Surprising cheetah genetics

An in-depth study of genes from wild and captive cheetahs is leading to new conservation strategies as well as questions and controversy

David Wildt and Mitchell Bush were surprised. Wildt, a reproductive physiologist at the National Zoo in Washington, DC, and Bush, the zoo's chief veterinarian, were examining sperm samples from male cheetahs (*Acinonyx jubatus*) at the De Wildt Research Center near Pretoria, South Africa. They had gone to South Africa to collect sperm to artificially inseminate female cheetahs in US zoos.

But those sperm samples, Wildt says, turned out to be "atrocious." For one thing, many of the sperm's flagella were strangely bent or coiled. In fact, up to 70% of the cheetahs' sperm were abnormal. In most animals, Wildt says, more than 20% abnormal sperm is a clinical sign of infertility. Moreover, the sperm concentration was very low, only ten percent that of domestic housecats.

These findings, made in 1981, launched an extensive study of cheetah genetics that has surprised not only Wildt and Bush, but also many others in the scientific and wildlife conservation communities. The results have led to new conservation directions offering hope for this endangered species' continued survival as well as to some unanswered questions and controversy.

Perplexed by their findings, Wildt and Bush sent blood samples they had taken at the same time as the sperm to Stephen O'Brien, a geneticist at the National Cancer Institute (NCI). O'Brien, who heads NCI's viral carcinogenesis laboratory in Frederick, Maryland, has long been interested in retroviruses and oncogenes in human and feline leukemias. Over the following two years, O'Brien tested blood samples from 55 cheetahs in various US, European, and South African zoos. The animals were all either caught wild or born to parents from the Transvaal and Namibia regions of south and southwestern Africa, two of the only areas where cheetahs still exist in relative abundance outside national parks.

Using gel electrophoresis, O'Brien separated 52 blood proteins to determine genetic differences in the amino acid sequences of individual cheetahs. The results shocked him. Nearly all of the 250 other species similarly tested so far, he says, show a genetic diversity of 10–50%. The cheetahs, however, had no significant differences in their genetic material no matter which zoo or geographic area they came from. "Their genes were all the same," O'Brien says. "They looked like highly inbred laboratory mice or domestic livestock. I had never seen a wild population with so little genetic variation."

