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Could plague, a largely dormant disease, come roaring back to kill again? Easily, if the antibiotic-resistant strains created by Soviet bioweapons experts happened to fall into the wrong hands.

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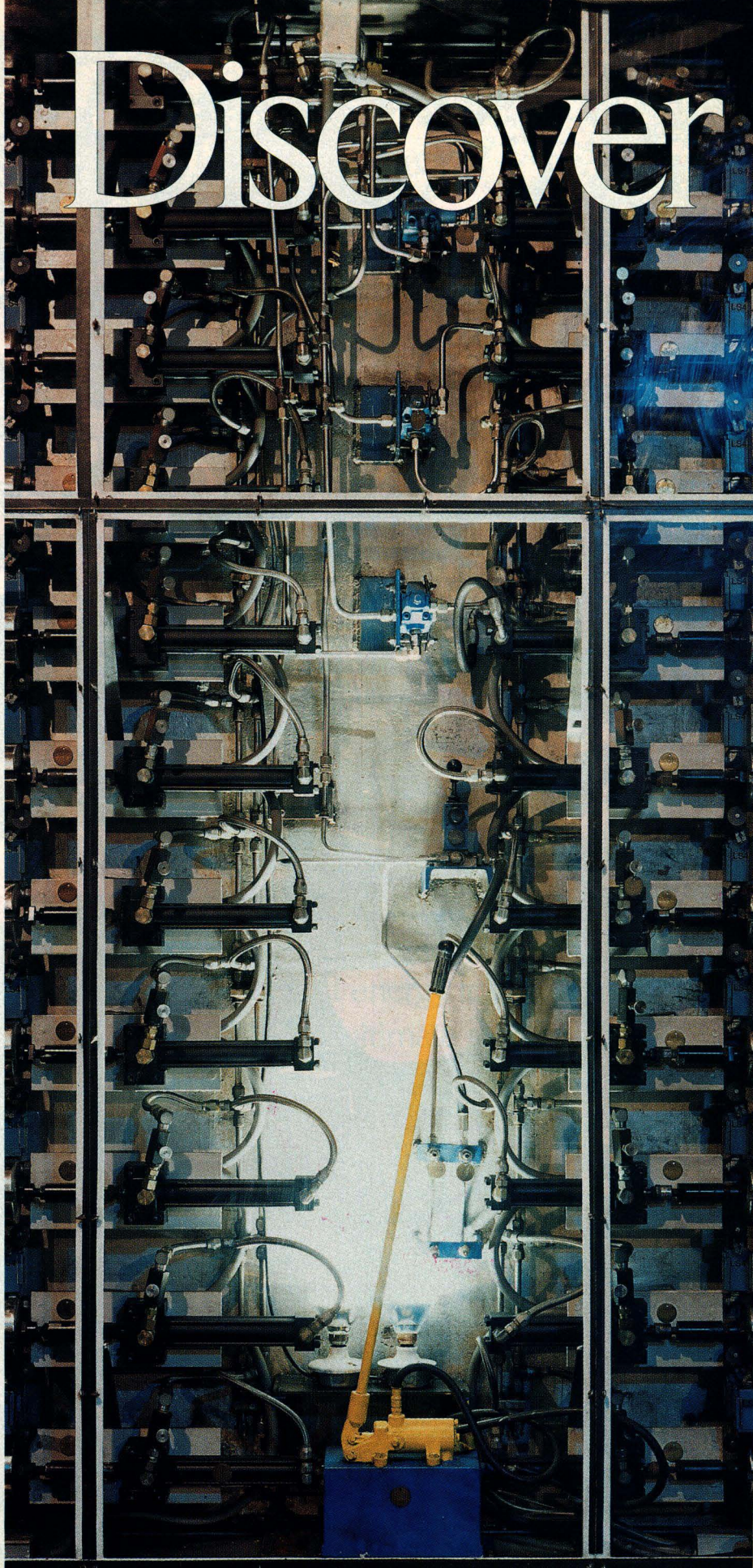
**RIGHT: A STEEL BLAST DOOR AT THE ENTRANCE TO THE NORAD COMMAND CENTER IN CHEYENNE MOUNTAIN, COLORADO, WAS BUILT TO WITHSTAND A 30-MEGATON NUCLEAR STRIKE. SEE PAGE 58.**

