VOL.1 | 2016

THE SCIENCE OF Saving Species

SAN DIEGO ZOO GLOBAL
INSTITUTE FOR CONSERVATION RESEARCH

The Future Is Now:
Saving the Northern White Rhinoceros

With only three remaining in the world, the last, best hope for northern white rhinos now rests with 12 precious cell lines preserved in the Frozen Zoo® and new assisted reproduction methods.

It's a wonderful time to be a geneticist. The development of new tools in the fields of genetics is rapidly expanding opportunities for addressing conservation problems. In the interests of the future, we should carefully pursue approaches that can offer an alternative to extinction—and we should not delay.

–Oliver A. Ryder, Ph.D.

Welcome to *The Science of Saving Species* newsletter!

San Diego Zoo Global's new vision to end extinction inspired us to create a dynamic redesign of *Conservation Update*. We've added more pages and given our scientists more room to share their passion—and explain the science—behind the new technologies and protocols they use every day to ensure a sustainable future for endangered species. For more stories about wildlife conservation breakthroughs from our hardworking team at the Institute for Conservation Research, visit us at **institute.sandiegozoo.org**.

How You Can Help

Our field research teams all over the world rely on the generosity of donors like you to help achieve San Diego Zoo Global's vision to lead the fight against extinction. To learn ways you can help, please call Maggie Aleksic at 760-747-8702, option 2, ext. 5762, or email **maleksic@sandiegozoo.org**.

ON THE COVER: Southern white rhinos at the Lewa Wildlife Conservancy in Kenya, one of San Diego Zoo Global's partners.

A Bold New Vision

By Oliver A. Ryder, Ph.D., Kleberg Chair, Director of Genetics

WHEN A SPECIES IS DOWN TO ITS LAST FEW INDIVIDUALS, IS THERE A WAY TO SAVE IT? AT THE INSTITUTE FOR CONSERVATION RESEARCH, WE BELIEVE WE MUST TRY, SO WE HAVE SPENT THE PAST YEAR LAUNCHING A GROUNDBREAKING, MULTIFACETED EFFORT TO RESCUE THE CRITICALLY ENDANGERED NORTHERN WHITE RHINOCEROS. WITH THE LOSS OF THE SAFARI PARK'S BELOVED NOLA LAST NOVEMBER, ONLY THREE NORTHERN WHITE RHINOS REMAIN IN A PROTECTED RESERVE IN KENYA, ALL OF THEM TOO OLD TO BREED NATURALLY.

It might seem that this rhino is doomed to extinction but for the fact that, in 1975, our research team had the foresight to begin saving critical genetic material in the Frozen Zoo®, well before technologies like PCR diagnostics and genome sequencing were invented. A sign above the Frozen Zoo® stated, "We must preserve things for reasons we do not yet understand." In its frigid depths, we have safeguarded the viable cells of 12 northern white rhinos, 8 of which are unrelated. Through genetic rescue, we now have the opportunity to use the living cells of these 12 animals to give the northern white rhino a second chance at survival.

However, cells alone do not constitute a crash of rhinos. The challenge ahead requires that the frozen cells be used to produce viable animals. Every organism starts its life (or lives its life) as a single cell, as has dramatically been shown by the ability to clone animals from single cells. Remarkably, methods are available to

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THE STEM CELLS WILL BE USED TO PRODUCE REPRODUCTIVE CELLS—EGGS AND

continued from page 2

induce cells, such as those in the Frozen Zoo®, to successfully go through the entire process of development, from embryo to reproducing adult, by reprogramming them to be pluripotent stem cells—cells capable of making any tissue in the body. Our colleagues in Dr. Jeanne Loring's lab at The Scripps Research Institute have accomplished just that with northern white rhino cells from the Frozen Zoo®. We are now working together to reprogram the cells of all 12 northern white rhinos to become stem cells.

The stem cells will be used to produce reproductive cells—eggs and sperm—a challenging, even daunting task, but not an impossible one. All the steps we propose for the rescue of the northern white rhinoceros—take cells from adult animals, induce them to become stem cells that are then directed to become eggs and sperm, produce embryos by *in vitro* fertilization, transplant these into surrogate southern white rhino mothers, and produce healthy northern white rhino offspring that can reproduce naturally—have been accomplished in a model animal, the laboratory mouse. It will not be easy to accomplish the same things in the rhinoceros, but we believe we must try.

