

Study discovers how beetle shells harden

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Kansas State University researchers think their discovery of the enzyme involved in the hardening of a beetle's exoskeleton or cuticle could lead not only to better pest control, but also help create similar strong, lightweight materials for use in aircraft and armor.

After a beetle first molts, its exoskeleton is soft and hydrated. Somehow, it dries out and forms a hard, stiff exoskeleton. Since the 1940s, scientists have wondered which enzyme among several possible candidates was involved in the hardening process.

The K-State researchers have found that by knocking out an enzyme called laccase-2, cuticle tanning, the process of hardening and pigmentation, can be prevented in the red flour beetle, *Tribolium castaneum*.

A paper, to be released the week of Aug. 1 in the Proceedings of the National Academy of Sciences, presents the research results. The K-State researchers are Yasuyuki Arakane, research associate in biochemistry; Subbaratnam Muthukrishnan, professor of biochemistry; Richard Beeman, adjunct professor of entomology; Michael Kanost, professor and head of the department of biochemistry; and Karl Kramer, adjunct professor emeritus of biochemistry.

Kramer said K-State researchers wanted to find out what happens between the times when the cuticle is soft and when it is hard. They studied the cuticle's composition and how the components interacted to give it stiffness, flexibility and lightness. The main components in the

cuticle are proteins and chitin, which also are found in crustaceans and other invertebrates.

The researchers knew one of two classes of oxidative enzymes, tyrosinases or laccases, is likely responsible for catalyzing the exoskeleton's hardening by cross-linking cuticular proteins, Kanost said.

"When we knocked out tyrosinase, everything was normal," Kramer said. "When we knocked out laccase-2, we prevented tanning from taking place."

When the laccase-2 gene was not expressed, the newly formed cuticle remained soft and white instead of becoming hard and dark-colored. These results indicated which protein was responsible for the hard shell's formation, Kanost said.

The identification of laccase-2 as the catalyst for cuticle tanning opens up possibilities of targeting this protein as a way of weakening the beetle's physical defenses against mechanical, chemical and biological injuries, Muthukrishnan said. Better insecticides could be developed as a result of having a more insect-specific target like laccase-2, Kramer said.

"Gaining knowledge about a molecular process required for insect development, but absent from humans and other vertebrate animals, such as cuticle tanning, may be useful for developing new, bio-rational methods for controlling pest insect populations," Kanost said.

Armed with this new information, a number of practical applications are possible. Materials based on the chemistry of the insect exoskeleton could be developed to make lightweight materials for aircraft and military armor, Kramer said.

"I sometimes speculate that we might help K-State coach Bill Snyder

develop better football helmets and shoulder pads for his players," he said.

Collaborative research with scientists at the University of Kansas is in the beginning stages to analyze quantitatively the mechanical properties of insect cuticles and to perform cuticle protein cross-linking experiments that are catalyzed by insect laccase, Kramer said. KU scientists will test the strength of the synthetic cross-linked biopolymers that are created. This could be used for the development of strong, lightweight materials.

Both Beeman and Kramer also work at the Grain Marketing and Production Research Center, Agricultural Research Service, United States Department of Agriculture, in Manhattan.

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