



POLICY FORUM: ECOLOGY

DNA Banks for Endangered Animal Species

Oliver A. Ryder, Anne McLaren, Sydney Brenner, Ya-Ping Zhang, Kurt Benirschke

Although the loss of biodiversity resulting from extinctions is receiving increasing attention, the accompanying decrease in access to genetic resources has not been widely discussed. The threat of extinction for many species, both known and as yet undiscovered, grows ever greater as whole ecosystems vanish, human populations proliferate, and human-mediated interference increases. Even though efforts to compile listings of threatened plants and animals are developing (1), current views of timing of recovery from anthropogenic extinctions suggest that many millions of years will be required for replenishment to present-day levels (2).

The establishment and scientific management of nature reserves and national parks in as many parts of the world as possible is imperative. This is especially true for the tropics, where the largest numbers of threatened and endangered species may reside. Major efforts to preserve biological diversity are under way through habitat conservation; other *in situ* support; living collections as maintained in botanical gardens, arboreta, and zoological parks; gamete and seed banks for species of agricultural interest; and the amassing and documentation of museum specimens. However, relatively little effort has been made to collect and document DNA specimens as genetic resources. Yet, the most fundamental derivative of a species is the information in its genome, currently most easily preserved in the form of purified high-molecular-weight DNA. Although the lack of emphasis on genetic resource collections, especially DNA banks, may be considered appropriate in the context of current conservation priorities, the lack of knowledge about their existence and

the biodiversity they encompass hinders current study, may place inappropriate pressure on natural populations, and leaves the present generation ignorant about a portion of its legacy to the future.

Genetic resource collections now provide essential tools that facilitate scientific study. For example, the comparisons of intraspecific diversity between humans, chimpanzees, and gorillas are enriching our understanding of human evolu-

tion (3). Studies of mitochondrial diversity have been used in coalescent dating to determine the evolutionary time back to a single ancestor for the cheetah (4) and for felids. In behavioral ecology, scientists interested in determining whether female sea turtles return to the same beaches when nesting have relied on DNA restriction fragment length polymorphisms (RFLPs) (5).

In the foreseeable future, DNA sequencing will be fully automated, and our descendants will be able rapidly to derive the sequence of any organism whose DNA has been appropriately collected and stockpiled. If sufficient genomes are available, they will be able not only to reconstruct what the organism was like, but also what its evolutionary relationships were, how specific genes arose to encode proteins that perform specialized functions, and how regulatory programming evolved. The medical value of such DNA banks should not be overlooked and will undoubtedly provide materials that will contribute to advances in medicine and pharmaceutical development. Determination of ancestral character states for single nucleotide polymorphisms (SNPs) assists in interpreting the influence of selection on loci within the human genome (6).

Assessment of genetic resources can serve a valuable planning purpose in the overall conservation effort, as well as in determination and management of the well-being of species and the richness of ecosystems. Examination of genetic diversity of

the Florida panther led to specific intervention to increase genetic diversity (7). Studies of evolutionary systematics using DNA markers have provided new insights, such as the origin of whales (8), and assist in identifying appropriate units for conservation in many taxa, for example, crocodilians (9), and the African cichlids (10).

Black rhinos are declining in Africa at alarming rates; there were about 65,000 throughout Africa in 1970, but there were only about 2600 in 1999 (11). As part of the effort to assess the problem, extensive analyses of mitochondrial DNA diversity and nuclear microsatellite polymorphism are currently being performed to distinguish between the Southern and Eastern black rhino populations (12). Phylogeographic studies are also continuing for the giant panda (13).

International Whaling Commission efforts to conserve cetaceans by regulating commercial and scientific whaling activities include prohibitions on taking some species, such as blue whales and all species of dolphins.

The ongoing monitoring of commercial products to make sure that dolphin meat and certain prohibited species of whales are not included depends on forensic DNA analyses, which require reference DNA collections (14).

Captive breeding provides an insurance policy and, for some species, may be the only hope of survival. It requires consideration of small population vulnerabilities to preserve high levels of genetic diversity. In some instances, input from reproductive physiologists to promote the establishment of pregnancies, for example, by artificial insemination, contributes in a major way to the genetic preservation goals. Cryopreservation of gametes and embryos has a role to play; whereas in the future, nuclear replacement cloning from established cell lines might prove of value. If the cell lines were made before numbers dropped dangerously low, the earlier level of genetic diversity might be restored (15).

As one example of ongoing use of genetic information, breeding efforts to save the California condor have been significantly aided by minisatellite RFLP variation from which kinship could be inferred (16) (see the figure). This is a species that had to be placed into captivity in order to save it from extinction (17). Risks of genetic diseases are important considerations in the management and survival of endangered species, which typically have small population sizes (18). A

dEbate!

