Marked. Stained projection neurons (green) in a fruit fly brain connect to glomeruli (bright green, foreground) and to higher brain areas (fainter green lines to the left).

input to guide them, as happens in the visual cortex. Alternatively, instructions may be programmed into the projection neurons from their birth—which is what Luo's team found.

The 150 or so projection neurons are born sequentially from cells called neuroblasts. Graduate students Greg Jefferis and Lisa Marin marked projection neurons, using a shock of heat to turn on a marker gene in only those neurons being born during the heat shock. By varying the timing of the heat shock, the researchers could label different neurons in the birth sequence.

The researchers heat-shocked thousands of fly larvae at various times in development. They found that the neurons that made connections with particular glomeruli always seemed to be born at roughly the same time. That suggested that, from the time a projection neuron is born, it knows which glomerulus to connect to. In a separate analysis, the team labeled neuroblasts, which then transferred the label to all of their progeny born after that time. In flies labeled early, a full set of glomeruli showed up. As the labeling time moved later, one by one the marked glomeruli disappeared. "They drop out in a defined sequence," says Luo. "There is no exception." The two analyses, Katz states, provide "absolutely compelling" evidence that the projection neurons get their marching orders at birth, based on when during development the neuron emerges.

Indeed, the picture drawn by both papers is that "the organization of this very complicated sensory system seems to be highly predetermined," says Rockefeller's Vosshall. And to many researchers, that makes a lot of sense. Smells are tightly linked to many instincts. If a young mouse had to learn from experience that the smell of coyote urine means danger, Vosshall notes, it may not get a second chance to use that information. It would be much more adaptive, she argues, for the coyote-urine smell to be hardwired into the animal's fear center by natural selection.

–MARCIA BARINAGA

ZOO BIOLOGY

A Fertile Mind on Wildlife Conservation's Front Lines

Renowned for getting captive elephants to breed, Thomas Hildebrandt and his team are extending their prowess to scores of other rare species

BERLIN—Few people in the world are as intimately familiar with the elephant cervix as Thomas Hildebrandt. That particular knowledge may not make the 37-year-old veterinarian a hit at a cocktail party, but it has won him admirers in zoos around the world. Hildebrandt and his colleagues at the Institute for Zoo Biology and Wildlife Research (IZW) in Berlin have pioneered an innovative approach—artificial insemination (AI) technology aided by ultrasound—to impregnate half a dozen elephants, raising hope that zoos will be able to maintain their populations of these hard-to-breed creatures.

Hildebrandt's ultrasound techniques "have revolutionized our ability to assess elephant reproductive health," says Janine Brown of the Smithsonian National Zoological Park in Washington, D.C., where an elephant named Shanthi is expected to give birth next month—thanks to the "Berlin Boys," as the IZW team is known.

These days, the team's prowess at making babies is in high demand. Scores of zoo elephants are nearing the end of their life-spans, while recent laws have made it nearly impossible to import endangered species from the wild. Without AI, say several experts, zoo elephants could all but disappear within 2 decades. Other rare animals are also falling for the charms of these high-tech love doctors. Hildebrandt and colleagues Frank Göritz and Robert Hermes have used their ultrasound instruments to probe the internal organs of more than 200 species, including giant pandas, Komodo dragons, endangered European brown hares, and even invertebrates: Hildebrandt has used his technique to sex an octopus at Washington's National Zoo. "He'll ultrasound just about anything that lives or crawls," says Richard Montali, head of the National Zoo's pathology department.

The face of elephant husbandry. Thomas Hildebrandt and his probe have become intimate with the reproductive systems of animals ranging from Komodo dragons to octopuses.

The team is currently working its charms on the rare white rhinoceros. This rhino has been hunted nearly to extinction for its horn, rumored to have aphrodisiac powers. Such power has sadly eluded white rhinos in captivity, where they have managed to breed successfully only a handful of times. The IZW researchers have pioneered techniques for both sperm collection and artificial insemination in the rhino, and they are waiting to hear whether an animal that they treated last month is pregnant. The Berlin Boys "are a sterling example of how science can work to the benefit of endangered species," says Michael Keele of the Oregon Zoo in Portland, coordinator of the national species survival program for elephants in the United States.

