Three ways to create offspring

BY **ROBIN MCKIE**
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Scientists are working on three different techniques for creating offspring from the cells of endangered species.

Nuclear transfer
This technology was used to create Dolly the sheep and involves transferring the nucleus of an adult mammal cell into a developing egg cell whose own nucleus has been removed. Scientists have cloned two endangered types of cattle — the gaur and the banteng — this way but have found the technique is associated with early deaths and malformations.

Mixing cells
This technique would involve mixing stem cells from an endangered animal with those of a more common, but related species. Offspring from this mix would then be selectively mated to breed out the genes of the common animal and leave only a purebred version of the endangered creature.

Creating sperm and eggs
By removing stem cells from an animal, scientists hope that they will one day be able to create sperm and eggs of that species. These could then be used, in a petri dish, to create embryos that could then be implanted in a related species.

Zoo aims to save endangered species from extinction

San Diego Zoo began collecting skin samples from rare animals in 1972 in the hope they might be used to protect these endangered species in the future. A breakthrough in stem-cell technology means that day is getting closer

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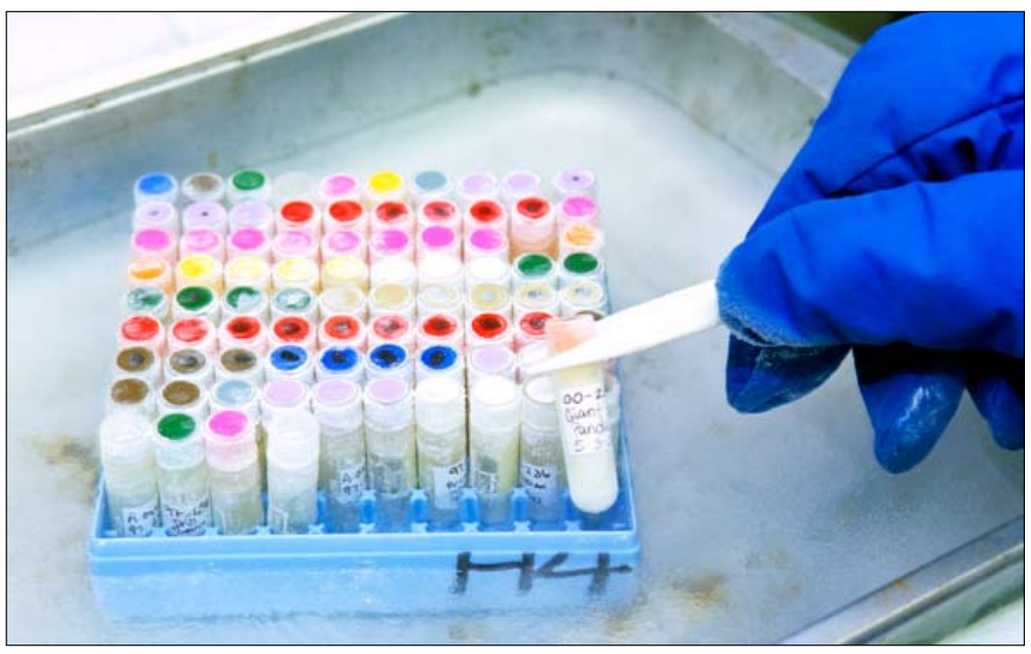
THE GUARDIAN, LONDON



Top: A northern white rhinoceros grazes at San Diego Zoo in San Diego, California. Skin cells from 12 white rhinos and 8,400 other animals are stored at the Frozen Zoo laboratory in the zoo's Institute for Conservation Research. The hope is that the cells can one day be used to create cloned animals and replenish endangered species. Above: Test tubes containing the frozen tissue of endangered animals are held in stasis at San Diego Zoo's Frozen Zoo laboratory in San Diego, California. PHOTOS: BLOOMBERG

The inside of a metal box filled with liquid nitrogen and frozen to minus 173°C is hardly the ideal habitat for a large African mammal. But, as a test tube is fished out of the container amid a billowing cloud of white gas, a note written on its side is unequivocal about its contents. "This is a northern white rhino," says scientist Inbar Ben-Nun as she reads out the label and holds the freezing vial with thick gloves.

