GENOME RESOURCE BANKING

FOR WILD SPECIES CONSERVATION

An Overview, A Strategy, A Draft Policy Statement
And
Background Documentation

SINGAPORE

September 1991

CAPTIVE BREEDING SPECIALIST GROUP (CBSG)
SPECIES SURVIVAL COMMISSION (SSC)
WORLD CONSERVATION UNION (IUCN)

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Cryopreservation and Banking of Animal Germ Plasm for Species Conservation: An Imperative for Action by the Captive Breeding Specialist Group

W.F. Rall, J.D. Ballou and D.E. Wildt
National Zoological Park, Smithsonian Institution
Washington, DC 20008-2598 USA

SUMMARY

Conservation efforts for rare animal species currently focus on programs to protect populations in natural habitat (in situ) and in captivity (ex situ). The ultimate aim of both approaches is to maximize both global biodiversity and genetic diversity. The systematic cryopreservation and banking of germ plasm from free-living and captive populations provide new opportunities to control and manage bio- and genetic diversity. Despite the widely acknowledged benefits of this approach, the development of genetic resource banking programs is hampered by the lack of a mechanism to integrate this activity with other conservation activities.

We propose that the CBSG act immediately to provide leadership for international coordination. The CBSG should assume responsibility for developing programs that encourage germ plasm banking as an integral component of in situ and ex situ conservation efforts. Specifically, the CBSG should: 1) draft and seek adoption of an IUCN Position Statement on the role of germ plasm banking in management and research programs to conserve endangered species; 2) establish a Genetic Resource Banking Oversight Committee to formulate global guidelines for the establishment, operation and review of animal genetic resource banking programs; and 3) develop a formal process that would assist the development of Genetic Resource Banking Action Plans. It is likely that extensive regional and international planning is required to establish and operate such banking programs and ensure the ultimate utility of the banked materials.

INTRODUCTION

Increasing numbers of species face extinction in their native habitat usually as a result of the direct or indirect actions of man. The survival of a species in the wild is thought to depend on a secure native habitat that is sufficiently large to support a population meeting certain genetic and demographic requirements (Soule 1987). Most of the important requirements are related to the properties and characteristics of the population as a whole, such as its size, life-history characteristics and the nature of the gene pool contained therein. The latter, especially genetic variations (i.e. polymorphism) within populations or communities of individuals, plays an important role in many of the critical biological processes related to species conservation, including

extinction (Ehrlich and Ehrlich 1981), inbreeding depression (Ralls et al. 1988), speciation (Templeton 1989) and natural selection (Frankel and Soule 1981). The loss of biological resources as embodied in species resulting from aeons of evolutionary adaptations is recognized as a major international concern. For this reason, it is generally recognized that every possible avenue should be taken to conserve bio- and genetic-diversity (Wilson 1988).

Conservation efforts consist of both: 1) 'in situ' conservation programs that protect and manage animal populations within their natural, native habitat; and 2) 'ex situ' conservation programs that remove individuals, gametes or embryos from wild populations for controlled breeding and management in captivity. Although habitat protection is acknowledged to be the most efficient approach for conserving bio-and genetic-diversity, for some species, in situ conservation alone can not be relied upon to ensure the long-term viability of species at risk (Conway 1988, Soule 1991). Continued human population growth and the biopolitical, environmental and social consequences of that growth require ex situ approaches as critical components of integrated conservation (McNeely et al. 1990).

Currently, ex situ efforts for animal species at risk of extinction focus on captive propagation (Soule et al. 1986, Foose et al. 1986). The immediate goal of such programs is to manage populations of a species so as to retain maximum genetic diversity. Ultimately, such captive populations would serve as a source of individuals for release into restored habitat or to infuse genetic diversity into inbred, free-living populations. This can be accomplished only if a significant fraction of the overall genetic diversity existent in the wild population is incorporated into and retained by the captive population. Most captive breeding programs seek to maintain 90% of the captive population's initial genetic diversity for 200 years (Ballou 1991), as recommended by Soule et al (1986). Unfortunately, the world's zoos and bioparks do not have sufficient capacity to house the numbers of animals needed to meet the habitat crisis facing wild animals. For example, estimates suggest that space currently is available in North America for only about 100 mammalian species in populations large enough to meet the required genetic and demographic goals (Conway, 1987). This compares to the 815 mammalian species estimated by Soule et al. (1986) that would require captive propagation programs during the next 200 years.

UTILITY OF GENETIC RESOURCE BANKS FOR IN SITU AND EX SITU CONSERVATION PROGRAMS

The efficiency and efficacy of captive breeding can be increased many-fold by applying recent advances in reproductive biotechniques (Wildt 1989, 1991). Perhaps the most important advance is germ

plasm cryopreservation or the low-temperature storage and banking of spermatozoa, embryos and oocytes. Germ plasm cryopreservation currently plays an important role in domestic livestock agriculture, especially in the international movement of disease-free, genetically-superior individuals. The development of banks of cryopreserved germ plasm for nondomesticated species offers many important advantages for conserving and managing the genetic diversity within existing populations. Specifically, an animal genetic resource bank:

1. Reduces the number of animals that must be maintained in captivity by extending the generation interval of a species indefinitely. Thus, the genetic diversity of a founder does not die with the animal, but remains viable and available for use in future generations.

2. Provides a high degree of security against the loss of diversity or entire species from epidemics, natural disasters and social/political upheavals.

3. Serves a vital, interactive role between in situ and ex situ conservation programs. Such interactions prevent unintended selection pressures in captivity, preserve new diversity resulting from natural evolutionary processes in free-living populations, and permit 'infusions' of genetic diversity into fragmented populations suffering from genetic drift or inbreeding depression. This strategy also eliminates the need to remove additional animals from the wild or introduce captive animals into wild free-living populations.

4. Provides a method for improving food production and the economy of local communities by inter-species hybridization with domesticated species (e.g. hybridization of rare species of cattle with domesticated breeds).

5. Allows ready access to systematic collections of rare biological specimens for research in conservation biology or other 'life' sciences.

The importance of germ plasm resource banks for conserving the genetic diversity of wild fauna has been recognized since the first reports of successful cryopreservation of spermatozoa (Polge et al. 1949) and mammalian embryos (Whittingham et al. 1972). Over the past two decades, reports of various public- and privately-sponsored task forces have stressed the need for germ plasm repository programs to be established for conservation purposes. These include:

1. Conservation of Germplasm Resources: An Imperative. National Research Council, National Academy of Sciences, Washington DC, USA, 1978.
2. Animal Genetic Resources: Conservation and Management.

Proceedings of the FAO/UNEP Technical Consultation, FAO Animal Production and Health Paper No. 24, Rome Italy, 1981.
3. Animal Germplasm Preservation and Utilization in Agriculture. Council for Agricultural Science and Technology, Report No. 101, September 1984, Ames, Iowa, USA.
4. U.S. Strategy on the Conservation of Biological Diversity. Interagency Task Force Report, U.S. Agency for International Development, Washington DC, USA, 1985.
5. Technologies to Maintain Biological Diversity. U.S. Congress, Office of Technology Assessment, Report OTA-F-330, U.S. Government Printing Office, Washington DC, USA, 1987.
6. Research Priorities for Single Species Conservation Biology. A workshop sponsored by the U.S. National Science Foundation, Washington, DC, 1989.

STATEMENT OF THE PROBLEM

Despite all the publicity directed at the issues of declining habitat, species extinction, loss of genetic diversity and the potential contributions of germ plasm banking, it is remarkable that no organized programs exist to sample, evaluate, cryopreserve, maintain and use germ plasm from wild animal species. Furthermore, there are no guidelines for establishing such germ plasm banking programs or integrating them with other conservation programs. As yet, no single organization with a role in the international coordination of conservation efforts has provided guidance or oversight.

There are several organizational and procedural matters that must be addressed before the full potential of genetic resource banks can be realized for international conservation purposes. We propose that the CBSG immediately provide a leadership role to remedy the lack of international oversight and coordination. The CBSG should assume responsibility for developing programs that encourage germ plasm banking as an integral component of in situ and ex situ conservation efforts. Specifically, the CBSG should: 1) draft and seek adoption of an IUCN Position Statement on the role of germ plasm banking in management and research programs to conserve endangered species; 2) establish a Genetic Resource Banking Oversight Committee to formulate global guidelines for the establishment, operation and review of animal genetic resource banking programs; and 3) develop a formal process that would assist the development of Genetic Resource Banking Action Plans. Other important elements of these overall activities include the coordination of activities within the Species Survival Commission to identify species conservation programs that would benefit from germ plasm banking, and assisting efforts to secure sources of funding for international germ plasm banking activities. Discussion of each of these critical needs follows.

ENCOURAGE INTERNATIONAL GERM PLASM BANKING ACTIVITIES

Germ plasm banking activities can best be encouraged by education programs to inform the public, conservation managers and conservation researchers of the benefits resulting from the systematic banking of genetic resources. Examples of current applications and the conservation and research benefits of germ plasm banking can be drawn from type-culture collections of microorganisms and cell cultures (Colwell 1976, Edwards 1988), the commercial cattle breeding industry (Seidel, G.E. 1990) and banks of embryos from genetically-defined strains of laboratory rodents (Mobraaten 1981).

Ongoing international programs for the ex situ conservation of plant genetic resources provide a useful model (Cohen et al. 1991). Efforts for developing collections of crop germ plasm are well advanced. International coordination of crop germ plasm conservation is provided by the International Board for Plant Genetic Resources (IBPGR) and the Consultative Group on International Agricultural Research (CGIAR). At present, 14 major agricultural research centers have been established in developing regions, each developing base collections of germ plasm for the major food crops. Funding for these activities is approximately US$300 million per year. Comparable efforts for domestic animal species are modest. Currently there is no 'International Board of Animal Genetic Resources' to coordinate international efforts to conserve agriculturally-important sources of animal germ plasm. However, the Food and Agriculture Organization (FAO) of the United Nations has established an initiative to establish germ plasm banks in developing regions. Coordination of FAO and wild animal conservation and germ plasm banking activities would be best provided through the CBSG.

IUCN POSITION STATEMENT ON ANIMAL GENETIC RESOURCE BANKING

One method of highlighting the potential benefits of active genetic resource banking programs is to seek an official position statement by the IUCN. The statement should be drafted jointly by the CBSG and the Chairman of the Species Survival Commission (SSC) of the IUCN. Information and review of the statement should be solicited from other SSC Specialist Groups prior to submission to the IUCN for approval. We suggest that the statement emphasize the importance of coordinated in situ and ex situ conservation programs for endangered species. The role of germ plasm banking in preserving important sources of genetic diversity and in providing a means for moving genetic diversity between captive and free-living populations should be stated. The CBSG should be designated to be responsible for oversight of germ plasm banking activities within the Species Survival Commission. Finally, the CBSG should be directed to coordinate and review international aspects of banking programs for nondomesticated animal species.

FORMULATE GLOBAL GUIDELINES FOR THE ESTABLISHMENT, OPERATION AND REVIEW OF ANIMAL GENETIC RESOURCE BANKS

A key factor to ensuring the success of animal genetic resource banks (GRBs) is to ensure that they are established using rigorous scientific criteria and state-of-the-art technology. Because limited resources are available, difficult choices will need to be made on which species can derive the maximum benefit from this approach. At present no guidelines exist to assist in formulating action plans for establishing and operating a genetic resource bank.

To assist the CBSG in developing such guidelines, we suggest the following sequence as a first attempt to address many of the important issues. This working plan was modified from one suggested recently by one of us (Rall 1992).

GENETIC RESOURCE BANKING OVERSIGHT COMMITTEE

STEP 1. The first step in establishing integrated GRBs is to establish a GRB Oversight Committee under the auspices of the CBSG. This committee should be composed of 8 to 15 members. The composition must include one or more experts from each of the following areas: 1) cryobiologist; 2) reproductive physiologist; 3) population biologist; 4) geneticist; 5) veterinarian; 6) *in situ* conservation biologist; 7) *ex situ* conservation manager; and 8) the chairmen of regional cryopreservation task force committees. Furthermore, the chairmen (or their representative) of all SSC specialist groups should serve as *ad hoc* members.

STEP 2. The second step is to define the responsibilities of the committee and formulate a formal process for establishing GRBs. We propose five basic missions for the GRB Oversight Committee:

1. Coordinate GRB activities within the SSC and regional propagation groups. The GRB Oversight Committee would assist SSC taxon Specialist Groups, regional taxon advisory and captive propagation groups achieve their goals of conserving rare species. This can be accomplished by integrating the consideration of GRBs directly into the framework of strategic planning processes of population viability assessment and conservation action plan (PVA/CAP) workshops. These activities require that an expert resource network be established to provide advise on all technical matters related to GRBs and their utility.

2. Establish guidelines for identifying candidate taxa, species or populations that would benefit from a GRB program. These guidelines should be detailed and assist in the development of strategic GRB Action Plans for conserving specific animal populations. The single most important consideration is to ensure that there is a defined conservation goal that requires the collection and storage

of biological materials. This requires that a integrated plan for a goal-oriented conservation program be established prior to initiating banking activities. We list three scenarios below to illustrate our proposed process.

3. Provide expert technical assistance to the appropriate taxon groups to assist in the development of GRB Action Plans. This would include identifying institutions with an interest in providing long-term repository storage space or local/regional assistance in collecting and preserving material. Furthermore, the GRB Oversight Committee would work with the CBSG, the SSC Financial Development Officer and other interested organization to identify sources for supporting international GRB activities. Proposals for funding might be submitted individually or jointly with these and other organizations to private foundations, national research granting agencies and multinational organizations.

4. Provide a mechanism for the review of proposed GRB Action Plans. Plans that meet recommended requirements should be approved formally by the CBSG. (Formal 'sanction' may assist in the securing of external funding.)

5. Develop a periodic review process for individual GRB programs. This would be best accomplished by shared responsibility with the appropriate regional GRB Task Force Committee. For example, the annual reports of individual GRBs could be presented by the chair of the appropriate regional GRB Task force for review of recent progress, problems and future directions of banking activities.

THREE SCENARIOS OF APPROPRIATE GENETIC RESOURCE BANKING PROGRAMS

Scenario 1. An ongoing captive propagation program seeks to increase safety and management options for maintaining genetic diversity in a population, and achieve the same goals with fewer animals. We propose that such a population would be a candidate for a GRB program if the following minimum requirements are met:

a. Populations in captivity and/or the wild must be potentially viable by demographic and genetic criteria. This information is best obtained from a recent population viability assessment (PVA).

b. Ongoing captive propagation (e.g. SSP, EEP), studbook and conservation research programs have been established for the candidate animal population(s).

c. The current level of success of captive breeding must be sufficient to provide reasonable assurance that GRB-associated reproductive biotechniques will be successful.

d. Animals with known genetic backgrounds should be available to serve as founders of a GRB.

e. Sufficient numbers of 'surplus' females and males must be available to act as recipients to demonstrate the viability of cryopreserved germ plasm and serve as a source of material for

research and protocol development.

f. The effects of potential restrictions on the importation and exportation of animals and animal products must be evaluated.

g. And other factors as appropriate for the specific candidate species or population.

Scenario 2. An animal population has declined to low numbers (<100) and is expected to recover slowly. The population is expected to lose heterozygosity rapidly (>0.5% per generation and be subjected to genetic drift. A propagation/management plan has been initiated with the goals of protecting current levels of genetic diversity, preventing the loss of diversity in specific elderly founders and increasing the size of the population. We propose that such a population would be a candidate for an emergency GRB program if the following minimum requirements are met:

a. The populations must be potentially viable by demographic and genetic criteria. This information is best obtained from a recent population viability assessment (PVA).

b. There is a reasonable expectation that captive propagation will be successful. For example, a taxonomically-related species or subspecies has been successfully bred in captivity.

c. There is a reasonable expectation that GRB-associated reproductive techniques (e.g. germ plasm collection and cryopreservation, artificial insemination, embryo transfer) will be successful. For example, these procedures have been successfully applied in a taxonomically-related species or subspecies.

d. And other factors as appropriate for the specific candidate species or population.

Scenario 3. A free-living population has declined rapidly and satisfies the 'critical' or 'endangered' categories of the Mace-Lande criteria for threatened taxa (Mace and Lande 1991). Factors leading to the decline have been identified and a management plan has been initiated to maintain the population at low numbers (<2000) for many generations (5 to 20) before an increase in population size is expected. The population remains at risk to a further rapid decline that may reduce genetic diversity to unacceptable levels. One management goal is to develop a secure ex situ program to provide a reinfusion of genetic diversity in the event of a future decline. We propose that such a population would be a candidate for a GRB program if the following minimum requirements are met:

a. There is a reasonable expectation that GRB-associated reproductive techniques (e.g. germ plasm collection and cryopreservation, artificial insemination, embryo transfer) will be successful. For example, these procedures have been successfully applied in a taxonomically-related species or subspecies.

b. Animals with known or identifiable genetic backgrounds should be available to serve as founders of a GRB.

c. Sufficient numbers of 'surplus' females and males must be available to act as recipients to demonstrate the viability of cryopreserved germ plasm and serve as a source of material for

research and protocol development.

d. And other factors as appropriate for the specific candidate species or population.

DEVELOPMENT OF ACTION PLANS FOR GENETIC RESOURCE BANKS

STEP 3. The primary responsibility for developing GRB Action Plans properly resides with those groups with specific responsibilities for in situ and ex situ conservation of specific taxa, species and populations (e.g. taxon Specialist Groups, Taxon Advisory Groups and regional captive propagation groups). These groups should be encouraged to include the development of GRBs as an integral component in their strategic conservation planning (e.g. Captive Action Plans, Taxon Action Plans). The first step in the process occurs when a group identifies a specific conservation goal for a taxon, species or population that requires the collection and storage of biological materials. The needs and characteristics of the candidate animal population(s) would be evaluated in terms of the requirements listed in the appropriate scenario listed above. If analysis of these factors suggest that conservation efforts would be enhanced or ensured by a GRB program, the group would petition the CBSG of their intent to develop such an action plan.

The GRB Oversight Committee would review the petition and, if approved, would assist the conservation group in organizing a working session meeting to further evaluate the conservation needs and develop a detailed action plan. The role of the Oversight Committee would be to identify technical experts who can assist in this effort. The specific goals of the meeting would be to:

1. Assemble and evaluate available information on the life-, reproductive- and genetic histories of ex situ and in situ populations of interest. Much of this information would be available for recent propagation/management (e.g. SSP, TAG) and PVA materials.

2. Evaluate the efficiency and efficacy of reproductive technologies for the candidate species, such as artificial insemination, embryo transfer, in vitro fertilization, gamete and embryo cryopreservation and collection of spermatozoa, oocytes and embryos. Areas requiring further research or development would be identified.

3. Identify the types of biological material requiring storage. It should be noted that a wide variety of different biological materials might be cryopreserved and stored depending on the goals and needs of the conservation program (see Table 1).

4. Specify the appropriate protocols for banking activities. These include:

a. The criteria used to select material(s) for accession, determine the quantity of material from each donor and identify

appropriate uses of the material.

b. Procedures for collection, processing, cryopreservation, shipping, thawing and other treatments. The minimum quality control standards for each process and overall viability would be identified.

c. The appropriate repository equipment, facilities, security and management systems that ensure the ultimate utility of the banked materials would be identified.

d. If any of the above items are unknown, specific areas requiring further research should be identified.

5. Determine the location of the primary repository for storage of cryopreserved materials and secondary backup sites.

6. Develop strategies for the use of banked materials in breeding and conservation research programs.

7. Identify sources of funding for the GRB Action Plan.

If analysis of the these factors indicates that a GRB program would benefit conservation, the petitioning organization would prepare a written Action Plan for developing a GRB program.

REVIEW AND APPROVAL OF PROPOSED ACTION PLAN FOR A GENETIC RESOURCE BANK

STEP 4. Identifying the appropriate authority for reviewing GRB Action Plans is complicated by the overlapping purviews of national, regional and international organizations and their animal propagation/management programs. We suggest that the proposed GRB Oversight Committee is the most appropriate organization because of the very nature and responsibilities of the CBSG. First, by definition, genetic resource banking programs represent a form of ex situ captive propagation. Second, GRB activities are international in that technical experts and populations of most rare species are located on several continents. Third, GRB programs require integration with other in situ and ex situ conservation programs. However, in many cases, regional cryopreservation task force committees will play an important role in regional coordination and development of these programs. In those cases, we propose that the GRB Action Plan be reviewed by both the regional banking authority and CBSG GRB Oversight Committee. After approval, the plan would be implemented and collection, storage and use of biological materials can begin.

CONCLUSION

The development of animal Genetic Resource Banks offers unique opportunities to control and manipulate the effects of time in the management and conservation of rare species. The ideas proposed

here are intended to help stimulate discussion about the process on a formal basis. Many important questions remain to be resolved, including the translation of banking germ plasm into live offspring. However, many recent reports of successes using artificial breeding techniques indicate the potential of reproductive biotechnology. The further development of strategies proposed here will ensure that GRBs are not merely an interesting idea or static warehouses of biological materials but facilitators for conservation.

Table 1. Biological Materials for Germ Plasm Banking and Conservation Research.

Material Type	Long-term Storage Conditions	Examples of Potential Uses
Sperm, oocytes	below -130°C[a]	Controlled breeding; international shipment; gene banking
Embryos	below -130°C	Control of generation interval and gene flow; population amplification; international shipment
Cell lines	below -130°C	Genetic and physiological research
DNA		Molecular biology:
-Isolated	dried, 4°C[b]	Sequence detection and identification (e.g. by PCR)
-Isolated and frozen tissues	below -60°C[c]	Pedigree determination; genomic and mitochondrial libraries
Serum, plasma	below -60°C	Disease status (detection of microbial antibodies and disease organisms); endocrine status (measure hormones or hormonal metabolites)
Urine, milk	below -60°C	Endocrine and health status (measure hormonal and other metabolites)

[a]Liquid nitrogen refrigerator.
[b]Refrigerator or cold room.
[c]Low-temperature mechanical refrigerator.
[from Rall 1992, with modifications]

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POPULATION BIOLOGY GUIDELINES FOR USE OF GENOME BANKING AS A MANAGEMENT TOOL FOR CONSERVATION OF WILD CATTLE POPULATIONS

Omaha Zoo, 13-16 June 1991

INTRODUCTION

Genome banking offers the opportunity to expand the scope, time span, scale, security, and economy of programs for conservation of species and of within species genetic diversity. As populations of wild cattle species are fragmented in distribution and reduced in numbers, genetic diversity is lost and the populations become increasingly vulnerable to extinction. Some of the species are vulnerable to hybridization with domestic cattle or domesticated stock of the wild species. Cryopreservation of representative samples of genomic materials from the wild populations will allow indefinite preservation of presently available diversity and protect against extinction. These materials also may be used for the genetic management of living wild and captive populations now and in the future.

Formulation of goals and objectives for a genome banking program are necessary for development of sampling and utilization strategies to guide: (1) selection of an optimal representation of the genetic diversity, (2) collection and storage of an adequate amount of material, and (3) distribution and use of the appropriate materials. These materials may then be used to assist restoration of extinct wild populations, genetically supplement small living wild populations, assist in the exchange of genetic material between previously connected wild populations, and support smaller captive populations with indefinite retention of presently available genetic diversity.

The utilization of genome banks as part of an integrated program for management of living wild and captive populations may allow retention of a larger fraction of the present genetic diversity in the wild populations with smaller living captive populations. It also may be possible to distribute embryos to other sites without removing animals from threatened populations. These might then be transferred to surrogate hosts to produce living populations

as a basis for further expansion of the genome bank, introductions to other sites, or supplementation of the wild population. The living population could receive periodic infusions from the genome bank to replace diversity lost by drift or to maintain a closer correspondence to the genetic composition of the wild population. The cryopreserved materials will allow indefinite (thousands of years) retention of the present day genetic diversity which will significantly modify current goals for captive conservation programs based upon 90% retention of genetic diversity for 100 or 200 years in the captive populations.

This capability to retain more diversity with smaller living captive populations should allow a dramatic (4-10 fold) expansion of the number of species or evolutionary significant units that might be supported with living captive populations and genome banks. This expansion in the number of species to be managed will greatly increase our need for systematic data collection, analysis, and distribution and for simpler development of species management plans. The addition of another mode for protection of species against loss should further secure them from extinction from catastrophic events and the impacts of continuing loss of habitat quantity and quality.

GOALS

1. Long term conservation of species, of genetically distinct populations, and genetic diversity within species.

2. Provide a resource for enhancement and development of agricultural breeds.

3. Transfer of cryopreservation, reproductive, and molecular genetic technology to collaborators in the range country of each species from which specimens are collected. One intent of this program would be to support genome banking of these species in the countries of origin.

OBJECTIVES

1. Develop models for optimal sampling of the wild and captive populations across space and time to meet program goals for genetic representation and for use over the proposed time span of the program.

2. Develop models for optimal use of the banked materials to preserve the genetic diversity of the wild and captive populations, to support the living wild and captive populations, and as a resource for reintroductions to replace extinct populations.

3. Develop models for the amounts of materials to collect and store from

individual animals in the captive populations and from animals in the wild populations.

4. Delineate the species distribution and population structure based upon concordance of geographic distribution, molecular genetics, and morphology.

5. Define a sampling strategy for collection of genetic materials (semen, ova, embryos) from the wild populations of the species to provide representation of 98% of the presently available species genetic diversity.

6. Determine if there has been introgression of genes from domestic cattle into the present wild populations of each of the species of wild cattle. If so, then determine on a case by case basis what strategy will be employed to sample these populations.

7. Evaluate whether there have been human caused movements of genetic materials between wild populations of each wild cattle species. If so, then determine on a case by case basis what strategy will be employed to sample these populations.

8. Conduct a workshop: a) to review the distribution, numbers,and threatened status (according to the Mace-Lande criteria; Appendix I) of all populations of wild cattle; b) to recommend conservation actions for each population in terms of protection and management needs, need for a Population Viability Analysis, need for establishment of a captive population, and need for establishment of a genome banking program.

9. Develop procedures and resources for transfer of the cryopreservation, reproductive, and molecular genetic technologies to specialists in each of the range countries of the wild cattle species.

10. Initiate a process to identify interested collaborators to explore the potential usefulness of these materials for enhancement and development of domestic breeds of cattle for use in the range countries and elsewhere.

SELECTION STRATEGY FROM SOURCE POPULATIONS

Wild populations.

Identify populations for genome banking based on:

1. Level of threat (Mace-Lande criteria)
2. Availability of collaborators, resources, and animals

3. Cooperation and interest of range country wildlife officials

Animals from populations living in nature are especially valuable gamete/embryo donors for three primary reasons:

1. They can be objectively selected from all parts of the species range and collectively represent the existing (remaining allelic) diversity for that species as a whole. In each case, care must be taken in the sampling strategy to avoid artifacts (i.e. artificial movement of individuals by humans)

2. It can be argued that the genetic makeup of adult animals from healthy populations in nature is, by definition, the current ideal. Perpetual reproductive access to these genomes provides the antidote to genetic drift (i.e. reduced "fitness") both in skewed gene frequencies and in absolute loss for both multi-generation captive populations and bottle-necked wild populations.

3. It is not always sufficient to only utilize captive animals since: 1) founder populations are frequently small and an inadequate sample; 2) we rarely have access to many of the founders, access only to descendants means an immediate loss of genetic diversity; 3) founders are commonly of unknown province (i.e. are they representative of the geographic range and range of diversity; are they litter mates)

It is recommended that appropriate sampling methods be developed for selecting the number and location of wild donors for continuing collections in future generations. It is also recommended that:

1. Wild donors be permanently identified (e.g., tagged, transponder implant etc.)

2. Blood and other tissue samples be stored from each donor for comparative (multi-generational) genetic studies as well as for epidemiological work both present and future.

Captive Populations.

Founder animals: It is recommended that the degree of relatedness between living founders (i.e., wild caught animals) be assessed with modern molecular genetic techniques. Founder animals are automatically given highest priority for gamete/embryo banking for many of the same reasons wild specimens are valued (see above), and

Captive-born animals: Careful analyses of the pedigrees of the current population are necessary to select gamete/embryo donors of captive-born origin for banking. Two criteria

for selection have been initially identified:

1. Mean Kinship calculated from the gene drop analysis (Lacy, 1991) using studbook data. The Mean Kinship is the average relatedness of each individual to every other animal in the population, and is calculated based on the kinship coefficient, using the additive matrix and pedigree data. Priority is positively correlated with mean kinship coefficient. Mean kinship will always rank parents higher than offspring.

2. The need to identify relationships among individuals listed in the high priority category to avoid over-representation of family lineages (i.e. siblings)

Additional scenarios may need to be examined for adequate assessment on a case by case basis.

Sample Sizes.

The number of selected donors will generally range between ten and twenty-five individuals. Collectively, the individual donors will represent a "percent of the genetic diversity retained" figure that is greater or equal to that of the current captive population as a whole. A model needs to be developed to evaluate and identify: 1) additional individuals for collection and 2) utilization of offspring produced by the frozen germ plasm.

MATERIALS TO BE BANKED

1. Reproductive management
   a. Semen
   b. Ova (as technology advances)
   c. Embryos

2. Genetic and disease management
   a. Fibroblasts in cell culture
   b. Serum

Until such time that ova can be reliably cryopreserved it is recommended that oocytes/ova be <u>in vitro</u> matured/fertilized and resulting embryos frozen.

AMOUNT OF SEMEN TO BANK

1. Quantity of semen stored should be sufficient to:

a. Provide duplicate samples for storage in geographically separate banks for security reasons, each bank providing sufficient insemination and/or embryo transfer opportunities to allow:

1) achievement of genome resource bank utilization goals,
2) protection against catastrophes,
3) establishment of genome resource bank in range country, and
4) full restoration of the original genetic diversity of the donor population in living form at least three times (generally meaning the potential of producing 30-50 living offspring x3).

2. Development of a formula for calculating the number of insemination opportunities (i.e., minimum sperm for conception per straw) and/or embryos will require values for the following variables:

a. Post-thaw viability.
b. Range of ejaculate quality (viable sperm per ml).
c. Samples required for testing.
d. Degradation through time (e.g., background radiation).
e. Female conception and birth rates using cryopreserved gene plasm.
f. Neonatal mortality.

STRATEGIES FOR COLLECTING MATERIALS:

Methodical/deliberate: Species specific programs need to be established in order to effectively collect the required genetic material from specified individuals or populations for the genome bank within the next year. This program would identify the logistics encountered for collecting semen, and nonsurgical collections of ova and embryos. Ova collected will be identified for either: 1) cryopreservation or 2) IVF and resulting embryos frozen.

Rescue/salvage: In order to protect against the loss of valuable genetic material a program needs to be developed for opportunistic gamete recovery from individuals. Circumstances under which material can be collected will include: 1) ovariohysterectomies, euthanasia, natural deaths (captive populations) and 2) human induced mortalities of

individuals in the wild (i.e. road kills, regulated hunting).

DEVELOPMENT OF COMPUTER SIMULATION MODELS

Projects:

1. Sampling strategies for wild and captive populations through time.

2. Designing explicit program goals, i.e. defining program length and levels of genetic diversity to be maintained.

3. Sperm/ova banks.

4. Embryo banks.

5. Interactive management between wild and captive populations including banks.

Computer simulations are designed to provide a basis for making decisions about the genetic management of both wild and captive populations. Simulations are intended to be used to explore and define the effects that different utilization and management strategies will have on the genetic composition of a population.