New insights into the reproductive biology of rhinos, along with advanced reproductive technologies, will provide a road map for assisted reproduction methods. At the new Nikita Kahn Rhino Rescue Center at the Safari Park, we now have six young female southern white rhinos that will become surrogate mothers for northern white rhino embryos. These are bold, hopeful steps, yet this approach is the only way to

Nola the northern white rhino.
Photo courtesy of Helene Hoffman

SPERM—A CHALLENGING, EVEN DAUNTING TASK, BUT NOT AN IMPOSSIBLE ONE.

prevent extinction of the northern white rhinoceros.

First, it is important to know whether the Frozen Zoo® cell lines represent sufficient genetic diversity to have the potential to one day produce a viable herd of animals. We have now compared the genome-wide variation of the northern white rhinoceros cell cultures in the Frozen Zoo® to its close relative, the southern white rhinoceros, in what is the first effort of its kind: to assess the potential for recovery of a unique taxon by assessing its gene pool diversity. Our geneticists are examining levels of genetic diversity of northern and southern white rhinos in a unique effort to evaluate the amount of genetic variations—the depth of the gene pool—as a measure of the possibility for recovering a species literally at the point of extinction.

Southern white rhinos went through a genetic bottleneck a century ago, when numbers fell to between 30 to 100 animals. This catastrophic drop in the population was countered by impassioned efforts to preserve and protect the remaining animals. This led to a small herd of 18 coming to the Safari Park to begin a breeding program in 1971, with 96 calves born over the years. Today, there are more than 20,000 southern white rhinos in existence, and the success of this effort is a 20th century conservation milestone.

By producing northern white rhinos from genetic samples of individuals in the Frozen Zoo®, we may initiate a vigorous population. Here at San Diego Zoo Global, we are committed to pursuing this hopeful possibility.

SAVING ENDANGERED SPECIES IS DAUNTING ENOUGH, EVEN WHEN THERE IS A SMALL POPULATION OF REPRODUCING ANIMALS. BUT TO BRING A SPECIES BACK FROM THE BRINK OF EXTINCTION WHEN THERE ARE NO FEMALES CAPABLE OF REPRODUCTION MIGHT BE CONSIDERED WISHFUL THINKING. THIS IS THE CHALLENGE WE FACE WITH THE NORTHERN WHITE RHINO, AS THE ONLY TWO FEMALES REMAINING HAVE PHYSICAL LIMITATIONS THAT PREVENT THEM FROM GIVING BIRTH! SO HOW WOULD WE BEGIN TO FORMULATE A PLAN TO SAVE THE NORTHERN WHITE RHINO? WITH A MEETING, OF COURSE.

BUILDING A RHINOCEROS: *Some Assembly Required*

By Barbara Durrant, Ph.D., Henshaw Chair, Director of Reproductive Physiology

Safari Park curators, animal care managers, collection managers, veterinarians, and Institute scientists gathered to explore the realities and possibilities of committing several years of our lives to the enormous task of saving the northern white rhino. With the outline of a plan we met with San Diego Zoo Global's President and CEO Doug Myers, who posed all the right questions, including asking each one of us if we were "all in." With our unanimous "yes," he took a leap of faith, promised his support, and we jumped the broom together.

Acknowledging that we alone could not accomplish all the project entails, we began to assemble a dream team of collaborators. From the field of reproduction we selected experts from zoos, research institutes, universities, and private veterinary practices from the United States, Europe, and South Africa. Each specialist immediately agreed, and we convened at the Safari Park for four days of research reviews and discussions, then further defined our long-term plan.

Our role as reproductive specialists is to develop the assisted reproductive techniques needed to produce northern white rhino embryos *in vitro* and transfer them to the closely related southern white rhino surrogates for gestation. Sounds simple, right? Actually, it's rather complicated. First, we need reproductively capable southern white rhinos to carry those precious northern white rhino embryos. But many southern white rhinos born into managed populations do not reproduce reliably, so the next step was to import six young females from South Africa, build them a facility, and hire specialized keeper/trainers. These keepers immediately began collecting samples for hormone analysis to assess the reproductive cycles of the new rhinos. They also began training the rhinos to stand in a specially designed chute, allowing regular ultrasound exams so our new postdoctoral fellow and I can document ovarian activity and pregnancy.