Ben-Nun is holding no ordinary scientific sample. For the frozen cells in that test tube could one day give rise to baby northern white rhinos and help save the species from extinction. They would be living specimens of one of the most endangered species on Earth, who after a few months would be trotting into wildlife parks, and maybe, just maybe, helping repopulate their kind on the African grasslands. No wonder that the place where the sample came from is called the Frozen Zoo.

The Frozen Zoo was founded in 1972 at San Diego Zoo's Institute for Conservation Research as a repository for skin-cell samples from rare and endangered species. At the time that the first samples were collected and put into deep freeze it was not really known how they would be used and genetic technology was in its infancy. But there was a sense that one day some unknown scientific advance might make use of them and it was better to be safe than sorry. Now, thanks to a team at the nearby Scripps Research Institute, that day has come a lot closer.

Genetic scientists at Scripps, working from an anonymous-looking building in a business park in San Diego's northern suburbs, have succeeded in taking samples of skin cells from the Frozen Zoo and turning them into a culture of special cells known as induced pluripotent stem cells. Stem cells are a sort of all-purpose building block of life that can then become any other sort of cell. By creating induced pluripotent stem cells from a species it is now theoretically possible to use them to create egg cells and sperm cells. Those two could then be combined via in vitro fertilization to form a viable embryo. And long-dead animals whose species are almost extinct could create new life. The breakthrough, so far, has come with creating induced pluripotent stem cells for the silver-maned drill monkey, a primate native to just a few parts of West Africa and which is the continent's most endangered monkey. On June 1 this year, the stem cells morphed into brain cells, proving their viability.

"The Frozen Zoo was a wonderful idea. They just thought: 'Well, something might happen, so we should preserve some samples for the future,'" says Jeanne Loring, who is leading the Scripps team of which Ben-Nun is a part. "This is the first time that there has been something that we can do."

The implications of Loring's breakthrough are clear for those trying to save endangered animals. If the technology is perfected and induced pluripotent stem cell cultures can be established for many of the species held in the Frozen Zoo, then conservationists will not just have to rely on preventing extinction by coaxing a few remaining individuals to breed. Instead, cell lines preserved in the Frozen Zoo can be added to the possible gene pool, increasing the chances of healthy reproduction.

"If we could use animals that were already dead ... to generate sperm and eggs then we can use those individuals to create greater genetic diversity. I see it as being possible. I see no scientific barrier," Loring says.

It has also raised another prospect among some observers: that of a *Jurassic Park* scenario. If viable cell samples could be harvested from the remains of extinct animal species, such as stuffed Tasmanian tigers in museums or the woolly mammoth corpses dug up from the Siberian tundra, then perhaps scientists would one day be able to reverse extinction. It is not a prospect that many scientists involved want to encourage. But ever since news of Loring's work with the drill monkey cells was revealed, the *Jurassic Park* headlines have been coming thick and fast.

Loring's lab at Scripps holds samples from the northern white rhino and the drill monkey, but the real Frozen Zoo, just a few kilometers away, is on a much larger scale. Housed in a building inside San Diego Zoo, its freezers contain samples from 8,400 animals, representing more than 800 species. They include Gobi bears, endangered cattle breeds such as gaurs and bantengs, mountain gorillas, pandas, a California gray whale and condors. The entire gigantic menagerie is housed in four deep-freeze tanks, representing a staggeringly important slice of some of the world's most rare wildlife.

Oliver Ryder, the geneticist who heads the Frozen Zoo program, welcomes the news of Loring's work, which itself built on a breakthrough in 2007 by Japanese scientist Shinya Yamanaka. For Ryder it is confirmation that the zoo's founding as a sort of "bet" on the science of the future now has great prospects of paying off. "We wondered if one day pigs would fly. Well, now pigs are flying. I am very excited by the results," Ryder says.

