

Genetics for wildlife conservation

DNA analysis of species and subspecies provides information not available via binoculars

Scott Baker maneuvered his rubber boat closer to a pod of humpback whales diving in the murky waters off Alaska's southeastern coast. As the boat drew within 20 feet or so, Baker reached for his crossbow and arrow. Carefully, he took aim at the nearest whale and fired. The arrow struck home—it's hard to miss a target the size of a humpback whale at close range, Baker admits—driving the dart tip into the animal's side.

The specially designed dart quickly removes a small piece of tissue from the whale, about the size of a cigarette filter, then pops out. The dart appears to disturb the whale only briefly, Baker says. Attached to a float, the dart bobs up and down with the waves until Baker retrieves it a few minutes later.

Back in the laboratory, he uses the tissue samples, each weighing about a gram, to analyze how whaling and geographic separation have affected the genetics of humpback whales. "We are trying to shed some light on these animals," says Baker, a behavioral ecologist at the National Cancer Institute's (NCI) viral carcinogenesis laboratory in Frederick, Maryland. He studies the social organization and behavior of humpback whales. "Molecular genetics provides us with a whole new window into their lives."

Indeed, Baker's research on humpback whales symbolizes the burgeoning role genetics plays in wildlife biology today. From whales and rhinos to tamarins and gazelles, geneticists and evolutionary biologists are using studies of mitochondrial and nuclear DNA to learn more about the basic

biology of wild animals.

"There is a great ignorance concerning the genetics of most species," says Oliver Ryder, a research geneticist at the San Diego Zoo's Center for Reproduction of Endangered Species. "By understanding their genetics better we will better understand how to conserve them."

The new focus on genetics in wildlife biology stems, in large part, from pioneering research on cheetahs in the early 1980s by Stephen O'Brien, chief of NCI's viral carcinogenesis laboratory, and David Wildt and Mitchell Bush of the National Zoo in Washington, DC (*BioScience* 36: 358-362). O'Brien and his colleagues

found cheetahs to be extremely genetically invariable, a result that surprised many scientists and wildlife conservationists.

That finding has led to similar studies on an ever-increasing number of captive and free-ranging species by several government, university, and zoo labs. Together, their work is helping to resolve taxonomic issues, explain reproductive problems, assess the risk of disease epidemics, and suggest conservation strategies.

Using genetics to better understand and conserve species is still in its infancy, O'Brien says. Genetic studies sometimes yield seemingly contradictory results, so conclusions based on



Genetic material from all seven bear species, including the spectacled bear (*Tremarctos ornatus*) has been analyzed. Photo: Susan Raich, © Lincoln Park Zoological Society.

by Jeffrey P. Cohn

only one study may be premature.

Genetics can help explain certain biological phenomena, but it cannot predict their consequences. Nor can it substitute for traditional wildlife studies. Yet, O'Brien notes, "There is a lot you can't see through binoculars."

Starting with pandas and lions

For starters, O'Brien was asked in 1983 to answer who sired a giant panda cub that died shortly after birth at the National Zoo. O'Brien not only identified the zoo's own male as the cub's father, thereby proving his fertility, but also may have resolved a long-standing dispute among taxonomists. Are giant pandas raccoons, as most scientists had thought, or bears, as their popular image suggests?

O'Brien's research and that of other geneticists assumes that related species diverge from a common ancestor due to accumulations of random mutations in the genetic material of isolated populations. The genetic changes occur over time in a steady,

clocklike manner and are roughly proportional to the time elapsed since two species shared a common fore-runner. By analyzing amino acid sequences in particular proteins, geneticists can determine the relationship between species and estimate when they diverged.

After comparing genetic material from giant and red pandas, raccoons, and coatis, plus all seven bear species, O'Brien concluded that bears and raccoons diverged 30 million to 50 million years ago. He further found that red pandas separated from the ancestor of today's raccoons and coatis a few million years later, some 10 million years before giant pandas diverged from the other bears. Thus, O'Brien says, giant pandas are indeed bears.

Meanwhile, Paul Joslin was not quite sure whether the "Asiatic lions" in US zoos were really Asiatic lions. Joslin, then assistant director of Chicago's Brookfield Zoo and now an independent zoo consultant, had noticed that many of the lion males lacked a telltale belly fold, a flap of

loose skin on their undersides that distinguishes Asiatic from African lions. He was also worried that increased infant mortality among "Asiatic lions" in US zoos signaled inbreeding problems.

To address Joslin's concerns, O'Brien and Janice Martenson, a researcher in O'Brien's lab, analyzed blood proteins taken from wild and zoo-born Asiatic lions. As with cheetahs earlier, they found no genetic differences among individual Asiatic lions in India. But they did find differences among Asiatic lions in US zoos.

