A beetle chemical defense gland offers clues about how complex organs evolve

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Rove beetles are among the chemists of the insect world, concocting noxious compounds within their bodies that are weaponized to ward off predators, enabling the beetles to survive in leaf litter and soil in ecosystems across the planet. On December 9 in the journal *Cell*, investigators studying a species of rove beetle report how two distinct cell types have come together to form a specialized gland for making and secreting these defensive cocktails. The work has implications for mapping out the evolution of more sophisticated organs found across the animal kingdom, including in humans.

"These beetles are fantastic models for understanding how new kinds of ecological relationships emerge during evolution through changes at the molecular, cellular, and behavioral levels," says senior author Joseph Parker of the California Institute of Technology. "As part of this question, we're very interested in how rove beetles have pieced together these glandular structures in their abdomens, which are made of different cell types that work together. These structures are the embodiment of a major conundrum: how complex organs evolve that are often composed of many different cell types that appear to seamlessly cooperate with each other. How this cooperativity emerges during evolution is challenging to explain."

Parker's lab focuses on rove beetles in part because of their ability to carve out niches for themselves in many different ecosystems, from in the dirt to inside ant colonies. One way they've been able to survive in the presence of other insects, such as ants, is through glands in their abdomen that release a defensive chemical compound that triggers pain receptors. The beetles have a supremely flexible body and can smear these chemical cocktails directly onto predators to defend themselves.

The species of rove beetle that was the focus of this research, *Dalotia coriaria*, has what's called a tergal gland in its abdomen that releases a cocktail made of two compound types: benzoquinones, which are highly toxic but solids on their own, and solvents, a fatty acid-derived blend of an alkane and three esters. The latter compounds by themselves are benign, but they weaponize the benzoquinones by dissolving them.

Parker's group investigated the tergal gland and found two cell types that were engaged in a biosynthetic division of labor. "One cell type makes the benzoquinones and the other makes the solvents," Parker says. "Both are needed to create a functional secretion that confers adaptive value."

In the study, the investigators used single-cell transcriptomics of the beetles' abdominal segments to uncover novel enzyme pathways that enable the creation of these substances in each cell type. They then used these findings to dig deeper, exploring how each cell type's pathway was constructed from components that functioned in other more ancient cell types elsewhere in the beetle. "We were able to discover the biosynthetic pathways in each cell type and could then ask how these pathways were stitched together during evolution," Parker notes.

Remarkably, one of the cell types—the solvent cells that make the alkane and esters—was found to be a hybrid of cells comprising the beetle's exoskeleton and two ancient metabolic cell types that make and store lipids and produce pheromones. "The beetle has recruited a major gene expression program from these ancient metabolic cell types and installed it into a patch of cuticle, creating a gland," Parker says.

Further experiments—including placing the beetles into battle arenas with ants—revealed that when either the solvent or benzoquinone pathway was knocked down, the beetles lost their defensive capabilities. This suggested that under natural selection, both cell types are needed to confer the beetles' chemical defense system. The investigators also found that the compound made by the tergal gland has antimicrobial properties, further raising the adaptive value of the gland.

The authors think the gland evolved via coevolution between the two cell types. "The solvent cells created a niche for a second cell type to produce the solid benzoquinones, which could dissolve in the alkane and esters. A highly toxic secretion emerged that massively raised the gland's adaptive value, locking the two cell types into a unit where they cooperate. In essence, a new organ emerged," Parker says.

"Across the animal tree of life, you see complex multicellular organs that are composed of many different cell types functioning collectively," Parker concludes. "Think of something like the mammalian eye, which has about 70 different cell types all functioning together to enable our visual system. The scenario we find playing out in the tergal gland—an organ made of only two cell types—you can imagine could go through further rounds as cell types create niches for new ones to be added, eventually generating really elaborate multicellular complexity."

More information: Joseph Parker, Evolutionary assembly of cooperating cell types in an animal chemical defense system, *Cell* (2021). DOI: 10.1016/j.cell.2021.11.014. www.cell.com/cell/fulltext/S0092-8674(21)01329-5

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