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Genome Resource Banks

Living collections for biodiversity conservation

David E. Wildt, William F. Rall, John K. Critser, Steven L. Monfort, and Ulysses S. Seal

David E. Wildt (e-mail: dewildt@shentel.net) is program head and a codirector of NOAHS (New Opportunities in Animal Health Sciences), and Steven L. Monfort is an endocrinologist and research veterinarian in the Reproductive Physiology Program, at the Conservation & Research Center at the National Zoological Park, Smithsonian Institution, Front Royal, VA 22630. William F. Rall is a cryobiologist in the Embryo Cryopreservation Unit of the National Center for Research Resources at the National Institutes of Health, Bethesda, MD 20892-5590. John K. Critser is scientific director of the Cryobiology Research Institute at the Advanced Fertility Institute, Methodist Outpatient Center North, Indianapolis, IN 46202. Ulysses S. Seal is chairman of the Conservation Breeding Specialist Group of the IUCN-World Conservation Union's Species Survival Commission, Apple Valley, MN 55124.

Biological diversity is the key to maintaining life as we know it (Wilson 1992). However, rapidly growing human populations place extraordinary pressures on ecosystems, such as large-scale environmental destruction, habitat conversion, habitat fragmentation, and pollution. One reaction to these problems has been the emergence of conservation biology, an assemblage of scientific disciplines that are focused on sustaining biodiversity through a cooperative synthesis of ideas, information, and approaches.

Virtually all conservation biologists agree that habitat preservation is the best way to conserve biodiversity. Setting aside large tracts of land so that they are free from human interference can protect many species, but this approach has also been characterized as "quaint" given the realities of the current and future world (Soulé 1992). Some battles for maintaining large, protected ecosystems have already been lost. One species that has been adversely affected by habitat loss is the tiger (*Panthera tigris*): Of the eight tiger subspecies that were recognized as endangered in 1969 by the IUCN-World Conservation Union, three (*Panthera tigris vigata, P. t. sondaica,* and *P. t. balica*) are now extinct, and one (*P. t. amoyensis*, the South China tiger) is critically endangered, with fewer than 80 individuals remaining in the wild (Table 1; Jackson 1993). Moreover, fewer than 400 Siberian tigers (*P. t. altaica*) exist in isolated pockets of eastern Russia, northeast China, and North Korea (Jackson 1993). Fewer than 500 wild Sumatran tigers (*P. t. sumatrae*) live on Sumatra, largely in five fragmented protected areas (Tilson and Brady 1992).

Table 1. Estimated numbers of tigers in nature and in zoos.

Panthera tigris **subspecies**	**Nature**[a]	**Zoos**
P. t. altaica	250-400	623[b]
P. t. sumatrae	<500	245[b]
P. t. tigris	3000-5300	297[b]
P. t. amoyensis	30-80	50[c]
P. t. corbetti	800-1400	68[d]

[a]Jackson 1993
[b]Mueller and Wesul 1995
[c]Yinghong 1995
[d]Tilson et al. 1995

Approximately 250 tigers are needed in a population to sustain adequate genetic diversity, which is defined as 90% of the current gene variation for the next 100 years (Foose 1987). But achieving this target for tigers in nature would require a habitat free of human disruption that is at least 25,000-50,000 km^2 (Foose 1987), or approximately the combined areas of Maryland and New Jersey. The reality for tigers and many other species is that there is too little contiguous space left to sustain free-living populations. As habitats become smaller and more isolated, the species occupying these habitats also become more vulnerable to inbreeding depression, disease epidemics, natural disasters, and social and political change.

Other approaches for helping to preserve biodiversity have been proposed. Soulé (1991) has described a "biospatial hierarchy" to protect biodiversity, beginning at the top with whole ecosystems (in situ protection) and proceeding down through communities, species, populations (ex situ zoo breeding programs), and eventually to cryobanked biomaterials. In this article, we refer to this last strategy as *genome resource banks* (GRBs), which are repositories of systematically collected germ plasm (gametes), embryos, blood products, tissue, and DNA for defined conservation programs.

Existing genome resource banks

The concept of GRBs is not new. Already, biomaterials from farmed crop plants and animals are stored systematically, a development driven largely by economics and by the desire to ensure secure food sources. Large-scale, organized repositories of germ plasm are more common for plants than for animals, in part because it is technically easier to store plant than animal germ plasm (i.e., seeds of many plants can simply be dried and stored in refrigerators at 4 °C or -20 °C; Wildt 1997). Animal agriculture has, however, benefited from large-scale sperm and embryo cryostorage for the purpose of improving meat and milk production in domestic livestock. In addition, a few programs bank biomaterials from animal models used in biomedical research to protect the long-term availability of standard genotypes of mice, rats, hamsters, rabbits, cats, and dogs, ensuring that researchers are working with uniform animal models that give repeatable results.

