They seem the picture of health, these lionesses hunting in an extinct volcano. But cut off within its walls, they are threatened by an unseen foe – inbreeding.

CAPTIVES IN THE WILD

Article and photographs by CRAIG PACKER

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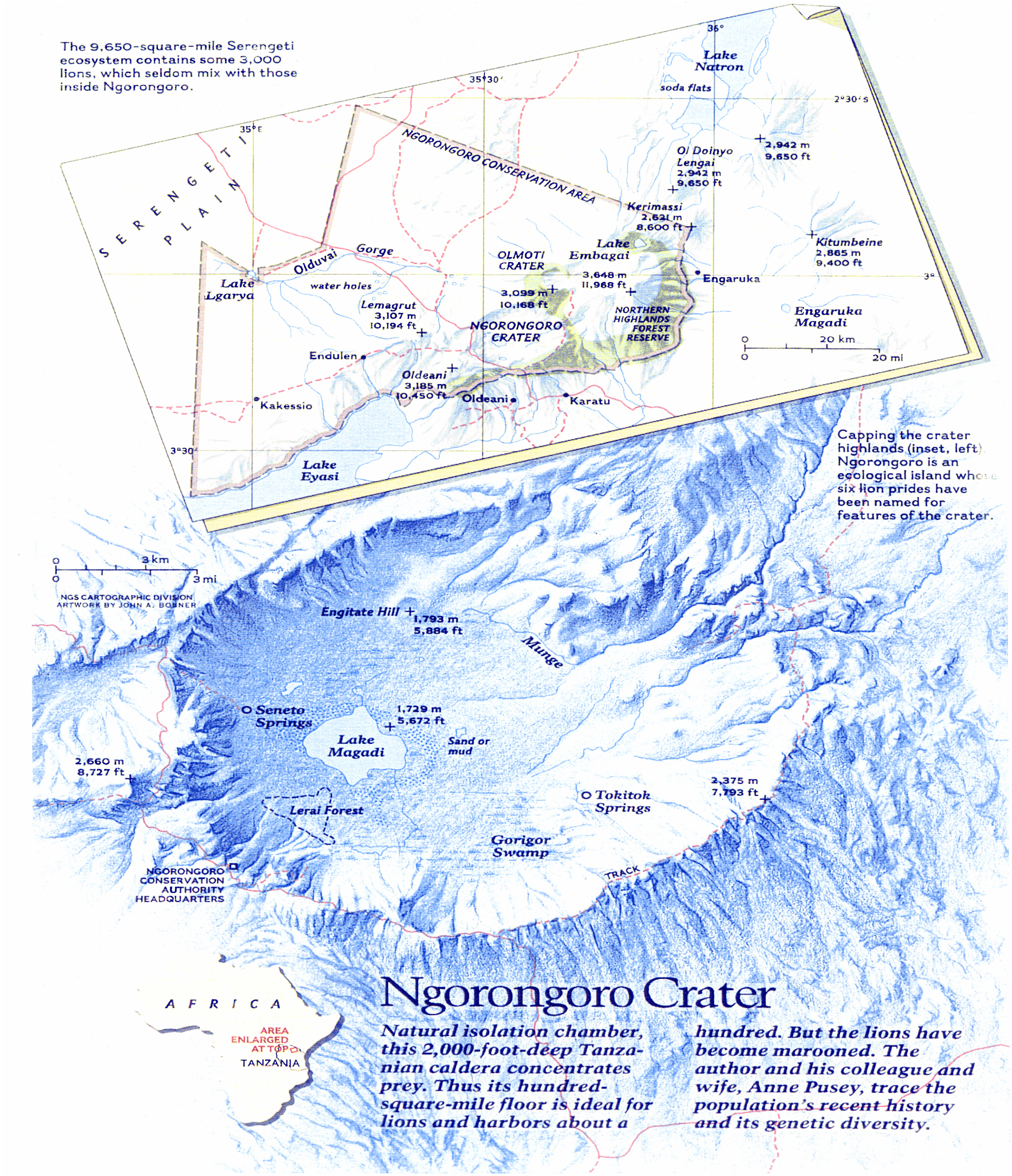


The 9,650-square-mile Serengeti ecosystem contains some 3,000 lions, which seldom mix with those inside Ngorongoro.

