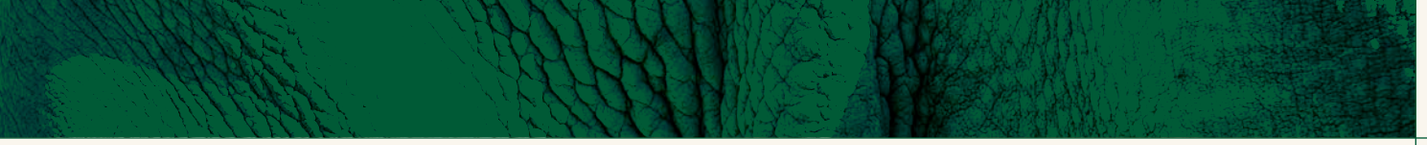
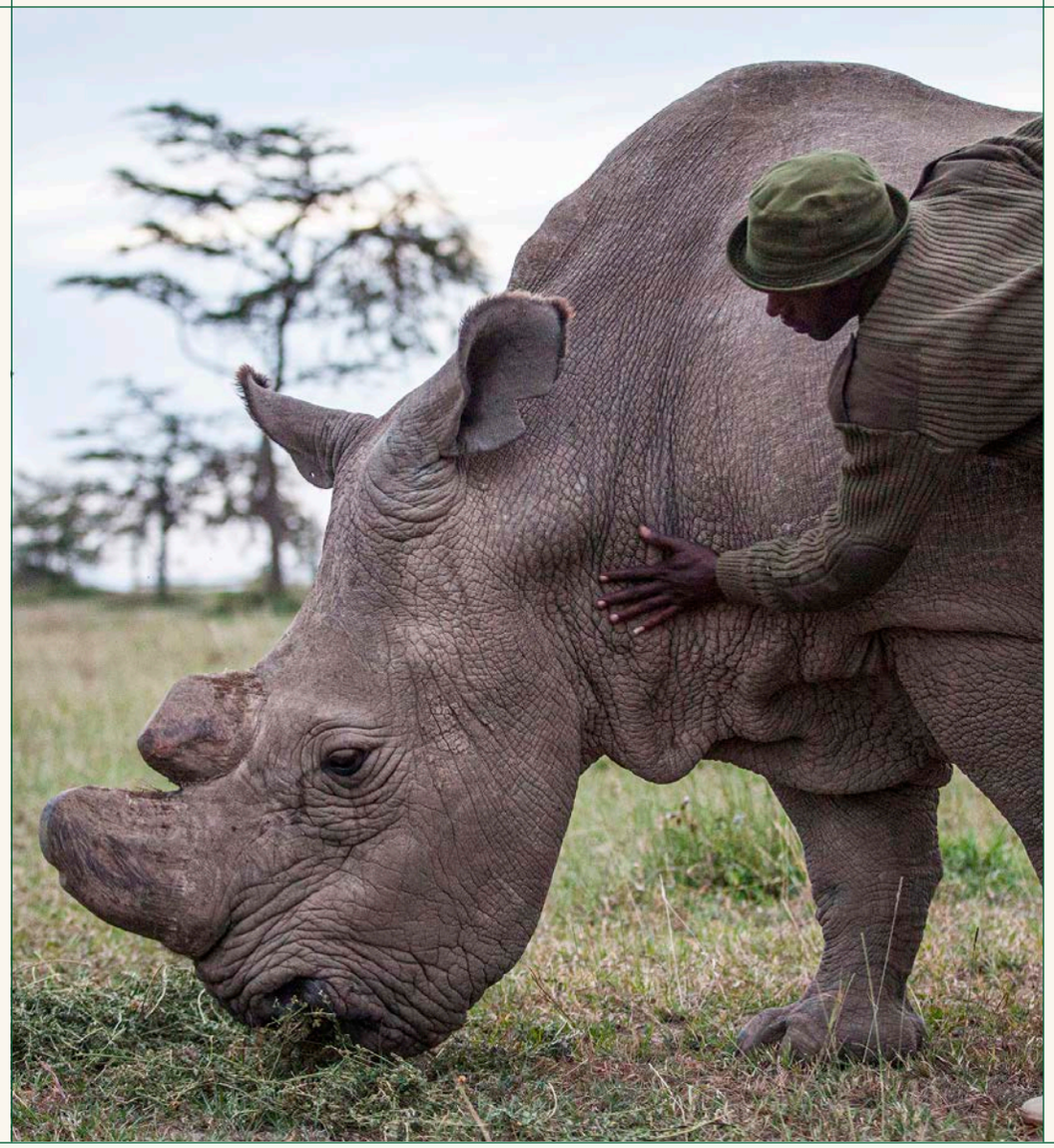