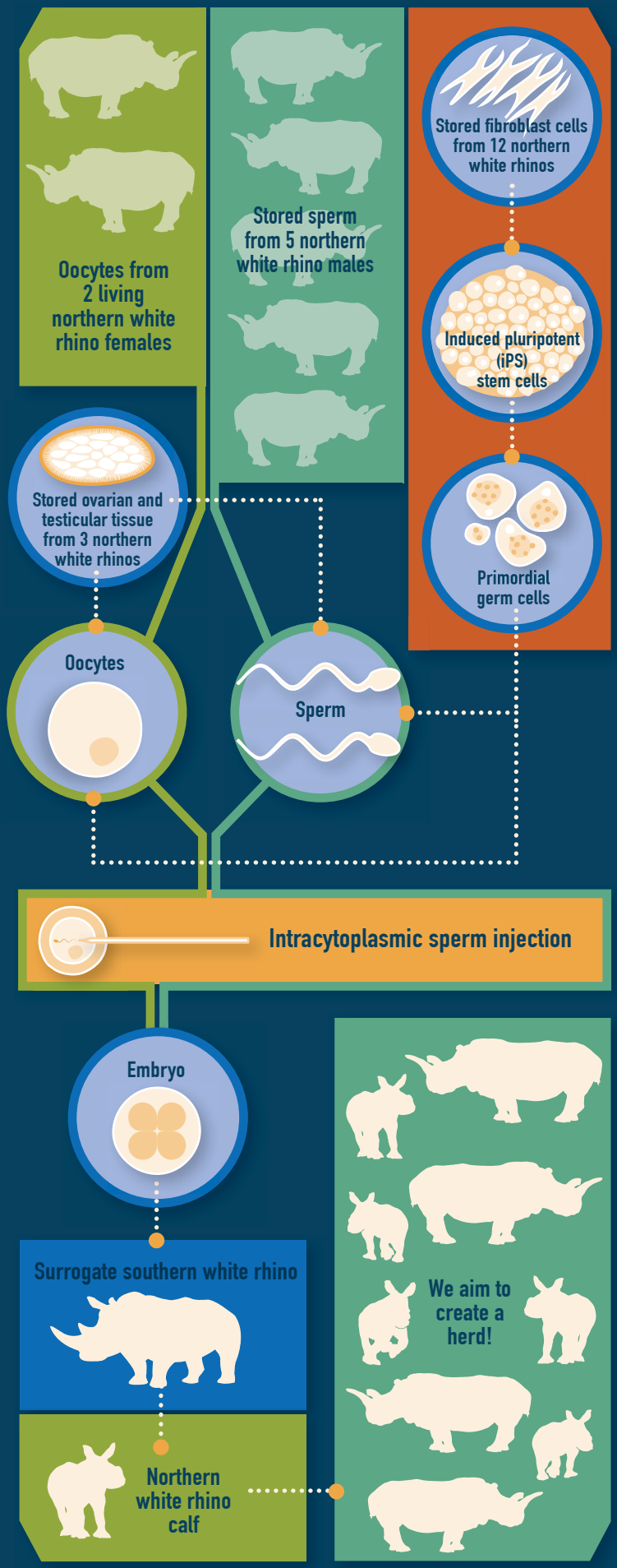
The opportunity to closely follow the growth of follicles containing oocytes, ovulation, and pregnancy is essential to the assisted reproduction project. A successful embryo transfer of a northern white rhino embryo into a southern white rhino requires a deep understanding of the physiological processes and *in vitro* culture requirements for ova maturation and fertilization, including intracytoplasmic sperm injection and embryo development to the blastocyst stage, when embryo transfer is most successful.