"We were surprised," Martenson recalls. "At first we thought, 'Great! There is some genetic diversity [among Asiatic lions],' but then we learned that African lions had been allowed to mate with Asiatic lions in some Indian zoos. The diversity we were seeing came from African-lion genes."

O'Brien and Martenson eventually found that almost all Asiatic lions in US zoos and some in Asian ones as well were hybrids. As a result, US zoos agreed to suspend mating Asiatic lions and allow the hybrid animals to die off naturally. In the future, zoos will breed only pure Asiatic lions, which Joslin hopes to import later this year from India, where only approximately 250 survive in the wild.

Hybrid animals also concerned Bruce Read, curator of mammals at the St. Louis Zoo. Read worried that wild cattle from southeast Asia had interbred with domestic cattle, thereby changing the genetics of animals in US zoos. The three wild cattle species—gaur, banteng, and kouprey—are considered endangered or threatened in the wild. Zoo programs to breed them have only recently begun.

Read asked Scott Davis, a geneticist at Texas A&M University in College Station, to solve the riddle. Davis analyzed DNA protein markers from gaur, banteng, and domestic cattle. He found the three species to be genetically distinct with no signs of hybridization among zoo animals. Genetic analyses of kouprey, which at



Ancestors of red pandas (*Ailurus fulgens*) diverged from those of raccoons 10 million years before the ancestors of giant pandas diverged from those of other bears. Photo © New York Zoological Society.

Mitochondrial messages

As killer African bees spread northward from Brazil, where they escaped 27 years ago, scientists and beekeepers need to know whether they will remain aggressive and supplant other species or mellow out as they mingle with the more docile European honeybees already present in Central America. Other biologists half a world away are trying to piece together the family tree of an extinct animal. Once widespread in Australia, was the marsupial wolf more closely related to an extinct group of South American carnivorous marsupials or to those of Australia? Physical evidence—tooth characteristics and a pelvic trait—suggests a South American connection. Molecular evidence from protein comparison argues for Australian relatives.

To answer such questions in evolutionary and population biology, researchers increasingly turn to molecular techniques that tease clues from DNA. Sometimes the best clues come not from the chromosomes, but from DNA in the mitochondria, cellular structures outside the nucleus.

Approximately the same size and shape as bacteria, and commonly known as the cell's powerhouse, the numerous mitochondria provide most of the cellular energy. Like bacteria, they contain circular DNA and reproduce by dividing in two. Their DNA is useful to researchers for several reasons. First, mitochondrial DNA (mtDNA) is inherited only from the mother, so it does not undergo the scrambling that nuclear DNA experiences during recombination in normal sexual reproduction. Changes in mtDNA are therefore due only to mutation, not reproductive shuffling. Second, mitochondria do not have error-detection and repair mechanisms as do nuclei, so mtDNA mutates approximately ten times faster than chromosomal DNA. Consequently, it evolves faster. Finally, mtDNA is compact, having few examples of duplications or noncoding regions. Size of mtDNA varies within and between species, but generally it carries fewer than 40 genes. These characteristics make mtDNA a particularly appropriate marker for tracing recent evolutionary history, including colonizations or founder events, introductions, and bottlenecks and geographic divisions in populations.

Researchers can derive simple measures of genetic distance by comparing mtDNA sequences and restriction fragment patterns. Restriction enzymes cut DNA at unique sites, and the resulting fragments furnish an indirect indicator of sequence changes. Calculations of mutation rates can provide a molecular clock, which can be used to construct genealogical trees or to determine the amount of time that has passed since individuals or species last shared a common female ancestor. Using such techniques, some scientists have advanced the controversial proposition that a common female ancestor—dubbed Eve—of all modern humans lived some 200,000 years ago in Africa.

Apparently the killer bees advancing north through Mexico at greater than 300 miles per year also have maternal roots in Africa. Two groups of researchers (H. Glenn Hall and his colleagues at the University of Florida in Gainesville and Deborah Roan Smith and her colleagues at the University of Michigan in Ann Arbor) have sampled wild bee swarms from several places in Central and South America and found predominantly African-type mtDNA (*BioScience* 39: 583). African queens must be leading the migrating front, which is expected to reach Texas this year. How these bees supplant the established European honeybee populations remains unclear.

A relatively recent addition to the biologist's toolbox can extend DNA analysis methods to extinct animals such as the marsupial wolf. Polymerase chain reaction (PCR) can increase the number of particular segments of intact DNA in a sample—even in the presence of a vast excess of degraded DNA (Arnheim page 174 this issue). Using this selective amplification process, researchers have examined genes from museum specimens preserved a variety of ways and from archaeological remains, including an Egyptian mummy, a 13,000-year-old sloth, and a 7000-year-old human preserved in Florida peat bog.