The $20 Million Bioengineering Gambit to

Save the Northern White Rhino



Sudan and one of his caretakers at the Ol Pejeta Conservancy in Kenya, in one of the last photos taken before the rhino's death.

→ We have the science and technology to bring animals back from extinction. But should we use it?

BY ANDREW ZALESKI

PHOTOGRAPHY BY **DAMON CASAREZ**

The day before he was euthanized by veterinarians in March of 2018, Sudan collapsed in the dirt at the Ol Pejeta Conservancy in Kenya, where he had lived since 2009. He was worn out and in pain.

Thomas Hildebrandt, second from right, and other BioRescue scientists locate oocytes in a female northern white rhino in January 2021.

COURTESY LEIBNIZ INSTITUTE FOR ZOO AND WILDLIFE RESEARCH; PREVIOUS SPREAD: THE WASHINGTON POST / GETTY IMAGES

AT AGE 45, SUDAN WAS THE FINAL progenitor of Earth's most endangered animal species: the northern white rhinoceros. As the last male northern white in the world, he was both a global icon for conservation and a two-and-a-half-ton target—because the horn of even the most precious rhino is not safe from poachers. He lived out his final years under 24/7 armed protection at the conservancy, along with two of his female relatives.

Half a world away, Barbara Durrant felt it. She had never met Sudan, but she knew Nola. Most people in San Diego knew Nola, though not the way Durrant did. Nola was a northern white rhinoceros, one of only four that remained by the middle of the last decade, along with Sudan and his kin. She lived at the Nikita Kahn Rhino Rescue Center, located at the San Diego Zoo Safari Park, about 30 miles north of the city, and not far from where Durrant reports to work every day at the zoo's Wildlife Biodiversity Bank.

Nola had also been euthanized, after age and infection caught up with her, in 2015. She was 41.

"She was just the most amazing animal," says Durrant, recalling Nola's wide mouth, her skin the color of clay stone, and her distinctive horn, which curved toward the ground. "It's not only losing that animal that you know personally and you love; it's another step in losing the whole species."

Durrant is director of reproductive sciences at the San Diego Zoo Wildlife Alliance and one of a handful of scientists around the world who are trying to save the northern white rhino. In Europe, another group, under the direction of wildlife researcher Thomas Hildebrandt, is also working on the problem. And while their scientific approaches may be slightly divergent, the scientists' end goal is the same: to rescue the northern white rhino before the bell of extinction rings.

Hildebrandt is the project head for BioRescue, an international consortium of scientists and conservationists. His group is harvesting eggs from female rhinos in Kenya; eventually the team hopes to create embryos using the frozen sperm of long-deceased northern white rhino males.

Meanwhile, Durrant's team in San Diego is undertaking an ambitious bioengineering challenge. Inside the Wildlife Biodiversity Bank is the Frozen Zoo, a cryopreserve where 10,000 still-living skin cells from 1,100 different animal species are stored in tanks of liquid nitrogen at extremely low temperatures. Among them are 12 cell lines taken from 12 different northern white rhinos, dating back to 1979.