**ON THE COVER: STEPHAN'S QUINTET, A GROUPING OF GALAXIES SEEN IN A HUBBLE IMAGE, ARE JUST FIVE OF THE MILLION GALAXIES TO BE MAPPED IN COLOR AND EXHAUSTIVE 3-D BY THE SLOAN DIGITAL SKY SURVEY. PHOTOGRAPH COURTESY OF NASA.**

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PHOTOGRAPH BY TONY LAW

# Discover

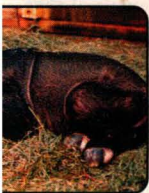




ON AN OVERPOPULATED EARTH OF DWINDLING HABITATS AND DISAPPEARING



# How to breed a 2,000 pound



SPECIES, ZOOKEEPERS TURN TO MATCHMAKING

IN THE BESTIARY OF DESIRE, the rhinoceros is more of a sex object than its glowering visage and hunker-down physique would suggest. In some parts of Asia, powdered rhino horn is regarded as both panacea and aphrodisiac. Long association of the rhino with sexual potency has even filtered into our popular culture, spawning such herbal sexual tonics as Wild Rhino, Rhino Sex Enhancement Tablets, and Rhino Cola. ¶ But life as a sex symbol has done little for the actual love life of the average rhino, particularly the Sumatran rhino. Little is known about this creature, the smallest of the five species of rhinos and among the most endangered of all mammals. We do know that despite their intimidating size and fierce reputation, the retiring, hirsute herbivores live solitary lives browsing their way from mudhole to mudhole in the

On September 13, Emi, shown some 14 months pregnant in July (opposite), delivered a healthy, 73-pound male calf at the Cincinnati Zoo. He is the first Sumatran rhino born in captivity in over a century—the last was born at the Calcutta Zoo in 1889. The calf's arrival is even more exciting because Emi had miscarried several times in the first three months of pregnancy. The series of inset photos above were taken when the calf was less than an hour old. Both parents are on loan from the Indonesian government—which will have the honor of naming the new calf in an upcoming ceremony. Emi became acquainted with her male partner, Ipuh, about five years ago at the Cincinnati Zoo. Lack of understanding about how ovulation is triggered in the Sumatran rhino had hindered the breeding effort. Only mating with a male will trigger the ovary to release an egg for fertilization.

BY ERIK NESS PHOTOGRAPHY BY JEFF MERMELSTEIN

## 'You light candles, you rub Buddha's belly, you pray things work'

rain forest. When they're not in the mood, which is most of the time, individuals avoid one another. Mating seems to be luck-of-the-draw, occurring whenever a male and a female happen to meet at just the right time.

This strategy worked fine for millions of years, but it isn't working too well today. Rhinos used to range from Borneo and Sumatra to the foothills of the Himalayas; now only an estimated 300 survive in reserves and isolated regions in Sumatra, the Malay Peninsula, and Borneo. The problem is not strictly habitat loss; there is still enough forest in this region to support perhaps 10,000 rhinos. The threat is the price of rhino horn. At \$25,000 a pound, the average Sumatran rhino wanders through life with a \$50,000 bounty on its head.

To procreate, a rhino must not only elude poachers; it must also find an opposite-sex and like-minded rhino of similar good fortune. So in 1984 rhino specialists decided to play



This ocelot kitten was conceived in the lab using sperm and a stored egg. The resulting embryo was put into the uterus of a female ocelot.

matchmaker for "doomed" Sumatran rhinos—those either isolated from other rhinos or inhabiting unprotected areas.

The effort began with high hopes. Captive propagation was already working with three other species of rhinos. Eventually 40 doomed animals were taken from the wild and shipped to zoos in Asia and the West. "The ultimate objective is to use the captive populations to reestablish or reinvigorate wild populations," says Tom Foose of the International Rhino Foundation. Nobody expected it to be easy—only one calf has ever been born and bred in captivity—but neither did they expect disaster.

**T**HE QUEST TO PRESERVE BIODIVERSITY IS A COMPLICATED bid to safeguard the legacy of evolution. Because each species has evolved distinctive mating and reproductive strategies, breeding endangered animals in captivity requires a respect for their sexual needs and preferences that is worthy of Dr. Ruth. Sometimes it's all about a setting that creates the mood: Some cranes, for example, know it's time to breed by the day length and other

seasonal markers such as heavy rainfalls. Sometimes it's about group dynamics: Flamingos will breed only when surrounded by enough birds to mimic the huge flocks they form in the wild. Shelter is important too: Red pandas prefer multiple nest boxes; black-footed ferrets prefer prairie-dog burrows.