Studies have been initiated in designing simulation models that begin addressing the issue of how to utilize the genetic material in sperm banks and the impact of different utilization schemes on the management of the living captive and wild populations (Appendix II). Conceptually, ova banks can be modelled as sperm banks with the knowledge that genetic material from individual females will be more limited. To-date, little effort has been directed to evaluate quantitatively the role of embryo banks in the genetic management of captive and wild populations. The use of embryo banks will influence the utilization of the bank, since the embryo genome bank will represent a diploid versus a haploid population.

As part of the metapopulation genetic management of populations it is necessary not only for living captive and wild populations to interact but also for interaction with respect to genome banks. Simulation programs provide an infinite source of options to evaluate and allow for refine modelling of such interactions. Suitable models need to be developed within the next 1-2 years to guide programs. These models will be dynamic and continually evolving as additional new information becomes available.

Prepared by: Leslie Johnston, Ulie Seal, Dan Wharton, and Jean Brennan with contributions from Jon Ballou and Bob Lacy.

RESEARCH PRIORITIES FOR SINGLE SPECIES CONSERVATION BIOLOGY

Workshop

13-16 November 1988

Sponsored by the National Science Foundation and
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Organized by

David E. Wildt, National Zoological Park
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and

Ulysses S. Seal, V. A. Medical Center
Minneapolis, MN

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FINAL REPORT

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Contents

INTRODUCTION

Past and ongoing human activities threaten biodiversity over the entire planet. We are in the midst of the largest biological experiment ever performed. It dwarfs projects like eradicating smallpox, sequencing the human genome, building a superconducting super-collider, or constructing a space platform. The experiment is the exponential growth of the human population to the present 5 billion, perhaps 10 billion in 40 years, with resulting fragmentation, disassembly, and simplification of ecosystems. The world has never before supported so many members of a single species with such enormous ecological demands. This single experiment is so enormous that a new discipline involving more than 30 academic fields is emerging to study its consequences and reduce its impact.

The great experiment is a great gamble with high stakes - at risk is our quality of life and the quantity of other life. The momentum and inertia of ongoing population growth and pressure on natural resources argue against any quick solution to the increasing erosion of species diversity. This loss of diversity, although greatest in the species-rich tropics, also is widespread in temperate regions of the industrial north. Witness the recent loss in North America of the free-ranging California condor, black-footed ferret, and red wolf, and the extinction of the dusky seaside sparrow.

During the past 8 years, the preservation of species by captive breeding, the development of species survival plans, recovery plans, and population viability analyses, and the release of captive-born animals into wild habitat have identified common research needs. These perceptions led to the organization of this workshop on Research Priorities for Single Species Conservation Biology. A workshop on general research priorities in conservation biology, conducted in April 1988 by M. Soule and K. Ralls, provided a broad framework for addressing the global crisis. We chose to focus on single species approaches including captive as well as wild species populations. Natural populations and release biology, small population biology, reproductive biology, and the effects of stress and disease were identified as high priorities deserving discussion. Twenty-seven invited participants responded with enthusiasm. This document summarizes the workshop discussions and recommendations.

The report is organized into 6 sections. Section I provides a summary of the 4 topic areas. Sections II - V address the common priorities that emerged independently in each task group for the respective topic areas. Additional recommendations also were made by each task group which could greatly facilitate the overall success of conservation biology programs. A recurrent theme was the need for global monitoring and improved databases, a topic area considered so important as to add an additional discussion section (VI) which also focussed on need and common problems.

SECTION I. SUMMARY

Natural Populations and Release Biology

Conservation Biology is multidisciplinary in nature, sharing a fundamental structure with many branches of the life sciences. It's overall goal is to understand taxa and natural and human systems sufficiently to permit modifications allowing maintenance of biodiversity. Natural population studies addressing a broad spectrum of disciplines are preferrable including population dynamics, habitat assessment, interspecific interactions, physiological ecology, reproduction, behavior, spatial requirements, and disease. However, only long-term, multi-generational projects can yield answers to many key questions. Priority should be given to rational and strategic sampling so that generalizations can be made from a few well-conducted studies. Reintroductions will be increasingly important to bolster numbers, genetic diversity, and geographical distribution of threatened species. The great strengths of reintroduction are its power to rally public support for conservation and to test hypotheses about managed ecosystems.

Small Population Biology

The basic issue of population biology pertaining to species conservation is the understanding of processes which dictate why some species survive and some become extinct. More strictly formulated, it is critical to identify those factors which influence the viability of modern and historic populations, species, and subspecies. The 5 most important areas warranting attention are studies of population vital rates, population-genetic structure, the interaction of genetics and demographics, the impact of catastrophes, and the influence of habitat patterns. The primary focus should be on the many new tools associated with molecular biology, physiology, and theoretical population biology. When combined with field studies of target species, these techniques offer infinite promise for revealing the ecological and evolutionary processes responsible for controlling species survival and extinction.

Reproduction

Maintenance of species diversity depends on the process of reproduction. Habitat destruction usually results in deleterious demographic, epidemiological, physiological, and behavioral changes which compromise reproductive performance. Rare species, and especially those in chronic decline, deserve immediate attention to identify and characterize factors influencing reproduction. Results are important from a basic and comparative knowledge perspective and will be dependent on studies of reproductive demography, basic reproductive patterns, the influence of nutrition, season and disease, the monitoring and control of ovulation-gestation-parturition, gamete and developmental biology, and the collection, evaluation, and long-term storage of genetic material including gametes and embryos. The information derived can be applied to improving and formulating plans for enhancing propagation either by natural or artificial methods. The long-range benefits of this strategy will be a greater understanding of reproductive diversity as it relates to speciation and evolution.

Stress and Disease

Wild taxa are forced to survive and thrive in rapidly diminishing natural habitats and artificial, captive environments. One measure of adaptability in either environment is the ability to reproduce. Ultimate success is measured by the ability of offspring to achieve sexual maturity and continue to reproduce for species perpetuation. This criterion is valid for mammals, birds, fish, reptiles, amphibians, and invertebrates. Both stress and disease can severely compromise an animal's ability to reproduce. Presently, no effective measures are available for qualifying or quantitating the effects of stress on physiological or behavioral performance or survival ability. To understand the impact of this topic on conservation biology, research efforts should be directed at characterizing "normal" population data, developing quantitative methods for assessing stress, evaluating the etiology of infectious disease, determining disease prevalence within populations, and defining the interrelationships of stress or disease with behavior, reproduction, nutrition, demography, and various environmental factors.

SECTION IV. REPRODUCTIVE BIOLOGY - DETAILED REPORT

Overview

The database defining reproduction for most animal species is limited or nonexistent. Yet, biotechniques for maintaining genetic diversity and enhancing captive propagation must be based on a thorough understanding of reproductive biology, especially physiology. This information can be obtained only by conducting both basic and applied research.

Characterizing reproductive demographics is the first step to identifying species or populations in crisis and providing an informational base for properly designing later studies. Demographic and reproductive status within and among species will be useful for understanding the evolutionary mechanisms influencing biological function and phylogenetic relationships. On a practical level, this knowledge will be important for documenting reproductive competence and identifying infertile animals which will be unable to contribute to breeding populations.

Basic reproductive characteristics of a species can be valuable in formulating propagation masterplans which can translate known physiological and behavioral information into improved husbandry and, if necessary, artificial breeding programs. The types of studies needed are diverse and include documenting mechanisms spanning the events from gamete formation to reproductive senescence. The influences of environment (e.g. season, nutrition) and our ability to understand and control reproductive processes (e.g. hormonal induction of ovulation/parturition, artificial insemination, in vitro fertilization) will be vital to ultimately assuring both species preservation and genetic variability within existing populations. Cryobiology will have a major impact on conserving genetic diversity. Used judiciously, a resource of frozen semen and embryos could be used interactively with living populations to periodically infuse genetic material from captive or wild animal stocks or to instill captive populations with thawed genes from previous generations.

Information gained from parallel studies of domestic and laboratory animal models certainly will be useful and may offer hypotheses that can be extrapolated to wild species. However, there is little doubt that most species, even those closely related, demonstrate unique features making species-specific research mandatory. Speciation often is accompanied by significant modifications in reproductive processes, a natural strategy for maintaining species integrity. From a practical perspective, this characteristic reduces the possibility of applying a specific concept or technique across species and, therefore, necessitates more overall research. From a positive viewpoint, these reproductive adaptations provide a unique informational resource for studying evolutionary change.

Both the phenomena to be studied and the methods to be used are so complex as to mandate major multidisciplinary and interdisciplinary efforts among reproductive physiologists, population biologists, ethologists, anatomists, geneticists, cell and molecular biologists, bioengineers, biochemists, and biophysicists. It also is necessary to extend reproductive biology research beyond species solely maintained in captivity. Physiological studies of free-ranging wildlife are possible and will provide comparative norms for captive conspecifics, insights into failed captive breeding programs, and new and potentially exciting perspectives in the most fundamental of biological processes, reproduction.

Research Priorities

Characterization of Reproductive Demography. Demographic data which include age-class, sex, and reproductive status are critical for formulating research strategies for a specific population. This information will provide both the justification and focus for later investigations and will aid in generating hypothesis-driven research proposals.

Characterization of Basic Reproductive Physiology. Fundamental reproductive information is essential for the captive propagation of rare species by natural or artificial methods. Because of the vast array of reproductive strategies even among closely related taxa, it is necessary to examine specific reproductive processes within species. When collected comparatively from a variety of species, this information will contribute to our understanding of speciation in the context of evolution. Essential areas of study include pubertal onset, seasonality, ovulatory mechanisms (spontaneous versus induced), the ovulatory cycle, ejaculatory norms, temporal patterns in gamete transport, implantation, social/sexual behavior, gestation, parturition, postpartum fertility, and litter or clutch characteristics.

Interaction of Nutrition and Reproduction. Nutrition has a major, multi-level impact on reproductive processes in all captive or free-living species. Quantity of nutrition regulates growth and, thus, pubertal onset. In concert with environmental cues, nutrition can alter the reproductive season and the time of postpartum breeding as well as postnatal survival. Even during periods of adequate nutrition, a marked increase in nutrient intake can enhance ovulation rate (the "flushing" effect). Quality of nutrition also is important because, under some circumstances, plant factors are known to enhance or reduce fertility. Specific nutritional requirements need to be identified for both wild and captive animal populations. More research also is required into mechanisms whereby nutrition affects reproductive

function.

Influences of Environment on Reproduction. Under natural conditions most species are seasonal breeders, a strategy conducive to offspring survival. The annual breeding rhythm often is synchronized with season by environmental cues, especially photoperiod in temperate climates. Changes in daylength, temperature, specific dietary factors, and social cues (sensory) that inhibit or enhance reproduction must be studied on a comparative basis. Additionally, parallel studies of disease, parasitism, or inadequate habitat on reproductive performance require further study.

Prediction and Detection of Ovulation. Predicting ovulation allows the precise timing of either natural or artificial breeding and the collection of fertilizable ova or embryos. Methods to estimate the ovulatory event must be developed on a species-to-species basis.

Induction of Ovulation. Hormonal induction of ovulation often is the prerequisite to artificial breeding. However, the choice of specific hormone, the pharmacologic dose, and the duration of treatment generally is species-specific. Further research is warranted to understand the variability in ovarian response both within and among species and to improve exogenous hormone delivery systems.

Monitoring and Control of the Estrous Cycle, Gestation, and Parturition. Poor conception rates, pregnancy loss, and neonatal mortality are major contributors to reproductive inefficiency. Therefore, the monitoring of cyclic patterns and gestation will be valuable for (1) improving fertility, (2) safeguarding existing pregnancies, (3) predicting the birth event, and (4) increasing neonatal survival. These objectives can be achieved by understanding the comparative endocrinology of pregnancy and parturition. Presently for intractable species, urinary hormone assessments offer a unique and atraumatic approach for identifying this fundamental information and, thus, should receive high priority. Likewise, measuring hormones in blood has been valuable for investigating acute hormone dynamics and species-specific mechanisms of endocrine control. Although more invasive, such studies have proven safe (due to modern anesthesia) and, therefore, also should be encouraged.

Collection and Evaluation of Germ Plasm. Current methods of semen collection are not universally effective for all species. Atraumatic methods are needed for safely collecting eggs and embryos from rare species. New methods for animal restraint which do not influence gamete quality or compromise animal well-being must be developed. Additional efforts should be directed to in vivo and in vitro assessments of gamete and embryo viability.

Artificial Insemination. Artificial insemination is an effective procedure for improving reproductive efficiency and alleviating certain types of infertility in domestic animals and humans. For wildlife species, this approach also circumvents the problem of sexual incompatibility or the absence of a male. Artificial insemination could be especially important for (1) ensuring reproduction between genetically valuable but behaviorally incompatible animal pairs, (2) eliminating the risk of animal transport while allowing reproduction between geographically dispersed individuals, and (3) providing a major avenue for using using frozen germ plasm to infuse genes from wild stocks into captive populations.

Maximizing the success of artificial insemination in wild taxa will require studies of sperm maturation, in vitro processing to enhance viability, and cell transport to ensure that sufficient sperm are present at the fertilization site at the proper time.

Gamete Biology. Further research in cell biology and gamete biochemistry is essential to enhance our understanding of gamete maturation, sperm capacitation, the acrosome reaction, and fertilization. This information is vital to implementing strategies for in vitro fertilization, embryo transfer, and the salvage of germ cells from reproductive senescent animals or those which die unexpectedly.

Developmental Biology. When natural reproduction fails, it is mandatory to apply more complex approaches, most of which require a sophisticated understanding of developmental biology. Prerequisite studies are needed on basic gamete interaction, placentation, maternal recognition, totipotency, and differentiation. This information will provide the framework for more detailed investigations of in vitro fertilization and embryogenesis, findings which then can be applied to even more advanced biotechniques. Those deserving study include interspecific and intergeneric embryo transfer, cloning, chimerism, and gene transfer.

Preservation of Genetic Resources. The cryopreservation and storage of gametes and embryos at low temperatures (-196^{o}C) offers unique opportunities for facilitating the propagation of wild species and ensuring the conservation of genetic diversity. The development of banks of cryopreserved germ plasm will (1) reduce the number of animals needed to ensure high levels of genetic diversity, (2) facilitate the infusion of germ plasm from wild populations into captive breeding programs, and (3) provide insurance against the loss of diversity from epidemics, natural disasters, and social/political upheavals. Similarly, DNA and tissue banks permit retrospective genetic analysis of founder animals. Research is needed to develop simple, alternative preservation approaches including vitrification, freeze-drying, and cold storage (4 or -20^{o}C).

Additional Suggestions and Recommendations

1. Animals are available in too few numbers to allow making scientifically sound conclusions. Investigating reproductive problems in rare animals often is compromised by low numbers of experimental subjects. Decisions may have to be based on data from less than ideal sample sizes, representing varying environmental backgrounds. To avoid this problem, research efforts should be initiated long before a species approaches extinction. In those situations where animal numbers are low, research protocols should be judged on the basis of optimal use of the available resource, rather than being compared with some idealized standard of experimental design.
2. International programs are necessary. The widely dispersed geographic distribution of species requires international research programs that must be conducted in foreign countries. To facilitate these efforts, formal international agreements and collaborative projects should be encouraged and engendered.
3. Training programs are necessary. The diversity of reproductive biology and the many problems which might be associated with reproductive failure necessitates specialized education in wildlife biology, modern biotechnical fields, and classical training in comparative reproductive biology. This need can be resolved through the development of

training grants, internships, and fellowships to support the education of talented young professionals.

SECTION VI. GLOBAL MONITORING AND DATABASES

Overview

Aldo Leopold stated "The first rule of intelligent tinkering is to save all the pieces." This standard, of course, is far from being met. By not even developing reliable estimates of the numbers of pieces being lost, there is a major failure to perform badly-needed science. By not assembling and distributing data sets, scarce resources are failing to be mobilized. Estimates of the number of species now on Earth vary by an order of magnitude. Predictions of the rate of loss of habitats including rain forests has been intensely controversial. Estimates of the fraction of global photosynthesis now diverted for human purposes vary enormously as do species extinction rates.

To strengthen both the empirical base and theoretical content of Conservation Biology, there is a strong need to monitor habitats and taxa and record detailed information in a global database.

Research Priorities

Habitat monitoring and databases. Centralized records are needed for the "great experiment", the exponential growth of the human population. This requires a mechanism whereby time series data on the remaining extent of the various habitat types around the world can be recorded and made universally available. This likely will require remote sensing with some selected ground truth validation. Portions of such strategies already are in sporadic existence (e.g. UNEP's GRID in Nairobi, The Nature Conservancy, The Sierra Club) but each data set is fragmentary and essentially a single time-point. No trendlines can be drawn.

The value of the data is related directly to those with access and skills to use it. The information not only needs to be collected, but also made readily available to both researchers and policy-makers worldwide. One approach might be the creation of a Geographical Information System (GIS) which could be facilitated through a network of common desktop microcomputers. This approach would enhance data assembly because users with local data could effectively review and update the central database.

Taxon data sets. Defining a species is a challenge, but species are better-defined measurable units than habitats or communities. As such, species play a key role in developing and testing conservation biology theory. Species also are the units usually protected by law and policy. Assembled knowledge of what is happening to species-level populations is critical, although generally such information is sorely lacking. A central database is needed to maintain and distribute the best available taxon-level information.

When possible, the data must include time-series for range and population size estimates. Costs would be lower and accuracy greater if users with special local knowledge had ready access to GIS-type subsets of relevant data which could be periodically up-dated in the field using modest microcomputer equipment. The IUCN/WWF/UNEP and the World and Conservation Monitoring Center (WCMC) now provide limited data, but access is restricted, timeliness inadequate, and the information largely is text with few accurate numbers.

At a secondary level, research and conservation strategies for hundreds, perhaps thousands of species will include and benefit from captive populations. Therefore, central, accessible, and distributable data sets are needed for global populations of animals. The International Species Information System (ISIS), in addition to approximately 100 single-species "studbooks", fulfills some of these needs. ISIS, although expanding rapidly, presently records data on only half the world's zoological specimens. Furthermore, wide distribution of ISIS and studbook data sets is only beginning.

Population dynamics (demography and genetics). Knowledge obtained from improved monitoring and assembly of global data likely will be the only warning of serious difficulties - more detailed knowledge organized by conservation biologists likely will provide our only solutions. Measuring status and trends in wildlife populations is fundamental. Developing simulation and analytic models that integrate demography, genetic, and environmental variation is needed immediately.

Selecting illustrative cases. Choice of model, target, or reference species and populations is necessary. Focus should be on rare, endangered, "keystone", and "flagship" species. Priority should be given to a rational and strategic sampling of cases, so that generalizations can be built from a modest number of well-performed studies. The new tools of molecular biology, physiology, and theoretical population biology, when combined with field studies of target, representative species, offer much promise for revealing the ecological and evolutionary processes that control the survival and extinction of species on earth.

Long-term studies. The value of Conservation Biology research compounds with duration. Only long-term, multi-generational projects can yield values for some of the key variables.

Genetic Resource Banks for Endangered Species - Information Needs

Nathan R. Flesness, Executive Director
International Species Information System (ISIS)

Preservation of the genetic resources of endangered species often involves sophisticated technologies. In the excitement about the technology, it is important not to overlook the fact that the preserved DNA, held in whatever tissue form, is only as valuable as what you know about it.

The intrinsic value of the DNA-containing tissue, as a tool for enhanced reproduction of live specimens, source of genes for genetic transplants, or as a DNA sequence archive for research, is determined by the associated information on the sample. It is obvious that samples (of whatever kind) must be reliably associated with information about what taxon they are from, which individual living specimen, collected where - or of what ancestry. It is also obvious that reliable information about the collection of materials - i.e. sample inventory - is critical for efficient access and use of the valuable Genetic Resource Bank by the diverse audience which is interested.

Extensive experience is in hand for the last 17 years for one kind of genetic resource bank - living captive specimens. The experience includes both zoological institutions and (for chimpanzees) primate research centers. ISIS presently tracks origin/provenance/pedigree, birth date, sex, move history, death date, death circumstances / autopsy results, and clinical chemistry/hematology on captive specimens in participating facilities; this assembled data then readily allows calculation of pooled inventory for over 112,000 live specimens of 4,200 taxa in 350 institutions in 37 countries. The database includes an archive of information on over 200,000 additional specimens ancestral to the current live inventory. Approximately 30% or 33,000 registered live specimens, are of presently endangered or threatened species. Available and essential information services include pooled inventory and species and specimen pedigree/provenance tracing. An essential part of the network are ISIS-developed PC software tools which operate as a distributed system within the individual facilities for maintaining local databases and communicating with the central information pool.

Considerable acquired experience with both zoological institutions and primate research centers indicates that local perspectives usually will dominate local data-recording. Adequate data quality and integrity can be established only through a broad-scale integrated information system which returns both short- and long-term valuable services to those holding individual collections of Genetic Resource Bank materials. Similar experiences suggest similar solutions for endangered plants as well as animals (Dr. Kerry Walter, Center for Plant Conservation, pers. comm.). Such broad-scale systems require

clear establishment of minimum specimen data standards, and are greatly facilitated by central development of PC applications software which can be distributed, to be used on-site for genetic resource bank collection maintenance, and also to facilitate pooling data across multiple banks.

A program to foster sound and coherent development of Genetic Resource Banks for Endangered Species must offer the maximum value for the resources invested. This can best be accomplished by including development of adequate information systems as a necessary component, and by building on or with existing genetic information networks.

LESLIE J.
A. Morales
L. Dolan
V. GEIST
E- Schwedt
D- MIQUELLE
J- DOLAN
D. Moore
D. Muller-Schwarze
A. Gardner
D. Wharton

Genetic Resource Banks and Reproductive Technology for Wildlife Conservation

David E. Wildt, Ph.D., Ulysses S. Seal, Ph.D.* and William F. Rall, Ph.D.

National Zoological Park
Smithsonian Institution
Washington, DC 20008

and

*Captive Breeding Specialist Group
World Conservation Union
12101 Johnny Cake Ridge Road
Apple Valley, MN 55124

Telephone for correspondence - (202) 673-4793
Telefax - (202) 673-4733

Introduction

Successful reproduction is the essence of species existence and, thus, the preservation of bio- and genetic-diversity. Therefore, reproductive physiology plays an essential role in the emerging field of conservation biology. Cryobiology, or low temperature science, will dictate our ability to successfully store animal germplasm, tissues and DNA which will be vital to preserving both species and genetic variation. Within each of these disciplines, scientists can wield an arsenal of techniques sometimes categorized under the rather board terms of "assisted reproduction" or "biotechnology". For more than 2 decades, there has been much hyperbolic debate about the potential uses of artificial insemination (AI), embryo transfer (ET), in vitro fertilization (IVF) and "frozen zoos" for the preservation of species. But, the saving of species implies the production of offspring, and most of the pregnancies generated in various wildlife species using assisted reproduction have been one-time events (Wildt et al., 1991). Many artificial breeding attempts also have failed and gone unreported (Wildt et al., 1986).

Although reproductive biotechnology, including the use of frozen gametes and embryos, has not as yet contributed to practical conservation, great strides have been made, enough to suggest that within the next decade artificial breeding will find management application, at least for some wildlife species. If the ultimate goal is to maintain the planet's bio- and genetic diversity, then we contend that organized genetic resource banks, containing sperm, oocytes, embryos, tissue and DNA, certainly can compliment and enhance other ongoing conservation efforts. The benefits of such a strategy are enormous, and a primary objective of this chapter is to discuss this potential. We also provide evidence of real scientific progress in terms of what works (and has not worked) in the laboratory for wildlife species. Even if all of these technical advancements were ignored, biologists have benefited immeasurably from their mistakes. Those of us interested in this field now are much more in awe of the overall challenge but much more confident about the strategies needed to allow biotechnology to contribute to conservation. This also is an exciting time from an organizational perspective. As will be described, systematic networks and formalized

programs are in place, and national and international cooperation is the norm for dealing with captive and wild species in crisis.

Developing Strategies for Conservation (Cross-Institutional Cooperation - Getting Organized)

The viability of our wildlife is interdependent on the level of genetic variation within species, populations and individuals. Captive wildlife populations are especially vulnerable to additional losses in genetic diversity. Prior to the 1980's, zoos paid little attention to which individual animals were breeding. As a result, many populations became genetically stagnant, a finding which became alarmingly apparent to the zoo community in 1979. Ralls et al., in that year, discovered an extremely high incidence of inbreeding in 29 zoo species which correlated directly with neonatal mortality. Subsequent studies confirmed that creeping genetic monomorphism was adversely affecting the health of many captive as well as free-living species. Coincidentally, the American Association of Zoological Parks and Aquariums instigated the concept of Species Survival Plans (SSPs) and, more recently, Taxon Advisory Groups (TAGs) to assist in managing species, populations and individuals for the purpose of ensuring genetic health. The SSP and TAG programs have been bolstered by the Captive Breeding Specialist Group (CBSG) working under the auspices of the IUCN (or World Conservation Union). The CBSG mission is global, and its goals are to: 1) organize a global network of people and resources; 2) collect, analyze and distribute information; 3) develop global captive breeding programs; and 4) integrate management programs for captive and wild populations (Ulie, Do you have a reference to use here?). The success of the SSPs, TAGs and CBSG largely relies on the volunteer efforts of a coherent assembly of experts which includes reproductive specialists. We contend that the development of successful genetic resource banks will be highly dependent on the ability of these specific groups to: 1) interact to formulate sound plans based on real science; and 2) translate research findings into an applied benefit, that is, the conservation of species and genetic diversity. Because of its global

mission, the CBSG (at the time of this writing) is developing formal guidelines for the eventual development of genetic resource banks for wildlife species.

A Call for Genetic Resource Banking

For more than 12 years, there has been a consistent demand for establishing genetic resource banks for wildlife species.

- "New agencies should be established, or existing agencies charged, with the preservation of particular germ plasm resources. Funds should be provided to support these agencies and to train the personnel necessary for the maintenance of these essential resources. What is done for domestic species (e.g., AI, sperm and blastocyst freezing, and implantation) should be done for all species reproducing in captivity." (Conservation of Germ Plasm Resources: An Imperative, National Research Council, Report of Committee on Germ Plasm Resources, National Academy of Sciences, Washington, DC, 1978.)
- "Establishment of a program in the U.S. to coordinate the management of animal germ plasm resources would be in the national interest." (Animal Germplasm Preservation and Utilization in Agriculture, Council for Agricultural Science and Technology, Report No. 101, Ames, Iowa, September 1984.)
- "Preservation of germ plasm requires that institutions should be developed and/or strengthened for collection, maintenance and dissemination of genetic resources." (U.S. Strategy on the Conservation of Biological Diversity, Inter-Agency Task Force Report to Congress, U.S. Agency for International Development, Washington, DC, 1985.)
- "A program of research could be administered through the National Science Foundation and channel funds to both basic studies on the reproductive biology and cryobiology of wild animals and to applied studies on the control of reproduction, AI and ET. Another approach could be establishing a few centers for study of the reproductive biology of wild animals. These centers could serve as focuses for programs of basic and applied research. They should be sufficiently

well-funded to allow broad programs of research on-site as well as extramural research with cooperating institutions. These centers could likewise serve as repositories for frozen gametes and embryos from endangered populations". (Technologies to Maintain Biological Diversity, U.S. Congress, Office of Technology Assessment, Report No. OTA-F-330, U.S. Government Printing Office, March, 1987.)

- "High priorities studies include . . . gamete and developmental biology and the collection, evaluation and long-term storage of genetic material including gametes and embryos. Successful cryobiology will have a major impact on conserving genetic diversity. A resource of frozen semen and embryos could be used interactively with living populations to periodically infuse genetic material from captive or wild animal stocks or to instill captive populations with thawed genes from previous generations. The cryopreservation and storage of haploid gametes and diploid embryos and cell cultures at low temperatures (-196°C) offers unique opportunities for facilitating the propagation of wild species and ensuring the conservation of genetic diversity." (Research Priorities for Single Species Conservation Biology, a workshop sponsored by the National Science Foundation and the National Zoological Park, Washington, DC, 1989.)

The potential of such resources already has been demonstrated by the successful preservation of germ plasm from important domestic food animals, companion animals and crop plants. Private enterprise as well as some governmental actions have been initiated to collect, protect and use these agricultural resources. For example, the commercial distribution of frozen cattle semen has been in place for more than 30 years. Recently, similar commercial efforts have spread to the routine use of frozen cattle embryos, frozen rodent embryos (for biomedical research) and frozen sperm from purebred dogs. Both private and federal institutions have begun to systematically store biological material from specific animal and plant genotypes. As three examples: 1) the Jackson Laboratory and the National Institutes of Health maintain large storage facilities for frozen embryos collected from hundreds of genotypes of mice used as animal models for biomedical research; 2) the United States Department of Agriculture maintains a repository for

plant seeds from crop species; and 3) The American Type Culture Collection functions to acquire, preserve and distribute characterized strains of bacteria, fungi, protozoa, algae, viruses, cell/tissue cultures and the creations of recombinant DNA technology.

Justification and Potential Utility of Genetic Resource Banks

In light of the publicity directed at dwindling habitat, the loss of species and genetic diversity and the potential of artificial breeding technology, it is remarkable that no organized effort exists, either in the U.S. or elsewhere, to sample, evaluate, cryopreserve, catalog, maintain and use germ plasm from wild animal and plant species.

- In practice, a Genetic Resource Bank would not be merely a static warehouse of biological material but would serve a vital, interactive role between living populations of captive and free-living species. These interactions are required to prevent undesirable selection pressures in captivity, preserve new diversity resulting from natural evolutionary processes and allow small or fragmented populations to receive "infusions" of genetic diversity from cryopreserved germ plasm.
- The combination of frozen gametes and embryos and reproductive techniques such as AI, ET and IVF offers unique opportunities for improving the efficiency of captive breeding programs. Cryopreservation of germ plasm extends the generation interval of a species indefinitely. The genetic diversity of the founder does not die with the animal, but remains viable and available for future generations.
- Germ plasm banking has the effect of reducing the number of animals needed to ensure that high levels of genetic diversity are retained within a population. This reduces the capital and operating costs of zoo breeding programs and provides space for other species at risk for extinction.

- Other benefits include the incorporation of germ plasm from wild stocks into captive breeding programs without removing animals from the wild, and the insurance banking offers against the loss of diversity or entire species from epidemics, natural disasters and social/political upheaval.
- Transporting frozen sperm or embryos eliminates the considerable risks associated with the transport or exchange of live animals.
- The judicious interspecies use of germ plasm (e.g., hybridization of rare species of wild cattle with common cattle) may provide avenues for improving the genetics, food production and general agri-economy of developing countries.
- Technologies developed for animal and plant germ plasm can be used to expand the genetic bank to include other biologicals including tissues, blood products and DNA. These new elements also can provide a service repository allowing more wide-spread access to rare specimens.

Utility of Genetic Resource Banks for Both Habitat and Single Species Conservationists

Conservation biologists constantly debate the relative merits of saving habitats versus species (Wildt, 1990). Habitat proponents suggest that representative ecosystems can be identified and permanently isolated from human interference. This approach focuses on the long range problem and protects the ecosystem and the many species within it. The problem, of course, is in choosing which habitats to preserve since many species inevitably will be excluded. Additionally, those species most susceptible to extinction (i.e. large-sized predators at the top of the food chain) often require extraordinary large home ranges. Therefore, it may be too expensive or socially disruptive to provide sufficiently-sized natural reserves for such animals. The contrasting view is to preserve single species, an approach exemplified by captive breeding programs. In this scenario, animals are maintained in a semi-controlled environment, and modern science is charged with identifying and manipulating the factors influencing reproductive success. In theory and given modern management techniques, rare species should thrive in captivity. But single species

conservationists encounter many of the same problems that plague programs designed to save native habitats. The shear cost of establishing "naturalistic" captive environments and prioritizing which species deserve the most attention are major concerns. These proponents also are faced with the paradoxical threat of unbridled success, that is, that captive breeding may increase animal numbers to the point that all captive habitat is saturated and then overwhelmed.