Capping the crater highlands (inset, left), Ngorongoro is an ecological island whose six lion prides have been named for features of the crater.

Ngorongoro Crater

Natural isolation chamber, this 2,000-foot-deep Tanzanian caldera concentrates prey. Thus its hundred-square-mile floor is ideal for lions and harbors about a hundred. But the lions have become marooned. The author and his colleague and wife, Anne Pusey, trace the population's recent history and its genetic diversity.

IT WAS only midmorning, but the January sun was so intense that the six lions had already melted for the day. Stretched out on the short grass, they looked like flood victims. Suddenly an impressive black mane appeared out of the yellow wreckage, but the lion was facing the wrong direction. We wanted to photograph his face before he collapsed back into a deep sleep. I craned out the window of the Land Rover, pointed my camera, and whispered to my colleague and wife, Anne Pusey, "Quick-make a noise." She cupped her hands and bleated like a wildebeest calf. The male looked hungrily toward her and gave me his profile. "Got it!" The photograph provided us with our first clue to a scientific mystery that would take us ten years to solve.

Ngorongoro Crater, one of the world's largest calderas, lies at the eastern edge of Tanzania's Serengeti Plain. The crater floor, while providing a life of plenty for the most densely packed lion population in Africa, is really only a small island of lion habitat. Anne and I suspected that the well-fed appearance of the lions might conceal the genetic vulnerabilities of a small population subjected to repeated inbreeding.

Close inbreeding can cause a significant reduction in reproduction and infant survival. A major goal for people who manage captive-breeding programs is to minimize the incidence of inbreeding within their study areas. Many wild populations of large vertebrates are also at risk, because they have become isolated as a result of habitat fragmentation. Here was a population that appeared to be naturally isolated, so it could provide important insights into the long-term consequences of inbreeding.

But what was the precise history of the crater lions? To answer this question, we needed to construct their family tree over at least five generations. We would have to track down every lion that had lived in the crater for the past quarter century.

On our first day in the crater, in January 1979, Anne and I were armed with a set of lion identification cards from an earlier pair of researchers, Jeannette Hanby and husband David Bygott. They had carefully cataloged and named each lion they saw on the crater floor from 1975 to 1978. Each ID card consisted of a series of closeup photographs on one side and a stylized drawing of the lion's face on the other; the drawing emphasized markings on the individual's face, including scars, ear notches, and the whisker spots on either side of its muzzle.

Whisker spots are less conspicuous than other markings, but they are the Morse code of lion identification, a permanent signature on each individual as unique as a fingerprint. Notches and scars accumulate with age, but whisker spots never change and are distinct in even the smallest cubs.

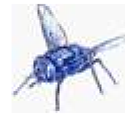
Although identifying drowsy lions is often tedious, individual recognition is essential for unraveling the life history of any animal. Lions live in complex social groups – prides – and lead complex social lives. A lion pride usually consists of six adult females, their dependent offspring, and a “coalition” of two or more adult males. At maturity, daughters either stay with their mothers or form a new pride nearby; sons form coalitions that disperse to neighboring prides. Males compete intensely - even fatally - against other coalitions to gain mating rights in a pride and are rarely able to withstand challengers for more than two years. Consequently, although females may live 17 years, males are lucky to live 12.

THE CRATER HIGHLANDS of northern Tanzania make a dramatic impression on even the most jaded traveler. Ngorongoro is more than ten miles across and 5,000 feet from the floor to the tops of nearby peaks. To drive into the crater, visitors must descend a rocky track that drops 2,000 feet in about two miles. Halfway down, sprawling constellations of black stars scattered across the plains below finally become recognizable as wildebeests and zebras, the principal species in a remarkably high density of large herbivores.

Our intense interest in the crater lions derives from this geography. Although a number of game trails run in and out of the crater and the lions sometimes do scale the heights, to leave the crater for significant periods would be to leave the largest larder in Africa. Other parts of Africa may boast larger herds of prey, but those regions lie within ecosystems where animal migrations impose an annual routine of feast or famine on the lions. In contrast, the rich volcanic soil, moderate rainfall, and seasonal flooding in the crater allow grazers to remain resident year-round. As a result, the hundred-square-mile area supports the highest density of large carnivores in Africa. However, even though the crater lions are densely packed, the breeding population is in fact rather small. Nearly a hundred lions live on the crater floor, but only about 30 of these are adults.

