



CREW ReView



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

Fall 2009

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.)





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Five Endangered Flora Bound for the CryoBioBank's Frozen Garden

The Plant Research Division was recently awarded a Conservation Project Support Grant from the Institute of Museum and Library Services (IMLS) that will support the cryopreservation of multiple genetic lines of five endangered plant species in tissue culture at CREW. Avon Park harebells (*Crotalaria avonensis*), Four-petal pawpaw (*Asimina tetramera*), Beautiful pawpaw (*Deeringothamnus pulchellus*), and Rugel's pawpaw (*Deeringothamnus rugelii*) are found only in Florida, while Todsen's pennyroyal (*Hedeoma todsenii*) is native only to two mountain ranges in New Mexico. Seed banking is not an option for long-term germplasm storage of these species because the seeds either do not survive storage or are not adequately produced. Therefore, with previous IMLS support, tissue culture propagation and cryopreservation methods were developed at CREW for these species and are now being used to preserve tissues from many different individual plants (genotypes) in the Frozen Garden of CREW's CryoBioBank. However, maintaining over 200 individual lines in culture requires significant labor and resources. The new IMLS grant will provide \$150,000 over two years in support of this effort. Furthermore, the process and methodologies will be evaluated and improved, thereby increasing the efficiency and lowering the costs of future work. This, in turn, should encourage the application of tissue banking for the estimated 5000 endangered species worldwide for which seed banking is not possible.



Culture of *Crotalaria avonensis* from which tiny shoot tips are removed for freezing.

CREW Repeats Groundbreaking Artificial Insemination Success in the Indian Rhino with CryoBioBanked Sperm

In 2006, a team of CREW scientists, veterinarians and CZBG Rhino Keepers led by Dr. Monica Stoops achieved the first successful pregnancy by artificial insemination (AI) in an Indian rhino and the first in any rhino species using frozen-thawed sperm. The sperm sample had been collected from a rhino at the Wilds in Cumberland, Ohio and stored frozen in CREW's CryoBioBank for two years prior to its use for inseminating the female rhino Nikki. Unfortunately, after completing a term pregnancy, Nikki's parturition ended tragically with the delivery of a stillborn calf. Indian rhino studbook records indicate that first time Indian rhino moms over the age of ten, like Nikki, have a 50% chance of delivering a stillborn calf. However, these statistics did little to abate the devastation felt by all involved with the groundbreaking achievement that ended so sadly. However, the team had to look to the future and be assuaged by the fact that female Indian rhinos experiencing a stillbirth following their first pregnancy have successful live births with subsequent pregnancies. Despite their disappointment, it was not long before the staff was ready to try and repeat the successful AI procedure. Success did not happen overnight, but the team persisted and in June 2009, Nikki conceived again following the insemination of frozen-thawed sperm collected from a rhino at the Bronx Zoo in 2005, and stored in CREW's CryoBioBank for 4 years.



Ultrasound image of Nikki's 2 month old fetus.

To date, Nikki's pregnancy is progressing well and weekly ultrasound exams reveal a healthy, growing rhino baby. Nikki's pregnancy demonstrates that the science of AI for the Indian rhino is repeatable. In addition, more than one male rhino's sperm sample in the CryoBioBank has proven fertile. AI can be used to improve the genetic health of captive Indian rhinos by infusing genes from non- or under-represented rhinos. Therefore, CREW celebrates Nikki's second pregnancy as a solid step towards ensuring the long-term sustainability of this species and its genetic diversity. (Project supported by a grant from the Institute of Museum and Library Services.)



Disease model kittens produced following IVF and embryo transfer using semen stored ~10 years in CREW's CryoBioBank.

