The Case of the Cloned Cats

Genetic Savings and Clone, Inc., is a private company that describes itself as "the world's leader in the cloning of exceptional pets." Genetic Savings and Clone (GSC) funded research at Texas A&M University that led to the 2002 birth of the world's first cloned cat, a healthy kitten named CC, as in "CopyCat" or "Carbon Copy". (At left is a picture of CC when she was a kitten.) GSC is now offering cat cloning services to the public on a limited basis. In May 2004, GSC plans to begin cloning nine cats, with the resulting kittens delivered to their clients by November 2004. (Three of the cats to be cloned belong to GSC employees.)

Some people think this is a great service to offer to people who love their pets. Others object to the commercial availability of cat cloning because of potential problems for the cloned animals and other considerations.

This case study will help you understand the science of cloning, the reasons why some people think this is a valuable and ethically defensible service, and the social and ethical considerations that have led some people to oppose the commercial availability of pet cloning services.

CC at 7 weeks old with scientists Mark Westhusin and Tae Young of the College of Veterinary Medicine at Texas A&M University.

Worksheet for small groups

Keep track of any further information you would need to answer these questions. How would you discover that information?

1. What do I need to do in order to get my cat cloned? How much does it cost? Why is it so expensive?

2. Why do some people want to clone their cats? Are some reasons for cloning cats better than others? Why or why not?

3. How does cloning work? Why doesn't CC look like her genetic donor, Rainbow?

4. Can cloning cats help people or animals in any way? If so, how? Are benefits expected from the commercial cloning of pets?

5. Would commercial cloning of pets harm people or animals in any way? If so, how?

6. Why do some people think that commercial cat cloning is unethical? What do you think of their arguments?

7. What responses can you think of to claims that cat cloning is unethical? Are these responses convincing? Why or why not?

8. In your own opinion, is it ethical to clone cats? Why or why not?

The First Nine Lives Extravaganza

World's First Cat Clones Now Available

Genetic Savings & Clone is pleased to announce the start of our cat cloning service – the first time pet cloning has been offered to the public. If you're interested in being one of the world's first cat fanciers to clone your feline, we invite you to contact us at your earliest convenience before our 2004 cat cloning capacity is filled.

Offer Details
GSC funded "Operation CopyCat" which produced "CC," the world's first cat clone in 2001. Today, CC is a healthy & adorable two-year-old cat. Our current technology is far more advanced than that used to produce CC, and we're confident that the clones we produce for our clients will be consistently healthy and bear striking resemblance to their genetic donors.

Despite our many refinements, feline cloning is still complex, time-consuming and costly. The cloning, pregnancy and weaning processes take approximately 6 months from start to finish – the final stage being delivery of the clones to their new families. This May, we'll begin cloning 9 cats and expect to deliver the clones by November 2004.

This year our cloning capacity will be very limited, with an estimated maximum of nine 9 total felines. This total includes 3 cats owned by the cat-loving staff at GSC, and 6 cats owned by our clients. Next year, we expect to increase our feline cloning capacity, and launch our canine cloning service.

Special Features & Benefits
Cat fanciers who take advantage of "The First Nine Lives Experience" will receive numerous benefits including:

- **The GSC Guarantee** – Each clone will strongly resemble the genetic donor and be completely healthy. If you are unsatisfied with your clone(s), you'll receive a full refund.
- **Anonymity or Publicity** - You may elect to remain completely anonymous or be featured in the publicity generated by this offer.
- **GSC Extravaganza** – You and a guest will receive, an all-expense-paid trip to attend a feline clone presentation party at our headquarters in Sausalito, California on the San Francisco Bay. At the party, our CEO Lou Hawthorne and Chief Scientist Dr. Irina Polejaeva will personally present your clone to you. You'll also have the privilege of witnessing the presentation of clones to the other participants in "The First Nine Lives Extravaganza." This party should prove to be quite an interesting & exotic event! After the party, you and the other clients will attend a special dinner with the GSC staff.
- **Cloning Video** – GSC will produce and provide you with a video of the cloning process, birth of your clone, presentation party and dinner as a personal keepsake and remembrance. Your personal video will be provided in both VHS and DVD formats.

Contact Us
"The First Nine Lives Extravaganza" is a unique, once-in-a-lifetime opportunity! We have already received great interest and worldwide publicity. To participate in this groundbreaking event, please contact us for further details and pricing information.

Availability & Pricing

8 Clones Reserved - Only 1 Available

Genetic Savings & Clone's feline cloning services will be available on February 1, 2004 through The First Nine Lives Extravaganza promotion. Availability will be very limited in 2004 - we'll only clone 9 cats in the entire year. 3 of these cats will be owned by GSC staff and the remaining 6 will be offered to the public.