When we took over from the Bygotts in 1979, we had two reasons to believe that the crater lions were inbred. First, records indicated that no new lions had entered the crater in the previous four years. Second, and even more important, was a report suggesting that the number of crater lions-and therefore the genetic richness of the group-had been devastated by a plague of biting flies in 1962.

During 1961 and 1962, according to Henry Fosbrooke, then conservator of the Ngorongoro Conservation Area, exceptionally heavy rains permitted the biting fly *Stomoxys calcitrans* (inset) to breed constantly for more than six months. By May 1962 the crater had switched from heaven to hell for the lions. Most lions became emaciated and covered with festering sores. Many sought shelter by climbing trees or hiding in hyena burrows, and eventually they became so ill they were no longer able to hunt. By the time the rains finally abated, Fosbrooke estimated that the population of at least 70 lions had been reduced to about ten.



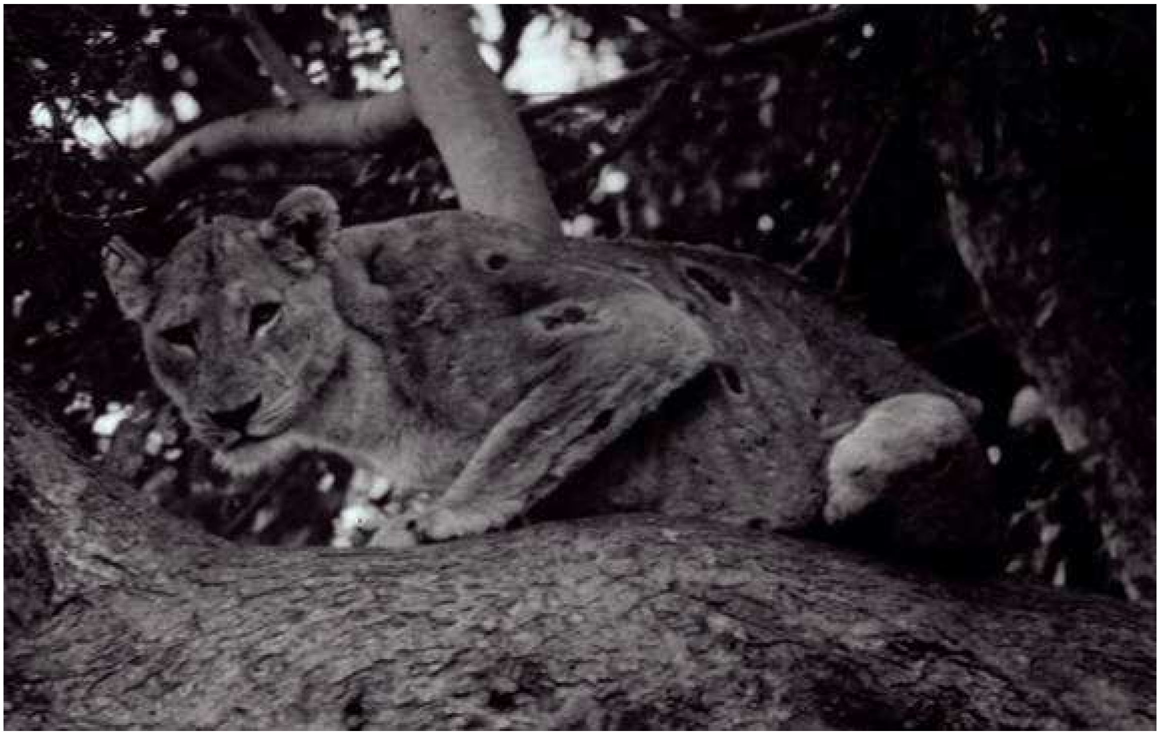
But what happened next? Had a large number of unrelated immigrants resettled the crater, or were today's lions all descended from Fosbrooke's few survivors? With such a small number of ancestors, the population might indeed be seriously inbred. How could we link those earlier records with our own? The biting fly plague occurred so long ago it seemed impossible to trace the current population back that far.

