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MEETING REVIEW

Cryobanking of viable biomaterials: implementation of new strategies for conservation purposes

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Abstract

Cryobanking, the freezing of biological specimens to maintain their integrity for a variety of anticipated and unanticipated uses, offers unique opportunities to advance the basic knowledge of biological systems and their evolution. Notably, cryobanking provides a crucial opportunity to support conservation efforts for endangered species. Historically, cryobanking has been developed mostly in response to human economic and medical needs — these needs must now be extended to biodiversity conservation. Reproduction technologies utilizing cryobanked gametes, embryos and somatic cells are already vital components of endangered species recovery efforts. Advances in modern biological research (e.g. stem cell research, genomics and proteomics) are already drawing heavily on cryobanked specimens, and future needs are anticipated to be immense. The challenges of developing and applying cryobanking for a broader diversity of species were addressed at an international conference held at Trier University (Germany) in June 2008. However, the magnitude of the potential benefits of cryobanking stood in stark contrast to the lack of substantial resources available for this area of strategic interest for biological science — and society at large. The meeting at Trier established a foundation for a strong global incentive to cryobank threatened species. The establishment of an Amphibian Ark cryobanking programme offers the first opportunity for global cooperation to achieve the cryobanking of the threatened species from an entire vertebrate class.

Keywords: amphibian conservation, conservation biology, cryobanking, endangered species, genome resource bank, viable biomaterials

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Report

Almost 17 years after the 1992 United Nations Conference on Environment and Development in Rio de Janeiro and ratification of the Convention on Biological Diversity (CBD), we now realize that the 2010 Target to achieve a significant reduction of the current rate of biodiversity loss at the regional, national and global level cannot be reached. Important biological information disappears forever with the loss of each species. However, if we are to be successful in preserving a mere handful of critically endangered species, it is clear that they will need to be intensively managed using a variety of *in situ* and *ex situ* approaches. Hence, more action and, especially, more strategies are needed that involve an alliance of all stakeholders.

These strategies span different approaches. A fundamental cornerstone in conservation is the identification of genetic lineages at different taxonomic levels. Short-term evolutionary consequences for rare or threatened species can be identified by recent and historic DNA samples to map the distribution of contemporary and past levels of genetic variation (Metcalf *et al*. 2007). The management of small populations of endangered animals, in both *in situ* or *ex situ* programmes, needs information on the genetic diversity of current populations (Leberg & Firmin 2008; Morgan *et al*. 2008). To support conservation breeding programmes, a genetically representative store of viable germ cells is needed to ensure the maintenance of the genetic diversity of the source population within the conservation breeding populations. Hence, with respect to the high number of threatened species, there is an immense need for the information that can be extracted from biological samples, and a need for germ cells themselves in managing small populations. But how can all this be handled?

In this context, the challenges of establishing a network for conservation purposes, and of developing and applying cryobanking technology for a broader diversity of species, were addressed at an international conference held at Trier University (Germany) in June 2008. Researchers from zoos and experts in stem cell research, cryopreservation technology and conservation biology discussed how cryobanking could be organized on a global scale to support the ongoing efforts to sustain genetic diversity.

Most *ex situ* approaches that focus on supporting *in situ* conservation measures via captive breeding rely on viable samples (Ehmcke & Schlatt in press; González *et al*. 2008). Access to high-quality biomaterials is a major obstacle that limits the success of these projects and this topic was vigorously discussed at the conference. Specimens that are traditionally stored in alcohol or that find their home as animal preparations in museums are not adequate for use in reproduction or in studies that answer questions arising in genomics, proteomics, gene regulation and transcriptome processes. Alternatively, cryopreservation of samples, a process where specimen are stored at temperatures below –146 °C and therefore are almost protected from chemical and physical changes, has huge potential to satisfy the requirements of modern biology and *ex situ* conservation efforts. Thus, viable cryopreserved cell lines set up from small biopsies contain the whole genome and proteome that is required in modern biological research. They can also be sampled in a nondestructive way by regenerating the material whenever it is used and therefore provide an almost infinite source of material for retrospective studies (Watson & Holt 2001). These abilities were clearly demonstrated at the conference in discussions of already established cryobanks like the global Frozen Ark Project (Nottingham, UK) (Clarke 2009), its participant the Frozen Zoo (San Diego, USA) (Ryder 2005) and other projects (Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany; Cryo-Brehm, Lübeck, Sulzbach, Germany).

Assisted reproductive technologies like artificial insemination, *in vitro* fertilization and embryo transfer are already vital components of conservation breeding programmes (reviewed in Holt & Pickard 1999). Here, cryobanking of gametes and embryos offers the possibility to exchange genetic information within and between populations although the donor is already dead. This will increase the genetic pool of *in situ* populations and will help to counter the loss of genetic diversity, and therefore inhibits the potential tendency towards the loss of fitness through inbreeding effects. The once critically endangered black-footed ferret, *Mustela nigripes,* and the wild European mouflon, *Ovis orientalis musimon,* are remarkable examples of collaboration between conservation biologists, reproductive physiologists and cryobiologists. Here, cryobanked samples helped to establish new founder populations to manage these populations, and subsequently to reintroduce them into the wild to support *in situ* measures (Miller *et al*. 1996; Ptak *et al*. 2002). Nevertheless, the question arises: is cryobanking practical for conservation measures considering the wide range of species?

