoff between horns and eyes in *Onthophagus acuminatus*. Males with long horns have proportionately smaller eyes than males with shorter horns. Images have been colorized to highlight the eyes and scaled so that the beetles are shown as approximately the same size.



generating (Alberch 1982, Oster et al. 1988, Arnold 1992).

If developmental processes were infinitely labile, then any phenotypic variant should be possible, and, in theory, such a population should be capable of evolving in any direction. Developmental phenomena often are not infinitely flexible, however, and certain morphologies may be mechanistically much easier to produce than others. In fact, in some cases, specific morphologies (or combinations of traits) are extremely difficult—or even impossible—to produce, given a particular developmental mechanism. In these cases, the translation of genotype to phenotype leads to a biased production of morphologies, so that some forms are produced far more readily than others. By biasing the generation of phenotypes within a population, developmental processes can influence the likelihood of various directions of evolution, thus biasing or constraining the evolution of those lineages (Alberch 1982, Arnold 1992). Our developmental studies (e.g., Nijhout and Emlen 1998) concerning the regulation of expression of beetle horns suggest that constraints operate to limit the range of possible beetle morphologies.

Beetle horns versus beetle eyes. The phenotypic expression of horns is not independent of all other morphological traits. In natural populations of both *O. acuminatus* and *O. taurus*, horns are negatively correlated with eyes; males with unusually long horns also have relatively small eyes, and males with small or no horns have relatively large eyes (Figure 8; Nijhout and Emlen 1998). In addition, males in general have smaller eyes than females, which do not produce horns (Douglas J. Emlen, unpublished observations). Such negative phenotypic correlations can result from a number of causal factors (e.g., Falconer 1989). For example, this pattern could result from horns and eyes exhibiting inverse but *independent* responses to the larval environment. In this case, evolutionary changes in one of the traits (e.g., horn enlargement) need not affect the expression of the other trait (eyes), and it would be inaccurate to consider the present association among horns

and eyes as indication of a constraint on the evolution of either trait. An alternative possibility is that this phenotypic correlation results from a limitation in the capacity of the underlying regulatory mechanism to generate morphological variants. For example, if a resource allocation tradeoff mandates a negative correlation between horn length and eye size, then any evolutionary modification to the relative size of one of the traits will necessarily influence the expression of the other. Such a finding would indicate a bias in the generation of phenotypic variation because individuals with large horns *and* large eyes would be difficult to produce. It would also constitute a true source of constraint on the evolution of beetle shape because enlargement of one trait would occur only at the expense of the other.

To examine whether the negative phenotypic correlation between horns and eyes reflects a limitation in the range of phenotypes that can be generated by this regulatory mechanism, my collaborator and I used three separate experimental methods (JH application, diet manipulation, and artificial selection) to manipulate male allocation to horns during development (Figure 9; Emlen 1996, Nijhout and Emlen 1998). Each experiment generated populations of males that differed in the relative lengths of their horns. In all three cases, males induced to produce relatively long horns had smaller eyes, and vice versa.

The first two of these methods (JH application and diet manipulation) operated within a single generation of beetles. Topical applications of methoprene (a JH mimic) to feeding-stage larvae and manipulations of larval diet quality each affected the relative length of horns produced by developing males (Emlen 1997b, Nijhout and Emlen