Researchers have prepared mtDNA from tissues of century-old museum specimens of the marsupial wolf, as well as from related groups of marsupials. After comparing the DNA sequence of a particular gene and weighing all the evidence statistically, Richard H. Thomas of the University of Nottingham, United Kingdom, concluded that the marsupial wolf is more closely related to Australian marsupial carnivores than to those of South America. Resemblances in physical characteristics, they decided, must be due to convergent evolution.

Unfortunately, mtDNA properties defined for one group of organisms do not necessarily apply to other groups. For instance, mutation rates vary. Nevertheless, when coupled with other molecular techniques, studies of mtDNA provide fertile ground for interaction among such fields as molecular, evolutionary, and population biology.

—Carolyn Strange

Carolyn Strange is a science writer in Saratoga, CA.

probably fewer than 100 left in the wild are the most endangered of the wild cattle, must await the arrival of blood samples from the first captive animals in Asia, maybe later this year or next year, Davis says.

How to divide primates into species

In other work, Lisa Forman wanted to know how the three types of lion tamarins (golden, golden-headed, and golden-rumped or black) are related. These New World monkeys are sometimes listed by taxonomists as separate species, although more frequently as subspecies. All three live in widely separated remnants of Brazil's coastal rain forests. Squirrel-sized, they look much alike except for their coloring. And altogether they are thought to total less than a thousand animals in the wild.

Forman, then a postdoctoral fellow at the National Zoo and now with Cellmark Diagnostics, a private DNA diagnostics firm in Germantown, Maryland, found little genetic variation among the three lion tamarins. At 45 of the 47 structural gene loci she analyzed, all three lion tamarins were virtually indistinguishable. Mitochondrial analysis (see box page 169) found only a few differences.

"Genetically, you cannot differentiate the three lion tamarins," Forman says. "They are essentially one species." She speculates that the species had little genetic variation within it before the three subspecies became separated, as Brazil's coastal rain forests shrunk to 2% of their former expanse.

"My studies show what can happen to a species when it becomes geographically separated due to the breakup of its habitat," Forman says. Still, she adds, the small amount of genetic variation that does exist may be important to the tamarins' ability to survive and thus should be preserved in captive breeding and wildlife management programs.

Forman's lion tamarin results differed markedly from work on another

primate, orangutans. Dianne Janczewski, another O'Brien colleague, analyzed genetic material taken from some orangs originally from Borneo, others from Sumatra, and still others that were hybrids born in zoos. Janczewski found the two orang subspecies to be as genetically distinct from each other as are common and pygmy chimpanzees, which are considered separate species.

Mingling rhinos

Similar work with sometimes equally unexpected results has been done on rhinoceroses. These genetic studies are particularly relevant to efforts to save the world's rapidly declining rhino populations in the face of rampant poaching in Africa and habitat loss in Asia.

Consider Africa's black rhino, whose numbers have plummeted from 60,000 twenty years ago to 3500 today (*BioScience* 38: 740-744). Taxonomists list six or seven subspecies, depending on which authority is cited, based on morphological studies of museum specimens. At least one subspecies is now extinct, and only two still have substantial numbers left. Should the subspecies be kept apart, as conservationists originally planned, to preserve their unique genes, or should they be allowed to mingle to maximize the species' genetic variation?

The answer seems to depend on what type of genetic study is done. One study of black rhinos from Kenya, Zimbabwe, and South Africa was conducted by Mary Ashley, an evolutionary biologist at Lake Forest College in Illinois. Ashley, who worked under Don Melnick at Columbia University, found almost no genetic differences in the mitochondrial or nuclear DNA of rhinos.

But, using chromosomal analyses, the San Diego Zoo's Oliver Ryder has found differences among black rhino subspecies. The findings, which he says are preliminary, suggest that although genetic changes occurred recently, at least two black rhino sub-

species are indeed distinct and evolving separately.

Among Africa's white rhinos, chromosomal analyses by David Woodruff, professor of biology and coordinator of the conservation science project at the University of California at San Diego, have found little detectable genetic variation. The findings are not surprising given the population bottleneck white rhinos underwent earlier this century when overhunting reduced their numbers to approximately 100. There are now more than 5000 white rhinos, most in South Africa.

More surprising were Woodruff's finding of almost no chromosomal differences between South Africa's white rhinos and a remnant herd of 22 white rhinos in Zaire's Garamba National Park in northern Africa. Taxonomists have long viewed the southern and northern white rhinos, geographically separated by two thousand miles, as distinct subspecies.

In contrast to the black rhino studies, in which chromosomal analyses found genetic differences that mitochondrial ones did not, Ryder showed genetic differences do exist between northern and southern white rhinos by using mitochondrial DNA. His findings support separate subspecies taxonomy.