Transgenic technology (the ability to incorporate novel genes into injected embryos) has also resulted in the production of thousands of new animal models. Although this technology has exponentially increased research opportunities, the byproduct is a proliferation of animal colonies that require expensive housing and care. One solution is to cryopreserve sperm and embryos from genotypes that are not in use currently but are potentially valuable in the future. The Jackson Laboratory in Bar Harbor, Maine, and the National Institutes of Health in Bethesda, Maryland, collect and cryostore embryos and, occasionally, sperm from animal models, usually rodents. Customized collections have also been developed for microorganisms that are used in the environmental, food, and biomedical industries to produce products valued at tens of billions of dollars (Cunning-ham 1994). The American Type Culture Collection (ATCC) in Rockville, Maryland, is the research community's leader in cold storage, with more than 80,000 cultures of algae, protozoa, bacteria, bacteriophages, cell lines, hybridomas, fungi, yeasts, recombinant DNA materials, and viruses available for worldwide distribution.

Reproductive technologies associated with GRBs

The usefulness of banked germ plasm and embryos from humans, livestock, and laboratory animals depends on assisted reproduction procedures, which include tools such as artificial insemination (AI), embryo transfer, and in vitro (test-tube) fertilization (IVF). These procedures are sometimes enhanced by other techniques, such as intracytoplasmic sperm injection (ICSI), in which an individual sperm cell is microinjected into an oocyte to create an embryo. Because a single semen sample often contains millions (or billions) of sperm, this sample has almost unlimited fertilization potential, assuming that oocytes are available and that ICSI is successful.

The ability to artifically inseminate a female to produce young was demonstrated more than 200 years ago by the Italian priest Spallanzani, using the dog (Asdell 1977). But AI is more complicated than merely randomly placing sperm into a female. Basic knowledge of reproduction and timing are essential. One must know, for example, when a female ovulates and where in the reproductive tract is the most appropriate site to deposit sperm. Using cryopreserved sperm adds more challenges. Gametes are delicate, water-filled cells, and if their structure and function are to survive cryopreservation, they must be properly dehydrated and rehydrated through the use of cryoprotectants (compounds that stabilize cell membranes) and precise cooling and thawing rates that minimize ice-induced cell lysis. Sperm cryopreservation procedures must be modified and optimized for each species (Watson 1995).

Nevertheless, AI is simpler than other reproductive techniques. Embryo transfer involves the collection of embryos from a donor female (either after mating or after AI), followed by the implantation of the embryos into a surrogate female at the appropriate stage of her reproductive cycle. The benefit of embryo transfer is, in theory, that a hormone-stimulated donor will ovulate many more ova than normal, most of which have the potential of becoming embryos. These embryos can be frozen or immediately transferred to one or more surrogates that gestate the embryos to term, with the offspring having the original donor's genotype. For example, a cow usually produces only one calf per pregnancy. However, a genetically valuable female that is given hormones can produce many embryos, each of which can develop in the reproductive tract of a less valuable cow. By avoiding pregnancy, the original donor can be used to produce even more embryos to rapidly proliferate her valuable genes. The significant challenges to cryo-preserving embryos include determining both the embryonic developmental stage that is most likely to withstand freezing, as well as the best cryoprotectants and optimal rates of cooling and thawing to avoid membrane lysis and embryo death.

Even more complex is IVF, whereby sperm are mixed in a culture dish with oocytes that are aspirated directly from a female's ovaries using a needle guided by fiber optic or ultrasonic techniques. Given the appropriate culture conditions, any embryos that result can then be either cultured and transferred to a recipient host or cryopreserved for later transfer. The challenges are similar to embryo transfer except that additional knowledge is required to safely collect good-quality oocytes and sperm and appropriately process them to stimulate fertilization and development.

In theory, it should also be possible to store unfertilized oocytes. However, unfertilized oocytes are much harder to cryopreserve than embryos because the haploid female gamete is highly susceptible to chilling injury and cryoprotectant toxicity (Parks and Ruffing 1996). This susceptibility is thought to be associated with the unique characteristics of mature oocytes, namely their large size and the presence of a meiotic spindle and cortical granules in the ooplasm (Candy et al. 1994).

Application of GRBs to wildlife species

Given the historical success of storing and using biomaterials from livestock and the growing popularity of this approach for management of laboratory animals and microorganisms, it is natural to consider the potential advantages of cryobiology for protecting and conserving wildlife. Technological advances once used only for humans and domestic animals are now finding relevance for wild animal species and are increasing the need for the systematic collection and storage of biomaterials. Organized banking of biomaterials from wildlife species could provide important benefits and options to biotic managers.