First, we need sperm and ova. It is unlikely that we will have "natural" ova or sperm from a living northern white rhino, so we must develop *in vitro* techniques with southern white rhino sperm and ova until such time northern white gametes may be generated from stem cells. Next, we will develop a method to extract ova from rhino ovaries, a technique called ovum pickup, which is used to collect ova for *in vitro* fertilization in humans, cattle, and horses. The southern white rhino oocytes will be fertilized with sperm from genetically valuable males to produce embryos, which will be transferred to other southern white rhinos. The embryo transfer technique, which requires exact synchronization of the embryo and the recipient uterus, has never before been attempted in any rhino species. Once we master this technique in southern white rhinos, we will be ready to transfer northern white rhino embryos into these experienced mothers.

The northern white rhino project is off to a thunderous start and gaining momentum. We have the opportunity to understand and document the intricacies of the southern white rhino's reproductive cycle in detail never before possible. The offspring produced from the six new southern white rhinos through assisted reproductive technologies will represent a welcome infusion of new genes into the North American population. And, perhaps more importantly, the knowledge gained will be applicable not only to the northern white rhino but also to the black rhino, the greater one-horned rhino, and the critically endangered Sumatran and Javan rhinos. We are committed to a sustainable future for all rhino species.

BUILDING A NORTHERN WHITE RHINO

NATURAL GAMETES ..... CONSTRUCTED GAMETES



The rhino project is one of the most ambitious undertakings in San Diego Zoo Global history. I'm proud to be part of an organization that is willing to take on conservation challenges of this magnitude. The birth of a northern white rhino calf will be a joyous occasion for the team and will represent a tremendous step forward in the fight to end extinction.

– Barbara Durrant, Ph.D.

Diet Matters

By Christopher Tubbs, Ph.D.,
Scientist, Reproductive Physiology

ON APRIL 2, 2016, we welcomed our newest southern white rhinoceros calf, named Masamba—which means leaves or vegetables in Yao, a Bantu language—to the Safari Park. While every rhino calf is a welcome addition to our herd, we hope that Masamba's birth signifies a breakthrough in the longstanding problem of sluggish reproduction of southern white rhinos born in zoos.

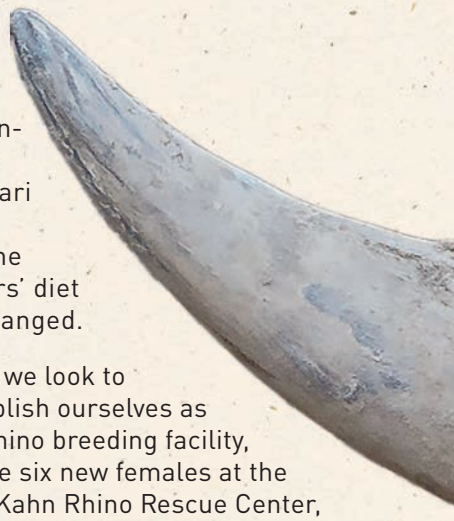
With 96 calves born at the Safari Park since 1972, it is the top southern white rhino breeding facility in the world. However, we and other institutions have seen a significant decline in births over time, as females born in zoos have not reproduced as well as females born in the wild. We decided to investigate the possible role of diet, specifically chemicals called phytoestrogens, in tackling this problem.

Phytoestrogens get their name because they mimic the hormone estrogen and can interfere with normal reproduction. We focused on them because they are produced by plants common in zoo diets, like soy and alfalfa, and because the reproductive problems we have seen in southern white rhinos are similar to those observed in other species eating high phytoestrogen diets. This can affect animals in two ways. In adults, hormone cycles can change and tumors can develop within the reproductive tract, all common to female southern white rhinos in zoos. When soy and alfalfa are removed from diets, normal reproductive function typically returns. The second effect occurs when a pregnant female eats a diet high in phytoestrogens, exposing her fetus to these chemicals and resulting in poor fertility that can be permanent in her daughter.

Studying how phytoestrogens affect rhinos requires unique approaches. For example, we can't directly test their effects on rhinos. Time is also an issue, given that rhinos take five to six years to reach maturity, have a 16-month gestation, and usually wait two to three years between births. Using a lab-based approach, we looked at how phytoestrogens interact with southern white rhino estrogen receptors to understand how they might interfere with normal hormone function. This gave us our first clue that phytoestrogens might be a problem, as southern white rhino receptors interacted with the chemicals more strongly than receptors of other rhino species that eat similar diets yet still reproduce well.

