Diversity in the Weapons of Sexual Selection: Horn Evolution in Dung Beetles

DOUGLAS EMLEN

Animal weapons take a breathtaking variety of forms, from the antlers of a bull elk, caribou, or moose, to the claws of crabs, the distended jaws of stag beetles, or the long tusks of a mastodon, walrus, or narwhal (Figure 1). All of these weapons are thought to have arisen from sexual selection: an evolutionary history of intense competition by males over access to a limited number of reproductive females. These structures are impressive because of their exaggerated sizes (a fiddler crab's claw can be more than half of his total body weight), but especially because of their diversity. Even closely related species of deer can differ dramatically in the type or shape of their antlers, and similarly diverse variations in weapon form characterize crabs, shrimp, sheep, lizards, bugs, and beetles. Although the basic functions of animal weapons have been well understood for decades (e.g., red deer stags use their antlers in fights over harems of females), the incredible diversity of animal weapons remains a mystery. It turns out that we know astonishingly little about how or why such weapons have diverged in form. Here, I relate an empirical journey into this void by describing a series of experiments and several very different approaches that my colleagues and I have used to study one specific class of animal weapons: beetle horns.

Literally tens of thousands of beetle species bear horns (Figure 2). Typically, these horns are expressed in male beetles (females sometimes have rudimentary horns, but they often lack horns entirely), and, in every species studied to date, these horns are used by males in battles over reproductive access to females. Many species of horned beetle (e.g., rhinoceros beetles) fight over sap that oozes from the trunks of trees where females visit to feed; other species fight over branches or bamboo shoots, or over burrows excavated into the stems of plants or into the soil.

In this essay, I will focus on one genus of horned beetles, a genus of dung beetles called *Onthophagus*. Males in most species of this genus are armed with horns, and, relative to their body sizes, these horns can be enormous—sometimes more than 15% of the total weight of the animal. There are more than

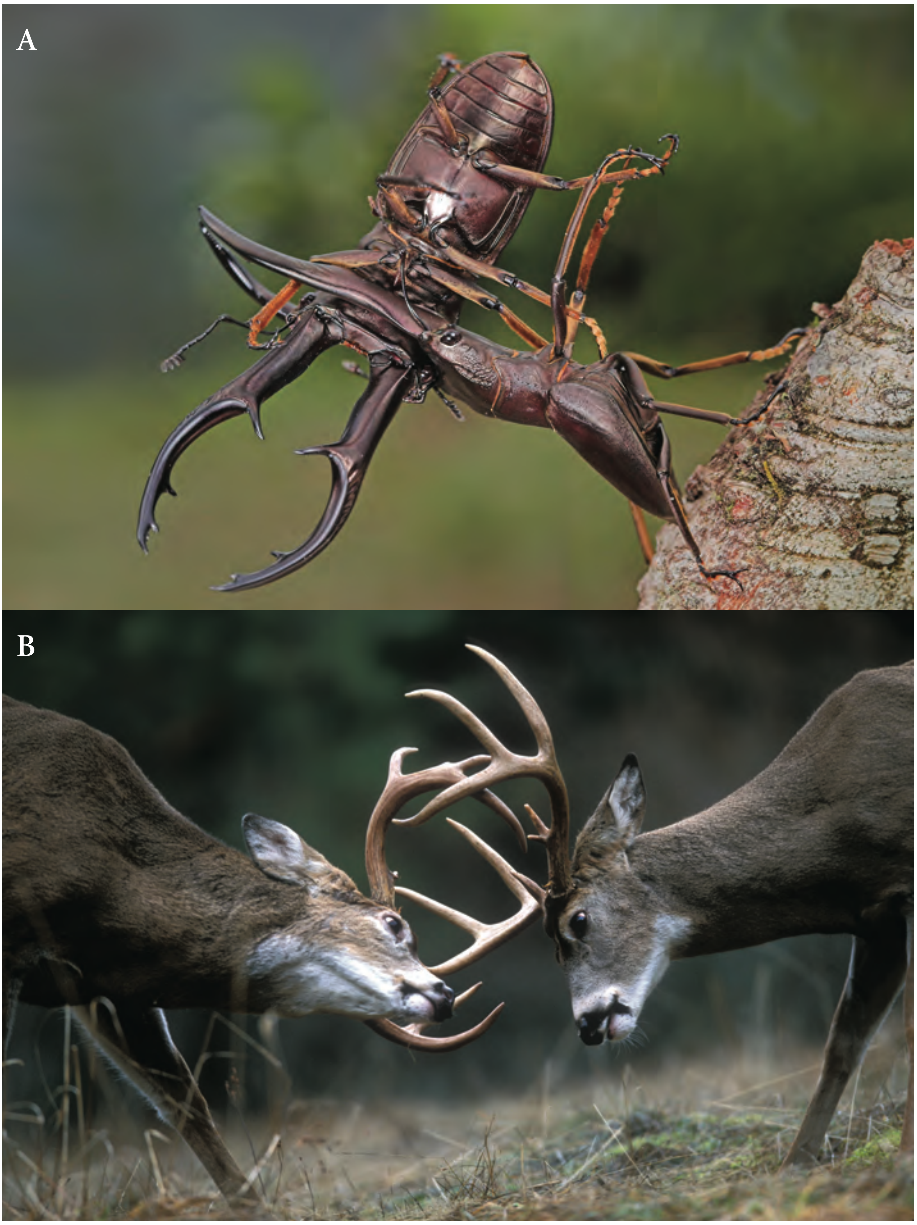


FIGURE 1 Stag battles. (a) Lucanid beetles, *Cyclommatus metallifer*. (Igor Siwanowicz) (b)Whitetail deer, *Odocoileus virginianus.* (Donald M. Jones)

FIGURE 2 Beetle horns.

2,000 species already described in this genus, and the morphological characters most divergent among these species are the horns. Onthophagine horns vary in size, they vary in shape, and they even vary in the physical location on the body from which they grow.

FIGURE 3 shows a front view of the head and thorax of each of 17 species of *Onthophagus* beetles, with the horns color-coded according to their respective physical locations. Horns can extend from the back of the head (blue). They can extend from the middle and front of the head (red and purple, respectively), and they can extend from the center or sides of the thorax (green or orange). Thus, in beetles, there are at least five developmentally distinct physical locations for horns, and different species can exhibit all possible combinations of these horn types.