Pricing
The all-inclusive price for each feline cloned is $50,000.00 USD. To increase efficiency, we'll likely clone & transfer multiple embryos of each cat to a surrogate, which could potentially result in the birth of more than one clone. There will be no additional charge for multiple clones that are born and delivered to clients participating in this offer.

Participation in The First Nine Lives Extravaganza is on a first-come, first-serve basis. To be included, we must receive a completed Cloning Services Agreement along with payment for 50% of the purchase price before we reach our production capacity. The balance of the purchase price must be paid at the time your clone is delivered to you.

Future Availability
In 2005, our feline cloning capacity will increase and we'll also begin to offer dog cloning services. At this time, we are unable to accurately forecast our future cloning capacity for next year - it will likely continue to be fairly small.

After our cloning capacity increases to a point where economies of scale and efficiency are realized, the price of our services will decrease. At which point, our pet cloning services will be sold primarily through partnerships with certified veterinary resellers.

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OUR CLIENTS

Our Clients & Their Cats

"We had Smokey neutered at a young age. We wanted some of his offspring, so when we heard about cloning we thought it was just the perfect thing for us to do."

—*Mary Ann Daniel*

Mary Ann Daniel
Costa Mesa, California

"In November 1984, Smokey appeared on my kitchen ledge. We fell in love with him immediately, and took him in. Now he's almost 19. What makes him special are eye contact, communication, and interpersonal skills that we have not found very often in cats, and we've had many of them over the years.

Roland, Mary Ann, and Smokey

We had Smokey neutered at a young age. We wanted some of his offspring, so when we heard about cloning we thought it was just the perfect thing for us to do. We want to have a cat that has some of his characteristics. Not necessarily all of them, but something.

Our vet gave Smokey a local anesthetic and took a small piece of tissue from the inside of his mouth. It didn't hurt him. I picked him up in a couple of hours from the vet, and everything was fine. Then the sample was sent in to GSC.

We've spread the word around. The people that we've spoken about this to are animal lovers and they see it as an opportunity to at least have some of the characteristics of their cat perpetuated. We're proud of it.

Over the course of our lives we have adopted many animals from animal shelters, and they've all been wonderful. The fact is, Smokey is one in a million.

He accompanies us when we go to our neighbors and waits outside for us. He walks us home. All these things show that there's a bond there beyond the normal cat relationships that we've had with our other cats."

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LAZARON BioTechnologies LLC — Applied Genetic Engineering for Animal Health

BENGAL

Richard Parker – Mentor, Ohio

Bengal was a beloved pet and was unique among cats; His personality was more like that of a dog. My friends were sad to learn that cancer got the better of him and I miss him every day. I am excited about the prospect that a little piece of him still lives on and could one day become a new living being. I find it fascinating that science has progressed to where this could be possible. I would be very interested to see if the cloned kitten would follow the same habits and behaviors that Bengal exhibited. He lived fourteen and a half years and they were great fun for me.

Contact Lazaron: (888) 882-8918 or (225) 334-6988

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LAZARON BioTechnologies LLC — Applied Genetic Engineering for Animal Health

MAX

Pamela Joseph & Robert Brinker – Aspen, Colorado

Max was a human spirit, an old soul, one cool cat, and our little man – a special being. He was in the studio with us every day and slept on the pillow between us, with his head against ours and his paws on our shoulders. He was highly vocal and would talk all the time. He was the star of our life. His best friend was Nell, our Rotweiller, and they would often play and rest together. Max was a great hunter and fierce warrior. We felt in attempting to clone Max, we were performing a positive gesture that would honor his memory. We realize that Max's special spirit may not return, but we hope that "Maxson", his clone, will embody some characteristics of this wonderful, one-of-a-kind cat.

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LAZARON BioTechnologies LLC — Applied Genetic Engineering for Animal Health

JAZZ

A rare African Wildcat believed to be the ancestor of the domestic cat
Audubon Institute - New Orleans, Louisiana

Jazz is a very special African wildcat. Not only was his birth a result of cross species embryo transfer, he is now the donor parent of the world's first cloned African wildcat, Ditto.

