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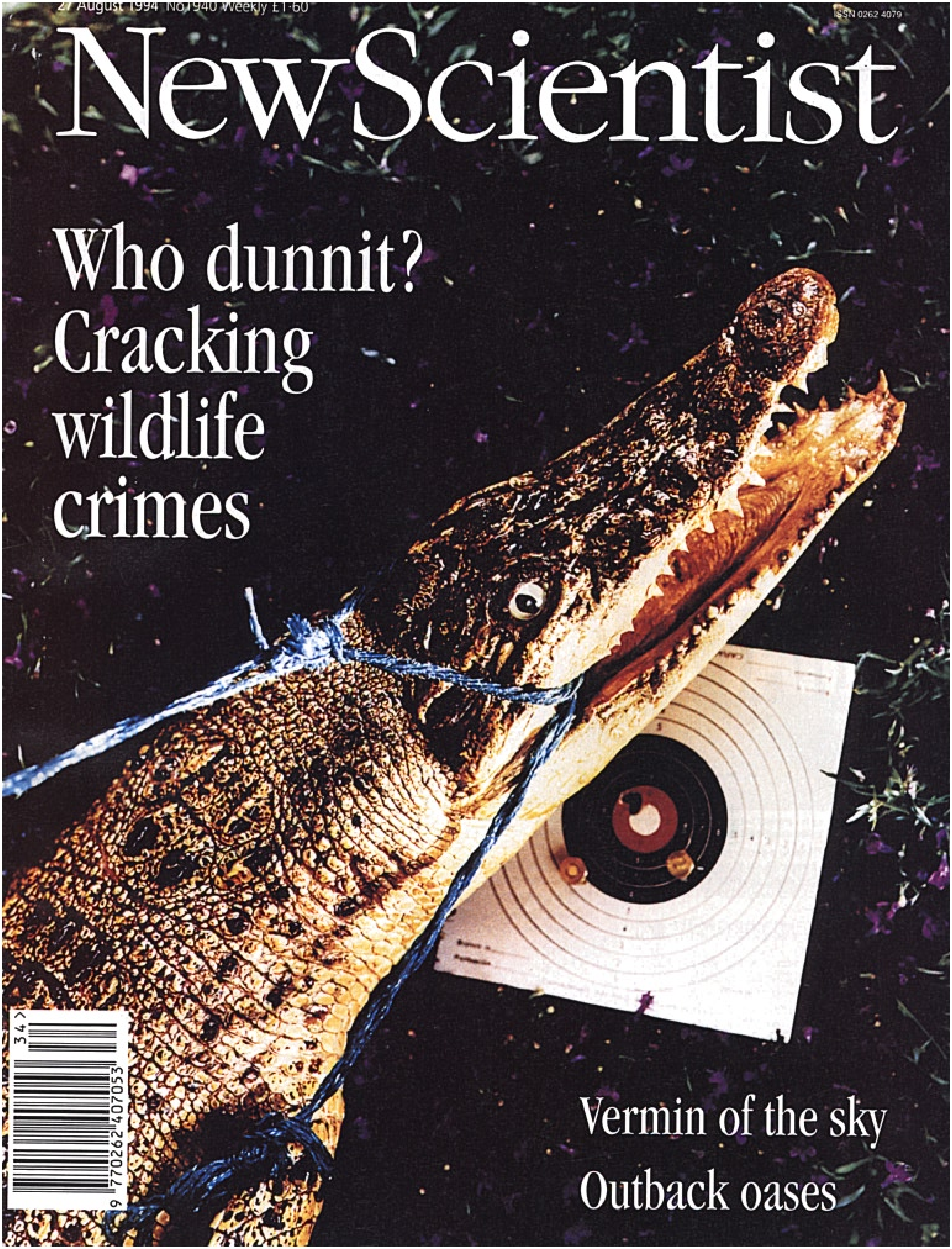