In order to characterize the historical transformations in horn morphology that have occurred over the evolutionary history of these beetles, my colleagues and I constructed a phylogeny using DNA sequence data from 48 different species. Phylogenies cluster species into groups based on the similarities of their DNA, and they can be used to estimate the historical transitions by

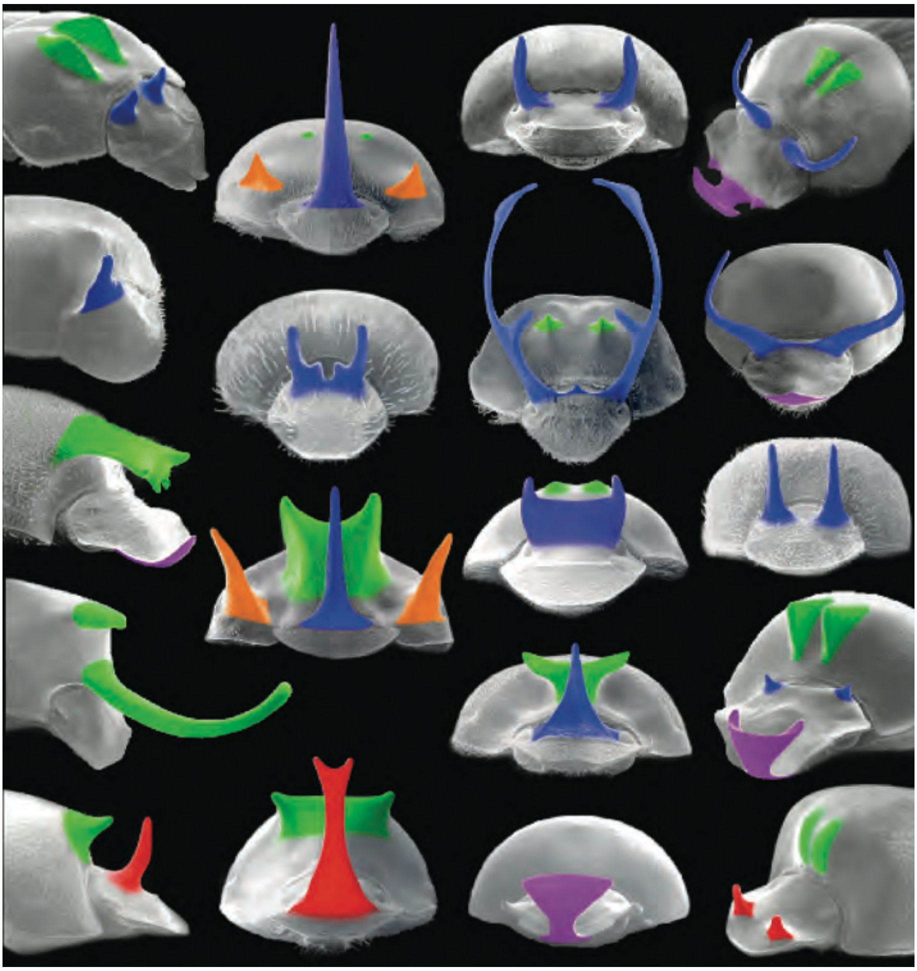


FIGURE 3 Weapon diversity in the dung beetle genus *Onthophagus* (horns colored based on their physical location).

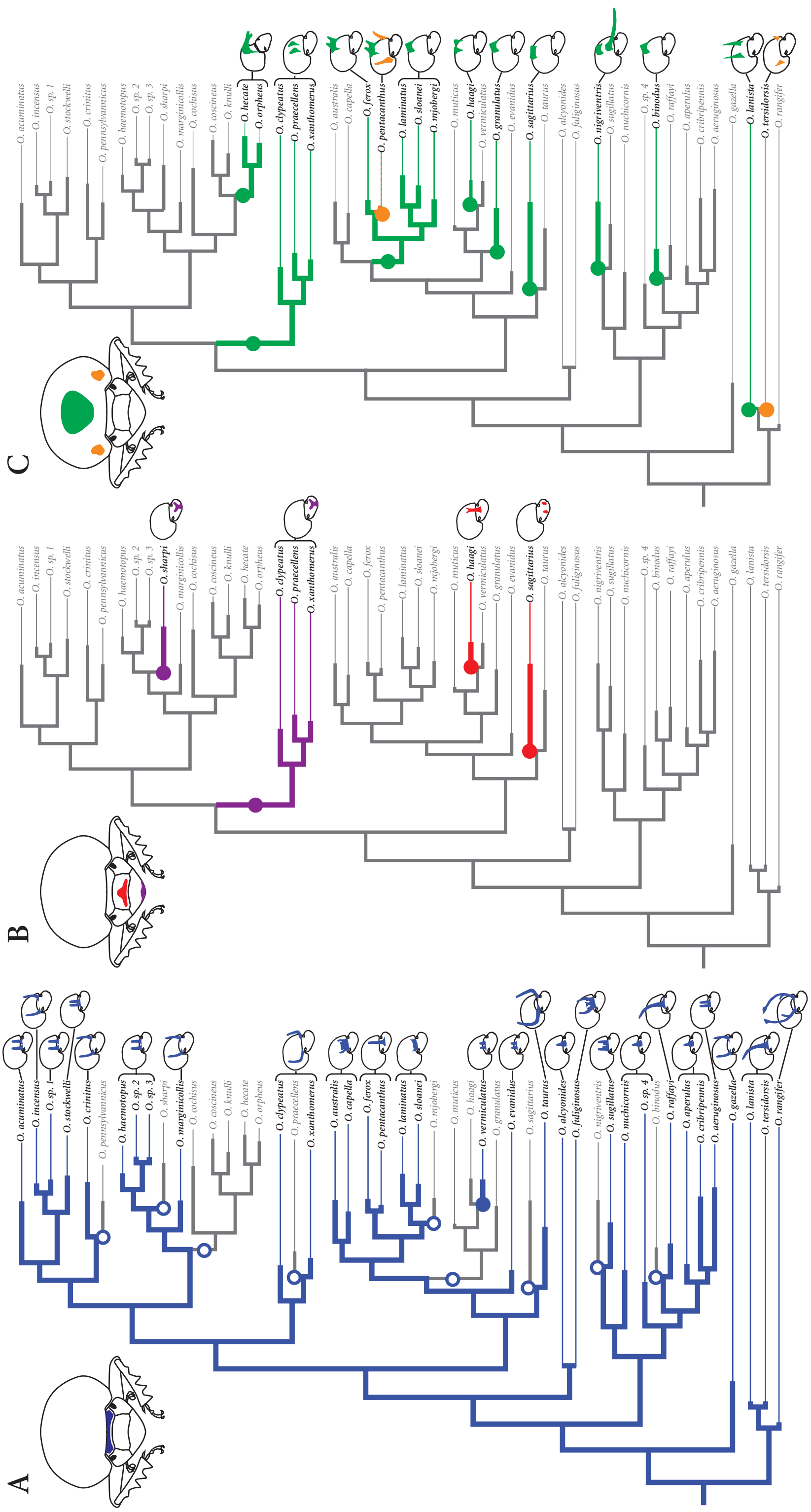
which ancestral populations were divided, modified, and divided again, until they became the diverse arrays of species that we observe around us today. The result is a phylogenetic "tree"—a nested series of branches depicting the ancient evolutionary trajectories of the organisms in question. We can use phylogenies as a backdrop to trace the evolutionary histories of specific traits of interest (e.g. the horns of beetles).

We first used our tree to ask a very basic question: did beetle horns evolve only a few times or did they evolve many times? In other words, are these large, complex weapons difficult to attain (in which case we would expect to see one or only a few instances in which horns arose in the evolutionary tree) or are they much more evolutionarily labile, cropping up and disappearing repeatedly in the evolutionary history of the beetles? (We later use this same phylogenetic tree to ask a different sort of question—whether the evolution of horns was correlated with specific ecological situations or habitats encountered by the beetles).

