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Digging for Answers

STUDY OF EVOLUTION IN DUNG BEETLES //BY WENFEI TONG

Artist rendering of male dung beetles gaurding the entrance to caves.

Photo taken by Professor Emlen of the eyes of the Dung Beetles.

Imagine yourself as a knight in shining armor, valiantly guarding the entrance of a long subterranean tunnel at the end of which your ladylove is waiting. Although you would have to shrink to the size of a pea to enter one of these tunnels, this is not " Alice in Wonderland", but the equally exciting underground world of the dung beetle genus *Onthophagus* . With over 2,000 named species, and at least half as many waiting to be described, these beetles are found on every continent except Antarctica , and feed on almost any type of dung you might imagine.

Professor Douglas Emlen began his intimate relationship with these tiny dung-specialists as a graduate student in Princeton's Department of Ecology and Evolutionary Biology, and studied them for several years on Barro Colorado Island in Panama . Almost ten years after leaving Princeton , he is still patiently watching as the beetles burrow industriously into the soil within special glass observation chambers. "The beetles are my ticket to travel", he said with infectious enthusiasm, and proceeded to describe the unglamorous process of beetle collecting, which involves grubbing about in dung piles. "When I first met my wife, I was too embarrassed to tell her I studied dung beetles because I was worried she would think I was a loser". Contrary to his earlier qualms, Emlen is already a professor in the University of Montana , and a recent winner of the Presidential Early Career Award. Armed with surgical gloves and wet wipes, he and his wife have traveled to Ecuador , South Africa , Panama and Australia to collect dung beetles for the laboratory collection.

Despite inhabiting a wide variety of habitats, all onthophagine dung beetles dig tunnels in the soil. A female will laboriously collect fragments of dung for the tunnel, fashion them into balls, and lay a single egg in each ball. Emlen has observed a female making over fifty separate trips to the surface before amassing enough dung for a single ball. Apart from these risky excursions, she will then remain in the relative safety of her underground home, while males jostle for the chance to mate with her.

Could these pitched battles between males explain the evolution of enormous weapons in the form of horns? In the species *O. nigriventris* , a male's horns are longer than the rest of his body. Darwin was the first to propose that weapons such as antlers might be the product of sexual selection, in which intense competition between males for a chance to mate drives the evolution of larger and larger weapons. In contrast to elaborate sexually selected ornaments like the peacock's tail, dung beetle horns are not merely displayed for the aesthetic benefit of females, but used in violent skirmishes as males attempt to gain sole access to a female by guarding the entrance of her tunnel. Emlen's experiments have shown that the combatant with larger horns almost invariably wins in fights between males of the same body size. Clearly, big horns are selected for because they enable males to win battles over females and thus sire more offspring.

However, not all males of the same species will have horns, and while bigger males have bigger horns, the smaller males almost never do. The reasons for this are obvious when one considers the costs associated with large horns. Dung beetles are built like miniature bulldozers, with compact, ovoid bodies covered in smooth hard armor. Elaborate protrusions often seem to hamper a male's attempts to even enter a tunnel let alone maneuver within one, and so the costs of having horns might well outweigh their benefits to a small male who is unlikely to win battles with larger males. In addition, horns may increase the risk of predation and starvation by impeding movement and increasing the energetic costs of flight.

Instead, small, hornless males seem to invest their limited resources in longer sperm and disproportionately larger testes, compensating for their inability to guard a female by ejaculating more seminal fluid per copulation. In this way, small males may increase the odds of fertilizing an egg each time they manage to sneak past their larger competitors who mate more often but possibly with less efficiency. When unable to scuttle past their larger opponents, sneaker males may even dig side tunnels into a female's chamber, where they are often detected by the resident guard and booted out. In the midst of all this rivalry, the female seems equally willing to mate with any male who manages to reach her, and it is likely that the competition continues within her, between the sperm of rival males. Thus the tradeoffs associated with horns may explain why males have evolved the two alternate mating strategies of guarding and sneaking.

What causes a male to become a guard or a sneak? After getting his PhD from Princeton, Emlen investigated this question with Fredrik Nijhout of Duke University , an expert on the mechanisms of insect development. It turns out that horn size is not an inherited trait, but one that is linked to body size via a developmental switch. Bigger males produce bigger horns because they grew up in more nutritious dung balls. This is because beetle horns develop at the end of the feeding stage, when larvae have already attained their final body size, so beyond a particular threshold size, horn growth will be stimulated, while below that threshold, barely any horns grow.