We then sampled diets from nine southern white rhino breeding institutions, measuring their phytoestrogen content. For wild-born southern white rhinos, no relationship was seen between a diet high in phytoestrogens and fertility, but we did see a significant negative relationship for captive-born females, which were less likely to reproduce later in life. With that discovery, our Nutritional Services team developed a new pellet low in phytoestrogens for our southern white rhinos.

So could changing diets restore fertility for captive-born southern white rhinos? My response used to be probably not, although now Masamba has caused me to reconsider. Despite breeding regularly for the last 10 years, his mom, Holly, failed to conceive. That changed about one year after a diet change was made using the new pellet, giving us hope that some females can be helped. In fact, in 2015 we had three rhino pregnancies at the Safari Park—after the mothers' diet was changed.



And as we look to reestablish ourselves as a top rhino breeding facility, with the six new females at the Nikita Kahn Rhino Rescue Center, we are optimistic that we can avoid repeating history so all female southern white rhinos born here will reproduce as successfully as their mothers.

Now more than ever, rhinos need our help. I am excited to be part of this project because it gives us an invaluable opportunity to learn about their reproductive biology and apply that knowledge to saving their species.

–Christopher Tubbs, Ph.D.

Good News:

SAFARI PARK RHINOCEROS BIRTHS

Greater one-horned rhino: 72

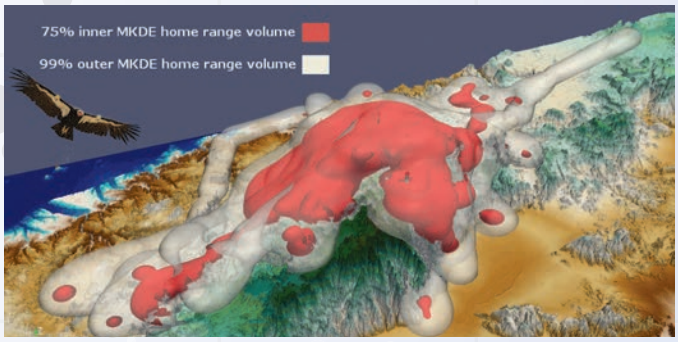
Southern white rhino: 96

Eastern black rhino: 14

Conservation Achievements

HONORS AND AWARDS

Dr. James Sheppard, along with colleagues at the United States Geological Survey and the San Diego Supercomputer Center, developed a 3-D California condor home range model that won the annual BMC Ecology Image Competition in the Theoretical Ecology and Models category.



Dr. Bruce Rideout received a Presidential Service Award from the American Association of Zoo Veterinarians in recognition of his assistance in developing procedures for evaluating research grant requests submitted to the Wild Animal Health Fund.

Dr. Megan McCarthy, our Steel Endowed Pathology Resident, was awarded first place in the Diagnostic Pathology Category of the American College of Veterinary Pathologists' Young Investigator Award for her poster presentation on canine esophageal disease.

Malenoh Ndimbe, Central Africa Program Monitoring Coordinator, won the best student intern prize at the Student Conference on Conservation Science held at Cambridge University, UK, for her work on chimpanzee population densities.

HIGHLIGHTED PUBLICATIONS

Felton, R. G., C. C. Steiner, B. S. Durrant, D. H. Keisler, M. M. Milnes, and **C. W. Tubbs**. 2015. Identification of California condor estrogen receptors 1 and 2 and their activation by endocrine disrupting chemicals. *Endocrinology* 156: 4448-4457.

This study explores the degree to which California condors living in coastal environments may be exposed to endocrine disrupting contaminants that cause reproductive problems, including eggshell thinning, by feeding on carcasses of marine mammals.

O'Connor, D. A., B. Butt, and J. B. Foufopoulos. 2015. Foraging ecologies of giraffe (*Giraffa camelopardalis reticulata*) and camels (*Camelus dromedarius*) in northern Kenya: Effects of habitat structure and possibilities for competition? *African Journal of Ecology* 53: 183-193.