"We are very excited about the possibility of using the cloning technology to propagate Jazz and other endangered species", said Dr. Betsy Dresser, Audubon Institute's Senior Vice-President for Research. Jazz is an African wildcat (Felis silvestris libyca) that is found in sub-Saharan Africa. It is generally recognized as the ancestor of the domestic cat (Felis catus). Jazz is currently living at the Audubon Institute in New Orleans, Louisiana. Jazz is also special because he was born as the result of an inter-species embryo transfer. A frozen-thawed embryo of an African wildcat was transferred to a domestic cat. The domestic cat subsequently gave birth to the wildcat, a male who weighed 128 grams at birth. This is the first time such a procedure utilizing a frozen-thawed embryo has been successful. The birth of this wildcat kitten is the latest in a series of projects pioneered by Audubon Institute's Senior Vice-President for Research Dr. Betsy Dresser and Senior Scientist Dr. C. Earle Pope, world-renowned leaders in the field of assisted reproduction technology for endangered species. To learn more about Jazz, visit the Audubon Institute Center for Reproduction of Endangered Species site.

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Cloning Process

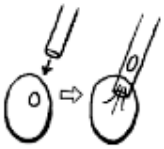
Pet cloning is very complex and involves many inter-connected processes that are species-specific; more processes are required to clone a dog than a cat. Because we have more scientific processes to perfect to clone dogs, dog cloning will be available after cat cloning. Many of the processes we used to clone CC, the world's first cloned cat included:

1. The cloning process begins with gene banking. A veterinarian takes a small tissue biopsy from the cat to be cloned, also know as the genetic donor.

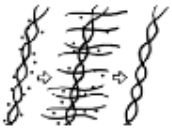
2. The tissue biopsy is transported in a refrigerated container called a BioBox to our gene bank...

3. ...where it's then cultured (grown). The cells are then cryopreserved in liquid nitrogen, where they can be stored indefinately.

4. A cat egg (oocyte) is enucleated, which means the genetic material is removed. The egg is then ready to receive the genes of the genetic donor.



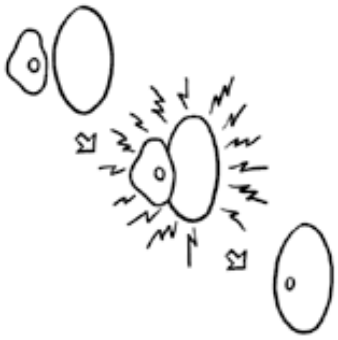
5. Prior to transfer to the egg, the donor cell is pre-treated with proteins that remove factors associated with differentiation. Differentiation is the process by which cells get "assigned" to develop into cells that have specialized functions, such as muscle cells, neurons, skin cells etc. By dedifferentiating the donor cell, we can increase the likelihood that it will lead to a healthy clone.



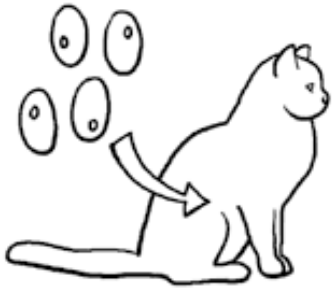
CC - World's First Cloned Cat

CC's was the result of research funded by GSC at Texas A&M University

6. The treated donor cell is combined with the enucleated egg by electrofusion, resulting in a single-celled cloned embryo, ready for transfer into a surrogate mother.



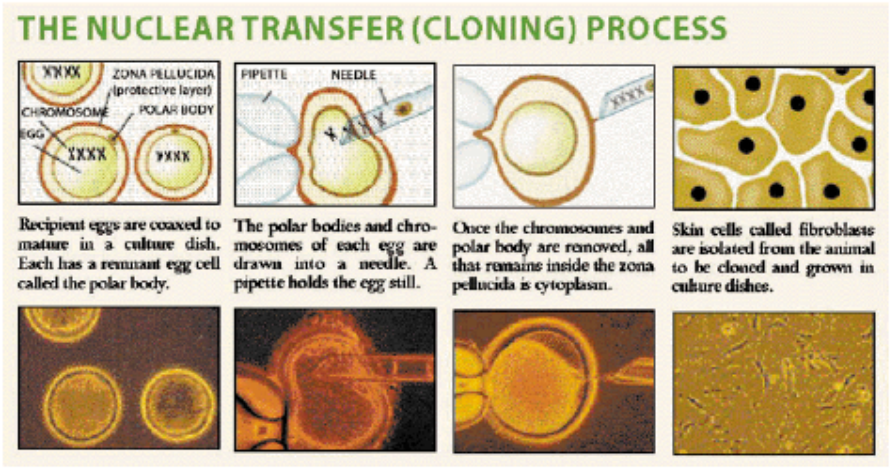
7. To become a surrogate mother, a cat must be in heat, or estrus. When a cat goes into estrus, we insert cloned embryos into her oviduct, resulting in pregnancy.