Easy, inexpensive movement of genetic material among living populations. One goal of conservation managers is to maintain healthy, genetically diverse animal and plant populations. GRBs can mitigate the effects of unnatural selection pressures, genetic drift, and inbreeding depression by providing a source of germ plasm (i.e., of new genes) that can be infused into small or fragmented populations. For example, rather than transporting stress-sensitive wild animals from one site to another, genetic heterogeneity could be maintained by shipping germ plasm or embryos. It may also be technically feasible to artificially inseminate free-living females with sperm from males of other wild, or even captive, populations. The latter approach has already been made part of the conservation management plan for Sumatran tigers living in highly fragmented Indonesian habitats (Tilson and Brady 1992). Moreover, a GRB could reduce or eliminate the need to remove animals from the wild to support captive populations. Instead, "surplus" germ plasm would be collected, leaving the entire wild population in nature, where its presence helps to protect native habitat.

Insurance. Small populations are vulnerable to environmental catastrophes, political upheavals, and disease outbreaks. The detrimental effects on small populations of perturbations ranging from oil spills to wars to viral epidemics are well known. For instance, the black-footed ferret (*Mustela nigripes*) nearly became extinct in the American West, first due to extermination of its prey (prairie dogs) by ranchers and then to an epizootic outbreak of canine distemper in the 1980s (Seal et al. 1988). In retrospect, a random sampling of germ plasm from the wild population before the disease epidemic could have saved more of the original genetic diversity, which is now lost forever. The black-footed ferret is not unique-most of Earth's biodiversity exists in habitats that are sensitive to epizootic disease outbreaks and drastic shifts in human social and political structure. Systematic "snapshots" of biomaterials would help to preserve extant, wild genetic diversity in a living archive. These biomaterials then could be rederived in emergencies, such as near extinction.

Extended generation interval. Genetic diversity is lost only when animals are no longer available to reproduce (Ballou 1992). As long as viable cryopreserved sperm or embryos remain stored, genes do not die with the animal. Banked germ plasm can be used long into the future. For example, cattle sperm that have been cryopreserved for 37 years retain fertilization capacity (Leibo 1994).

Increased efficiency of captive breeding. Many rare species in zoos are managed strictly to maximize genetic diversity as part of cooperative zoo breeding programs (Hutchins and Wiese 1991). Curators routinely identify desirable animal pairings (e.g., Animal A should be mated with Animal B) on the basis of the need to retain a target level of genetic diversity for the species. However, these individuals will not necessarily be sexually compatible. Like people, nonhuman animals have sexual partner preferences, and it is not unusual for "ideal" matings to fail after animals have been shipped long distances. Therefore, when rare species are bred strictly on the basis of genetics, there will always be a need for assistance, perhaps using cryopreserved germ plasm or embryos and assisted breeding techniques such as AI, embryo transfer, and IVF.

Resolving space problems by reducing the number of living animals needed to meet genetic diversity targets (Ballou 1992). One of the greatest challenges faced by modern zoos is that too little space is available to maintain too many species, subspecies, and populations. Thousands of species deserve attention, but zoos have only enough space to potentially conserve fewer than 1000 unique taxa by conventional breeding programs (Conway 1986). GRBs could make cooperative zoo breeding programs more efficient. For example, as many as 300 animal spaces are needed to retain 90% of the existing diversity in each regional tiger population that is managed by cooperative zoo programs. Using cryo-preserved sperm and AI, only 160 spaces would be needed because the sperm of selected males would be available from liquid nitrogen, rather than from living animals (Johnston and Lacy 1991, 1995). Newly created space could be reallocated to other species in crisis.

A resource for blood, tissue, and DNA. Usually the concept of frozen repositories of biomaterials is associated with germ plasm and embryos. But collecting and storing blood and tissues is also important because these materials can be processed into serum, plasma, blood cells, DNA, and tissue and cell cultures. These biomaterials have wide-ranging applications for studying genetic variation, phylogeny, paternity, and the processes underlying diversity, such as gene flow, selection, and mating. Molecular techniques have been used to address many practical conservation issues, including measuring the extent of hybridization in the endangered Florida panther (*Felis concolor coryi*) and assessing the impact of 200 years of slaughter of humpback whales (*Megaptera novaeanglia*) on levels of modern genetic diversity (O'Brien 1994a, 1994b). All of these analyses required the collection and storage of blood products and tissues. These biomaterials are also important for practical health and survival issues. For instance, blood samples are a resource for developing indices of clinical well-being. Biomaterials that are cryopreserved over time can be screened to identify the onset and cause of a particular epidemic in natural or captive populations. One illustration is the recent epizootic outbreak of canine distemper that was fatal to as many as 30% of the lions (*Panthera leo*) in the Serengeti ecosystem in Tanzania (Roelke-Parker et al. 1996). Because blood samples had been periodically collected and cryopreserved for over a decade, scientists had the raw materials to determine the time between the emergence of this morbillivirus and the onset of lion morbidity and mortality.