In 1970 a Canadian scientist, John Elliott, had begun a threeyear study of the crater population. He reported that lion numbers had recovered to their former level by 1972 and that the lions were distributed in three prides. However, none of the five prides identified by the Bygotts in 1975 were clearly descended from Elliott's three prides.

The Bygotts had sent photographs of whisker spots to Elliott, but he was unable to recognize any of their animals because his records were based on the blotches on the lions' noses. Unfortunately, nose blotches change with age, and distinctive blotches in Elliott's era had become obscure three years later.

We were stymied. However, by 1985 our studies led us to conclude that the crater lions really were isolated: Every lion in the population had been born on the crater floor. Transient males might show up for short periods but were soon evicted by large coalitions of crater-born males.

Leveler of lions, bloodsucking flies torment a male during a relatively minor 1990 outbreak. In 1962 heavy rains made the fly population explode - a catastrophe for the lions.



Sore-ridden 1962 fly victims fled to the trees in desperate attempts to escape their tormentors. The besieged lions died by the score; a population of 70 was reduced to a handful (photo by Henry Fosbrooke).

In addition, the level of inbreeding in the crater had been raised by the unusual success of a single coalition of six males. These males controlled virtually the entire crater floor for eight years, from 1976 to 1984, and hence fathered most cubs in the population. Consequently, sons of these males dispersed to new prides composed largely of their own sisters and cousins.

In 1984 we invited Steve O'Brien at the National Cancer Institute and his colleagues from Washington's National Zoo to conduct genetic studies of the crater lions. Because of his research on feline leukemia O'Brien had surveyed the genetics of several different cat species. In the course of these surveys he discovered that cheetahs from all over the world are nearly identical in their genetic composition. Such a low level of genetic diversity probably results from an extensive history of extreme inbreeding.

We assessed the genetics and reproductive physiology of the crater lions by comparing them with the nearby Serengeti lions. Our long-term studies of the Serengeti population had shown that close inbreeding is almost nonexistent in that vast area. Our team collected blood samples from dozens of lions in both populations, immobilizing each lion with a dart gun and drawing a small amount of blood while it peacefully dozed. At the same time, a number of the males were electro-ejaculated.

Analysis of the genetics of the crater lions' blood enzymes suggested extensive inbreeding. The lions' estimated genetic diversity was much lower than that of their Serengeti counterparts. In addition, the semen samples from the crater males showed that they had levels of sperm abnormality twice as high as the Serengeti males, another indication of inbreeding.

More eager than ever to reconstruct the crater's recent history, I visited Henry Fosbrooke in 1986 to look at photographs of the survivors of the 1960s biting fly plague. Now in his 80s, Fosbrooke is still an active conservationist. His house is on the rim of Duluti Crater near Arusha, Tanzania, and on a clear day you can see Mount Kilimanjaro through one window and have a splendid view of Lake Duluti through another.

The weather was hazy when I visited, but his photographs were remarkably clear. "Here are the four females that repopulated the crater," he said as he handed me a pile of contact sheets. I was delighted to discover that I could not only pick out the whisker spots of each lion but I could also discern the pride's composition; several identifiable individuals could often be seen in the same photo. But were these the sole founders of the crater population?


Ear notches: Squabbles while feeding can result in permanent and identifiable nicks.

Unusual eye color can mark an individual's identity.

Long-lasting scars, on the face or elsewhere, aid researchers.

Whisker-spot patterns are unique, present at birth, and never change. Researchers note the position of the top-most cluster of spots relative to the uniform row below.

PAINTING BY GREG HARLIN

Noses show distinctive pink or mottled blotches in young. In adults the nose turns black.

Teeth may be missing, broken, crooked, or yellowed, thereby providing readily visible clues.

A lion who's who

Which lions survived the flies? How was the crater repopulated, and with how much genetic diversity? Only by tracking down the identity of every lion since the plague of flies could Packer and Pusey answer those critical questions.