Respond online
http://www.sciencemag.org/cgi/content/summary/288/5464/275



Genetic information is being used to save the California condor from extinction.

O. A. Ryder is at the Center for Reproduction of Endangered Species, Zoological Society of San Diego, CA 92112-0551, USA. E-mail: oryder@ucsd.edu A. McLaren is at Wellcome/CRC Institute, University of Cambridge, CB2 1QR, UK. E-mail: A. McLaren@welc.cam.ac.uk S. Brenner is at the Molecular Sciences Institute, Berkeley, CA 94704, USA. E-mail: sbrenner@molsci.org Y.-P. Zhang is at the Key Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China. E-mail: zhangyp@public.km.yu.cn K. Benirschke is at the Department of Pathology, University of California, San Diego, CA 92093, USA. E-mail: kbenirsc@ucsd.edu

CREDIT: S. LAFRANCE/ACADEMY OF NATURAL SCIENCES

heritable chondrodystrophy has been identified in California condors (19), and its management through identification of a linked marker to detect carriers may be feasible, in part, because of banked DNA samples. DNA banking has also been crucial for the preservation of Przewalski's wild horses through breeding programs that are attempting to conserve a limited gene pool derived from 12 wild-caught individuals.

Such strategies may succeed in saving a small fraction of endangered species, at least for a time. Resources for conservation are limited, and many species are found only in inaccessible places, in impoverished countries.

The work of museums in botanical gardens and conservation of biological diversity through the collection, identification, cataloging of specimens, and, in some instances, tissues, is an underpinning effort. However, for many species of both vertebrate and invertebrate animals, if nothing more is done, our descendants will be left with little else than brief descriptions in scientific papers and voucher specimens in museums with only limited use for comparative genomic studies.

Although efforts are under way to establish integrated databases, we suggest that emphasis be placed on banking genetic resources even as the strategic cataloging efforts are under way. Currently, a limited and relatively uncoordinated effort exists to preserve tissues, gametes, and frozen viable cells, although some catalogs of genetic resource specimens are currently available online (20). A laudable effort is being made to organize seed banks for plants (21).

The technical aspects of saving DNA are straightforward. Any tissue samples can be simply stored at -70°C or in liquid nitrogen. DNA can be isolated from fresh or frozen tissue samples, and purified DNA may be preserved for hundreds to thousands of years at room temperature, provided it is kept dry, for example, in a closed vial of inert gas.

We urge that a coordinated worldwide attempt be made to store, for every endangered animal species, samples of DNA, DNA libraries, or frozen cells or tissues that could readily yield DNA. This might comprise, under current estimates, at least 5200 recognized species (22). There will, of course, be many difficult issues to address. Access to endangered species consistent with their protection will require consideration by the signatories of the Convention on International Trade in Endangered Species, which has tended to impede transfer of specimens for biological research. Intellectual property considerations and sovereignty issues under Article

15 of the Convention on Biological Diversity (23), sharing of rights to access and potential benefits, need for duplicate collections, and international coordination are controversial issues that need to be addressed. Strategies for organizing international coordination need to be developed. We recognize that local communities and national governments have rights over their natural resources, including genetic resources. Conservation at every level must always involve local human populations.

Our proposal is first to establish a means of collecting information on DNA banking efforts for endangered species of animals and, second, to encourage efforts to bank documented DNA specimens for the future. Efforts in this direction are already under way, largely adjunct to particular medical or agricultural research projects. For example, collections of DNA are held at the Center for Reproduction of Endangered Species of the San Diego Zoo, the Laboratory of Genomic Diversity at the U.S. National Cancer Institute, and the Kunming Institute of Zoology from a variety of mammals, birds, and freshwater fishes. Collections of DNA from agricultural plants and animals are being assembled and distributed (24), and DNA specimens from botanical collections are also being made available (25).

Although it is difficult to estimate accurately costs for a global effort, an annual cost per site for labor and materials in the United States for technical assistance, supplies, and freezers for unprocessed specimens might amount to \$50,000 to \$80,000. Benford (17) has estimated costs for saving examples for future analysis of species in rainforests by storage in liquid nitrogen at about \$20 million per year. DNA banks for recognized endangered species of animals would be less inclusive, but would also be less costly and of more immediate practical value.