FIGURE 4 shows the best supported phylogenetic tree, onto which we have reconstructed evolutionary gains and losses of each of the five different types of horns. These ancestral reconstructions are based on the distribution of traits among living species. If two species that are most closely related to each other (called "sister species") have the same type of horn, then we assume that their common ancestor did as well. When sister taxa (either living species or inferred ancestors) have horns of a different type, we employ an algorithm that minimizes the number of evolutionary transitions that are inferred to have occurred. We accept this as our working hypothesis, rather than using a reconstruction that implies a greater number of evolutionary changes.

To make our reconstructions of horn evolution easier to see, the tree is printed three separate times (FIGURE 4, PARTS A–C). Beetle lineages that our data suggest had a particular horn are shown in bold, with the color corresponding to the type of horn in question; lineages that we infer lacked the horn are shown in gray. From this mapping of horn states onto our phylogenetic tree, we see that horns at the back of the head (blue) appear to have been present in the ancestors of this genus (branches at the base—left—of the tree), and today they occur in males of most extant taxa. Yet there have been numerous losses of this horn type (nine losses, and one instance of regaining the lost horn, to be specific). Horns on the middle of the head (red) and on the front of the head (purple) each appear to have been gained two separate times, and we see even more independent gains of horns on the center (green) and sides (orange) of the thorax (nine and two gains, respectively). All told, this initial sampling of only 48 species of one genus (a mere 2% of the species in this genus) revealed 25 changes in the physical location of horns, with at least 15 independent gains of novel horn types,

FIGURE 4 provides just a taste of the full explosion in beetle form because it considers only one type of variation in weapon expression: changes in the physical location of the horns. Horns also vary extensively in overall size and shape. If we look at any of the different types of horns—for example, the ones protruding from the base of the head—we see repeated transformations in the size and shape of those horns (FIGURE 5), and this applies to all of the other horn types as well. Clearly, the horns of beetles have diverged prolifically in form.


A
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O. crinitus
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O. haemotopus
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O. sharpi
O. marginicollis
O. cochisus
O. coscineus
O. knulli
O. hecate
O. orpheus
O. clypeatus
O. praecellens
O. xanthomerus
O. australis
O. capella
O. ferox
O. pentacanthus
O. laminatus
O. sloanei
O. mjobergi
O. muticus
O. haagi
O. vermiculatus
O. granulatus
O. evanidus
O. sagittarius
O. taurus
O. alcyonides
O. fuliginosus
O. nigriventris
O. sugillatus
O. nuchicornis
O. sp. 4
O. binodus
O. raffayi
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O. cribripennis
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O. gazella
O. lanista
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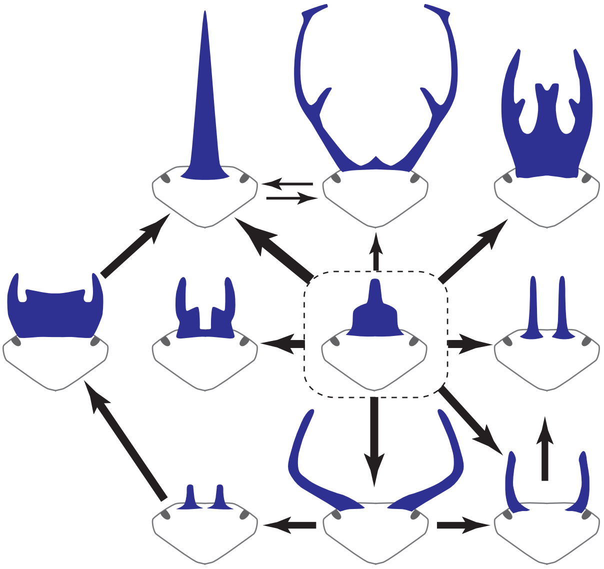


Figure 4 (facing page) *Onthophagus* phylogenetic tree showing evolutionary gains and losses of male horns. (a) Horns at the back of the head (blue); (b) Horns on the middle (red) or front (purple) of the head; (c) horns on the middle (green) or sides (orange) of the thorax. Closed circles = gains of new horns; open circles = loss of ancestral horn. Figure 5 Diversity in horn shape. Arrows depict evolutionary transitions in head horn morphology revealed by our phylogeny.

It turns out that there have been numerous spectacular evolutionary radiations in animal weapon form. Indeed, we see dramatic divergence in the shape of antlers in deer and in the horns of antelope, chameleons, and dinosaurs, and we see similar divergence in the shapes of the claws and chelipeds of isopods and crabs, and even in the spiny protrusions on the exoskeletons of trilobites. But why should these structures be so diverse? In each of these animal groups, the form of the weapon has changed dramatically so that even very closely related species differ in the details of their armaments—where they point, how they curve, twist, or branch, and, in many animals, where on the body they develop. Why don't all deer species have similar antlers, and why don't all horned beetles have the same type of horn? To begin to address this question for the beetles, we turned to the natural histories of these animals, to examine how beetles actually use their horns.

In all of the beetle species that have been studied thus far, males use their horns in fights over access to females, and the single best predictor for which species have these huge weapons and which do not is the substrate on which their contests occur. Almost without exception, species with large horns fight over linear, or otherwise spatially restricted, resources. In some cases (e.g., in many of the dynastine "rhinoceros" beetles), it is branches of trees, or new shoots of bamboo; in other cases, it is localized wound sites with oozing sap on the sides of trees; and in still others, the fights occur inside tunnels, such as hollowed-out stems of sugar cane. Spatially restricted, defensible resources appear to be a critical prerequisite for the evolution of weapons in insects.

For members of the genus *Onthophagus*, this means tunnels beneath dung. Whether in the Australian outback, the African savannah, or a tropical wet forest, all *Onthophagus* species do the same basic thing: females dig tunnels into the soil directly beneath pieces of dung, and males guard the entrances to these tunnels (FIGURE 6). Males fight vigorously with other males over access to tunnels, and males that are successful in defending a tunnel will mate repeatedly with the resident female. Male beetles use their horns in these fights—males brace themselves against the tunnel walls, and use their horns to block, pry, or dislodge intruder males—and males with the longest horns usually win these contests. This can translate into a fertilization advantage for males with the longest horns, with the result that large males experience positive, directional sexual selection for increases in horn length. Specifically, success at these encounters depends on a male's horn length relative to the horn lengths of his rivals. This simple fact has profound implications for horn evolution, because there will be no set "optimum" horn size. As one increase in horn length spreads through the population (because these males are best at defending their tunnels and siring progeny), it will reset the baseline horn length in that population, and, subsequently, will favor the next increase. This, then, will favor yet another increase in horn length, and so on, in an "arms race" that can lead to a continuing escalation in investment by males into the production of horns.

In fact, we see evidence for just such a history of intense sexual selection and weapon escalation. Our phylogeny suggests that the size of the beetles' horns has increased repeatedly, in some cases leading to truly outrageous proportions. In three separate lineages, horn lengths now exceed the total length of the rest of the male's body. The number of horns has also increased repeatedly—at least 21 separate times—resulting in species with two, three, or as many as five different horn types.