The Sumatran rhino effort was like a bad date from the get-go. Malaysia captured mostly females, while Sumatra captured mostly males. Political dynamics led each home site—Indonesia, the Malay Peninsula, and Sabah—to go it largely alone. Many animals were in poor health, and when a gender-balancing exchange eventually did take place, the male subsequently died. "Virtually all of the animals that have been captured by the program have evidence of encounters with poachers, either very bad snare wounds or scars," says Foose. "One animal that recently died was discovered to have a bullet embedded in her lungs from before the time of her capture 15 years ago."

Compounding the challenge was an ignorance of Sumatran rhinos and their needs. The San Diego Zoo lost three animals—two in only four days. And survival in captivity poses its own problems. Accustomed to the deep shade of the understory in a rain forest, many of the rhinos developed eye problems when their new enclosures exposed them to hours of direct sunlight. Diet also proved to be tricky. At the Cincinnati Zoo, a male Sumatran named Ipuh lost several hundred pounds and eventually stopped eating altogether. The zoo mounted a costly rescue bid, flying in fresh ficus browse from California and Florida. Ipuh lifted his head just at the smell of the greenery, then ate for two straight days. The price of feeding this new fare to the zoo's three Sumatrans is \$100,000 a year.

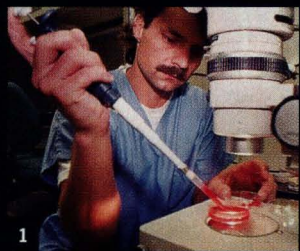
All of this might have been tolerable if the program were generating calves. It wasn't. Not only are Sumatran rhinos solitary, but the females give little apparent indication of estrus. And when a male and female are put together at the wrong time, fighting ensues. One female that died of liver failure was found at autopsy to have broken ribs from abortive trysts. "For a long time in a lot of places, people just didn't place animals together because they were afraid of what went on," says Foose.

With 24 of the original 40 rhinos dead and no calves born, most of the remaining Sumatrans were shipped back home and placed in more spacious outdoor preserves. In Cincinnati, three remained: Ipuh, Rapunzel, and Emi.

VISITORS TO THE CENTER FOR RESEARCH OF ENDANGERED WILDLIFE (CREW) at the Cincinnati Zoo & Botanical Garden are treated to one of the more bizarre spectacles of modern conservation. Through a sky-lit well, one can look down on a half-dozen large metal canisters stenciled "Frozen Zoo & Garden." This is cryodiversity—each vessel of liquid nitrogen cradling sperm, eggs, and embryos, the genetic essence of the rarest creatures on Earth. If all goes as planned, this DNA reserve will someday help reestablish viable wild populations, or at least deliver a dose of diversity to a genetically threadbare species.

Just 15 years ago high-tech fertility tricks were integral to

## 'We need to do it using **whatever tools** are available to us'



1) Bill Swanson, a researcher at the Cincinnati Zoo, removes blood from a biopsy of a domestic cat's follicles, the egg-nurturing structures in the ovary. 2) After the blood is extracted, the eggs in the sample are easier to detect. 3) Terri Roth, who directs the zoo's endangered wildlife research program, opens up one of the chilled canisters containing sperm samples and frozen embryos. 4) Samples stored in liquid nitrogen cooled to  $-374$  degrees Fahrenheit remain viable indefinitely. They are often used successfully after two to five years in storage.

plans for breeding hundreds of critically endangered species in captivity. But a variety of setbacks in zoos and in the wild have forced researchers to rethink that strategy. Assisted reproduction is now seen as only one tool of captive breeding, which is in turn just one part of an overall conservation plan.

"We do not ever promote technology as a way to save species instead of saving habitat, because that's ridiculous," says CREW director Terri Roth. "If our role is to preserve species [until] the wild is safe again and we can put them back out there, we need to do it using whatever tools are available to us."

The toolbox is diverse: In a two-day visit I observed the small CREW staff ultrasound a rhino, collect and analyze rhino blood, collect and analyze sperm of the common American toad, collect eggs from two domestic cats, and then freeze the cats' eggs for later experimental use. Working with model species such as the domestic cat and common toad is particularly useful. An endangered animal is too rare to put at risk with a new procedure. Even repeated handling is discouraged.