Economic opportunities for improving the agricultural economy and the quality of life, especially when wild species hybridize with domesticated livestock. For example, in Southeast Asia wild cattle occasionally leave their dense forest habitat and mate with common domestic cattle to produce hybrid calves (Vietmeyer 1983). The resulting hybrid vigor makes wild cattle species attractive for boosting the quality of local meat-producing stocks. A GRB would not only protect the biodiversity of native wild cattle stocks through the long-term preservation of sperm, but also enhance animal agriculture through the shared use of germ plasm with local cattle producers.

Thus, the benefits of GRBs for wild animals arise largely from the need to effectively manage small populations in nature or in captivity. Scientists routinely survey and collect animals and plants as study specimens. Cryobanked germ plasm, embryos, tissue, blood, and DNA would be another type of collection, but one with considerably more conservation potential than provided by the conventional collections of objects, plants, and animals found in traditional museums, botanical gardens, and zoos (Figure 1).

Germ plasm cryopreservation for wildlife

Several laboratories have proven the biological viability of cryopreserved sperm or embryos for species as diverse as the bighorn sheep (*Ovis canadensis*), the chimpanzee (*Pan troglodytes*), and the giant panda (*Ailuropoda

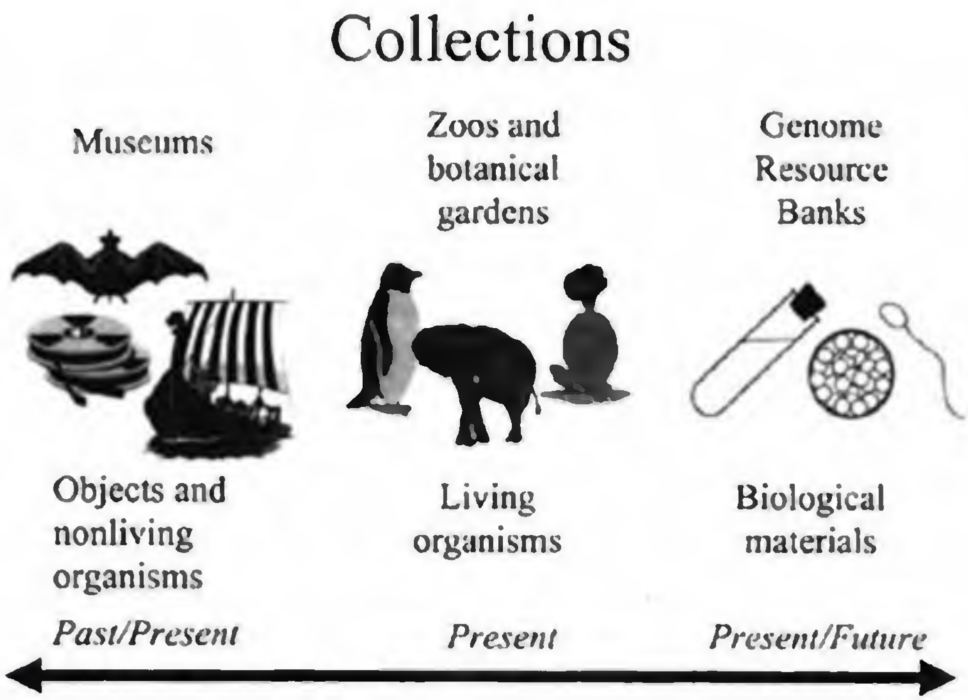

Figure 1: Various collections of biological materials. Whereas traditional museums, zoos, and botanical gardens collect materials from the past and present, genome resource banks (GRBs) offer enormous opportunities for the future.

melanoleuca; Wildt et al. 1993). We have taken this approach further by demonstrating that cryopreserved sperm can be used to help manage small populations. One example is the Eld's deer (*Cervus eldi*), which is endangered throughout its natural range in Southeast Asia (Figure 2). Specialists in assisted reproduction, cryobiology, endocrinology, animal husbandry, veterinary medicine, and behavior collaborated to develop conditions that allow AI to succeed. So far, 15 fawns have been born from AI using cryopreserved sperm from genetically valuable males (Figure 2; Monfort et al. 1993), and half of them have been translocated to the Singapore Zoo to begin a captive breeding and education program in a region that is close to their original habitat.

Figure 2: Eld's deer fawns produced by artificial insemination with frozen-thawed sperm from genetically valuable sires. The Eld's deer is an endangered species that is indigenous to Southeast Asia.