There is no doubt that habitats and single species should be salvaged simultaneously and that the cryopreservation of biological materials can play a major role in both types of conservation. For example, free-living animals produce excessive germ plasm which can be recovered safely, cryopreserved and used to infuse captive populations with genetic vigor while eliminating the need to remove more animals from the wild. Likewise, cryobiology offers a resolution to the problem of limited captive breeding space. It simply is more cost-effective to preserve genetic material from populations, genotypes and individuals at low temperatures, eventually re-deriving these species when needed.

Overview on State-of-the-Art Reproductive Biotechnology including Cryopreservation of Germ Plasm and Embryos

In humans and domestic animals, successes with germ plasm cryostorage, AI, ET and IVF were the result of considerable basic research which was translated into significant improvements in reproductive performance. In reality, these advances have sparked a revolution in combating human infertility and improving livestock production (Wildt, 1989). For wildlife species (including vertebrates and invertebrates), these techniques could be useful for enhancing propagation and sustaining current levels of bio- and genetic diversity. This speculation is bolstered by considering the surge of interest and the development of effective and practical strategies for improving captive breeding of rare animal species over the past 10 years. Witness the formulation of species survival/action plans and the evolution of the population viability analysis which allows wildlife managers (those concerned with both the wild and captivity) to

work together to (1) assess species/population robustness and (2) generate recovery plans for the future based on scientific fact (Seal and Foose, 1983; Foose, 1987; Ulie, do you have other good references we could insert here?). This progress, combined with the actual release of captive-born animals (e.g., golden lion tamarins, red wolves, bison, Arabian oryx Kleiman review article) into wild habitats have revealed one common theme, there is an all-out need for more quality research (Wildt, 1989).

Logic dictates that considerable emphasis be placed on sustaining existing biodiversity using cryopreservation technology since any further delay only will accentuate ongoing and relentless losses in species and genetic variability. The actual details for using frozen gametes and embryos for producing wildlife offspring have yet to be developed for many species. Nevertheless, sufficient technology and in vitro test procedures are available to ensure that most frozen germ plasm is biologically competent and eventually useful (Wildt, 1989). Another argument for developing genetic resource banks first is that this approach naturally spawns and mandates related research in other reproductive, biomedical and veterinary fields.

Reproductive biology is a complex discipline, and the actual applied use of a genetic resource bank will inevitably require interactive research among many disciplines and the development of new technologies. These, in turn, will generate massive data sets which will help scientists fully comprehend the fundamental biology of rare species, almost all of which have never been studied. For example, the "simple" collection and cryostorage of Siberian tiger sperm will require interaction among: 1) the Species Survival Plan Coordinator and SSP Propagation Committee (to choose which animals to use); 2) population biologists (to help determine the number of samples to preserve to maximize genetic representation); 3) veterinarians (responsible for developing optimal anesthesia); 3) biotechnical engineers (responsible for developing equipment to collect sperm); 4) gamete biologists (talented in sperm collection, processing, and evaluation); 5) cryobiologists (skilled in the freezing, packaging and storing of sperm); and 6) registrars (responsible for maintaining computerized catalogs detailing the pedigree of the sperm donor and the location, amount and distribution of the germ plasm). The actual use of the frozen

sperm will stimulate even further research into an array of exciting areas (e.g., establishing the length of the estrous cycle; studying the impact of seasonality on reproductive performance; identifying and synchronizing estrus; predicting ovulation; developing techniques for AI, IVF and ET; diagnosing pregnancy and impending parturition). Therefore, genetic resource banks will serve as the incentive for developing and expanding other biological disciplines ranging from fundamental reproductive biology to genetic management of rare populations to applied aspects of veterinary medicine to global monitoring/computerized information systems.

Potential benefits of AI, IVF or ET to wildlife conservation. The benefits of a genetic resource bank can be realized only if AI, IVF and ET methods are available for the species of interest. AI is valuable for ensuring reproduction between behaviorally incompatible pairs, eliminating the risks of animal transport and providing an avenue for infusing genes from wild stocks into genetically-stagnant, captive populations.

From an applied perspective, IVF has the potential of resolving many of the serious problems routinely encountered in modern captive-breeding programs (e.g., achieving representation from animals failing to reproduce because of behavioral peculiarities, physical handicaps or stress susceptibility) (Wildt, 1990). IVF also is highly attractive because it requires neither detection of overt estrus nor direct interaction between the male and female. In the context of a frozen germ plasm resource, an IVF program could be used to infuse gametes collected from free-living animals into captive populations. There also has been recent advances in related technology which allows recovering and maturing early stage ovarian oocytes in vitro, an approach which could be useful for salvaging genetic material from rare animals that die abruptly. Theoretically, with cryostored germ plasm, IVF could be combined with gamete maturation to produce embryos and then offspring from deceased individuals. Pilot studies already have been successful with laboratory rodents and farm livestock (see reviews, Johnston et al., 1989, 1991).

For wildlife species, ET offers the possibility of accelerating the number of offspring produced and using females incapable of reproducing because of age or physical /medical handicaps. In conjunction with embryo freezing, ET could help preserve the combined genetic

component of an individual in suspended (frozen) animation, thereby offering an approach for reintroducing available genetic material into later generations. The potential of ET increases if embryos can survive interspecies ET, that is, develop and be born to surrogate mothers of a more common species or closely-related genus.

Current realities to the use of cryopreserved germ plasm and AI, IVF and ET in mammalian species. Although the possibilities of using frozen wildlife germ plasm and embryos in concert with AI, IVF or ET are staggering, most successes have been limited to farm livestock and laboratory animals. Table 1 lists the 28 species in which AI with frozen-thawed sperm has resulted in live-born offspring. IVF has been successful in a total of 13 species, and, in almost all cases, the young born were conceived using fresh (non-frozen) gametes (Table 2). Table 3 includes the 14 species in which frozen-thawed and transferred embryos have resulted in live-born offspring. Considering that more than 4,000 mammalian species inhabit our planet, it is obvious that the potential of reproductive biotechnology has been tested in an infinitesimal fraction of wild taxa.

The lack of application is the direct result of insufficient or, in most cases, nonexistent funding for both basic and applied wildlife research. Amazingly, no centralized organization has evolved to compel zoos to participate in a genetic resource conservation program (like the Species Survival Plans). Even so, there is a general consensus that reproductive biotechnology could be used to better preserve genetic diversity and assist in captive propagation given that species reproductive norms were known (Wildt, 1989). In this respect, this same strategy was used to make the use of frozen sperm and embryos feasible in farm livestock. For example, the conventional use of AI and embryo transfer in domestic cattle became routine only after years of research into gamete biology and fundamental reproductive processes. This substantial progress could only be made after millions of research dollars were provided by federal, commercial and private sources.

Encouraging progress with wildlife species. Basic research strategies, similar to those used in livestock, laboratory animals and humans, are possible and have been applied to wildlife species (primarily on limited or pilot basis) by a few pioneering institutions. Advances in wildlife

and zoo veterinary medicine now permit routine and safe animal anesthesia which allows blood sampling (for endocrine monitoring; Seal et al., 1985; Wildt et al., 1988; Brown et al., 1991a,b,c), electroejaculation (for semen collection; Howard et al., 1986), uterine catheterization (for nonsurgical embryo collection/transfer; Schiewe et al., 1991; Schiewe, 1991), laparoscopy (for oocyte recovery; Miller et al., 1990; Donoghue et al., 1990) and ultrasound (for ovulation and pregnancy diagnosis; Donoghue et al., 1990). Other novel and exciting techniques have been developed to facilitate the eventual practical use of genetic material. The hormonal status of many wildlife species now can be tracked by measuring hormonal metabolites in voided urine or feces (Monfort et al., 1990, 1991a,b; Lasley and Kirkpatrick, 1991; Wasser et al., 1991). This innovative approach, which eliminates the stresses associated with blood sampling under anesthesia, has been used to document seasonality, the estrous cycle, time of ovulation and even predict pregnancy and impending birth. Simple, cost-effective enzyme-linked immunosorbent (ELISA) assays can be used to predict critical events (like ovulation from a urine sample) and offer exciting means of assessing endocrine function under field conditions. New approaches, originally developed for assessing human fertility potential, also are finding application in wildlife species. "Heterologous" IVF systems are available whereby hamster or domestic cat oocytes can be used to test the penetrating or fertilizing capability of sperm collected from other species (Howard and Wildt, 1990). These in vitro assays of sperm viability will be important in testing the biological competence of frozen-thawed, wildlife sperm. Lastly, reproductive biotechnology is beginning to contribute significantly to nonmammalian and invertebrate species. Some progress has been made in the long-term storage of fish gametes (especially salmon and rainbow trout sperm; Schmidt-Baulain and Holtz, 1989 Bill, please insert the appropriate references of Thorgaard, Cloud, others) and mollusc (Gallardo et al., 1988; Bill we need all the authors for the Literature Citation) embryos. Insects also are benefiting as illustrated by continued progress in the freeze storage of, for example, honey bee semen and the recent successful cryopreservation of Drosophila embryos (Steponkus et al., 1990).

As demonstrated in Tables 1 to 3, there is similar tantalizing evidence in other wildlife species. The live births of any wildlife species as a result of biotechnology and the use of fresh or frozen germ plasm are laudable. However, certain events are particularly worthy of note as they either: 1) demonstrate a biological "first" for wildlife species; 2) illustrate the ability to apply techniques developed for farm livestock to wild counterparts; or 3) focus on a particularly difficult taxon which has received little or no research attention. Such milestones could include the birth of live offspring following:

- intraspecies embryo transfer in a baboon (first successful ET in a wildlife species; Kraemer et al., 19??);
- IVF in a baboon followed by embryo transfer (first successful IVF in a wildlife species; Clayton and Kuehl, 1984);
- AI of a puma using fresh sperm (Moore et al., 1981)
- interspecies embryo transfer in the gaur (to Holstein cow; Stover and Evans, 1984), eland (to cow; Dresser et al., 1982), bongo (to eland; Dresser et al., 1985), zebra (to domestic horse; BILL, REF?) and Przewalski's horse (to domestic horse; Summers et al., 1987)
- intraspecies embryo transfer in the eland (Dresser et al., 1984), oryx (REF??), bongo (REF???) and suni antelope (JGH REF???)
- AI of a giant panda using frozen-thawed sperm (Moore et al., 1984)
- intraspecies transfer of frozen-thawed marmoset embryos (Summers et al., 1987)
- AI of blackbuck with fresh or thawed sperm (Holt et al., 1988)
- IVF of Indian desert cat oocytes followed by embryo transfer to the domestic cat (Pope et al., 1989)
- AI of gaur with frozen-thawed sperm (HENRY DOORLY ZOO GET REF)
- IVF of tiger oocytes followed by embryo transfer to a surrogate tiger (Donoghue et al., 1990)
- AI of black-footed ferrets with frozen-thawed sperm (Howard et al., unpublished)

Although cryopreserved genetic material played a role in only five of these events, there is no biological reason why frozen gametes and embryos could be not be used successfully to extend these early accomplishments.

Regardless of how a genetic resource is developed for a given species and which technique is used, some sperm and embryos always fail to survive freezing and thawing. Although much effort has been directed at evaluating various cryoprotectants and cooling rates for semen and embryos, the ease (or difficulty) of achieving post-thaw survival is, in part, dictated by species or genotype within species. In some cases, techniques that work well with one or more species fail or are only partially effective in a taxonomically-related counterpart (Wildt et al., 1991). Perhaps most significant are findings that genotypes within a species can respond differently to a standardized protocol (Schmidt et al., 1985). For example, when 4- to 8-cell mouse embryos from 27 different genotypes were frozen and thawed using a regimented procedure, survival ranged from 27.4 to 75.2% (Schmidt et al., 1987).

Even given such observations, it is likely that only modest research will be needed for some species, and progress will benefit through the use of existing knowledge and common laboratory animal models (Wildt et al., 1986). There is, however, an almost complete lack of information on sperm and embryos for a vast number of species, suggesting that much more basic research will be needed. Also of concern is the potential introduction and spread of diseases to domestic livestock and other wild stocks that could occur with the international transport of poorly-monitored gametes and embryos (Schiewe, 1991). These factors must be taken into consideration during the first steps of formulating a genetic resource bank.

Recommendations for Establishing a Genetic Resource Banks

Logic allows us to conclude that there is (1) an immediate need to maintain as much of the earth's bio- and genetic diversity as presently exists and (2) that genetic resource banks in combination with assisted reproductive technology has enormous potential. We also assert that

significant progress has been made in beginning to adapt human/livestock technology to wildlife species. Certainly, compared to only a few years ago, we have increased our understanding of fundamental reproductive processes exponentially for a variety of wildlife species. Furthermore, although these strategies have not yet had a practical conservation effect, live offspring from endangered species have been produced, despite an almost total lack of appropriate federal funding. Therefore, it is exactly the right time to begin considering the formal development of such resources for conservation. The interactive intramural and extramural features of a genetic bank infrastructure certainly are open to debate, but, in our opinion, the following characteristics are essential.

- First, progress will be accelerated by supporting existing institutions, preferably those with multidisciplinary team talents. Organizations with professionals and technicians with combined skills in the fields of (domestic and nondomestic species) reproductive physiology, cryobiology, low temperature (cold storage), gamete function, embryology, AI, IVF, ET, endocrinology, veterinary medicine (especially anesthesia), disease transmission via germ plasm, population biology, genetic management, field studies and/or computer programming, are highly worthy of consideration.
- The research record of the staff should reflect an appreciation for the importance of basic research as a prerequisite to practical collection and subsequent utilization of the genetic materials.
- The institutions should have earned independent recognition and/or be closely affiliated with other organizations of national and international repute and have demonstrated a record of leadership in conservation issues, scientific research and training.
- The institutions should have international contacts and existing collaborations with conservation organizations, research institutions and governmental/private wildlife authorities world-wide. Such relationships are a prerequisite to coordinating, organizing and gaining access to special or rare wildlife populations for genetic material recovery, storage and distribution. Special

collaborative talents will be required to ensure that species, populations and individuals in crisis receive first priority attention.

The issue of number and size of genetic resource banks needs to be addressed. It may be most appropriate to establish a single national center supported by multiple regional banks located throughout the U.S. and abroad. These smaller institutions will be crucial to securing appropriate samples of genetic material unique to specific geographic locations. Regional centers could support the mission of the national bank by providing half the frozen sample to the central repository while maintaining half locally for "insurance-safety" purposes. Regional banks also could be encouraged to conduct research, much of which will be based on innovations and advances made at the national bank level.

Most importantly, there must be considerable emphasis placed on both basic and applied research by the various banking institutions. Areas of high priority include:

- Defining the effect of species on the efficiency of freezing sperm, ova and embryos (a strategy which will determine how well existing technology can be applied immediately to rare species);
- Identifying the best cryoprotectants and cooling and thawing processes for a particular species of interest;
- Developing accurate laboratory approaches for testing the viability of thawed germ plasm, embryos, tissues and DNA;
- Conducting detailed, longitudinal studies on the many species-specific factors that influence the production of live offspring from thawed material;
- Improving testing and processing procedures to ensure that the transport of germ plasm does not contribute to disease transmission.

Certainly, two additional critical questions to be addressed in this decade will be (1) which species should be targeted and (2) what will be the source of funding for these very costly ventures? For now, those species or taxa managed under an SSP/TAG authority should receive considerable attention. The fact that an organized effort exists argues that there is general

agreement that the species is experiencing difficulty and deserves high priority. Unfortunately, species priority also will be dictated by the continued discovery of species or subspecies approaching extirpation. Federal research monies often magically appear when species numbers decrease to levels which almost ensure extinction. The goal should be not to rely on last ditch scientific heroics, but to use the scientific method to understand species biology before extinction becomes reality.

Certainly, establishing and maintaining genetic resource banks will be expensive, requiring long-term, financial commitments. Funding will needed for: 1) capital development; 2) the support of existing staff and the hiring new talent; 3) purchasing equipment and supplies to permit the safe collection, processing, long-term storage and active use of animal materials; 4) transporting and supporting research teams charged with collecting and preserving material; and 5) conducting basic and applied research. There is no "National Institutes of Health (NIH) for Wildlife Species", although developing a federally-supported National Institutes of the Environment (NIE) presently is being debated. An NIE likely would provide extramural support for wildlife research, much like the NIH does for human and animal model studies. Also, hopefully the National Science Foundation will increase the amount of money in "Basic Research in Conservation and Restoration Biology", a funding program initiated in 1990. Nonetheless, this issue is so crucial, that it is essential that congress and appropriate federal agencies finally attend to what is rapidly becoming an almost historic plea to conserve our wildlife heritage by these promising strategies.

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Table 1. Mammalian species in which offspring have been produced by AI and frozen-thawed sperm.

Domesticated	Non-Domesticated
Cattle	Fox
Sheep	Wolf
Horse	Addax
Pig	Blackbuck
Rabbit	White-tailed deer
Dog	Fallow deer
Goat	Chimpanzee
Cat	Bighorn sheep
Mouse	Red deer
Water buffalo	Bison
Domestic ferret	Siberian ferret
Human	Giant panda
	Gaur
	Wapiti
	Reindeer
	Black-footed ferret

Table 2. Mammalian species in which offspring have been produced by IVF followed by embryo transfer.

Domesticated	Non-Domesticated
mouse	baboon
rabbit	rhesus monkey
rat	cynomolgus monkey
human	Indian desert cat
cattle	tiger
pig	marmoset
sheep	
cat	

Table 3. Mammalian species in which offspring have been produced using frozen-thawed and transferred embryos.

Domesticated	Non-Domesticated
mouse	eland
rat	baboon
rabbit	cynomolgus monkey
cow	marmoset
sheep	
pig	
cat	
goat	
horse	
human	

UTILIZATION OF SPERM BANKS TO MAINTAIN GENETIC DIVERSITY IN CAPTIVE POPULATIONS OF WILD CATTLE

Leslie A. Johnston and Robert C. Lacy

INTRODUCTION

As a result of habitat fragmentation, wild cattle species of Southeast Asia are either threatened or endangered in their indigenous habitats, and soon will become extinct unless preservation efforts are undertaken immediately. Gaur, (Bos gaurus), a critically endangered species of wild cattle, historically ranged throughout Asia. Today, there are only remnant populations in Nepal, India, Thailand, Laos, Burma, Cambodia, Vietnam and Malaysia. Gaur, which are shy forest-dwelling creatures, are reluctant to use cleared areas as migration corridors to other forested areas. As a result, sub-populations are becoming highly isolated, resulting in genetic and demographic instability.

Recently, captive propagation has become an integral component of the conservation strategy to maintain wild populations through: 1) enhanced preservation of genetic diversity; 2) protecting gene pools against the effects of environmental and demographic fluctuations in the wild and 3) providing animals for reinforcement of wild populations or establishment of new wild populations. Soule et al. has suggested that approximately 815 mammalian species will all but disappear unless they are maintained in captivity [1]. In order to manage captive populations, effective breeding sizes need to be adequate to achieve specific genetic and demographic goals. In many cases this requires a minimum of 200 to 800 individuals/species maintained in captivity. Currently, there is enough space in zoos worldwide to house only 100 mammal species in populations large enough to be self-sustaining demographically and genetically [2].

Ballou has recently discussed the benefits of germ plasm cryopreservation (sperm, ova, embryos) for captive management programs [3]. Cryopreserved germ plasm enables the captive program to: 1) maintain a population's original genetic diversity indefinitely; 2) extend generation length of individuals/populations and 3) decrease the number of individuals required to achieve the defined genetic goals. Long term preservation of genetic diversity will enable the population to continue evolving through time. In addition, increasing generation length will provide fewer opportunities for losing genetic diversity. The combined effect of preserving genetic diversity and extending generation length is the ability to reduce population numbers and at the same time achieve genetic goals. Consequently, cryopreservation programs will allow additional space to become available for other species.

While there has been much speculation on the effectiveness of gamete cryopreservation as a conservation tool (ranging from discussions of completely frozen zoos to dismissal of the concept), little effort has been made to evaluate quantitatively

the role that reproductive technologies can play in wildlife conservation. Development of sampling and utilization strategies to guide selection of an optimal representation of the genetic diversity are required for a genome banking program. In this paper, we present a quantitative assessment of the likely efficacy of sperm banking in furthering the goals of the captive breeding program for gaur. Using computer simulation models, we examined a scheme proposed for the preservation of the gene pool of the North American gaur herd, and make recommendations for the optimal use of the sperm in the captive program.

Conceptually we have proposed the use of three sperm banks for gaur. The first one is established with semen collected from genetically valuable males in the current captive population. The second one is established with semen from wild-caught males. The third is a mirror of the captive population through time, with genetic exchange between the bank and the living population. The first and second banks will be used to replenish the living population. The intent of the third bank is to be used primarily as a reserve in case the other two banks are destroyed.

Frozen sperm banks offer promise for preserving much of the genetic variation in populations of wild cattle. Unlike the situation for most endangered species, reliable artificial insemination (AI) techniques already exist for cattle and are being applied to other bovid species, including gaur [4].

Gaur were brought into captivity between 1958-65, and in 1981, the North American Gaur Species Survival Plan was formed. Presently, the herd consists of 162 animals descended from 8 founders. Pedigree analysis indicates that the captive herd has 89.4% of the gene diversity (heterozygosity expected under Hardy-Weinberg equilibrium) of the ancestral wild stock from which founders were obtained. It is estimated that over 900 animals will be required to maintain this level of diversity through the next 100 years. Currently, there is limited space for expanding the herd beyond 150 individuals; therefore it is critical that a semen cryopreservation program is designed in order to limit further decline of genetic diversity.

The following variables were examined during individual simulations between the living gaur population and semen cryo-bank to assess their effects on retention of genetic diversity: 1) effective population size of the living herd; 2) sex ratio of the herd; 3) number of offspring required from each bank for each generation; 4) semen usage interval; 5) the minimum number of males required to establish the captive bank and 6) the interaction of both the captive and wild bank.

Methods

Computer simulations were done by using a modified version of Lacy's GENESIM computer simulation program of genetic diversity [5].

To simulate the fate of two alleles at a genetic locus, with the interactions of frozen banks, the program:

1. Prompts the user to enter the number of described conditions for a population: number of generations, population

size, sex ratio, initial gene frequency at a hypothetical locus (defining the initial gene diversity) and the use of any of 3 sperm banks. If any of the banks are selected, the user is prompted to enter the initial gene frequency of the bank, the generation usage of the bank, number of offspring produced per generation. The use of sperm bank 3 also allows the user to define how many males are used to establish the bank and the number of males each generation to be used to augment the bank. The simulation program assumes that each sperm sample can be infinitely extended, so that the lack of sufficient frozen sperm does not interfere with planned matings.

2. Creates a population of living diploid individuals, assigning 2 alleles to each individual with the probability p that each allele is of one type (A), where p is the initial allele frequency as specified by the user.

3. Either: selects two parents at random from the living population, or selects a male from the bank and female from the living population. The decision to select two living parents vs. a female and frozen sperm from a sperm bank is determined by the designated frequency of use of each sperm bank.

4. Randomly selects one allele from each of the two parents and assigns that allele pair to the offspring.

5. Calculates allele frequencies and % expected heterozygosity that would be observed if the population was in Hardy-Weinberg equilibrium.

6. Steps through the specified number of generations, randomly mating all females of the herd.

7. Repeats the simulation 1000 times to generate a distribution of outcomes.

8. Averages overall heterozygosity for each generation over all repetitions.

RESULTS

Population size:

One of the primary benefits to the use of frozen germ plasm is the ability to reduce the population size and still achieve the genetic goals. Figure 1 compares the genetic variation present after 1000 years in the living herd with genetic replenishment from either of two cryo-banks (wild, captive) or without either bank, using populations of sizes: 40, 80, 120, 160, 200 and 500 individuals. As expected, without infusion of genetic material, genetic diversity was dependent upon population size. The mean heterozygosity dropped to a low of 18.0% (after 1000 years with an Ne = 40) and with Ne = 500 was maintained at 78.9%. By using the captive bank every generation, the herd was able to retain 80.9% to 82.9% heterozygosity (SE = 0.61-0.52) over 125 generations (=1000 years). Utilization of the wild bank enabled the herd to increase its heterozygosity from 89.2% to above 90%. The population was able to maintain heterozygosity levels at 91% for Ne = 40, 80, 120, 160 and 200. With a effective population size of 500, the mean heterozygosity retained was 92% (SE = 0.31). All of the following simulations

assumed N=80.

Sex ratios:

Frozen banks, in principle, genetically augment the living population size. However, the use of sperm banks will increase only the effective male population size. While adding males to the breeding pool through the use of their sperm in the banks, the genetically effective population size (and the retention of genetic variation) might be increased by an offsetting skew of the living population toward females. Therefore, to manage the population effectively, it is important to determine the optimal sex ratio of the living population. Figure 2 depicts the percent of genetic diversity retained for 1000 years with either: 1) genetic replenishment from the wild or captive bank or 2) no infusion at all using sex ratios (proportion male) of: 0.1, 0.2, 0.3, 0.4, and 0.5. Without the use of either frozen bank, retention of genetic diversity was detrimentally influenced by sex ratios lower than 0.5. This is expected to occur since the small number of individuals of one sex will act as a bottleneck because half of the genetic information inherited by the next generation comes from each sex. With either the wild or captive cryo-bank replenishing the living population, the retention of genetic diversity appears almost independent of sex ratio. The mean heterozygosity ranges from 87.4% (SE = 0.50) to 91.5% (SE = 0.34) when semen from the wild bank is used. With the captive bank replenishing the living population, mean heterozygosity ranges from 77.6% to 81.6%. However, there is a slight advantage to skewing the ratio toward 0.3 when using the wild bank.

Number of offspring:

Figure 3 shows the effect on genetic diversity retained by producing different numbers of offspring every generation from each cryo-bank. The following number of offspring produced were tested; 2, 4, 6, 8 and 10. Regardless of which bank was in use, 2 offspring per generation was insufficient to maintain heterozygosity above either 80% (captive bank - 75.1%) or 90% (wild bank - 86.3%). Producing 4 offspring per generation from the captive bank will enable the population to retain 81.7% genetic diversity. Additional offspring will maintain the population between 83.6% to 85.8% (n=6 and 10, respectively). If genes are only brought in from the original captive herd (bank 2), diversity can never increase above 89.4%. Production of 4 offspring using the wild cryo-bank will maintain heterozygosity at 91.4%. However, production of 8 to 10 offspring using the wild bank will capture much of the diversity in the wild population, increasing heterozygosity to 95.5% and 96.2%, respectively.

Semen Usage Interval:

To assist in planning the amount of semen to be cryopreserved, it is important to determine how often during the course of management semen is required for optimal utilization of

the frozen banks. Generation intervals for semen usage were tested and the results are shown in Figure 4. Regardless of whether the living population is replenished with semen from the captive or wild bank, it is optimal to utilize the banks every generation (82.0%, 91.2%, respectively). Although, even with occasional use of either cryo-bank, it is still substantially better to use a bank on a limited basis, than no bank at all (recall that the population declines to just 38% heterozygosity when no bank is used).

Number of Captive Males to Establish a Bank:

For any management scheme to be set in place it is critical to determine the required number of males from which sperm needs to be frozen to utilize the frozen bank efficiently and to maintain maximum genetic diversity. The number of males tested to establish the cryo-bank from the captive population ranged from 5 to 100. A minimum of 20 males are required to maintain most of the heterozygosity present in the initial population (Figure 5). After banking sperm from 30 males, the incorporation of additional animals is unnecessary, and even with 100 males heterozygosity remains at virtually the same level as with 30 (80.7% vs 80.9%).

Interaction between captive and wild banks:

The establishment of the captive bank is operationally easier than establishing a wild bank. Therefore, to maximize resource and effort, there is the need to assess the benefits of using the wild bank immediately or in the future, assuming the captive bank is already functional. Usage of the captive bank alone will maintain genetic diversity at ~80% relative to the original wild population. Figure 6 shows the results of initially using the captive bank every generation and then incorporating the wild bank after 5, 10, 15, 20, 40, 60, 80, 100 and 120 generations. Once the wild bank is incorporated, the captive bank will no longer be used. It is apparent that as long as the captive bank is replenishing the living population, it is possible to postpone using the wild bank until at least generation 80 and still maintain 91.6% heterozygosity after 1000 years. From the data, (not shown), it appears that once the wild bank is initiated, it takes approximately 5 to 10 generations to increase the genetic diversity of the living population to greater than 90% of the wild population.

Discussion

In the preservation of maximum biological diversity, the use of reproductive technology may be the last resort. It has been estimated that the demographic winter and human control of wilderness areas will last at least the next 500-1000 years [1]. When human attitudes change and habitat regeneration becomes a goal of society, it will take a minimum of centuries to replenish the ecosystems destroyed in tropical and temperate regions. As a consequence, zoos will need to maintain the genetic and

demographic health of their charges for many centuries or millennia. Many Species Survival Plans provide management of populations with genetic goals of 90% for the next 100-200 years. It is estimated that after that time, advances in reproductive technology will be able to extend the program to at least 1000 years. Because reproductive technologies have already been well developed for cattle, we can now begin managing wild cattle species for that 1000 year goal.

The major problem facing long-term management of small populations is the loss of genetic diversity through drift. Genetic drift can be controlled by the maintenance of large breeding populations in which all animals contribute equally to future generations. In many cases, immigration of individuals into subpopulations can reduce genetic drift. In captive and, increasingly, in wild populations, the number of breeding individuals is so reduced and populations are so isolated that even short-term maintenance of genetic diversity is placed in jeopardy. Therefore, strategies will need to be designed to eliminate additional loss of heterozygosity. Sperm banks will increasingly play an important role in small population management by preserving original germ plasm indefinitely and re-introducing this material (as immigrants) through time to counter genetic drift.

With the modelling simulations shown, it is possible to sustain a genetically healthy gaur population for the next 1000 years with a sperm banking program. The loss of genetic variation is affected by a number of factors including: the number of breeding individuals in a population, sex ratio and number of offspring produced from AI. Excluding demographic constraints, it is possible to retain maximum genetic diversity with as few as 40 individuals in the population when offspring are produced from semen from either the captive or wild bank. In fact, the retention of genetic variation becomes relatively insensitive to the captive population size when genetic material can be regularly infused from one or more sperm banks. The capability to retain more diversity with smaller living captive populations should allow a dramatic expansion of the number of species that might be supported with living captive populations and genome banks.

When the wild bank is in use, there is also a tendency for optimal retention of genetic diversity when the sex ratio is skewed toward 0.3. The use of gametes from sperm banks in the production of offspring skews the effective sex ratio toward males. This effect can be countered by skewing the sex ratio of the living population toward females. It is possible that since the wild cryo-bank is genetically more unique than the living population, it is optimal to reduce the number of living and not as "genetically unique" males even more than might be estimated based on the number of frozen sperm samples utilized each generation. However, genetic diversity is affected detrimentally if the sex ratio is reduced further, as a result of decreased number of breeding animals (Ne).

