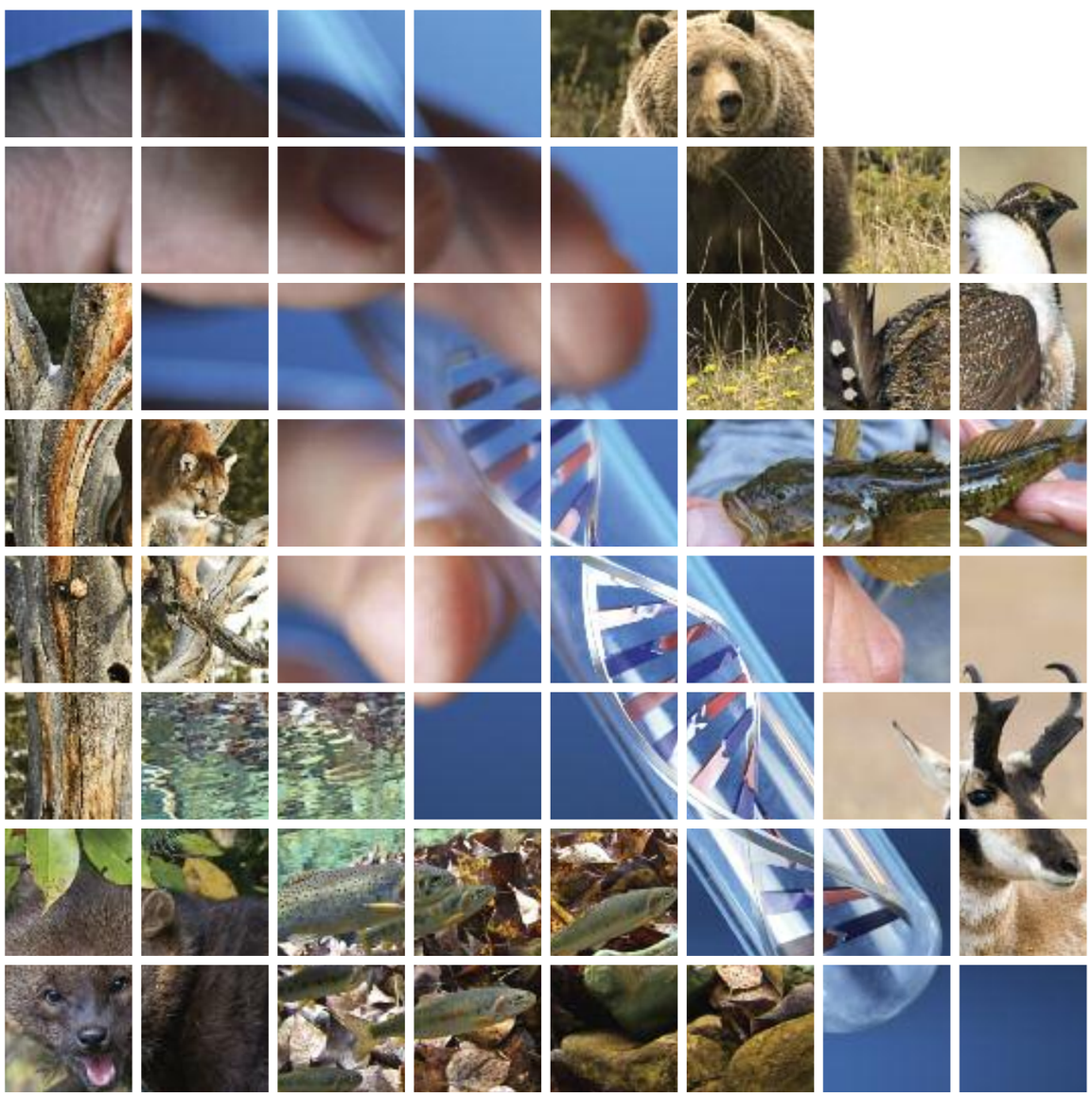
READING AN ANIMAL'S "FINGERPRINTS"

DNA science improves fish and wildlife conservation, management, and law enforcement. BY TED BREWER



IMAGES: SHUTTERSTOCK; USFWS; MONTANA FWP

In the mid-2000s, University of Montana biological sciences professor Kerry Foresman led a study on river otters in the Upper Clark Fork drainage. The aim? To learn if the otter population, which had taken a severe hit during decades of heavy metal contamination from old upstream mining waste, was rebounding, as suspected. And, if so, where the otters might be coming from.

