

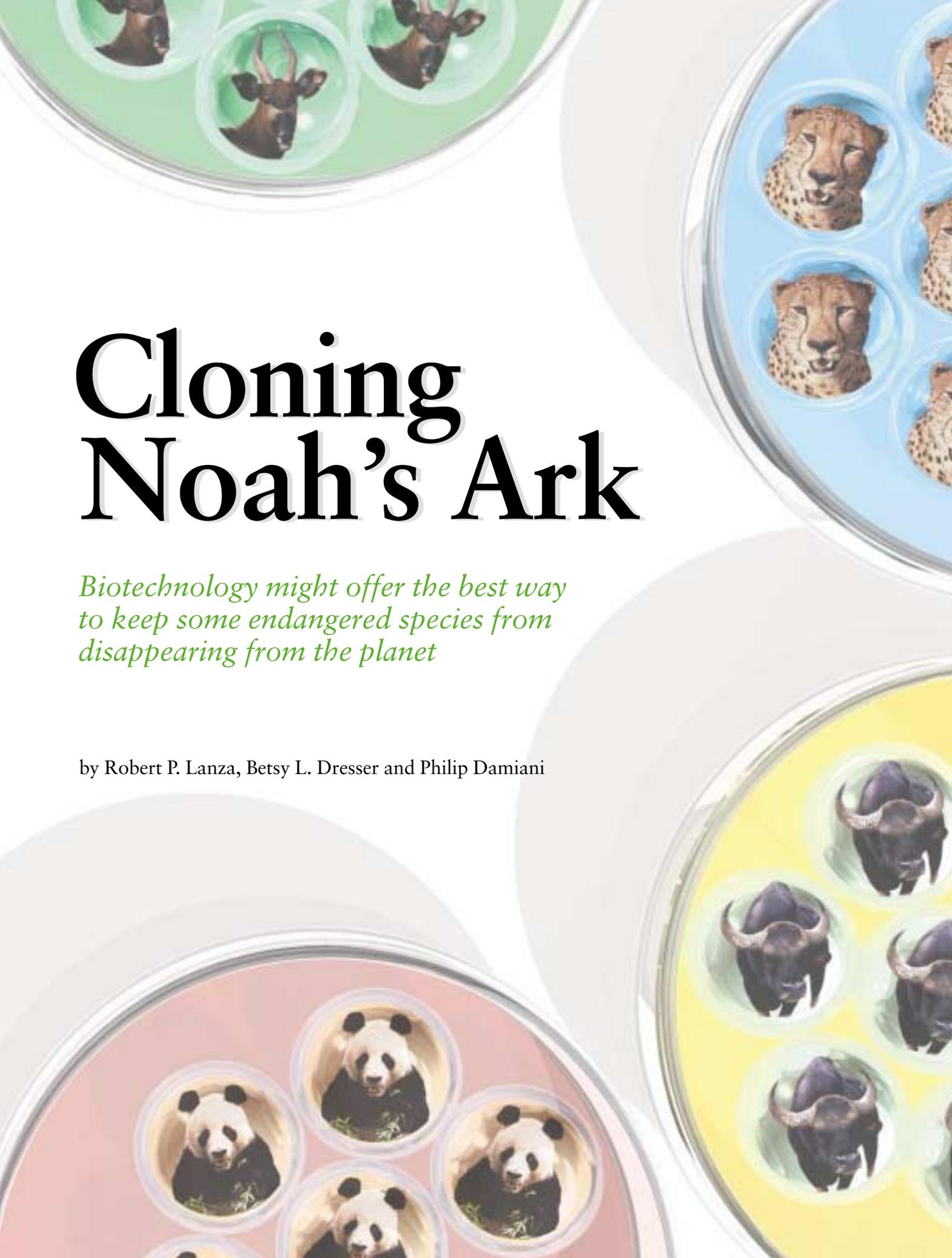
In late November a humble Iowa cow is slated to give birth to the world's first cloned endangered species, a baby bull to be named Noah. Noah is a gaur: a member of a species of large oxlike animals that are now rare in their homelands of India, Indochina and southeast Asia. These one-ton bovines have been hunted for sport for generations. More recently the gaur's habitats of forests, bamboo jungles and grasslands have dwindled to the point that only roughly 36,000 are thought to remain in the wild. The World Conservation Union-IUCN Red Data Book lists the gaur as endangered, and trade in live gaur or gaur products—whether horns, hides or hooves—is banned by the Convention on International Trade in Endangered Species (CITES).