Based on the generation length for gaur (T=8), essentially one offspring is required to be produced from either the wild or captive bank each year to maintain maximum genetic diversity. It

is also worthwhile to note that although it is optimal to use either bank every generation, there is still benefit to the living population if sperm banks can be used only intermittently. It is also important to note that once the wild bank becomes functional, use of the captive bank will no longer benefit the living population. Also, a third bank, which would mirror of the living population, will only be utilized if either of the other two banks fail. The genetic representation of this bank will be intermediate between either of the other two banks and the living population.

In many instances, it will be impossible to depend upon a wild bank initially; therefore efforts should be made to establish a captive bank before additional genetic diversity is lost. Currently the North American gaur herd consists of 59 males. In order to establish an effective captive bank, one-third of these males are required to be banked (see Fig. 5). The selection of which males to use will be based on the Mean Kinship values derived from pedigree analysis. The most genetically valuable males are those with the lowest mean kinship to the living population, i.e., those with the rarest genes. Mean Kinship is the average relatedness of each individual to every other animal in the population, and is calculated from pedigree data. An animal with high average relatedness will share alleles with a number of other individuals in the population, and conversely an individual with no relatives will have an average relatedness of 0.

Finally, these simulations are based on a number of simplifying assumptions, including random mating. Through the use of existing management programs [GENES, Lacy], based on pedigree analysis, it may be possible to optimize mating strategies to produce more genetically valuable animals than expected with a random system. Conversely, unexpectedly poor breeding by some animals could lead to more rapid losses of genetic diversity than projected by the simulations.

Based on these simulations, initial management plans would consist of: (1) continued maintenance of a small demographically stable captive population; (2) collection of sperm from 20-30 genetically valuable males present in the captive population; (3) initiation of cryopreservation of sperm from individuals in wild populations; and (4) interactive management of the living herds and the sperm banks.

Although these simulations focused on the captive population, wild populations are facing the same threats. Eventually this type of management strategy may be required to sustain healthy wild populations. It is apparent that there is a wide range of possible strategies, and refined modelling of options is important as a part of adaptive program management. Although this is a simple model, in any genetic management scheme it is important to analyze the expected consequences of the program and determine whether it will achieve its desired goals before the program is implemented. Properly utilized, sperm banks can be very effective tools for preserving genetic diversity in a living population, and for introducing gene diversity from additional subpopulations.

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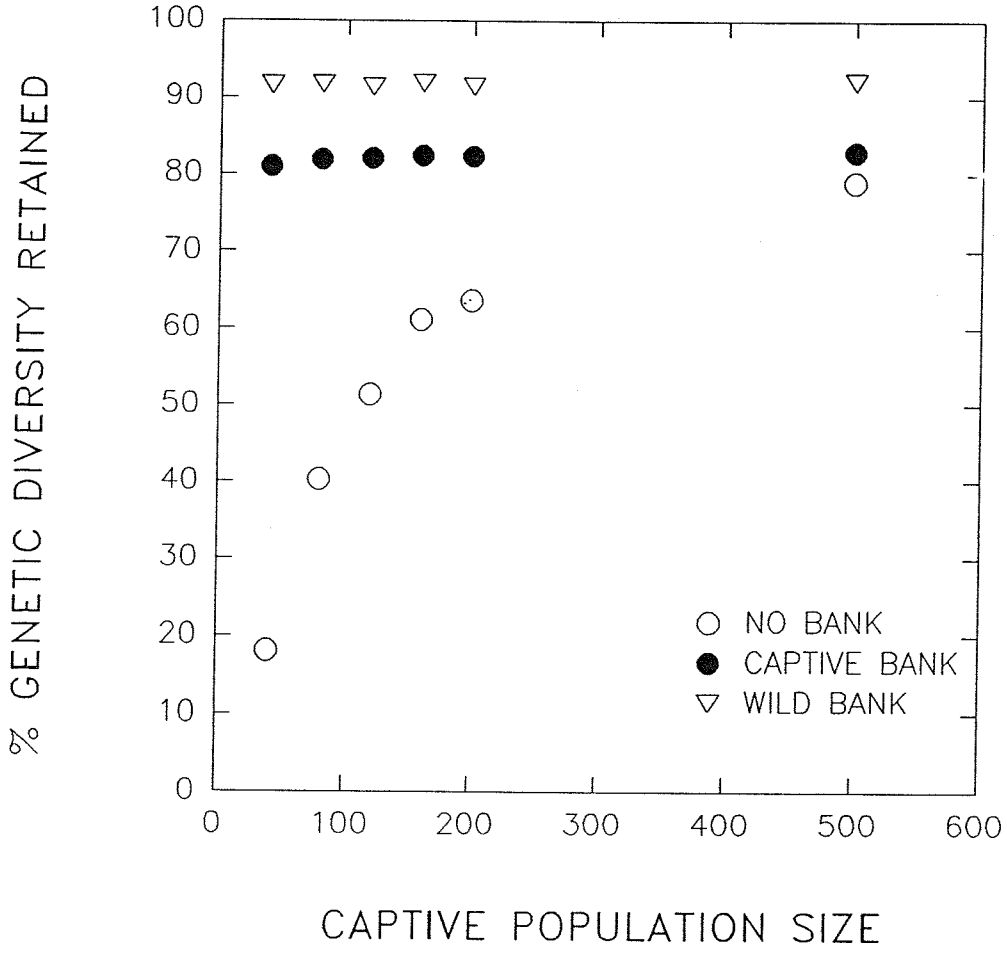
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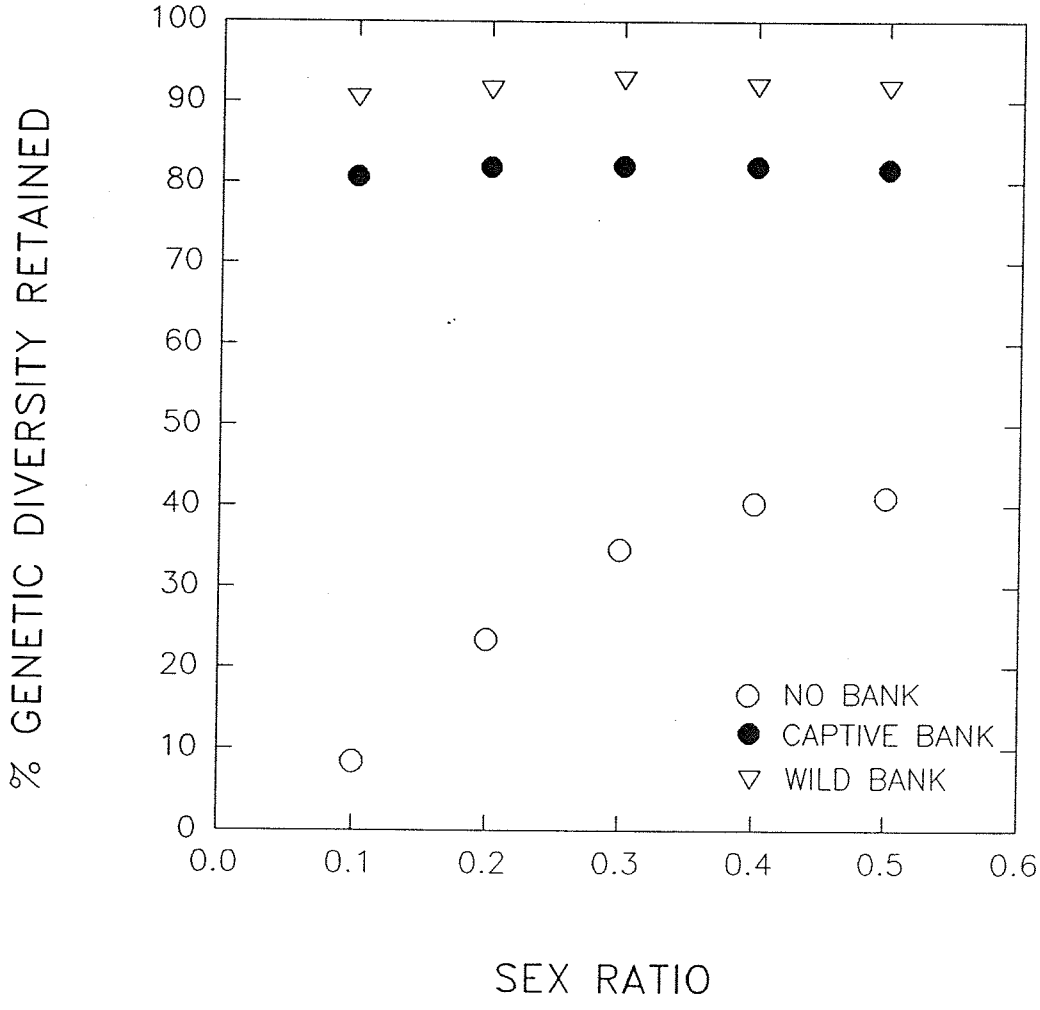
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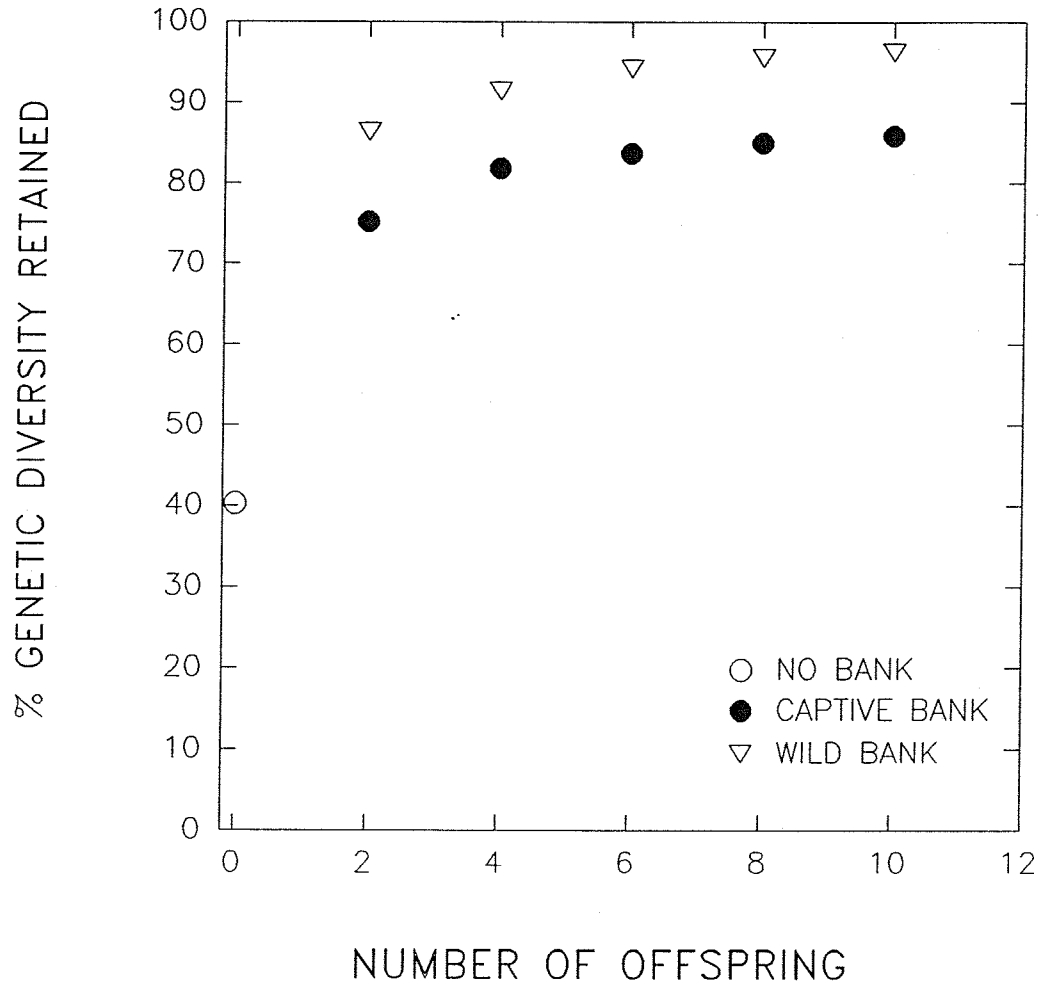
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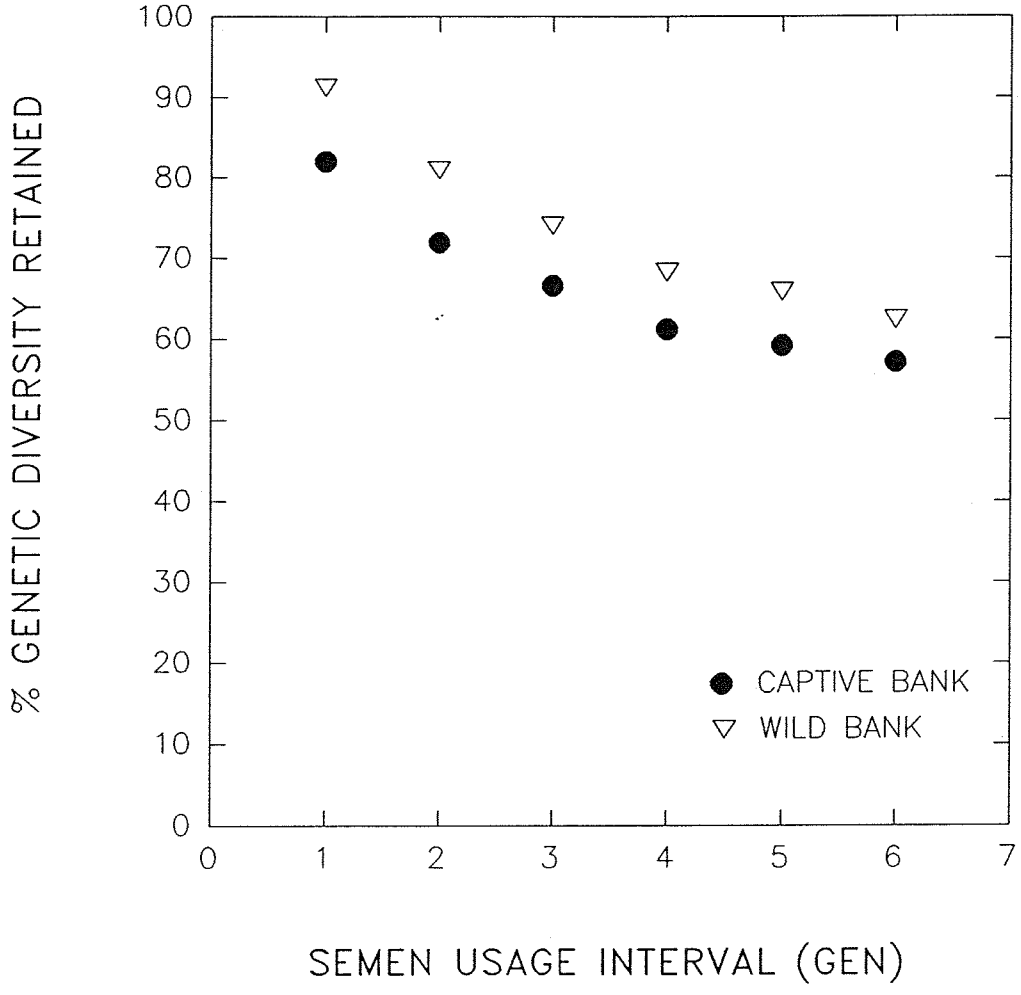
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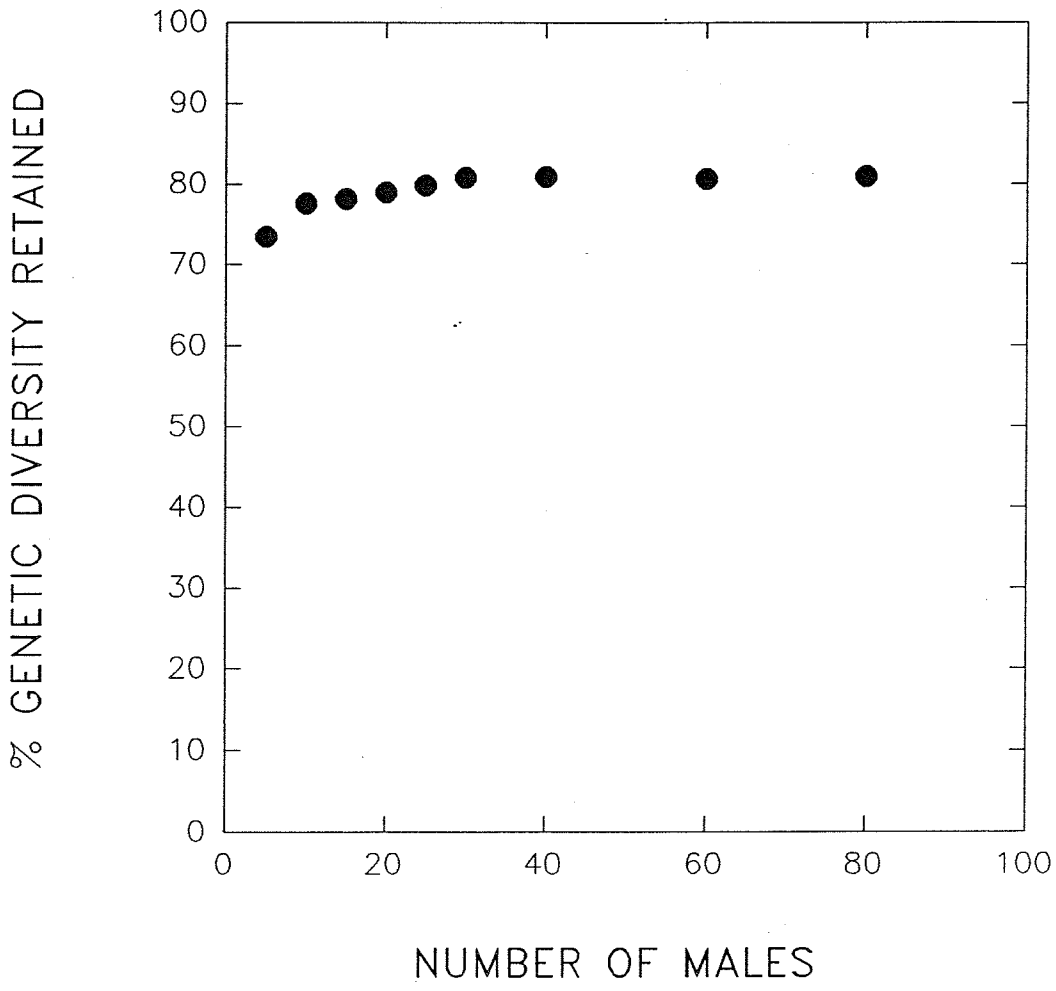
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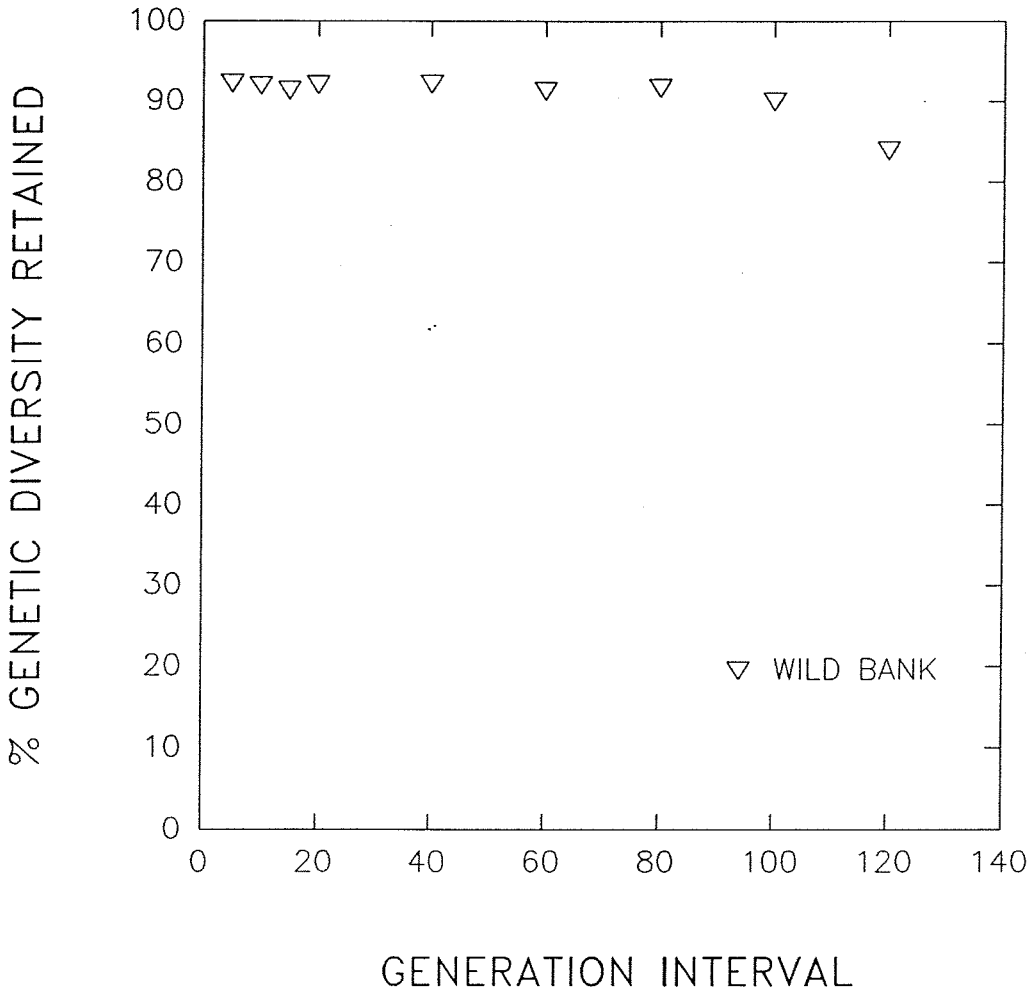
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WILD BANK
GENERATION INTERVAL

POTENTIAL CONTRIBUTION OF CRYOPRESERVED GERM PLASM TO THE PRESERVATION OF GENETIC DIVERSITY AND CONSERVATION OF ENDANGERED SPECIES IN CAPTIVITY

JONATHAN D. BALLOU
DEPARTMENT OF ZOOLOGICAL RESEARCH
NATIONAL ZOOLOGICAL PARK
WASHINGTON, D.C. 20008

RUNNING TITLE: PRESERVATION OF GENETIC DIVERSITY

ABSTRACT

Demographic and genetic objectives of captive propagation programs for endangered species focus on establishing demographically secure populations that maintain adequate levels of genetic diversity. Long term storage and utilization of cryopreserved germ plasm could extend the population's generation length and allow higher levels of genetic variation to be maintained in smaller populations. Since fewer breeding animals would be needed, more species would be 'rescued' from extinction using the cage facilities currently available at existing institutions. Doubling generation lengths for callitrichid primates through use of cryopreservation could almost triple the number of species that could be rescued in world zoos. Additionally, long term cryopreservation would allow for a third population, that of the frozen zoo. Three-way exchange of germ plasm from germ plasm banks to captive and wild populations would increase genetic diversity at reduced risk and expense. Advances in reproductive technology and better understanding of the reproductive physiology of these animal populations are necessary to permit routine application of artificial insemination and embryo transfer using frozen-stored germ plasm.

INTRODUCTION

Prior to 1970, zoos were primarily interested in maintaining collections consisting of many different species with few individuals of each species. As animals became increasingly more difficult to obtain from the wild and as the conservation ethic grew the focus on breeding species in captivity increased. By the mid-1970's zoos held fewer numbers of species, but more individuals of each species. Captive propagation now plays a vital role in species conservation and the continued survival of many large vertebrate species may ultimately depend on captive propagation efforts (8).

Soule et al. (22) estimate that approximately 815 mammal species will be in need of captive propagation programs within the next 200 years. However, Conway (6) estimated that with the space currently used to house mammal species in world zoos, only 100 mammal species could be preserved in populations large enough to meet genetic and demographic requirements. As the technology stands now, zoos simply do not have the capacity to meet the conservation crises facing wild populations. Since captive resources (cage spaces) are limited, the actual population size established for each species may be a compromise between the number of animals needed to achieve specific genetic goals and a number that allocates a more equitable distribution among similar endangered species competing for these cage spaces (6). Many

more species could be maintained if cryopreservation and infusion of stored germ plasm could be utilized in captive propagation efforts.

The purpose of this paper is to outline strategies captive management programs can use to genetically and demographically manage captive populations for long-term conservation and to discuss the primary effect cryopreservation of germ plasm may have on the conservation of biological diversity.

CHALLENGES TO SMALL CAPTIVE POPULATIONS

Demographic instability, inbreeding problems and loss of genetic diversity are significant problems facing the continued survival of small captive populations. Captive populations of severely endangered species are often dispersed among different zoos. Conway (6) estimated that 76% of the 2750 mammal, bird and reptile species held in captivity had fewer than 25 individuals; 18% had only one or two. Their small numbers and wide distribution place these species at especially high risk of extinction (9). Very small populations are particularly vulnerable to demographic stochasticity. Random fluctuations in birth and death rates or sex ratio distortion, while insignificant in large populations, can drive small populations to extinction simply by reducing the population below numbers capable of recovery.

Inbreeding depression can also adversely affect the viability of small populations. The deleterious effects of inbreeding are well known in domestic livestock and laboratory animals (27). Data on the deleterious effects of inbreeding in exotic wildlife are sparse (17) and most data come primarily from surveys of captive populations (18). In a survey of 45 captive mammals, mortality of inbred offspring was higher than non-inbred offspring in all but three populations (19)

Small populations also rapidly lose genetic diversity, Genetic diversity is lost each generation at a rate inversely proportional to the effective size of the population (Figure 1). Loss of genetic diversity adversely affects both the short and long-term fitness of the population. In the long-term, loss of diversity reduces the population's ability to evolve and adapt through natural selection. In the short-term, there is a general, but not universal, relationship between levels of genetic variation and various fitness components including disease resistance (1) and reproductive potential (26). Retention of this diversity maintains fitness in these populations and allows for future management options.

CAPTIVE PROPAGATION PROGRAMS

The goal of conservation oriented captive propagation programs is to maintain genetic diversity in a stable population (7). Soule et al. (22) recommend captive breeding programs

attempt to maintain 90% of the population's genetic diversity of 200 years. Since retention of genetic diversity is dependent on population size, it is possible to calculate the number of animals required to achieve this objective. It is also a function of the generation length of the species, the number of individuals that founded the populations, and the populations's growth rate (3). Each population will require a unique size to achieve this recommended goal.

Demographic management objectives are to establish a stable population of this critical size and maintain the population at zero population growth. Population stability is achieved through monitoring the age distribution of the population and conducting life table analyses to determine age-specific survival and reproductive rates. From these analyses, it is possible to calculate the number and ages of animals to breed to achieve these objectives.

Genetic management objectives focus primarily on which individuals to breed to maximize retention of genetic diversity. Pedigree analyses determine how the founder's genes are distributed within the population's gene pool (13, 24). Often a small proportion of founders are responsible for most of the early breeding and these founders genes will be disproportionally represented in the current population. Breeding recommendations identify priority animals for reproduction to remedy this disproportionate distribution of founder genes.

Demographic and genetic recommendations are integrated into a management plan for the species. Currently, the American Association of Zoological Parks and Aquariums oversees 55 such Species Survival Plans and similar programs exist in the United Kingdom, Europe, Australia, New Zealand and Japan.

CONTRIBUTION OF CRYOPRESERVED GERM PLASM

Long-term storage of germ plasm through cryopreservation, followed by infusion of the germ plasm back into future generations could substantially increase generation lengths of populations. Cryopreservation of germ plasm extends the lifetime of an individual for as long as its stored germ plasm lasts. By periodically infusing genetic material back into the living population, either through stored semen, ova or embryos, the individual(s) contributing the germ plasm can continue to provide genetic material well into the future.

The generation length is defined as the average amount of time it takes an individual to replace itself in the population: the longer the animal reproduces, the larger its contribution to the population's generation length. Extending generation length decreases the number of generations occurring over a given time period. This provides fewer opportunities for losing genetic diversity. Figure 2 illustrates the effects of increasing

generation length on maintaining levels of genetic diversity over a fixed time period (200 years). Populations with longer generation lengths can retain more of the population's original diversity than populations with shorter generation lengths. With cryopreservation, propagation goals could lengthen generation times and retrain higher levels of genetic diversity (e.g., 95% rather than 90% heterozygosity retained over 200 years.

Alternatively, captive propagation can benefit from the effect of lengthening generation time by establishing larger numbers of smaller populations. Extending generation times will allow smaller populations to achieve the same genetic goals as larger populations with shorter generations. Figure 3 illustrates the effect of generation length on population sizes required to maintain 90% of the genetic diversity for 200 years. Lengthening generation length can significantly reduce the population size required to achieve this goal. Facilities become available for other species with similar captive needs and the number of species benefiting from captive propagation can be increase.

The advantage of extending generation length can be illustrated using callitrichids (marmosets and tamarins) as an example. The current worldwide capacity of zoos to house callitrichids is approximately 2800 individuals (Table 1). Twenty-five of 35 known species (and subspecies) are currently kept in captivity. Ten are endangered. Generation length among

the group varies, however, six years is probably a rough estimate for most callitrichids. Population sizes required to maintain 90% of the population's genetic diversity for 200 years for these species varies between 500 and 700 (depending on founder number and population growth rate). Division of available resources by need allows only 4 to 5 (11%) species of callitrichids to be preserved.

Figure 4 illustrates the increase in number of callitrichid species that zoos could preserve by increasing generation length through cryopreservation. Doubling the generation length (from 6 to 12 years) increases the number of species that can be preserved from five to 13 since population sizes need only be about 200 animals. Tripling the generation length to 18 years enables 22 species to be preserved with 125 individuals each. Reducing population sizes any further places the population at high demographic risk. Any additional increases in generation length resulting from cryopreservation should be used to retain more genetic diversity rather than reduce population size.

DISCUSSION

The reality of cryopreservation, with the concurrent necessary advances in reproductive technology, will clearly have a direct and critical impact on the contribution of captive management to single-species conservation efforts. A number of authors have discussed the potential contribution of cyrobiology

to the maintenance of genetic diversity in captive gene pools (14, 22, 20). Ballou (2) discusses some of the genetic considerations involved in identifying individuals to contribute to germplasm banks. However, perhaps the most important application of cyropreservation is that it can substantially extend generation lengths of captive populations thereby allowing smaller populations to retain higher levels of genetic diversity. Smaller populations of each species permits more species to be included in the limited zoo "ark."

The effect of cryopreservation on generation length will depend directly on how much germ plasm can be preserved and how effectively it can be restored to the population. Taken to the (unrealistic) extreme, if unlimited amounts of cryopreserved germ plasm are available, generation length no longer becomes an issue in captive management programs and essentially 100% of a population's genetic diversity can be maintained indefinitely.

Additionally, the reality of long-term cryopreservation would allow a third 'population' to be considered - that of the frozen zoo (5). Three-way gene exchange between germplasm banks, wild and captive populations would increase the gene pool and effective size of the entire species. It would also facilitate two-way exchange of germplasm between wild and captive populations by reducing the logistics, expense and risk of

capturing and transporting individuals between populations. In cases where concerns of disease transmission restrict movement of living individuals (wild cattle), transportation of cryopreserved germplasm may be the only way in which new genetic material is brought into the captive population.

Cryopreservation can only make a contribution if advances in reproductive technology would permit routine application of artificial insemination and embryo transfer of stored germplasm in a wide range of exotic species. Unfortunately, we are not yet at the stage where cryopreservation can be routinely applied in captive propagation programs. Fundamental understanding of the basic reproductive physiology does not exist for most exotic species and there is clearly a need to continue and increase basic research in this area in order for this biotechnology to be realistically and fully applied to conservation problems.