To the best of our knowledge there have been no systematic attempts to coordinate or even to register these efforts, let alone to extend them to endangered species worldwide. We therefore propose to set up a Web-based method to register DNA banks. Such a register should include taxonomic information, number of accessions, geographic reference to collection location and/or pedigree information (for example, from a zoo studbook), and who is responsible for the collection. This would reveal the extent of what exists already and would help to structure the conservation community's thinking about the magnitude of the task.

Participation in generating a listing of banked DNA specimens from endangered species of animals would not require mak-

ing samples available. However, as a first practical step, a catalog of DNA specimens might build interest in creation of genomic libraries for a wide variety of studies. We encourage suggestions about the design of the Web site for collecting information on DNA collections from endangered animal species and participation of the scientific and conservation communities in the undertaking of this effort. Individuals and institutions interested in receiving additional information, including the announcement of the Web site for collecting information about DNA collections may contact DNA_banks@sandiegozoo.org.

References and Notes

- 1996 International Conservation Union (IUCN) List of Threatened Animals; IUCN List of Threatened Plants; National Biological Information Infrastructure (www.nbi.gov) (1997); H. Saarenmaa, The Global Biodiversity Information Facility: Architectural and Implementation Issues (European Environmental Agency Tech. Rep. 34, Copenhagen, 1999), 34 pp. (www.eionet.eu.int/gbif/gbif-implementation-latest.html).
- J. W. Kirchner and A. Weil, *Nature* **404**, 177 (2000).
- M. Ruvolo, *Annu. Rev. Anthropol.* **26**, 515 (1997); P. Gagneux et al., *Proc. Natl. Acad. Sci. U.S.A.* **96**, 5077 (1999).
- M. Menotti-Raymond and S. J. O'Brien, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3172 (1993).
- B. W. Bowen et al., *Evolution* **48**, 1820 (1994).
- J. G. Hacia et al., *Nature Genet.* **22**, 164 (1999).
- P. W. Hedrick, *Conserv. Biol.* **9**, 996 (1995); M. E. Roelke, J. S. Martenson, S. J. O'Brien, *Curr. Biol.* **3**, 340 (1993); S. J. O'Brien et al., *Natl. Geogr. Res.* **6**, 485 (1990).
- M. Shimamura et al., *Nature* **388**, 666 (1997).
- G. Amato, J. Gatesy, P. Brazaitis, in *Molecular Evolution: Approaches and Applications*, R. DeSalle and B. Schierwater, Eds. (Birkhauser, Basel, Switzerland, 1998), pp. 177–189.
- W. E. Mayer, H. Tichy, J. Klein, *Heredity* **80**, 702 (1998).
- See www.rhinos-irf.org.
- M. Jama, R. R. Ramey, O. Ryder, M. Houck, R. Aman, unpublished data.
- Y. Zhang et al., *Science China Ser. C. Life Sci.* **40**, 210 (1997).
- S. R. Palumbi and F. Cipriano, *J. Hered.* **89**, 459 (1998); C. S. Baker, F. Cipriano, S. R. Palumbi, *Mol. Ecol.* **5**, 671 (1996); F. Cipriano and S. R. Palumbi, *Nature* **397**, 307 (1999).
- O. A. Ryder and K. Benirschke, *Zoo Biol.* **16**, 295 (1997).
- C. J. Geyer, O. A. Ryder, L. G. Chemnick, E. A. Thompson, *Mol. Biol. Evol.* **10**, 571 (1993).
- G. Benford, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 11098 (1992).
- O. A. Ryder, *Nature* **331**, 396 (1988).
- K. Ralls, J. D. Ballou, B. A. Rideout, R. Frankham, *Anim. Conserv.*, in press.
- RIKEN Gene Bank (www.rtc.riken.go.jp); American Type Culture Collection (www.atcc.org).
- Millennium Seed Bank (www.rbgekew.org.uk/seedbank/msb.html).
- List of Threatened Animals, Wildlife Conservation and Monitoring Centre (www.wcmc.org.uk).
- Convention on Biological Diversity (www.biodiv.org/chm/conv/default.htm).
- Japanese Ministry of Agriculture, Forestry, and Fisheries (<http://bank.dna.affrc.go.jp>).
- Missouri Botanical Garden, St. Louis, MO, USA (www.mobot.org/MOBOT/research/DNAdocs/dnabank.html).
- This initiative is recognized as part of the International Year of the Biosphere (IBOY), an activity of DIVERSITAS, a creation of UNESCO and the International Council of Scientific Unions (ICSU), and forms part of the effort to engender international cooperation in research and decision-making as a critical element for solving global environmental problems such as the accelerating loss of biodiversity. We thank J. Soberon, R. Medellin, and an anonymous reviewer for helpful comments and suggestions.