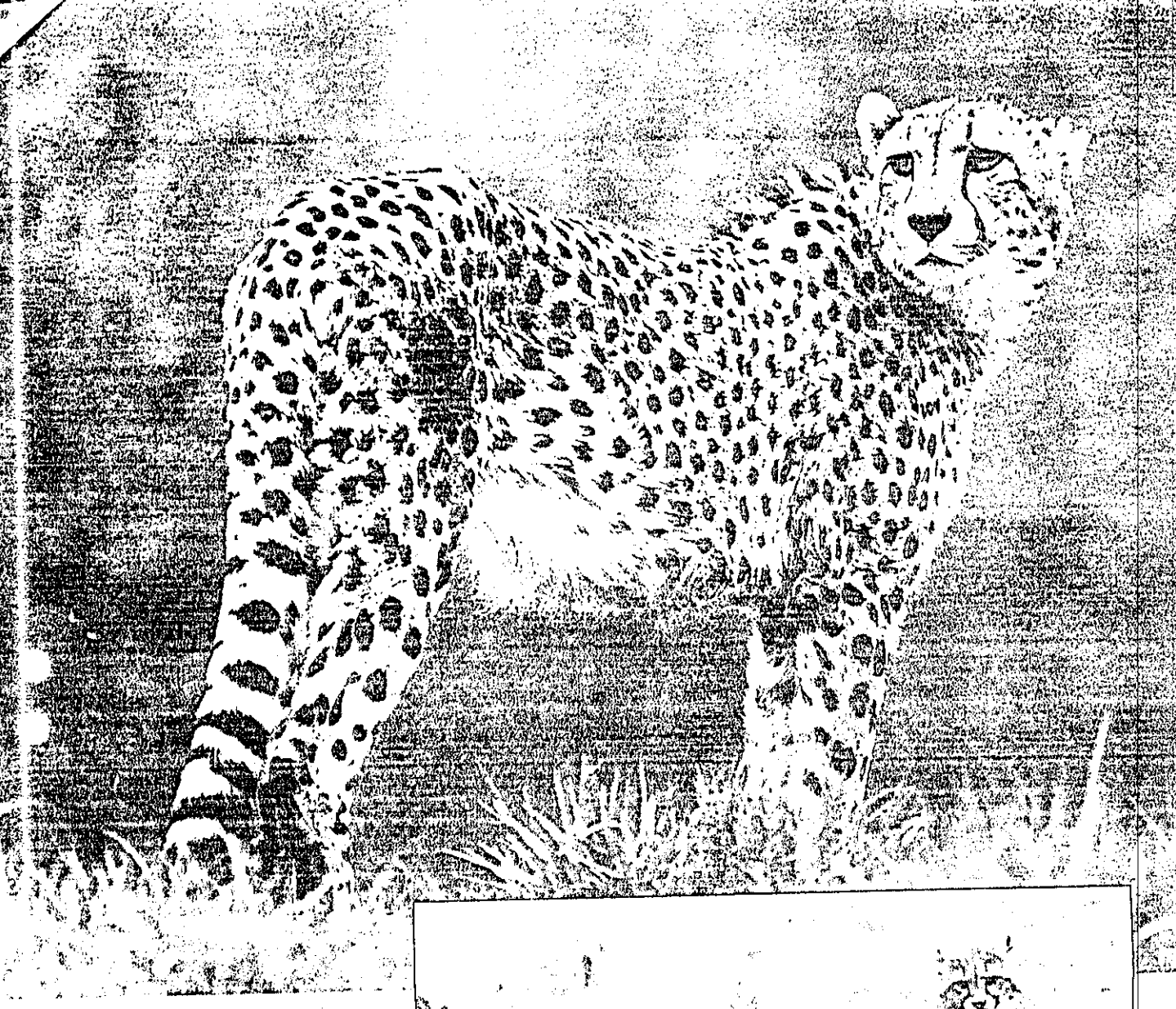
Expanding his research, O'Brien next studied 33 cheetah skulls kept at the Field Museum of Natural History in Chicago, the American Museum of Natural History in New York, and the Smithsonian's Museum of Natural History in Washington, DC, to see how similar they were. The theory of genetic homeostasis, he explains, predicts that genetic diversity prevents physiological extremes and that species lacking diversity, such as inbred livestock, show greater skeletal dissimilarities than genetically diverse ones. Once again, the results startled him: Comparing cheetah skulls with those of leopards (*Panthera pardus*), ocelots (*Felis pardalis*), and margays (*Felis wiedi*)—cats known to be genetically diverse—O'Brien found significantly more asymmetry in the cheetahs. Moreover, most of the cheetah skulls he examined came from eastern, not southern or southwestern, Africa, suggesting that genetic invariability could be widespread.

In related research, Laurie Marker, curator of cheetahs at Wildlife Safari in Winston, Oregon, examined infant mortality rates for captive-born cheetahs. She found that 29% of cheetah cubs die within six months; only half reach adulthood. While precise infant mortality data on other captive felines are scarce, Marker says that "you just don't see that in the other large cats."

Marker and O'Brien then compared the cheetah's infant mortality rate with that of 28 other animals previously studied by Katherine Ralls and Jonathan Ballou of the National Zoo (*BioScience* 34: 606–610, 612). They found that more cheetahs die at an early age than any other species examined except reindeer and dikdiks, small African antelopes. Furthermore, there was no significant difference in mortality between cubs born to inbred parents and those born to unrelated ones. That was "not surprising," O'Brien and his colleagues wrote (*Science* 227: 1428–1434), given the cheetah's lack of genetic diversity.