With this in mind, disadvantages of different freezing methods, sample types and their potentials were discussed. Participants of the workshop agreed that, although a lot of protocols already exist for making cryopreservation feasible for the huge set of endangered species, many questions still remain unanswered.

To maximize the potential of cryobanking, the participants pointed out that standardized sampling procedures and preservation techniques should be applied, and stored samples should be supported by an accessible database. Besides all the scientific problems, fundraising, biosafety and quarantine, current regulatory requirements of CBD and CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), as well as the sharing of information and benefits were considered.

A special roundtable discussion focused on the urgent need to cryobank viable nuclei or cells for amphibian conservation breeding programmes since they are one of the most threatened groups of vertebrates (more than 500 species are expected to require conservation breeding programmes; Mendelson *et al*. 2006). The Amphibian Ark is a joint effort of WAZA (World Association of Zoos and Aquariums), IUCN/SSC Conservation Breeding Specialist Group (CBSG) and IUCN/SSC Amphibian Specialist Group (ASG). It implements the *ex situ* components of the IUCN Amphibian Conservation Action Plan (Gascon *et al*. 2007) and recognizes cryobanking as an essential technique to support conservation and associated research. The first step to developing a network for the cryobanking of amphibians was the establishment of an Amphibian Ark Cryobanking Advisory Committee (AACAC), which has been launched by the participants of the Trier roundtable discussion.

As the Trier conference revealed, many biological samples are already stored in cryobanks around the world, but most of these banks have been established to serve the research interests of the respective founder institutes. It has been pointed out that, although there is a general agreement that stored biological materials are needed and that organized cryobanking would help global scientific research activities, the extent of efforts that focus on strategic banking is clearly inadequate. Therefore, all participants of the Trier meeting clearly agreed that viable biomaterials have to be preserved for future generations for ethical and beneficial purposes. To further these principles in theory and practice, a subsequent meeting is planned in the third quarter of 2009. One goal of this meeting will be to broaden the range of participants and to extend the global reach of the cryobanking network.

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Dominik Lermen studied Applied Environment Sciences. He is now PhD student at the department of Biogeography at Trier University and is working on the development of protocols to isolate and cryopreserve skin derived stem cell like cells of endangered mammals to support conservation measures. His main scientific interests are conservation biology, ecology, cryobiology and cell biology with focus on the in vitro differentiation potential of adult stem cells. Brunhilde Blömeke is interested in the impact of cryopreservation on viability of immune cells. Robert Brown's research focuses on the development of reproduction technologies for amphibian conservation. Ann Clarke is interested in the immunological relationships between the mammalian mother and her embyro and the long term preservation of genetic resources from endangered animals. Paul W. Dyce is biochemist. His research interests focus on the differentiation potential of somatic stem cells in vitro. Thomas Fixemer is involved in the 'Cryo-Brehm' project. Günther Fuhr leads the 'Cryo-Brehm, the German Biobank for Wild Animals' which is based on the stem cell and cryotechnology research for the long term storage of viable multipotent cells of endangered species. William V. Holt's research interests focus on the ability to store spermatozoa and its use in captive breeding programmes further in understanding the cellular mechanisms involved in the processes which lead to fertilisation. He is involved in many international efforts to promote genome resource banking for conservation. Katarina Jewgenow is interested in reproduction biology of non-domestic animals, in particular gametogenesis in felid species and contraception of wildlife and feral animals. Rhiannon E. Lloyd's research focuses on the development of assisted reproduction techniques (ART), including biomaterials banking for amphibians, the study of sperm-oviduct interactions in mammals from both applied and theoretical perspectives, and the study of the regulation of mitochondrial DNA transmission, and its effects on development, following ARTs. Stefan Lötters is interested in amphibian biogeography and conservation. Martin Paulus is interested in biomonitoring an environmental Specimen Banking. Gordon McGregor Reid is interested in conservation of threatened, Red-listed animals through zoo and aquarium breeding programmes and fieldwork as well as the preparation and preservation of biological materials in support of this. Daniel H. Rapoport's personal research interests concern the development of new technologies for analyzing, handling, storing and propagation of stem cells and other complex cell culture systems. David Rawson research focuses on the development of protocols for the cryopreservation of embryos and gametes of fish and lower vertebrates, and on the impact of cryo-protocols on cellular function and genetic expression. Jennifer Ringleb's research focuses on the preservation of gametes from felid species, in-vitro techniques and cryobanking. Oliver A. Ryder leads San Diego Zoo's effort to preserve frozen, viable cell cultures from threatened and endangered species (The Frozen Zoo®), that now encompasses more than 8,600 individuals of over 700 vertebrate taxa. Gabirele Spörl is involved in cryobiological fundamental research according to the development of cryoprotocols for amphibian eggs. Thomas Schmitt's research interests are molecular and classical biogeography as well as ecology, evolutionary biology, taxonomy and conservation biology. Michael Veith's scientific focus is on ecology, evolution and conservation. Paul Müller's broad scientific interests include conservation biology and cryobiology. Thomas Fixemer is involved in the 'Cryo-Brehm' project and supports the conservation of threatened farm animals.