Yet some handling is unavoidable. Jimmy, a white rhino under Roth's care, has desirable genes and the perfect mate. But he has a bad hip. And when you are expected to scramble more than a ton of torso atop your mate, a bad hip is equivalent to erectile dysfunction. To preserve Jimmy's genes, Roth must collect semen, which is not standard zoocraft. Roth's team has tried manual stimulation, but that was unsuccessful. Next they will try electro-ejaculation, which is pretty much what it sounds like. But the probe is still in the experimental stage, and to test it, the subject must be anesthetized. Putting large mammals under is not a casual undertaking, even less so when the animal is critically endangered. So Roth waits for a nearby zoo to anesthetize an Indian rhino to treat his bad feet. Then she drives 60 miles to test her latest model rhino electro-ejaculation probe.

Such technologies may help breeders sidestep one of the oldest sexual problems on the books: incompatibility. Scientists use genetic analysis and family trees to figure out which pairing would best achieve the overall goal of genetic diversity. But nothing can guarantee mutual interest. "You light

candles, you rub Buddha's belly, you pray things work," says Diana Weinhardt of the Houston Zoo, who helps manage the North American population of spectacled bears. Weinhardt recalls one female paired with a younger prospect: "Every time this male came near her, she would just scream."

Sometimes success is just moments away when tragedy strikes. Stuart Wells, now a cheetah specialist at the National Zoo in Washington, D.C., recalls one cat under his care years ago in Phoenix. Ndala had been introduced daily to a male who beat her up, and not surprisingly, she soon avoided males. When Wells met her he was developing a system designed to simulate the nomadic rhythm of cheetahs in the wild. He kept males and females separate, mixing them only when they showed mutual interest. Under this regime Ndala slowly gained confidence, choosing to fight aggressive males rather than run. After a year she finally bred, but she made one mistake. "She flopped," says Wells, describing how Ndala lay on her side during intercourse. "That flop response is submissive. She triggered an instinctive, aggressive response in the male, and he killed her. He choked her. It was so depressing."

With some animals, the very problems pushing an animal to extinction plague captive propagation as well. This is especially true for amphibians. All over the world, they face habitat loss and other threats: pollution, ozone depletion, and chytridiomycosis, a fungal infection. Wyoming toads from the Laramie basin are bred in zoos for restocking in the wild, and keepers can overcome most problems. But the chytrid fungus still stalks them even in the herpetarium.

In the end, a successful breeding can be completely dependent on human wiles. In the early 1980s the International Crane Foundation (ICF) in Baraboo, Wisconsin, was trying to get chicks from Tex, a whooping crane that had been reared in captivity. "Tex got pretty old before she saw other whooping cranes," explains curator of birds Scott Swengel. "The older a crane gets before it sees another opposite-sex individual, the harder it is to pair it. She was hopeless by the time we got her. She was completely in love with men." Inseminating Tex

artificially was possible, but it would work only if she created a fertile egg. And ICF cofounder George Archibald understood that Tex would produce that egg only if she thought she was paired. Part of the pair-bonding ritual in cranes is dancing, and Tex clearly liked George, so he set up a small shack on the prairie where he could work and dance like a crane with her as best he could. Behold, with a little assisted insemination and a lot of bonding, Tex laid her first fertile egg. Just 24 days after it hatched, Tex was killed by a raccoon.

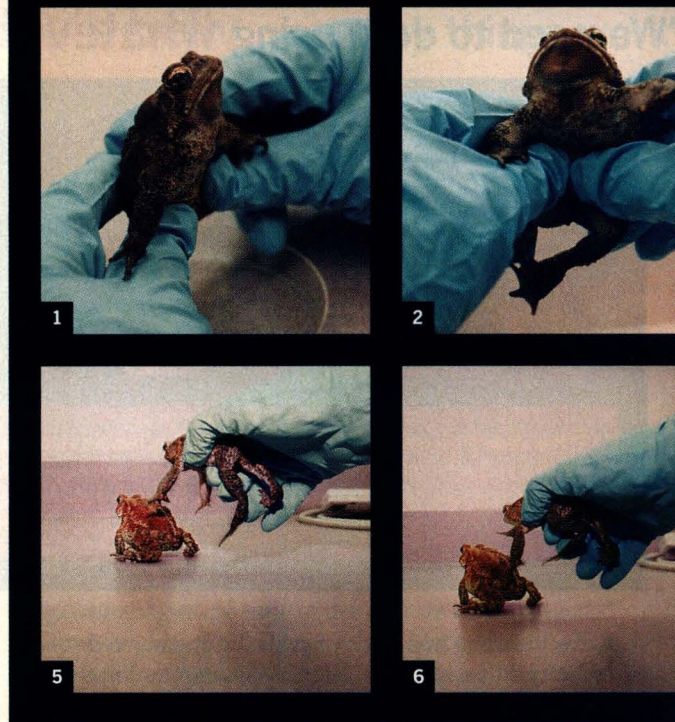
**V**ISITORS TO THE LAB OF JOGAYLE HOWARD ARE GREETED at the door by a series of eight ice-tinted photos. One is unmistakable: the slender biotic vessel of a sperm cell. The rest are jumbled messes—some of the sperm are coiled, some are bent, some are swimming backward. “None of these fertilize eggs. They don’t bind to eggs, they don’t penetrate, they don’t fertilize,” says Howard, a theriogenologist, or veterinary reproductive specialist, at the National Zoo. Sperm misfits make up about 70 percent of cheetah sperm, which helps explain why breeding cheetahs remains one of the biggest challenges in the zoo world.