The cheetah provides another example of the use of assisted reproduction to manage a small population. For centuries, this species has reproduced poorly in zoos. More than a decade of reproductive, genetic, and medical studies in the field and in zoos were needed to obtain the essential physiological data to consistently produce cheetah cubs by AI (Howard et al. 1992). Nine cheetah litters have been born from artificially inseminated females, including two recent births in North America sired with sperm from wild-caught males of Namibia; the sperm had been cryopreserved and shipped transcontinentally (Figure 3). Although the sperm donors were released and later found killed, their genes remain available in frozen sperm that is now known to be capable of producing offspring.

The ability to effectively cryopreserve sperm from this species eliminates the need to ever remove another cheetah from the wild to support zoo breeding programs. Meanwhile, the GRB provides a reserve of disease-free gametes that could help to restore the cheetah population in Namibia in the event that a local catastrophe severely depletes the population.

The Eld's deer and cheetah examples are the first indications that reproductive technology can assist in the practical management of endangered species. Until recently, wildlife was rarely propagated successfully by AI, embryo transfer, or IVF. Progress has been slow because reproductive techniques that were developed for cattle or humans cannot be readily applied to animals as different as gorillas (*Gorilla gorilla*), orinoco crocodiles (*Crocodylus intermedius*), and Puerto Rican parrots (*Amazona vittata*). Biological mechanisms regulating reproductive fitness among taxa are diverse, varying greatly even among closely related species (Wildt et al. 1992). The detailed mechanisms of reproduction are known for fewer than 100 species, many of them livestock and laboratory animals. Intensive study of the reproductive biology of wild animals, especially those that are unrelated to laboratory and livestock animals, is needed to generate appropriate basic knowledge so that AI, embryo transfer, and IVF can be used, when necessary, to help manage endangered populations.

Figure 3: Cheetah cub produced by artificial insemination at the Rio Grande Zoo in Albuquerque, New Mexico, with frozen -thawed sperm that was collected from a wild, free -ranging cheetah in Namibia and then transported trans-continentally.

All aspects of reproductive biology require attention, especially the sensitivity of gametes and embryos of different taxa to the stresses of freezing and thawing. In some cases, only minor refinements will be needed to ensure that a cryopreserved sample is viable on thawing. However, many scientific advances and new techniques will be required to overcome inevitable problems. For example, it is necessary to develop the ability to cryopreserve oocytes. And because most gamete biologists are mammal-centric, sophisticated cryopreservation studies need to be extended to the gametes and embryos from taxa whose reproductive biology has been largely ignored, including birds, reptiles, amphibians, fishes, and invertebrates.

Many species will present formidable challenges. For example, AI with frozen-thawed sperm is relatively common in the breeding and management of fish (Harvey 1993), but fish embryos have never been cryopreserved successfully, despite vigorous attempts. Again, basic technological research is needed to provide the answers. For example, magnetic resonance imaging (a technique that is commonly used in the assessment of human health) has recently revealed a major obstacle to the cryopreservation of fish embryos-namely, a multinucleated layer of nonyolky cytoplasm (the yolk syncytial layer) that prevents adequate movement of cryoprotectant compounds into and out of the embryo (Hagedorn et al. 1996).

Practical implementation of GRBs for conservation

Collecting and storing biomaterials from diverse species is one challenge, but their judicious use is quite another. The most serious potential pitfall is the development of spurious, disorganized collections that have no particular conservation purpose and are unlinked to nature. Repositories should also not become static warehouses of biodiversity or, worse, "gene morgues" that are composed of dead or nonviable materials (Goodman 1990).

How can GRBs be implemented for conservation? The Conservation Breeding Specialist Group (CBSG) of the IUCN-World Conservation Union's Species Survival Commission has addressed this question as a part of its overall mission. CBSG, with a worldwide network of 700 members, serves as a neutral catalyst and facilitator for conservation planning for animals, plants, and habitats (and maintains a Web site at http://www.cbsg.org/). CBSG has helped in developing GRB processes in two ways. First, it facilitates workshops in biologically rich regions throughout the world whose participants prioritize those species deserving conservation attention and then recommend detailed strategies to ensure their recovery (Ellis and Seal 1995, Westley and Vredenburg 1997). Thus, CBSG helps to objectively identify species that could benefit from a host of conservation approaches, including repositories of cryopreserved biomaterials. CBSG workshop participants have recommended GRBs for conservation of the cheetah and lion in Namibia and the giant panda in China.

Second, CBSG has provoked international awareness and debate about the usefulness of organized biological repositories as part of conservation planning and action (Bartels and Wildt 1994, Wildt and Seal 1995). A GRB "Action Plan" concept has been formulated to steer stakeholders through the many pitfalls associated with cryobanking activities. Specific guidelines have been developed on writing an action plan (CBSG 1992). Furthermore, a prototype GRB action plan has been developed for the tiger (Wildt et al. 1995), a species chosen because of its precarious status in nature and its charisma (which will attract attention to the process) and because an enormous amount of information is available on the biology and assisted reproduction of the tiger, which would allow a GRB to be effective. This exercise has been useful because it has allowed biologists to identify and address the many complexities that are inherent to developing and implementing a practical repository for any given species.