The chemical signal that links body size to horn development is juvenile hormone (JH), which is positively correlated with larval size and nutritional conditions. The more nutritious the dung ball, the larger a larva will grow, and the higher the circulating levels of JH will be. By experimentally raising JH levels during the critical 30-hour window for horn growth, Emlen induced tiny *O. taurus* males to produce horns, showing that it is this level of circulating JH that determines whether or not a male will have horns. In another experiment, the diet was manipulated, so that well-fed larvae were large and grew horns, but malnourished ones were small, and did not. While at Princeton , Emlen used intensive artificial selection experiments to show that the link between horn size and body size can be shifted by changing the threshold level of JH needed to stimulate horn development in epidermal cells. Thus different species of dung beetle appear to have different size thresholds that trigger horn production in response to a critical JH level.

Why might different species have different thresholds, and why might the horns appear in such a diversity of sizes, shapes and locations on the body? In the JH application, diet manipulation and artificial selection experiments, horn size was positively correlated with body size, and horn growth was only triggered after a critical threshold body size was reached. Emlen thought that since horns make flying costly, horns size might limit wing size, so that smaller beetles should have proportionately larger wings, while their larger, horned counterparts would have relatively small wings. To his dismay, neither of his experimental species showed a significant relationship between wing size and horn size.

However, these two species do experience a trade-off between eye and horn size, so perhaps there is an indirect link between beetle ecology and morphology after all. Both of these species are diurnal, so the costs of impaired vision may be less severe than for a nocturnal species. In contrast, other beetle species that specialize in a habitat where sight is more important than flight may have evolved a tradeoff between wings and horns instead. Emlen hypothesizes that different species of beetles may minimize the functional costs of horn production by evolving a tradeoff between horn size and the development of the structure that is least crucial in their particular environment. This tradeoff may explain why beetles in a diverse range of habitats all over the world might have evolved such a varied array of horn types.

The structure that develops inversely with horn size is almost invariably adjacent to the horns, suggesting that developmental mechanisms are the proximate cause of this trade-off. Consequently, a species that can best afford a trade-off between antennae and horns should grow horns near the antennae. Sure enough, the two species that Emlen studied grow their horns next to their eyes, and a similar link between ecological niche, horn location and developmental trade-off has also been found in several other dung beetle species.

In the process of studying these small and smelly insects, Emlen has become a major driving force in the relatively new field of "evo-devo", in which an understanding of developmental mechanisms has enriched out understanding of evolutionary biology and sexual selection theory. When I asked him what advice he might have for graduate students in biology, he replied with two things that he learnt from the beetles, and from Princeton . "Learn to ask the right questions that allow you to tease apart alternative explanations", and "Let the animal show you what's going on". His own experiences with the beetles taught Emlen that "no matter how hard you try, you inevitably end up in a rut, and the key to being a good biologist is to step back and see that the animal is always right. It takes something unexpected, where you just don't have the answers you want, to make you sit down and look at things from a new perspective, and figure out where you were wrong."

One of these formative experiences occurred while Emlen was still in Princeton , looking at the results of his artificial selection experiment, in which seasonal changes in monkey dung had caused fluctuations in the diet quality his beetles received. The result was a strange and unexpected set of zigzags across his graph of horn size plotted against body size. Yet once the periodic changes in diet had been factored out, the lines of the selected and wild-type strain diverged beautifully, resulting in the 1 st publication on plastic allometry. "Hidden in that pile of rubble was a signal, and the signal was beautiful. What I really want to teach my students is how to find that signal, because as a biologist, things aren't supposed to work. If you go through graduate school, and everything works, you've been cheated, because the most exciting thing about biology is trying to explain why things didn't turn out the way you expected them to."

Most of the questions about dung beetles have yet to be answered. In addition to working on a phylogeny and looking at the placement of horns in relation to other structures, Emlen is currently investigating the evolutionary significance of female horns in the rare cases where females instead of males produce horns. Before long, we will know even more about the genus *Onthophagus* that has it's tiny representatives living in almost everyone of our backyards all over the world.

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