Foresman asked Montana Fish, Wildlife & Parks for tissue samples from otters taken by trappers in the drainage. He also collected samples of otters trapped in neighboring river drainages, including in Idaho. DNA from the samples would tell Foresman where these otters and their recent ancestors might have originated.

The analysis revealed something intriguing: The genetic signature from some otters trapped in western Montana bore an unmistakable relationship with the genetic signature of those in Idaho's Lochsa River drainage. That meant that one or more river otters born in the Lochsa had, at one time, traveled several miles by land, climbing Lolo Pass in the northern Bitterroot Mountains and dropping down Lolo Creek to the Bitterroot River.

"People have suggested [that a migration by land] could occur," says Foresman. "They've seen otters walking through the woods, but there's never been any evidence before this that otter dispersal over mountain ranges in Montana resulted in breeding and genetic exchange."

DNA analysis has proved to be a vital new tool that allows wildlife and fisheries researchers to obtain more information faster. The process identifies not only an animal's species and sex, but also the individual and its relatedness to other individuals in a certain area or across a region. "Because of the DNA technology, I can finally answer a lot of questions I started asking 20 years ago," Foresman says.

Better science, better decisions

At the U.S. Forest Service's Rocky Mountain Research Station on the University of Montana (UM) campus, lab technicians working under conservation genetics team leader Michael Schwartz are extracting and analyzing DNA from blood, tissue, scat, hair, bone, and other types of samples sent from all over the United States and parts of Canada. On any given day, the technicians could be analyzing DNA samples from an Oregon pika, a South Dakota mountain lion, or a fisher that lived 100 years ago in western Montana.

Using tweezers, lab tech Kelly Morgan places a tissue sample from a sage-grouse into a centrifuge tube. About the size of a aspirin, the sample is part of a study gauging the bird's genetics in eastern Montana. She adds drops of purified soap and enzymes to the tube and places it in a heating block overnight. This process breaks open the tissue's cells and releases the deoxyribonucleic acid, or DNA, from the cells' nuclei. After extracting the DNA strands, lab technicians then sequence the precise order of the sage-grouse's genes, creating a printout that looks like an enlarged barcode. This is the individual animal's DNA "fingerprint" or "signature."

Before DNA analysis became a tool of fish and wildlife studies, biologists had to physically capture and mark animals to estimate, for example, how many mountain lions inhabit the Blackfoot River drainage or which individual grizzly bears reside in the Northern Continental Divide Ecosystem. Both species are elusive and often live in hard to reach areas. Someone trying to tally the number of individuals in a population could easily wind up counting the same lion or bear twice (if it hadn't been marked) and many not at all. Projects relying on physical capture could take up to a decade, with extremely high costs, making widespread application unfeasible.

Lacking accurate abundance and density estimates, Montana Fish, Wildlife & Parks wildlife officials sometimes struggled to determine whether certain populations were declining (requiring special protections) or increasing (able to withstand greater hunting or trapping harvest). "These advances in molecular genetics and new, faster methods for extracting DNA give us a much more accurate sense of how many animals are out there," says Justin Gude, head of wildlife research for FWP.

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As an example, Gude points to the finding in January by FWP and UM researchers that the estimated number of adult mountain lions in Montana's southern Bitterroot Valley is two

WELCOME TO MONTANA In the mid-2000s, researchers found Idaho otter DNA in otters living in western Montana. That meant one or more otters born in the Lochsa Basin had once crossed Lolo Pass in the northern Bitterroot Mountains and dropped down Lolo Creek to the Bitterroot River.

to four times greater than previously thought. "Our earlier estimates came from radio telemetry, a method that could give a minimum count only for resident lions that didn't disperse widely," he says. "But now, using DNA analysis, we've been able to estimate the size of the Bitterroot population with far greater accuracy in a way that also includes dispersing and transient lions."