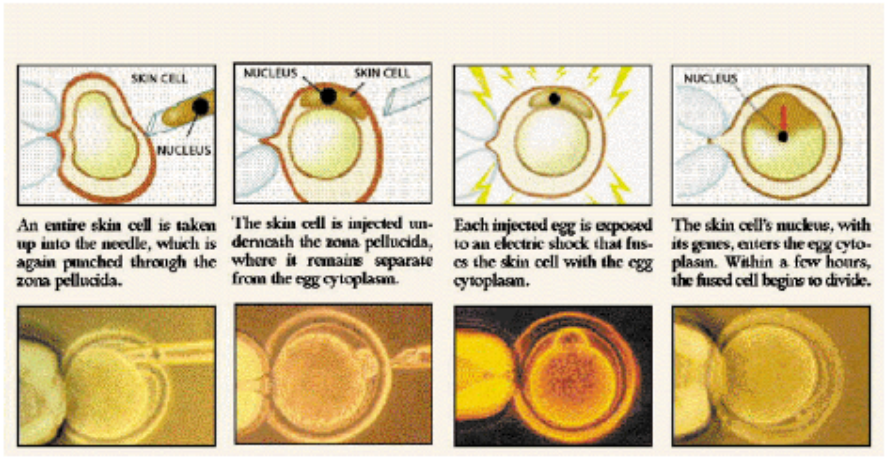


8. The surrogate carries the pregnancy to term and gives birth to kittens who are clones of the original genetic donor.

November 17, 2000

The Nuclear Transferring (Cloning) Process





Back to Article (Cloning Noah's Ark)

ABOUT CLONING

X-linked Inactivation

CC's genetic donor, Rainbow, is a calico domestic shorthair, while CC is a tiger-tabby domestic shorthair. What gives? Shouldn't CC be a calico too? The answer to this question sheds light on a fascinating and less-than-fully-understood issue called "X-linked inactivation."

First of all, calicos are almost always female, which means they have two X-chromosomes (versus the male's XY). One of these X chromosomes contains a gene for orange coat color and the other contains a gene for black coat color (white patches are specified by a different set of genes which are not relevant here).

For reasons which are not fully understood, as the embryo develops, a phenomenon called "X-linked inactivation" occurs, in which one or the other X-chromosome in every cell in the Calico embryo becomes randomly inactivated. If the specific X-chromosome containing the gene for orange coat color becomes inactivated, that cell will go on to produce black coat color (assuming it becomes a coat follicle cell). The inverse is true if the X-chromosome containing the gene for black coat color becomes inactivated.

Given that the inactivation is random, one would expect a very fine distribution of orange and black hairs within the coat, but for reasons which are not germane here, the inactivation occurs in larger patches of orange and black.

"Mosaicism" is the term for distribution of different cell types within a single organism. Mosaicism is three-dimensional, meaning that the inactivation of orange or black-producing genes occurs within cells throughout the calico's body regardless of whether the cells have anything to do with production of the animal's coat. Thus, even the specific cumulus cell used to clone CC would have been inactivated for either orange or black coat color.

If the nuclear transfer process were to reset the inactivated X-chromosome the way it resets the nuclear differentiation, then one might expect to see a calico clone with a calico coat. On the other hand, if nuclear transfer does not reset X-activation then one would expect to see a clone with a black coat if the donor cell used had an orange coat gene on the inactivated X-chromosome, and conversely one would expect a clone with an orange coat if the donor cell used had an black coat gene on the inactivated X-chromosome.

The fact that CC has no orange in her coat is consistent both with the theory that nuclear transfer does not reset X-activation, and also that the cumulus cell used had an orange coat gene on the inactivated X-chromosome.

ABOUT CLONING

Physical & Behavioral Resemblance

"In general, cats seem to have genetically determined personality types. It doesn't seem to make a whole lot of difference what the environment is. Shyness, being hostile, being friendly, being bold — they seem to be inherited."
--Dr. Katherine Houpt, director of the Animal Behavior Clinic Cornell University College of Veterinary Medicine

Cloning: What's Possible?

Most clones strongly resemble their genetic donors, like identical twins. A pet born through cloning will have the same genes, a similar appearance, and the same predisposition to health, intelligence, agility, and temperament as the genetic donor. For the lucky person who owns an exceptional pet, cloning is as close as you can get to "lightning striking twice."

But cloning does not bring a dead animal back to life, nor does it produce an animal that has the memories of another animal. Such feats — exciting to some people, horrifying to others — are the stuff of science fiction.

On this page, we discuss the similarities and differences between clones and their genetic donors.

