

The following is an excerpt from a chapter from *Tears of the Cheetah* by Dr. Stephen O'Brien, medical geneticist and conservationist. His science adventure stories cover the earth's most endangered and beloved wildlife. While many may think poaching and habitat loss are the direct cause of possible extinct, few understand that lack of genetic diversity may spell doom for many of these animals now.

Note: Words highlighted in purple may be new vocabulary words. Words highlighted in blue may be new science words.

Prides and Prejudice

Section One

The spring of 1962 was particularly wet in Ngorongoro, leading to the unprecedented **proliferation** of a bloodsucking fly, *Stomoxys calcitrans*. Huge swarms of these insects converged on the lions, causing skin lesions, **exsanguinating** them, and leaving **emaciated** lions hardly able to hunt. According to Henry Fosbrooke, conservator of the Ngorongoro Crater during the outbreak, the population plummeted from a high of one hundred lions to a low of ten individuals, as tormented lions perished or ran from the crater in horror.

Packer was fascinated with this event because it was a real-time population bottleneck in an isolated population. He reckoned that he could reconstruct the entire recovery of the lions with a pedigree based on pride association and individual identification. Craig and Ann had developed an elaborate lion identification scheme based on the patterns of whisker spots that allowed them to recognize and track individual lions. Every field biologist who had tracked lions in the crater had taken close-up photographs of each lion they saw, providing a photo record of the living crater lions since 1962. To back these up, the Packers put out classified ads in African tourist magazines for amateur lion photos from the thousands of people who had visited the crater between 1962 and 1978, just before he and Ann began their oversight. He figured that nearly every tourist who spotted a lion in the Ngorongoro Crater would try to take a picture! And he was right.

It took years of painstaking analysis and sorting through thousands of lion photographs for Craig to trace the lions' recovery since the *Stomoxys* **plague**. But eventually he determined that all the modern crater lions were descended from fifteen founders, eight survivors of the *Stomoxys* plague and seven immigrant males who wandered into the crater in the few years following the epidemic. It was difficult to know the fathers of cubs precisely, but the pride mothers could sometimes, but not always, be identified by cub-rearing association. For the first time, a pedigree of close inbreeding following a defined population bottleneck had been documented precisely. We were very anxious to examine whether a molecular genetics and reproductive analysis of the Serengeti and Ngorongoro Crater lions would affirm the bottleneck's effects and reveal more details about the history of these lions. ‘

The National Geographic Society bankrolled our team's expedition to Tanzania in 1987. Eight of us boarded the plane for East Africa along with twelve huge trunks of veterinary drugs, blood- and skin-sample processing supplies, bush clothing, and very precious nutrients unavailable on the Serengeti Plain, like peanut butter, apricots, and Triscuits. We landed in Nairobi, where we purchased more supplies, organized vehicles,



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gathered multiple spare tires, and were joined once more by wildlife photographer Karl Ammann. Karl's curiosity and **unfettered** eagerness to help in all areas more than made up for his lack of scientific training. He had proved invaluable in our previous safaris looking for cheetahs in the Masai Mara and the Serengeti. Also, he could spot cheetahs and lions one hundred times better than anyone else we knew.

Our convoy set off for Seronera, a tiny village in the center of the Serengeti Park that researchers like Craig and Ann used as a home base. Craig had previously installed radio collars on a dozen lions. Since lions sleep all day, they were usually easy to find, dart, collect biological specimens from, and resuscitate. The vets would always wait for the lions to recover enough to walk, run, and defend themselves before leaving. After a week of very long days in the Serengeti we had specimens from twenty-seven lions, enough for an evaluation. We headed next for the Ngorongoro Crater.

Up at dawn each morning, Craig and the two wildlife vets, Mitch Bush from Washington's National Zoo and Don Janssen of San Diego Zoo, went out in search of lions to dart. The first day went very well; three lions were darted, sampled, and recovered. We relaxed with hot showers and a gin at our fancy safari camp, tired from a long, hot day wrestling lions for their bodily fluids. Then it all unraveled.