Current goals and management objectives already assume that cryopreservation for most captive exotic animals will be a reality in the future. The recommendation of Soule et al. (22) that 90% of a population's genetic diversity be maintained for 200 years is based on the assumption that reproductive technology should be available by that time. At that point, captive populations of reduced size can be managed with the aid of cryobiology. Without it, zoos will have insufficient resources to confront the pressing demand for captive propagation of species endangered in the wild.

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FIGURE LEGENDS

Figure 1. Loss of genetic diversity (heterozygosity) over time in populations with various effective population sizes.

Figure 2. Loss of genetic diversity over a 200 year period in two populations of equal size (20) but with different generation lengths.

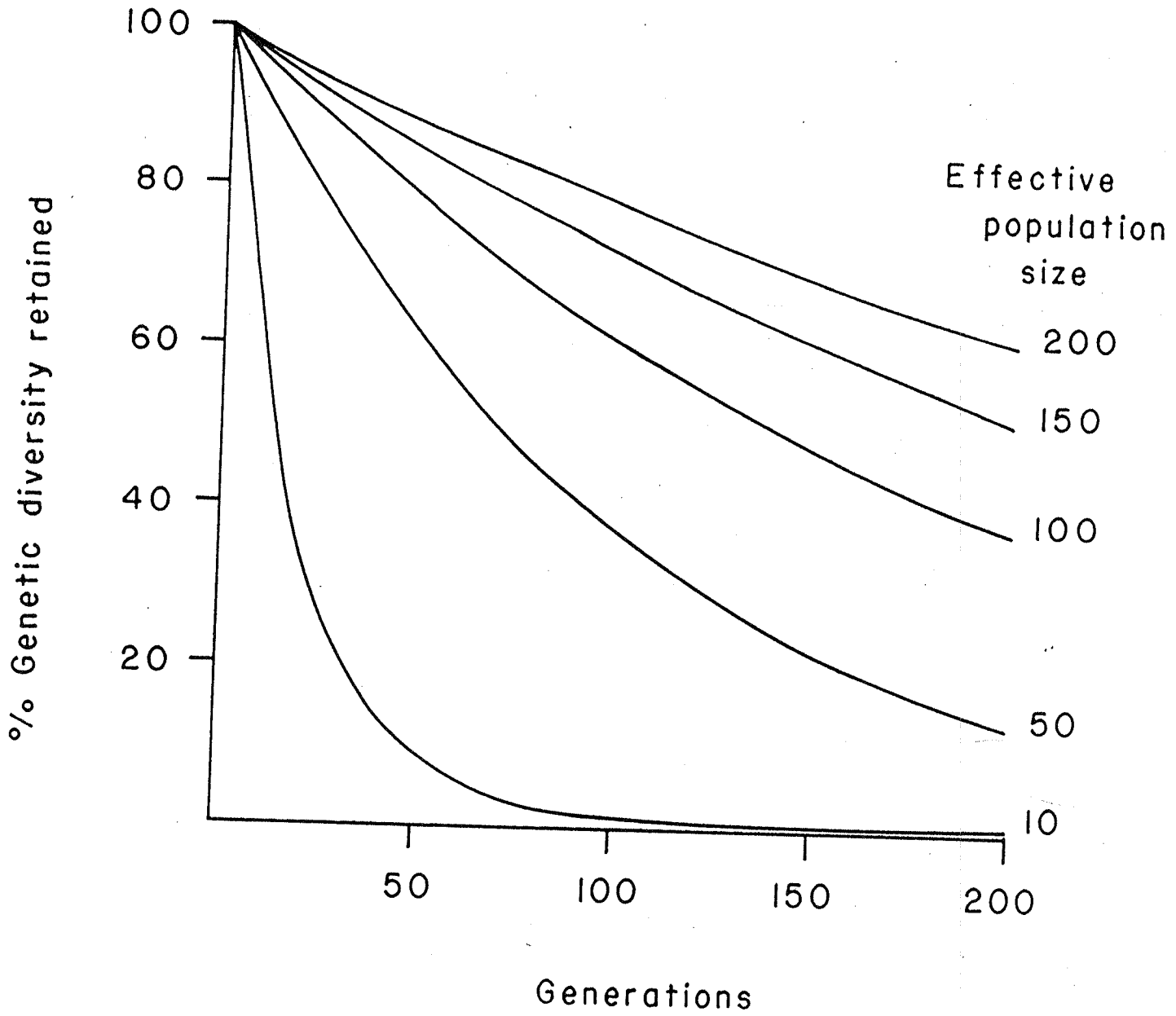
Figure 3. Effect of generation time on population sizes required to maintain 90% of a population's genetic diversity over a 200 year period. Calculations are based on founding populations of 20 individuals and an annual growth rate of 10%. A population with generation length of 5 years requires over 800 individual to achieve this goal while a population with generation length of 10 years requires only 250 individuals.

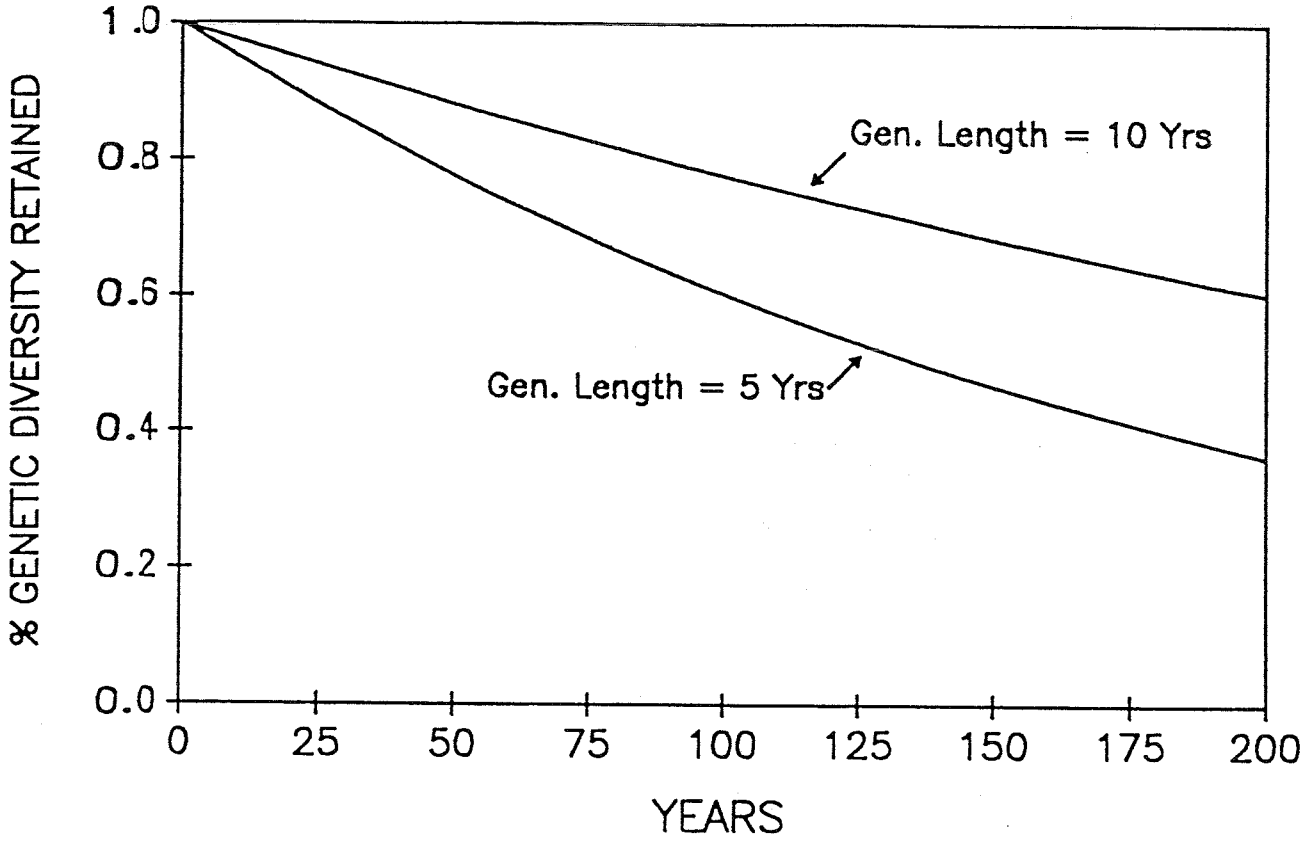
Figure 4. Increase in number of callitrichid species zoological insitutions can preserve by increasing generation length through cryopreservation of germplasm and reproductive technology. Based on global zoo capacity for 2800 callitrichids (Table 1).

Table 1. Numbers and status of callitrichid species in zoological parks worldwide. Sources: Individual Species Studbooks; ISIS = International Species Inventory System (10); IZY = International Zoo Yearbook (16).

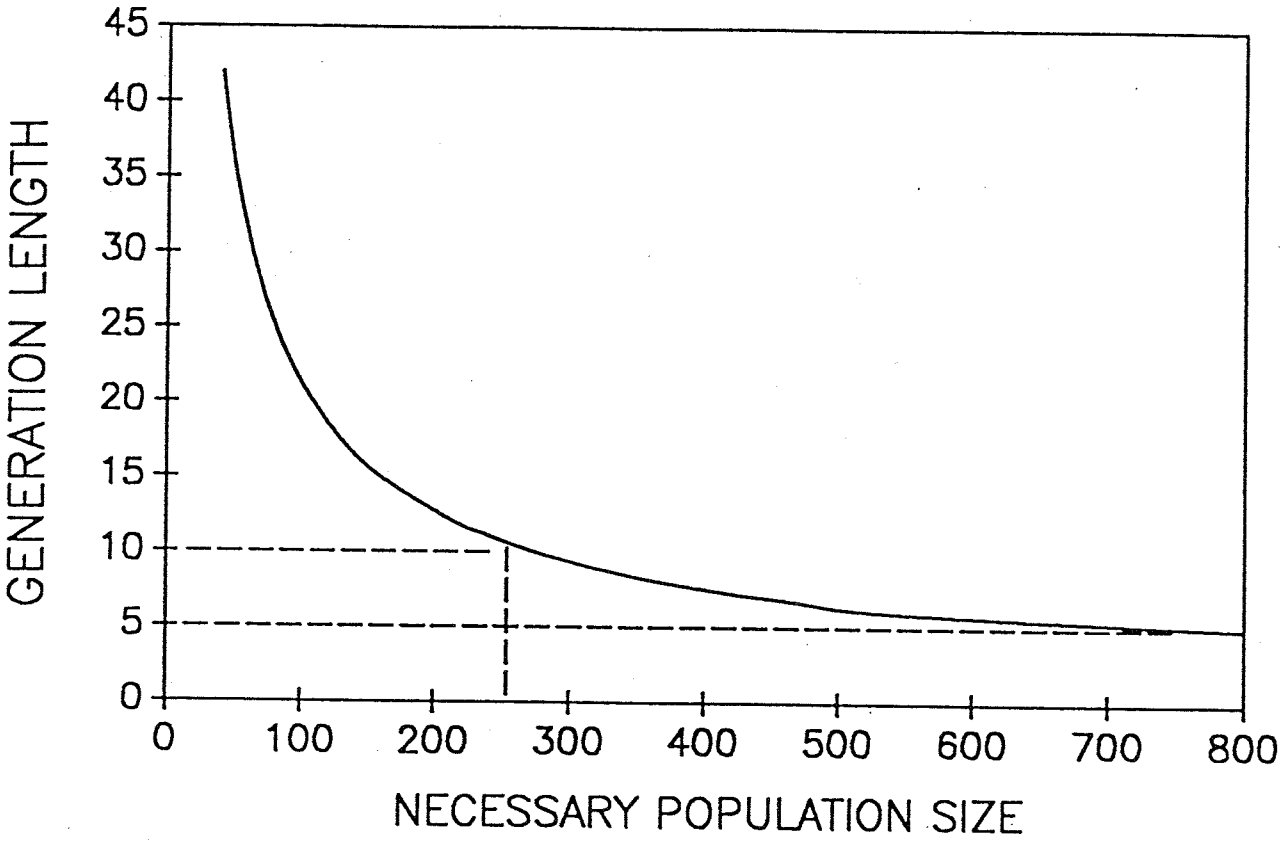
GENUS	COMMON NAME	RED DATA[1] ENDANGERED STATUS	# INDIVIDUALS	SOURCE*
Callimico	Goeldi's Monkey	R	326	25
Callithrix	Black Tailed Marmoset	V	79	10,16
	White-Eared Marmoset	E	12	16
	White-Fronted Marmoset		88	10,16
	Common Marmoset		346	10,16
	Black-Eared Marmoset		10	10,16
	Pygmy Marmoset		142	12
	Buff-Headed Marmoset	E	1	16
	Golden Lion Tamarin	E	520	4
	Golden-Headed Lion Tamarin	E	157	15
	Black Lion Tamarin	E	57	21
Saguinus	Brown-Headed Tamarin		71	10,16
	Geoffroy's Tamarin		69	10
	Red-Manteled Tamarin		59	10,16
	Emperor Tamarin	I	107	10,16
	Emperor Tamarin		64	10
	Red-chested Moustached		109	10,16
	White-footed Tamarin	V	4	10
	Red-Handed Tamarin		51	10,16
	Moustached Tamarin		27	10
	Black and Red Tamarin		2	10,16
	Cotton-Headed Tamarin		487	23
	Pied Tamarin	I	11	16
	Geoffrey's Saddle-back Tamarin		5	IZY
	TOTAL		2822	

1. IUCN (11). E = Endangered; V = Vulnerable; R = Rare, I = Indeterminate.

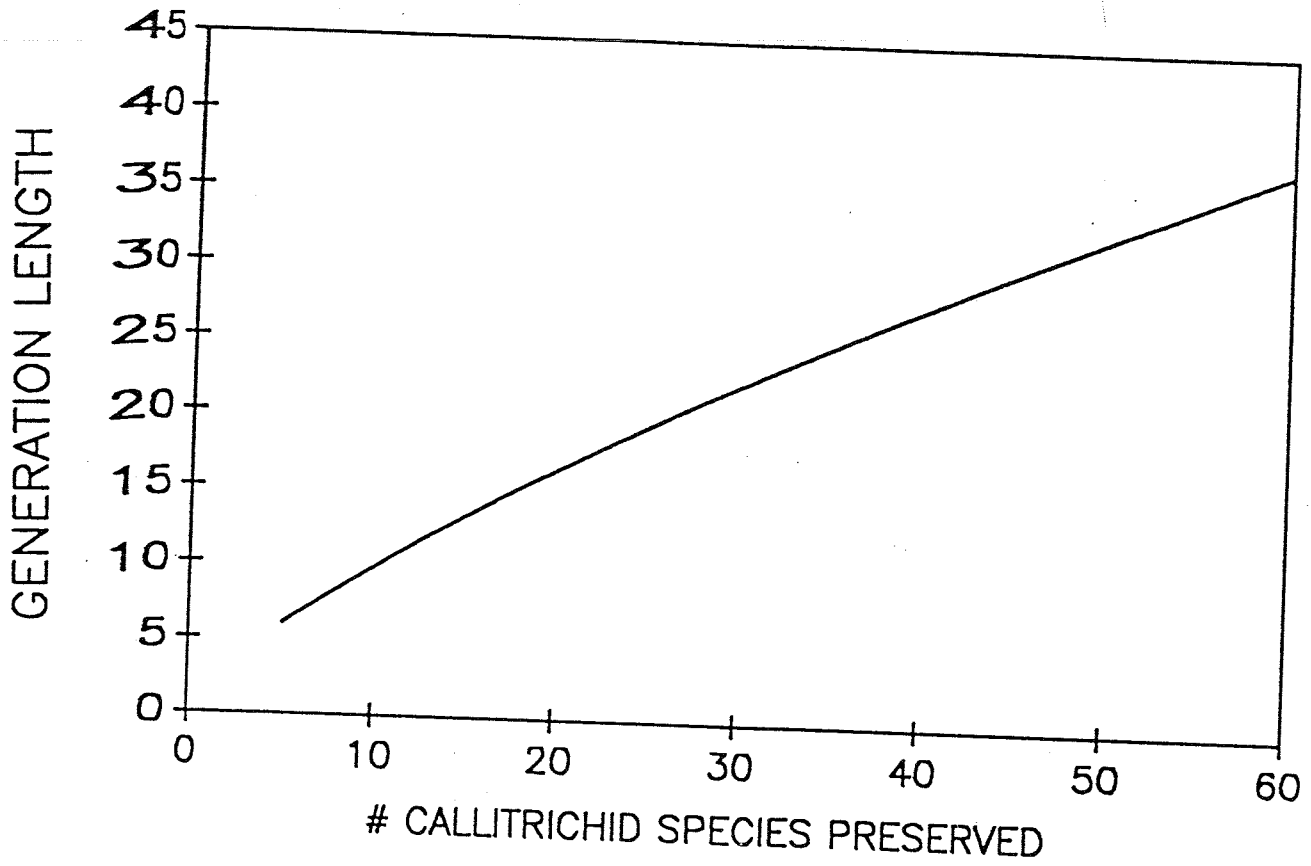

100
80
60
40
20
% Genetic diversity retained
Effective
population
size
200
150
100
50
10
50
100
150
200
Generations


% GENETIC DIVERSITY RETAINED
1.0
0.8
0.6
0.4
0.2
0.0
0
25
50
75
100
125
150
175
200
YEARS
Gen. Length = 10 Yrs
Gen. Length = 5 Yrs

Effect of Generation Length on Population
Sizes Required to Retain 90% Gen. Diversity for 200 Years



Effect of Increased Generation Time on
Number of Callitrichid Species Preserved



ESTABLISHING GENETIC RESOURCE BANKS FOR ENDANGERED SPECIES

Prepared for the
National Research Council Panel on Biodiversity

Dr. David E. Wildt, Head, Reproductive Physiology Program, National Zoological Park, Smithsonian Institution

Dr. William F. Rall, Cryobiologist, National Zoological Park, Smithsonian Institution

Jonathan Ballou, Population Biologist, National Zoological Park, Smithsonian Institution

Nathan Flesness, Director, International Species Information System

Dr. U. S. Seal, Chairman, Captive Breeding Specialist Group (CBSG), Species Survival Commission, World Conservation Union (IUCN)

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Executive Summary

There has emerged on the rapidly changing face of our planet a pattern of increasing numbers of animal and plant species reduced in distribution, populations, and numbers. Even species relatively numerous in total numbers are being fragmented into small isolates with no exchange between them. Small populations face genetic, demographic, and environmental risks as a result of their size and isolation. In the extreme case a species is reduced to one population, oscillating and shrinking in numbers, losing genic diversity, and subject to abrupt reductions or extinction through the random whims of natural events or man. The extreme has become commonplace with intensively managed wild and captive populations the only short term hope for survival for hundreds of plant and vertebrate species worldwide. The challenge with invertebrate species, with perhaps millions at risk, has been difficult to even formulate.

Demographic and genetic objectives of recovery programs in the wild and captive propagation programs for endangered species focus on establishing demographically secure populations that maintain adequate levels of genetic diversity. Long term storage and utilization of cryopreserved germ plasm could extend the population's generation length and allow higher levels of genetic variation to be maintained in smaller populations. Since fewer breeding animals would be needed for captive programs, more species would be 'rescued' from extinction using captive facilities currently available at existing institutions. Doubling of generation lengths for some species could almost triple the number of species that could be rescued in zoos. Additionally, long term cryopreservation would allow for a third population, that of the frozen gene pool. Three-way exchange of germ plasm from germ plasm banks to captive and wild populations would increase genetic diversity at reduced risk and expense. Advances in reproductive technology and better understanding of basic reproductive processes are necessary to permit routine application of these technologies.

A Genetic Resource Bank should be established to facilitate international efforts to preserve rare and threatened animal and plant biodiversity. Funding is required to support a National Genetic Resource Bank. The Bank's mission should be to: 1) acquire, store, use and disseminate viable germ plasm (sperm, ova, embryos, seeds), tissues and DNA collected from wild species of animals and plants; 2) conduct basic research in (a) low temperature biology and (b) storage and use of germ plasm, embryos, tissues and DNA from wildlife species; and 3) simultaneously perform interactive research in reproductive biology and artificial breeding to help maintain biodiversity and propagate endangered animals and plants. To encourage genetic diversity, research efforts should be directed at free-living, wild taxa, especially the transfer of germ plasm into genetically stagnant populations. Because of the magnitude of the crisis, immediate efforts also should be initiated to secure federal and international funding for developing Regional Genetic Resource Banks to be located throughout the U.S. and abroad. The national and regional banks would work interactively, sharing expertise and resources and coordinating and ensuring the long-term storage and dissemination of rare genetic material.

The Importance of Genetic Resource Banks

Various public- and privately-sponsored task forces have emphasized the importance of developing strategies for maintaining genetic diversity in wild fauna and flora. For more than 12 years, there has been a consistent demand for establishing genetic resource banks for wildlife species.

- "New agencies should be established, or existing agencies charged, with the preservation of particular germ plasm resources. Funds should be provided to support these agencies and to train the personnel necessary for the maintenance of these essential resources. What is done for domestic species (e.g., artificial insemination, sperm and blastocyst freezing, and implantation) should be done for all species reproducing in captivity." (Conservation of Germ Plasm Resources: An Imperative, National Research Council, Report of Committee on Germ Plasm Resources, National Academy of Sciences, Washington, DC, 1978.)

- "Establishment of a program in the U.S. to coordinate the management of animal germ plasm resources would be in the national interest." (Animal Germplasm Preservation and Utilization in Agriculture, Council for Agricultural Science and Technology, Report No. 101, Ames, Iowa, September 1984.)

- "Preservation of germ plasm requires that institutions should be developed and/or strengthened for collection, maintenance and dissemination of genetic resources." (U.S. Strategy on the Conservation of Biological Diversity, Inter-Agency Task Force Report to Congress, U.S. Agency for International Development, Washington, DC, 1985.)

- "A program of research could be administered through the National Science Foundation and channel funds to both basic studies on the reproductive biology and cryobiology of wild animals and to applied studies on the control of reproduction, artificial insemination and embryo transfer. Another approach could be establishing a few centers for study of the reproductive biology of wild animals. These centers could serve as focuses for programs of basic and applied research. They should be sufficiently well-funded to allow broad programs of research on-site as well as extramural research with cooperating institutions. These centers could likewise serve as repositories for frozen gametes and embryos from endangered populations". (Technologies to Maintain Biological Diversity, U.S. Congress, Office of Technology Assessment, Report No. OTA-F-330, U.S. Government Printing Office, March, 1987.)

• "High priorities studies include . . . gamete and developmental biology and the collection, evaluation and long-term storage of genetic material including gametes and embryos. Successful cryobiology will have a major impact on conserving genetic diversity. A resource of frozen semen and embryos could be used interactively with living populations to periodically infuse genetic material from captive or wild animal stocks or to instill captive populations with thawed genes from previous generations. The cryopreservation and storage of haploid gametes and diploid embryos and cell cultures at low temperatures (-196oC) offers unique opportunities for facilitating the propagation of wild species and ensuring the conservation of genetic diversity. The development of banks of cryopreserved germ plasm will: 1) reduce the number of animals needed to ensure high levels of genetic diversity; 2) facilitate the infusion of germ plasm from wild populations into captive breeding programs; and 3) provide insurance against the loss of diversity from epidemics, natural disasters and social/political upheavals. Similarly, DNA and tissue banks permit retrospective genetic analysis of founder animals. Research is needed to develop simple, alternative preservation approaches including vitrification, freeze-drying and cold storage." (Research Priorities for Single Species Conservation Biology, a workshop sponsored by the National Science Foundation and the National Zoological Park, Washington, DC, 1989.)

The time is right to finally act on these recommendations. There is an overall renewed public consciousness about environmental issues, and recent advances in "biotechnologies" make the actual utility of genetic resource banks more a reality than ever. The potential of such resources already has been demonstrated by the successful preservation of germ plasm from important domestic food animals, companion animals and crop plants. Private enterprise as well as some governmental actions have been initiated to collect, protect and use these agricultural resources. For example, the commercial distribution of frozen cattle semen has been in place for more than 30 years. Recently, similar commercial efforts have spread to the routine use of frozen cattle embryos, frozen rodent embryos (for biomedical research) and frozen sperm from purebred dogs. Both private and federal institutions have begun to systematically store biological material from specific animal and plant genotypes. As three examples: 1) the Jackson Laboratory and the National Institutes of Health maintain large storage facilities for frozen embryos collected from hundreds of genotypes of mice used as animal models for biomedical research; 2) the United States Department of Agriculture maintains a repository for plant seeds from crop species; and 3) The American Type Culture Collection functions to acquire, preserve and distribute characterized strains of bacteria, fungi, protozoa, algae, viruses, cell/tissue cultures and the creations of recombinant DNA technology.

The Justification and Potential Utility of Genetic Resource Banks

During the past decade, there has been much concern about the loss of genetic diversity in wild taxa. It has been speculated that certain biotechniques like gamete (sperm and ovum) and embryo cryopreservation could be instrumental in assisting in the conservation and management of wildlife species. In light of the publicity directed at dwindling habitat, the loss of species and genetic diversity and the potential of artificial breeding technology, it is remarkable that no organized effort exists, either in the U.S. or elsewhere, to sample, evaluate, cryopreserve, catalog, maintain and use germ plasm from wild animal and plant species.

- In practice, a Genetic Resource Bank would not be merely a static warehouse of biological material but would serve a vital, interactive role between living populations of captive and free-living species. These interactions are required to prevent undesirable selection pressures in captivity, preserve new diversity resulting from natural evolutionary processes and allow small or fragmented populations to receive "infusions" of genetic diversity from cryopreserved germ plasm.

- The combination of frozen gametes and embryos and reproductive techniques such as artificial insemination, in vitro fertilization and embryo transfer offers unique opportunities for improving the efficiency of captive breeding programs. Cryopreservation of germ plasm extends the generation interval of a species indefinitely. The genetic diversity of the founder does not die with the animal, but remains viable and available for future generations.

- Germ plasm banking has the effect of reducing the number of animals needed to ensure that high levels of genetic diversity are retained within a population. This reduces the capital and operating costs of zoo breeding programs and provides space for other species at risk for extinction.

- Other benefits include the incorporation of germ plasm from wild stocks into captive breeding programs without removing animals from the wild, and the insurance banking offers against the loss of diversity or entire species from epidemics, natural disasters and social/political upheaval.

- Transporting frozen sperm or embryos eliminates the considerable risks associated with the transport or exchange of live animals.

- The judicious interspecies use of germ plasm (e.g., hybridization of rare species of wild cattle with common cattle) may provide avenues for improving the genetics, food production and general agri-economy of developing countries.

• Technologies developed for animal and plant germ plasm can be used to expand the genetic bank to include other biologicals including tissues, blood products and DNA. These new elements also can provide a service repository allowing more wide-spread access to rare specimens.

Background - An Argument for Single Species Conservation Biology in Concert with A Genetic Resource Bank

The biodiversity of the planet is disappearing at an alarming rate. Most extinctions are human-induced and caused by the fragmentation, destruction, exploitation and pollution of natural habitats. Of particular significance is the emergence of a new field - Conservation Biology - which seeks to define and understand the factors affecting ecosystems and controlling species survival. Conservation biology is interdisciplinary and relies on a synthesis of ideas, information and approaches from a wide variety of scientific and geopolitical fields. One vital component is reproduction, the process on which maintenance of all inter- and intra-species diversity depends.

Conservation biologists constantly debate the relative merits of saving habitats versus species. Habitat proponents suggest that representative ecosystems cam be identified and permanently isolated from human interference. This approach focuses on the long range problem and protects the ecosystem and the many species within it. In an undisturbed natural habitat, most species are likely to fulfill their essential needs and flourish. The problem, of course, is in choosing which habitats to preserve since many species inevitably will be excluded. Additionally, those species most susceptible to extinction (i.e. large-sized predators at the top of the food chain) often require extraordinary large home ranges. Therefore, it may be too expensive or socially disruptive to provide sufficiently-sized natural reserves for such animals. The contrasting view is to preserve single species, an approach exemplified by captive breeding programs. In this scenario, animals are maintained in a semi-controlled environment, and modern science is charged with identifying and manipulating the factors influencing reproductive success. In theory and given modern management techniques, rare species should thrive in captivity. But single species conservationists encounter many of the same problems that plague programs designed to save native habitats. The shear cost of establishing "naturalistic" captive environments and prioritizing which species deserve the most attention are major concerns. These proponents also are faced with the paradoxical threat of unbridled success, that is, that captive breeding may increase animal numbers to the point that all captive habitat is saturated and then overwhelmed.

There is no doubt that habitats and single species should be salvaged simultaneously and that the cryopreservation of biological materials can play a major role in both types of conservation.

For example, free-living animals produce excessive germ plasm which can be recovered safely, cryopreserved and used to infuse captive populations with genetic vigor while eliminating the need to remove more animals from the wild. Likewise, cryobiology offers a resolution to the problem of limited captive breeding space. It simply is more cost-effective to preserve genetic material from populations, genotypes and individuals at low temperatures, eventually rederiving these species when needed.

An Overview on State-of-the-Art Reproductive Biotechnology including Cryopreservation and Use of Germ Plasm and Embryos

Basic reproductive research has sparked a revolution in combating human infertility and improving livestock production. Reproductive biotechniques no longer are exclusively useful to humans, domestic and laboratory species. The current biodiversity crisis mandates that more fundamental science be directed at unconventional species, most of which have never been studied. These taxa conceal a wealth of new information on biological phenomena ranging from basic gamete physiology to reproductive adaptations to speciation. In humans and domestic animals, reproductive biotechniques (germ plasm cryostorage, artificial insemination, in vitro fertilization, embryo micromanipulation and embryo transfer) were the direct result of basic research and, in turn, became essential to achieving even greater improvements in reproductive performance. For wildlife species (including vertebrates, invertebrates and plants), these techniques could be used to enhance propagation and sustain current levels of bio- and genetic diversity.

During the past decade, there has been a surge of interest in developing captive breeding programs for preserving rare animal species. The formulation of species survival plans, recovery plans and population viability analyses and the release of captive-born animals (e.g., golden lion tamarins, red wolves, bison, Arabian oryx) into wild habitats have revealed one common theme, there is an all-out need for more quality research. The question then becomes what types of research are of highest priority?

Logic dictates that considerable emphasis be placed on sustaining existing biodiversity using cryopreservation technology since any further delay only will accentuate ongoing and relentless losses in species and genetic variability. The actual details for using frozen gametes and embryos for producing wildlife offspring have yet to be developed for many species. Nevertheless, sufficient technology and in vitro (laboratory) test procedures are available to ensure that most frozen germ plasm is biological competent and eventually useful. Another argument for

developing genetic resource banks first is that this approach naturally spawns and mandates related research efforts in other reproductive, biomedical and veterinary fields. Reproductive biology is a complex discipline consisting of many components ranging from behavior to endocrinology to sophisticated micromanipulations of the embryonic genome. The actual applied use of a genetic resource bank will inevitably require interactive research among many disciplines and the development of new technologies. These, in turn, will generate massive data sets which will help scientists fully comprehend the fundamental biology of rare species, almost all of which have never been studied. For example, the "simple" collection and cryostorage of Siberian tiger sperm will require interaction among: 1) the Species Survival Plan Coordinator (to choose which animals to use); 2) veterinarians (responsible for developing optimal anesthesia); 3) biotechnical engineers (responsible for developing equipment to collect sperm); 4) gamete biologists (talented in sperm collection, processing, and evaluation); 5) cryobiologists (skilled in the freezing, packaging and storing of sperm); and 6) registrars (responsible for maintaining computerized catalogs detailing the pedigree of the sperm donor and the location, amount and distribution of the germ plasm). The actual use of the frozen sperm will stimulate even further research into an array of exciting areas (e.g., establishing the length of the estrous cycle; studying the impact of seasonality on reproductive performance; identifying and synchronizing estrus; predicting ovulation; developing techniques for artificial insemination, in vitro fertilization and embryo transfer; diagnosing pregnancy and impending parturition). Therefore, genetic resource banks will serve as the incentive for developing and expanding other biological disciplines ranging from fundamental reproductive biology to genetic management of rare populations to applied aspects of veterinary medicine to global monitoring/computerized information systems.