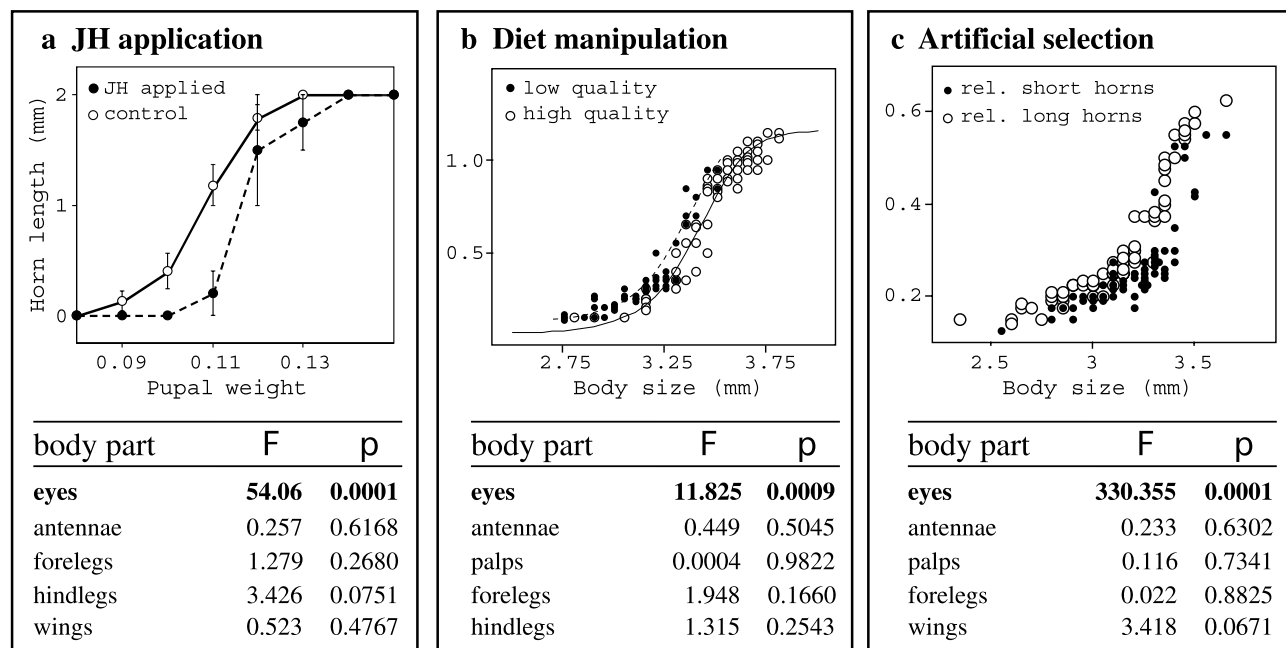


Figure 9. Perturbation of male horn expression and correlated effects on other traits. (a) Topical application of a juvenile hormone mimic (methoprene) to early third instar larvae of *Onthophagus taurus*. (b) Manipulation of the quality of larval diet in *Onthophagus acuminatus*. (c) Artificial selection on the relative length of male horns in *O. acuminatus*. All of these treatments caused males to shift their allocation to horns during development. In each case, experimental manipulation of male allocation to horns was accompanied by inverse changes in the relative sizes of male (but not female) eyes. Other traits were not affected (F-values are from analyses of covariance, with body size [prothorax width] as covariate and treatments as indicated). Modified from Nijhout and Emlen (1998).

1998). By altering male investment in horns during growth, these experiments revealed a developmental linkage between these two traits. Horn expression was inversely coupled with eye expression, and perturbing the expression of one trait influenced expression of the other. The third experiment altered male investment in horns across generations by artificially selecting on the relative length of the horns. This evolutionary change in male investment in horns also brought about an inversely correlated response in the eyes: males selected for unusually long horns also had small eyes, whereas males selected for unusually short horns had large eyes. This result indicated that the negative correlation between horns and eyes can bias the morphological evolution of these traits.

The observation of a negative genetic correlation between male horns and male eyes indicates that there is a limit to the range of phenotypes that can be produced; males with long horns and small eyes are quite easy to produce, as are males with small horns and large eyes, but males with long horns *and* large eyes are very difficult to generate. This paucity of certain shapes in beetles may explain why evolution of relative horn length also has brought about a correlated (although inverse) change in relative eye size. It also suggests that there may be an upper limit to enlargement of male horns and illustrates one way that a developmental process may bias or constrain evolution.

