Photo of mountain lion tracks © L. Sweanor

DNA analysis fo conser

The new millennium finds us at the very beginning of a genetic revolution - Dolly the cloned sheep, DNA-based medical diagnostic and treatments, the Human Genome Project. Over the past two decades, tools for genetic discovery have been developed and refined. But, only recently have these discoveries allowed us to

By Holly Ernest

examine the DNA (Deoxyribonucleic acid) of wildlife and to use this information in the protection of natural populations of plants and animals. Geneticists, wildlife veterinarians, game wardens, and wildlife biologists work together using new DNA technologies. With this technology we may answer questions involving mountain lion presence, numbers, and movements that were difficult, if not impossible to answer in the past. Using genetic techniques to collect information will not only address the following questions, but many more as well.

- Since mountain lions can travel long distances and may be able to mate with others living distantly, are all mountain lions in California closely related, forming one big population? Or are there distinct groupings of lions that may warrant special attention?
- Mountain lions inhabiting the Santa Ana Mountain Ranges in Southern California may be losing migration corridors because of highways and residential developments that inhibit animal movements to and from other areas. Are these lions becoming genetically inbred by having too few mating partners?
- Wardens confiscate a piece of gall bladder tissue from a person trying to sell it as a special medicine. Did the tissue come from a mountain lion making this a case of illegal take?
- Seven sheep in Mendocino County are found dead of predator wounds. Did one or more mountain lions kill them? If a mountain lion is killed on depredation permit near the sheep flock, did it kill the sheep?
- A predator kills several endangered bighorn sheep in the desert mountains of San Diego and Riverside counties. Did one or more mountain lions kill them? Do some lions living in bighorn habitat kill more bighorn sheep than other lions?
- The eastern cougar, once roaming U.S. east of the Mississippi has been listed as extinct for decades (with the exception of a few existing Florida panthers). But Eastern fish and wildlife agencies report increases in cougar sightings. Are these truly cougars, or are they cases of mistaken identity (coyotes, dogs, bobcats, lynx, or even domestic cats)?
- A California Department of Fish and Game (DFG) wildlife biologist wants to know if the radio-collared female mountain lion that she has been following might be the mother of another female observed five miles to the south. And is the male lion wearing another radio collar the father?

So how do we find the answers? Consider first what "genetics" means. Genetics is the science of heredity (traits transferred from

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one generation to the next) and variation (differences between individuals). DNA is the genetic blueprint contained within our cells, and the cells of all plants and animals. Even viruses, the tiniest of life forms have some type of nucleic acid, either DNA or its cousin molecule, ribonucleic acid (RNA). DNA contains the information to build a mountain lion, or human, from the fertilized egg to its adult form. Bodily processes including digestion of food, blood pressure, processing of fat, behavior, and even aging are guided and modified by information translated from our DNA. No two mountain lions in nature will have exactly the same DNA unless they are identical twins.

DNA is located in two main places in animal cells: in the nucleus and in the mitochondria (the little "power houses" of the cell). In the nucleus, there are duplicate sets of double stranded DNA molecules. One half of the nuclear DNA in a mountain lion is inherited from the mother and one half from the father. The mother's DNA combines with the father's to give each mountain lion a uniquely different DNA blueprint. Paternity (or maternity) tests make use of the way that nuclear DNA strands are inherited from father or mother to offspring. Pieces of DNA from a child or mountain lion cub and her potential parents can be examined to see if they share DNA. Unlike the nucleus, the mitochondria contain a small circle of DNA that is inherited only from the mother.

The DNA in one mountain lion contains the information equivalent to a library containing thousands of books. To study DNA which is so complex and has so much information, scientists look at one sentence out of one book at a time. Using DNA extraction and duplication techniques, subtle variations between individuals can be detected. David Foran and his colleagues at the University of California at Santa Cruz have used DNA from mitochondria to determine the presence of several secretive carnivore species. By extracting and analyzing DNA from scat (fecal samples) collected from the field, they are able to document the presence of animals including mountain lions, bobcats, and coyotes.

Conserving a specific group of animals or plants requires knowing what *is* and what *is not* a member of that group. Accurate identification of groups of organisms into species, subspecies, and populations is particularly important in the case of endangered species. Mountain lions of North and South America (also known as cougars, pumas, and including Florida panthers) are grouped as the species *Puma concolor* (also, *Felis concolor*, "cat of one color"). The species has been divided into 32 subspecies (regionally distinct groups). Subspecies divisions were assigned decades ago by biologists who noted regional differences in morphologic characteristics (form and structure of an organisms including fur color, skull shape and size, etc). Morphologic characters are influenced by the DNA blueprint, but also by climate and habitat (the local environment occupied by an organism) in which the mountain lion lives. Lions living in tropical climates tend to be smaller, have lighter smaller skulls than lions living in cold climates. Lions with reddish fur are more likely seen in the tropics, brownish fur in deserts, and darker brown-colored fur in Pacific coastal forests. But there is great individual variation, often with lions of all sizes and fur color inhabiting the same area. Grouping lions based on observable features like fur and skull may not be the most accurate method.