But Ryder does not appreciate some of the wilder headlines that have sprung from the potential implications of the research. The words "*Jurassic Park*" get short shrift from the plainspoken scientist. He has little time for those who advocate bringing back long-dead species or those fringe figures who dream one day of recreating a dinosaur just like in Steven Spielberg's movie. Apart from the fact that the science of extracting viable DNA for such animals is virtually impossible, he believes it distracts from the Frozen Zoo's primary aim: to stop species becoming extinct in the first place. "What would be the benefit of bringing back something that has been extinct for some 10,000 years? It is intriguing and evocative but it plays to human hubris. What's the motivation? Is this for personal benefit or society saying: 'We have arcane powers and the world is our oyster,'" he says.

When it comes to species still on the brink, with perhaps just a few individuals left, however, Ryder is insistent that humanity has a duty to save them and that the Frozen Zoo can play a crucial role. Especially close to Ryder's heart is one of the species that Loring is working on: the northern white rhino. There are just eight of the animals left alive on Earth and not all of them are viable breeders. But, if Loring's work succeeds in creating northern white rhino induced pluripotent stem cells and then turning them into sperm and eggs, that gene pool can be deepened again.

It is a race against time. Unlike with the drill monkey, Loring's efforts with rhino cells have not yet worked. But at least Loring thinks she knows why. The drill monkey samples were coaxed into becoming induced pluripotent stem cells using viruses loaded with carefully selected human genes that can trigger that reaction. Loring suspects it worked with drill monkeys because — as fellow primates — they are genetically close enough to humans for the introduced human genes to work properly. Rhinos, she thinks, may be too distantly related. However, she plans to try again, this time perhaps using genes from a closer animal relative to the rhino, the horse.

Ryder makes no secret of how emotionally attached he is to saving the northern white rhino while there are still living animals, rather than just reviving some later entirely from a test tube. He recalls witnessing the birth of a female northern white rhino more than 20 years ago and watching it being introduced to its herd: something that would be lost for ever if the last northern white rhino died before Loring's technology is perfected. "I saw her meet the rest of the rhino herd. There was a clear sense of how to meet the baby. If we wait until there are no white rhinos and then one is created from a test tube, to whom are we going to introduce it?" he says. "My feelings about the rhino come straight from the heart. I am not ready to give up on this rhino."

Sadly, it is already too late for other species. The Frozen Zoo already holds samples from animals that are now extinct. One such is the po'ouli bird, a species of honeycreeper that lived in Hawaii and was only discovered in 1973. Unfortunately, the last recorded sighting of the po'ouli was in 2004, and it is thought to be extinct, assailed by habitat loss and the introduction of disease by humans. Now it resides only in the Frozen Zoo in the form of its skin cells preserved and frozen. Ryder, sticking with his belief that there is no point in rescuing the already extinct, hopes instead that studying the po'ouli bird's genes will help conservationists prevent other related and endangered species from following the same path. "Maybe we cannot bring back the po'ouli, but we can use its secrets to help others," he says.

Ryder believes the importance of the Frozen Zoo cannot be overestimated in the face of the vast pressures that humanity is putting on the creatures with which it shares the planet. In fact the Frozen Zoo's collection of samples is so valuable that a secret duplicate collection has been established in case a natural or manmade disaster were to strike the original. "No time that people have kept something safe in just one place has it worked. This is a globally important depository and its importance is not going to decrease. Over time there is going to be a big disaster. So we have to insure against that," he says. He is also keen on reaching out to other, smaller frozen zoos that exist elsewhere, such as one at the Audubon Nature Institute in New Orleans and one at the University of Nottingham. He hopes one day a global network of frozen zoos will be established to provide the ultimate insurance policy to carry the Earth's rarest animal species into the future. "Having a duplicate site is an important step but in the long run we need to have a global network," he explains. "The future will thank the present generation for saving what we can save. We have to look beyond the current moment. People who are not yet born will greatly appreciate what we can do."

That opinion holds true for Loring, too. Her success in creating induced pluripotent stem cells has the potential to unlock the whole Frozen Zoo as a powerful tool for breeding and conservation. She is already thinking of getting a third species from the zoo to add to the Scripps research on drill monkeys and the northern white rhino. She, too, is seeing the big picture and says there is a moral imperative to use the animals kept in the Frozen Zoo to preserve rare species as part of a living, breathing global ecosystem.

"The idea of doing it has become a reality," Loring says.