In 1979 they began using lion identification cards (right) with pictures of individuals at various ages, noting field marks such as whisker spots and ear notches (above) that can pinpoint lions. They contacted preceding researchers who had photographed or drawn the same lions or their forebears. They also solicited photographs taken by tourists, receiving hundreds to help fill in the gaps.

Eventually their catalog put faces on more than 500 individuals, most now dead. And their detective work determined that all of today's crater lions descend from only 15 lions that either survived the flies or invaded Ngorongoro shortly thereafter.

MUNGE

128 *National Geographic, April 1992* *Captives in the Wild*

FOSBROOKE'S LIONS represented the beginning of the crater story, and we knew every lion that lived there from 1975 onward. Then I suddenly realized that tourists and filmmakers had been photographing the crater lions daily since the road into the crater was completed in 1959. Thus, distributed over the entire world was a complete photographic record of the lions' recovery from the *Stomoxys* plague.

Here was the problem: In 1975 there were five sets of breeding females and three coalitions of breeding males. The goal was to trace the origins of these eight groups and their connection to Fosbrooke's survivors. Our method would be to assemble and organize all the lion photographs we could find.

BY EARLY 1988 we had received several hundred tourist photographs from around the world. John Elliott sent in his records, and Harvard University biologist Dick Estes supplied a large batch of lion photographs taken from 1963 to 1965. A population reconstruction seemed possible after all.

In the meantime, Steve O'Brien furnished us with the results of a second genetic survey. Disturbingly, it revealed a striking lack of genetic variability in the crater lions' immune defense systems. This loss of genetic variability could render a population especially susceptible to an epidemic.

DNA-fingerprinting studies by Dennis Gilbert have shown that mating success among male lions is highly unequal. The crater population contained many adult males, but only a few fathered most of the offspring. The breeding population was even smaller than we thought. The time had come to pull together all our materials.

First, we looked at Elliott's ID files. In addition to nose flecks, he had also recorded ear notches and whisker spots. Now much of the period between 1972 and 1975 made sense.

Next, we focused on Estes' and Fosbrooke's photographs, covering 1963 through 1966. Fosbrooke's founding pride consisting of four females had large batches of cubs in 1963 and 1965. Daughters from both batches eventually established their own prides, and several survived until the Bygotts' era. Thus two of Elliott's three prides originated from Fosbrooke's group of four females.

Then it was time to go over the photographs from tourists and professional photographers. This was unexpectedly rewarding. Several pictures from 1959 included one of Fosbrooke's founding foursome. Because female lions associate only with their close female kin, all four must have been born on the crater floor and managed to survive the biting fly plague. Estes' earliest photographs showed that a single male was resident with these females, and he too had been photographed in 1959. Another survivor! This lion had fathered the 1963 cohort of cubs but was evicted by a pair of males in late 1964. These latter two males did not match up with any of the many males photographed in 1959, so they must have entered the crater from elsewhere. In late 1964 Fosbrooke first photographed a group of young males on the crater floor. They would have been small cubs at the time of the plague and thus could not have survived the disaster. They too must have been immigrants.

But to get a precise idea of the genetic composition of the contemporary lion population, we had to account for *all* the lions. We were nowhere near completion. We had a good grip on the population in 1963-66, 1970-72, and 1975 onward, but there were numerous missing links between these periods. The most serious gap was in the late sixties, and we began searching for more photographs. Then we recalled a conversation we had had with Jane Goodall.

Since a mother will carry only her own cub, maternity is certain (opposite). This photograph also recorded the whisker spot patterns of both. In 1968 scientists used an inflatable lion (left, taken by Joan Root) to test the reactions of real cats to it. Two males look askance after upending the intruder. To Packer and Pusey the results of the experiment didn't matter- but knowing the date of the photograph did. They had been searching for one pride's resident males at that time. Here they were.

Mating pairs have too many common ancestors. After the 1962 plague of flies, seven males entered the crater. But since 1969 no new males have contributed to the gene pool.

Jane and filmmaker Hugo van Lawick had spent most of 1968-69 in the crater conducting research for their book *Innocent Killers.* Jane told us, "There was a French-Canadian scientist who was studying the lions while we were there. I was impressed that he could identify every lion in the crater by the whisker spots." Unfortunately she could not recall his name.

