

'Cyclops' beetles hint at solution to 'chicken-and-egg' problem in novel trait evolution

August 23 2016



Heads of horned and cyclopic beetles of the genus *Onthophagus* are shown. After knocking out the gene *otd1*, the cyclopic beetle (right) lost the horn but gained a pair of small compound eyes in the center of the head. Credit: Indiana University

Beetles with cyclops eyes have given Indiana University scientists insight into how new traits may evolve through the recruitment of existing genes—even if these genes are already carrying out critical functions.

The study, reported in the *Proceedings of the Royal Society B*, was led by Eduardo Zattara, a postdoctoral researcher in the IU Bloomington College of Arts and Sciences' Department of Biology. It was published in tandem with another study led by Hannah Busey, an undergraduate student researcher at IU Bloomington and 2016 Goldwater fellow, which appeared in the *Journal of Experimental Zoology*.

The discovery was made after switching off orthodenticle genes in horned [beetles](#) of the genus *Onthophagus*, also known as dung beetles. Knocking out these genes caused drastic changes in the insects' head structure, including the loss of horns—a recently evolved structure used for male combat over access to females—as well as the growth of [compound eyes](#) in a completely unexpected place: the top center of the head.

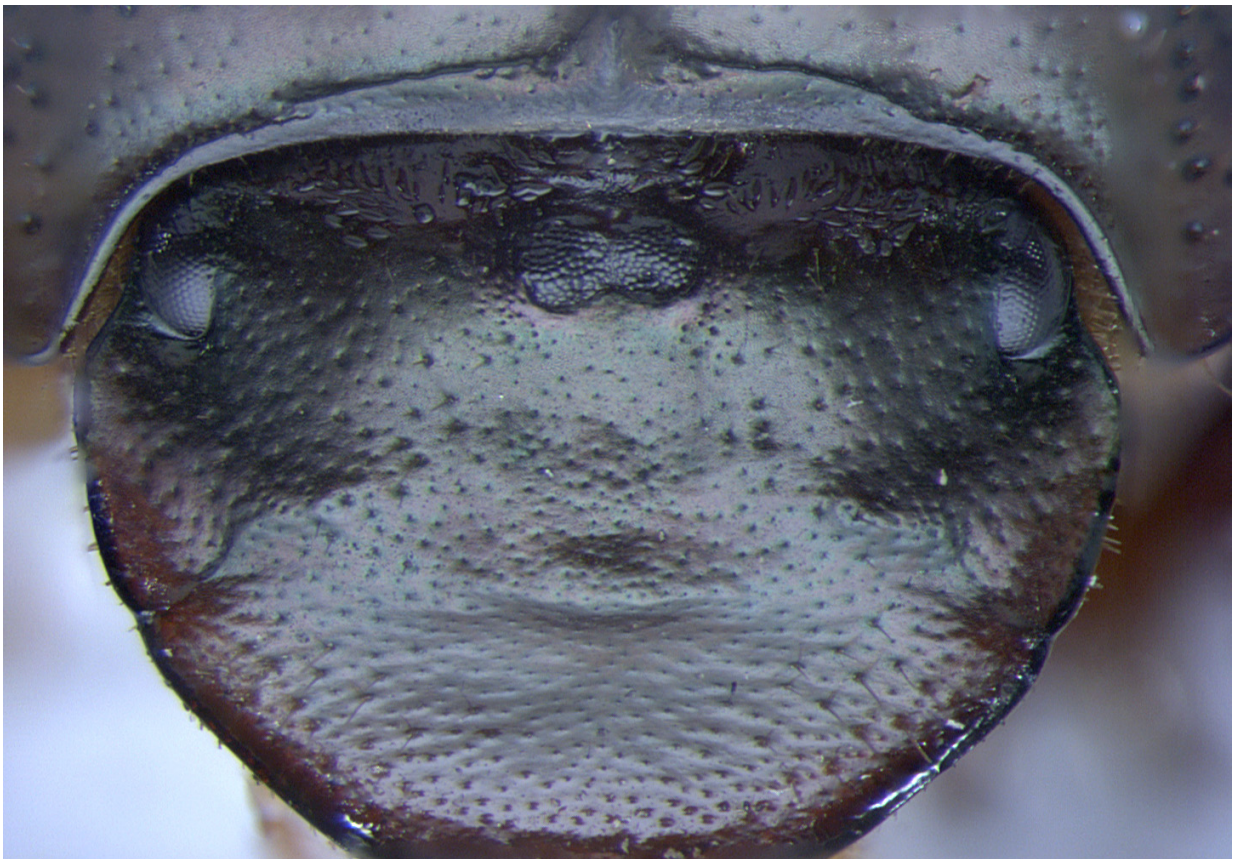
The results were specific to *Onthophagus*; the same changes did not produce the same effects in *Tribolium*, or [flour beetles](#), which do not have horns.