A park ranger appeared at our camp early the next morning and informed us that the park conservator, Mr. Joseph Kayera, had ordered the lion collection stopped immediately. The ranger gave no explanation, just simply delivered the order. I climbed aboard our rented Land Rover, took a Motorola hand radio, and began the treacherous two-mile rocky "up road" from the crater floor to the crater ledge where the park office was located. Kayera was not in and I was told that his busy schedule might prevent him from seeing me today, or at all, for that matter. I insisted that I would wait cheerfully.

Day became dusk, then evening, and still no conservator, so I made my way to the Ngorongoro Park Lodge and booked a room. The lodge is a beautiful luxury hotel with a view of the entire crater floor. At the bar sipping Tusker ale that night, a group of tom-bus drivers revealed the source of our troubles. A princess from the Netherlands had visited the day before and became extremely distressed upon seeing a bunch of characters "molesting" the lions in the crater. She was not sure what was happening, but the lions certainly looked dead to her. The princess, of course, shared her horror with the conservator. My work was cut out for me.

The next morning I radioed the camp with the grim news and headed for the park office. Kayera appeared at around ten A.M., but he was too busy to see me. I waited patiently until four P.M., when he invited me into his office. He welcomed me and proceeded to describe in some detail his disdain for the way that arrogant American researcher Dr. Craig Packer was carrying out his program. He never sought permission for collars, did not report his findings, harassed the lions, frightened the tourists, and, just like all the other expatriates, was insensitive to local regulations and culture. Kayera was furious. He did not really know or care much who I was, but by association with Packer, he assumed I was no good for this place either. The princess's complaint was simply the straw that broke the camel's back. The project was cancelled! Go home! Now! Please!

I was tired, intimidated, and stunned. But I decided to tell him what he wanted to hear, Yes, Dr. Packer was not perfect, certainly arrogant and insensitive. Of course he should communicate more with his Tanzanian hosts. But I implored him to understand that this project was bigger than all of that. The veterinary assessment alone would benefit the animals and inform managers. The anesthesia and sampling were extremely safe. So far every lion had recovered properly; none had died. I promised to redress his fears, side with him on all fights against Craig. (I assumed Craig would forgive me for the sake of the project.) I promised to explain calmly to any visiting tourist how a biomedical evaluation was an invaluable management

and research tool of great benefit to the lions.

By nine P.M. he had softened: the project could continue, but only for one day at a time and with the requirement that I report back to him daily on all aspects. I thanked him for his insight and wisdom, went to the crater's edge, and radioed the team to go ahead. For the next several days Kayera and I became friendly as I spent many hours detailing progress and problems. The Dutch princess left Tanzania and our team collected samples from sixteen crater lions, enough for a very close analysis.

Once back in the States, Wildt, Bush, and I were quick to examine the specimens. We first looked at the Serengeti lions, which displayed a great deal of genetic diversity, easily as much as **outbred** house cats or other wild cats like ocelots or leopards. Measures of DNA variation at the lions' MHC, the gene complex we had measured by skin grafting in cheetahs, were also highly variable. Instead of performing skin grafts (hardly an option with wild lions), we measured the lions' MHC with a technique called "**restriction fragment length polymorphism**," RFLP, which tracks DNA sequence differences in the genes that encode MHC proteins.

As we suspected, the crater lions presented a very different story. They had 50% less overall molecular genetic diversity than the prides of the Serengeti. In other words, their 1962 population bottleneck had cost the population half of its **endemic** genetic variation. Dave Wildt found that sperm counts of crater males were only 60% of that of Serengeti lions. The larger outbred Serengeti lion population had about 25% abnormal sperm ... a sign of rather healthy sperm, while crater lions had twice that number. The hormone profiles run on the lions' serum samples showed a dramatic difference as well. Serum **testosterone** levels of Serengeti lions were three times higher than the testosterone levels in crater males. Testosterone is a critical hormone produced in the testes that **mediates** sperm development. Low testosterone concentration in crater lions was the likely reason for the elevated frequency of **malformed** sperm. Inbreeding after the 1962 bottleneck was having the effect on the crater lions we had feared and Craig's elaborate pedigree reconstruction had predicted.