I phoned everyone who might have known this early lionologist, but no one could help me. Then while reading George Schaller's book on the Serengeti lions, I noticed a passage on the crater lions in which he cited "P. DesMeules, personal communication."

But how could I find P. DesMeules 20 years later? All I knew about him was that he must have been a scientist. If he wrote a scientific account of his research, it would be found in any university library. I went to the library and immediately struck gold: nearly a dozen citations to P. DesMeules, including one for a Canadian wildlife journal in 1968. I literally ran to the stacks. The paper was by Pierre DesMeules of the Canadian Wildlife Service. "Current address: Box 1, Ngorongoro Crater, Tanzania."

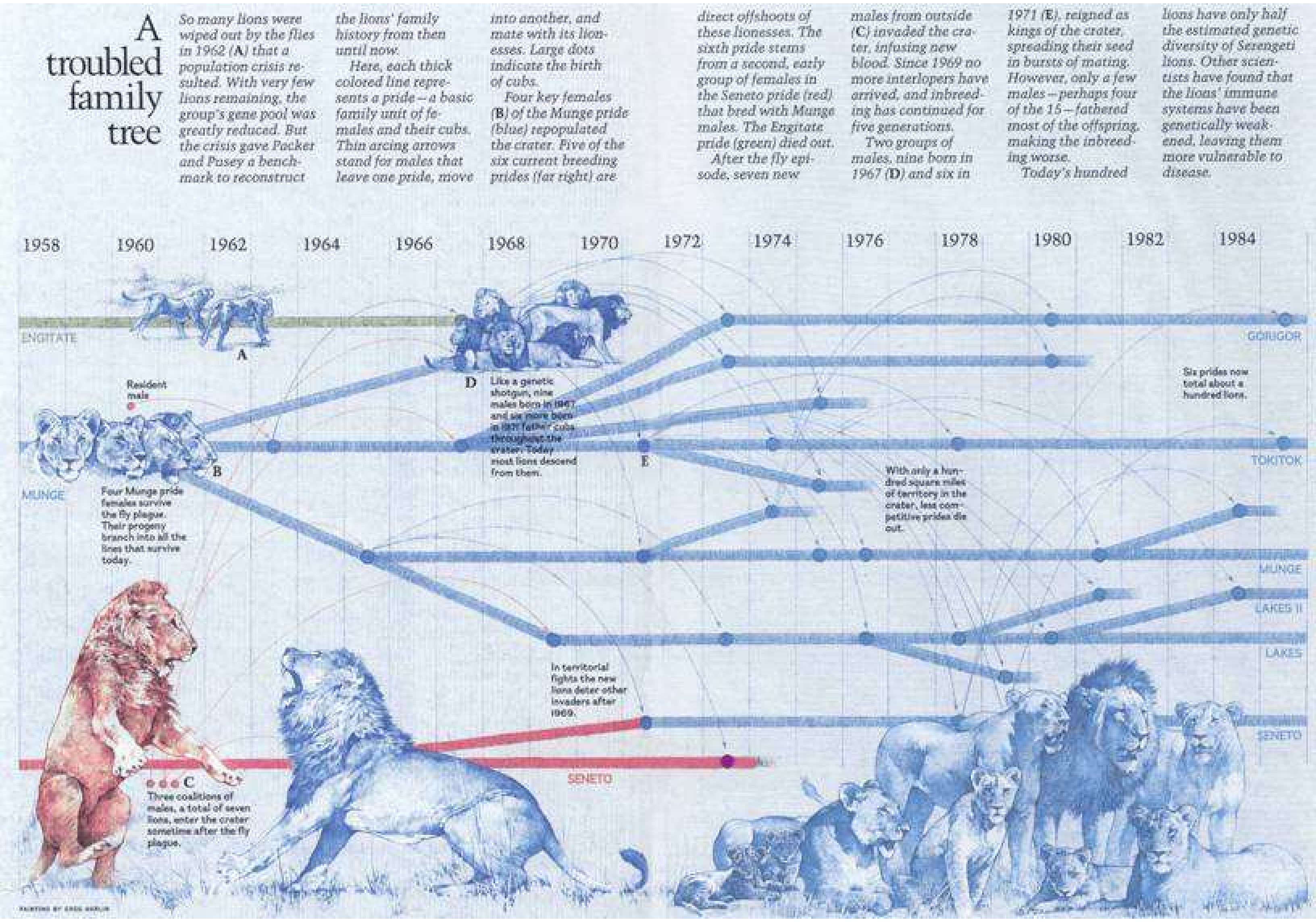
I had my man, but where was he now? A second paper revealed that he had become the senior scientist for Parks Canada in the Quebec region. The park service advised that he had recently retired, and his former secretary told me he now lived near the small village of Sacré-Coeur de Marie.