CryoBioBanking of Rare Domestic Cat Populations

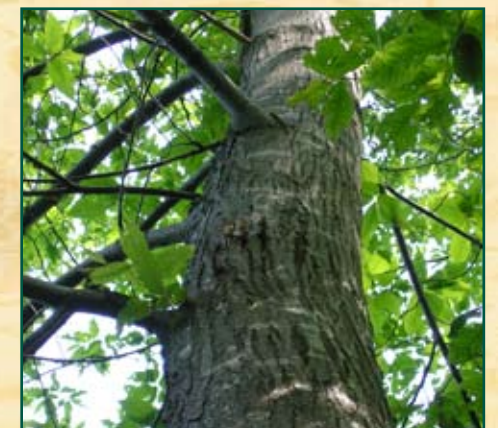
Much of our research at CREW is focused on improving natural breeding and developing assisted reproduction, such as in vitro fertilization (IVF), sperm and embryo cryopreservation and embryo transfer, for conserving threatened wildlife species. With felids, we are fortunate to have the domestic cat as a research model for endangered nondomestic cats. Our recent successes with artificial insemination and frozen embryo transfer in Brazilian ocelots are directly attributable to knowledge gained from our domestic cat studies, funded primarily by the National Institutes of Health (NIH). Another objective of this NIH-funded research has been to apply these reproductive technologies to the management

and preservation of other rare felids, specifically domestic cat models of hereditary disease maintained at several veterinary and medical schools. During the past few years, CREW scientists have collected and cryopreserved semen and embryos from more than 80 cats representing 24 disease models. Using IVF and embryo transfer with these frozen samples, we have produced multiple offspring in seven cat models now, including three that existed only within CREW's CryoBioBank and no longer as living populations. Some of these embryo transfer kittens were subsequently returned to the veterinary schools at the University of Pennsylvania, University of California and Michigan State University to form the basis of their new breeding populations. This CryoBioBank for domestic cats allows university veterinarians and other scientists to maintain their invaluable research models indefinitely within liquid nitrogen tanks while devoting more of their limited resources to studying those hereditary diseases that can afflict both cats and humans. (Supported by NIH grant R24RR015388)

CREW's CryoBioBank "Survivor" – the American Chestnut Pollen Story

The American chestnut (*Castanea dentata*) was once a dominant tree in the Eastern U.S. forests, but in the early 20th century, it was almost eradicated by the chestnut blight fungus. The American Chestnut Foundation (ACF) is working to develop a blight-resistant American chestnut tree by backcrossing it with the disease resistant Chinese chestnut in hopes of someday restoring the tree to its native forests. Meanwhile, there is a need to store genetic material from this species, but the seeds are short-lived and do not survive traditional seed banking methods. Therefore, 16 years ago, CREW post-doctoral researcher Dr. Leslie Leverone initiated a long-term experiment in chestnut germplasm storage. The ACF sent valuable lines of chestnut pollen to CREW where they were divided and stored under three conditions: 4°C (refrigerator temperature), -20°C (low temperature freezer), and in liquid nitrogen in CREW's CryoBioBank Frozen Garden (-196°C) for future use in breeding programs.

The pollen has remained undisturbed until this year when CREW researchers tested it for viability. Pollen was removed from storage, placed in a sugar solution at 31°C overnight and then examined under a microscope for germination. All of the samples stored in the CryoBioBank were viable, whereas none of the samples at -20°C and 4°C survived. American chestnut pollen is typically very short-lived, losing viability within a few weeks of harvesting, but now CREW scientists have demonstrated that liquid nitrogen storage vastly extends the life of this pollen. The pollen samples from valuable chestnut genotypes stored in CREW's CryoBioBank Frozen Garden have been preserved successfully and can be used in developing breeding strategies for preserving and ultimately restoring this majestic tree in the future. (Viability testing of American chestnut pollen was made possible by a grant from Scripps Howard, which supports our student co-op/intern program.)



The American chestnut tree that was once a dominant tree in the Eastern U.S. forests.



In Loving Memory of Emi

Emi, the Sumatran rhino that made history by producing three calves at the Cincinnati Zoo from 2001-2008, passed away in her sleep on Sept. 5, 2009. No animal at the zoo was more beloved than this amazing rhino who contributed more to saving her species than any other Sumatran rhino in the world. Because Emi was so docile and amiable, CREW scientists were able to study her in depth and unravel the mysteries of Sumatran rhino reproduction which led to our successful breeding program...the only one in the world. Although she left us too early, Emi's legacy lives on in her three beautiful healthy calves. Emi's first born, Andalas, was returned to Sumatra to serve as the catalyst for a breeding program in the species' native land. Her only daughter, Suci, remains at the Cincinnati Zoo and is fast-approaching puberty and her youngest son, Harapan, is growing up in Florida at the White Oak Conservation Center. Those of us who had the privilege to work with Emi will never forget her playful personality, her spirit when facing her mate, Ipuh, or the care she demonstrated when raising her calves. Emi may have left us too early, but the memories she created will last a lifetime and will provide endless inspiration to those of us striving to save the Sumatran rhino.