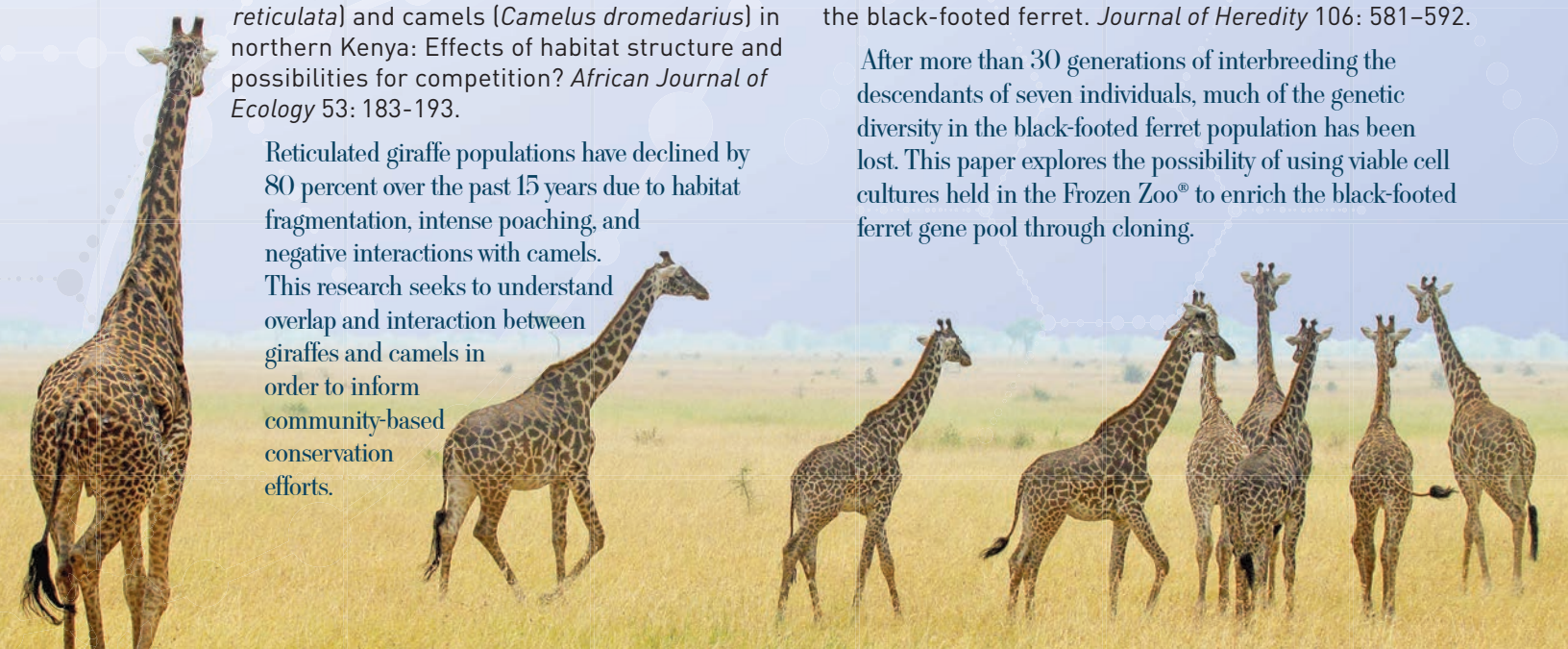
Reticulated giraffe populations have declined by 80 percent over the past 15 years due to habitat fragmentation, intense poaching, and negative interactions with camels. This research seeks to understand overlap and interaction between giraffes and camels in order to inform community-based conservation efforts.

Pasachnik, S. A., R. Carreras De Leon, and Y. M. Leon. 2016. Protected only on paper? Three case studies from protected areas in the Dominican Republic. *Caribbean Naturalist* 30: 1-19.

This paper evaluates three case studies from the Dominican Republic, focusing on conflicts between the country's national protected areas system and agriculture, tourism, and the charcoal trade, and concluding that park designations are an important first step to build upon.