I called and introduced myself. DesMeules confirmed that he had lived in the crater from late 1968 until the beginning of 1970. He had identified every lion on the crater floor, and he had several thousand photographs of the crater lions.

Four days later we shook hands in a small village in southern Quebec amidst the autumn foliage. I turned my back on the brilliant colors to examine the bonanza of black-and-white photographs of some 60 lions that had all died more than a decade ago.

And what photographs! DesMeules had recorded the whisker patterns on both sides of each lion's face. I could confirm all the links between Elliott and the Bygotts and fill in many more gaps. By the next day I was sure that three groups of females had survived the biting fly plague. One group died out. The other two groups founded the three Elliott prides and hence all five of the Bygott prides. Fosbrooke's group of four females were the ancestors of all six current prides.

The females were now sorted out, but the males were still unclear. I would eventually discover the origins of one large coalition from DesMeules's photographs. But there were two gaps left: the origin of a coalition of nine males that had already left their natal pride by the time DesMeules arrived and the identity of the fathers of one other set of females.



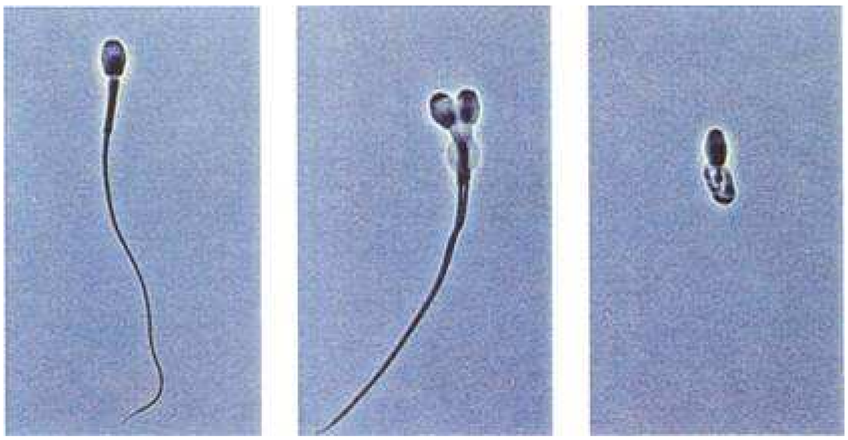
Back to Africa. Our dearest friend in Nairobi, Barbie Allen, renewed our request for pictures from wildlife photographers, but now over a much narrower time interval: early 1968. When filmmaker Joan Root sent us photographs from January 1968, we struck gold once more. Joan had gone to the crater to film some experiments, and the "experimental subjects" turned out to be the pride for which we needed to know the identity of the resident males. From Joan's photos we could see that the two males were the survivors of the coalition first seen by Fosbrooke in 1964. These two were later resident in several other prides and thus were the male ancestors for many in the subsequent population.

During the same trip Joan had photographed the remnants of Fosbrooke's original group of four females. They had had one last batch of cubs in 1967, and these included the coalition of nine males first seen as subadults by DesMeules. Our quest was over.

WE NOW KNOW that the entire crater poplation is descended from 15 animals. Eight of these had survived the plague; the rest were males that may have entered the crater from the Serengeti. The plague removed so many adult males from the crater that fresh blood was able to enter. Once the residents resumed breeding, they had several large sets of sons that monopolized the crater prides and kept further immigrant males out. Thus the current crater population has been subject to close inbreeding since 1969, about five lion generations.