Who dunnit? Cracking wildlife crimes

Vermin of the sky

Outback oases

WITNESS for the DECEASED

The trade in endangered species is an international problem. Stephanie Pain reports from a unique laboratory dedicated to solving wildlife crimes

HE WAS rich enough to go big game hunting in Brazil, and foolish enough to record his exploits on film. He also made the mistake of underestimating how much his soon to be ex-wife disliked him. She sent the photographs to an agent of the US wildlife service, who found them rather interesting. As well as picturing the wealthy hunter with a jaguar he had just killed, they showed the numberplate of his hired Land-Rover. Later shots, taken back home in Texas, showed the hunter with a half-stuffed jaguar, and then with the beast on display. It looked like a straightforward case.

The jaguar is protected by an international treaty which bans all trade in any part of the animal. The man's crime under US law was to import the dead cat into the country. But to make sure he had a watertight case, the wildlife agent sent the pictures to the National Fish and Wildlife Forensics Laboratory in Ashland, Oregon. Was there any way of confirming that the stuffed cat in Texas was the one killed in Brazil?

Unfortunately for the hunter, one jaguar's spots are never quite the same as another's. All the forensic team in Oregon had to do was blow up the photographs and match up the patterns of spots. The man was fined and sentenced to a long stretch of community service.

For the scientists at the Oregon laboratory this is about as simple a case as they will ever have to tackle. The laboratory, set up in 1989, is the only one in the world dedicated to investigating crimes that breach the laws protecting wildlife. Its investigators often have nothing more to go on than a scrap of down, a spot of blood or a piece of skin altered beyond recognition. And while police crime labs have just one species, *Homo sapiens*, to deal with, the Oregon scientists have to cope with around 30 000 vertebrates, not to mention the occasional invertebrate.

But this is just what makes their work so enthralling. "We are creating a whole new field of science," says Ken Goddard, the ex-policeman who now heads the laboratory. In essence, that science is an alternative taxonomy. "The laboratory's job is to find new characteristics for defining species so that we can tell what animals the bits and pieces come from," says Goddard. The characteristics must be unique to each species. This applies