Wisely, S. M., **O. A. Ryder**, R. M. Santymire, J. F. Engelhardt, and B. J. Novak. 2015. A road map for 21st century genetic restoration: Gene pool enrichment of the black-footed ferret. *Journal of Heredity* 106: 581–592.

After more than 30 generations of interbreeding the descendants of seven individuals, much of the genetic diversity in the black-footed ferret population has been lost. This paper explores the possibility of using viable cell cultures held in the Frozen Zoo® to enrich the black-footed ferret gene pool through cloning.



Gifts & Grants

THE INSTITUTE FOR CONSERVATION RESEARCH IS GRATEFUL TO THE FOLLOWING FOR THEIR INVESTMENTS IN ENDANGERED SPECIES CONSERVATION:

- Anonymous donors for their gifts to support capacity building efforts in Africa and Asia.
- The Beckman Coulter Foundation for sponsoring a Conservation Education Lab visit by Fullerton Union High School students.
- The Ellen Browning Scripps Foundation for their grant to challenge donors to support the Rhino Rescue initiative.
- Lisa and Frank Chapman for their support of the Human Dimensions of Wildlife program in Kenya.
- Nan C. Katona for an endowment gift in support of Conservation Education at the Institute for Conservation Research.
- The Steven and Carole Weinberg Foundation for their grants in support of the Rhino Rescue initiative.

GIFTS FROM THE HEART

With time running out to save the northern white rhino species, benefactor and animal advocate Nikita Kahn came forward with a transformational gift to build the Rhino Rescue Center at the Safari Park: ***"I am very proud to be a part of this important effort to save these majestic animals."*** Ms. Kahn and other generous donors helped build the state-of-the-art facility and attended a reception and ribbon-cutting event on May 18. Our research team is working to develop new technologies, such as assisted reproduction, that will eventually benefit all five endangered rhino species. The first step is to work with six new southern white rhino females that arrived there in November as we partner with scientists worldwide to understand all aspects of a rhino's reproductive physiology. This is a challenging project with the goal of establishing sustainable northern white rhino populations at the Safari Park.

Nikita Kahn with her namesake at the Nikita Kahn Rhino Rescue Center ribbon-cutting ceremony on May 18.

Thank You!

We honored our five lead donors by naming one of the new southern white rhinos for each of them. The donors had an opportunity to see their namesake at the reception, including one named Amani through a San Diego Zoo Global naming contest.

Nikita Kahn

Nikita

Annenberg Foundation

Wallis

Seaver Institute

Victoria

Dr. Helene Hoffman and Frank Trousdale

Helene

Livia and Biz Stone

Livia

WHAT'S News

APPLIED ANIMAL ECOLOGY
Our team traveled to Svalbard, Norway, to initiate a study of denning behavior in wild polar bears using specially designed surveillance cameras.

BEHAVIORAL ECOLOGY
Our researchers collected data from canopy and ground-level camera traps in Peru's Tambopata National Reserve, recording an abundance of large mammals compared with more heavily hunted sites.

CONSERVATION EDUCATION
We partnered with Scripps Institution of Oceanography in Mexico to assess perceptions of vaquita conservation and potential impacts on environmental management.

CONSERVATION PARTNERSHIP DEVELOPMENT
We organized the second Little Green Guards Conservation Forum and associated camera-trap training workshop in Guizhou, China.

GENETICS
Our team is using whole-genome sequencing of the critically endangered alala to study disease immunity, contribute to genetic management, and identify preferred candidates for reintroduction to the wild.

PLANT CONSERVATION
We finalized an agreement with The Nature Conservancy to assist with development and maintenance of a Tecate cypress field gene bank that will serve as a seed source for wild populations.

REPRODUCTIVE PHYSIOLOGY
Our researchers successfully matured and fertilized South African cheetah oocytes *in vitro*, a first for the lab and a direct demonstration of the Frozen Zoo®'s potential to preserve genetic diversity over time.

WILDLIFE DISEASE LABORATORIES
We assisted the Ishaqbini Hirola Community Conservancy in northeastern Kenya with a disease surveillance and prevention strategy for the highly endangered hirola antelope.

THE SCIENCE OF Saving Species

SAN DIEGO ZOO INSTITUTE FOR CONSERVATION RESEARCH

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