Science Gives Ivory a Sense of Identity

DNA fingerprinting and isotope analyses may help solve the African ivory conundrum by identifying the locales where tusks come from

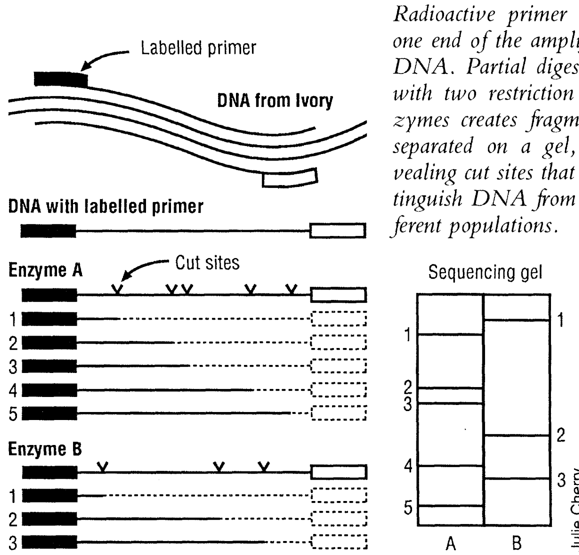
FOR MONTHS NOW, ELEPHANT TUSKS have sat center stage during an acrimonious debate: how might the community of nations reconcile its desire to save from poachers the remaining elephants north of Zimbabwe with the legitimate wishes of Botswana, South Africa, and Zimbabwe to trade ivory? In those countries, at least, elephants are thriving and their ivory could be an important source of cash.

As *Science* predicted (*Science*, 6 October, p. 1246), in October the member countries of the Convention on International Trade in Endangered Species (CITES) overcame their internal divisions and voted to ban all trade in ivory for at least 2 years. So far, the ban is holding up, thanks in part to the cooperation of the southern African exporters and Japan, the major importer.

But they are waiting for a reconsideration of the question, promised for 1991, that may allow some ivory trade to resume. As they do so, the dilemma on many minds is: how might ivory culled from well-managed southern herds be distinguished from the illicit ivory peddled by the poachers? One tusk, after all, looks pretty much like another.

Enter the scientists: it may be possible to pinpoint just where in Africa a tusk originated. An animal's DNA and its diet can both provide the information needed to ensure that only ivory from secure populations enters trade.

In many species, the DNA of populations that do not interbreed much is detectably different and labels the populations. David Western, director of Wildlife Conservation International at the New York Zoological Society, was aware of the new techniques of DNA fingerprinting that police detectives have been using to tie murder and rape suspects to their crimes. He asked John C. Patton, an expert on DNA identification techniques at Washington University in St. Louis, to work with Nick Georgiadis, a wildlife biologist with experience in East Africa, to see whether the same methods might also work to trace the origins of elephant tusks.



Patton's procedure. *Radioactive primer tags one end of the amplified DNA. Partial digestion with two restriction enzymes creates fragments separated on a gel, revealing cut sites that distinguish DNA from different populations.*

First, Patton and Georgiadis had to discover whether they could obtain usable amounts of DNA from tusks. As soon as an animal dies, its DNA starts degrading, chopped up by enzymes in the animal's own cells and in putrefying bacteria. But sun-dried tissue can contain surprisingly intact strands. So Georgiadis visited the ivory rooms of Dar es Salaam in Tanzania and Nairobi in Kenya—where ivory confiscated from poachers as well as that from natural carcasses is stored—in search of the bits of meat that poachers sometimes leave on the tusks when they hack them from the elephant's head. About one in five tusks was "dirty," and Georgiadis gathered 100 samples from tusks collected at known locations.

Back at the laboratory in St. Louis, three-quarters of the samples, especially those consisting of "tough, amber-colored tissue," proved to contain high-quality DNA, with fragments several thousand base pairs in length. So it *is* possible to get DNA from tusks. Phase two of the operation, which is just getting under way, is to discover whether that DNA will reveal differences between elephants from different populations.

There are two basic techniques, fingerprints and restriction maps. Both use restriction enzymes, to cut the DNA at specific recognition sites, and radioactive probes, to identify the fragments created, but the emphasis of the two approaches differs.