Physical Resemblance

There is no question that clones bear a close physical resemblance to their genetic donors, as demonstrated by the hundreds of clones produced to date in various species. One notable exception:

CC, the world's first cat clone, has coat colorings that differ from those of Rainbow, her genetic donor. CC left some observers wondering whether cat clones in particular would always look so different from their donors. But CC, a calico, represents an unusual case. Due to an effect known as X-linked inactivation, described here, the clones of calico cats will always look different from their donors.

However, in any other breed of cat, and all breeds of dog, a clone should look like the identical twin of its genetic donor.

Behavioral Resemblance

The question of behavioral resemblance, popularly termed "personality," between clones and their donors is much more hotly contested than physical resemblance.

There are numerous theories of animal behavior. The most widely acknowledged view is that animal behavior is determined by a mix of genetic and environmental factors. Two behavioral traits with strong genetic bases are intelligence and temperament, as is clearly demonstrated by differences in these traits between dog breeds such as Border Collie, Golden Retriever, and Pit Bull. Although environmental factors can shape the behavior of individuals to a certain degree, anyone who has worked with these breeds knows that their behavioral responses, including herding in Collies, retrieving in Retrievers, and aggression in Pit Bulls, are hardwired to a large degree. It is not illogical to assume that specific behaviors in

Cloning *is* and *isn't* resurrection

Cloning is not the resurrection of the individual animal being cloned, whose consciousness and memories will die with the individual. Cloning cannot raise an animal Lazarus-like from the dead, nor can cloning perpetuate an individual as though immortal.

And yet, cloning IS a form of resurrection on a genetic level: the individual dies, yet a new individual is born possessing the exact genome of the deceased individual. In this sense, cloning does resurrect a specific genome, though not a specific consciousness.

It's important for anyone considering cloning a pet to understand what is and is not possible through cloning. For more information, see our FAQ section.

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SOCIAL BENEFITS

Animal Health Research

Canine physiology in general and canine reproductive physiology in particular are black holes in science. Biological research on other species has been heavily underwritten by corporations with vested interests in the efficient reproduction of those species. For instance, agribusiness underwrites research on livestock reproduction, and the human fertility industry underwrites research on human reproductive therapies.

Unfortunately, until GSC came on the scene, grants to study any aspect of canine physiology were few and far between (cats are better understood because they've been used extensively as lab animals). In fact, the original $2.3M Missyplicity grant was the largest that any of the Texas A&M scientists working on the project had even heard of in the field of canine physiology.

One of the prime benefits of GSC's dog cloning research is that it will increase understanding of the dog. These wonderful animals are ostensibly our "best friends," yet we know less about their physiology, especially their reproductive physiology, than just about any other common mammal. Ultimately, our research is likely to contribute to cures for common canine diseases, regardless of whether the diseases are current research targets of GSC.

Also, the process of developing canine and feline cloning technology generates information useful in the development of effective pharmacological contraceptive and sterilization methods to reduce cat and dog overpopulation. We currently underwrite a major pet contraceptive/sterilization research effort at the University of Virginia, and in the future will be supporting their effort with our own research data in addition to our funds.

Finally, there are enough similarities between mammals that fresh, innovative research into one species tends to advance understanding of other species as well. Although scientific progress resulting from canine cloning research will primarily benefit the dog, it's also true that by improving our understanding of mammalian ova maturation, nuclear reprogramming, estrus cycling, and other key aspects of canine cloning we may also someday improve human medical knowledge.

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SOCIAL BENEFITS

Endangered Species

Every day, approximately 100 living species disappear due to hunting, poaching, competition between humans and other animals for scarce resources, and, most of all, loss of habitat. With the advent of cloning, wildlife conservationists have a new tool in their efforts to protect endangered species from extinction. Cloning is the new last line of defense for these animals; after habitat preservation, poaching control, and captive breeding.

Although those other methods are more efficient than cloning, they don't always succeed (for more information about this, see "What impact will cloning have on genetic diversity?" in our Ethical FAQs). In those instances, cloning may be the only way to prevent the loss of a species forever.

The canine and feline cloning research being done at Genetic Savings & Clone may improve the technologies available for the cloning of endangered species. We are developing non-profit partnerships to provide technical know-how on canine reproduction (especially *in vitro* fertilization, IVF, and cloning) to organizations that work to repopulate endangered canids, including varieties of wolves, foxes, and wild dogs.

The first threatened animal to be cloned was the cattle-like Asian gaur (*Bos frontalis*). Advanced Cell Technology (ACT), a Massachusetts-based company, used its experience in cattle cloning to produce a gaur clone using a cow as a surrogate mother (when cloning a threatened species, researchers perform an "interspecies embryo transfer," which means the cloned embryo is transferred to a surrogate mother of a different, though related, species). Noah, the gaur, was born in January 2001, and died of dysentery two days later.