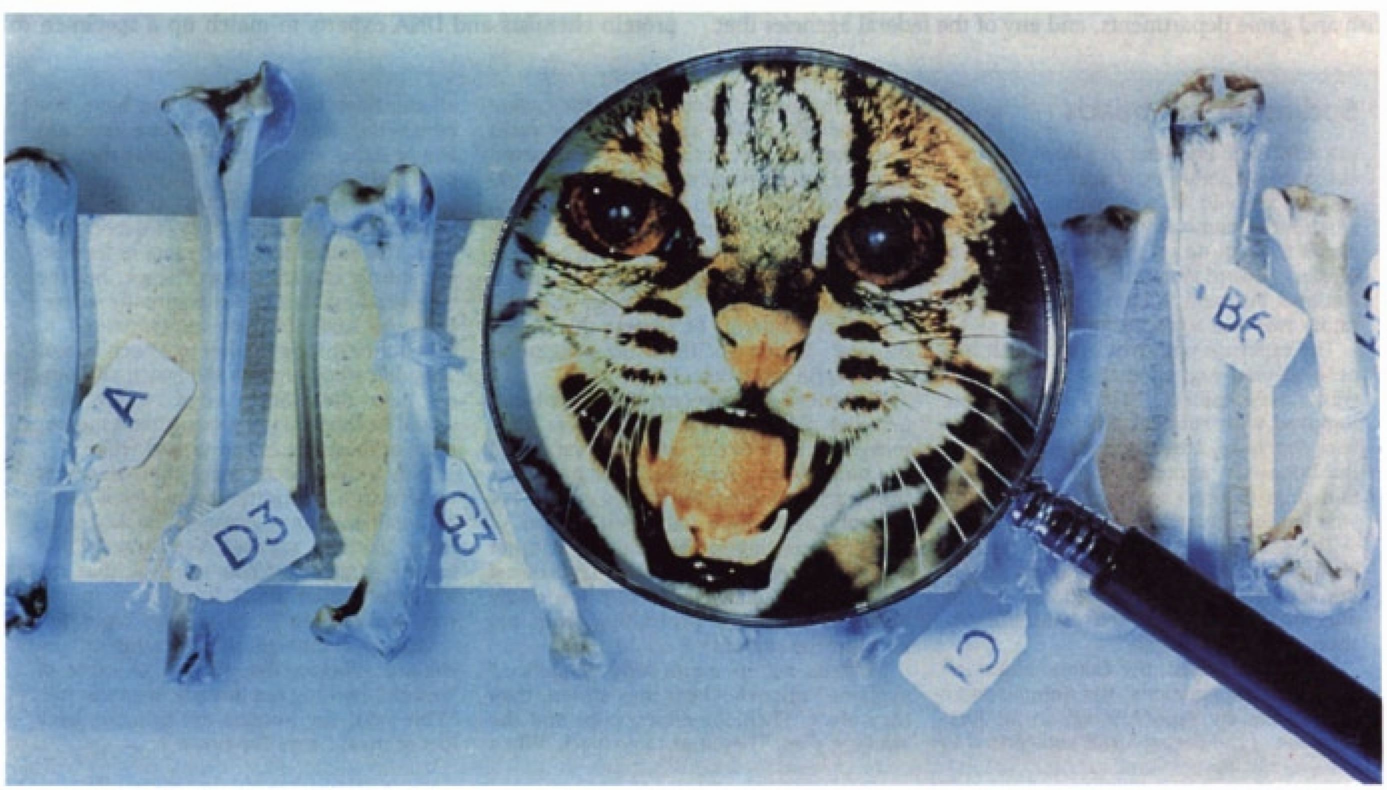

Illustration: John Morris

tissue from an animal whose identity they know.

Before the laboratory opened, wildlife agents had to rely on museums and university researchers for help in identifying parts of animals. The arrangement was not ideal. "Museum staff are not good at doing things fast—and they tend to disappear up the Amazon for months," says Stephen Busack, head of the morphology department. As a result, many cases ran out of time and had to be abandoned. "The laboratory produces a court-defensible species identification within a reasonable time, mostly within two weeks of receipt," says Busack.

A typical week's work for Beth Ann Sabo, Ashland's bird expert, might revolve around three feet, a headless torso and some down left in a trap. Bonnie Yates, the mammal expert, might receive a few hairs stuck to a bloody knife blade, a handful of bones or a tiger-claw charm. With practice, she can match even these small samples to creatures in the reference collection. Under a microscope, the tiny barbules of a down feather or the structure of a mammalian hair are characteristic of a species.

Busack's own field, reptiles and amphibians, presents special problems. Most evidence arrives in the shape of snakeskin shoes from China, lizardskin watch straps, and crocodile shoes and bags. "There are 168 species of snake in China that could be made into shoes," says Busack. "If they are tanned and dyed, they lose their characteristic pigmentation and it is virtually impossible to identify the species."

He is working on a system for identifying reptiles from the shapes and patterns of their scales, which might be the only identifiable feature that remains after the skin has been processed. Watch straps, for example, are often made from the skins of monitor lizards, some of which are protected and some not. Busack is confident that he can distinguish between 20 of the 30 or so species of monitor lizard on the basis of the shape of the dorsal scale. But only when he has found distinguishing characters in the 10 or so remaining species will he be able to say with certainty which animal a piece of skin came from.

If there is no morphology left in the evidence, the researchers have to glean information by other means. This could mean passing the case down the corridor to the serologists, who might try to extract some proteins or a sample of DNA. Sometimes it ends up in the "criminalistics" laboratory, where a team equipped with electron microscopes, spectrometers and other high-tech instruments may be able to identify a species from its chemical signature. "If they don't know what it is, we get it," says Mary-Jacque Mann, the lab's electron microscopist.