Thus, the tunneling behavior ubiquitous among these beetles sets the stage for their mating system, and it generates what appears to have been consistent, and intense, directional sexual selection for increased investment in weapons. This observation may help to explain three things: why so many dung beetle species have horns, why these horns can be so enormous, and why so many species have multiple horns. The big question that still remains, however, is why

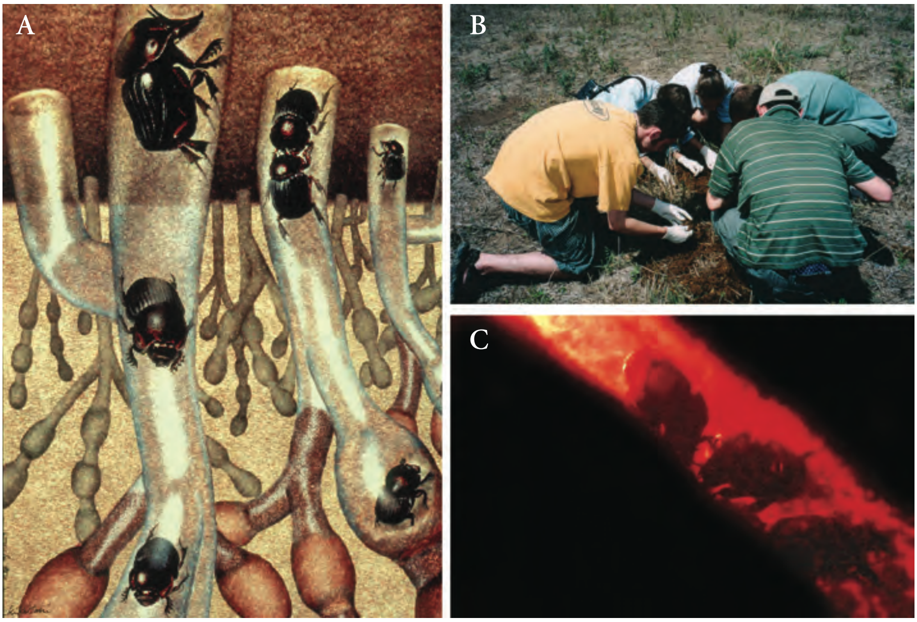


FIGURE 6 (a) Female *Onthophagus* dung beetles bury dung inside tunnels to use as provisioning for eggs. Large-horned males fight to guard tunnels from rival males. Small hornless males sometimes sneak into guarded tunnels (using a side tunnel) to mate with the female on the sly. (U. Kikutani) (b) Collecting dung beetles in Africa. (Don Christian) (c) Males in battle inside a tunnel—note female to the right. (Doug Emlen).

are beetle horns diverse? It is not at all clear why the types of horns that are produced should change. Yet the physical location of horns has changed many times. The ancestral horn type was lost at least nine separate times, and novel horns were gained at least 15 times, resulting in species with everything from no horns to multiple horns, and all possible combinations of horn types. Today, even closely related species differ markedly in the shape and physical location of their horns. What historical factors led to this incredible diversity in weapon morphology?

In the following sections, I discuss two alternative possible explanations. Both of these hypotheses explore associations between specific horn morphologies and various aspects of beetle habitat or ecology. They look at how selection may have (or may not have) shaped patterns of horn evolution in such a way as to generate divergence in horn morphology—changes from one horn type to another.

The first putative mechanism focuses on the function, or the benefits, of particular horn types. We've just seen what these horns do—how, and in what contexts, they are used. Could selection on the utility (or the function) of horns have favored changes in form from one horn type to another? If there were differences in the utility of horns across environments, then it is possible that colonization of new or different habitats could have generated selection for new or different horn morphologies. The critical prerequisites for this are either that horn types function differently—they do different things in different environments—or that the horn types performing best in one environment not be the same as the horn types performing best in other environments, leading ultimately to the evolution of functional associations between horn morphology and ecology.

This perspective has been applied to a variety of other animals to explain diversity in sexually selected structures. Color ornamentation in male fish, for example, may stand out from backgrounds differentially, depending on the characteristics of each specific stream habitat. In such a situation, female preferences for conspicuous males may have generated habitat-associated divergence in ornamentation. Similarly, for ungulates, changes both in habitat and in body size are thought to have led to shifts in the style of combat between males, and this is thought to have favored divergence in the shape of their weapons (e.g., antlers or horns).

To apply this logic to the beetles, we must consider the habitats within which horns function. Specifically, we have to ask whether the mating system (including the function of the horns) varies from habitat to habitat, and whether the horn types performing best in one environment differ from the horn types performing best in other environments. However, when we do this, we run into a problem. These beetles do indeed inhabit an incredible breadth of habitats, and they feed on almost every type of dung imaginable: deer, toad, bison, zebra, monkey, kangaroo, elephant—you name it. If it produces dung in any respectable quantity, then there are probably *Onthophagus* species that feed on it.

The problem is that all of this variation in habitat and ecological circumstances may be irrelevant to the function of beetle horns. Habitat does not affect the mating system, or the social or physical context in which the horns are used. Once these beetles find dung, they all do the same thing: they dig tunnels into the soil below, and all of the fighting and mating takes place inside these tunnels below ground. This means that, irrespective of continent, climate, or habitat, all of these beetles use their horns in the same setting: inside cylindrical tunnels below ground.

Surely all tunnels are not precisely the same. A tunnel in the hard desert soil of the Australian Outback is going to differ somewhat from a tunnel in a tropical wet forest. But we are still talking about tunnels: dark, cylindrical underground tubes that must be blocked to exclude other males, and over which males fight for control. The crucial point here is that beetle horns in the Australian outback do the same basic thing as beetle horns in a tropical forest. So, the horn types do not appear to have different functions, and the immediate ecological contexts in which horns are used appear to be very similar across species. This contrasts markedly with the situation observed in guppies and ungulates, and it is not what we would expect if habitat-specific selection for divergent horn functions were driving this massive radiation in weapon morphology. I will not argue that there are no functional associations between the type of horn and the habitat or context in which it is used, merely that we have been unable to find any compelling patterns for the different horn types among dung beetles thus far. But this situation has caused us to turn our attention to an alternative hypothesis.

Horns may incur costs differently across habitats, with some horn types being more expensive than others in each ecological situation. In this case, selection to minimize the costs of horn expression could have led to evolutionary shifts in horn location. This mechanism is similar to our first hypothesis, in that it predicts a match between horn morphology and ecology, with one very important difference. Here, the predicted evolutionary associations have nothing to do with the actual function, or benefit, of horns; instead, they focus on costs. The critical prerequisites for this mechanism are that the costs of horns must not be the same for all horn types, and that some horn types must be more expensive than others in each environment.

What kinds of costs could meet these criteria? For the past decade, my colleagues and I have been studying the development of beetles, including their metamorphic transformations from larvae to adults. These studies have permitted us to explore how, where, and when the horns grow, how horn growth is regulated, and, most recently, which genes and pathways control horn growth. These explorations into mechanisms led us to just such a cost of horn expression. It arises because of two key aspects of horn development: when the horns grow, and where the horns grow.