For more information about ACT, visit their web site.

The Center for the Reproduction of Endangered Species (CRES) at the San Diego Zoo has been developing a "Frozen Zoo" of viable cell samples since 1976 that now includes more than 350 species and sub-species. In April 2003, cells stored at this Frozen Zoo were used to produce a pair of cloned Javan bantengs (*Bos javanicus*, a rare cattle-like species). Standard beef cows served as the surrogate mothers. ACT performed the nuclear transfer procedure and Iowa-based Trans Ova Genetics performed the embryo transfer.

Find out more about CRES at their web site.

The Audubon Center for Research of Endangered Species in New Orleans also maintains a gene bank of endangered species (less than a dozen such collections exist worldwide). The Center's research is showing how cryopreserved biological materials can be transported around the world for use in the reproduction of endangered species. In November 1999, the Center produced the world's first interspecies transfer using an embryo that had been frozen and thawed. "Cayenne," a domestic house cat, gave birth to "Jazz," an African wildcat (Felis silvestris).

For more information about the Center, visit their web site.

The Thai government has funded research at the Suranaree University of Technology for the cloning of two vulnerable species, the marbled cat (*Pardofelis marmorata*) and the serow (*Capricornis sumatraensis*). Cells for this research come from members of those species housed in state zoos. The project will run for five years and, if successful, will be extended to other species.

These various research projects demonstrate that the use of cloning in wildlife preservation is becoming a reality. Furthermore, various zoos are engaged in research in embryo freezing, *in vitro* fertilization, and other technologies with cloning-related applications.

Meanwhile, GSC has begun to subsidize the gene banking of endangered relatives of the cat and dog. We gene bank one male and one female member of these species at no charge, and we gene bank additional members at reduced rates. Please contact us for more information.

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SOCIAL BENEFITS

Working Dogs

Genetically exceptional, highly trained dogs perform valuable services for humans, such as assisting people with disabilities, rescuing disaster victims, and detecting arson and explosives. Only certain individual dogs within specific breeds have the right mix of sensitivity, intelligence, temperament, and other qualities to successfully perform these jobs. Furthermore, less than half the dogs entering assistance or other working dog training programs end up being placed with clients; the others are rejected for health and/or behavioral reasons.

Once a suitable dog is placed, it may be able to provide assistance for eight or nine years. Or it may become ill, lame or die long before that, in some cases due to genetic disorders including progressive retinal atrophy, hip dysplasia, thyroid problems, etc. When an assistance dog becomes unable to perform due to early illness or death, a client faces the burden of coping with that loss as well as coping with training and acclimating to a new dog. By providing clients with clones of the healthiest, best-performing assistance and other working dogs, we will reduce the frequency and duration of the transitional periods.

Genetic Savings & Clone is collaborating with several programs engaged in the training and/or breeding of working dogs, and is underwriting on a case-by-case basis the gene banking of the most exceptional individuals. We have completed gene banking several such dogs, and are looking forward to cloning them in the future, and testing their performance relative to their genetic donors.

If you have one or more world-class working dogs, please contact us to discuss gene banking and possible cloning. We use a sliding scale for determining cost, from zero to full price, based both on your resources as well as a qualitative assessment of the dog.

"Since many working dog programs drop over 50% of their dogs due to poor temperament, health problems or lack of working aptitude, why not store the DNA of exceptional workers? This will no doubt lead to a higher success rate, because the clones' DNA will have 'the right stuff.'"

—Glenn Martyn, Executive Director PawsAbilities - Canine Partners for People with Disabilities

November 19, 2000

Cloning Noah's Ark

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

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, mooing 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.

WHAT ABOUT ROVER?

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 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 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 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 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 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 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.

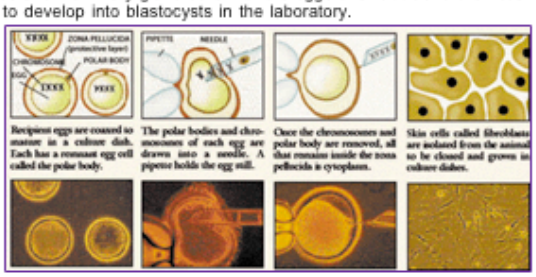
Panda-monium

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 fumblings 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 endangered 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 August 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.