For the serologists, "evidence" can be distinctly unpleasant—a pan of half-cooked venison, a pair of trainers splashed with blood, or a pile of guts found rotting in the woods. "It may not be something you want to look at before lunch," says Bob Hoesch. Frozen meat is ideal to work with because freezing preserves the proteins. Cooked meats, such as sausage or salami suspected of containing illegal venison, pose more of a problem.

'Espinoza noticed that the pattern of fine, curved lines that radiate from the centre of sections through tusks were slightly different in elephants and mammoths'

Photographs: Madison Crotts

Hoesch applies standard techniques of immunology and protein chemistry to identify the source of a sample of meat or blood. To narrow down the family, he relies on immunodiffusion, testing his sample of protein against antisera from a number of animals until he gets a reaction that indicates a match. To home in on the species, he turns to electrophoresis, a technique based on the movement of charged particles through a gel. Because proteins are manufactured under genetic control, they vary from species to species. The point in the gel at which the protein comes to a halt can be used as a distinguishing feature of the species. Even proteins from closely related species will translate into variations in electric charge that are large enough to influence the protein's progress. For example, the enzyme glucose phosphate isomerase from a black-tailed deer travels farther than the version of this enzyme found in white-tailed deer, mule deer and moose. A second test, with superoxidase dismutase, separates the moose from white-tailed deer and mule deer. A third run, for erythrocyte acid phosphatase, picks out the mule deer.

The unit is building up a database of marker proteins that can be used to differentiate between species. One marker per species is not enough when samples are in poor condition and the proteins are badly degraded. The idea is to produce a suite of markers that will allow an unassailable identification.

A sophisticated toolkit

The three DNA specialists add a range of sophisticated techniques to the identification toolkit. As one animal's genetic material is never quite the same as another's, DNA sequences can reveal more than just the identity of the species. It can allow spots of blood at the scene of a killing to be matched to the meat in a suspect's freezer. It can tell you how many animals were killed and what sex they were. The biochemists can even compare an animal's DNA profile with those of its putative parents, which comes in useful in cases where a dealer claims that a rare animal being offered for sale was bred in captivity.

"DNA technology is our most powerful tool," says Goddard. Using the polymerase chain reaction, the DNA team can multiply a piece of genetic material millions of times. What started as a tiny scrap of DNA from the hide of a long-dead animal or a weathered piece of bone can provide enough information to go to work on. But there are still problems to be overcome. An element of subjectivity is involved in matching the "bar codes" of DNA profiles: a human observer compares the bands from two samples and decides if they match, and genetic fingerprint evidence is always presented in terms of the probability of a match.

Steve Fain and his team of DNA specialists would like to be able provide juries with evidence that they have an exact match that leaves no room for doubt. So they are looking for DNA sequences that are characteristic of a species and which can be picked out by standard probes acting on a single piece of genetic material. "We would like to find the 'Rosetta piece' for each species," says Fain. He hopes to find those telltale features in repeating sections of DNA called "microsatellites", which accumulate mutations very quickly. Once you know the variations that characterise a species—or even a population—it would be simple to check the unknown with probes designed to pick out the differences.

As with other techniques for identifying an animal, the DNA experts must have a standard to compare their unknown samples against. So far the lab can cope with any North American species of deer, bears, wolves, wild sheep and mountain goats, but it will be many years before they have all the species covered. "We need to move into birds, especially parrots," says Fain. The trade in live birds is worth $15 million a year in the US. Rare parrots from South America fetch high prices on the black market. Often they are smuggled into the country as eggs or chicks, which are impossible to identify by normal means. The usual strategy is to incubate the eggs and wait for the birds to develop their distinctive adult plumage. If DNA technology could be applied to samples from eggs that do not hatch it would immediately identify any protected species.