The action plan is a written document containing explicit information (Figure 4) that justifies the bank on the basis of both in situ and ex situ conservation. It also provides relevant information on species biology (life history and natural reproduction), on animal numbers in the wild and in captivity, and on the accessibility of these animals for donating to the GRB (there is no point to developing a repository if managers are reluctant to allow sampling of biomaterials).

Figure 4: Factors that need to be considered in developing a GRB for an animal taxon or species, with an emphasis on reproductive biomaterials.

One goal of the planning process is to determine the type and amount of biomaterials to collect, store, and use to achieve genetic management targets. For example, how many sperm must be collected from a given male of a given genetic value that is living in a given population? Computer simulations allow many of these issues to be addressed, using information about the reproductive biology of the species combined with specific conservation goals. Such analyses show that even the intermittent, occasional use of frozen sperm can greatly enhance a manager's ability to achieve genetic goals (Johnston and Lacy 1995, Wildt and Seal 1994). These analyses further reveal the need for three banks for each species, one designated an "in perpetuity" repository (for use only when the species approaches extinction), another for routinely managing living animals, both in situ and ex situ, and yet another containing biomaterials available for research (i.e., nonpropagation purposes).

The action plan also deals with the technical aspects of collection, storage and use, research needs, funding of the GRB, and proprietary issues. In the case of ownership of rare biomaterials, participants from the CBSG workshops have agreed that nations have sole responsibility for determining the ownership and value of their genetic resources within international and legal limits. First, a country always has the option of declining to provide samples to any GRB program. Second, biomaterials should ideally be donated to GRB programs, thereby separating the resource from commercial interests. There is reluctance to commercialize genetic materials, the fear being that adding a price to a sperm sample or a vial of DNA will provoke nefarious trade in endangered species products. Third, some proportion of every collection should be placed in a central GRB within the region in which it is collected, and it should be managed by an appropriate species specialist group or management committee. Fourth, ownership of germ plasm and embryos should remain with the owner of the donor animal (i.e., the nation, institution, or individual), unless transferred to a species coordinator or a management committee (the preferred options). Decisions about apportionment of offspring resulting from the use of these biomaterials should be made by the owners of the donor and recipient at the time when the sperm, ova, or embryos are used. Finally, right and title to all other biomaterials (the tissues, blood products, DNA, and other bodily fluids or excretions) should be assigned by the owner of the donor to a regional coordinator or species specialist group that can apportion these materials to those specialists who are best able to generate the knowledge that will contribute to conservation and protection.

Cryoconservation: high priorities for the future

Wide-ranging dialogues, facilitated by organizations such as CBSG, about the ramifications of GRBs and how the process could proceed are a positive first step. For a wildlife GRB to be an effective conservation tool, there must be an organizational configuration that involves all stakeholders, strong science, a written strategy, and global cooperation. Specific, high-priority needs include:

- More knowledge and support. Mastery of the modern use of cryopreserved sperm and embryos in cattle breeding was built on three decades of research involving hundreds of animal scientists, thousands of experimental cattle, and millions of dollars of federal, state, and industrial funding. The burst of growth in strategies to combat human infertility can also be traced to a biomedical community that was both entrepreneurial and responsive to a human health need. Hundreds of fertility clinics emerged that generated massive amounts of new data; the inevitable result has been that many children are born from assisted reproduction. Thus, for both cattle and humans, the success of using cryo-preserved germ plasm was predicated on enormous financial support for basic and applied research.

Similar resources must be applied to high-priority wildlife species. Several conservation models already mentioned (e.g., Eld's deer and cheetahs) have proven the value of multidisciplinary and integrative studies: Technology can be used with some reliability in these species to positively effect management of small populations. One confounding factor will continue to be species-specific differences that almost always will require studies to understand the reproductive biology of both males and females and how their germ plasm and embryos respond to cryopreservation (Wildt et al. 1992). Sperm and embryos that appear viable after thawing but somehow later fail to result in offspring are another dilemma. Is the problem sublethal cellular injury? Or is it, rather, failure to create a suitable in utero environment in the host female?