Key to the team's success is ultrasonic imaging, used in human medicine to visualize organs without penetrating the skin. A probe emits high-frequency sound waves, which tissues absorb or reflect to different degrees depending on their density. Although technicians can image a fetus by moving the probe outside a woman's abdomen, an elephant's skin and muscle layers are so thick that the sound waves cannot penetrate to the internal organs. Researchers can glimpse the animal's reproductive tract only from a closer vantage point: inside the digestive tract. Donning a shoulder-length glove, one of the team members inserts the specially designed ultrasound probe through the rectum and more than a meter into an elephant's colon and points it toward the reproductive organs. Although endoscopy exams are noto-

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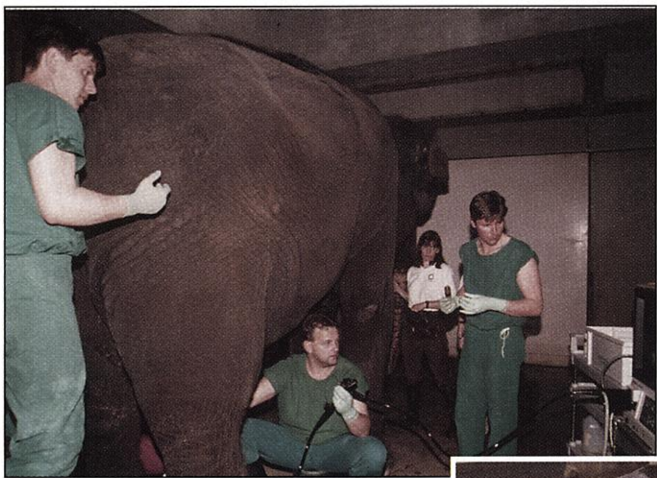
riously uncomfortable for people, most animals, if distracted by food, tolerate the procedure well.

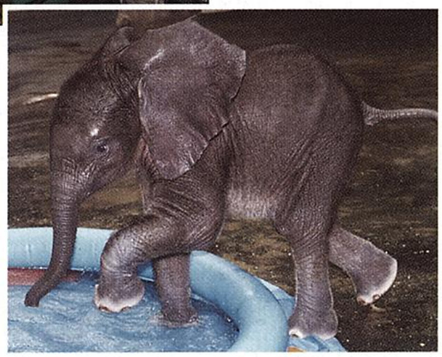
The sonogram is displayed on a TV monitor and inside a special helmet Hildebrandt designed to follow the probe even from a contortionist's position behind a 6-ton beast. To the untrained eye, the images seem an abstract blur, but years of painstaking correlation between the fuzzy shapes and postmortem dissections have allowed the IZW team to spot even subtle abnormalities. Less than a decade ago, says Montali, ovaries "were obscure structures you could only see in a dead elephant." Using ultrasound, however, the IZW team has diagnosed ovarian cysts and uterine polyps and tumors that have prevented females from reproducing. It turns out that such abnormalities "occur with a frightening regularity" in captive elephants, says Keele. Hildebrandt estimates that one in four females has some sort of reproductive pathology. He suspects that allowing females to ovulate for many years without a pregnancy might be part of the problem. In the wild, he notes, female elephants spend most of their adult lives either pregnant or nursing a calf and produce very different levels of some hormones than females in captivity do.

But female physical maladies explain only part of captive elephants' low fertility rate. Bulls housed together, for example, tend to have poorer quality semen than do bulls kept alone or with females. Indeed, scientists are just beginning to unravel the complex biological phenomena that may doom zoo populations of elephants.