What has a developmental perspective contributed? It is possible to describe these same sources of bias without understanding how the relevant traits develop or how their growth is regulated. Measures of the phenotypes alone, especially in conjunction with controlled breeding designs or knowledge of parentage, can reveal nonrandom associations among traits—that is, correlations between the expression of one trait and that of others (e.g., Falconer 1989, Roff 1997). Quantitative genetics theory permits us to predict the effect of trait associations on the short-term evolutionary response to selection, using these measures of genetic correlations among traits (Falconer 1989, Roff 1997). What additional insights are gained with the developmental perspective? What, for example, have we learned that could not have been learned from the artificial selection experiment alone?

The observed response to artificial selection was sufficient to reveal a negative genetic correlation between male horns and male eyes, and from this information it is possible to quantify the extent to which a tradeoff between horns and eyes will constrain the independent evolution of either trait (e.g., enlargement of horns may be hindered by the inverse effects on eyes). But this result is not generalizable; it cannot legitimately be applied to other populations or other species. The developmental perspective, by contrast, helps reveal why the negative genetic correlation

exists—that is, why it is the eyes, rather than some other trait or traits, that are affected by horn growth. Characterizing how horns influence the expression of eyes in *O. taurus* and *O. acuminatus* provides a foundation for predicting how similar tradeoffs may be manifest in related species.

Beetle horns versus other traits. In *O. taurus* and *O. acuminatus*, horns and eyes appear to interact because these traits grow in close physical proximity; they are adjacent to each other in developing larvae (Nijhout and Emlen 1998). Although the resource or signaling factor leading to interactions among morphological traits has not been identified (for review, see Stern and Emlen 1999), the behavior of this signaling factor is similar in several insect groups. Traits in close physical proximity interact and/or compete with each other more directly than traits that are more distant from each other (Klingenberg and Nijhout 1998, Nijhout and Emlen 1998).

This simple observation has several implications. For beetles, it means that exaggerated horns should affect the expression of adjoining or neighboring structures. For species such as *O. acuminatus* and *O. taurus*, the negative genetic correlation between horns and eyes probably results because horns and eyes develop in close proximity (at the base of the head; blue region in Figure 7a). But what about other beetle species? Related species possess all types of horns (Figure 1), and many of these horns are not physically adjacent to the eyes.

Consequently, the tradeoffs for other species should be very different. Species with large thoracic horns should show negative correlations between horns and wings because thoracic horns and wings develop in close proximity in larvae (green region in Figure 7a). Indeed, this tradeoff between horns and wings has been documented in a related family of horned beetles: Phenotypic measures of four species of dynastid beetles with large thoracic horns showed negative correlations between wings and horns; horned males had proportionately smaller wings than hornless males or females (Kawano 1995, 1997). Similarly, species with horns on the clypeus are predicted to show tradeoffs between horns and antennae or mouthparts, because these structures develop adjacent to the clypeal horns (red region in Figure 7a).

Although horn evolution in all of these species may be constrained by the same type of negative interaction with neighboring structures, the precise nature of the constraint is predicted to differ depending on the physical location of the horns. This pattern could not have been predicted from the artificial selection experiment alone. In fact, had we tried to generalize from that result, the prediction would have been horn versus eye tradeoffs for all beetle species expressing horns. Thus, an understanding of the mechanism generating correlations among traits in two species made it possible to predict how similar biases may be manifest in other taxa. A variety of complex, species-specific predictions all arose from a relatively basic

understanding of the developmental phenomenon.

In summary, when genetic correlations among morphological traits are identified using quantitative genetic techniques alone (e.g., controlled breeding designs), the reasons for the trait correlations remain obscure. Although these tools may be effective for describing existing biases to short-term evolution within a focal population, they are not transferable to other populations or other species. Only by beginning to explore aspects of the development of a trait can we begin to identify the causes of bias or correlation among traits. And only with this insight can we begin to generalize—to transfer our results from one population to other populations or other species.