The actual use of animal germ plasm requires either artificial insemination (AI) or in vitro fertilization (IVF). The use of embryos requires embryo transfer (ET) technology.

For wildlife species, AI could be valuable for ensuring reproduction between valuable but behaviorally incompatible pairs, eliminating the risks of animal transport and providing an avenue for infusing genes from wild stocks into captive populations, many of which have become genetically stagnant.

IVF potentially is one of the most powerful tools available for artificial breeding of rare species. From an applied perspective, IVF has the potential of resolving many of the serious problems routinely encountered in modern captive-breeding programs. In theory, the genetic material from rare animals that fail to reproduce because of behavioral peculiarities, physical handicaps or stress susceptibility could be reintroduced into captive populations using IVF. This

procedure also is highly attractive because it requires neither detection of overt estrus nor direct interaction between the male and female. In the context of a frozen germ plasm resource, an IVF program could be used to infuse gametes collected from free-living animals into captive populations. Finally, recent advances in the ability to recover and mature early stage oocytes in vitro could offer an approach for salvaging genetic material from rare animals that die abruptly. Theoretically, with cryostored germ plasm, IVF could be combined with gamete maturation to produce embryos and then offspring from deceased individuals. Pilot studies already have been successful with laboratory rodents and farm livestock.

For wildlife species, ET offers the possibility of accelerating the number of offspring produced and using females incapable of reproducing because of age or physical /medical handicaps. In conjunction with embryo freezing, ET could help preserve the combined genetic component of an individual in suspended (frozen) animation, thereby offering an approach for reintroducing available genetic material into later generations. The potential of ET is increased if embryos can survive interspecies ET, that is, develop and be born to surrogate mothers of a more common species or closely-related genus.

Although the possibilities of using frozen wildlife germ plasm and embryos in concert with AI, IVF or ET is staggering, most successes have been limited to farm livestock and laboratory animals. Table 1 lists the 28 species in which AI with frozen-thawed sperm has resulted in live-born offspring. IVF has been successful in a total of 13 species, and, in almost all cases, the young born were conceived using fresh (non-frozen) gametes (Table 2). Table 3 includes the 14 species in which frozen-thawed and transferred embryos have resulted in live-born offspring.

Table 1. Mammalian species in which offspring have been produced by AI and frozen-thawed sperm.

Domesticated	Non-Domesticated
Cattle	Fox
Sheep	Wolf
Horse	Addax
Pig	Blackbuck
Rabbit	White-tailed deer
Dog	Fallow deer
Goat	Chimpanzee
Cat	Bighorn sheep
Mouse	Red deer

Water buffalo	Bison
Domestic ferret	Siberian ferret
Human	Giant panda
	Gaur
	Wapiti
	Reindeer
	Addax

Table 2. Mammalian species in which offspring have been produced by IVF followed by embryo transfer.

Domesticated	Non-Domesticated
mouse	baboon
rabbit	rhesus monkey
rat	cynomolgus monkey
human	Indian desert cat
cattle	tiger
pig	marmoset
sheep	
cat	

Table 3. Mammalian species in which offspring have been produced using frozen-thawed and transferred embryos.

Domesticated	Non-Domesticated
mouse	eland
rat	baboon
rabbit	cynomolgus monkey
cow	marmoset
sheep	
pig	
cat	
goat	
horse	
human	

Considering that more than 4,000 mammalian species inhabit our planet, it is obvious that the potential of reproductive biotechnology has been tested in an infinitesimal fraction of wild taxa.

The lack of application is the direct result of insufficient or, in most cases, nonexistent funding for both basic and applied wildlife research. Historically, there also has been notoriously poor coordination of similar efforts among zoos. Amazingly, no centralized organization has evolved to compel zoos to participate in a genetic resource conservation program (like the Species Survival Plans developed through the American Association of Zoological Parks and Aquariums). Even so, there is a general consensus that reproductive biotechnology could be used to better preserve genetic diversity and assist in captive propagation given that species reproductive norms were known. In this respect, this same strategy was used to make the use of frozen sperm and embryos feasible in farm livestock. For example, the conventional use of AI and embryo transfer in domestic cattle became routine only after years of research into gamete biology and fundamental reproductive processes. This substantial progress could only be made after millions of research dollars were provided by federal, commercial and private sources.

Similar basic research strategies are possible and have been applied to wildlife species (primarily on limited or pilot basis) by a few pioneering institutions. Advances in wildlife and zoo veterinary medicine now permit routine and safe animal anesthesia which allows blood sampling (for endocrine monitoring), electroejaculation (for semen collection), uterine catheterization (for nonsurgical embryo collection/transfer), laparoscopy (for oocyte recovery) and ultrasound (for ovulation and pregnancy diagnosis). Other novel and exciting techniques have been developed to facilitate the eventual practical use of genetic material. The hormonal status of many wildlife species now can be tracked by measuring hormonal metabolites in voided urine or feces. This innovative approach, which eliminates the stresses associated with blood sampling under anesthesia, has been used to document the estrous cycle, time of ovulation and even predict pregnancy and impending birth. Simple, cost-effective enzyme-linked immunosorbent (ELISA) assays can be used to predict critical events (like ovulation from a urine sample) and offer exciting means of assessing endocrine function under field conditions. New approaches, originally developed for assessing human fertility potential, also are finding application in wildlife species. "Heterologous" IVF systems are available whereby hamster or domestic cat oocytes can be used to test the penetrating or fertilizing capability of sperm collected from other species. These in vitro assays of sperm viability will be important in testing the biological competence of frozen-thawed, wildlife sperm. Lastly, reproductive biotechnology is beginning to contribute significantly to nonmammalian and invertebrate species. Some progress has been made in the long-term storage of fish gametes (especially salmon and rainbow trout sperm) and mollusc embryos. Insects also are benefiting as illustrated by continued progress in the freeze storage of, for example, honey bee semen and the recent successful cryopreservation of Drosophila embryos. As demonstrated in Tables 1 to 3, there is similar tantalizing evidence in other wildlife species. The live births of any

wildlife species as a result of biotechnology and the use of fresh or frozen germ plasm are laudable. However, certain events are particularly worthy of note as they either: 1) demonstrate a biological "first" for wildlife species; 2) illustrate the ability to apply techniques developed for farm livestock to wild counterparts; or 3) focus on a particularly difficult taxon which has received little or no research attention. Such milestones could include the birth of live offspring following:

- intraspecies embryo transfer in a baboon (first successful ET in a wildlife species);
- IVF in a baboon followed by embryo transfer (first successful IVF in a wildlife species);
- AI of a puma using fresh sperm;
- intraspecies embryo transfer in the eland, oryx, bongo and suni antelope;
- interspecies embryo transfer in the gaur (to Holstein cow), zebra (to domestic horse), Przewalski's horse (to domestic horse) and bongo (to eland);
- AI of a giant panda using frozen-thawed sperm;
- intraspecies transfer of frozen-thawed marmoset embryos;
- IVF of Indian desert cat oocytes followed by embryo transfer to the domestic cat;
- AI of gaur with frozen-thawed sperm;
- IVF of tiger oocytes followed by embryo transfer to a surrogate tiger.

Although cryopreserved genetic material played a role in only four of these events, there is no biological reason why frozen gametes and embryos could be not be used successfully to extend these early accomplishments.

Regardless of species and technique used, some sperm and embryos will inevitably fail to survive freezing and thawing. Although a great deal of research has been directed at cryoprotective solutions and cooling rates for semen and embryos, it now is apparent that these factors can vary with species. Basic formulae for freezing sperm and embryos are available, and it is likely that only modest research will be needed for some species. However, there is an almost complete lack of information on sperm and embryos for a vast number of species, suggesting that much more basic research will be needed. Also of concern is the potential introduction and spread of diseases to domestic livestock and other wild stocks that could occur with the international transport of poorly-monitored gametes and embryos. These factors must be taken into consideration in the formulation of genetic resource banks.

An Overview on State-of-the-Art Biotechnology for Cryopreserving Plant Genetic Resources

During the past two decades, a complex system of inter-related organizations have evolved to develop and implement programs to survey, collect and protect plant genetic resources on both a national and international scale. Compared to the preservation of animal germ plasm, this effort has been much better organized and coordinated. This largely is attributable to the International Board for Plant Genetic Resources (IBPGR). The IBPGR was formed in 1974 to create and coordinate a global network of plant germ plasm conservation centers. Current efforts focus on economically important crop species and their wild relatives. The IBPGR receives funds from the World Bank, The Food and Agriculture Organization of the United Nations (U.N.) and the U.N. Development Program. Membership of the Board includes the three major sponsors, 14 donor governments, three regional development funds, the European Economic Community, private foundations (Ford, Rockefeller, Kellogg), the International Development Research Center (Ottawa) and two representatives from each of the five major developing regions. These members also serve on the Consultative Group on International Agricultural Research (CGIAR), a consortium of nearly 50 governments, international organizations and foundations that support a system of 13 major agricultural research centers in developing countries. CGIAR currently provides about $275 million/year for food crop and livestock research that benefits developing countries. As a result of these activities, an international network of approximately 60 research institutions serve as base collections of germ plasm for the major food crops.

The lead organization in this country in plant germ plasm preservation is the U.S. Department of Agriculture. Most current U.S. efforts are in the form of seed banks or field collections of living plants that must be maintained by vegetative propagation. Brief information on other U.S. and international organizations are provided in A Partial List of Federal, Commercial and Private Institutions Involved in the Preservation of Genetic Material (see below).

Different technologies are available for preserving unique sources of plant germ plasm including seeds, field and greenhouse collections of living plants, pollen banks, in vitro plantlet and tissue culture and the cryopreservation of plant tissues that are capable of regenerating into whole plants. Unfortunately, no single approach is appropriate for all types of plant materials due to the diverse range of morphological, physiological and genetic properties of plant species.

Seed Storage:

One phase of the life cycle of many plant species provides a convenient means for storing natural propagules of plants, namely seeds. Seeds often remain viable for years when dried to a moisture content of 5 to 8% and stored at low temperatures. Seeds commonly are divided into two groups: 1) orthodox seed that withstand a reduction in moisture content and storage at low temperature; and 2) recalcitrant seeds that cannot survive a reduction in moisture content. Most of the major temperate food crops produce orthodox seed and many can be stored indefinitely at liquid nitrogen temperature (-196°C) after drying. Many important tropical crop species, aquatic plants and some temperate tree species produce recalcitrant seed that cannot be stored in seed banks.

Three other biological limitations may restrict the application of seed storage for the conservation of plant germ plasm. First, a significant number of important crop species (potato, apple, citrus) require vegetative propagation for the retention of specific economic traits or qualities. Second, suboptimal environmental conditions, physiological barriers or genetic deficiencies may prevent some plants from producing seed. Furthermore, some common cultivars of plants (bananas) are sterile. A third biological limitation is the presence of a long juvenile stage in some species which makes seed collection difficult. This is of particular concern for important forest tree species.

In Vitro Culture:

In vitro maintenance is defined as the aseptic culture of cells, tissues, organs or plantlets under defined laboratory conditions. One major advantage of this approach is that many plant pathogens can be eliminated from infected plants when cultures are initiated from intact isolated meristems. In vitro cultures can be divided into two broad categories based on the degree of organization of the plant material.

1. Plantlet cultures. The in vitro propagation of large numbers of identical clones of a plant is termed micro-propagation. In this approach, the primary shoot tip and axiliary shoot tips (buds) associated with each leaf are excised and placed on a nutrient medium. The genetic stability of plantlets produced by this procedure is remarkably high, and there are reports of successive subculturing over a 12 year period with no obvious deterioration or genetic changes. The rate of metabolism of shoot cultures can be decreased by placing the cultures at low temperatures. The major advantage of such cold storage is to reduce the labor and materials for long-term maintenance by increasing the interval between subcultures. Successful cold storage of in vitro cultures has

been reported for up to 6 years. There are some reports of successful cryopreservation of shoot tips and meristems but current procedures often exhibit variable rates of success. Unfortunately insufficient data are available on whether a single protocol can be applied to maintain diverse sources of plant germ plasm or whether regenerated plants are normal after prolonged low temperature storage.

2. Tissue and cell cultures. Plant tissue cultures (callus cultures) are initiated in vitro from proliferating masses of largely unorganized cells arising from wounds on differentiated plant tissues. It is possible to initiate callus cultures from many species of plants and to further isolate and subculture this tissue on fresh medium. When cultures are transferred into liquid medium, callus may form a suspension of single cells or multicell aggregates. Sometimes it is possible to regenerate whole plants from tissue cultures using defined environmental, nutritional and/or hormonal treatments. All types of plant organs and tissues (stems, roots, leaves, floral organs) have been used to initiate callus cultures. Two properties of plant tissue and cell cultures are of particular concern for plant germ plasm preservation. First, the vigor and regenerative potential of the material decreases over time. Second, genetic changes frequently are found in plants regenerated from callus cultures. Although such genetic variation provides a powerful approach for plant breeders, it is unacceptable when the objective is to preserve a specific genotype. For these reasons, in vitro plantlets derived directly from buds or shoot tips are considered more suitable than callus cultures for maintaining germ plasm in vitro.

An Overview on State-of-the-Art Biotechnology for Cryopreserving Plant Tissue Cultures

Although procedures for the cryopreservation and storage of microbes and animals cell cultures became well-established in the early 1960s, significant progress in applying this biotechnology to plant tissue cultures did not occur until late in that decade. True cryopreservation of plant tissue cultures when defined in terms of regeneration of whole plants after thawing was first reported in 1973. Subsequent research indicated that the ease of cryopreserving plant material depends on complex interactions between morphological, physiological and cryobiological factors. In general, the larger the size and complexity of the tissue, the lower the likelihood of success. Therefore, although significant progress has been made in the cryopreservation of plant cell suspensions and plant pollen, applications of this procedure to shoot tips and meristems yield lower and more variable results. There are several reports of the regeneration of plants from cryopreserved shoot tips and meristems but in most cases some abnormal growth and callusing occurs after thawing.

An Overview on State-of-the-Art Biotechnology for Preserving Cultured Cells and DNA

Animal tissues and cultured cells currently do not offer a means for propagating genetic diversity. However, they do represent important material useful for supporting basic and applied research. Methods are available for establishing frozen banks of living tissues and cells so that the material remains useful for studies involving systematics, evolutionary biology, comparative biochemistry, comparative physiology and developmental biology. These studies can be crucial for determining the level of genetic diversity within a species, population or individual. This information , in turn, can direct specific conservation and cryopreservation efforts. Presently, access to cell lines from diverse taxa is extremely limited and, when samples are available, they rarely originate from more than one or two individuals. Studies on population genetics require cells from many different individuals of a species.

There are a few collections of frozen mammalian cell cultures in the U.S.. The largest is maintained by the American Type Culture Collection, but the emphasis at this institution is on human- and rodent-derived cells. Small collections of frozen cells obtained from wildlife species have been established at the San Diego Zoo, the National Zoological Park, the National Cancer Institute_s Laboratory of Viral Carcinogenesis and the University of Texas (at Houston).

Banks of nuclear and mitochondrial DNA isolated from wildlife species can serve as an alternative resource for molecular genetic studies directed at conservation. The cost and effort required to establish a bank of DNA are much less than that for frozen cells, but DNA alone provides less information on gene expression from living cells. DNA banks from individuals and families at high risk for inherited disease have been established as valuable resources for developing diagnostic tests and therapeutic treatments. Modest banks of human DNA have been established at the American Type Culture Collection, the National Institutes of Health and the Centre d'Etude du Polymorphisme Humain (Paris, France). Presently, there are no organized programs for collecting and maintaining DNA banks from wild animal species.

A Partial List of Federal, Commercial and Private Institutions Involved in the Preservation of Genetic Material
(in alphabetical order)

American Type Culture Collection (ATCC), Rockville, MD (nonprofit organization which acquires, preserves and distributes characterized strains of microorganisms, viruses, cell/tissue

cultures and the creations of recombinant DNA technology; also is a leader in developing computerized record systems for stored biologicals).

Center for Plant Conservation, Jamaica Plains, MA (nonprofit organization that has established a network of botanical gardens and arboreta to conserve rare North American wild plant species).

Cincinnati Zoo, Cincinnati, OH (zoological park conducting basic and applied research in sperm and embryo freezing of domestic and nondomestic species).

Henry Doorly Zoo, Omaha, NE (zoological park conducting basic and applied research in sperm freezing in nondomestic species).

Jackson Laboratory, Bar Harbor, ME (nonprofit organization conducting basic and applied research in mouse embryo freezing; maintains a major frozen embryo repository).

Metro-Toronto Zoo, Toronto, Canada (zoological park conducting basic and applied research in sperm and embryo freezing in domestic and nondomestic species).

Minnesota Zoo/University of Minnesota, Minneapolis, MN (zoological park/academic institution conducting basic and applied research in sperm freezing in domestic and nondomestic species).

National Cancer Institute, Laboratory of Viral Carcinogenesis, Frederick, MD (federal institution which maintains selected cell lines from wildlife species).

National Institutes of Health Embryo Cryopreservation Unit, Bethesda, MD (federal institution conducting basic and applied research in embryo freezing in laboratory rodents including mice, rats and rabbits; maintains a major repository for frozen mouse embryos).

National Science Foundation, Washington, DC (federal institution providing support for some important genetic plant and microorganism stock collections).

National Zoological Park, Smithsonian Institution, Washington, DC (zoological institution conducting basic and applied research in sperm and embryo freezing in domestic and nondomestic species; establishes cell lines from wildlife species).

San Diego Zoo, San Diego, CA (zoological park conducting basic and applied research in sperm and tissue freezing in nondomestic species).

Seed Savers Exchange, (organization of hobbyists that collect heirloom and endangered vegetable varieties).

Texas A&M University, Department of Veterinary Physiology and Pharmacology, College Station, TX (academic institution conducting basic and applied research in sperm and embryo freezing in domestic and nondomestic species).

U.S. National Plant Germplasm System (NPGS) [NPGS is a network of cooperating federal, state, private agencies and research facilities that acquire and maintain crop germ plasm. Maintains some wild species but this is a secondary function and usually involves relatives of cultivated crops or economically valuable plants. Composed of six elements including: 1. the National Plant Germplasm Committee which coordinates research and service efforts of the federal and state agricultural experiment stations; 2. the National Seed Storage Laboratory in Fort Collins, CO which is the nation_s primary long-term storage facility for seeds; 3. the Crop Advisory Committee which advises on acquisition, maintenance and use of genetic resources; 4. the U.S.D.A./A.R.S. Plant Genetics and Germplasm Institute in Bethesda, MD which manages various germ plasm acquisition and introductions; 5. Four Regional Plant Introduction Stations which maintain germ plasm from specific crops; and 6. Nine National Clonal Germ Plasm Repositories which maintain living plant collections of species that must be propagated vegetatively (fruit and nut species)].

Zoological Society of London, London, England (zoological institution conducting basic and applied research in sperm and embryo freezing in domestic and nondomestic species).

Recommendations for Establishing a Genetic Resource Bank(s)

1. Immediate funding should be provided to support an institution whose objectives and research accomplishments are oriented to conservation biology and the preservation of biodiversity. This organization would be designated the National Genetic Resource Bank.

- Progress will be accelerated by supporting an existing institution, preferably one with multidisciplinary team talents. Organizations with professionals and technicians with combined skills in the fields of animal (domestic and nondomestic species) or plant reproductive physiology, cryobiology, low temperature (cold storage), gamete function, embryology, artificial insemination, embryo transfer, in vitro fertilization, endocrinology, veterinary medicine (especially anesthesia), disease transmission via germ plasm, population biology, genetic management, field studies and/or computer programming, are highly worthy of consideration. Since it is unlikely that any single organization would have either the expertise or physical space to maintain a frozen repository of germ plasm/embryos, plants/seeds and tissue/DNA, separate organizations should be responsible for each of these three biologicals. Each would serve as a separate Division (one for animal germ plasm, one for plants, one for tissue/DNA) within the National Genetic Resource Bank.

- The research record of the staff should reflect an appreciation for the importance of basic research as a prerequisite to practical collection and subsequent utilization of the genetic materials.

- The institution should have earned independent recognition and/or be closely affiliated with other organizations of national and international repute and have demonstrated a record of leadership in conservation issues, scientific research and training.

- The institution should have international contacts and existing collaborations with conservation organizations, research institutions and governmental/private wildlife authorities world-wide. Such relationships are a prerequisite to coordinating, organizing and gaining access to special or rare wildlife populations for genetic material recovery, storage and distribution. Special collaborative talents will be required to ensure that species, populations and individuals in crisis receive first priority attention.

- Funds should be used to: 1) support existing staff and/or hire new talent; 2) purchase equipment and supplies to permit the safe collection, processing, long-term storage and active use of germ plasm and DNA; 3) transport and support research teams to permit the collection and preservation

of rare genetic material; and 4) conduct basic and applied research (as outlined in recommendation #3 below).

2. Additional federal and private funding should be identified and appropriated to begin developing regional genetic resource banks throughout the U.S. and abroad. These smaller institutions will be crucial to securing appropriate samples of genetic material unique to specific geographic locations. Regional centers should support the mission of the National Genetic Resource Bank by providing half the frozen sample to the central repository while maintaining half locally for "insurance-safety" purposes. Regional banks also should be encouraged to conduct research, much of which will be based on innovations and advances made at the National Genetic Resource Bank.

3. Considerable emphasis must be placed on both basic and applied research by the Genetic Resource Bank institution. Areas of high priority include:

- Defining the effect of species on the efficiency of freezing sperm, ova, embryos and seeds (a strategy which will determine how well existing technology can be applied immediately to rare species);

- Identifying the best cryoprotectants and cooling and thawing processes for a particular species of interest;

- Developing accurate laboratory approaches for testing the viability of thawed germ plasm, embryos, seeds, tissues and DNA;

- Conducting detailed, longitudinal studies on the many species-specific factors that influence the production of live offspring from thawed material;

- Improving testing and processing procedures to ensure that the transport of germ plasm does not contribute to disease transmission.

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Resources of Plant Germplasm

Conserving the world's biological diversity has emerged as a matter of international concern. Annually, many species are disappearing. Of particular importance are plant species related to those employed in agriculture. Some of the plants that produce little or no food possess genetic traits that enable them to withstand stress, pests, or diseases of many kinds. In the United States, most of the plants grown in agriculture have been bred to resist many known hazards. However, it is inevitable that destructive capabilities of pests and disease organisms will evolve. When such contingencies arise, plant breeders seek to incorporate germplasm having the necessary resistant characteristics. Preserving biodiversity of relevant plant species is in effect an inexpensive insurance policy to safeguard future low-cost supplies of food.

In this matter, the National Plant Germplasm System (NPGS) of the United States has an important role. It has responsibility for about 400,000 accessions of 8,700 species. Additional accessions are held by commercial breeders, universities, other organizations, and individuals. Most species are not originally native to the United States. The major crop plants were brought here by immigrants or plant collectors. Among those who brought seeds to the United States were Benjamin Franklin and Thomas Jefferson. Later, U.S. botanists roamed the world collecting many thousands of specimens. In recent decades much of the new germplasm has been acquired through exchanges with organizations or individuals elsewhere. Other countries, including Russia, China, and India, have substantial collections. However, the United States is reputed to have the most extensive one.

In the United States only a small fraction of the varieties of plants is grown intensively. Most of the germplasm is safeguarded in the special facilities of the NPGS. Under special conditions seeds can remain viable for many years. However, circumstances in many small tropical countries are such that heavy losses would occur rather quickly. Seeds must be dried to a moisture content of about 6% and then maintained in cold storage. A new facility located at Fort Collins, Colorado, will provide vaults cooled to liquid nitrogen temperatures. About 230,000 accessions are stored there. Special problems of germplasm preservation arise when seeds behave poorly after desiccation or when plants do not produce useful seeds. In any event, it is necessary from time to time to propagate the germplasm accessions. This activity is conducted at a substantial number of places, including Ames, Iowa; Geneva, New York; Griffin, Georgia; and Pullman, Washington. These four regional centers are responsible for management, regeneration, characterization, evaluation, and distribution of more than a third of the accessions of the national system.

Until the recent past, the primary motivation for collecting and maintaining germplasm was to ensure the self-sufficiency of U.S. agriculture. In effect, the effort has been broadened. Today, the United States is the world's largest distributor of plant germplasm. Each year, the NPGS supplies, free of charge, 230,000 samples from its collections to more than 100 nations. When seeds are involved, a sufficient number are provided to plant a row 15 meters long. The NPGS is rendering an important service both domestically and internationally. However, a recent report* of the Board of Agriculture of the National Research Council, which described NPGS in detail, also indicated that both the nation and the world could be even better served. The NPGS is a loose network of facilities that is administratively under the Agricultural Research Service of the Department of Agriculture. It is in effect an orphan. The NRC report recommended a substantial improvement in the status of NPGS and increases in its multifaceted efforts to manage some of the world's most precious genetic resources.

There is a basis for expectation that the situation may be improved. Under terms of congressional legislation (S2830-386), the structure and objectives of a National Genetic Resources Program have been outlined. The provisions of the act are broad and constructive in mandating research on genetic materials. The provisions also include, "Make available upon request without charge and without regard to the country from which the request originates the genetic material which the program originates." The next step in the process will be a report by the secretary of agriculture, due 1 November 1991, to Congress, followed by implementing appropriations.—PHILIP H. ABELSON

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Ex Situ Conservation of Plant Genetic Resources: Global Development and Environmental Concerns

JOEL I. COHEN,* J. TREVOR WILLIAMS, DONALD L. PLUCKNETT, HENRY SHANDS

Conservation of plant genetic resources is achieved by protection of populations in nature (in situ) or by preservation of samples in gene banks (ex situ). The latter are essential for users of germplasm who need ready access. Ex situ conservation also acts as a back-up for certain segments of diversity that might otherwise be lost in nature and in human-dominated ecosystems. The two methods are complementary, yet better understanding of this interrelation and the role of ex situ conservation in global environmental considerations is needed. Inclusion of ex situ conservation efforts within current environmental policies conserving global diversity would focus greater international attention on the safeguarding of these efforts.

CONSERVATION OF PLANT DIVERSITY CAN BE ACHIEVED IN A number of complementary ways: conservation of whole plants in their native ecosystems or conservation of samples of a plant's genetic diversity and of endangered species. Frequently, one method acts as a back-up to another, and the degree of emphasis placed on a particular method depends on a specific strategy developed to fulfill conservation aims and uses.

Donor agencies have increasingly incorporated environmental considerations in international development activities of the past decade. When these considerations include conservation, support is generally provided for protection of plants in situ because of the urgent need to protect ecosystems in face of imminent change.

Conservation of samples of plants away from their field habitats is considered to be ex situ. This has been most directly relevant to crop

J. I. Cohen is biotechnology and genetic resource specialist, Office of Agriculture, Agency for International Development, Washington, DC, 20523. J. T. Williams is director, International Program for Tropical Tree Crops, International Fund for Agricultural Research, Arlington, VA 22209. D. L. Plucknett is scientific adviser, Consultative Group on International Agricultural Research, World Bank, Washington, DC 20433. H. Shands is national program leader for germplasm, U.S. Department of Agriculture, Beltsville, MD 20705.

*To whom correspondence should be addressed.

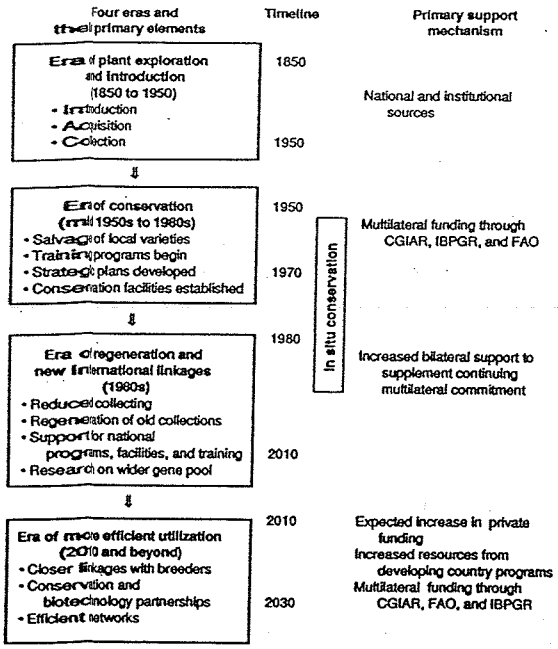

Fig. 1. Four eras of ex situ genetic resource conservation and use, with timeline of conservation events.

genetic resources because of utility for crop improvement. The methodology for crops developed over the past 25 years is now being applied to other sectors, such as forest reserves or endangered wild flora. We examine the status of ex situ conservation of useful plants and global efforts to conserve biological resources.

Development Assistance for in Situ and ex Situ Conservation

In recent years, support has increased more for in situ than for ex situ conservation, despite the complementarity of these methods (*1*). For example, material conserved ex situ is of great relevance to the rehabilitation of in situ sites, and for the provision of genetic materials for the management of areas protected in situ (*2*). However, competition has existed between the proponents of the two strategies in view of different intellectual perspectives and limited funds. For example, of the $37.5 million expended by the United States in 1987 for conservation of biodiversity, only a little over 1% was used for ex situ projects, excluding contributions to the international system of gene banks (*3*). The United Nations Environment Program (UNEP) has expressed concern about biodiversity—its committee developing an international convention for conservation of biodiversity has emphasized equality of in situ and ex situ approaches. It has requested support to establish centers for ex situ conservation, particularly to conserve samples for restoration of ecosystems (*4*).

Genetic resource conservation is a long-term activity with a large initial investment and continuing cost. Enhancement of agricultural production has received preferential support and, because of this and the few immediately tangible benefits (such as employment) of ex situ conservation, the latter has received lower priority (*5*).

Among international funding agencies and foundations, there is also a belief that the ex situ activities are being fully attended to elsewhere, through funding of commodity-based International Agricultural Research Centers (IARCs) of the Consultative Group for International Agricultural Research (CGIAR), the International Board for Plant Genetic Resources (IBPGR), and the Food and Agriculture Organization (FAO) of the United Nations, or by international coordinating mechanisms or through activities of national programs conducted by the U.S. Department of Agriculture (USDA). This erroneous perception lowers the priority of activities designed to ensure germplasm conservation and the need to consider ex situ conservation in national environmental policy.

The FAO has been instrumental in drawing global attention to the need for collection and conservation of crop and forest genetic resources. Its intergovernmental commission on plant genetic resources might help dispel misconceptions as its future reports providing global overviews and recommendations become more widely recognized (*6*).