Perhaps the most important evidence of this lack of diversity came when O'Brien, Wildt, and Bush tested the cheetah's major histocompatibility complex, a set of genes that code for cell surface antigens. The researchers placed reciprocal skin grafts on 14 cheetahs at the Pretoria and Johannesburg zoos in South Africa and at Wildlife Safari. Six pairs were unrelated animals and the seventh, siblings. Normally, except among inbred mice and identical twins, such

by Jeffrey P. Cohn



A variety of blood sample and other analyses of cheetahs in US, European, and South African zoos and in the wild have revealed a surprising lack of genetic diversity; a possible consequence of this low variability is difficulty breeding the animals in captivity as well as high infant mortality rates. Photos: Jay Golden, courtesy Wildlife Safari.

skin grafts are rejected within 10–12 days. But all the cheetah grafts took. Only three were eventually rejected, and those rejections occurred at least 50 days after the original graft.

In recent years, researchers have observed a striking consequence of the cheetah's lack of genetic diversity. An apparently healthy cheetah that came to Wildlife Safari in July 1982 developed feline infectious peritonitis (FIP), a viral disease that infects all cat species, and died shortly afterwards. Examination of blood samples showed that none of Wildlife Safari's other cheetahs had antibodies for FIP at that time. By 1983, however, when the epidemic was in full swing, all of the cats had both antibodies and symptoms. In the next 18 months, another 17 cheetahs, half those at Wildlife Safari, died of FIP. Else-

where, FIP claimed cheetahs at several locations, including Fota Wildlife Park in Ireland, Fossil Rim Wildlife Ranch in Texas, and the San Diego Zoo. In contrast, among domestic cats FIP typically kills only about 5% of an affected population and rarely more than 25%. "The cheetahs showed a homogeneous response to the virus that further confirms their lack of genetic diversity," O'Brien says.

In a program sponsored by White Oak Plantation, a private breeding center for rare animals near Jacksonville, Florida, zoos are now sending cheetah blood samples to James Evermann, a clinical virologist at Washington State University. Nearly 25% have antibodies for FIP, Evermann says. "We may have two populations of captive cheetahs," he adds, "those with no antibodies that can be moved safely between zoos and those that have antibodies and should not be moved."

Causes of genetic invariability

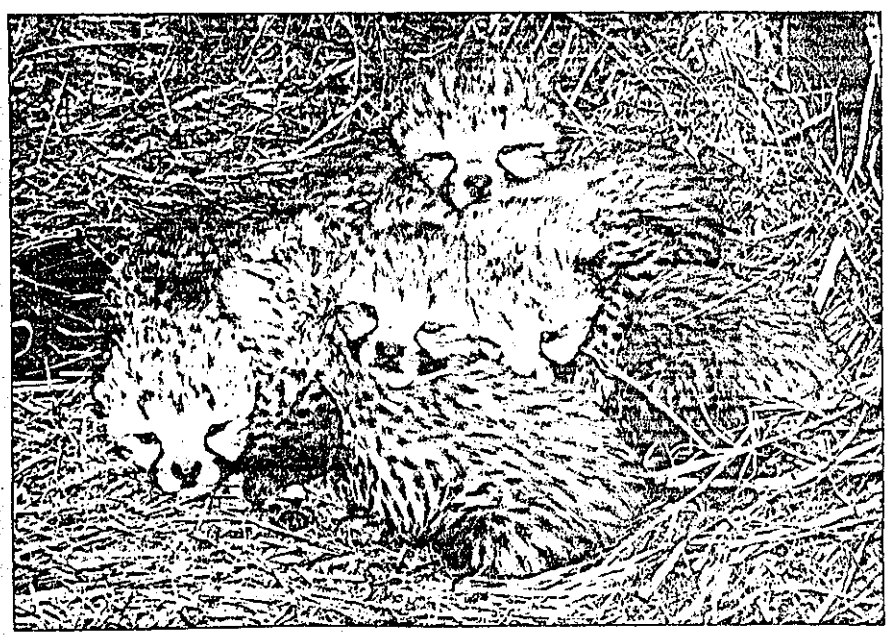
What caused cheetahs to become virtually genetically identical? No one knows for sure, but O'Brien thinks that the animals experienced a population bottleneck about 10,000 years ago. At that time there were several cheetah species living in North America, Europe, and Asia as well as in Africa. All except today's cheetah became extinct, along with 75% of all mammals in some areas, at the end of the Pleistocene. The population bottleneck, which O'Brien says may have happened more than once, could have resulted when plummeting populations were reduced to a mere handful of breeders. Cheetah populations eventually recovered, at least in Africa, but only by inbreeding over many generations.