Swimming upstream. When the Berlin Wall opened on 9 November 1989, tens of thousands of East Berliners streamed toward the celebrations at the Brandenburg Gate. But Hildebrandt was urgently bicycling the opposite way toward the Tierpark, East Berlin's zoo. That evening the IZW graduate student needed to give a hormone injection to a rare yak to try to induce it to produce multiple fertile eggs—and thanks to the timely injection, it did. The end of the Cold War ushered in many more opportunities for Hildebrandt, who stayed on at the IZW, one of the few institutes to emerge unscathed from the reorganization of eastern German science after reunification.

The experiment that changed Hildebrandt's life, meanwhile, had begun a year earlier. He and his colleagues had been attempting to perfect an ovary transfer technique that required them to perform surgery on fetal goats. Often the trauma of surgery would cause a doe to abort, so the team sought the assistance of doctors who use ultrasound to perform minimally invasive surgery on humans. Hildebrandt quickly saw the technique's broader potential. "I was fascinated with this tool that would let us explore internal organs," he says. He first tried the technique to image the reproductive tract of macaque monkeys but soon wondered if it might be useful for use with heftier beasts too.





Where no man has gone before. The Berlin Boys examine an elephant's ovaries; their talents produced Abu (*inset*) in Vienna last April.

Hildebrandt was especially eager to try his hand with elephants. But the technical challenges of imaging from the inside of such a large animal were daunting. He had to design special probes, now patented, which could be maneuvered into the animal's rectum and aimed in the right direction. It took more than 2 years for the IZW group to perfect the procedure.

From the start, Hildebrandt hoped ultrasound might aid efforts to artificially inseminate elephants. AI is a potential boon to elephant keepers, for an obvious reason: It is much easier to ship sperm than an elephant. The procedure has long been used to breed cattle and other livestock, but for decades no one had gotten it to work in elephants. Again, technical difficulties hindered the attempts: Ideally, the sperm should be deposited inside the 2-centimeter-wide elephant cervix, which is situated considerably more than an arm's length—more than 1 meter—from the opening of the so-called vestibule, a combined urinary-genital opening that prevents a straight shot to the cervix.

But Hildebrandt's new ultrasound tools, coupled with a better understanding of the estrus cycle of female elephants, enabled two groups to succeed. In 1997, Dennis Schmitt of Southwest Missouri State University and the Dickerson Park Zoo in Springfield, Missouri, announced the first successful use of AI on an Asian elephant. A few months later, the Berlin researchers confirmed that their AI procedure had impregnated a 20-year-old African female at the Indianapolis Zoo. Two years later, both animals gave birth to healthy babies.

Elephant AI is no easy feat. The sperm often doesn't survive freezing and thawing, so the process is carefully choreographed. When monitoring of a female's hormones and ovaries shows she is fertile, a team member travels to the chosen bull to collect fresh sperm and then jets off to the recipient female. Once the sperm arrives, the team uses the ultrasound probe to monitor the placement of a catheter—attached to a custom-designed endoscope—on its long journey to the cervix to deposit the sperm.

The team members enjoy the unusual profile that comes with the job. "You can imagine the response when the flight attendant asks, 'What's in the special case?' and the answer is, 'Elephant semen,' " says Harald Schwammer, vice director of the Vienna Zoo. There, the IZW team's AI techniques led to the birth of a healthy bull last spring.

The Berlin Boys happily share their AI legerdemain, spending nearly half the year on the road. This month they're in Tanzania, collecting sperm from wild elephant bulls for cryopreservation experiments and using ultrasound to check the reproductive health of elephants in Selous National Park.

If their research on sperm preservation bears fruit, it would give a huge boost to the prospects of the captive African elephant population, enabling managers to supplement the shrinking gene pool with "wild-caught" sperm. But the work in zoos also benefits wild populations, Hildebrandt says. Elephants hold our fascination as few other species do, so they play a charismatic role in teaching the public about conservation, he says. And attempts at conservation without understanding each species' reproductive pattern are doomed to fail—a lesson from Hildebrandt's own unique brand of intimacy.

–GRETCHEN VOGEL

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