Beetles pass through three larval stages and a pupal stage before molting into an adult (FIGURE 7). Most of the larval period is devoted to feeding, and this is when all growth in overall body size occurs. But then the animals stop feeding, and they actually purge their guts of all contents in preparation for metamorphosis. The end of the final larval stage (when the animals have emptied their guts) and all of the pupal stage are nonfeeding parts of the life cycle. Animals are “closed systems” at this time, and all of the metamorphic transformation occurs using resources that were set aside prior to the purging of their guts.

It turns out that all of the major adult structures grow during this nonfeeding period, including the eyes, wings, antennae, genitalia, and horns. These structures form from localized clusters of cells that begin a burst of proliferation and growth right at the end of the larval period. The horns grow as long

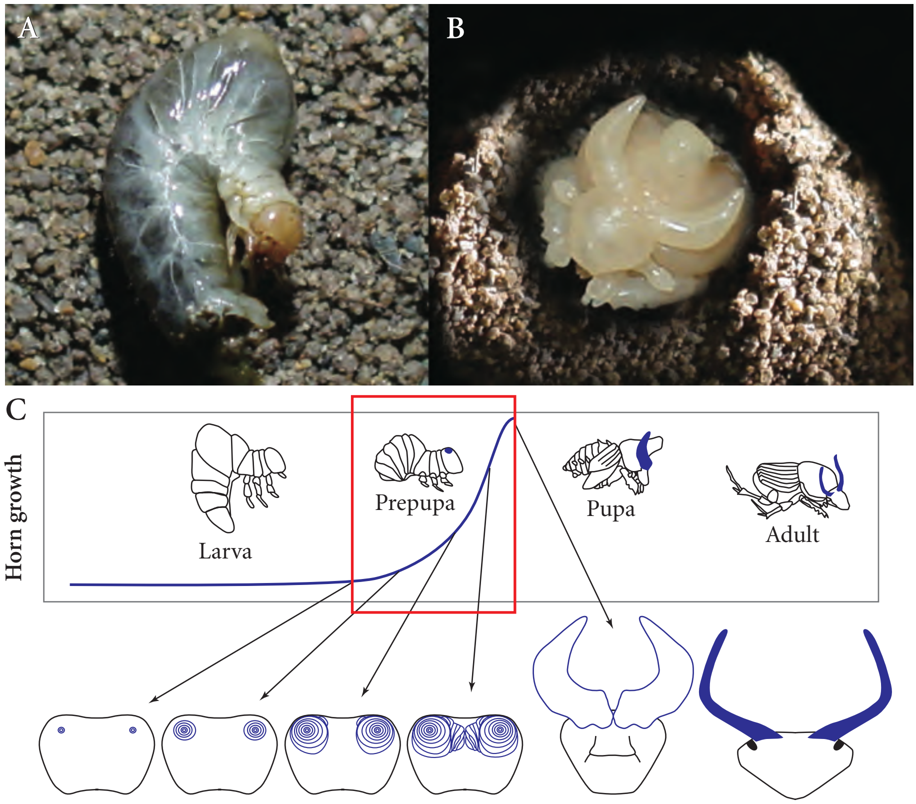
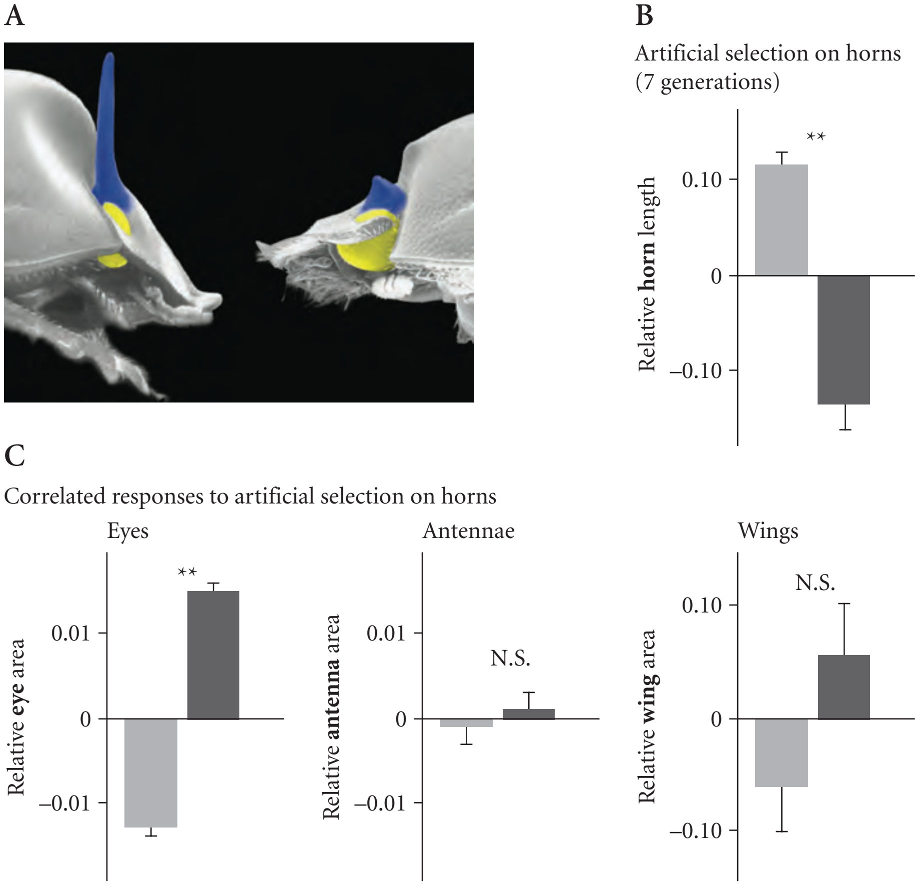


FIGURE 7 Development of beetle horns. (a), (b) larva and pupa of *Onthophagus taurus*; (c) growth of horns (blue) is concentrated at the end of the larval period when the animals have stopped feeding (prepupa period, red box).

tubes of epidermis that remain trapped beneath the larval exoskeleton, or cuticle, and these unfurl to take their full form when the animal sheds this outer cuticle and molts into a pupa. FIGURE 7 shows what these developing horns look like in the species *Onthophagus taurus*. From this image it should be clear that the horns are massive outgrowths. They arise from rapid cell proliferation in very specific localized regions of the larval epidermis. All of the adult structures undergo similar concentrated bursts of cell proliferation at this same time, and all of this growth occurs while animals are not feeding—that is, these structures must complete their growth using nutrients that were stored prior to the onset of metamorphosis.

Well, perhaps not surprisingly, horn growth can be associated with trade-offs. Because of when, during ontogeny, these structures develop, growth of beetle horns can stunt the relative sizes of other morphological structures, including antennae, wings, and eyes. The first tradeoff that we identified was between horn growth and eye size: males with the longest horns also had the smallest eyes (Figure 8a).