A population crash is not the only possible explanation, however. Wildlife biologist George Frame, who studied cheetahs in east Africa during the 1970s, says they may have lost their genetic variation through intense natural selection to become highly specialized predators. Such an explanation, he says, is "equally as plausible" as O'Brien's.

O'Brien disagrees. Although conceding that the theory is at least possible, he points to the cheetah's difficulties competing with lions and other large predators, to the fact that at least three other cheetah species are known to have lived—making today's cheetah not unique—and to evidence that cheetahs still suffer from deleterious genes that cause high infant mortality and susceptibility to disease. But Oliver Ryder, a geneticist at the San Diego Zoo, argues that the cheetah's high infant mortality and failure to breed well in zoos may instead result from nutritional and disease problems of captivity. George Frame seconds that view, noting that cheetahs seem to breed well and suffer no undue cub losses in the wild.

Another criticism of the bottleneck explanation comes from Michael Gilpin, a biologist at the University of California, San Diego. He argues that a widely distributed, territorial, and slowly reproducing species like the cheetah "would have to have only ten individuals breeding for 25 generations to reach such a level of genetic invariability" using the population bottleneck theory. "They would go extinct first," he says. Instead, Gilpin suggests that cheetahs colonizing Africa from Asia may have come from a small geographic area with a limited gene pool. Applying the genetics concept of metapopulation to the cheetah, Gilpin says a patchily distributed population with frequent local extinctions and subsequent recolonizations could lose its genetic variability without suffering serious losses from inbreeding.

Whatever the causes of invariability, wildlife biologists and geneticists agree that because genetic diversity is essential for species to adapt to environmental change, the lack of such diversity could cause a species to become extinct. They are puzzled, however, over how the cheetah has survived 10,000 years after losing its diversity. O'Brien believes that the most detrimental effects of inbreeding—birth defects, low reproductive vigor, and inability to adapt to environmental change—most likely appeared soon after the population bot-



Cheetah cubs. Photo: Jay Golden, courtesy Wildlife Safari.

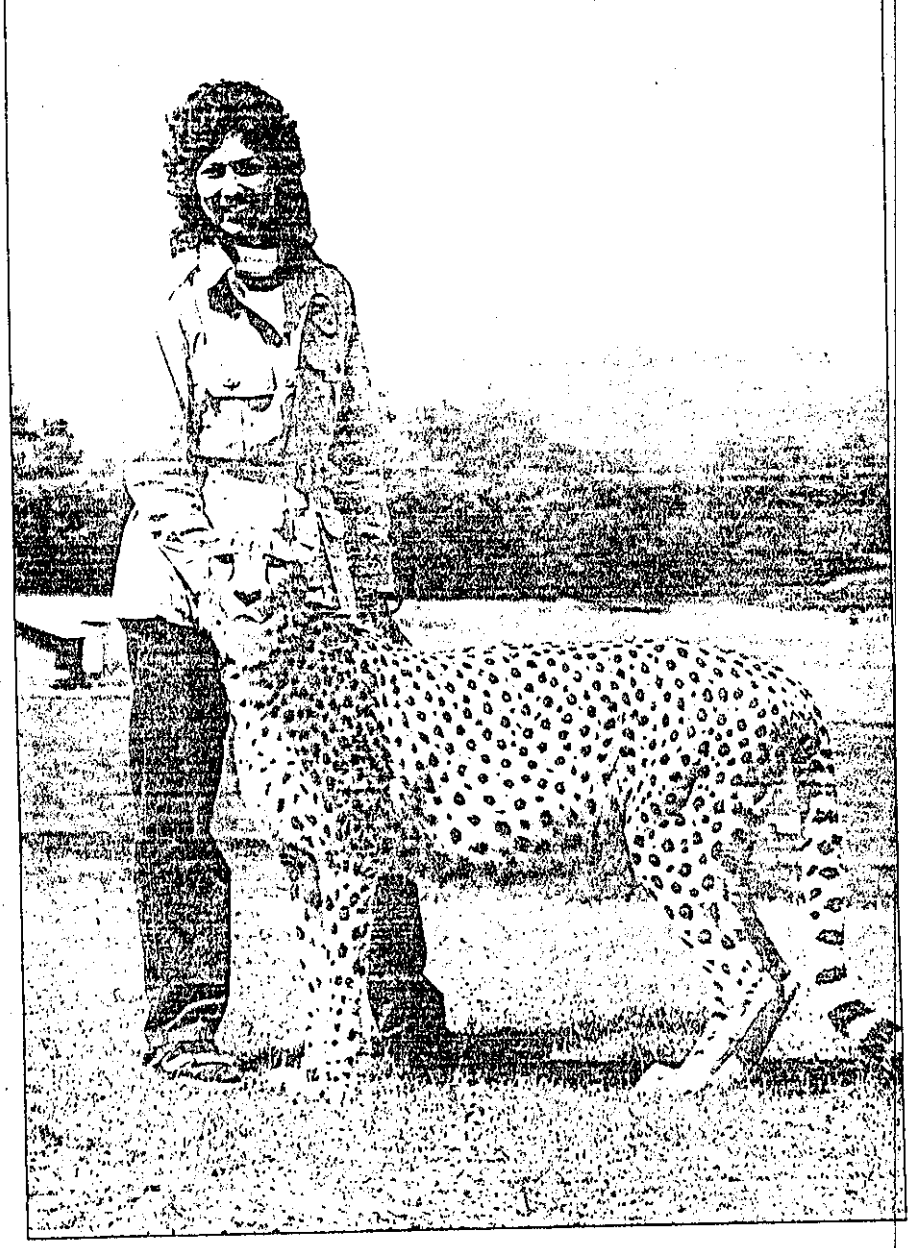
tleneck. "The cheetahs that died early probably took the lion's share of deleterious genes with them," he says.