Maybe the most crucial technological advance in DNA analysis has been polymerase chain reaction (PCR). This biochemical technology enables laboratory technicians to make millions of DNA copies, much like our bodies do, from the few strands of DNA that researchers extract from scat, hair, vomit, feathers, eggshells, bone, and even fish cells floating in a scoop of creek water. Before PCR, scientists relied entirely on blood and tissue samples for obtaining DNA. That required finding and then obtaining samples directly from the animals that biologists were studying, a particular challenge with secretive species and large predators.

PCR has made collecting DNA much less invasive and obtrusive, allowing researchers to collect far more samples than before. Now biologists often need only gather scat from the landscape (sometimes found with the help of specially trained dogs) or collect samples from snag stations, in which barbed wire strung around stinky bait catches hair of curious bears and other wildlife.

Ted Brewer is a writer in Helena.

Because DNA shows which particular lion or other animal left the scat or hair, researchers can use statistical models to count the number of individuals in an area, providing wildlife managers with reliable abundance and density estimates.

Aiding bull trout and westslope cutts

DNA analysis for fish and wildlife grew out of a federally funded project that began in 1990 to "map" the human genome, the complete set of genetic information for *Homo sapiens*. "The technology starts in human medicine, advances to human forensics and population genetics, and within a couple of years we're adapting it to wildlife," Schwartz says. "We draft behind the Genome Project, behind the medicine. We just pick the technology off."

Fisheries scientists also are running with the new technology. Biologists with the USFWS, working with FWP, Avista Utilities, and others, are capturing migrating adult bull trout below Cabinet Gorge Dam on the Clark Fork River. They then use "rapid genetic assessment" to determine how far above the dam to move the native fish so the trout are more likely to spawn in natal waters.

Two other bull trout conservation efforts using DNA analysis are in the Clearwater Chain of Lakes in the lower Seeley-Swan Valley and in the Blackfoot River drainage. There, FWP biologists are sampling bull trout in spawning tributaries to learn where fish that live in the various lakes and the mainstem Blackfoot River originate. "This helps us identify the most critical spawning tributaries for conservation," says Robb Leary, FWP fish conservation geneticist.

FWP biologists also use DNA analysis to identify where the purest strains of westslope cutthroat live. Leary says the agency can then focus limited conservation dollars on those critical habitats rather than watersheds where native fish are too genetically diluted by hybridization with rainbows.

Biologists with FWP and the Rocky Mountain Research Station have developed genetic "markers" for native cutthroat trout, native redband trout, and non-native rainbow trout. This gives them a better idea of how the various species use their range, where they intersect, and how to prevent hybridization that threatens the genetic purity of indigenous species.

For instance, Bostwick Creek in the Gallatin drainage historically contained only pure-strain westslope cutthroats. Recently cutthroats that have hybridized with rainbow trout ("cutt-bows") have entered the creek from downstream. To conserve the native fish, biologists are capturing and tagging trout, from which they take tissue samples to send to a laboratory. "If the samples contain genetic markers showing that the trout are hybrids, biologists can go back and remove those particular fish from the stream population," Leary says.

AN ENTIRE BEAR IN A SINGLE HAIR Using what's known as PCR biochemical technology, scientists produce millions of DNA copies from just a few strands of DNA in a hair, feather, or fish scale to identify an animal's identity and genetic makeup.

CLOCKWISE FROM LEFT: BRUCE BECKER; MONTANA FWP; SHUTTERSTOCK

DNA analysis also helps FWP nab poachers. "We regularly see DNA matches between the remains of poached carcasses and various items in a suspect's possession," says Mike Korn, assistant chief of the FWP Enforcement Division. For instance, in spring 2012 several elk were illegally killed near Thompson Falls, the meat cut out and carcasses abandoned. By matching DNA from tissue at the crime scene with meat he later obtained, local warden Tom Chianelli obtained a search warrant that resulted in three poachers eventually confessing to the crime, permanently losing their hunting privileges, and paying $18,000 in fines and restitution. In another case, a headless bull elk carcass was found near Hardin. Human DNA on a rope-handled knife discovered at the crime scene by game warden Shane Yaskus matched that of a local criminal, who pled guilty to illegally possessing a bull elk.