With complete reproductive records of the population since 1963, we could test whether inbreeding has lowered the productivity of the population. We now estimate that the crater lions have lost about 10 percent of their genetic diversity over the past 20 years. Our estimates of this decline are closely correlated with a reduction in reproductive rates in the crater lions, although it is too soon to be certain of a direct link. If inbreeding has indeed caused this reduction, then reproductive rates in the crater population will continue to decline in the future unless new males are once again able to enter the crater.

The crater lions also show somewhat lower genetic diversity than can be attributed solely to the effects of the *Stomoxys* plague. The crater has been naturally isolated for millennia, and its lions may have undergone several cycles of isolation, decline, and repopulation. It is possible that the previous population was highly susceptible to the depredations of the biting flies precisely because they were already quite inbred.



Ominous telltales, sperm from crater males (middle and right) show abnormalities when compared with a normal sample. Reproductive physiologist David Wildt and his colleagues at Washington's National Zoo found structural deformities in more than half the sperm of each male tested, strong evidence of inbreeding. The continuous decline of genetic diversity since 1969 is perhaps linked to a falling reproductive rate. Photos by David Wildt and Jo Gayle Howard.

I visited Henry Fosbrooke again in October 1990. When I told him that I suspected the crater lions had been through previous periods of inbreeding and genetic decline, he led me into his large library and said, "You should read these." They were accounts of big-game expeditions that went into the crater in the early twenties. During two weeks in 1922 one hunting party bagged seven adult lions and badly wounded another three. The last expedition was in 1924, when five more lions were killed. Considering that there are never more than about 30 adult lions in the crater and that most of the wounded animals probably died as well, the breeding population must have been severely reduced. Our genetic assays more than 60 years later may well have revealed the results of this onslaught.

THE SERENGETI and Ngorongoro were declared wildlife sanctuaries in the late twenties to protect the lions from further hunting. Ngorongoro Crater became a world heritage site in 1979 in recognition of its special significance as a microcosm of African savanna. The popular appeal of charismatic carnivores such as lions has often led to the conservation of habitat that sustains a host of other species. But living at the top of the food chain inevitably means that predators often end up in small, threatened populations.

The history of the crater lions may represent the future for many other large vertebrates. Increased human habitation around Africa's national parks has formed virtually impermeable boundaries, and recently many species have become isolated in small populations, making them even more vulnerable to environmental catastrophe. Add to this the effects of close inbreeding, and many small populations may well be caught in a downward spiral.

Perpetuating these populations will require more than just protecting them from hunters and poachers. The crater lions are conspicuous and have therefore proved surprisingly easy to monitor. The fates of most other small populations will run their course undetected.

A trio of males patrol their crater territory. Ironically, they are strong enough to deter what their population most needs - the entry of outside lions with new genes.

GENETIC EROSION

A Global Dilemma

By STEPHEN J. O'BRIEN
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The Ngorongoro lions are isolated geographically, but they are not alone in their genetic impasse.

Around the world, wildlife populations are shrinking into fragmented islands amid a sea of human expansion. Only 30 to 50 Florida panthers cling to survival. Before a captive breeding program began, the black-footed ferret was down to 17 animals. In India, fewer than 250 Asiatic lions remain.

Before conducting genetic studies for Craig Packer's lion project, I examined the genetic history of the cheetah, whose range once spanned the globe. I was amazed to find that every one of today's 20,000 cheetahs is genetically almost identical. They descend from survivors of a near-extinction catastrophe that resulted in generations of close inbreeding 10,000 years ago.

These and other species share something important with the Ngorongoro lions' population bottleneck. It creates a shrinking gene pool that leaves fewer and fewer mating partners. What are the genetic implications?

The animals become part of a high-stakes poker game - with a crooked dealer. After beginning with a 52-card deck, the players wind up with, say, five cards that they are dealt over and over.

As they begin to inbreed, congenital defects appear, both physical and reproductive. Often abnormal sperm increase; infertility rises; the birthrate falls. Most perilous in the long run, each animal's immune defense system is weakened.

Thus, even if an endangered species in a bottleneck can withstand whatever human development may be eating away at its habitat, it still faces the threat of an epidemic that could well be fatal to the entire population.