As recently as two decades ago, the next step amounted to the stuff of science fiction: taking those skin cells, reprogramming them into sperm and egg, combining them in a test tube, and then implanting that embryo into a surrogate host. Recreating a whole new northern white rhino. And then another, and another, and then, once nature took its course, dozens more. Breathing life back into that which is dead. De-extinction, in other words, the purposeful resurrection of animals that have died off. Animals like Sudan.

"People are seeing a species go extinct right before their eyes," says Durrant. "Can we really even make a dent? The answer is, well, we have to. We have to do this."

Astronomical costs and enormous risks stand in the way. An investment of at least $20 million is required to realize the ultimate goal of reconstituting a population of wild northern white rhinoceroses. Retrieving oocytes (eggs) is a delicate endeavor, because if scientists puncture blood vessels near the uterus, the animal will bleed to death. And preserving a species through bioengineering is a fraught, messy process, one that calls into question the sophistication of current reproduction techniques and the ethics of meddling with nature.

If the project succeeds, it would be a scientific breakthrough like no other. What was once outside the realm of possibility is almost within our grasp. At some point in the not-too-distant future, a rhinoceros calf—a cultivated northern white—may very well take its first steps.

OF THE WORLD'S FIVE RHINO SPECIES, the northern white—one of two subspecies of white rhinos—drew the short straw. Northern whites once roamed East and Central Africa, enjoying an herbivorous lifestyle with few natural predators. Humans prized them for their horns, which can grow to over four feet. In Europe circa 1900, rhino horn was fashioned into ornamental accoutrements, like walking sticks and pistol grips. It remains a common ingredient in traditional Chinese medicine, which prescribes powdered rhino horn mixed with boiling water as a cure for fever, gout, and rheumatism.

Poaching and war rapidly thinned the northern whites' numbers, from the thousands to the hundreds to the tens. Nola arrived in San Diego in 1989; by the end of that decade, fewer than 40 remained in the northeast corner of what is today the Democratic Republic of the Congo. The last northern white in the wild was spotted in 2006. By then, the only survivors were those that had been relocated to zoos in the 1970s. They included Sudan; his daughter, Najin; her daughter, Fatu; and another bull, Suni, who were all taken to Kenya's Ol Pejeta Conservancy in 2009. They were the eligible breeders, yet no calves were born. Suni died four years before Sudan.

Four became three, then three became two, so now only Najin and Fatu remain. They are old and getting older, and even if they could mate, veterinarians have

→ "The Frozen Zoo is a cryopreserve where 10,000 still-living skin cells from 1,100 different animal species are stored in tanks of liquid nitrogen."

determined that neither is capable of carrying a pregnancy to term. It's a foregone conclusion then, yes? The line of northern white rhinos dies with Najin and Fatu.

"Sometimes we feel kind of helpless," says Durrant. "We're battling such a huge wave of extinction."

Southern white rhinos, on the other hand, largely escaped their cousins' misfortune. There were fewer than 100 remaining in the late 1800s, but a tenacious conservation effort followed and continues today. More than 20,000 of these rhinos currently roam the earth, mostly in South Africa. The San Diego Zoo Wildlife Alliance has six females, which will play a crucial role in its effort to produce a pure northern white rhino. Summarizing the idea is easy enough: An embryo made of northern white sperm and egg is implanted into a surrogate—a female southern white rhino. Sixteen months later, a northern white calf is born.

Durrant and her colleagues have already cleared several hurdles in the past five years. Using ultrasound technology, the team deciphered the inner workings of the rhino's reproductive system. Mapping the cervix was a key first step. A rhino cervix is a tight, convoluted maze of rings. Navigating it can be tricky. To practice, the zoo artificially inseminated two southern white females in 2018 using preserved southern white male sperm. Two healthy calves, Edward and Future, were born in 2019.

→ "I can say pretty clearly that this would be the first time a robot has ever been used in animals like this."