New challenges to survival

Today, however, the cheetah faces new challenges to its survival in the wild. The species has disappeared completely from Asia—probably in the 1940s from India and perhaps as late as the 1970s from Iran. It is also nearly extinct in the sub-Saharan north and northeastern Africa, and researchers are concerned that cheetahs may be losing out to rising lion populations in some African national parks. There are now only between 5000 and 25,000 wild cheetahs worldwide.

Also troubling is the fact that cheetahs have long been notoriously difficult to breed in captivity. Despite 4000 years of use as "hunting leopards" by various Egyptian, Sumerian, Indian, Moghul, and European potentates, no cheetahs were known to have given birth in captivity since the sixteenth century until a female at the Philadelphia Zoo bore one litter in 1956. Even after that, no captive-born cubs survived more than a few days until 1960, and no second-generation captive-born were produced until 1976.

Recently, zoos have redoubled their efforts to breed cheetahs in captivity, and beginning in the 1970s, several zoos in the United States and elsewhere began to mate the animals successfully. Undoubtedly the most successful has been Wildlife Safari, a 600-acre wildlife park in southern Oregon created by California realtor Frank Hart to combine a tourist attraction with a center for breeding endangered species. One reason for the park's success at breeding cheetahs has been the personal zeal of Laurie Marker, the center's director of education. Although she has no formal training in wildlife management or biology, Marker has dedicated herself to the cheetahs, studying their ways and coaxing them to breed. When necessary, she has hand raised cubs and even took one to Africa for two and a half months, teaching it to hunt as a wild animal.



Laurie Marker has contributed significantly to Wildlife Safari's efforts to breed cheetahs; since 1973, 97 cubs have been born at the center. Photo: Jay Golden, courtesy Wildlife Safari.

Whatever the reasons, the cheetahs have responded. Starting with one male and three females, 97 cheetahs have been born at Wildlife Safari since 1973. Most of the 69 surviving cubs have been shipped to other zoos and wildlife parks. With 17 cheetahs today, Wildlife Safari has one of the world's largest collections.

But Marker still worries about the cheetah's future in captivity. Although there are now more than 200 cheetahs in 35 North American zoos, 29 died while only 18 were born in 1985. Seven of these also died before reaching adulthood. Further, only 6 of the 39 founder animals are still alive, and just 7 males are now breeding. Most important, only 10–15% of all wild-caught cheetahs have ever bred in captivity.