Photo by National Geographic, Robert Clark

Propagating Some of Nature's Most Intricate, Endangered Beauties

Many people are fascinated by orchids. Their elaborate flower structure and unique relationships with pollinators have made them objects of beauty and scientific study. An orchid is a species within the Orchidaceae, the largest family of flowering plants encompassing over 30,000 species, many of which are adapted to unique ecological niches and endangered from habitat loss and over-collecting.

At CREW, the Plant Research Division is developing and implementing propagation protocols for several rare orchids including three species from south Florida: Carter's orchid (*Basiphyllaea corallicola*), Cowhorn orchid (*Cyrtopodium punctatum*), and Young palm orchid (*Tropidia polystachya*). The tiny seeds of these orchids have been cultured at CREW for germination and use by collaborators at Fairchild Tropical Botanic Garden. One orchid pod may contain thousands of tiny orchid seeds devoid of food reserves. In nature, these seeds receive nutrients through fungal associations, but in the



Cowhorn orchid

lab, seeds are germinated under sterile conditions on a nutrient medium. Both the Carter's orchid and the Cowhorn orchid have germinated readily in culture producing many seedlings, but the Young palm orchid has proven more challenging, and different methods must be tested when more seed is available.

CREW's scientists have also successfully propagated two local orchid species, the Kentucky lady slipper (*Cypripedium kentuckiense*) and Showy lady slipper (*Cypripedium reginae*). The latter is not federally endangered, but was last seen in Hamilton County in the mid-1800s. CREW is working with the Hamilton County Parks to propagate and re-establish this beautiful species in the county for all to enjoy. However, beauty is in the eye of the beholder, and CREW is equally concerned about less visibly striking species on the brink of extinction. After all, concern for biodiversity is blind, but don't tell the exquisite orchids.

Suddenly Awash in Fishing Cats



Fishing cat kittens at the Cincinnati Zoo.

In 2003, Thai field ecologist Passanan (Namfon) Cutter began searching diligently for any evidence of fishing cats (*Prionailurus viverrinus*) in the last remaining wetland areas of Thailand. A mere four years later, she finally obtained her first camera trap photograph of a wild fishing cat. Not a terribly auspicious beginning for this aspiring Master's degree student at the University of Minnesota but, more ominously, also a likely reflection of the increasing scarcity of this aquatic cat species in the wild. In early 2009, Namfon expanded her field survey to Sam Roi Yot National Park using 10 new camera traps provided by CREW. Over a four month period, she obtained photographs of 15 individual fishing cats at this new site and, with assistance of several Thai veterinarians, captured and anesthetized six cats for placement of radiocollars. In fact, this unexpected abundance of fishing cats outstripped her ra-

diocollar supply, requiring an emergency infusion of equipment funding from U.S. zoos. Monitoring of these radiocollared cats is allowing Namfon to conduct the first comprehensive ecological assessment of this endangered species in Southeast Asia (see www.fishingcatproject.info for more details). Locally, the Cincinnati Zoo & Botanical Garden has experienced its own fishing cat population surge with the birth of three kittens in the Cat House; the first fishing cat kittens born here since 1993. The kittens' father and mother are descended from wild Thai and Cambodian fishing cats, respectively, making these three kittens extremely valuable genetically as the distant 'out-of-town' relatives of Namfon's radiocollared cats.