When female rhinos are ovulating, circulating estrogen helps relax the rings of the cervical tissue. For that reason, Durrant and her team were able to inseminate the zoo's rhinos by hand. The future embryo transfer, however, will be much tougher. Once the team has produced a viable pure northern white rhino embryo, they will stimulate ovulation in one of the southern white rhinos residing at the Safari Park. Then they'll have to wait another 10 days to let the embryo mature in vitro before implantation. But the surrogate's estrogen levels will have decreased by then, causing her cervix to tighten once more. Navigating it by hand will be impossible, because the risk of severely damaging cervical tissue is too great. Instead, Durrant and her team are currently collaborating with roboticists at the University of California San Diego to develop a workaround.

"I can say pretty clearly that this would be the first time a robot has ever really been used in animals in any kind of major computation effort like this," says Michael Yip, a professor of electrical and computer engineering at UCSD and director of the Advanced Robotics and Controls Laboratory.

Yip's lab is outfitting a noodlelike catheter with miniaturized robotic controls. Imagine a tiny metal cylinder, thinner than the circumference of a headphone jack and sheathed in a flexible filament. A camera on one end will give zoo workers a view of where they're going, while a PlayStation-like controller will bend the catheter with sub-millimeter precision—enough to ensure that they can navigate the rings without scraping tissue or puncturing blood vessels.

"We'll do very little, if any, tissue damage, but we'll be able to get through that tightened-down cervix," Durrant says.

In March 2020, Durrant completed the zoo's first oocyte pickups. Because the scientists had already done the ultrasound mapping, they had a clear idea of where the ovaries and follicles were located.

Eggs were collected from each of their six southern white females using a four-foot-long double-lumen (two channeled) needle, which is capable of flushing out the follicles and sucking out the oocytes. They collected a total of 22; in the lab, each oocyte was fertilized with a single sperm. In the end, while half of the fertilized oocytes matured, none developed into blastocysts, the final stage of embryo growth. But the effort allowed the researchers to start piecing together some novel rhino science: What nutrients do rhino embryos need, in vitro, to mature?

This was a critical juncture in the team's de-extinction work, as valuable practice for the fertilization procedure to come. You don't transfer an embryo on the initial try. Fail to navigate the cervical maze, and you might damage tissue, imperiling the pregnancy. Fail to mature a reprogrammed egg into a blastocyst, and there's no embryo to even transfer. Everything Durrant's team has done with southern whites is a dress rehearsal for the premiere event, when it finally comes time to make a southern white female the surrogate mother of the main character: a northern white rhino embryo.

The task of generating the sperm and egg falls to the San Diego Zoo Wildlife Alliance's Marisa Korody, a conservation geneticist who is trying to create stem cells from the functionally extinct northern white rhinos. She starts with cryopreserved fibroblasts, cells that compose the connective structural tissue of all animals. The Frozen Zoo has fibroblasts generated from skin samples of 12 different northern whites—eight of which are unrelated—that contain enough genetic diversity to save the species. These fibroblasts are then reprogrammed into induced pluripotent stem cells—that is, cells that can turn into any cell type in the body. By directing these stem cells to specific developmental paths, the researchers can generate primordial germ cells, precursors to what eventually become sperm and eggs.

This is as far as the science goes—at least for now, and at least with rhinos. Korody is optimistic as she's managed to generate the germ cells. Generating northern white rhino sperm and northern white rhino egg, though, is a long-term process, one that involves figuring out the hormones and growth signals needed to get the germ cells to differentiate further.

"Maybe in 10 years or so, we'll be close," she says.

It's a different strategy from the one Thomas Hildebrandt and BioRescue are focused on right now. While the team in San Diego is trying to generate northern white rhino embryos from cells, BioRescue is attempting to fertilize eggs collected from Fatu and Najin with cryopreserved northern white rhino sperm.

"We can use this approach to transfer the embryos into a southern white rhino surrogate, and then let the calf grow up with Najin and Fatu," says Hildebrandt, who also leads the department of reproduction management at the Leibniz Institute for Zoo and Wildlife Research in Germany.