Photo © L. Sweanor

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Above, a female mountain lion is monitored while sedated. Below, collecting scat (fecal matter) for later DNA analysis.

Photo © Jim Zuehl and Bambi Collins

DNA analyses help. Dr. Melanie Culver, University of Maryland, recently completed a DNA analysis of 31 of the 32 cougar subspecies. She found that cougars differ very little from each other, especially in North America, so that fewer subspecies are warranted. At the University of California, Davis, (UCD) with help from Culver and her colleagues at the National Institutes of Health, we are examining samples of hide and feces collected in the eastern U.S. for characteristic mountain lion DNA signatures. Wildlife officials want to know if natural populations of *Puma concolor* once again exist in the eastern states. The situation is complicated by the release to the wild of captive mountain lions (both legal release under the jurisdiction of state and federal agencies and illegal releases by unknown individuals). DNA analysis will help officials to properly classify and manage mountain lions discovered in their states.

In California, some of our research is more problem specific. Collaborating with the DFG, California Department of Parks and Recreation, and fellow researchers at the UCD, current efforts include examining DNA to study the predation of endangered desert bighorn sheep of the California Peninsular Ranges by mountain lions. When a predator kills its prey, it often leaves a handy fecal sample near the kill site. DNA extracted from these samples not only indicates whether the sample came from a mountain lion, but also identifies DNA type so that individual lions can be differentiated. This information helps estimate the number of mountain lions that use bighorn as food, and to determine whether certain lions kill bighorn more than others.

In other projects at UCD, DNA analyses helps to identify and count lions traveling near hiking trails in Yosemite and Redwood National parks. With help from the Yosemite Fund and United States Geological Service Biological Resource Division, mountain lions were counted by genetically typing lions captured for radiotelemetry studies and fecal samples collected from park trails. DNA extracted from bite wounds (containing lion saliva) on prey carcasses, has identified a mountain lion responsible for inflicting fatal wounds in domestic sheep at the University of California Hopland Research and Extension Center in Mendocino County. The genetic signature from the bite wounds on the sheep matched that of a lion killed on depredation permit nearby. Geneticists working with the DFG

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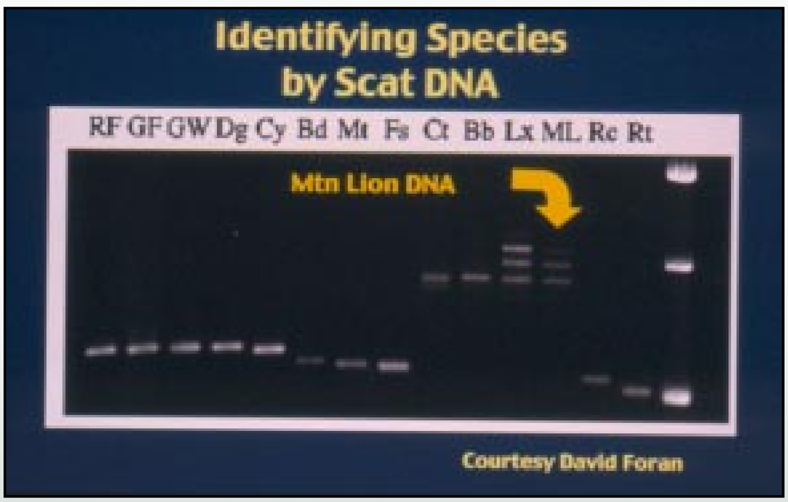
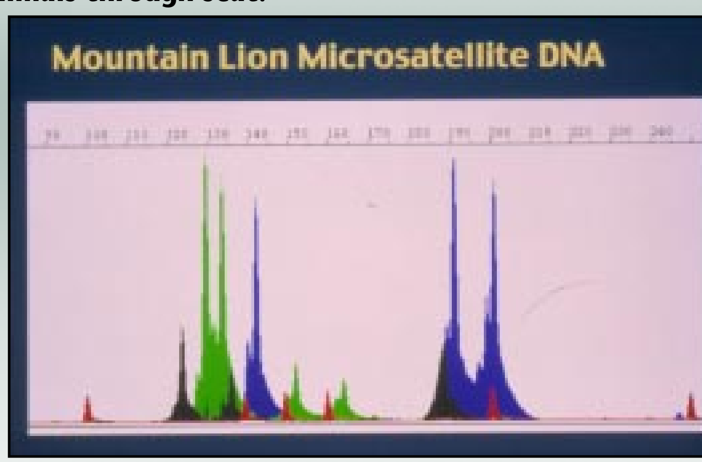


Graphic © Wildlife Society

David Foran's mitochondrial DNA work for identifying animals through scat.