Pallas' Cats, Toxoplasma and the Island of Mongolia

Mongolia is a land of extremes. This Central Asian country, with the lowest human population density in the world, is characterized by rolling steppes with an average altitude of ~4000 feet, frigid winters (as low as -40°F) and very little rain (less than 12 inches annually), and is inhabited by a diversity of rare wildlife, including the Pallas' cat (*Otocolobus manul*). Having evolved in this extreme environment, Pallas' cats are undoubtedly unique as the only species in their genus. They also are peculiarly susceptible to dying of toxoplasmosis, caused by a normally harmless parasite (*Toxoplasma gondii*) of domestic and most nondomestic cats. In recent CREW research, we have been delving deeper into the causes of this unusual susceptibility. In June 2009, we obtained blood samples from 150 Mongolians, 147 domestic sheep and goats and 45 wild rodents in Mongolia to evaluate for antibodies against *Toxoplasma*. Amazingly, not a single sample tested positive; in the U.S., similar studies typically find 20-30% of humans, 30-70% of sheep and goats, and 10% of rodents have anti-*Toxoplasma* antibodies. These findings suggest that Mongolia is most similar to several island nations, such as Madagascar and Australia, where a historical absence of cats prevented *Toxoplasma* from ever becoming established, resulting in the native lemurs and kangaroos having little natural resistance. The Mongolian extremes (high altitude, cold temperatures, minimal moisture) combined with a lack of domestic cats and limited numbers of wild cats had a similar biogeographical impact – creating what is essentially a *Toxoplasma*-free island in the heart of Asia. Because Pallas' cats have been rarely, if ever, exposed to this parasite in the wild, they, like the other island species, never developed innate immunity and remain extremely vulnerable to toxoplasmosis as a consequence.



Dr. Bill Swanson collecting blood samples from goats in Mongolia.

SAVING SPECIES WITH SCIENCE®

Samples preserved in CREW's CryoBioBank represent valuable genetic material from species and individuals across the globe.



Cumberland Sandwort



Endangered Plants



Black Rhino



Guenon



Indian Rhino



Sand Cat



Pallas' Cat



Fishing Cat



Asian Small Clawed Otter



Sumatran Rhino



River Otter



White Rhino



Okapi



Black-Footed Cat



Brazilian Ocelot



Gorilla



Cheetah



CINCINNATI ZOO & BOTANICAL GARDEN



CryoBioBank -196 L
-320 F

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Project list

Africa

- African Violet
- Black-Footed Cat
- Black Rhinoceros
- Cheetah
- Guenon and Mangabey
- Lowland Gorilla
- Okapi
- Sahelo-Saharan Antelope
- White Rhinoceros

Asia

- Asian Small Clawed Otter
- Fishing Cat
- Indian Rhino
- Pallas' Cat
- Sand Cat
- South East Asian Flying Fox (Megabat)
- Sumatran Elephant
- Sumatran Rhino

Central/South America

- Brazilian Ocelot and Tigrina
- Blue-throated Macaw
- Chilean Penguin
- Giant Otter
- Blue and Gold Macaw
- Spotted-neck Otter

North America

- Alaskan Seabirds
- Autumn Buttercup
- Black Warrior Waterdog
- Cumberland Sandwort
- Endangered Plants
- Florida Manatee
- Florida Rare Plants
- Grand Valley Reclamation Project
- Local Flora
- Mill Creek Restoration
- Northern Wild Monkshood
- Polar Bear
- River Otter

● Species in the CryoBioBank



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.



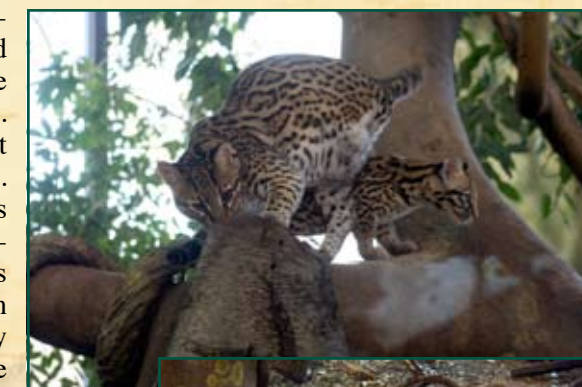
If you are interested in learning what it is like to work at CREW, there are a couple of popular CREW education offerings you and your family may want to consider. *Scientists for the Future* is an overnight program for



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).



BOOK CHAPTERS

Pence VC, S Murray, L Whitham, D Cloward, H Barnes and R Van Buren. 2008. Supplementation of the autumn buttercup population in Utah, USA, using in vitro propagated plants. In: Soorae PS (ed.). Global Re-introduction Perspectives. Abu Dhabi, UAE: IUCN/SSC Re-introduction Specialist Group; Pp. 239-243.