But if all goes as predicted, in a few weeks a spindly-legged little Noah will trot in a new day in the conservation of his kind as well as in the preservation of many other endangered species. Perhaps most important, he will be living, mooring proof that one animal can carry and give birth to the exact genetic duplicate, or clone, of an animal of a different species. And Noah will be just the first creature up the ramp of the ark of endangered species that we and other scientists are currently attempting to clone: plans are under way to clone the African bongo antelope, the Sumatran tiger and that favorite of zoo lovers, the reluctant-to-reproduce giant panda. Cloning could also reincarnate some species that are already extinct—most immediately, perhaps, the bucardo mountain goat of Spain. The last bucardo—a female—died of a smashed skull when a tree fell on it early this year, but Spanish scientists have preserved some of its cells.

Advances in cloning offer a way to preserve and propagate endangered species that reproduce poorly in zoos until their habitats can be restored and they can be reintroduced to the wild. Cloning's main power, however, is that it allows researchers to introduce new genes back into the gene pool of a species that has few remaining animals. Most zoos are not equipped to collect and cryopreserve semen; similarly, eggs are difficult to obtain and are damaged by freezing. But by cloning animals whose body cells have been preserved, scientists can keep the genes of that individual alive, maintaining (and in some instances increasing) the overall genetic diversity of endangered populations of that species.

Nevertheless, some conservation biologists have been slow to recognize the benefits of basic assisted reproduction strategies, such as in vitro fertilization, and have been hesitant to consider cloning. Although we agree that every effort should be made to preserve wild spaces for the incredible diversity of life that inhabits this planet, in some cases either the battle has already been lost or its outcome looks dire. Cloning technology is not a panacea, but it offers the opportunity to save some of the species that contribute to that diversity.

A clone still requires a mother, however, and very few conservationists

The background of the page features several overlapping petri dishes. The top-left dish is green and contains three deer heads. The top-right dish is blue and contains three cheetah heads. The bottom-right dish is yellow and contains three bison heads. The bottom-left dish is pink and contains three panda heads. The dishes are arranged in a circular pattern, suggesting a laboratory or cloning environment.

Cloning Noah's Ark

*Biotechnology might offer the best way
to keep some endangered species from
disappearing from the planet*

by Robert P. Lanza, Betsy L. Dresser and Philip Damiani

would advocate rounding up wild female endangered animals for that purpose or subjecting a precious zoo resident of the same species to the rigors of assisted reproduction and surrogate motherhood. That means that to clone an endangered species, researchers such as ourselves must solve the problem of how to get cells from two different species to yield the clone of one.

A Gaur Is Born

It is a deceptively simple-looking process. A needle jabs through the protective layer, or zona pellucida, surrounding an egg that hours ago resided in a living ovary. In one deft movement, a research assistant uses it to suck out the egg's nucleus—which contains the majority of a cell's genetic material—leaving behind

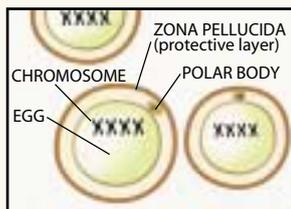
Advanced Cell Technology (ACT) in Worcester, Mass., had to fuse skin cells taken from a male gaur with 692 enucleated cow eggs. As we report in the current issue of the journal *Cloning*, of those 692 cloned early embryos, only 81 grew in the laboratory into blastocysts, balls of 100 or so cells that are sufficiently developed to implant for gestation. We ended up inserting 42 blastocysts into 32 cows, but only eight became pregnant. We removed the fetuses from two of the pregnant cows for scientific analysis; four other animals experienced spontaneous abortions in the second or third month of the usual nine-month pregnancy; and the seventh cow had a very unexpected late-term spontaneous abortion in August.