Fingerprints use a radioactive probe to locate specific stretches of DNA, released by cutting with a restriction enzyme, which vary in length from individual to individual. Restriction maps seek the presence or absence of short sequences, the recognition sites of various restriction enzymes, throughout the DNA. Radioactive probes identify the fragments. In both cases, the fragments are measured by running them through an electrophoretic gel.

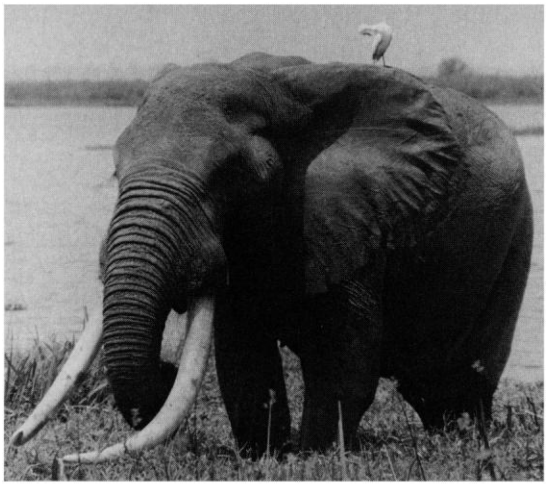
The question is: will either technique reveal any features of the DNA that can reliably be used to discriminate tusks from different populations?

The quick and dirty answer is yes: "One of the gels we ran last night gave us what we want," said Georgiadis in a telephone interview last week. "This marker differentiates between Kenya and Tanzania. It's what we've been looking for, and I'm sure we'll find more."

Patton, meanwhile, has been developing an entirely new approach to restriction mapping and using it first to analyze the elephants' DNA. Conventional restriction mapping has some disadvantages, he notes. It requires several digests with different restriction enzymes to detect details of the DNA. And that requires the use of several radioactive probes to reveal the landmarks. "We are bathed in radiation, which is a real pain," says Patton, whose new technique cuts down on the radioactivity and "takes out about two-thirds of the lab work."

Patton uses the polymerase chain reaction (PCR) to amplify the specific DNA segment he is interested in. That generates fragments with ends that he has defined. More importantly, he can label one end of the amplified fragment by using a radioactive primer. The next step is a partial digest; a small quantity of restriction enzyme, allowed to work for a short time, does not cut every site it could. Instead, it creates a set of fragments of increasing length, each defined at one end by the radioactive primer. The fragments can be run on an ordinary sequencing gel, which separates the bits out according to length, and the fragments produced by different enzymes can be run in adjacent lanes. "The result," says Patton, "is an instant mapping of sites down to virtually a nucleotide by nucleotide level with a minimal level of exposure to radiation."

In his first series of experiments, Patton has amplified part of a gene encoding a "zinc finger protein." This gene plays a part in determining gender and certainly differs between males and females. Patton compared



Leonard Lee Rue III/Animals, Animals

Big bull. *Almost gone from East Africa.*

the amplified stretches from two known females with those from four animals of unknown gender. "Three of the four animals show bands that are unlike anything found in the known females," Patton told *Science*. Those three are probably males. In the future it may be possible not only to tell which population of elephants a tusk came from, but also the gender of the animal that carried it, invaluable information for those attempting to conserve and manage the elephants.

A completely different approach to pinpointing the origins of ivory is to ask what the elephant that produced it ate. One way to do that is to look at the proportions of the various stable isotopes of atoms in the tusk. Nikolaas van de Merwe, Clay Professor of Scientific Archaeology at Harvard University, has been doing just that.

Van de Merwe is an expert on diagnosing the diet of ancient animals by looking at isotopes in their fossil remains. His current project came about when he went to South Africa this past summer to look at some fossils. People there knew that he had examined elephant bones and ivory on a small scale; with the CITES conference looming, and the ban on ivory in the offing, the South Africans asked van de Merwe if he could apply his techniques to locate the areas where an elephant lived. "The thing needed sorting out quickly," van de Merwe said, so he coordinated efforts at laboratories in Johannesburg and Cape Town. "The techniques are straightforward," says van de Merwe. "It's just a different problem."