To test whether horn growth was in fact responsible for these reductions in eye size, we needed a way to perturb the development of the horns so that we could observe whether altered patterns of male allocation to horns were reflected in the final sizes of the eyes, and it turned out that we had a perfect way to do this. Several years ago, I had conducted an artificial-selection experiment that shifted the relative sizes of male horns in the Panamanian species *Onthophagus acuminatus*. For seven successive beetle generations and in six



Artificial selection on horns (7 generations)

Correlated responses to artificial selection on horns

Figure 8 (a) Tradeoff between horns (blue) and eyes (yellow) in *Onthophagus acuminatus.* (b) Artificial selection on relative horn length generated populations of males with unusually long (gray) and short (black) horns for their respective body sizes. (c) These evolutionary changes in horn length were accompanied by inverse correlated changes in relative eye size, but not in antenna or wing size.

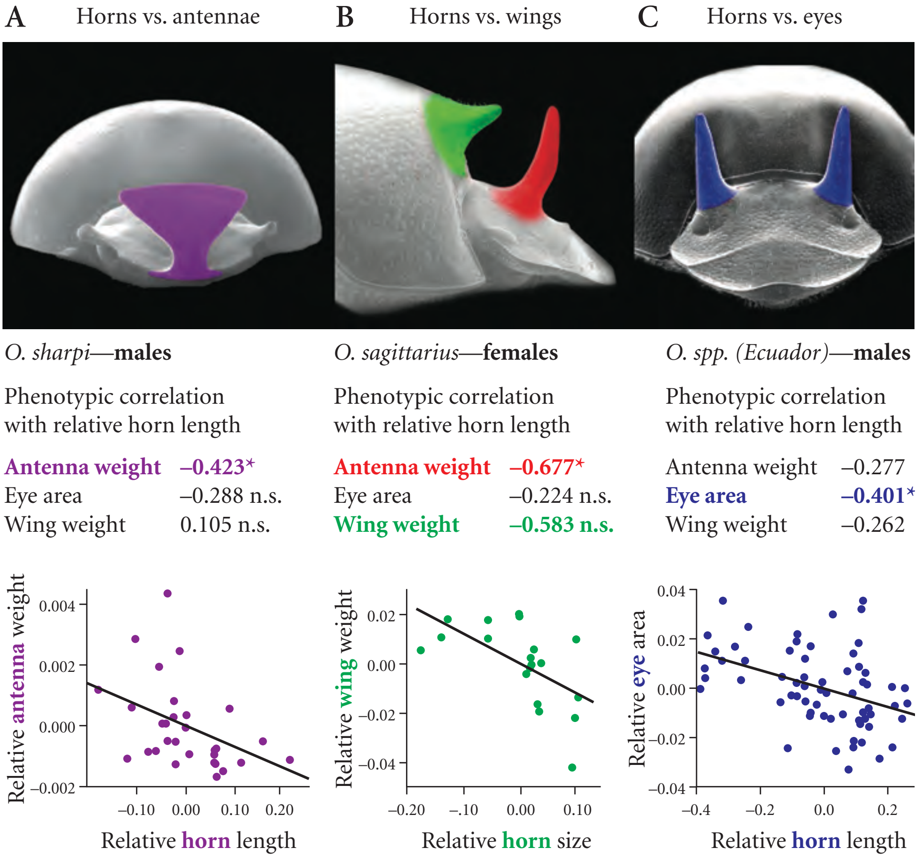
separate (populations (lines), I permitted only those males with the longest horns relative to their body sizes to breed (lines 1 and 2), or only those males with the shortest horns relative to their body sizes to breed (lines 3 and 4). (Two additional lines were kept as control lines, with males selected at random with respect to horn size.) After seven generations of this experiment, the relative horn lengths of males had diverged significantly (FIGURE 8B), so that, across the same range of body sizes, males selected for long horns (gray bar) had proportionately longer horns than males selected for short horns (black bar).

At that time, I had been interested only in the horns, so I did not measure any other traits (such as eyes). But I still had the dried beetle specimens from that experiment in boxes on a shelf, so I now returned to these same animals, and this time I measured the sizes of several additional structures. I predicted that if eye size was not involved in a tradeoff with horns, then eyes should have been unaffected by the genetic changes in horn length resulting from this experiment. If, on the other hand, horn growth had negatively impacted eye growth, then the observed genetic changes in horn length should also have been associated with inverse changes in relative size of the eyes, so that, at the completion of the experiment, males artificially selected for longer horns had smaller eyes than males selected for shorter horns. FIGURE 8C shows that this is exactly what we found: experimentally produced evolutionary changes in horn size had inverse, correlated effects on the size of eyes. Thus, horn growth can reduce the relative sizes of other morphological structures. But the question remains: how could this cost of horn growth favor evolutionary divergence of horns?

For this step we must consider where the horns develop, because growth of the horns appears to impact primarily physically adjacent (or nearby) structures. In the first two species that we studied, male horns grew at the base of the head, and this is a developmental location adjacent to growing eyes. This meant that the structures most likely to be impacted by horn growth were the eyes. But horns don't just grow at the base of the head in this genus of beetles. In fact, we've already seen that there are species with horns that grow from the center or the front of the head, locations more adjacent to growing antennae; and there are species with horns that grow on the thorax, a developmental location adjacent to growing wings. So this suggested that trade offs might not be the same in all beetle taxa. Even though the same basic process applies, and horn growth trades off with the growth of nearby structures, interspecific variation in the physical location of the horns might mean that the particular structures affected by horn growth (e.g., eyes, antennae, or wings) could vary across taxa.

FIGURE 9 shows data from three species that suggests that this is indeed the case. The first species (*Onthophagus sharpi,* also from Panama) has horns that extend like big spatulas from the front of the face (purple). Because males of this species do not have horns at the back of the head or on the thorax, we predicted a tradeoff between horns and antennae (because the closest traits to the horns are the antennae—they are not visible in this picture, but they extend from under the middle of the faceplate, near the base of the horn). When we examined patterns of morphological variation in a natural population of this species, we found that the males with the longest horns did have the smallest antennae.

FIGURE 9B shows a species that produces two horns, one that is centrally located forward on the head (red) and a second, cone-shaped horn that extends from the thorax (green). This is an extraordinary species for testing our ideas because these horns are actually produced by the females, rather than the males. In this species, the two closest structures are the antennae, which lie just under and a bit forward of the head horns, and the wings, which develop next



O. sharpi—**males**

Phenotypic correlation with relative horn length

Antenna weight	**–0.423***
Eye area	–0.288 n.s.
Wing weight	0.105 n.s.

O. sagittarius—**females**

Phenotypic correlation with relative horn length

Antenna weight	**–0.677***
Eye area	–0.224 n.s.
Wing weight	**–0.583 n.s.**

O. spp. (Ecuador)—**males**

Phenotypic correlation with relative horn length

Antenna weight	–0.277
Eye area	**–0.401***
Wing weight	–0.262

FIGURE 9 Tradeoffs between horns and antennae, wings, and eyes in *Onthophagus* beetles.

to the thoracic horn. When we looked at natural populations of these beetles, we found strong negative correlations between horns and each of these traits: females with the longest horns had relatively smaller antennae, and also relatively smaller wings. (Males of this species have a broad bulge on their thorax and two small "tusks" on their heads; none of these male features is as large as the female horns, and none exhibits tradeoffs with other morphological structures.)