The statistics of the efficiency of cloning reflect the fact that the technology is

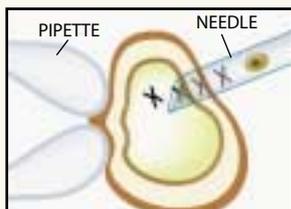
we are now working to understand. They are also a function of the vagaries of assisted reproduction technology.

Accordingly, we expect that the first few endangered species to be cloned will be those whose reproduction has already been well studied. Several zoos and conservation societies—including the Audubon Institute Center for Research of Endangered Species (AICRES) in New Orleans, which is led by one of us (Dresser)—have probed the reproductive biology of a range of endangered species, with some notable successes. Last November, for example, Dresser and her colleagues reported the first transplantation of a previously frozen embryo of an endangered animal into another species that resulted in a live birth. In this case, an ordinary house cat gave birth to an African wildcat, a

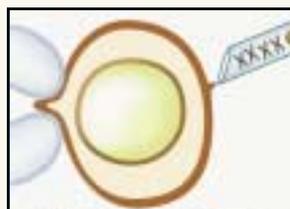
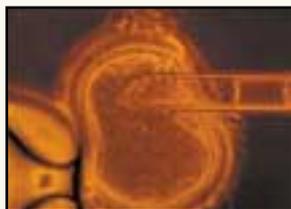
THE NUCLEAR TRANSFER (CLONING) PROCESS



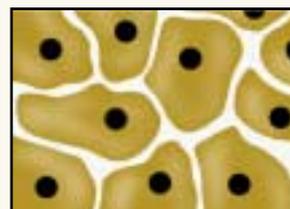
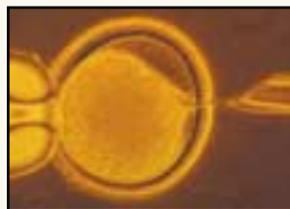
Recipient eggs are coaxied to mature in a culture dish. Each has a remnant egg cell called the polar body.



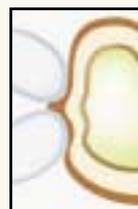
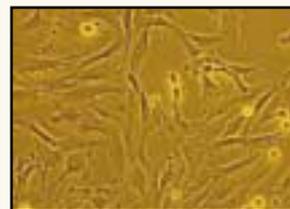
The polar bodies and chromosomes of each egg are drawn into a needle. A pipette holds the egg still.



Once the chromosomes and polar body are removed, all that remains inside the zona pellucida is cytoplasm.



Skin cells called fibroblasts are isolated from the animal to be cloned and grown in culture dishes.



An entire skin cell is pushed up into the zona pellucida again using a pipette.



only a sac of gel called cytoplasm. Next he uses a second needle to inject another whole cell under the egg's outer layer. With the flip of an electric switch, the cloning is complete: the electrical pulse fuses the introduced cell to the egg, and the early embryo begins to divide. In a few days, it will become a mass of cells large enough to implant into the uterus of a surrogate-mother animal previously treated with hormones. In a matter of months, that surrogate mother will give birth to a clone.

In practice, though, this technique—which scientists call nuclear transfer—is not so easy. To create Noah, we at

still as much an art as it is a science—particularly when it involves transplanting an embryo into another species. Scientists, including those of us at ACT, have had the highest success rates cloning domestic cattle implanted into cows of the same species. But even in this instance we have had to work hard to produce just a few animals. For every 100 cow eggs we fuse with adult cattle cells, we can expect only between 15 and 20 to produce blastocysts. And only roughly 10 percent of those—one or two—yield live births.

The numbers reflect difficulties with the nuclear transfer process itself, which

species that has declined in some areas.

So far, beyond the African wildcat and the gaur, we and others have accomplished interspecies embryo transfers in four additional cases: an Indian desert cat into a domestic cat; a bongo antelope into a more common African antelope called an eland; a mouflon sheep into a domestic sheep; and a rare red deer into a common white-tailed deer. All yielded live births. We hope that the studies of felines will pave the way for cloning the cheetah, of which only roughly 12,000 remain in southern Africa. The prolonged courtship behavior of cheetahs requires substantial

territory, a possible explanation for why the animals have bred so poorly in zoos and yet another reason to fear their extinction as their habitat shrinks.