Species survival plan

To address these concerns, the American Association of Zoological Parks and Aquariums (AAZPA) created a species survival plan for the cheetah in 1983. "We had no team effort before for cheetahs," Marker says. "There was just a sort of random breeding." With that in mind, Marker developed a stud book and pedigree chart for all captive-born cheetahs in North America. The pedigree chart includes information on the inbreeding coefficients for each animal.

Next, the AAZPA cheetah committee, chaired by Schmidt, plans to publish handbooks on breeding and management techniques. The books will include recommended diets, medical procedures, enclosures, and observation methods. The committee will then develop standardized laboratory tests for zoos to follow and a blood serum storage bank at NCI. Finally, Schmidt plans to issue a biannual newsletter to keep zoos informed.

Despite this progress and the confidence of Marker and Schmidt, several problems remain. Some zoos, Schmidt says, are reluctant to send or receive cheetahs from other zoos for fear of transmitting feline infectious peritonitis. Nor does anyone know the best procedures for breeding cheetahs in captivity. Another problem is the loss of founder animals and the fact that few wild-caught cheetahs breed in captivity, leaving few choices for future mating arrangements. To remedy this situation, Marker hopes to diversify the captive gene pool by importing wild-caught cheetahs from southwestern Africa, animals that would otherwise be shot by ranchers. "We would like to find out if there are some genetic differences out there," Schmidt says.

O'Brien would like to see the work go even further. He recommends that zoos mate cheetahs from southern Africa with those from eastern Africa, as has been done at the London Zoo's Whipsnade Park. He says that mutations may have occurred in east African populations that would add some genetic variability to the species.

A snag in these plans occurred in 1983, when a proposal to import wild-caught cheetahs was denied by the US Fish and Wildlife Service (FWS), which must grant permits under the Endangered Species Act and the Convention on International Trade in Endangered Species (CITES) before zoos can import rare animals. Richard Robinson, head of FWS's permit office, says suspicions that animal dealers may have been trying to create a market, plus concern over importing animals that would not add genetic diversity to the captive population, led to the denial. A new request, however, might be viewed differently, Robinson hints. "There is some merit to importing animals that are known depredators rather than just shooting them," he says. "The zoos should develop a well-defined plan that documents on a national basis how many cheetahs are needed to prevent inbreeding." That, says Schmidt, is a prime goal of the AAZPA's species survival plan.

In addition to these conservation problems, many questions raised by research on cheetah genetics remain unresolved. For example, does the lack of genetic diversity apply to cheetahs from areas other than southern or southwestern Africa? To investigate this, O'Brien, Wildt, and Bush went to Kenya and Tanzania in 1985 to collect sperm and blood samples from wild cheetahs. Final results are not yet available, but Wildt says the animals' sperm looks as abnormal as that of previously tested cheetahs.

Another question concerns whether other highly specialized species exhibit a lack of genetic diversity. One test of four giant pandas (*Ailuropoda melanoleuca*) showed a similar lack of variability, but O'Brien says no conclusions can be drawn from so small a sample. Elephant seals (*Mirounga angustirostris*), which did suffer a population bottleneck in the late nineteenth century, also show little genetic variation.

But perhaps the most important question is, given its lack of genetic diversity, can cheetahs avoid extinction? Geneticist O'Brien is optimistic: "The cheetah is a survivor," he says. "It has already lasted thousands of years despite a lack of genetic variation. Some of the things we see in the laboratory might not be so important to life in the wild. We are seeing just one side of a three-dimensional problem."

However these questions are ultimately answered, the geneticists' work has already aided zoos. "Now we have an idea of what cheetahs look like genetically," says Marker. The research has also "answered many questions for captive breeding," she adds, including why the animals reproduce poorly in captivity and why infant mortality rate and disease susceptibility are so high.

The research has also opened new avenues for scientific investigation. O'Brien, Wildt, and Bush are now beginning to look at clouded leopards (*Neofelis nebulosa*) and other wild cats to determine their degree of genetic diversity. And researchers elsewhere are applying the same methods to yet other species. Says Wildt, "We've demonstrated the importance of genetics to the management and survival of endangered species." □

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