In 2019, Hildebrandt's team accomplished a scientific first: It transferred a rhino embryo fertilized in vitro into the uterus of a female rhino. In this case, it was a southern white. As of July 2021, BioRescue has completed seven southern white rhino embryo transfers.

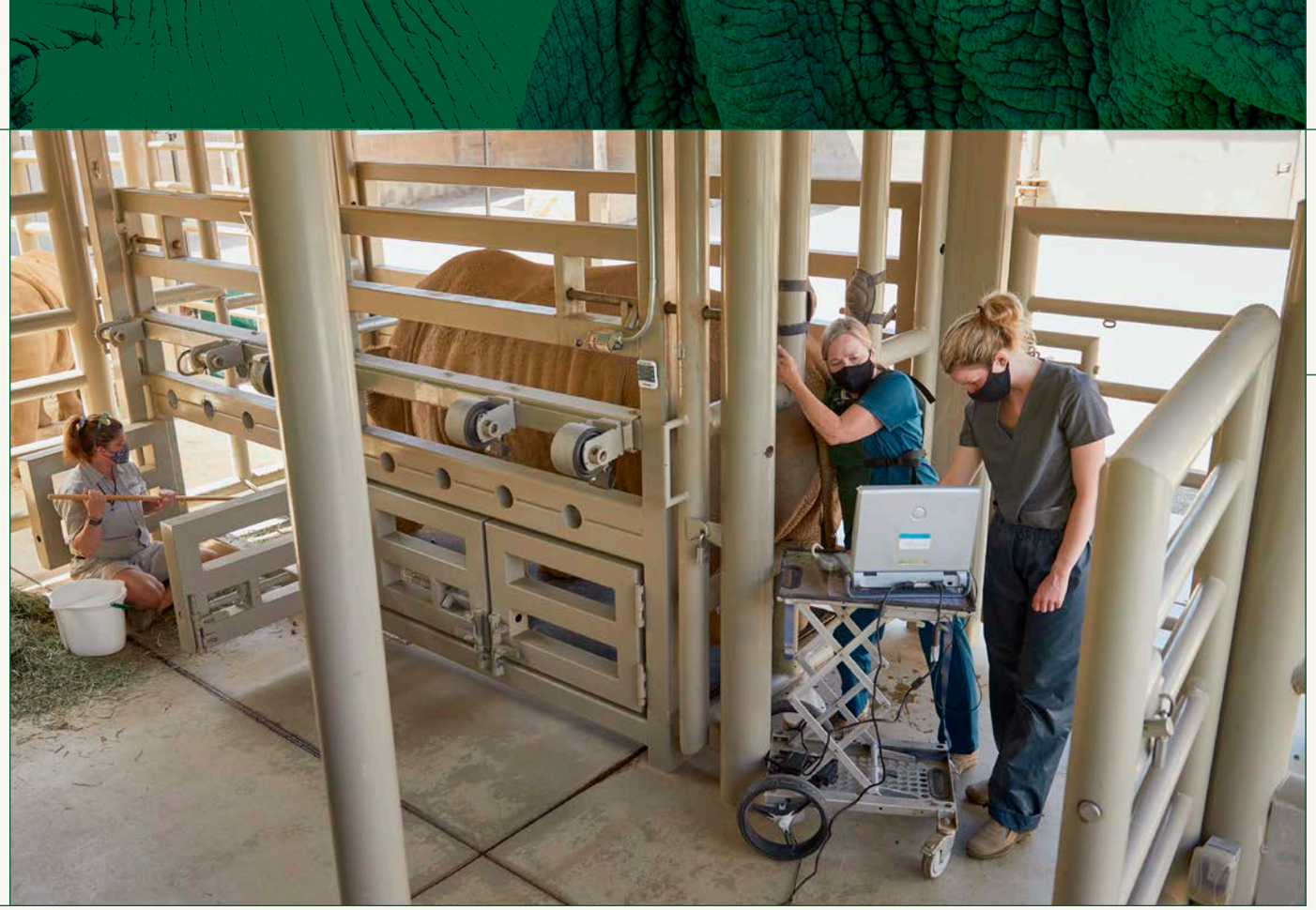
In the next few years, Hildebrandt says, BioRescue will be ready to transfer a northern white rhino embryo into a surrogate southern white female.