Panda-monium

One of the most exciting candidates for endangered-species cloning—the giant panda—has not yet been the subject of interspecies transfer experiments, but it has benefited from assisted reproduction technology. Following the well-publicized erotic fumbblings of the National Zoo's ill-fated panda pair, the late Ling-Ling and Hsing-Hsing, the San Diego Zoo turned to artificial insemination to make proud parents of its Bai Yun and Shi Shi. Baby Hua Mei was born in August 1999.

Giant pandas are such emblems of en-

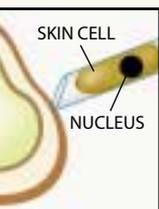
gust 1999 Dayuan Chen of the institute and his co-workers published a paper in the English-language journal *Science in China* announcing that they had fused panda skeletal muscle, uterus and mammary gland cells with the eggs of a rabbit and then coaxed the cloned cells to develop into blastocysts in the laboratory.

A rabbit, of course, is too small to serve as a surrogate mother for a giant panda. Instead ACT and the Chinese plan to turn to American black bears. As this issue of *Scientific American* goes to press, ACT is finalizing plans to obtain eggs from female black bears killed during this autumn's hunting season in the northeastern U.S. Together with the Chinese, ACT scientists hope to use these eggs and frozen cells from the late Hsing-Hsing or Ling-Ling to generate cloned giant panda embryos that can be

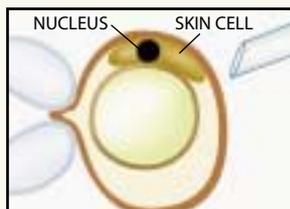
with AICRES and Louisiana State University) and their colleagues announced the birth of a bongo after moving very early embryos from a pregnant female bongo to an eland surrogate mother.

Most of the mountain subspecies of bongo—a medium-size antelope with vertical white stripes—live in captivity. According to the World Conservation Union—IUCN, the mountain bongo is endangered, with only 50 or so remaining in a small region of Kenya. In contrast, the 1999 Bongo International Studbook lists nearly 550 mountain bongo living in zoos throughout the world. The lowland bongo subspecies is slightly better off: it is listed as “near threatened” and has a population of perhaps several thousand scattered throughout central and western Africa.

A coalition of conservation organiza-



skin cell is taken with a needle, which is inserted through the zona pellucida.



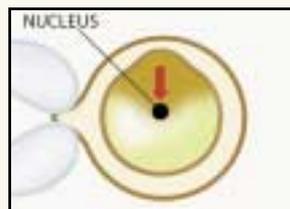
The skin cell is injected underneath the zona pellucida, where it remains separate from the egg cytoplasm.



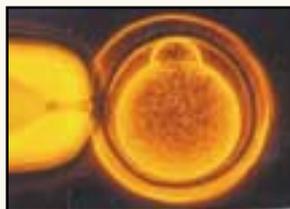
The process is repeated many times; it can require hundreds of nuclear transfers to obtain one animal.



Each injected egg is exposed to an electric shock that fuses the skin cell with the egg cytoplasm.



The skin cell's nucleus, with its genes, enters the egg cytoplasm. Within a few hours, the fused cell begins to divide.



dangered species that the World Wildlife Fund (WWF) uses one in its logo. According to a census that is now almost 20 years old, fewer than 1,000 pandas remain in their mountainous habitats of bamboo forest in southwest China. But some biologists think that the population might have rebounded a bit in some areas. The WWF expects to complete a census of China's pandas in mid-2002 to produce a better estimate.

In the meantime, we at ACT are discussing plans with the government of China to clone a giant panda. Chinese scientists have already made strides toward the goal of panda cloning. In Au-

gust 1999 Dayuan Chen of the institute and his co-workers published a paper in the English-language journal *Science in China* announcing that they had fused panda skeletal muscle, uterus and mammary gland cells with the eggs of a rabbit and then coaxed the cloned cells to develop into blastocysts in the laboratory.