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Specimen from 1896

DNA analysis is also allowing scientists to better understand the lives of wildlife that lived here hundreds or even thousands of years ago. By examining animal remains in ancient rodent dens, researchers are learning about short-faced bears, saber-toothed tigers, and woolly mammoths. DNA science has even upended a long-standing belief about Montana's fishers.

For much of the 20th century it was common knowledge that this cousin of the wolverine was extirpated (locally extinct) in Montana. Biologists attempted to restore fishers in the 1960s by releasing several dozen from British Columbia in Idaho and western Montana. Then, from 1989 to 1991, FWP translocated another 110 fishers, from Minnesota and Wisconsin, to Montana's Cabinet Mountains.

Some 20 years later, one of Kerry Foresman's graduate students (and current FWP wildlife biologist), Ray Vinkey, evaluated the success or failure of the fisher translocations. As Foresman had done with the otter study, Vinkey asked Schwartz's lab in Missoula to analyze DNA from tissue samples of legally trapped fishers stored by FWP. All the fishers across western and northwestern Montana possessed genetic signatures indicating they descended from fishers reintroduced from British Columbia and the Midwest. However, fishers from the mountains in western Montana also had genes not present in either of the source populations.

So where did they come from?

Working on a hunch, Vinkey and Schwartz decided to compare the trapped fishers' DNA with that from a fisher skull at Harvard University, a specimen originally collected in Montana near Lolo Pass in 1896. Schwartz flew to Harvard and brought back the specimen's nasal turbinate bone, from which he found enough trace blood to extract the DNA. His analysis indicated that fishers in western Montana mountains may be part of a native, historical population whose ancestors include the Montana fisher at Harvard. "We thought fishers were totally extirpated from the state," Foresman says. "Now we know they probably weren't and that we have a unique Montana population."

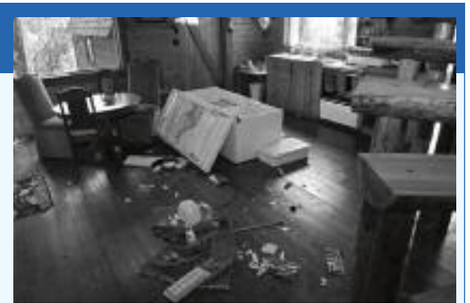
No doubt even more surprises about Montana's fish and wildlife await discovery in the DNA that scientists are right now collecting and analyzing.

SCIENCE SPARES MARAUDING BEAR LOOK-ALIKES

Had it not been for DNA fingerprinting, several innocent grizzly bears might have been unjustly euthanized for the misdeeds of one notorious bear, the Albino Basin grizzly.

FWP first recorded the infamous bear's DNA in 2004, when he wandered into a trap in the Swan Valley and was radio-collared. After shedding his collar, he appeared on the department's radar (and gained his name) a year later when he left behind fur in a hair snare that researchers had set in Albino Basin, deep in the Bob Marshall Wilderness.

From 2006 to 2009, FWP biologists investigating a series of cabin break-ins throughout the Bob Marshall and Swan Valley found hair and blood they were able to identify, through DNA fingerprinting, as belonging to the Albino Basin grizzly. In 2007 alone, he broke into at least ten different cabins in the Swan Valley near Condon. Unlike most bear burglars, he never returned to crime scenes, thereby evading traps FWP set near the break-ins afterward.



CRIME SCENE A cabin raided by the Albino Basin grizzly. The bear, known to focus on buildings containing white refrigerators, was eventually captured. After identification using DNA analysis, the grizzly was euthanized for the sake of public safety.

At various times that same year, grizzly specialist Tim Manley caught four male suspects whose paws were the same size as the tracks left at the vandalized cabins. Because FWP is obligated, for the sake of public safety, to euthanize bears that break into places where people live, biologists might have had to kill one or more of those grizzlies. But DNA analysis confirmed that none of the hair and blood of the four bears matched what was found at the break-ins, so Manley was able to release them.

The 20-year-old Albino Basin grizzly was finally tripped up in 2009, when he returned to the scene of his latest crime and landed in a trap Manley had set. A full body mount of the infamous grizzly is on display at the FWP regional office in Kalispell. Visitors are welcome.