Secure, long-term funding is rarely available for international programs because donors continually reassess priorities and redirect limited funds. Ex situ conservation programs coordinated through the CGIAR have a reasonable time horizon for funding because of the nature of the CGIAR's mandate and commitment from its member governments. Nonetheless, commodity-based CGIAR centers cannot carry the conservation responsibility alone, particularly for crops and plants that are outside center mandates and are responsibilities of national programs, many in the developing world (*7*).

In 1990, Congress asked the U.S. Agency for International Development (A.I.D.) to study the need for ex situ conservation of biological diversity and programs requiring support through A.I.D. assistance. Recommendations were developed, and congressional legislation authorized A.I.D. to initiate activities based on its report (*8*).

Four Eras of ex Situ Activities and Development Priorities

Practical action on ex situ genetic resource conservation and use can be divided into four major time eras (Fig. 1). These eras illustrate the evolution of ex situ conservation and apply to all organisms. In the first phase, utility is tested; in the second, a wide spectrum of genetic diversity is conserved because of its utility; in the third, long-term viability of the investment in collection is ensured; and in the fourth, there is enhanced exploitation, usually by breeding.

Era of plant exploration and introduction. Between 1850 and 1950, some of the most famous plant collectors traveled widely in search of useful and rare genetic resources to be collected and preserved in botanical gardens and germplasm collections. This era was dominated by plant collectors such as Frank Meyer, Wilson Popenoe, Nikolai Vavilov, and David Fairchild. It was a time of amassing collections by plant introduction, initiating quarantine systems, and studying plants taxonomically.

The remarkable work of early pioneer collectors and their contemporary successors has contributed to the central role that plant introduction plays in world agriculture. It has long been recognized that crop production in developed countries has depended on plant introduction. Even now, many developing countries, some undeniably rich in indigenous germplasm, are highly dependent on crops introduced from other nations: less than one-third of the crops produced in the developing countries of Africa and of the Americas are of local origin (*9*).

During this first era, three major components were recognized as essential to promote crop introduction: (i) access to an adequate germplasm base from national or international collections, or direct access by collection; (ii) national quarantine systems, including post-entry inspection, seed health testing, and facilities for growing-out samples; and (iii) effective national breeding and plant protection agencies working closely with conserved diversity in collections.

Era of conservation. During the 1960s, the Green Revolution became a recognized force in improving cereal yields and increasing production to keep food supplies ahead of population pressures. This was also the start of increased efforts to collect local varieties threatened to be displaced by the widespread adoption of new high-yielding varieties.

This new urgency in collecting responded to the needs of the decade and led to the development of ex situ conservation facilities, including base and medium-term storage facilities for seeds. In the United States, regional plant introduction stations established medium-term storage facilities in the late 1940s. Long-term seed storage (at −18°C) at the National Seed Storage Laboratory (NSSL) in Fort Collins, Colorado, did not occur until 1978. In 1974, IBPGR was established and given the responsibility of developing a world plant germplasm network with emphasis on food crops (and later adding forages). IBPGR assumed a central role in stimulating field collection and helping to establish effective operation of germplasm storage facilities internationally (*10*). More recently, global interest in conservation has been stimulated by the Botanic Gardens Conservation Secretariat and the Center for Plant Conservation, both with emphasis on endangered and threatened plants.

Much of the progress on crops during this conservation era came from support provided to the commodity-based centers of the CGIAR, which developed important global collections of major crop gene pools. These collections are generally competently handled and accessions are usually attainable, although inventory problems have led to some concern.

Crops for which the private sector traditionally plays an important role in collection, conservation, and use present special problems and concerns. Examples are tropical plantation or industrial crops such as sugarcane, rubber, oil palm, pineapple, and some pharmaceuticals. Examining how these industries support ex situ conservation could provide models for conservation of neglected crops, including medicinal plants and spices.

Era of regeneration and new international linkages. Genetic resources in storage requiring regeneration are sometimes best returned to original areas of collection for multiplication. Gene banks may need to develop international agreements to properly regenerate their materials and to minimize genetic drift. Such arrangements would enhance efforts to increase seed, ensure high quality, and aid evaluation.

International cooperation is the key to successful and comprehensive regeneration programs. Donors can work closely with national programs to initiate cooperative efforts. This era represents the juncture where bilateral assistance joins the progression of ongoing conservation activities (Fig. 1). It is the time to strengthen national programs so they can work more closely with global collections housed by IARCs, formalize local linkages with programs geared to wild flora, and modernize gene banks where state or local collections have been consolidated.

Era of more efficient use. It is recognized that some degree of use of collections has occurred in all three of the previous eras. However, utilization has been hampered by a number of factors, such as samples that are poorly characterized, samples with low or no viability, or samples too small to allow evaluation and distribution. If regeneration is the most important challenge in strengthening ex situ collections, efficient and wider use of accessions is the challenge of the early 21st century.

For conserved germplasm collections to be "user friendly," essential descriptive and screening work must be done and linked to "prebreeding" for crops. Also critical is linking use to improvement through biotechnology and conventional breeding research (*11*).

Recommendations for Action

Strategic planning for ex situ conservation. National strategic plans must take into consideration all aspects of biodiversity conservation; yet ex situ conservation, a particular need in the agricultural context, generally has not been included in biodiversity assessments. Because of the urgent need to apply technologies for ex situ conservation and regeneration, development assistance projects would benefit from ex situ planning included in national conservation and environmental strategies.

Ex situ programs require a clear policy framework and estimates of necessary development assistance. To obtain such assistance, development agencies must be informed of the following: (i) the central roles that ex situ collections of genetic diversity will have in broader environmental and conservation concerns, (ii) the need for national policies and programs for ex situ conservation in developing countries, and (iii) the total financial, physical, and biological resources required to ensure sustainable use of global biodiversity in world agriculture and for other purposes. IBPGR has a major coordinating role to play in this regard.

Where should official responsibility for conservation of diversity be placed at the national level? Frequently, ministries of environ-

Table 1. Comparison of the current status of ex situ conservation programs of crop plants in developing countries, the United States, and the IARCs.

Characteristics	Developing countries	U.S.	IARCs
Major regions of diversity represented within boundaries	Many	Limited	Some
Participation in international germplasm exchange	Limited*	Yes	Yes
Ex situ conservation policies developed	Very few	Yes	Usually
Fully functioning germplasm system	Limited	Yes	Usually
Long-term financial support	Very limited	Yes	Yes
Exploration interests	Yes	Yes	Yes
Long-term storage facilities	Limited	Yes	Yes
Regeneration capabilities	Limited†	Some§	Some‡
Regeneration sites	Some‡	Some‡	Some§
Data management expertise	Limited	Yes	Yes
Distribution capabilities	Limited	Yes	Yes
Able to train scientists	Limited	Limited§	Yes
Able to provide technical assistance	No‖	Limited§	Yes
Hold global base collections	Some	Yes	Yes
Operative germplasm quarantine facilities	Limited	Yes	Limited arrangements

*Often conditioned by national policies and by number of accessions with adequate material for distribution (see Tables 2 and 3). †Limitations imposed by lack of resources, coordinating mechanisms, and availability of appropriate sites. ‡No one genetic resources program has local access to a complete range of sites for regeneration. §Limited by availability of personnel and by conflicting responsibilities. ‖Except in very few cases.

ment, tourism, agriculture, forestry, and fisheries all may have programs related to genetic resource conservation. Such fragmentation causes problems, especially when linkages are difficult. This is often true for development assistance and explains the perception that there is little coordination, especially at global levels.

Better partnerships: Supporting priority ex situ programs. Partnerships between national and international programs would enhance the capacity of these programs to conserve and regenerate vulnerable ex situ collections. These partnerships are needed now if loss of diversity from existing national collections is to be avoided. Financial support from the United States would help to distribute the burden placed on the world community to conserve diversity and to ensure preservation of germplasm collections.

Inefficient conservation programs, including national programs that are not fully functional and that lack financial resources to preserve adequately genetic material, place a heavy burden on

Table 2. Percentage of accessions from crop germplasm collections available for distribution following regeneration. Values shown are numbers (of accessions or locations) and percents of totals; N/A, not available.

Center*	Crop	Accessions	Accessions able to regenerate per year	Accessions sufficient for distribution	Locations available for regeneration
AVRDC	Mungbean	5,273	1,000	3,200	2–5
	Pepper	3,471	250	1,000	2–5
	Soybean	12,303	1,000	8,000	2–5
	Tomato	3,814	350	3,000	2–5
	Total	24,861		15,200 (61%)	
CIAT	Phaseolus (bean)	25,000	1,800	20,000	3
	Tropical pastures	21,000	1,800	14,000	3
	Cassava	4,500	In vitro	In vitro	
	Total	50,500		34,000 (74%)	
CIMMYT	Barley	7,200	2,000	6,480	3
	Bread wheat	48,600	8,000	43,740	3–7
	Durum wheat	15,300	3,000	13,770	3–7
	Primitive wheat	4,320	4,000	3,888	3
	Triticale	11,700	3,000	10,530	3
	Wild relatives	2,700	500	2,430	3
	Total	89,820		80,838 (90%)	
	Maize	13,346	250	10,910	4–8
	Teosinte	93	In situ	N/A	
	Tripsacum	90	Clonal	N/A	1
	Total	13,529		10,910 (81%)	
CIP	Potato (clonal, in vitro, seed)	4,500	3,500 clones 300 seeds	3,500 seeds 650 clones	4–9
	Sweet potato (clonal, in vitro, seed)	5,507	2,000 clones 300 seeds	50 clones	1
	Total	10,007		4,200 (40%)	
ICARDA	Food legumes	17,900	2,000	10,740	1
	Total cereals	43,700	3,000	26,220	1
	Forage legumes	22,000	2,000	13,200	1
	Total	83,600		50,160 (60%)	
ICRISAT	Groundnut	12,841	1,500	12,500	10–15
	Pearl millet	21,919	2,000	21,800	10–15
	Pigeonpea	11,482	1,300	11,300	10–15
	Other millets	7,082	1,000	7,000	10–15
	Sorghum	32,890	2,600	32,600	10–15
	Chickpea	15,995	1,400	15,600	
	Total	102,209		100,800 (99%)	
IITA	Musa	412	412	412 (100%)	2–7
	Oryza species	12,500	4,500	9,000	3
	Vigna unguiculata	15,200	4,000	9,439	3
	Wild vigna	1,450	1,000	1,000	
	Total	29,562		19,851 (67%)	
IRRI	Rice	86,000	10,000	77,400 (90%)†	2
WARDA	Rice	5,430	1,000	3,000 (55%)	3–16

*AVRDC, Asian Vegetable Research and Development Center; CIAT, Centro International de Agricultura Tropical; CIP, International Potato Center; ICARDA, International Center for Agricultural Research in the Dry Areas; ICRISAT, International Crops Research Institute for the Semi-Arid Tropics; IITA, International Institute of Tropical Agriculture; and WARDA, West African Rice Development Association. †Of its total 86,000 accessions, IRRI has successfully canned 43,500 accessions for medium and long-term storage.

functioning programs. Improving the conservation capability of other national programs is important to the United States and to all national partners because of the global interdependence on germplasm (*5*) and the recognition that no single national system can ensure conservation of all plant diversity (*12*).

A.I.D. has initiated bilateral projects that support contributions of financial and technical resources from the United States to strengthen the capacity of national programs to collect, evaluate, conserve, and internationally exchange plant germplasm (*5, 13*). This promotes development of a more effective interface between the conservation of a developing nation's botanical resources and the use of these resources in crop improvement.

A comparison of ex situ collections in developing countries, in the United States, and in the IARCs identifies deficiencies that bilateral development and funding could rapidly improve (Table 1). Collaboration between programs could enable national ex situ programs in developing countries to join the global network of base collections (*14*) and could reverse the trend of unnecessarily large numbers of accessions being stored in developed countries.

Scientists involved with plant introduction and exchange interests will continue to be key stakeholders in ex situ conservation. Even though the golden era of exploration and introduction for most crop plants has passed, effective programs are needed for special plant and tree species and for new and novel crops. New germplasm must be introduced, preserved, and used so national programs can fulfill their role within countries they serve.

Community seed banks and international collections. The preservation of diversity, particularly of endangered plants and of traditional and heirloom varieties, is no longer solely the domain of large national and international programs. In recent years, seed saving exchanges and other nongovernmental organizations (NGOs), such as community and regional seed banks, have emerged to support and expand local efforts in global biodiversity conservation, in many cases linking traditional farmers with ex situ conservation programs. The motivations and tactics of grass-roots groups are different from those of more formal genetic conservation programs, yet they are an important complement to conserving genetic resources. For example, Seed Savers Exchange conserves and exchanges over 5000 native varieties (*15*). External evaluation, resulting in a determination that such programs do complement those of national strategic plans, may be needed before development assistance is provided to these newer efforts.

Expanding resources for improved management of collections. A review by the U.S. National Research Council (NRC) on the management of the U.S. National Plant Germplasm System (NPGS) stresses multiplication, regeneration, evaluation, characterization, documentation, and distribution (*16*). Of particular relevance to developing countries, and essential for the future well-being of samples (*17*), are improved storage facilities, expanded regeneration, and characterization. Samples conserved must represent a wide spectrum of population diversity; regeneration protocols must consider the type of material being regenerated to ensure sample integrity; and, for safety, samples must be duplicated at different sites while information and data are maintained. These practices must be implemented as older collections are transferred into modern conservation storage.

Regeneration protocols are only now being properly addressed or are yet to be implemented. Without increased funding for regeneration and preservation of collections, there will be no security, and some germplasm repositories may become germplasm morgues (*17*). Even in the United States, national germplasm banks have been built without adequate regeneration or evaluation funds (*16*).

Regeneration capacities, including the number of sites available for regeneration, of the CGIAR centers (Table 2) and the NPGS (Table 3) have been documented. The efforts made to ensure long-term viability illustrate a shift in emphasis to conservation coupled with active regeneration, especially since the 1980s (Fig. 1).

Table 3. Percentage of accessions from NSSL and regional plant introduction and conservation facilities available for distribution after regeneration. Values shown are numbers (of accessions or locations) and percents of totals.

Center	Crop: Facility*	Accessions	Accessions able to regenerate per year	Accessions sufficient for distribution	Locations available for regeneration
NPGS	Barley: NSSL†	721		436	
	NSGC	26,168	5,000	22,175	2
	Maize: NSSL	21,671		1,865	
	NCRPIS	8,783	450	7,000	2–7
	Peanut: NSSL	121		29	
	SRPIS	8,165	1,100	2,000	1–4
	Bean: NSSL	6,427		1,065	
	WRPIS	11,030	600	10,220	2–4
	Potato: NSSL	3,262		86	
	IR1	4,272	300	4,000	1
	Rice: NSSL	942		205	
	NSGC	16,010	5,000	13,899	1
	Sorghum: NSSL	15,043		8,797	
	SRPIS	17,604	2,000	15,600	4
	Tomato: NSSL	1,984		1,202	
	NERPIS	5,615	300	5,500	1
	Cowpea: NSSL	279		255	
	SRPIS	8,133	1,500	5,500	2
	Wheat: NSSL	1,597		619	
	NSGC	42,478	5,000	36,932	2
	Totals				
	NSSL	52,047		14,559 (28%)	
	Regional	148,258	21,250	122,826 (83%)	

*Acronyms for regional plant introduction and collection facilities are as follows: NSGC, National Small Grains Collection; NERPIS, Northeast Regional Plant Introduction Station; WRPIS, Western Regional Plant Introduction Station; NCRFPIS, North Central Regional Plant Introduction Station; IR1, Inter-Regional Potato Introduction Project; and SRPIS, Southern Regional Plant Introduction Station. †Values for NSSL indicate number of unique accessions not yet in the regional plant introduction facilities.

Among the IARCs, the percentage of accessions with sufficient material for distribution varies from 40 to 99% (Table 2). Of the crops sampled, the NPGS has 83% of total accessions held at regional introduction and conservation centers available for distribution and 28% at the NSSL that contain sufficient seed for distribution to other collections (Table 3). Much work has been accomplished, but many accessions need regeneration with greater attention placed on patterns of diversity encompassed by collections, especially heterozygous primitive material (*18*).

Human resource development. Major gaps exist worldwide to provide relevant training in ex situ conservation of plant genetic resources. For the NPGS to have a major role in specialized training, it must develop a significant international extension to its existing domestic mandate, as is consistent with NRC recommendations (*16*). An "International Coordinator" could be supported within the NPGS to facilitate bilateral training and research efforts and to overcome problems encountered, because there is no single NPGS location that provides all facilities, relevant specialists, and research needed to reinforce training (*8*).

More assistance should be provided for training and internships at universities and internationally recognized centers for germplasm conservation. Training should be available for scientists from developing countries and for staff from development assistance and conservation agencies. For example, the International Rice Research Institute (IRRI) has held three training courses, two of which were 12 months long and included practical, hands-on training plus studies on basic conservation principles. IBPGR, in conjunction with the University of Birmingham, sponsors an intensive 12-month course that has awarded more than 196 M.S. degrees (*7, 19*).

New international opportunities for coordination. A "World Strategy for Conserving Biodiversity" is being developed in a coordinated effort headed by three groups: International Union for the Conservation of Nature and Natural Resources, UNEP, and World Resources Institute (*20*). Program preparation is taking place for release of the strategy in 1992, in consultation with other international agencies. This coincides with the United Nations Conference on Environment and Development, which will also examine means to conserve global biological diversity (*21*). Coordinated efforts such as offer new means to inject concerns associated with ex situ preservation of diversity within a broader context and it is an opportunity to add conservation plans for germplasm of economic significance to the rationale for saving natural habitats and to ensure that development assistance can be carried out in accord with world environmental strategies.

Exploring Available Funding Options

Funding mechanisms for support of priority ex situ conservation initiatives should include "debt-for-development" swaps, expanded bilateral initiatives, and new joint efforts with the private sector that could enhance funding available to gene banks.

Funding through debt-for-development swaps would enhance ex situ programs while refinancing the national debt of developing countries. This procedure takes advantage of the discount at which debt sells in secondary markets. In the case of U.S. federal agency funding, swaps are accomplished through an NGO as the recipient of purchasing funds, then an appropriate exchange of funds with a host-country institution. Ex situ conservation presents NGOs with new challenges and opportunities for partnerships with donor agencies.

Bilateral assistance, based on prior recommendations and authorized legislation, will begin new initiatives. First, A.I.D. plans to support international cooperation to prevent further loss of endangered genetic resources of maize. This project will include 13 Latin American national programs, Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), and NPGS, and it will systematically regenerate the Latin American and Caribbean accessions of maize. Second, a comprehensive international training institute for conservation of plants and animals is being developed by the University of California, Davis. This course will include 30 participants from developing countries and approximately 60 from the United States.

Finally, some new opportunities for funding through the private sector may be derived from cooperative evaluation efforts based on promising accessions that have already been identified. Partnerships could include pharmaceutical, biotechnological, or agricultural companies that are prepared to make equitable legal arrangements to balance the control, movement, rights, and access to genetic resources requested. Arrangements between the private sector and the National Institutes of Health use material transfer agreements to control distribution of incoming and outgoing materials and Cooperative Research and Development Agreements to allow funding of complementary research (*22*).

Donor Concern Regarding ex Situ Conservation Options

There are several concerns that development agencies may raise before providing funds to national genetic resources programs. First, they must be convinced that the proposed activity does not duplicate germplasm efforts being conducted elsewhere. Second, the degree to which a proposed activity complements or enhances existing agricultural research and development activities in the host country will have to be considered. Development programs have only recently considered projects for the creation and management of national genetic resources programs, but indefinite support is not feasible.

The connection between projected loss of genetic resources and subsequent negative effects on national development is not well understood (*23*). Wider understanding by funding agencies will help efforts to integrate conservation and use of genetic resources of economic importance and will demonstrate conservation to be consistent with development initiatives by identifying enhanced economic benefits derived from the maintenance of biodiversity (*24*).

Finally, use of conserved germplasm in plant improvement programs must be better documented by organizations to produce clear data justifying the ex situ efforts needed. Some data are available (*25*), but, if ex situ preservation of diversity is to derive much greater and needed support, then use of collections through breeding and biotechnology will be a primary concern among development practitioners (*26*), along with the understanding that new technologies will be hampered without well-maintained germplasm collections.

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Research Articles

Atomic Structure of Acetylcholinesterase from *Torpedo californica:* A Prototypic Acetylcholine-Binding Protein

JOEL L. SUSSMAN,* MICHAL HAREL, FELIX FROLOW, CHRISTIAN OEFNER,† ADRIAN GOLDMAN,‡ LILLY TOKER, ISRAEL SILMAN*

The three-dimensional structure of acetylcholinesterase from *Torpedo californica* electric organ has been determined by x-ray analysis to 2.8 angstrom resolution. The form crystallized is the glycolipid-anchored homodimer that was purified subsequent to solubilization with a bacterial phosphatidylinositol-specific phospholipase C. The enzyme monomer is an α/β protein that contains 537 amino acids. It consists of a 12-stranded mixed β sheet surrounded by 14 α helices and bears a striking resemblance to several hydrolase structures including dienelactone hydrolase, serine carboxypeptidase-II, three neutral lipases, and haloalkane dehalogenase. The active site is unusual because it contains Glu, not Asp, in the Ser-His-acid catalytic triad and because the relation of the triad to the rest of the protein approximates a mirror image of that seen in the serine proteases. Furthermore, the active site lies near the bottom of a deep and narrow gorge that reaches halfway into the protein. Modeling of acetylcholine binding to the enzyme suggests that the quaternary ammonium ion is bound not to a negatively charged "anionic" site, but rather to some of the 14 aromatic residues that line the gorge.

THE PRINCIPAL BIOLOGICAL ROLE OF ACETYLCHOLINESTERASE (AChE, acetylcholine hydrolase, E.C. 3.1.1.7) is termination of impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine (ACh) (*1*).

$$CH_3COOCH_2CH_2N^+(CH_3)_3 + AChE \rightarrow CH_3CO\text{–}AChE$$
$$+ HOCH_2CH_2N^+(CH_3)_3 \rightarrow CH_3COO^- + H^+ + AChE$$

In keeping with this requirement, AChE has a remarkably high specific activity, especially for a serine hydrolase [for a review, see (*2*)], and functions at a rate approaching that of a diffusion-controlled reaction (*3*). The powerful acute toxicity of organophosphorus poisons (as well as of carbamates and sulfonyl halides, which function analogously) is primarily because they are potent inhibitors of AChE (*4*). They inhibit AChE by forming a covalent bond to a Ser residue in the active site (*2*). AChE inhibitors are used in treatment of various disorders such as myasthenia gravis and glaucoma (*5*), and their use has been proposed as a possible therapeutic approach in the management of Alzheimer's disease (*6*). Knowledge of the three-dimensional (3-D) structure of AChE is therefore

J. L. Sussmann, M. Harel, F. Frolow, C. Oefner, and A. Goldman are in the Department of Structural Chemistry and L. Toker and I. Silman are in the Department of Neurobiology at The Weizmann Institute of Science, Rehovot 76100, Israel.

*To whom correspondence should be addressed.
†Visiting scientist at the Weizmann Institute of Science. Permanent address: F. Hoffmann–La Roche Ltd., Central Research Unit, CH-4002 Basel, Switzerland.
‡Visiting scientist at the Weizmann Institute of Science. Permanent address: Waksman Institute, Rutgers University, New Brunswick, NJ 08855.

Keystone International Dialogue Series on Plant Genetic Resources

OSLO PLENARY SESSION

FINAL CONSENSUS REPORT: GLOBAL INITIATIVE FOR THE SECURITY AND SUSTAINABLE USE OF PLANT GENETIC RESOURCES

Third Plenary Session
31 May - 4 June, 1991
Oslo, Norway

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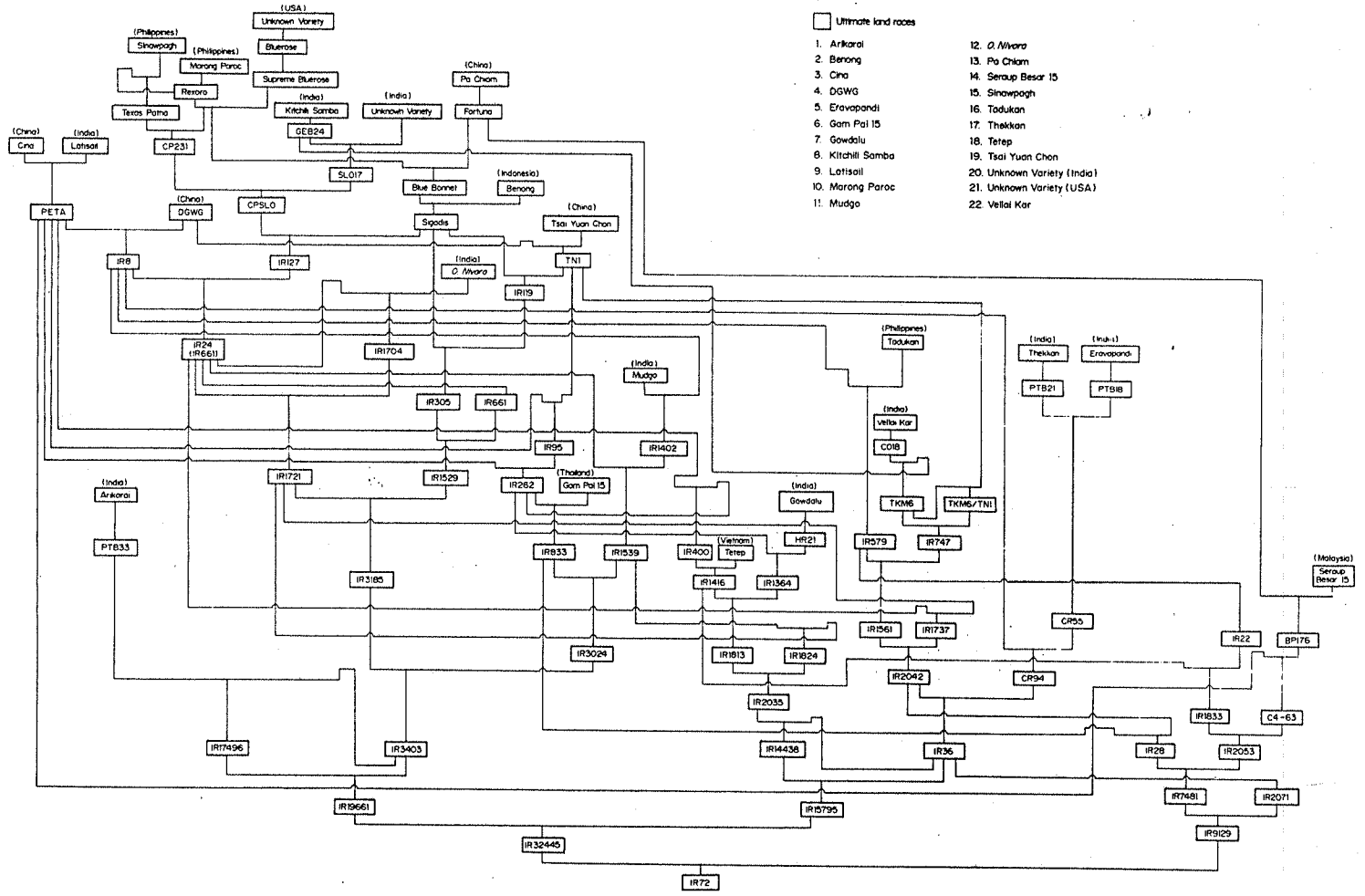
The Keystone Center, founded in 1975, is located in the Colorado Rocky Mountains, USA. The Center is a non-profit organization comprised of three divisions: 1) the Science and Public Policy Program, which facilitates the resolution of public policy conflicts through the use of a consensus dialogue approach; 2) the Science School, which provides residential natural science education programs for students of all ages; and 3) the Symposia on Molecular and Cellular Biology, which offers an annual series of colloquies addressing critical developments in science and research.

Pedigree of IR-72



Dialogue participants believe that the pedigree for IR-72 symbolizes the interdependent nature and importance of the conservation and utilization of the world's plant genetic resources. The pedigree of this modern variety of rice demonstrates the critical role that landraces from all over the world play in contributing to the range of genetic characteristics necessary to meet the world's future food requirements. As indicated in the chart above, twenty-two landraces contributed to the development of IR-72.

EXECUTIVE SUMMARY

Overview

In the third and final report of The Keystone International Dialogue Series on Plant Genetic Resources, the participants from the Oslo plenary session unanimously agreed on the need to alert the international community to the threat of genetic erosion. If the loss of plant genetic resources (PGR) continues unabated at the present rate, genetic options for needed changes in agricultural production in the future will be lost forever.

The Dialogue participants firmly believe that the current situation calls for a **Global Initiative for the Security and Sustainable Use of Plant Genetic Resources.** To be successful, the Global Initiative set forth in the report will require the joint efforts and involvement of all affected parties and institutions from all levels and from all parts of the world—including those who are contributors of germplasm, information, technology, funds, and systems of innovation.

The report represents a consensus of 41 individuals with a diversity of backgrounds and interests from 22 countries. The group was able to reach consensus on the content of the report, which is a summary of their discussions, findings and recommendations. The participants of the Oslo session, noting the urgency of the situation, stated affirmatively their commitment to take immediate and sustained action to make the Global PGR Initiative a reality.

The Dialogue group strongly recommends that the upcoming United Nations Conference on Environment and Development (UNCED 1992) consider plant genetic resources conservation as an important part of overall biological diversity conservation and as an integral component of "Agenda 21."

Gaps and Needs

Plant genetic resources provide the basic raw materials to adapt crops to: expanding biotic and abiotic stresses; changing consumer preferences; and possible changes in the environment, as may occur through global warming, rising sea levels, and depletion of the ozone layer. Crops will have to be adapted to sustainable forms of agriculture while maintaining increased productivity to feed a still growing world population.

The basic elements of an institutional framework for PGR conservation are in place at community, national, regional, and international levels. However, the system remains largely inadequate to provide the needed security of plant genetic resources due to a serious lack of funds and the need for improved institutional structures and implementation mechanisms at all levels.

The report identifies gaps in activity areas that must be addressed if the system is to cope with the urgency of the situation. Specifically, it will be necessary to improve and expand the current system in many areas, including:

- *ex situ* conservation, including collecting, storage and regeneration, documentation and information systems, germplasm evaluation and enhancement, and exchange;
- on-farm community conservation and utilization;
- *in situ* conservation;
- monitoring and early warning of genetic erosion in specific locations;
- development of techniques for sustainable advances in agricultural productivity; and
- research, training, and public education.

In all of these areas there is a necessity to enhance the linkages between the formal and informal sectors at the community, national, regional, and global levels.

Magnitude of the Funding Requirements to Meet the Needs

The centres of diversity of most crops important to world agriculture are located in the less developed regions of the tropics and the subtropics. Unfortunately, many of the countries in these areas cannot, by themselves, adequately meet the cost of conservation. Hence, preventing genetic erosion is an urgent international task that requires a sustained international commitment.

The Global PGR Initiative recommended in the Oslo Report will require substantial *additional* funding once the institutional framework discussed below is fully operational. It is estimated that the resources that will be required to strengthen existing programmes and institutions in the manner outlined above and described more fully in the report are on the order of US $300 million per annum on a sustainable basis. During the period of 1993-2000, which coincides with UNCED's "Agenda 21," it is estimated that US $1.5 billion will be required.