AICRES scientists hope to take advantage of the success with bongo antelope that one of us (Dresser) had while at the Cincinnati Zoo. In 1984 Dresser and Charles Earle Pope of the University of Alabama at Birmingham (now

tions in the U.S. and Kenya is now planning to send mountain bongo that have been bred in captivity to two sites in Kenya. And in a new approach to reintroducing a species, AICRES is working in Kenya to transfer frozen bongo embryos into eland surrogates. Cloning could support these efforts and possibly yield more bongo for reintroduction.

But what about animals that are already extinct? Chances are slim to nil that scientists will soon be able to clone dinosaurs, à la *Jurassic Park*, or woolly mammoths. The primary problem is the dearth of preserved tissue—and hence DNA. A group of researchers unearthed

WHAT ABOUT ROVER AND FLUFFY?

The list of domesticated animals that scientists have been able to clone so far includes sheep, cattle, goats and laboratory mice—and now, we expect, the gaur. Compared with that menagerie, you'd think that cloning an ordinary dog or cat would be a snap. Unfortunately, this has not been the case. Both of our research groups have created cloned cat embryos and have implanted them into female cats, but as this article goes to press, neither of our teams has yet obtained a full-term pregnancy. Dogs have presented even more problems.

But we anticipate success soon. At Advanced Cell Technology (ACT), we have undertaken a research program that uses cloning technology to propagate pets as well as service animals such as seeing-eye dogs for the blind, hearing dogs for the deaf, search-and-rescue dogs, and animals used for social therapy. Together with Louisiana State University, the Audubon Institute has teamed up with a company called Lazaron BioTechnologies in Baton Rouge, La., to clone pet dogs and cats.

A surprising number of people are interested in cloning their favorite deceased pet in the hope of getting an animal with similar behavioral characteristics. A good deal of a cat or dog's demeanor is thought to be genetically determined. Although one can argue that there are already plenty of cats and dogs in the world that need homes, people still use traditional breeding methods to try to reproduce a particularly desirable animal. Cloning could offer a more efficient alternative. It could be particularly important in the case of service animals. Currently, for instance, male seeing-eye dogs are neutered at an early age so that they can concentrate better during their expensive and rigorous training. So, unfortunately, even if a dog turns out to be very good at his job, he can't be bred to produce more like him.

Our efforts to clone pets could also pay off for endangered

species. We expect to be able to apply the information we obtain from cloning cats and dogs to preserving endangered felines and canines.

ACT and several other companies now offer pet cloning kits that veterinarians can use to preserve samples from a client's pet for possible future cloning. The kits contain materials for collecting a skin specimen and sending it back to a laboratory. Research assistants there use the tissue to establish a collection of pure, dividing cells called a cell line, which will be the source of donor cells for cloning.

ACT extracts eggs for the cloning procedure from reproductive tracts taken from animals that have been spayed by veterinarians. We remove the ovaries and carefully puncture all visible follicles to release the eggs. Then we collect the eggs and place them in a specialized maturation medium that contains hormones, proteins and nutrients. Once fully matured, the eggs are ready for the nuclear transfer procedure [see illustration on pages 86 and 87].

So far our main focus has been the domestic cat, primarily because its reproductive physiology has been well studied, and embryo transfers of early- and late-stage embryos have resulted in the birth of live kittens. Both ACT and the Audubon Institute have been able to establish systems for prompting cat eggs to mature in the lab and have consistently produced cloned embryos that are being transferred to recipients.

But dogs are a different story. The dog's reproductive physiology is unique among mammalian species. Dogs ovulate an immature egg that has a very long maturation time. This means that we need a different maturation system from the one we have used in cats and that we have fewer eggs to work with in the end. So Fluffy will probably have a leg up on Rover when it comes to cloning.

—R.P.L., B.L.D. and P.D.



SERVICE ANIMALS and pets might soon be cloned. In a new film, *The 6th Day*, grieving pet owners go to a company called RePet to copy their animals.

what they had hoped would be a well-preserved mammoth last year, but repeated freezing and thawing over the eons had poked holes in the creature's DNA, and molecular biologists have not yet found a feasible way of filling in such genetic gaps.

