



# CREW ReView



Lindner Center for Conservation and Research of Endangered Wildlife • Cincinnati Zoo & Botanical Garden

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## CryoBioBank



### Roth's Remarks CREW's CryoBioBank™

**Dr. Terri L. Roth**  
VP of Conservation & Science and Director of CREW

In the heart of the CREW building and vital to CREW's mission is the CryoBioBank. CREW's CryoBioBank consists of numerous specialized tanks filled with small vessels containing tiny animal and plant samples surrounded by liquid nitrogen. Cryopreserved at -320°F, these samples all but cease metabolic activity remaining viable in their suspended state for decades or even centuries. CREW's CryoBioBank is a safe haven for some of the world's most valuable and irreplaceable biological samples from rare and endangered plants and animals. For example, nowhere else in the world are viable Sumatran rhino spermatozoa preserved. Embryos from the imperiled Pallas' cat lay waiting to be thawed and transferred into recipients to carry on the genetic lineage of their deceased parents. And, cryopreserved tissue from the Northern Wild Monkshood offers the only hope for restoring a genetic line now extinct in the wild. To-date, samples from 75 animal and 210 plant species populate the tanks. Because CREW's mission focuses on propagating and genetically managing endangered plants and animals, the CryoBioBank primarily contains tissue and cells necessary for propagation. Animal samples encompass sperm, embryos and oocytes, whereas plant samples include shoot and root tips, pollen and seed. The CryoBioBank is a reservoir of genetic diversity, but it is not a static collection. Instead, samples are added to and removed from these tanks year-round as CREW scientists use them to further their research and to produce genetically valuable offspring and seedlings so desperately needed by rare populations in decline. CREW's CryoBioBank is not the answer to the extinction crisis but it is one more powerful tool in the arsenal needed to combat the world's ongoing loss of biodiversity. (CREW is very grateful to Weldco Incorporated for generously donating all the liquid nitrogen needed to maintain CREW's CryoBioBank over the past 4 years.)







## From Missiles to Mint

CREW and White Sands Missile Range (WSMR) have entered into a Cooperative Agreement to study and help conserve the endangered Todsen's pennyroyal (*Hedeoma todsenii*). This rare mint species grows in three clusters of populations: two clusters in the Sacramento Mountains and one cluster about 40 miles west, in the San Andres Mountains on White Sands Missile Range. Todsen's pennyroyal produces flowers, but little if any seed has been found from these populations. If it is propagating clonally, the question arises as to how much genetic diversity there is within and between populations in these three clusters. Through a grant funded by the U.S. Army, CREW is attempting to answer this question using the technique of RAPD analysis. CREW's preliminary results



Todsen's pennyroyal grows in the mountains of New Mexico.



Dr. Doug Winget, collecting shoot tips of Todsen's pennyroyal for genetic analysis and tissue culture at CREW.

from a few samples of the northern cluster of populations in the Sacramento Mountains indicate that there is some diversity within populations, but even more between populations.

With this promising start, CREW researchers returned to New Mexico this August to collect from additional populations on WSMR. The plants grow above 6200 feet in very isolated areas, requiring several hours of driving and difficult hiking to reach. Despite the challenges, samples from two areas of the San Andres cluster and one area of the northern Sacramento cluster were collected and sent to CREW. These were immediately put into culture and have initiated a number of new genetic lines for DNA analysis. As a further benefit, tissues from these lines can be cryopreserved and banked in CREW's CryoBioBank, since seeds are not available for seed banking.

Although this species is fairly isolated, its low numbers and low seed production put it at risk for loss through environmental changes or catastrophic events. The grant from the U.S. Army will provide a better understanding of this species and help secure its survival into the future. (Project supported by Department of the Army, U.S. Army Garrison White Sands.)