The historical reason for this is a mystery; the biological reason is not. About 10,000 years ago the cheetah population may have dwindled to just a few individuals. That genetic bottleneck produced cheetahs so genetically similar to one another that skin grafts between unrelated individuals—which are normally swiftly rejected in most other mammalian species—often take in cheetahs. The lack of genetic diversity produces many problems, including susceptibility to diseases, weak cubs that often die for no apparent reason, and poor sperm quality.

Researchers struggle to avoid re-creating problems like these as they cobble together their programs for captive breeding. But endangered species are, by definition, small populations facing their own genetic bottlenecks. Whatever animals we manage to shepherd through this age of extinction will face problems in captivity and, eventually, in the wild. Even in small populations with perfect breeding success, some genes will never make it into the next generation and are thus lost forever. And the ill effects of a small gene pool don’t take long to manifest. “One or two generations of inbreeding could cause a male to go from good semen quality to poor semen quality,” says Howard.

Fortunately, animals respond well to infusions of new blood. The Florida panther was so genetically depleted that the few dozen remaining animals had problems ranging from poor sperm quality to leaky hearts. Once biologists gave up on preserving them as a subspecies and began mating them with Texas pumas for a bit of genetic fresh air, the very first generation showed signs of improvement in sperm quality.

RHINOS ARE ONE OF THE OLDEST MAMMAL FAMILIES AND ONE OF the trickiest research subjects. “We don’t really have any domestic analogues,” says Tom Foose. The horse is similar to the rhino, but kinship is nowhere near as close as wild felines are to domestic cats, wild canids to domestic dogs, or even hippos to pigs. “We’ve got a lot of experience with species that are very similar to a lot of the wild exotic species that we work with, but we don’t have that with rhinos,” says Foose.



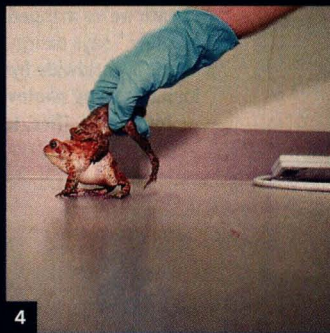
“It’s always the unexpected, with every rhino we’ve ever worked with,” adds Terri Roth. When Roth came to the Center for Research of Endangered Wildlife in 1996, she began an attempt to get a calf from either Emi or Rapunzel, the two female rhinos at the Cincinnati Zoo. The first order of business was to determine their reproductive cycles.

Using ultrasound, Roth found that Rapunzel, the older female, didn’t seem to have working ovaries. That left Emi. A couple of times a week, week after week, Roth took a look at Emi’s follicles, the structures that nurture eggs within the ovary. When the follicles reach a certain size, ovulation should occur: One of the follicles should pop, delivering a mature egg for fertilization. But the follicles kept growing without appearing to release an egg. Roth tried to arrange a few liaisons between Ipuh and Emi, but Roth’s hunches were wrong. “They pretty much completely ignored each other. It was clear it wasn’t the right day.”

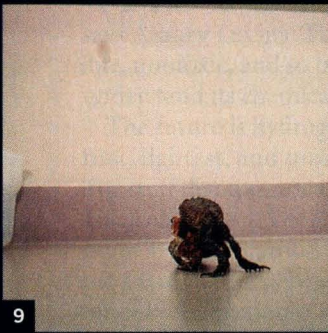
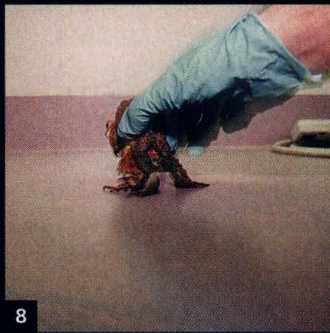
Next they tried closely monitored daily visits. They were lucky that Emi could outrun Ipuh, reducing her risk of injury should he grow belligerent. It was summer, and Ipuh spent much of the time sacked out in his pool, but “all of a sudden one day he was interested. Instead of flopping down in his pool, he started following her slowly around the enclosure. That was the first breeding attempt. He failed, but two days later was the first time I definitely saw ovulation,” Roth says.