In the criminalistics lab, evidence tends to be even further removed from the animal at the centre of the case. The creature's identity is usually reduced to a spectrum of chemicals or a pattern of lines in an electron microscope. One of the laboratory's early successes was to find a way to distinguish between ivory from elephants and from their extinct relatives, the mammoths. When trade in ivory was banned in 1989, dealers appeared to be able to lay their hands on unlimited supplies of mammoth tusks almost overnight—or so their customs declarations indicated. At the lab, Ed Espinoza and his team began to look for a way of distinguishing between the two types of ivory.

'For the serologists, "evidence" can be distinctly unpleasant. "It may not be something you want to look at before lunch," says Bob Hoesch'

Mammoth task

Espinoza and Mann noticed that the pattern of "Schreger lines"—the fine, curved lines that radiate from the centre of sections through the tusks—were slightly different in the living and extinct species. In elephants, these lines consistently meet at angles of more than 115°, while in mammoths they meet at an angle that is less than 90°.

Why should one angle point to mammoths and the other to elephants? The lines are created by slicing through the dentinal tubules, microscopic canals that radiate from the centre of the tusk. The distance between the tubules determines the angle at which the lines cross. Examination under a scanning electron microscope shows that in elephants the tubules are widely spaced; in mammoths they are packed closely together.

Once this was established, Espinoza and his colleagues devised a simple technique that agents could use to make seizures with confidence. "You take your object to the photocopier, set it on darker, enlarge it a bit and press the button," says Espinoza. Out of the machine comes a perfect reproduction of the Schreger lines. After measuring a few angles with a protractor, the inspector can decide whether to seize the item. The technique has been a huge success. Most of the ivory entering the US today comes, legally, from warthogs and hippopotamus teeth.

Espinoza's team also has to deal with the imported pills and potions that allegedly contain parts of endangered animals—the rhino-horn tea balls, tiger-bone plasters and so on. Testing for rhino horn has been a challenge because it is composed of keratin, the protein that hair, hooves and fingernails are made of. Early attempts to distinguish between keratin from different sources had little success. Then Espinoza and Mann tried a technique called Fourier transform infrared spectroscopy, which analyses the wavelengths at which different molecules absorb infrared light. This revealed a unique rhino "fingerprint".

The telltale spectrum belongs not to the keratin fibrils, which produce the same spectrum as sheep's wool, but to the "glue" that holds the fibrils together. The chemistry of this binder remains a mystery, but its characteristic spectrum allows the laboratory to detect rhino horn in concentrations as low as 1 per cent. It turns out that few of the substances that are supposed to contain rhino horn actually do. Most are fake. "Only wealthy and influential individuals in Asia get the real thing. Everyone else gets the rubbish," reckons Espinoza.

The same is true of products that supposedly contain tiger

'Stephen Busack is working on a system for identifying reptiles from their scales'

bone, which is used to treat inflammation. "Everything we've analysed so far does not even have bone in it," says Espinoza, so the laboratory has never had to go the extra step and identify which animal the bone comes from.

Bears have also suffered from the huge demand for medicines, based on bile from the black bear's gall bladder. Asian doctors prescribe bile medicines for liver complaints, gallstones and as a general tonic—for those who can afford it. In a pharmacy in Seoul, crystallised bile fetches twenty times the street price of heroin.

There are now so few black bears left in Asia that the search for gall bladders has shifted to North America. The US has around 700 000 black bears. A hunter might be able to sell a fig-sized gall bladder to a black market dealer for $150, but by the time it reaches Taiwan or Korea it could be worth $50 000. Black bears are not yet endangered in the US, and hunting them is still legal with a licence, but a sudden demand for gall bladders could quickly push them towards extinction. As recently as March, a group of Koreans was discovered hunting bears illegally in northern California. Before they were arrested, the hunters had killed at least 35 black bears for their gall bladders and paws.

Penis carving

To deal with cases like this, the forensics lab needed to find a way to tell whether a suspect gall bladder has come from a bear. The problem is that most gall bladders are dried until the tissue hardens and the bile salts turn to crystals, leaving no identifying proteins. Gall is made up of a collection of bile acids. Espinoza's team found that bear gall contains three distinctive bile acids, ursodeoxycholyltaurine, cholyltaurine and chenodeoxycholyltaurine. These form the bear's "bile signature": no other animals have all three acids. But nor, as it turned out, do most supposed "bear bile" remedies—the majority of impounded gall bladders come from domestic pigs.

