Paleoscatology: Prying DNA from dated dung

Poinar

Dung from an ice-age ground sloth.

Taking their clue from confectioners, molecular biologists have discovered a chemical trick for extracting ancient DNA from desiccated dung left by ice-age animals. The new procedure could help in a variety of biological endeavors, from studies of extinct saber-tooth cats to analyses of endangered living populations.

Past attempts to pull DNA from old feces have proved disappointing because investigators did not realize that the DNA was hidden inside caramelized sugar compounds, says Hendrik N. Poinar, a researcher at the Max-Planck-Institute for Evolutionary Anthropology in Munich. Poinar and his colleagues discovered this molecular sugarcoating after they initially failed to find any DNA in a sample of 20,000-year-old dung from an extinct ground sloth.

The researchers suspected that the DNA had degraded while the dung sat through the millennia in Gypsum Cave in Nevada. When they performed chemical tests, however, they found an abundance of long sugar compounds formed by a chemical transformation called the Maillard reaction—the same browning process that gives chocolate, bread crusts, and meat their distinctive, intense tastes. Confectioners have researched the Maillard reaction extensively, as have clinicians working with diabetic patients who have a problem with sugars condensing in their vascular system, says Poinar.

The researchers hypothesized that the long sugar chains had bound up and preserved much of the DNA in the dung. To liberate the genetic information, they added N-phenacylthiazolium bromide, a compound that cleaves the sugar links made by the Maillard reaction. They then isolated fragments of preserved DNA and copied them using the polymerase chain reaction technique, according to their report in the July 17 Science.

"Now we know that the Maillard reaction is beneficial not only to Nestlé but also to the fossil researchers," says Poinar.

The DNA recovered confirmed that the dung belonged to the extinct ground sloth, whose bones lie nearby in the cave. More importantly, it also revealed aspects of the sloth's diet. Poinar's group found eight types of plant DNA, the majority of which came from wild capers, mustard plants, and yucca. All these still grow in the vicinity of the cave, about 30 kilometers east of Las Vegas. The researchers also turned up DNA from a wild grape that requires surface springs and doesn't grow near the cave today. During the last ice age, the nearest springs would have been 10 to 20 km away, indicating that the sloth ranged far while foraging for food.

The new technique holds promise for other researchers who study DNA from dung, says Robert K. Wayne, a biologist at the University of California, Los Angeles. In many cases, biologists find it easier to obtain dung than to collect blood samples from wild animals. Endangered animals, he notes, are often hard to track and experience stress when captured. Researchers, however, have had trouble isolating DNA from feces of some species, and the new methods may improve results, Wayne says.

Poinar has tested his technique on dung as ancient as 40,000 years old. The process will only work with unfossilized material that has been preserved by desiccation, he says. Older coprolites, such as the recently discovered tyrannosaur dung (SN: 6/20/98, p. 391), have turned to stone and would not contain any DNA, he says. *—R. Monastersky*

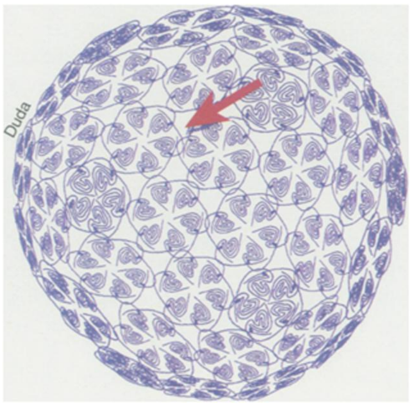
Protein chain mail offers armor for viruses

To Robert L. Duda, the protein shells protecting the DNA of viruses that infect bacteria evoke images of battles from long ago.

"When craftsmen in Japan made *katabira* for *ninjas* and the guildsmen of Europe forged *chainmail* for knights, they were unaware that their armor reflected an ancient biological design used by bacterial viruses. The fabric of interlocking rings of metal that shielded medieval warriors has a protein analog in [the bacterial virus] HK97," says the University of Pittsburgh biologist in the July 10 Cell.

Duda came to this unlikely connection while trying to explain puzzling data concerning the protein shell, or capsid, assembled by HK97. Like many viruses, this bacterial virus, or bacteriophage, creates its shell from hundreds of copies of a single protein (SN: 3/25/95, p. 186). The capsid forms through a multistep process in which five or six of these copies assemble into a ring, and the resulting pentamers and hexamers come together in a shell reminiscent of a soccer ball. The finished HK97 capsid has 72 faces: 60 hexamers and 12 pentamers.

When Duda and other researchers tried to dismantle this capsid into its components, they ended up with unusually large breakdown products. Capsids normally disassemble into individual proteins or into the pentamers or hexamers created during shell assembly. But HK97's capsid broke into pieces so large that they couldn't even be measured by traditional means.



In HK97's outer shell, each face intertwines (arrow, for example) with neighboring ones.

Duda initially thought that an undetected chemical bond was forming extra links between the capsid's many pentamers and hexamers, but experiments did not reveal such bonds. He then realized that the protein rings might not just chemically bond to each other but might also physically interlink like the metal rings of chain mail.

This intertwining apparently occurs right before the capsid proteins form into rings. "Imagine you're joining hands in a ring of six people," says Duda's colleague Roger Hendrix. "But before you join hands, you reach around the arms of the guys in a neighboring ring, and so you get linked rings."

"It's really neat stuff," says Peter E. Prevelige Jr. of the University of Alabama at Birmingham, who studies viral shell assembly. "People don't know how to fold proteins, let alone fold them, make them assemble, and then transform them into something as complex as this."

HK97 isn't alone in its chain mail strategy. "We've now found quite a few other bacteriophages that do this," says Hendrix. He and Duda suggest that the interlinking stabilizes the capsid.

Viruses that infect animal cells may not employ protein chain mail, notes Hendrix, because they must shed their shells to release their DNA. In contrast, bacteriophages inject their DNA into bacteria with a syringelike mechanism. Although Duda deduced the chain mail strategy from indirect experimental data, investigators plan to directly map HK97's capsid structure at atomic resolution by shining X rays through crystallized versions of it. That should soon determine whether bacteriophages are indeed pioneers in the wearing of chain mail. *—J. Travis*