Hidden costs of producing horns. The genetic correlations between horns and other traits reveal a previously unanticipated cost to the expression of beetle horns: the expression of horns reduces the relative size of adjoining traits. When these adjoining traits are themselves important to survival or reproductive success, then the fitness consequences of their reduction may offset the advantages gained by horn growth.

In *O. taurus* and *O. acuminatus*, the relatively small eyes in males with long horns result from reductions in the numbers of facets, or ommatidia, that make up the compound eye. Fewer facets means that these males will have both a smaller total visual field and poorer image resolution than males with larger eyes (analogous to watching a computer monitor with fewer pixels). The result is a tradeoff between male fighting ability and male vision. In other species, horn growth is predicted to reduce the relative size of a male's wings. Males with thoracic horns will be heavier (because of the horn), yet they may need to fly with disproportionately small wings. As a result, the flight efficiency of horned males should be reduced (see Dudley 1992), leading to a tradeoff between fighting ability and dispersal capacity. In still other species, horns may reduce the relative size of antennae or mouthparts. If males with horns at the front of the head have proportionately smaller antennae, then they may not be able to locate dung or females (both of which are located using olfactory cues) as effectively as males without horns. The most important point is that the type of tradeoff differs, depending on the location of the horns. The functional price that a male pays for producing horns should therefore depend on the type of horn produced.

Sexual selection can lead to rapid evolution of exaggerated weapons in males (reviewed in Andersson 1994). One look at a genus such as *Onthophagus* and it is clear that the evolution of weapons can follow many different trajectories, leading to radically different morphological outcomes (e.g., Figure 1). What the developmental perspective adds to this view of sexual selection is the realization that these different trajectories, once started, may carry with them very different consequences for the males, and that, as a

result, the weapons (horns) may be much more costly in some species than in others.

We can now ask the question: is the horn morphology manifest in each species an accident of history? Or do certain ecological or social situations favor horns in specific locations (e.g., at the back of the head, thorax, or front of the head)? For the first time, instead of just looking at the use of *horns* in each of these environments, we can also look at the other affected traits (eyes, wings, or antennae). These additional factors may help identify unanticipated—even counterintuitive—links between the diverse interspecific variation in onthophagine horn morphology and the equally diverse array of biotic and abiotic environments inhabited by the species in this genus.

For example, McIntyre and Caveney (1998) predicted that large eyes would be more important in nocturnal beetles than in diurnal ones, and in a comparative study of nine species of onitine dung beetles (Coleoptera: Scarabaeidae) they observed a significant correlation between the size of the compound eyes and daily patterns of flight activity. Their argument can be extended by suggesting that horns located at the base of the head, because they lead to reductions in eye area, may be more costly to nocturnal species than to diurnal ones. In this case, one testable prediction would be that within *Onthophagus*, nocturnal species would be less likely to have horns at the base of the head than diurnal species (unfortunately, McIntyre and Caveney did not provide descriptions of the horn morphologies of these species). Similar logic can be applied to the tradeoff between horns and wings. All dung beetles must fly from dung source to dung source, but this dispersal capacity may be more crucial in some habitats than in others. Species using dung that is sparsely or widely distributed may rely much more heavily on flight capabilities than similar species that use dung that is more densely or uniformly distributed. Thoracic horns, if they reduce flight efficiency, should be particularly costly in species that rely most heavily on dispersal, leading to another testable prediction relating ecology with horn morphology.

All of these potential costs to the expression of horns were recognized only as a result of studying the developmental mechanisms regulating the expression of the horns. In each of these examples, the proposed link between horn morphology and habitat has little to do with the shape or morphology of the horn itself. Instead, these predictions stem from developmental linkages arising between horns and adjoining traits. Thus, the integration of development with studies of evolution has revealed new and unanticipated avenues for future research. By revealing the interconnectedness of morphological traits, and thus the allocation tradeoffs associated with horn growth, studies of a developmental mechanism have shed new light on our understanding of the processes of sexual selection and the evolution of extravagant morphological weapons in beetles.

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