IN THE 55-MILLION-YEAR EVOLU-tionary history of the rhino, 10 years is nothing but a heartbeat. In the here and now, however, a decade is enough time to exacerbate an annihilation crisis that's already under way.

In 2019, a landmark report from the United Nations revealed that a million animal and plant species are careening toward extinction. A subsequent report issued by the World Wildlife Fund in 2020 indicated that

Barbara Durrant at the rhino habitat at the San Diego Zoo Safari Park's Nikita Kahn Rhino Rescue Center, June 2021.

Skin cell specimens from 12 different northern white rhinos are preserved in the Frozen Zoo.



Barbara Durrant, center, and her team perform an ultrasound on a southern white rhino at the San Diego Zoo Safari Park's Nikita Kahn Rhino Rescue Center in June 2021.

wildlife populations have declined by two-thirds in the past half century due to human activities; deforestation, insecticides, and poaching are all complicit. Various species we hardly think of but are nonetheless important for humans and ecosystems to thrive are in the crosshairs.

"If we can think of this as a leaky bucket right now, the bucket is pouring out water and more and more species are falling out," says Tierra Curry, a senior scientist with the nonprofit Center for Biological Diversity, based in Arizona. "Trying to put a couple more species back in the bucket isn't going to fix the problem."

Criticism of de-extinction efforts often begins with something like Curry's premise. Her preference would be to "fight like hell" for everything still alive. After all, the natural world is at the brink, but animals aren't the problem.

Instead, the ultimate problem is "uniquely and definitely humans," says Ross MacPhee, a curator in the mammalogy department of the American Museum of Natural History in New York City. There's no way to guarantee that a population of northern white rhinos wouldn't need around-the-clock protection the way Najin and Fatu do today. Southern white rhinos, despite their resurgence, are already considered a species on the way to endangered, as lust for rhino horn continues unabated. Some horns fetch a purse of $300,000. How much might a rare northern white rhino horn go for?

While the San Diego Zoo Wildlife Alliance hopes to generate a self-sustaining population of northern white rhinos back in part of their native range, Durrant says that would only happen if it's safe to put the animals there. But not making the effort isn't an option, she says.

"Everything is connected," says Durrant. "When you take any species, plant or animal, out of an ecosystem, it starts to unravel."

As it stands now, most of the African species of rhinos—the southern white and black rhinos—are concentrated mainly in southern Africa. Very few black rhinos are roaming around central Africa where northern white rhinos once predominated: The pointed mouth of the black rhino is good for eating branches and leaves, while the wide mouth of the white rhino is better adapted for grazing on grass.

Scientists interested in saving the northern white rhino often cite the good that such a keystone species provides. A megafauna creature like the white rhino directly and indirectly affects the well-being of dozens of other creatures. By eating long grass, they help keep vegetation at a reasonable level so predators can see their prey. Their feet carve avenues in the grass so prey can escape. Their droppings fertilize the grass and provide nutrients for insects. It's a tiny biosphere where nonhuman life thrives. Upset the balance, and that life has to migrate elsewhere. Maybe to urban ecosystems. Maybe carrying disease.

"The rhino hasn't failed in evolution. It's at the brink of extinction because humans have poached and killed it."

UNDER ANY OTHER CIRCUMSTANCES, A GROUP OF PEO-ple kneeling around Fatu inside the Ol Pejeta Conservancy would be a cause for concern. But on this Sunday in December 2020, the scientists and veterinarians in attendance were monitoring Fatu as she lay under general anesthesia. Near her backside was Hildebrandt. He was collecting eggs.