Moving on to Asia, geneticist Gary McCracken has found surprisingly high levels of genetic variability among Indian rhinos in Nepal's Royal Chitwan National Park. Numbering no more than 60 or so in 1960, Chitwan now protects approximately 400 of these prehistoric-looking beasts. Wildlife biologists studying Chitwan's rhinos had assumed that a population bottleneck, plus geographic isolation, would have yielded inbreeding.

"It was a very positive finding," says McCracken of the University of Tennessee at Knoxville. "A substantial portion of their original genetic diversity appears intact. That speaks well of their chances for long-term survival if they can continue to be protected from poaching."

The indeterminate gazelle

One species in captivity that has little or no genetic variability to lose has been Speke's gazelle. Little is known about these small antelopes. They inhabit rocky arid terrain in Ethiopia and Somalia, a war-torn region of East Africa. Their numbers in the wild are unknown, because no one has been able to census the population. Officially, the International Union for the Conservation of Nature lists Speke's gazelle as "indeterminate."

Until recently, the only Speke's gazelles known to be in captivity were at the St. Louis Zoo. They were breeding there, but the entire group descended from only four animals, says Read. Genetically diverse at first, by 1979 inbreeding had caused 80% of the young to die before reaching sexual maturity. As the remaining animals aged, the zoo's herd was in danger of extinction. There was no choice but to inbreed the animals, given their low numbers and lack of others to add genetic diversity.

As he was later to do for the wild cattle, Read turned to a geneticist for help. Alan Templeton of Washington University in St. Louis, whose previous work had focused on fruit flies, began studying the pedigree of each Speke's gazelle at the zoo.

Making a virtue out of necessity, Read and Templeton began mating proven healthy, albeit inbred, animals rather than allowing only the herd's dominant male to breed. Females were bred as rapidly and with as many males as possible to increase the herd's size and diversity quickly. "We were breeding for survival, not genetic diversity per se," Templeton says. "We figured animals that died [young] had the deleterious genes and those that lived [to maturity] did not."

It worked. There are now 25-30 Speke's gazelles in four US zoos. All

are healthy, Read says, and producing healthy offspring. As a result, he expects the herd size to double in the next five years. Moreover, Read has learned of a second captive herd on a private preserve in the Middle Eastern country of Qatar. He plans to import four animals from that herd later this year, and maybe more in subsequent years, to increase the genetic diversity of Speke's gazelles in US zoos.

Conservation plans that reflect genetics

Beyond individual species, geneticists are also analyzing groups of species to discern their relationships and devise conservation strategies. One such group are the Asiatic wild asses—kulan, onager, khur, kiang, and dziggetai. Found in open plains and arid regions from Syria to Mongolia, taxonomists have listed them as one species with five subspecies.

But analyses done by Oliver Ryder on captive kiang, khur, and onager show their chromosome numbers range from 50 to 56. "Mammals do not usually have such a range," Ryder says. "Maybe Asiatic wild asses are on their way to becoming different species." He recommends conservation plans that treat these animals separately.

Separate management may also be called for regarding gray foxes living on the channel islands off California's Pacific coast. Robert Wayne, a geneticist at the University of California at Los Angeles, has found these dwarf foxes to be genetically distinct from mainland gray foxes.

Taken together, the channel island gray foxes are rare but not endangered. But in some cases, gray foxes on individual channel islands are genetically distinct from those on other islands, Wayne says. If each island's foxes are viewed as a separate subspecies, as Wayne's preliminary data suggest, separate conservation plans may

be required.

On a discordant note, Wayne's other genetic studies have found evidence of hybridization between gray wolves and coyotes in Minnesota. Once almost extinct in the continental United States, gray wolves have been making a comeback since being declared endangered in 1967. The extent of the gray wolf-coyote hybridization and its implications for wolf recovery have yet to be determined, Wayne says.

Meanwhile, at sea, Baker has found humpback whales to be genetically subdivided based on the geographic range of different summer feeding populations. Whales that summer off Alaska's southeast coast, for example, have different mitochondrial genes than those living in California waters. And both differ genetically from humpback whales that summer in the Gulf of Maine in the Atlantic Ocean.

Baker found enough genetic similarities, however, to suggest that geographic isolation among humpback whale populations is not complete. He believes interbreeding occurs on winter grounds, especially in Hawaiian waters. In addition, although Alaska's humpbacks show a loss of genetic variation because of a population decline caused by whaling, those off California and in the Gulf of Maine do not.

In sum, genetics allows wildlife biologists to address questions of differences and similarities among species. But where those studies and their findings lead is still subject to much debate. "There are a lot of strong opinions based on little or no data," Ryder says. "Oftentimes we wind up guessing what to do. Genetics can help us know which biological units to preserve and how to do it." □

Jeffrey P. Cohn is a Washington, DC-based science writer who specializes in zoos and conservation.