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Section Two

The grim situation of the crater lions, however, would seem modest alongside the faraway lion population we would study next. The lions of the Gir Forest Sanctuary in the Gujarat state of western India comprised a **relict** population of about three hundred animals that were the sole survivors of the Asiatic subspecies of lion, *Panthera leo persica*. Formerly occupying a vast range from Turkey and the Arabian Peninsula on the east to western India/Pakistan on the west, the Asian subspecies was **extirpated** by agricultural development and rampant colonial big-game hunters. Census records from 1880 to 1920 show multiple periods, even multiple generations, when the population dropped to fewer than twenty individuals. Once lion hunting was outlawed in the Indian state of Junagadh in the 1920s, the population increased gradually to its present size. Today it occupies a sanctuary area, fourteen hundred square kilometers in size, in the Gujarat peninsula of western India.

Asiatic lions look different in several respects from African lions. They are a bit smaller, and most have a marked skin fold running along the length of their underbelly. Males have a much shortened mane, and about half of Asian lion skulls, including all of today's Gir lions, have a bony ridge in the cheekbone that crosses an opening for nerves to the eye called the "**infraorbital foramen**." In all other **felid** species and in African lions, the foramen is a single opening with no bridge.

These physical characteristics were originally thought to be adaptations or at least modifications associated with the long time that Asiatic lions were isolated from African lions. Our molecular genetic estimates would suggest that African and Asian lions have been isolated from each other for at least fifty thousand years. We now believe, however, that these physical traits in Asian lions are manifestations of extremely severe inbreeding in their very recent past. The evidence for our conclusions was encrypted in their genes.

Paul Joslin, the deputy director of the Brookfield Zoo in Chicago, had spent three years tracking lions in the Gir Forest Sanctuary, but he was more worried about the troubled population of captive Asiatic lions. In 1981, he had helped establish a Species Survival Plan (SSP) for Asian lions under the *auspices* of the Zoo Associations of America, Australia, and Europe. Captive Asiatic lions in Western zoos had been bred and managed as a population backup for the tiny wild population since the early 1980s. By 1989, 205 Asiatic lions were being bred in thirty-eight different zoos. Paul explained that he was concerned because many of the captive offspring did not show the diagnostic belly fold, the small mane, or the infraorbital foramen bridge. Also, the entire captive population was descended from only five founders, a tiny number that might constitute an inbreeding threat. Furthermore, the records of the precise parentage of those five

original founders were suspect, particularly since two founders came from an Indian zoo that was rumored to interbreed African lions with Asian lions. The Indian zoo officials denied the charge.

Paul offered to organize blood sample collections from the captive population and from the Sakkarbaug Zoo, just outside the Gir Forest in Junagadh, India. The Sakkarbaug Zoo lions were assuredly authentic, since they had all come right out of the adjacent Gir Forest.

The Gir Forest Sanctuary borders on heavy human settlements, inevitably leading to lion attacks on local citizens. Ten of about one hundred attacks in the 1980s were fatal. Wildlife agents captured all the man-eaters and placed them in the Sakkarbaug “breeding program” - a sort of maximum security penitentiary for the killer lions. Joslin wanted to find out for sure if his captive SSP lion population was “pure” or “hybrid.” I wanted to look at the Gir lions’ genes to compare them to their African cousins.

Bush, Wildt, Joslin and I set out for the Gir Forest in hopes of collecting authentic Gir lions both from the Sakkarbaug Zoo and from the Gir Sanctuary itself. The Sakkarbaug Zoo was overflowing with twenty-eight lions, largely because human attacks were on the rise. We collected blood, semen, tissue, and serum from all the Sakkarbaug animals carefully and quickly.

The free-ranging lions in the Gir Forest Sanctuary were more of a challenge. The teak forest habitat is dense, extremely arid, dusty, and malaria-infested. For the first time in our lives, our team was tracking lions on foot; the forest was too thick for a Land Rover. And this was a place with a dozen lion attacks on people each year. The park rangers carried only narrow wooden spears for protection -- no guns, blow darts, or pepper spray -- so when we would come across several lions lying in a creek bed, we were very cautious. Looking back now, it seems **inconceivable** that we were able to anesthetize and sample the lions, but somehow we did succeed with six males.