Finally, in still another species, males produce a pair of horns at the base of the head (blue). This is an as-yet unidentified species from lowland tropical forests of Ecuador, and the horns in this species are similar in location to the horns of the original two species that we used for our developmental studies. As in the earlier species, we predicted a tradeoff between horns and eyes, and this is exactly what we found. The relative sizes of male horns were negatively correlated with the relative sizes of male eyes: the males with the longest horns also had the smallest eyes.

Thus, these measurements of animals in three additional species all suggest that there are morphological tradeoffs associated with horn growth in this genus of beetles. Perhaps more importantly, these tradeoffs are not all the same. *The nature of the developmental tradeoff differs depending on the type of horn that is produced.* We have since learned of a few exceptions (i.e., some populations and species do not fit this general pattern), but, to a close approximation, horns at the front of the head grow at the expense of antennae, horns on the thorax grow at the expense of wings, and horns at the back of the head grow at the expense of eyes.

Why does this matter? It matters because these are not trivial components of the morphology of these beetles. We have to think about this from the perspective of the beetles. Each of these traits is associated with a critical, but different, animal function: olfaction, flight, and vision, respectively.

Olfaction is an essential sensory modality in dung beetles, because they find their food, and their mates, by smell. These beetles are extraordinarily adept at finding their dung resources, often arriving only seconds or minutes after the dung is deposited. Quick arrival is critical, because beetles in many habitats face intense competition for their food resource. Compromised antenna size could affect an individual beetle's ability to find food (and mates) quickly, and this could constitute a very important functional cost of an enlarged skeletal weapon such as a horn.

Reductions in wing size will affect flight. Wings are essential for dispersal, and both dung and females are commonly located in sparsely distributed patches. Male beetles with sizable horns will have more weight to carry, and if they also have proportionately smaller wings, then this could constitute a significant reduction in their dispersal capacity or their flight efficiency.

Finally, these beetles use their eyes for orientation and balance during flight. Nocturnal species, in particular, rely on very large eyes to see and fly under low-light conditions. Horns reduce eye size by almost 30%, and they may significantly impair the visual capacity of males.

Consequently, dung beetle horns have different functional consequences—or costs—depending on their physical location. This is our first prediction: the costs of the different horn types are not the same. What about the second prediction? Well, there is a potential link, albeit an indirect one, between horn morphology and ecology. As we have already seen, beetles of this genus live at all elevations and in all types of climates and habitats. This time, however, this diversity of habitats does matter. Beetles in all of these varied environments will surely need to smell, to fly, and to see. But the relative importance of these sensory and locomotory modalities may vary greatly depending on the specifics of each environment—how well smells carry in that environment, how dispersed the food resources are, and whether the beetles are active at night or in the day—with the result that some horn types may be much more costly than others in any particular environment.

If the relative cost of each of the horn types differs across selective regimes or habitats, then selection to minimize the functional costs of horn expression could have contributed to evolutionary diversification in horn morphology. As beetles colonized new food sources, or invaded new and different habitats, the relative costs of the different horn types may have shifted. In these populations, individuals producing horns that perform well but *cost less* in the new habitat may have replaced those that produced the older, more expensive weapon forms, leading to evolutionary changes in the physical locations of their horns.

We were able to collect some basic, ecologically relevant information for each of the species included in the phylogeny, and we can use this information to provide three preliminary tests of this mechanism of divergence, involving each of the proposed tradeoffs: horns versus antennae, horns versus wings, and horns versus eyes.

First, horns versus antennae: beetles use their antennae to detect odor plumes from dung, and odor plumes appear to persist longer in forest understory habitats than they do in windier, more open pastures and fields (one of the many unusual pieces of trivia gleaned from 15 years crawling around looking for dung beetles: dung dries out and odors evaporate rapidly in treeless terrain). We predicted that if dung odors were especially difficult to detect in open habitats (because the smells blow away), then horns on the head might be prohibitively costly in these environments (because the development of horns stunts the relative growth of antennae, the principle organ of smell). If we turn this logic around, we predict that head horns will be least costly in forests and most costly in pastures and fields.

If, historically, these costs influenced the patterns of evolution of beetle horns, then we would expect gains of head horns to have occurred preferentially in forest environments, rather than in pastures. To test this idea, we used our phylogeny to reconstruct behavioral shifts from beetles inhabiting open habitats (thin gray lines in FIGURE 10A) to forest habitats (thick black lines), and, in some cases, back again to the open. We then overlaid onto this tree all evolutionary gains of horns on the head (blue, purple, or red circles), and we tested whether gains of these particular horn types tended to occur on lineages of the tree that also were scored as living in forest habitats more often than would be expected by chance. Thus, these methods test for an evolutionary correlation between changes in horn morphology and changes in ecology. As predicted, gains of head horns tended to occur in lineages of beetles that also inhabited forests. Even though we only have five gains of these horns to work with, the fact that four out of these five coincided with branches on the tree that also were forest-dwelling is not a pattern that we would expect to occur by chance.

We can also do the countertest: what happens if we look at the other types of horns? In contrast with head horns, thorax horns are not expected to be more strongly associated with forests than with pastures, because their growth does not impact antenna size as strongly. When we map evolutionary gains of thorax horns onto this phylogeny (not shown in the figure), we see no association with forest habitats: only three of eleven gains of thorax horns occurred in beetles that inhabited forests.

What about the second proposed tradeoff, horns versus wings? Dung beetles use their wings to fly from one breeding location to another (e.g. from dung pad to dung pad), and the typical distance beetles need to fly between these breeding locations could alter the relative cost of horns on the thorax, if these horns stunt the growth of the wings. We do not yet have sufficient information to estimate accurately the typical dispersal distances for all of these species. But the next best thing—something we could measure—was population density. Density is not an ideal proxy for dispersal distances (for example, we do not have any reason to believe that densities now have anything to do with what occurred in the past), but it is the best we have to work with at this time. The logic is this: we predicted that beetles living in high-density populations would need to travel much shorter distances between breeding locations, on average, than beetles living in low-density populations (i.e., populations that are sparsely distributed). If our prediction were true, then horns on the thorax would be least costly to these superabundant species, because growth of the thorax horns comes at the expense of wings.

In this situation, if tradeoffs have influenced patterns of horn diversification, then gains of thorax horns should have been more likely in lineages with high