PEER-REVIEWED PUBLICATIONS

Bateman HL, JB Bond, M Campbell, M Barrie, G Riggs, B Snyder and **WF Swanson**. 2009. Characterization of basal seminal traits and reproductive endocrine profiles in North American river otters and Asian small-clawed otters. *Zoo Biology* 28: 107-126.

Steinetz B, S Lasano, F de Haas van Dorsser, S Glickman and **W Swanson**. 2009. Relaxin concentrations in serum and urine of endangered and crazy mixed-up species: new methods, uses and findings. In: Bryant-Greenwood GD, CA Bagnell and AD Bathgate (eds.). Relaxin and Related Peptides: Fifth International Conference. *Annals of the New York Academy of Sciences* 1160: 179-185.

Willerslev E, M Thomas, P Gilbert, J Binladen, SYW Ho, PF Campos, A Ratan, LP Tomsho, RR da Fonseca, A Sher, TV Kuznetsova, M Nowak-Kemp, **TL Roth**, W Miller and SC Schuster. 2009. Analysis of complete mitochondrial genomes from extinct and extant rhi-

noceroses reveals lack of phylogenetic resolution. *BioMed Central Evolutionary Biology* 9:95 (Pp. 1-11).

SCIENTIFIC PRESENTATIONS

Conforti VA, CH Adania, PG Gonzalez, C de Oliveira and **WF Swanson**. 2009. Novel recipient synchronization regimens for successful embryo transfer in the Brazilian ocelot following long-term frozen embryo storage. Proceedings of the International Embryo Transfer Society Annual Meeting. *Reproduction, Fertility and Development* 21:176-177. Poster presentation, San Diego, CA.

Pence VC. 2009. In vitro propagation and cryopreservation of south Florida endangered ferns: *Asplenium verecundum*, *Lomariopsis kunzeana*, *Thelypteris patens*, and *Trichomanes punctatum* var. *floridanum*. In *Vitro Cellular and Developmental Biology* 45: S66. Poster presentation, Society for In Vitro Biology, Charleston, SC.

Roth TL, DC Szymanski and ED Keyster. 2009. Effects of age, hormones and hibernation on breeding success in boreal toads (*Bufo borealis*). Proceedings of the International Embryo Transfer Society Annual Meeting. *Reproduction, Fertility and Development* 21: 181. Poster presentation, San Diego, CA.

Stoops MA, L Vollmer and TL Roth. 2009. Faecal steroid analysis for monitoring repro-

ductive function in polar bears (*Ursus maritimus*). *Reproduction, Fertility and Development* 21: 182. Poster presentation, San Diego, CA.

GRANTS AWARDED

Funding Source: Institute of Museum and Library Services. Project: Improving the Environment for Five Endangered Species Maintained in Tissue Culture at CREW. Role: Principal Investigators. Duration: 7/1/09 – 6/30/11. Amount: \$150,000.

Funding Source: U.S. Army. Project: Genetic Analysis and Cryopreservation of the Endangered Todsens's False Pennyroyal. Role: Principal Investigators. Duration: 7/1/09 – 6/30/12. Amount: up to \$50,000 total.

CREW Wish List

Plate Washer

As we start construction this year on our offsite Small Cat Reproduction Center (SCaRCe), we will need to begin acquiring equipment for its gamete biology and endocrine laboratories. One essential item needed for the SCaRCe endocrine lab is an automatic plate washer to rinse the plastic 96-well plates used during cat hormone assays. Cost: **\$1000**.

Large Capacity Bench Top Centrifuge

CREW's endocrinology lab is busier than ever with more than 1000 fecal samples processed for hormone analysis each month in species ranging from ocelots to polar bears to rhinos. A centrifuge is essential in this process, and the resulting data are critical to our reproductive studies and for assisting the zoo in its animal breeding efforts. Cost: **\$3,000**.

Fume Hood Filter

Fume hoods in CREW's laboratories protect the working scientists and help keep valuable plant and animal samples free from contamination. Over time, the filters in these hoods need to be replaced so that their efficacy is not compromised. Cost for filter and installation: **\$1,000**.