Over the past two years, with the permission of the Kenyan government, Hildebrandt and BioRescue have performed six separate egg pickups on Najin and Fatu. The latest one, in December 2020, yielded 14 oocytes from Fatu. Collection is done by anesthetizing the rhino and then inserting an ultrasound wand into the rectum. The wand is there only to provide a picture, a way to guide the needle that flushes out the rhino's follicles and grabs the eggs. Both times the eggs were rapidly transported to Avantea, an advanced biotechnology lab in Italy. There they were fertilized with frozen semen that had been extracted from Suni before he died. To date, BioRescue has cryopreserved nine embryos that combine northern white sperm and northern white egg.

It's a monumental step, one that represents the closest any group of scientists has come to bringing a northern white rhino calf into the world. Hildebrandt doesn't just consider it fascinating science; he likens it to a moral imperative. Picking and choosing which animals to de-extinct is easy when nature hasn't selected against them.

"The rhino hasn't failed in evolution. It's at the brink of extinction because humans have poached it and killed it," he says. "So it is actually our human responsibility to fix this problem, because we have caused it."

While BioRescue's current endeavor is separate from the work being conducted by Durrant, Korody, and others at the San Diego Zoo Wildlife Alliance, the two groups are working toward common goals. Hildebrandt and his counterparts in San Diego held the first international conference on rescuing the northern white rhino in 2015 in Vienna. He says the work being conducted on pluripotent stem cells in San Diego is an important component of the overall effort. BioRescue has created embryos made with eggs from Najin and Fatu and sperm from Suni; the embryos the San Diego team hopes to create will come from multiple other northern white rhinos, which will increase the genetic diversity of a future population. In turn, that should help improve the animals' overall health by serving as a safeguard against disease.

Yet Hildebrandt wants to bring a baby northern white rhino into the world as quickly as possible. While the two subspecies are related, northern white rhinos are wider, with straighter backs, flatter skulls, and a different neck structure. The differences are stark enough that a baby northern white rhino might not learn how to graze properly if it grows up in a herd of its southern white cousins. Hildebrandt wants the animal to socialize with Najin and Fatu before they, too, die. Sudan's granddaughter is only in her early 20s and still playful. Najin, on the other hand, is in her early 30s, and lives with a large tumor on her abdomen.

"There's a lot of things morphologically which are links to behaviors," says Hildebrandt. "The social knowledge, how to behave as a northern white rhino, is something we can preserve. But there is no way to do that unless we produce a calf very soon."

Still, a de-extinction project inevitably requires two finite resources: time and money. Hildebrandt thinks it will take about 20 years to reintroduce a healthy population of the animals back to Africa, at a cost of approximately $1 million per calf. But how much is one northern white rhino worth to the world?

DEPENDING ON BIORESCUE'S PROG-ress this year, there might be a baby northern white rhino walking with Najin and Fatu within two years. The bioengineering tools required to accomplish the incredible—resurrecting a herd of 6,000-pound animals—are here, in the hands of Durrant, Korody, Hildebrandt, and their teams.

We have the technology. We can rebuild them. Now comes the hardest question of all: Should we?

It's perhaps too soon to tell if a new birth in a species on the brink of extinction would be heralded as a success. After all, humans nearly killed off every northern white rhino in existence. What's to say people won't poach the animals for their horns, and do it flippantly, openly, even expectantly? You created a bunch of northern white rhinos before, we might cry out. Just do it again. This, we might incorrectly believe, is the promise of something like the Frozen Zoo. We preserve natural history, only to reanimate it according to our whims.

"Yes, science can save species. But don't rely on science to save species," says Durrant. "We can't do this for every species. We don't want to do this for every species. We want species to be preserved in their native habitats before they go extinct."

Cryopreservation and embryo transfers aren't blueprints for managing the planet. But they might preserve Sudan's legacy. If we're paying attention, maybe one new rhino will wake us up. PM