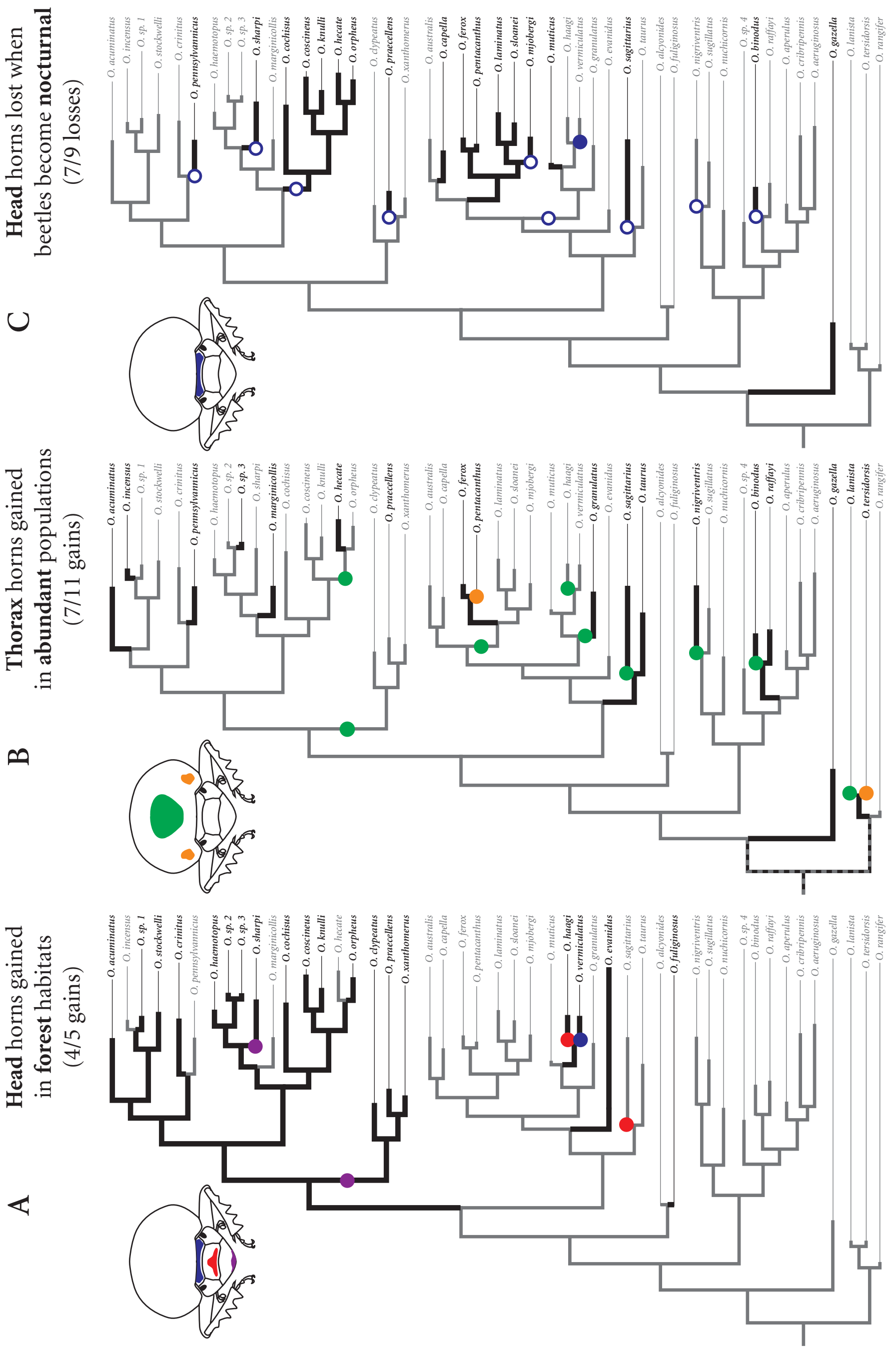


Figure 10 Phylogenetic evidence that tradeoffs (costs of horns) may have influenced the directions of horn evolution. Each panel tests for correlated evolution between changes in beetle behavior and/or habitat use (black versus gray branches), and gains or losses of horns (colored circles; closed = gains; open = losses).

population densities (e.g., abundant species) than in those with lower population densities (e.g., rare or sparsely distributed species). This is precisely what we found (FIGURE 10B). Seven of the eleven gains of thorax horns (green or orange circles) occurred in lineages scored as having high population densities (bold black bars), where such horns were predicted to be least costly. Here again, we can do the complementary test with head horns. Head horns do not trade off with wings, and we predict no associations with population abundance. As predicted, when we map gains of head horns (not shown), we find that these events are not associated with population density: only one out of the five gains of head horns occurred in lineages with high population densities.

Thus, evolutionary gains of head horns were concentrated on branches of the phylogeny with forest habitats, but not on branches with high population densities, and gains of thorax horns were concentrated on branches with high population densities, but not with forest habitats. Already we are starting to see hints of associations between morphology and beetle ecology, but the strongest evidence involves the tradeoff between horns and eyes.

Horns at the back of the head (in blue) reduce eye size, and eyes in these beetles are used for balance and orientation during flight. One widespread pattern in the animal world is that nocturnal species tend to have proportionally larger eyes than diurnal species (think, for example, of owls and cats), presumably because large eyes are better at light gathering and at dealing with low-light, or crepuscular, conditions. This pattern is definitely true for dung beetles, and, for this reason, we predicted that horns at the base of the head would be especially costly to species that fly at night.

FIGURE 10C shows lineages of beetles that fly during the daytime (thin gray lines) and those that fly at night (bold black lines). From this it should be clear that the ancestors of this genus probably flew in the daytime, but that at multiple times in their evolutionary history these beetles colonized the night. Overlaid onto this history is the evolution of horns at the back of the head (blue circles). Because this horn type was already present in the majority of sampled taxa (and was the ancestral horn type), we focused on evolutionary losses of this horn type (open circles) rather than gains (closed circles). We predicted that, if tradeoffs have influenced the evolutionary diversification of horns, then losses of head horns should be most likely in lineages that switched from diurnal to nocturnal flight behavior, because it is in these situations that the ancestral horn type would have been most costly. In fact, of the nine observed losses of this horn type, seven occurred on branches with nocturnal behavior. Consequently, this change in ecology was strongly correlated with changes in horn morphology: evolutionary changes in behavior—when in the day or night the beetles fly—coincided with changes in the physical location of the horns.

To summarize, we do see evolutionary associations between horn morphology and aspects of dung beetle ecology and behavior, and at least one of these patterns is very strong (FIGURE 10C). Thus, we can return to the big question of why beetle horn morphologies have diverged so dramatically in form. At least part of this diversity may have resulted from selection to minimize the relative costs of horn growth. Existing horns were lost primarily in those habitats in which they were most costly, and, where novel horns were gained, more often than not, the type of horn gained was the type predicted to be least expensive in that new environment.

One conclusion from these studies is that the costs of producing enlarged or exaggerated weapons may, in some instances, contribute to the evolutionary diversification of their form. In such cases, there may well be meaningful associations between trait morphology and ecology if you can figure out where to look. It is both interesting and surprising that, in these beetles, the only associations between weapon morphology and ecology that we have been able to find so far have nothing whatsoever to do with the function, or the benefits, of the horn itself. Instead, they depend on correlated structures and on the developmental costs of producing the horns. I think this serves as a healthy reminder that organisms have integrated phenotypes; we cannot just consider our focal traits (e.g., horns) as independent of the rest of the animal body. Here, developmental associations between horns and other traits may prove to be a big part of the story of beetle weaponry.

To conclude, I've shown you a lot about one genus of tiny beetles. It is a genus with spectacular morphological diversity, and, I think, it is a genus with a lot still to teach us about biology. In this essay I have introduced you to some of their diversity in form, to some of their behavior and biology, and to some of our attempts to explain the evolutionary radiation of their horns. But this essay is also about a much bigger point: that we still know very little about why the weapons of sexual selection are so stunningly diverse in form. This, I suspect, will keep us busy for a long time to come.

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