## Unveiling the Sumatran Rhino's Hidden Hormones

CREW animal scientists use non-invasive hormone monitoring to follow the reproductive cycles and diagnose pregnancy in many endangered animals in the Zoo's collection to assist with their management and breeding. Metabolites of estrogens and progestogens are typically the most important hormones measured in urine or feces that can easily be collected without disturbing the animal's normal routine. However, CREW scientists have learned (repeatedly) that no two species are alike, and despite the fact that estrogens and progestogens can be evaluated in the urine or feces of other rhinoceros species, estrogen metabolites have not been reliably detected in Sumatran rhino samples. In fact, even efforts to monitor estrogens in serum have provided little insight into the reproductive physiology of this unique species. Post-doctoral trainee, Dr. Mandi Vick, who joined CREW in May as a part of a large IMLS grant focused on the rhino and small cat *Signature* projects, is taking on the challenge of monitoring estrogens in the critically endangered Sumatran rhino. CREW is teaming up with collaborators at NIH to delineate the specific forms of estrogens predominantly produced by the Sumatran rhino. With that information in hand, it should be possible to develop a method of monitoring estrogen metabolites in urine or feces. Such a tool will be very valuable for timing both rhino pair introductions for natural breeding and artificial insemination procedures to enhance the success of the global captive breeding program. (Project supported by a grant from the Institute of Museum and Library Services.)



CREW's new Post-doctoral trainee, Dr. Mandi Vick, is studying Sumatran rhino hormones.

## The Secret is Out

CREW has often been touted as the "Zoo's best kept secret" but word is starting to get out about this hidden treasure. So far this year, presentations about CREW's research, conservation and education efforts have been shared with faculty and students at many local universities and colleges, employees of several business organizations, special clubs and as an element of nature and career days at various locations. On grounds, CREW has received far more building tour requests by schools than in previous years, even in the middle of summer, which has kept the CREW Volunteer Educators hopping. General CREW tours have also been offered as a special component of several Group Sales and Development events, and VIP behind-the-scenes tours are often available to the highest bidder at silent auctions during Zoo events. Slowly but surely, CREW's mission "Saving Species With Science®" is becoming known.



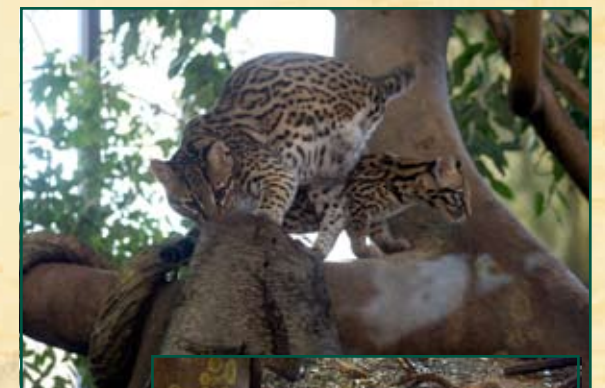
If you are interested in learning what it is like to work at CREW, there are a couple of popular CREW education offerings you and your family may want to consider. *Scientists for the Future* is an overnight program for

high school students and *Scientists for a Night* is an overnight program for adults of all ages. Both programs will be offered between September 2009 and May 2010 at CREW and will highlight the science of saving polar bears, rhinos and endangered plants and specifically how you can help. Please contact Bernadette Plair at (513) 569-8223 or bernadette.plair@cincinnati-zoo.org for further information.



## The Art of Ocelot Conservation

Assisted reproductive technology (ART) is a field replete with acronyms - AI (artificial insemination), IVF (in vitro fertilization) and ET (embryo transfer) - that are increasingly familiar to most people due to their growing popularity and success with infertile human couples. These acronyms have become common terms in the zoo field as well but still more for their conservation potential than any real applied value. Techniques that improve reproduction in humans or domestic animals such as cattle and dogs often don't translate nearly as well when extrapolated to nondomestic wildlife species. ART-related births in zoos are still so rare that they frequently receive considerable media attention but many actually never benefit the endangered populations managed by zoo-based Species Survival Plans (SSPs). One exception has been the application of ART to the Brazilian ocelot. CREW research has demonstrated that the transfer of frozen ocelot embryos, created by IVF, can be used to produce multiple pregnancies and healthy kittens that are genetic founders for the Ocelot SSP. Most recently, we have been assisting the Ocelot SSP in improving genetic diversity by conducting AI with Brazilian ocelot pairs that are physically or behaviorally unable to reproduce naturally. For example, in August 2008, CREW scientists performed an AI at Connecticut's Beardsley Zoo involving a three-legged female incapable of natural breeding and a behaviorally aggressive male, resulting in the birth of a healthy male kitten. Without CREW's assistance, this genetically-valuable kitten would never have been born. The Ocelot SSP has recommended the use of AI with several other non-reproducing ocelot pairs, allowing ART to contribute more substantially to the science of endangered species management.



Brazilian ocelot kitten (with mother) produced by artificial insemination (Beardsley Zoo).