THE FIRST STEPS OF A NUCLEAR TRANSFER PROCESS

CLICK HERE FOR ALL 8 STEPS

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 implanted into a female black bear now living in a zoo. A research group that includes veterinarians at Bear Country U.S.A. in Rapid City, S.D., has already demonstrated that black bears can give birth to transplanted embryos. They reported the successful birth of a black bear cub from an embryo transferred from one pregnant black bear to another last year in the journal Theriogenology.

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 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 organizations 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 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 resurrection. 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 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 costly 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.

Further Information:

PRESERVATION OF ENDANGERED SPECIES AND POPULATIONS: A ROLE FOR GENOME BANKING, SOMATIC CELL CLONING, AND ANDROGENESIS? Graham E. Corley-Smith and Bruce P. Brandhorst in *Molecular Reproduction and Development*, Vol. 53, No. 3, pages 363–367; July 1999.

BIODIVERSITY HOTSPOTS FOR CONSERVATION PRIORITIES. Norman Myers, Russell A. Mittermeier, Cristina G. Mittermeier, Gustavo A. B. da Fonseca and Jennifer Kent in *Nature*, Vol. 403, No. 6772, pages 853–858; February 24, 2000.

VANISHING BEFORE OUR EYES. E. O. Wilson in *Time* (special report on Earth Day 2000), pages 29–34; April–May 2000.

The Authors

ROBERT P. LANZA, BETSY L. DRESSER and PHILIP DAMIANI share an interest in reproductive biology and animals. Lanza is vice president of medical and scientific development at Advanced Cell Technology (ACT) in Worcester, Mass. He founded the South Meadow Pond and Wildlife Association in Worcester County and is a member of the conservation commission of Clinton Township. Dresser is senior vice president for research at the Audubon Institute and director of the Audubon Institute Center for Research of Endangered Species and the Freeport-McMoRan Audubon Species Survival Center, all in New Orleans. Damiani, a research scientist at ACT, is also a member of the International Embryo Transfer Society's committee on cryopreservation.

THE HUMANE SOCIETY OF THE UNITED STATES

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Cat Cloning is Wrong-Headed States The Humane Society of the United States

February 14, 2002

WASHINGTON - The Humane Society of the United States, the nation's largest animal protection organization, expressed strong disapproval with the practice of cloning cats, in the wake of reports from scientists at Texas A&M University who claim to have succeeded in completing this experiment.

The HSUS attempts to foster close bonds between people and their pets since research demonstrates that a break in this bond is the leading cause of pet overpopulation, which leads to the unnecessary deaths of millions of animals each year.

"We recognize that a person may have an extraordinary bond with an animal, and we encourage the development of these bonds. But, the fact is, there is no way to create a pet identical to the one who is gone. They are distinctive, and cloning cannot eliminate their uniqueness," said Wayne Pacelle, HSUS senior vice president.

"The Humane Society of the United States opposes pet cloning because it is dangerous for the animals involved, it serves no compelling social purpose, and it threatens to add to the pet overpopulation problem. It doesn't sit well with us to create animals through such extreme and experimental means when there are so many animals desperate for homes," adds Pacelle.

Cats outnumber dogs in animal shelters and most shelters have kittens as well as juvenile and adult available for adoption.

"The sad reality is that we outlive our pets in most cases and people who decide to have a pet should recognize this fact before they accept that responsibility," said Brian Sodergren of The HSUS' Companion Animals section.

"Keeping a pet is enriching for the people involved and it is life-saving for the animals," adds Pacelle. "Cloning animals strips away the altruistic component behind pet-keeping and reduces the experience to one of selfishness. There is something wonderful about providing hope and homes for animals in need."

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Watch the Video

- Pets for Life

News Release

For Immediate Release
August 13, 2002

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Cloned Animals Suffer Death, Deformities According to Leading Journal Articles

Washington, DC -- The latest edition of the journal Cloning and Stem Cells documents deaths and deformities suffered by cloned pigs at the University of Missouri and Texas A&M. The University of Missouri study, entitled "Phenotyping of Transgenic Cloned Pigs," cites "a high mortality rate among cloned piglets." Out of 10 born, five died or were destroyed by researchers due to defects such as heart failure, lameness, and anemia. The Texas A&M study, entitled "A Highly Efficient Method for Porcine Cloning by Nuclear Transfer Using In Vitro - Matured Oocytes," documents a 94% failure rate. Out of the 511 manipulated oocytes transferred, only 28 pigs came to term, one of which was still born. Additionally, "another of the 28 piglets was born lacking an anus and tail," a fatal condition called anal atresia. The study suggests that the deformity may have been introduced through the cloning process: "Was the genetic (or epigenetic) defect that led to the anal atresia introduced during the culture of the donor cell, or was it due to inappropriate nuclear reprogramming?"