Graphic © Holly Ernest

Above, top axis is basepair size of DNA from one mountain lion. DNA can also be extracted from saliva on prey carcasses.

used the same techniques to confirm that lions killed near the site of human fatalities in California and Canada were responsible for the bite wounds.

The population structure of mountain lion populations throughout California is also being examined by analyzing DNA from several hundred animal necropsies (animal equivalent of an autopsy) by the DFG veterinarians and wildlife biologists. Genetic analysis will provide estimates of animal migration and how that migrational movement can affect populations of mountain lions, their prey, and humans. This DNA database will also provide population information necessary to assign match probabilities to DNA fingerprints from mountain lion fecal samples. A match probability is the chance that any two lions will share the same genetic type for the DNA sections that are tested. These DNA probabilities are commonly used in human murder and paternity cases.

From a conservation biology standpoint, knowledge of the genetic structure provides a foundation on which to evaluate whether certain populations might be threatened with inbreeding and what impact this could have on the species as a whole. Knowledge of genetic structure is also important to understanding how diseases may spread through wildlife populations.

DNA technology is advancing very rapidly. DNA testing of wildlife by remote and noninvasive methods so that animals will not be disturbed is becoming more widely available. The future will see increasing use of fecal and hair DNA studies to count animals that are otherwise difficult to find in the wild. Cataloging the genetic variability that is present now and was present in the past (by DNA analysis of museum specimens), are some of the priorities for the future. For more information checkout these websites at:

- www.dir.yahoo.com/Science/Ecology/Molecular_Ecology
- www.bis.med.jhmi.edu/Dan/DOE/intro.html
- www.vetmed.ucdavis.edu/whc

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Photo © Esther Rubin

Radio collared bighorn sheep killed by a mountain lion.

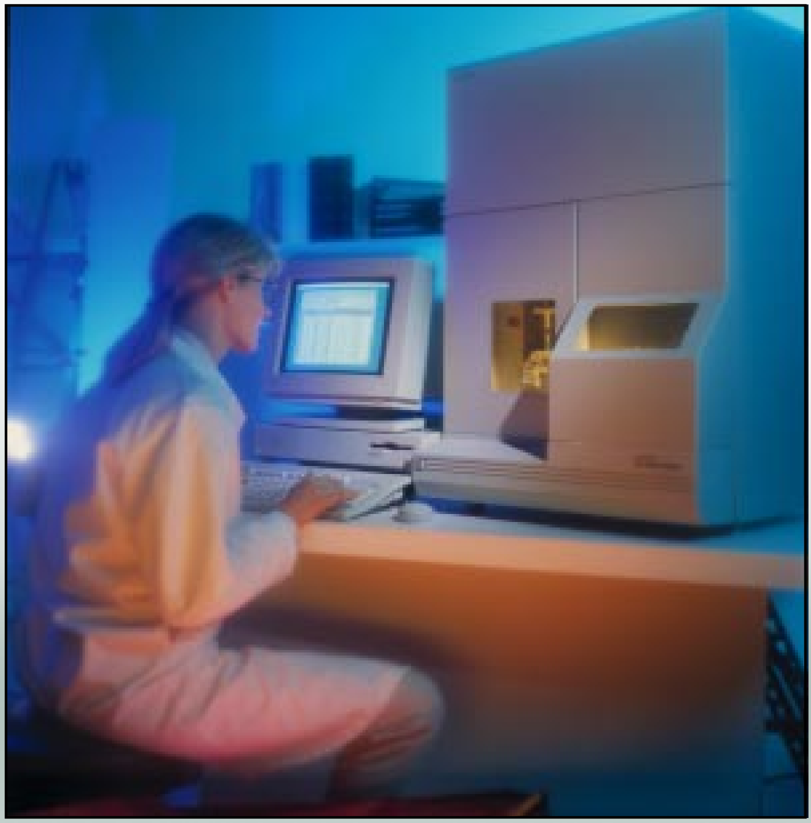

Photo © Perkin Elmer

Above, a DNA analyzer (ABI Prism 310 Genetic Analyzer). From a conservation biology standpoint, knowledge of the genetic structure provides a foundation on which to evaluate whether certain populations might be threatened with inbreeding and what impact this could have on the species as a whole. Knowledge of genetic structure is also important to understanding how diseases may spread through wildlife populations.