Sadly, a freak accident during our study in the Gir Forest led to a young lion’s death. The park rangers had lured a small group of lions to a clearing using a slaughtered gazelle for bait. Four lions were chewing on the carcass when we arrived. Bush darted a young male who jumped as the blow dart injected into his back leg. A nearby female, also eating, reacted to his jump with a swat and then bolted off into the forest. The darted male followed in hot pursuit. We moved slowly around the other lions and then took off on foot after the two lions. They had run over a mile on the dense dusty game trail to find perhaps the lowest point and the only body of water within miles, a tiny basin two meters wide and a foot deep. The tranquilized male succumbed to the drug precisely at the water hole, collapsed, dropped his head under the water, and drowned. He had died seconds before we arrived.

The moment was tragic and devastating to witness, even as we tried to persuade ourselves that the small risk we always take when we anesthetize wild animals served a greater good. Bush performed a complete autopsy. We filed a detailed written report of the accident. Although our Indian hosts were understanding, supportive, and even forgiving, we did not have the heart to continue the collections any further. We returned to the United States with samples from six wild males and twenty-eight captive Gir lions from the Sakkarbaug Zoo.

The genetic profile we drew from these samples was rather disturbing. Asiatic lions from the zoo or the forest had virtually zero measurable genetic diversity... This was much worse by far than the Ngorongoro Crater lions. ... Serengeti and even Ngorongoro crater lions showed rich diversity in DNA fingerprint patterns, but the Gir lions were all identical. It was as if they were all clones or identical twins. This was the most genetically uniform population we had ever observed. The Gir lions had even less variation and were more severely inbred than cheetahs ...



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The reproductive distress of the male Gir lions seemed to have translated into breeding troubles as well. Sakkarbaug lion pairs frequently failed to conceive or produced stillborn cubs. Cub mortality was much higher in Sakkarbaug compared to captive African lions in other zoos. Even the rare normal-appearing sperm ... were defective in fertilization tests attempted in Dave Wildt's laboratory.

Add to these reproductive defects the Asian lions' distinctive traits - the reduced mane, belly fold, and infraorbital foramen bridge -and we see a recipe for inbreeding depression. The genetic evidence for historic inbreeding was overwhelming and the cost of these events was unmistakable.

Remember the captive SSP population of Asian lions in America that Paul Joslin worried about? When we examined the genetic structure of that population we found it to be quite different from the Gir and Sakkarbaug lions. The Asiatic lion SSP population retained quite a bit of **intrinsic** genetic variation, in stark contrast to the genetically **monotonous** Gir lions. Also, the genetically variable "alleles" of the SSP animals were familiar ones, because we had seen them previously in African lions. When we examined the SSP lion pedigree carefully and tracked the inheritance of these variable African alleles, we became convinced that two of the five original founders of the SSP Asiatic lions were actually from Africa ... Thirty-eight zoos were engaged in amassing a "pure Asiatic" lion population that in truth derived from matings between African and Asian lion forebears. Oops!

Paul and other zoo managers of the Asiatic lion SSP were not happy. Their very successful breeding program, with far better **fecundity** and productivity than the "pure" Gir lions from Sakkarbaug, was a genetic mixture of two continental subspecies. As the bearer of bad news, I tried to emphasize what I believed was a positive spin on the revelation. Of course the SSP lions did well: They had inadvertently **ameliorated** all of the woes of inbreeding present in "pure" Asiatic lions. The pure Gir lions were intrinsically flawed, reproductively impaired, and weakened by generations of inbreeding. The SSP population offered a living testament to the real benefits of maximizing outbreeding. I urged the zoo community to **perpetuate** the SSP lions as an experimental population to allow further, more extensive scientific study and as a reminder of the benefits of good genetic management.

Nobody listened to me. My optimism notwithstanding, within a year of our disclosure, all the SSP lion "hybrids" were fitted with birth control implants! Nobody wanted to conserve a hybrid subspecies. This was my first, but not the last, close encounter with the politics of species and subspecies hybridization ...