"Deaths and deformities in cloned animals are the norm, not the exception, and these studies make plain once again that these creatures are suffering terribly in the process," said Wayne Pacelle, Senior Vice President of the Humane Society of the United States."

According to Dr. Ian Wilmut, co-creator of Dolly the sheep "the widespread problems associated with clones has [sic] led to questions as to whether any clone was entirely normal" ("Why no-one should be attempting to clone a child," Roslin Institute, www.roslin.ac.uk/publications/0001annrep/child.html). Even Dolly, the product of 277 failed attempts now suffers from arthritis and other symptoms of premature aging possibly caused by cloning.

At a time when political debate is heating up on the topic of whether or not to permit human cloning in the United States and many scientists are quick to proclaim their experiments a success, Dr. Wilmut also offers words of caution: "There is abundant evidence that cloning can and does go wrong and no justification for believing that this will not happen with humans."

The first cloned human pregnancies were reported in April and July of this year. "It should concern us all that scientists are trying to clone humans," said Larry Bohlen, Director of Health and Environment Programs at Friends of the Earth. "Given the evidence of almost certain harm human cloning should

be banned."

FoE is a national environmental organization dedicated to preserving the health and diversity of the planet and empowering citizens to have an influential voice in decisions affecting their environment. HSUS is a national organization with a mission of promoting the protection of all animals. HSUS is dedicated to creating a world where humans' relationship with animals is guided by compassion, a truly humane society in which animals are respected for their intrinsic value, and where the human-animal bond is strong.

-end-

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ETHICS & DISCUSSION

Code of Bioethics

1) GSC shall be run in the manner most likely to maximize public knowledge. Only those aspects of GSC's business involving proprietary technology and pivotal business strategies shall be kept confidential. GSC shall provide online forums for free and open debate of all issues related to GSC's activities.

2) Transgenic (gene-changing) work shall be conducted only after a thorough analysis of the respective benefits of the proposed transgenic work versus potential risk(s). We intend to make this analysis public.

3) GSC shall not produce cloned animals for anyone who seeks to use them for any purpose that's likely to result in physical or psychological harm to the clones, such as in product testing, competitive fighting, etc.

4) No proprietary data, personnel or other resources shall be knowingly shared with people or programs seeking to clone human beings.

5) GSC shall guarantee that its activities reduce the national population of unwanted dogs and cats by a greater degree than its cloning activities add to the problem. Methods for accomplishing this may include development of canine/feline pharmacological contraceptives and/or sterilants, direct or indirect purchase of ovaries from spay clinics, and/or direct or indirect donation of funds to shelter systems.

6) Baseline animal care at GSC shall at all times meet or exceed guidelines set forth by the Association For Assessment and Accreditation of Laboratory Animal Care (AAALAC), whose standards are available for review at www.aaalac.org.

7) GSC is committed to use of the most advanced embryo production and assessment technologies available, in order to minimize the risk of deformities or other problems in cloned offspring. GSC shall strive to reduce the risk of such problems in cloned offspring below the background risk of such problems in nature.

8) In the unlikely event that an animal is born with deformities or other problems, it shall only be euthanized if it is suffering or facing high probability of near-term suffering, and shall otherwise be placed in a loving home at GSC expense.

A Powerful Technology

Cloning is a powerful technology, with the potential for abuse. At the same time, cloning is simply the latest form of assisted reproduction, not all that different from artificial insemination and *in vitro* fertilization, which were also controversial when first introduced. To guide ourselves in the appropriate use of cloning technology, GSC has developed a Code of Bioethics. All GSC employees are contractually bound to follow this Code, which governs the treatment of all animals whether clones or surrogates, involved in the development of our technology as well as future applications of this technology.

While we don't believe in modifying this Code to suit our convenience, we also don't believe our Code should be immutable. As both our understanding and the nature of our work evolves, we will occasionally modify our Code in response. In the interest of transparency (Point 1 of our Code), prior versions of the Code are available for review:

- Missyplicity Project Code of Bioethics

- GSC Code of Bioethics, Pet Division (2000)

- GSC Code of Bioethics, Livestock Division (2000)

The Case of the Cloned Cats

Notes for Teachers

There are many more issues that can be covered using this case study. Here are some ideas:

1. Why is it more difficult to clone dogs than to clone cats?
2. Do the problems faced by scientists in cloning cats and dogs give us reasons to be concerned about cloning human beings? Why or why not?
3. Ask students to analyze a cartoon about cat cloning. One is reproduced below; more can be found at http://cagle.slate.msn.com/news/CloningCATS/main.asp