"We were amazed that shutting down a gene could not only turn off development of horns and major regions of the head, but also turn on the development of very complex structures such as compound eyes in a new location," Zattara said. "The fact that this doesn't happen in *Tribolium* is equally significant, as it suggests that orthodenticle genes have acquired a new function: to direct head and horn formation only in the highly modified head of horned beetles."

The use of *Onthophagus* as a model system for the evolution of novel traits has been pioneered by Armin Moczek, professor in the IU Bloomington Department of Biology, who is senior author on the papers. Work on *Tribolium* was conducted by David Linz and Yoshi Tomoyasu at Miami University.

Beetle embryos hatch as larvae, which grow and metamorphose into adult beetles. Many genes crucial to making the head of larvae during embryonic development are known from studies in *Tribolium*, but whether they were involved in making adult heads during metamorphosis was largely unknown.

In her study, Busey removed small patches of skin from the heads of larval *Onthophagus* and then traced where the adult heads were missing tissue.



After knocking down the gene *otd1*, this *Onthophagus sagittarius* beetle shows a pair of fused compound eyes at the top center of the head. Credit: Indiana University

"Using this microsurgical technique, we created a map showing which region of the larval head made each part of the adult head," she said.

"This allowed us to apply knowledge about *Tribolium* embryonic development to *Onthophagus*, because even though adult heads are very different between horned and flour beetles, the larval heads are quite similar."

Zattara's study used these results to select genes needed by embryos to build larval heads and switched them off to test whether they had any roles in building the head of adults.

Among the genes they selected was *orthodenticle*, or *otd*, which contributes to head development in simple invertebrates to complex mammals. If *otd* is deleted, most animal embryos will not develop a head or brain. Similarly, beetle embryos need *otd* to properly develop heads, but no larval or adult function was known.

But when Zattara and colleagues switched off *otd* genes in the larvae of two species of *Onthophagus*, they found *otd* had acquired a new function: reorganizing the head during metamorphosis, integrating the horns in the process.

They also found that switching off these genes shrank or eliminated the beetles' horns and associated head regions and, strikingly, induced development of "cyclopic" compound eyes at the top center of the head, where they aren't normally found in insects.

Although the same manipulations in *Tribolium* flour beetles did not affect head development or grow extra eyes, the IU scientists were surprised to find that *otd* genes were still expressed in the same location as larval and adult *Onthophagus*.

The results suggest that the lingering expression of genes in specific

tissues or life stages where they no longer have a function may comprise a "stepping stone" in recruiting those [genes](#) into making new traits.

"These studies provide a solution to an important 'chicken-and-egg problem' of modern evolutionary developmental biology," Zattara said. "For a gene to carry out a new function, it needs to find a way to be activated at the right time and location. But it is hard to come up with a good reason why a gene would become active in a new context without already carrying out some important function."

"Here we have a situation where a gene is already in the right place—the [head](#)—just not at the right time—the embryo instead of the adult," Moczek added. "By allowing the gene's availability to linger into later stages of development, it becomes easier to envision how it could then be eventually captured by evolution and used for a new function, such as the positioning of horns."

More information: Eduardo E. Zattara et al, Neofunctionalization of embryonic head patterning genes facilitates the positioning of novel traits on the dorsal head of adult beetles, *Proceedings of the Royal Society B: Biological Sciences* (2016). [DOI: 10.1098/rspb.2016.0824](https://doi.org/10.1098/rspb.2016.0824)

Provided by Indiana University

Citation: 'Cyclops' beetles hint at solution to 'chicken-and-egg' problem in novel trait evolution (2016, August 23) retrieved 20 April 2024 from <https://phys.org/news/2016-08-cyclops-beetles-hint-solution-chicken-and-egg.html>

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