If Emi is representative, the Sumatran rhino is an induced ovulator, unique among rhinos. “She was not ovulating because we were not breeding her. That makes sense. They don’t hang out in a herd. There’s no reason to ovulate if she doesn’t come into contact with the male,” says Roth.

In other words, some cue in the rhino mating colloquy triggers the egg’s release: When Emi is in estrus and put in Ipuh’s enclosure, he will immediately start following her, slowly, so that he doesn’t startle her. Sometimes she will begin cantering about,



Like many amphibians, American toads are facing problems in the wild: habitat loss, disease, and various forms of pollution. Cincinnati Zoo researchers study the biology of toad reproduction; they also mate toads to create progeny for restocking depleted populations in the wild. Images 1 and 2 show a researcher extracting sperm, which will be frozen for later use or study. Images 3 through 10 show frogs paired for mating. The male mounts the female and clasps her from behind, which helps release her eggs. Both eggs and sperm are shed simultaneously and fertilized externally. Each mating produces thousands of fertilized eggs.



## If not for **this extinction-era fertility cult**, there would probably be no California condor, **no bison**, no Wyoming toad

and he'll just walk, waiting for her to settle down. Then they'll walk nose to tail for about 15 minutes, she'll stop, and he'll mount her briefly three to five times. "On two occasions he's failed altogether and exhausted himself trying," says Roth. He stays mounted anywhere from 20 minutes up to as long as 50 minutes. "They pretty much sack out, both of them, after that."

The second breeding attempt was more successful. A pregnancy was announced, but the fetus was gone a week later. It was easy for Emi to get pregnant, but she kept losing the calf: five pregnancies, five spontaneous abortions.

Could crowding be a problem? The Sumatran rhino is solitary, and Emi lives practically on top of Ipuh and Rapunzel. The committee of biologists overseeing the project decided to try a progesterone supplement before taking the riskier step of moving any animals. Progesterone, a female hormone that helps control the development of the uterine lining, has been used on several black rhinos (and human females) without adverse effect to the offspring. With five lost pregnancies, Roth didn't celebrate until Emi delivered a healthy, 73-pound male calf on September 13, 475 days after conception.

Roth's team had prepared for problems such as a premature birth or difficulties nursing. But Emi took to motherhood with ease. "Her response to the calf was beautiful," says Roth. "She turned around and just looked at it with her ears forward and her eyes all bright. The expression on her face said, 'What is this? What did I just do?' It was so cute. Then she started licking it." The calf stood within hours and nursed constantly. Roth and her colleagues finally uncorked the champagne.

BREEDING ENDANGERED SPECIES IS A PECULIAR CONSERVATION NICHE with only a few inspiring successes, many unresolved ethical questions, and a serious backlog. If not for this extinction-era fertility cult, there would probably be no California condor, no bison, no black-footed ferret, no Guam rail, no Wyoming toad. And while these animals seem more secure than they were a decade ago, their futures in the wild are by no means guaranteed. Even support for captive breeding is uncertain. This past April, the secretary of the Smithsonian Institution announced plans to close its flagship center for captive breeding in Virginia. A loud outcry from conservation biologists scrapped the plan.

Even if Emi's great-grandcalves never go home, Roth hopes that what we've learned about managing and breeding small populations of animals will prove useful in preserving other threatened species in the wild. While some accuse the zoo community of focusing too narrowly on small captive populations, Roth is unapologetic. "Quite frankly, that's what we are going to be dealing with. We're going to have to mix wild and captive animals or we're going to lose genetic diversity. Assisted reproduction is a way to manage genes, but you have to know that the assisted reproduction can work."

"It enables you to maximize your options and minimize your regrets," says Foose, noting that three black rhinos, bred and reared in captivity, were released in South Africa this year. The work with Emi, he adds, will yield far more than a single calf. "A captive program is probably going to be vital if the species is going to survive. If the managed breeding program doesn't succeed, maybe the species doesn't survive." ❏