Reproductive scientists have also speculated about the potential of using a common species to enhance propagation of a rare species. The best example is interspecies or intergeneric embryo transfer, in which the embryos of an endangered species are gestated in the uterus of a different species. There have been a few isolated successes with this technique. Offspring have been born from gaur (*Bos gaurus)* embryos that were transferred to domestic cattle (Stover and Evans 1984); from bongo (*Tragelaphus euryceros*, an African bovid) embryos transferred to eland (*Taurotragus oryx,* another African bovid; Dresser et al. 1985); and from zebra (*Equus burchelli*) and Prze-walski's horse (*Equus przewalskii*) embryos transferred to domestic mares (Summers et al. 1987). But the lack of similar success stories for the past ten years suggests that biological compatibility between the trophoblast (the progenitor of the placenta) of the embryo and the uterine endometrium will prevent interspe-cies embryo transfer from becoming routine, at least using current technology. By contrast, intraspecies embryo transfer does have potential, so long as information is available about the time of ovulation and about ways to synchronize the donor and surrogate so that the uterus of the recipient accepts the donor's embryos. Basic research will be key to characterizing these mechanisms for every species of high interest.

Promising new approaches for assisted reproduction also need to be explored. For example, whole ovarian sections have been found to survive cryopreservation, with normal follicle growth and ovulation occurring after transplantation, often to a host whose immune responses are suppressed to keep her from rejecting the foreign ovary tissue. Oocytes released from such frozen-thawed material are viable; live births have resulted in mice and sheep after autologous transplants of ovarian grafts stored at -196 °C (Gosden et al. 1994, Gunasena et al. 1997, Harp et al. 1994). A second promising approach that may simplify applying germ plasm cryopreservation to diverse species is the ability to isolate and cryopreserve embryonic and spermatogonial stem cells (Avarbock et al. 1996, Campbell et al. 1996). Recently, such techniques have permitted cryopreserved rat testes cells to continue spermatogenesis when thawed and transplanted into im-muno-incompetent mice (Clouthier et al. 1996).

The bottom line is that basic and applied research in conservation biology must be strongly advocated at all levels, including through federal agencies such as the National Science Foundation and the proposed National Institute for the Environment (NIE). If established, the NIE would fund taxonomic and biogeographic surveys, studies of the roots of extinction, and the upkeep of ex situ and in situ genetic resources, including germ plasm repositories (Blockstein 1990, Hubbell 1993).

- Cooperation and training. A high priority for successful GRBs is the development of cooperative linkage at the level of global, regional, national, and local communities. GRBs must contribute positively to the global conservation of a taxon. Establishing a tiger GRB for the captive population in North America, for instance, does little to address the need to conserve the species in its range countries. Therefore, the tiger GRB Action Plan is only one component of a comprehensive conservation plan developed by all regions that are interested in preserving the species. Western countries also have most of the reproductive and banking specialists, whereas developing countries hold most of the world's biological richness. Cooperative GRB programs provide an opportunity for technology transfer that will enable developing countries to become self-sufficient in all available conservation strategies, including genome banking. Because GRBs depend on cooperation of scientists from many different disciplines (e.g., cryobiology, gamete biology, embryology, veterinary medicine, population biology, molecular genetics, captive breeding, and field biology), training could span many of the life sciences. The result would be conservation programs and repositories in the range countries of the species, with trained specialists regulating protection of, and access to, native resources.

- Birthing GRBs. Quantitative measures must be used to select species for banking. The workshop processes developed by CBSG assists in making first-cut prioritizations of taxa deserving attention (Ellis and Seal 1995). It then becomes the responsibility of those who manage individual habitats or species (both in situ and ex situ) to determine if, and when, genome banking would be a useful adjunct to other conservation initiatives.

Once the decision is made to implement a GRB, then the next challenge becomes genome banking infrastructure. If resources were limitless, formal regional GRB edifices-complexes that house many curators caring for vast amounts of living biomaterials from diverse biota-could be developed. But such an approach is naive in this era of constricted budgets and organizational downsizing. Moreover, a centralized GRB approach has not worked particularly well for others, as those who struggle to financially maintain centers that preserve orthodox seeds from valuable food plants will testify. We believe that the best strategy for now is to exploit already available infrastructure. Museums could add collections of frozen, living specimens, thereby adhering to (and, indeed, ingeniously amplifying) their archival and conservation mission. Crop seed banks and botanical gardens could expand activities to include more plant diversity. One excellent example is the Center for Plant Conservation (CPC), which is headquartered at the Missouri Botanical Garden. CPC is a consortium of 28 gardens and arboreta working together voluntarily to conserve rare native plants and to conduct research and education (CPC 1991). The Center's national collection of nearly 3.5 million seeds (of which one-third are cryopreserved) includes 494 taxa, of which 216 are federally listed as endangered or threatened.