Not all fakes end up in Espinoza's laboratory. One of the more inventive frauds was uncovered by the morphologists and serologists. The dubious items were allegedly dried tiger penises, which are in demand for their supposed aphrodisiac properties. A tiger's penis has two distinguishing features: a spiky tip and a distinctive bone, or baculum, to stiffen it. These looked like the real thing, right down to the spiky sculpturing. But when the morphologists rehydrated and dissected them no trace of a baculum could be found. The spikes had been created by cutting small nicks in the skin that curved back as the penis dried. Analysis of proteins from the tissue showed that it had come from a member of the cattle family. "There must be a cottage industry in penis carving somewhere," says Peter Dratch, coordinator of the protein unit.

Some of the animals the lab has to deal with are victims of a very different industry—American agriculture. A growing share of the caseload in Ashland stems from illegal poisonings of wildlife, often by farmers anxious to protect their livestock from predators. As well as killing the intended target—usually coyotes or eagles, some of which are protected—the poison can also kill animals such as bears that scavenge off the victims. Most cases involve carbofuran and aldicarb, which are so toxic many states have banned their use. Farmers often lace the bait with massive doses of poison: "There is so much poison you often get big die-offs of birds around a carcass," says Jo Ann Shafer, the poisons chemist.

When the bodies are delivered to Ashland, the first step is for the pathologist to determine if they have been poisoned. "Often the chemical is in such high concentrations there is no doubt what it is," says Shafer. But it is harder to say where the poison came from and what the bait was. The answer often lies in the animal's stomach or crop, and any meaty tissue found there is analysed by the serology team. Often this is no easy task. "Eagles prefer dead food, so it has usually been lying on the ground before they eat it," says Hoesch. "Then it's part digested by the time we get it." With luck, the pathologist might find a few hairs or a chewed feather in the gut, which the morphologists can work on.

Not all cases of poisoning are deliberate. When dead eagles started to turn up on the rubbish dump in a small Alaskan town, the chemists showed they had been poisoned by pentobarbital and the serologists showed that the birds' stomachs contained tissue from the cat family. This was puzzling. Eagles do not generally kill cats. Then someone remembered the local cat shelter. Further inquiries revealed that the poison was being used to put down unwanted cats, and instead of burying the dead animals staff at the shelter were disposing of them at the dump, where eagles scavenged. When the shelter began to bury its dead cats, the eagle population recovered.

The task of policing crimes against all the world's wildlife is impossibly large. Today, a small fraction of Ashland's caseload comes from abroad. But later this year, at the biannual meeting of CITES, the Convention on International Trade in Endangered Species, the laboratory will be officially opened up to the 122 countries that have signed the convention. Goddard looks forward to the time when the Ashland lab can hand over its databases and techniques for other scientists to use in the fight for wildlife in other regions of the world. The first to take up the offer might be India, which has signed an agreement of cooperation with the US government to set up a similar laboratory, with funding from both governments.

As forensic scientists, the staff at the laboratory have to be scrupulously nonpartisan. "We are not anti-hunting," says Mann. "We just enforce the hunting laws." Nor can they be seen to be some sort of green crusaders out to stop the trade in wildlife. The forensic lab's job is to analyse the evidence, not to win convictions. "We don't notch up successes," says Goddard. "We try to be objective and look at problems scientifically." The evidence can prove a suspect innocent as well as guilty.

One man who escaped conviction was not particularly pleased with the verdict. The laboratory proved that the walrus tusk he had bought in Spain for $3000 was made of plastic. Told he had been sold a fake, he breathed a sigh of relief. He paid for the tusk with his American Express card—so perhaps he could claim a refund? No need, said the lab. Plastic is perfectly legal. □