A similar difficulty has hobbled efforts by Australian scientists to clone a thylacine, or Tasmanian tiger, a wolflike marsupial that died out in the 1930s. Researchers at the Australian Museum in Sydney are attempting to clone cells from a thylacine pup that was preserved in alcohol in 1866, but the DNA is in such poor condition that they say they will have to reconstruct all of the animal's chromosomes.

The recently extinct bucardo may prove a more promising target for resur-

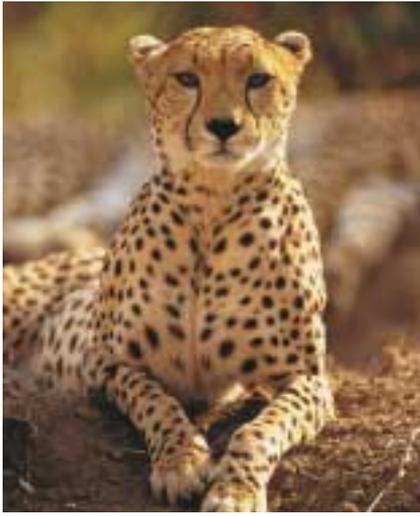
rection. ACT is arranging a collaboration with Alberto Fernández-Arias and José Folch of the Agricultural Research Service in Zaragoza, Spain. Fernández-Arias froze tissue from the last bucardo. He and Folch had tried for several years to preserve the mountain goat, which in the end was wiped out by poaching, habitat destruction and landslides. Last year they transferred embryos from a subspecies related to the bucardo to a domestic goat, yielding live kids.

But even if interspecies nuclear transfer succeeds for the bucardo, it will yield only a sorority of clones, because we have tissue from just one animal, a female. ACT plans to try to make a male by removing one copy of the X chromosome from one of the female bucardo's cells and using a tiny artificial cell called a

microsome to add a Y chromosome from a closely related goat species. The technology has been used by other researchers to manipulate human chromosomes, but it has never before been used for cloning. A nonprofit organization called the Soma Foundation has been established to help fund such efforts.

Why Clone?

Cloning endangered species is controversial, but we assert that it has an important place in plans to manage species that are in danger of extinction. Some researchers have argued against it, maintaining that it would restrict an already dwindling amount of genetic diversity for those species. Not so. We advocate the establishment of a worldwide



Left to right, clockwise: GERRY ELLIS/Minden Pictures (cheetah); CHARLES V. ANGELO/Photo Researchers, Inc. (bongo); FRANS LANTING/Minden Pictures (giant panda); JOHN CANCALOSI/Peter Arnold, Inc. (bucardo); ROLAND SEITRE/Peter Arnold, Inc. (gaur); MARTIN WENDLER/Peter Arnold, Inc. (ocelot)

network of repositories to hold frozen tissue from all the individuals of an endangered species from which it is possible to collect samples. Those cells—like the sperm and eggs now being collected in “frozen zoos” by a variety of zoological parks—could serve as a genetic trust for reconstituting entire populations of a given species. Such an enterprise would be relatively inexpensive: a typical three-foot freezer can hold more than 2,000 samples and uses just a few dollars of electricity per year. Currently only AICRES and the San Diego Zoo’s Center for Reproduction of Endangered Species maintain banks of frozen body

cells that could be used for cloning. Other critics claim that the practice could overshadow efforts to preserve habitat. We counter that while habitat preservation is the keystone of species conservation, some countries are too poor or too unstable to support sustainable conservation efforts. What is more, the continued growth of the human species will probably make it impossible to save enough habitat for some other species. Cloning by interspecies nuclear transfer offers the possibility of keeping the genetic stock of those species on hand without maintaining populations in captivity, which is a particularly cost-

CLONING CANDIDATES include (clockwise from upper left) the cheetah, bongo, giant panda, bucardo, gaur and ocelot.

ly enterprise in the case of large animals. Another argument against cloning endangered species is that it might siphon donor money away from habitat maintenance. But not all potential donors are willing to support efforts to stem the tide of habitat destruction. We should recognize that some who would otherwise not donate to preserve endangered species at all might want to support cloning or other assisted reproduction technologies. The time to act is now. SA

The Authors

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Further Information

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