This plant model could be useful in developing similar cooperative arrangements among wildlife managers and universities, commercial bull (cattle) studs, or even human fertility clinics that routinely cryopreserve biomaterials. Universities offer a particularly rich source of ideas and talents. Many university investigators struggle to find money for their biological studies and are constantly looking for new funding sources. If genome resource banking can be made attractive to donors from the public, private, and corporate sectors, then the academic community (the natural collectors of all kinds of biomaterials) would be highly motivated to become involved. CBSG has found worldwide interest among all of these groups. Perhaps a first step is a global electronic GRB network for coordinating interests and resources. A professional society concerned with conserving living genetic resources from wildlife, crops, and livestock could also be created. The benefits would go far beyond simply exchanging technical information, as widely divergent stakeholders interested in technology, agriculture, wildlife, animals, plants, and biopolitics would be given the opportunity to better understand one another's needs, while identifying common strategies to protect all valuable genetic resources.

GRBs could also be developed under the umbrella of the intensive species management strategies that now prevail in zoos. There are more than 80 species survival plans in North America, in which zoos work together to maintain maximum genetic heterogeneity in a given species (Hutchins and Wiese 1991). These efforts are occasionally linked to the range countries of species origin, with support provided to local zoos or field researchers. For example, the North American Cheetah Survival Plan has financially supported educational outreach and research in Namibia, including sperm collection and cryopreservation. For wild populations, GRB activities could be coordinated through IUCN-World Conservation Union taxon specialist groups that have a mandate to develop species or taxon action plans for both wild and captive populations. Superimposing gene banking on the process would be a natural way to strengthen the chain linking science and conservation.

- Safety. Standards for safe and effective monitoring, quality control, and long-term accessions support are essential. Protocols already in place for securing agricultural germ plasm and microorganisms will be useful prototypes. For example, the ATCC's ex situ management of microorganisms is a remarkable model of safe storage and tracking on a huge scale for many different organisms. Samples are stored in 57 liquid nitrogen refrigerators with a capacity of more than two million vials each, in 37 ultra-low temperature refrigerators (that hold more than 150,000 vials at -70 °C), or in four 4 °C cold rooms (900 square feet each), all of which are continuously monitored electronically for temperature changes. GRB operating procedures must comply with the highest quality control standards and have at least two storage locations to minimize loss in case of catastrophe. Quarantine programs will be mandatory to avoid introducing disease to wild or domestic stocks. More research will be needed on enhanced methods of pathogen detection and germ plasm treatment to reduce the risk of disease transfer.

- Databases. Any rare biomaterial from a living animal or plant is only as useful as the information available about that particular specimen-that is, without high-quality data on its type, quality, source, owner, and ancestry, the biomaterial can be worthless. The lives of zoo animals representing many wildlife species are now monitored in relational databases (Ballou 1991, Ballou and Lacy 1995), and similar programs are needed to track an animal's germ plasm, embryos, blood products, tissue, and DNA. The International Species Information System (ISIS), an international nonprofit organization based in Minneapolis, monitors the origin, provenance, pedigree, birth and death dates, sex, translocation information, and biomedical data for more than 250,000 living and 750,000 deceased animal specimens representing 6500 taxa at 495 institutions in 54 countries (Flesness et al. 1984).[1] Because of its vast experience with tracking whole animals and their pedigrees (i.e., by maintaining records on dead specimens), ISIS is the logical choice to develop software that will monitor stored biomaterials and meaningful data that are related to their collection (e.g., the history and pedigree of the donor and the quantity and quality of the specimen). ISIS is currently focusing on creating application software that allows relevant information on each biological specimen to be shared across continents, regions, countries, and locales. The result will be an integrated information system that allows GRB users to be confident of the origin and value of every stored biomaterial, thereby ensuring that any new data will benefit basic knowledge and hopefully conservation of biodiversity.

Conclusion

The scientific literature is filled with justifications for animal, plant, and microbial conservation, ranging from the need to have tigers in the natural food chain to the versatility of bacteria and fungi for environmental remediation and industrial production of enzymes, antibiotics, and alcohol. Beyond such pragmatic advantages, biodiversity should be conserved simply because the world provides all of humankind with a wondrous web of life.

But there is no single approach to protecting biodiversity. Effective conservation must take advantage of all reasonable opportunities. We have argued here that GRBs could contribute to protection. The reality is that organized banks of biomaterials will never replace conventional conservation approaches, such as habitat protection. Rather, GRBs would *assist* in preserving existing genetic diversity at the fundamental, self-replicating unit of biodiversity-the species. Systematically collecting and storing biomaterials from high-priority taxa provides insurance and increases future opportunities. Who knows the future value of these snapshots of biodiversity? What is certain is that failure to collect, to store, and to make this material accessible will contribute to a growing loss in genetic diversity and fewer discretionary options for the future. For these reasons alone, it is worth developing more grassroots programs in systematic sampling, storage, and use of germ plasm and biomaterials, especially for high-priority taxa.

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Return to the November 1997 Issue of BioScience