Data Loggers

Understanding the environment of plants is critical to adapting tissue culture and acclimatization conditions for endangered plants at CREW. Data loggers provide information on temperature and humidity conditions of plants at their exact locations in the wild. We would like three units. Cost: **\$200 per unit**.

Polar Bear Challenge

Our Polar Bear Challenge does not include a very cold winter day, a swimsuit and a cold pool to jump in, but what it does involve is your investment in CREW. We want you to be part of one of the biggest fundraising challenges we have faced to date. We are literally racing against the clock to raise funds to match a challenge grant awarded to us from The Shumaker Family Foundation. **The goal: \$35,000. The deadline: December 15, 2009.** As a world leader in reproductive science, CREW is doing amazing research that could improve the welfare of polar bears while facilitating the breeding program for this endangered species. If we succeed in meeting our polar bear challenge, the new funds will

allow us to expand our research effort and offer education programs about polar bears, but **we need your help**. Please consider making a gift of \$50 or more to CREW's Polar Bear Challenge. If you have any questions or would like additional information, contact Nita Douglas, (513) 487-3327 or e-mail to: nita.douglas@cincinnati-zoo.org. On behalf of the bears, we thank you in advance for helping us meet the challenge.



Want to Help Save Endangered Cat Species? Adopt a CREW Cat

The domestic cat is the most popular companion animal in the U.S. with more than 75 million cats living with their human caretakers. After barely 5000 years of domestication, domestic cats still share many physical and physiological characteristics with their wild cousins. For that reason, domestic cats at CREW serve as an invaluable model for our studies to better understand cat reproduction, improve natural breeding and develop assisted reproductive methods to help conserve endangered cat species. Domestic cats at CREW are provided with the best of care and many are adopted out to loving homes after two to three years in the cat colony. Some of our reproductive studies result in offspring so both kittens and adult cats are frequently in need of homes. For example, in our ongoing study testing a



Kayleen



Leah

Two of several available SFF (single furry female) - likes other cats, belly rubs and purring in your ear, and would love to be your future house cat.

contraceptive vaccine for controlling feral cat populations, we anticipate the birth of several litters of kittens in our non-contracepted control group. CREW cats are vaccinated against rabies and other common diseases and are neutered (if old enough) before adoption. These cats are well-adapted as indoor house cats and are incredibly affectionate and sociable, as dozens of Zoo staff and volunteers can attest after adopting CREW cats themselves. If you want to help CREW to conserve endangered cats, why not consider adopting one of the domestic cats that has contributed so much to our progress? If interested, please contact Jackie Newsom at CREW 513-569-8233 or jackie.newsom@cincinnati-zoo.org.

Join CREW in A Toast to the Wild

Due to the growing popularity of Wild about Wine and Zoo Brew, 2009 introduced A Toast to the Wild, sponsored by Key Bank and featuring five great events with all proceeds benefiting CREW. Zootini, a martini tasting event, kicked off A Toast to the Wild. This new event featured eight specialty martinis created by EQ the Cooking School @ The Party Source to highlight CREW's research and conservation success stories. Three Wild About Wine events were held throughout the summer and featured Constellation Brands wines sponsored by Kroger. We ended our tasting series with our ever popular Zoo Brew featuring Sam Adams brews. Beyond great martinis, diverse



wine selections and unique brews from Sam

Adams, each event features a great location in our Zoo, lots of animals, CREW scientist encounters, light appetizers from local area restaurants and live entertainment from a band or two. In 2009, the fast rising Irish band, The Script, gave an acoustical performance at Zootini to kick off A Toast to the Wild series and helped promote our Rhino Signature Conservation Project at the same time. The 2010 Wild about Wine evenings at the Cincinnati Zoo are scheduled for: May 6th, June 24th and Aug. 26th, with Zootini on March 18th and Zoo Brew on Oct. 14th. Advance tickets will go on sale in December. Be sure not to miss this wonderful evening event series by purchasing advance tickets online: www.cincinnati-zoo.org.



The Script vocalist, Danny O'Donoghue, promoted CREW's efforts to save rhinos during the band's guest appearance at the first 2009 Wild about Wine event.



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