Van de Merwe measures the proportions of the stable isotopes of three elements in the ivory, focusing especially on the ratio of carbon-13 to carbon-12. Tusks from different populations reliably turn out to have different ratios: why? "If you work up the data for biomass of grass versus trees and shrubs [where the elephants live], it tracks [the ratio of ^{13}C to ^{12}C in the tusks] just about linearly, for the simple reason that elephants like to browse and will eat grass if only pushed."

Behind van de Merwe's "simple reason" lies the complicated biochemistry of photosynthesis. Trees and shrubs use the so-called C_3 pathway to convert carbon dioxide to sugars and other organic compounds. Tropical grasses use the more efficient C_4 pathway. The two photosynthetic pathways have different effects on the ^{13}C:^{12}C ratio. The C_3 pathway discriminates against ^{13}C, so that in trees and shrubs the ratio is much lower than it is in C_4 plants. The differences work their way through to the carbon-containing compounds in the animals that eat the plants and provide a signature of the diet.

The carbon ratios give a pretty good indication of the amount of grass that the elephants have eaten, which depends on where they live, but they can sometimes be shaky about separating two locations. Determining the ratio of nitrogen-15 to nitrogen-14 offers another fix. The ratio of ^{15}N to ^{14}N correlates with rainfall, which further identifies the origin of the ivory.

"And if that doesn't sort it out for you unambiguously," says van de Merwe, "you go to strontium isotopes, which gives you the age of the geological substrate. Between those three we have not found any ambiguous identifications in 27 different areas that I've looked at so far."

So it appears very much as if, between them, DNA analysis and isotope signatures can be developed into workable identity cards for ivory. Will they be needed?

Richard Leakey, director of Kenya's Department of Wildlife Conservation and Management, says, "it's an expensive business, and I don't really think there is any need." According to Leakey, only a ban on ivory trade will save the elephants. "No matter how sophisticated our police force and surveillance, if there is a demand, the criminal element will outwit us."

As a result of the ban on imports to Europe and the United States, says Leakey, "there is no value to ivory in Kenya at this moment." That, and beefed-up anti-poaching patrols, allow Leakey to boast that no elephant has been killed inside a national park in the past 6½ months.

The southern African states will continue to cull elephants, and although they have agreed not to export ivory for the next 2 years, they hope that trade will resume some day. "I think it's the only thing that is going to conserve elephants outside national parks," says Nick Georgiadis. When that happens, proof of origin will be an essential element of the trade.

Van de Merwe agrees: "I think if there is going to be a legal ivory trade down the road, each pair of tusks that a government wants to bring to market would have to have a certificate with its isotopes. It would add a bit to the price of ivory, but not much." The estimated cost is about $300 per tusk.

But even when ivory poaching is under control, Leakey is adamant about resuming trade: "We do not intend ever to put Kenyan ivory back on the market."

■ JEREMY CHERFAS

Stehelin Persists in Nobel Protest

Can a fait accompli by the Nobel Committee be overthrown? Dominique Stehelin of the Centre National de la Recherche Scientifique at the Pasteur Institute in Lille, France, would certainly like to think so. When the 1989 Nobel Prize for Physiology or Medicine was announced in October, Stehelin, backed by other French scientists and officials, complained bitterly and publicly that the Nobel Committee did not see fit to award him a share of the prize with J. Michael Bishop and Harold Varmus of the University of California, San Francisco (*Science*, 20 October, p. 326).

Now he has taken his case directly to the Nobel Committee. In an open letter, which runs four single-spaced pages plus three pages of enclosures, Stehelin maintains that the Committee has committed an injustice "in excluding . . . the very person who carried out the crucial experiments" for which the prize was awarded. He summarizes his contributions to those experiments, which culminated in the discovery that the cancer-causing oncogenes found in certain animal viruses had in fact originated in the cells those viruses had infected.

And what does Stehelin hope to achieve in pursuing his claim? "I don't see why they shouldn't overturn their decision," he told *Science*, "but if they don't want to do that then they should change the wording [of the citation] so that it doesn't reflect so narrowly what I did in Mike Bishop's lab."

Overturning a Nobel decision would certainly be unprecedented, and this one seems destined to stand. Earlier, Jan Lindsten, the secretary of the Nobel Committee, told *Science* that the committee thought that Bishop and Varmus were the key persons in the discovery. But then Stehelin can always hope—at least until 10 December when this year's prize will be actually given out.

■ JEAN MARX