Ownership and Intellectual Property Rights

In addition to the gaps and needs associated directly with PGR conservation and utilization, the Dialogue group addressed issues related to ownership and intellectual property right (IPR) systems. The issue of IPR for plant genetic resources has fallen within the scope of wider discussions on IPR in the General Agreement on Trade and Tariffs (GATT) negotiations. If the GATT negotiations result in the strengthening of IPR within developing countries, this, in turn, might result in both the adoption of plant variety protection systems and the patenting of plants, animals, and the genetic materials that are contained in them. In previous Dialogue reports, the Dialogue group expressed strong concern about the imposition of IPR for plant genetic materials through the GATT or bilateral trade negotiations. Every country has the right to decide whether and to what extent they adopt IPR for PGR. No country should be pressed to do so. To date, the issue has received little attention and discussion by the GATT negotiators. The Dialogue group strongly recommends that the implications of IPR for PGR (as discussed in the Oslo Report) be given adequate discussion and evaluation by the negotiators, with input from national experts and other entities involved with PGR, before any GATT action is taken.

The group agreed that the impact of intellectual property rights on plant genetic resources must be reviewed locally before IPR is extended to plant genetic resources. Although IPR may have important value to stimulate innovation in certain market conditions, when applied to PGR it could have a negative impact on the farmer-breeders who still actively maintain important genetic diversity as part of their traditional activities. Developing countries choosing to implement a Plant Breeder's Rights (PBR) system should retain provisions allowing Farmer Plantback of protected varieties. This is especially important in developing countries where farmers cannot afford to buy seed every year or are not consistently reached by a seed distribution infrastructure and must therefore rely on seed saved from the previous season.

Institutional Structures and Implementation Mechanisms

In order to effectively implement the Global PGR Initiative it will be necessary to utilize, build upon, and improve existing institutional structures at the community, national, regional, and global levels. For certain critical components of this Initiative, especially the creation and maintenance of *ex situ* gene banks, there should be an especially heavy reliance on national level institutional structures and implementation mechanisms. The precise nature of these structures will undoubtedly vary. There is also a critical need for more effective linkages between the formal and informal systems at all levels.

The Dialogue group reached consensus regarding the need for a global mechanism designed to promote political and policy oversight, mobilization and distribution of funds, and implementation of well-defined tasks that fulfills the following basic criteria:

- It should have the confidence of all countries which are important repositories of PGR;

- It should inspire support from contributors of germplasm, information, funds, technology, and systems of innovation; and
- It should be capable of ensuring effective, economical, and timely implementation of approved programmes.

To achieve the above tasks the group believes that four major instruments will be needed. These are:

1. An **Intergovernmental Council** (IGC), based on the principle of one country, one vote, to discuss and decide on policies and priorities, and approve a biannual plan of action, programme of work, and budget. The IGC will include **Associate Members** to ensure inputs from the broader PGR community;
2. An **Executive Board** (EB) of the IGC that is authorized to take action on the implementation of the priorities specified in the approved plan of action;
3. A **Scientific and Technical Advisory Committee** (STAC) of independent professionals to provide the necessary scientific and technical advice and support to both IGC and its EB; and
4. A **PGR Trust Fund** operated as a special trust fund through a designated fiduciary agency.

An appropriate location that allows operational autonomy of the IGC/EB/STAC organizational structure will be essential to ensure the successful implementation of the Global PGR Initiative. With this and other criteria in mind, the Dialogue group concluded that at present there does not exist any ideal organization that completely fulfills all of the identified criteria. Several options are possible: FAO, due to its longstanding experience in this field and its existing intergovernmental Commission on PGR; an intergovernmental body that may emerge from the Biological Diversity Convention negotiations; an intergovernmental body that may emerge from the UNCED process; and the Consultative Group on International Agricultural Research (CGIAR), if it is able, as with the other options, to develop a policy-making structure along the lines of the Intergovernmental Council, Executive Board, and Science and Technology Advisory Committee which are described in more detail in the report.

Call for Immediate Action

The new Global PGR Initiative is designed to create the basis for a general cooperative venture based on mutual benefit. The Global PGR Initiative, taken as a whole, will inevitably create a new environment of trust and exchange. The purpose of the Global PGR Initiative is to act now to ensure conservation and use forever.

It is acknowledged that it may take some time for the PGR Initiative to become operational. Meanwhile, the tasks needing attention and financial support are urgent. These include: immediate assistance to existing gene banks located in Eastern Europe and Ethiopia; training of gene bank managers; infrastructure development for PGR conservation in developing countries which are centres of genetic diversity; and the development of a broader public awareness for PGR conservation. Given the immediate needs outlined above, the Dialogue group recommends that a suitable project proposal be prepared and submitted for consideration for support under the recently created Global Environment Facility (GEF) in view of the high priority accorded by GEF to the protection of biological diversity. The GEF is under the joint co-sponsorship of the International Bank for Reconstruction and Development (IBRD/World Bank), United Nations Environment Programme (UNEP), and the United Nations Development Programme (UNDP).

Every day's delay in pursuing this programme of action may result in a considerable loss of genetic variability in plants of current and potential use.

as a means of improving PGR conservation, utilization, and innovation. It is therefore desirable also to consider policy mechanisms which would provide incentives to informal innovation. In essence, these would encourage and/or reward farmers for their efforts to maintain and enhance crop diversity, and to develop varieties with better quality, improved yield, stress and disease resistance, and more efficient utilization of inputs.

As discussed elsewhere in this document, there is a great need for increased agronomic and economic research at the small farm level. There is a need to identify and describe constraints within the informal sector to enable better targeting of incentives. Research is needed to help solve technical constraints faced by informal innovation. One simple but potentially effective approach is the idea of a "Conservation Corps" which could include the provision of fellowships for young researchers interested in working at the farm level and training workshops for farmers interested in plant breeding, variety testing, and selection.

Improved market opportunities for a wider variety of produce and for better quality products also will serve as a direct reward to farmers for improvement of a diversity of varieties. Here again there is a role for different approaches such as improvements to market infrastructure (e.g., transportation of goods to markets, removing legal restrictions on local markets), information campaigns, varietal advertising, agricultural fairs to familiarize consumers, and market research.

Farmer cooperatives can support PGR work at the local level through the provision of germplasm, appropriate technologies, and training.

Unresolved Issues

The Dialogue group wishes to confirm its belief that the plant genetic resources community should continue to strive for greater transparency, mutual confidence and trust throughout the system, leading to a situation of increasing full access to plant genetic resources.

The following two concepts were received positively and with interest in holding further discussions from participants from industry and NGO backgrounds. Those participants more directly engaged in gene bank operations were less interested in pursuing discussions in these areas.

In the course of discussing the positive or negative impact of formal plant breeding in developing countries, participants saw value in encouraging an ongoing dialogue among various interest groups on the wider implications of the introduction of plant genetic resources in the form of advanced varieties. Such discussions could bring still greater trust and transparency to the community and allow the world to ensure the greatest benefit from PGR introductions.

The Keystone Dialogue considered ways to recognize the contribution made by all countries through their accessions now held in gene banks worldwide. For example, it was suggested that in the long-term every gene bank in the international system might provide each country contributing germplasm with an inventory of the accessions known to have originated in the contributing country. A revised inventory could be prepared every five years or at the time new collections are added. Despite general agreement on the desirability of making such information available, this would require a much better funded, more efficient, and better functioning series of PGR data banks than presently exist.

A further idea would incorporate licensing agreements giving more favorable access to commercialized materials derived from gene bank accessions to those who contributed the germplasm. Although such a scheme would recognize and thus encourage more active participation by "gene-rich" countries, it would impose considerable burdens on the gene banks and raise many difficulties for implementation.

There were several broadly shared and serious reservations about the desirability of trying to implement such proposals.

An Analysis of Gaps and Needs

A global system for the security of the world's PGR is developing, but the existing institutional capacity, structures, and programmes are generally inadequate and underfunded at all levels. An effective and responsive system

must be sustainable and ensure conservation and availability of PGR to meet future global needs for formal and informal systems of PGR conservation and utilization. The Dialogue group identified current gaps and weaknesses which must be remedied if the system is to cope with the urgency of the situation.

Conservation Strategies

PGR conservation efforts can be carried out in various ways: (a) *in situ* conservation in natural or original habitat; (b) *ex situ* conservation in gene banks as seed, tissue, or pollen, in field gene banks, or in other live collections; and (c) on-farm/community conservation.

In situ conservation. *In situ* conservation of plant genetic resources depends on eco-geographic surveys to determine the amount of diversity present, its current status, and established measures to ensure future assessments.

In situ conservation should be seen as complementary to *ex situ* conservation. This is very often not recognized.

Ex situ conservation. *Ex situ* conservation involves collecting, storage, and regeneration, documentation and information systems, evaluation, enhancement, and exchange.

•*Collecting*

The inadequacy of most current collections is widely recognized. Even in major crops, there are important areas of diversity that remain to be sampled, and some areas where past sampling was inadequate or faulty may need to be revisited.

Minor crops have been neglected and are in serious need of further collection.

• *Storage and Regeneration*

Many existing facilities lack modern storage. Even some global base collections are stored under inadequate or insecure conditions.

Much of the genetic erosion taking place today occurs inside gene banks because of lack of regeneration, poor storage or handling conditions, inadequate funding to ensure proper operation, lack of trained personnel, or managerial inefficiency. Additionally, wars and civil unrest threaten the security of collections.

Field gene banks, often used for perennial species (fruits, cash and plantation crops, and forestry) require large areas and are expensive to operate and difficult to manage because of demanding maintenance requirements. Many tropical crops cannot be stored in regular gene banks and therefore must rely on field gene banks or *in vitro* conservation.

To maintain the genetic integrity of samples and to ensure the security of existing collections, more funding for long-term storage and effective regeneration are urgently required.

• *Documentation and Information Systems*

Much material currently in *ex situ* conservation lacks documentation. No national or regional programme has fully documented all of its material.

Without documentation, much of this material will never be used. Valuable characteristics preserved in gene banks may be unknown to potential users.

Information systems at both national and international levels are currently inadequate and many fail to take full advantage of the opportunities offered by modern computer technology. Information about accessions gathered from farmers and based on their practical experience is often lacking in collections. Considerable data stored in numerous locations but not computerized are essentially unavailable to researchers and gene bank curators.

• *Evaluation and Enhancement*

Many programmes lack the ability or resources to evaluate or enhance the materials they are conserving. This is particularly true with minor crops. The result is incomplete usage of valuable genetic materials.

Evaluation should be carried out in suitable environments where more traits can be assessed. To improve their effectiveness and to improve utilization of PGR, many gene banks may need to take on increased responsibility for PGR enhancement.

• *Exchange*

In some cases the exchange of plant genetic resources remains problematic. Exchange can be hampered by a lack of capacity or resources of gene banks to answer all requests for material. Sometimes government decisions may hamper exchange. The current system cannot solve these problems without addressing a wide range of administrative and political issues.

There is also a critical need for additional quarantine facilities. While seed exchange is vital and valuable, exchange without adequate quarantine precautions could lead to the introduction of new pests and diseases.

On-farm/community conservation. On-farm/community conservation includes aspects of collecting, storage, and regeneration, appropriate documentation and information systems, evaluation, monitoring, research, training, and advocacy. *In situ* conservation of landraces is also emerging as a viable method of PGR conservation. Mutual benefits could be obtained by closer ties between the formal and informal sectors. For example, this could result in training of grass roots conservationists and the development of more efficient conservation systems, storage technologies, breeding methods, etc. The informal sector could also benefit from the careful and coordinated introduction of new genetic materials to expand options for local breeding endeavors. A "Conservation Corps" of young professionals could help provide technical support to community organizations.

Involvement at all levels in the decision-making process within the formal sector would bring benefits to both sectors and possibly enhance the ability of NGOs to utilize the resources of the formal sector, and advocate for improvements and additional support for both sectors.

Community level work is chronically underfunded. Genetic losses could be prevented in these programmes through provision of modest funding tied to specific conservation/utilization objectives.

Other Concerns That Require Special Attention

Monitoring and Early Warning. Genetic erosion is a stealthy process that may often go unnoticed until it is too late. This is often caused by a lack of information about the existing range of genetic diversity, and no timely warning of events that may affect such diversity (introduction of new varieties, crop failure, etc.). Loss of habitats is often not systematically recorded.

Not all genetic erosion can be prevented. However, at present no comprehensive or coordinated system exists which would provide an early warning of impending genetic erosion.

Sustainability. Many developing countries, particularly in South and Southeast Asia, experience high population pressure on land and water. This is also the region where a majority of the world's poor people live. Therefore, accelerated economic development is a must in such countries for meeting the minimum needs of rural and urban families for food, water, clothing, shelter and work. Nearly 70 percent of the population in these countries depend upon crop and animal husbandry, fisheries, forestry and agro-processing for earning their livelihood. Therefore, the gains achieved in agricultural advancement must not be at the expense of basic environmental assets like land, water and biological diversity.

It is in the development of techniques for sustainable advances in productivity that PGR plays a pivotal role. Using the naturally occurring genetic variability for producing new crop strains carrying a wide range of genes from landraces and wild species, it is possible to reduce the use of chemical pesticides and fertilizers.

Plant breeding for sustainable agriculture will need special genetic enhancement centres to create novel genetic combinations for distribution to local breeders, including farmers groups.

Research.

A better scientific understanding is required to solve the problems that hamper PGR conservation. This can only be achieved by an intensification of integrated and systematic research on technical and socioeconomic aspects of PRG conservation.

More research will also be necessary to reduce costs and achieve greater managerial efficiency in the establishment and maintenance of *in situ* and *ex situ* collections. Research is also required on techniques involved in assessing genetic variation, maintaining genetic integrity during seed regeneration, all aspects of gene bank management, novel storage techniques, pathology, seed health and quarantine, among others. To ensure wider use of genetic diversity, gene banks should increasingly become involved in the enhancement of plant genetic resources. Research is needed to demonstrate and make more effective the essential role of PGR in achieving sustainable agriculture. Special research effort should be directed at on-farm/community conservation and utilization.

Training.

An expanded and more effective gene bank system will require additional and better trained staff to implement PGR research and conservation.

Core segments should be included in the curricula of formal undergraduate and postgraduate courses, with provision for specialization. There is also a need for more in-service training at research institutions for researchers, technicians, students, and farmers. To involve the informal sector more effectively, special training efforts are needed in: on-farm techniques; on-farm conservation; land use management; vegetative propagation; communication; and other skills.

Public Education and Awareness.

The long-term commitment of substantial financial resources to PGR conservation will require the widespread understanding and support of the public, governmental, and private sectors.

Enlisting support from these diverse elements of society will require a well-orchestrated programme of information and communication concerning the activities, issues, and successes of the plant genetic resources community.

Although there have been substantial efforts to produce and catalog technical information concerning plant genetic resources for the scientific community, clear and concise presentation materials for policymakers and for educational programmes have been rare or produced on limited funds largely by the NGO community.

A multifaceted awareness programme, directed at appropriate audiences, is a necessary and critical component of a Global Plant Genetic Resources Initiative. The objectives should be to attract more interest in PGR management as a career, to educate policymakers, to enlist the support of specialized groups within the public sector, and to heighten general public awareness.

Generation of public awareness of PGR and its importance for food and livelihood security should be given a very high priority in the action programme.

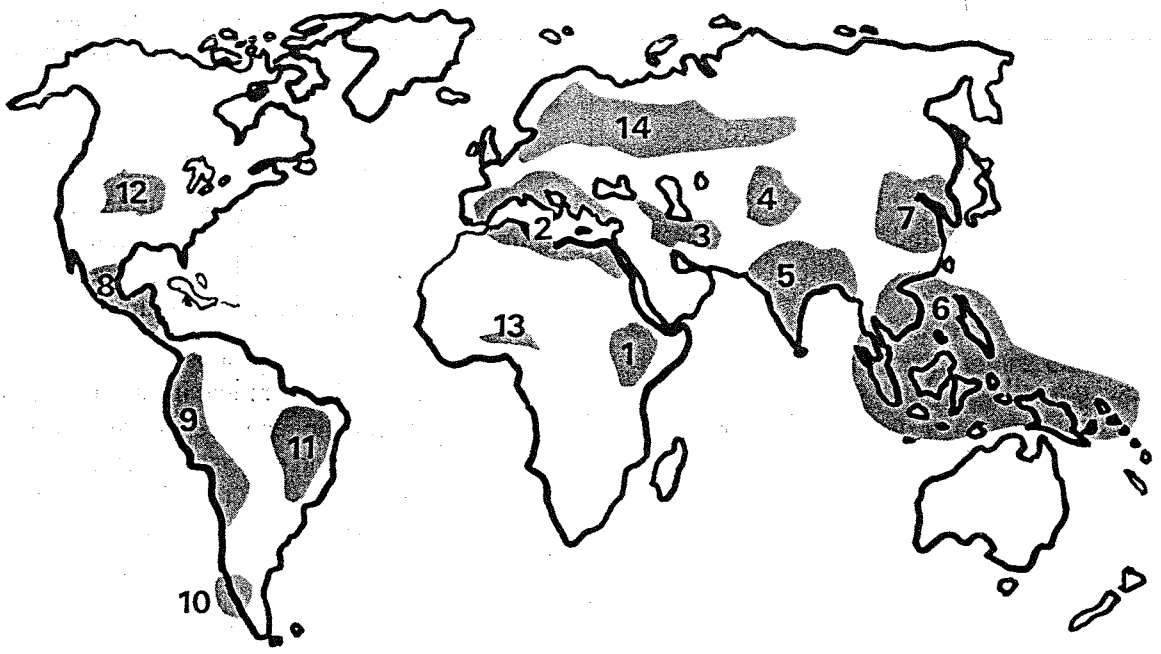
Magnitude of the Funding Requirements to Meet the Needs

Introduction

The participants of the Keystone Dialogue, representing programmes at all levels, used their collective experience and judgement in determining the funding needed to strengthen and sustain conservation and utilization of PGR. The proposed levels of additional fund-

Vavilov Centers of Plant Genetic Diversity

Areas of the World Where Food Crops Originated, and Where the Genetic Diversity of Those Crops Is Greatest



1. **Ethiopia**
   barley, coffee, sorghum
2. **Mediterranean**
   oats, olives, wheat
3. **Asia Minor**
   barley, lentil, oats, wheat
4. **Central Asia**
   apple, chickpeas, lentil
5. **Indo-Burma**
   eggplant, rice, yam
6. **Indo-Malaya**
   banana, coconut, sugar cane
7. **China**
   sorghum, millet, soybean
8. **Central America**
   bean, corn tomato
9. **Peru-Ecuador-Bolivia**
   bean, potato, squash
10. **Southern Chile**
    potato
11. **Brazil-Paraguay**
    peanut
12. **North America**
    sunflower
13. **West Africa**
    millet, sorghum
14. **Northern Europe**
    oats, rye

ing were developed by the Dialogue group to provide a sense of the magnitude of funding needs. The funding estimates outlined below were based on the best information available to the Dialogue group at the 31 May-4 June, 1991 Oslo Plenary session. These estimates are conservative and will require more detailed analysis. However, we are confident that the order of magnitude is realistic and indicative of real and urgent needs.

In calculating the needs for additional funding, certain assumptions were made. Central to a global activity are national programmes. Each country requires genetic variation in support of ongoing breeding programmes (working collections). This would seem to fall within the na-

tional responsibility. Additional funding would then be allocated on the basis of the importance of PGR found in the country beyond those short-term needs.

Consequently, major recipients would be located in the recognized centres of diversity. (See map on following page.) A national programme generally includes both a formal gene bank and community level activities. No attempt is made to separate the two in terms of funding as that would probably differ by country.

Most major industrialized countries fall outside centres of diversity and for that reason would not qualify as major recipients of funding. However, there is a question as to whether the level of economic wealth of a country should be taken into account. The concept of contributors of funds and contributors of germplasm may follow a general pattern now, but may do so less in the future as countries develop. Also, information, enhanced materials, and technology may become increasingly important as in-kind contributions to a global system, and may have to be taken into account. In addition, the roles and responsibilities of various elements of the global system will be evolving, as discussed elsewhere in this report. Many of these matters have not yet been resolved. Hence, financial requirements have been estimated only for the period 1993-2000. It is assumed that beyond that period the results of UNCED 1992 will have become operational and available funding determined, on the basis of in-depth analyses of the real needs.

Components Requiring Funding

All funding estimates discussed below are presented as percentages of available funds, which the Dialogue group believes should total US $300 million per annum (1991 US dollars) in the 1993-2000 period.

Present holdings of PGR, as documented in the IBPGR Database on *ex situ* conservation, number approximately 3.5 million accessions of which 50 percent are known to be stored under long- and medium-term conditions (i.e., at -10 to -20 degrees Celcius).

National Programmes. In calculating financial requirements to collect, store, maintain, and document a reasonable sample of still existing PGR diversity or seed in cold storage, a number of assumptions are made.

- Only part of the present day holdings are unique samples (probably less than 50 percent).
- All unique samples have to be stored in at least two gene banks.
- The number of unnecessary replicated samples over those in gene banks will approximately be balanced by additional samples still to be collected, and therefore the total number of accessions in collections will not exceed 4 million.
- The capital expenditure for adequate gene bank facilities is approximately $75 per sample.
- The cost of storage, maintenance, and documentation per sample is estimated to be on the order of $50 per sample, based on current costs of a number of gene bank programmes.

To conserve the total estimated world collection of 4 million unique samples at $50 per sample requires $200 million. Current expenditure is estimated at $75 million. Hence the added financial requirement per annum should be 43 percent of the Fund.

Field Gene Banks and In vitro Collection. A sizeable number of crops cannot be stored long term in cold storage. This includes vegetatively propagated crops, many tree species, and species with seeds that lose their viability rapidly under conventional storage conditions. These species have to be maintained in field collections and/or *in vitro* collections (tissue culture). At present, approximately 150,000 accessions are stored under these conditions with an estimated 2,000 accessions in *in vitro* collections. It is estimated that 10 percent of total future holdings will be in field gene bank collections.

It is difficult to provide a reliable estimate of the possible cost of a total world collection of such crops. However, the additional financial requirements for expanded collections is unlikely to be less than 6 percent of the Fund.

On-Farm Conservation. Current recorded expenditure for stimulating and facilitating on-farm and community conservation through NGOs is

Total Costs per Annum

National Programmes	43%
Field Gene Banks	6%
On-farm Conservation	6%
Supporting Activities	10%
Research	17%
Training	4%
Public Education & Awareness	8%
Subtotal	94%
Capital Investment/Annum from 1993-2000	6%
Total*	**100%**

estimated to be on the order of $7 million. A substantial increase would seem reasonable in view of expanding programmes in this area: 6 percent of the Fund should be allocated toward this end.

In Situ Conservation. It is assumed that *in situ* conservation of PGR will be an integral part of overall biodiversity conservation. To insure adequate conservation requires a number of activities including eco-geographical surveys, monitoring of important habitats, etc. These costs are included in the financial requirements for research.

Supporting Activities. Coordination and stimulation of the international system (FAO, IBPGR, networks, etc.) as well as an adequate intergovernmental implementation structure of the kind discussed below may require an additional amount equal to 10 percent of the Fund.

Research. Rationalization and provision for PGR conservation with an appropriate knowledge base may require 17 percent of the Fund.

Training. Provision of enough skilled personnel to execute various activities on plant genetic resources may require 4 percent of the Fund.

Public Education and Awareness. Generation of public awareness of PGR and their importance so that long-term conservation is secured may require 8 percent of the Fund.

Capital Investments. Of the current holdings approximately two million samples are stored in long-term or medium-term storage facilities. Hence, there is a need to build new gene banks or expand present facilities for another two million samples at $75 per sample. The total capital expenditure required therefore is 6 percent of the Fund.

The suggested annual additional funding requirement of $300 million may seem a large sum. It is an approximately four-fold increase in global funding of PGR conservation over current levels. Considering the present state of inadequacy of the current system, that would not seem excessive. The total annual global seed value at market prices was estimated to be $50 billion in the mid-1980s. Of this, $18 billion was from farmer-saved seed, the public sector contributed $17 billion, and the private sector $15 billion. Assuming that these figures are realistic, $300 million represents no more than 0.6 percent of that total value. If estimated in terms of global agricultural output, $300 million represents less than 0.002 percent.

An organizational structure is required to manage the fund. Specific recommendations are made in the section on Institutional Structures and Implementation Mechanisms. However, it should be clear that in addition, strong technical support is needed to: develop strategies and action programmes; coordinate activities within regions in all aspects of collection management and utilization; provide training; assist in institution building; stimulate community involvement; and so on. Many of these activities are presently carried out on a limited scale by the IBPGR. An expanded and institutionally reorganized IBPGR and appropriate regional and national gene banks might be considered for providing the necessary institutional support. It should have the confidence of the international PGR community and at the same time be accepted in the political arena.

It is clear that a build up of facilities and the development of human resources will determine the needs and the ability to utilize funds. For this period, a minimum total allocation of approximately $1.5 billion will be needed to lead into the 21st century. This figure probably represents the amount which can be efficiently expended in the seven year 1993-2000 period.

Conservation: Tactics for a Constant Crisis

MICHAEL E. SOULÉ

IS WILDLIFE CONSERVATION FAILING? IN THE UNITED STATES, species diversity appears to be declining at an accelerating rate (*1*). Even the Endangered Species Act of 1973 (ESA) has not significantly slowed the deterioration of the nation's biological estate, although this is largely the result of lack of support from the federal administration. Currently there are over 4000 species and subspecies recognized as candidates for endangered species status, but the listing process administered by the U.S. Fish and Wildlife Service is bogged down because of lack of funding. There are no recovery plans for nearly half of the 600 or so species in the United States that have been officially listed as threatened or endangered, and the score or so of recovering species is balanced by an equal number that may be extinct (*2*).

The situation is generally much worse in other nations. Biologists with extensive experience in developing countries are saying that by almost any quantitative standard conservation is failing, and that current approaches to conservation, such as traditional parks and reserves, are unlikely to succeed (*3, 4*). Worldwide, only about 3% of the land is set aside in 5000 nature reserves or protected areas (*5*), but many of these reserves are deteriorating (*6*). Because the moist tropics are far richer in species diversity than other biogeographic regions, and because deforestation will probably eliminate almost all of the tropical forests outside of protected areas by 2100 (*7*), biogeographers estimate that from 25 to 50% or more of tropical species will vanish in the next century or sooner (Fig. 1) (*8*). Even if humanity were to depart the earth, recovery of biotic diversity by evolutionary mechanisms would require millions of years, depending on how deep, taxonomically, the extinction crisis cuts (*9*).

Such dire predictions are now leading to a reappraisal of conservation's goals and tactics. In this article, I conclude that this reappraisal would be more fruitful if there were a deeper appreciation of the biological and social contexts of conservation actions, particularly how both biogeography and political geography dictate different conservation tactics in different situations. I also argue for an actuarial approach to the viability of protected areas—one that considers the social factors determining the half-life of nature reserves.

The Biospatial Hierarchy

Effective conservation is impossible without some knowledge of biotic (biological) diversity (biodiversity). For most scientific purposes, "life" is classified taxonomically, based on similarity and presumed evolutionary relationship. For purposes of protection, however, the living components of nature are usually classified in a "biospatial" hierarchy of nested sets. In practice, there are about five levels to this hierarchy: (i) whole systems at the landscape or ecosystems levels, (ii) assemblages (associations and communities), (iii) species, (iv) populations, and (v) genes (*10*). Place, not evolutionary relationship, is the basis for the biospatial hierarchy, because most conservation strategies are geographically anchored (*11, 12*).

The targets at the top of the biospatial hierarchy are ecosystems (or landscapes and seascapes making up interacting ecosystems), including such topographic features as entire drainages. A frequently cited example is the Yellowstone National Park region, including the adjacent Grand Teton National Park and other federally managed lands. Ideally, ecosystem conservation protects the contained biotic communities: habitats, species, populations, and genes, not to mention all ecological interactions, processes, and some of the traditional, human cultural practices that have been historically associated with the ecosystem.

At the second level, an arbitrary number of biotic assemblages can be defined within ecosystems, although the species themselves show little correlation in their distributions when climate changes (*13*). Nevertheless, state, federal, and international conservation programs often base their conservation strategies on the completion of the network of biotic community types—the so-called coarse-filter approach. The discovery of "gaps" in the network of assemblages is most often based on systems of biogeographic classification (*12, 14*).

The third biospatial level, species, is defined as groups of populations that routinely exchange genes or are phenotypically similar (*15*). The selection of protected areas is frequently based on the presence of one or more endangered species, often large-bodied or attractive ones. In addition, regions with high species diversity, such as tropical forests, coral reefs, or regions with large proportions of local endemic species, such as isolated mountain ranges or oceanic islands, are frequently identified as targets of conservation. Another reason for focusing on species is that the management of protected areas is often facilitated by attending to a relatively small number of so-called keystone or indicator species; these species may not be endangered themselves, but they are used to monitor the status of a much larger assemblage of species (*16–18*).

Next is populations. Populations, whether mobile or sedentary, are dynamic assemblages of individuals which maintain genetic and sometimes social information in lineages that may ramify and merge as individuals are born, reproduce, and die. Endangered populations, and those of species that mediate important ecological processes, are often targets of conservation, so that their viability is a major concern (*18, 19*). Theoretical treatments of population viability are influencing public policy, such as the debate over the spotted owl in the Pacific Northwest (*20*).

At the small end of the biospatial hierarchy of conservation targets are genes. Genes are sometimes conserved ex situ (*21, 22*) as seed collections, in tissue culture or germplasm collections, or as cryopreserved semen, ova, embryos, and tissues. The extraction of genes from nature annually produces multibillion dollar benefits for agriculture, biotechnology, and public health (*23*). In nature, genetic

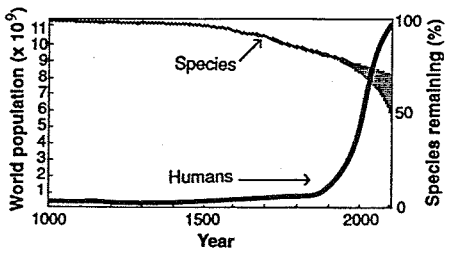


Fig. 1. The expected inverse correlation between human population size and the survival of species worldwide. Extinction rates depend on the size of the habitat fragment and occur at a decreasing rate as habitat fragments age. Anthropogenic extinctions before A.D. 1000 are ignored. The shape and width of the extinction curve reflect the uncertainty of the predictions; the curve is based in part on the assumption that most of the extinct species will be small organisms with geographically limited distributions.

The author is professor of conservation biology, Board of Environmental Studies, University of California, Santa Cruz, Santa Cruz, CA 95064.

variation maintains the fitness and evolutionary flexibility of natural populations (*16*). Reserves in seminatural areas have been set aside to preserve the wild relatives of commercially important plants, especially to protect genes and gene combinations providing resistance to pests, drought, and other climatic factors (*24*).

The Six Classes of Interference and the North-South Distinction

The five levels of the biospatial hierarchy—are being undermined by six major classes of human interference (*25*), as shown in Fig. 2. These six factors are (i) the loss of habitat; (ii) the fragmentation of habitat-producing deleterious area, edge, demographic, and genetic effects; (iii) overexploitation; (iv) the spread of exotic (introduced and alien) species and diseases; (v) air, soil, and water pollution; and (vi) climate change. These factors have all been discussed in great detail (*16, 19, 22, 26, 27*). The intensities of shading in the two parts of Fig. 2 are subjective, but suggest that the present and future hazards posed by the six factors are not equal in strength or concordant in rank across the range of conservation targets, or from economically poorer to economically richer nations.

Clearly the impact of a given factor depends on the time, the place, and the circumstances. As indicated in Fig. 2, economics, culture, as well as the temperate-tropical disparity in species diversity and other biogeographic patterns, explain the differences in biotic vulnerability between tropical, poor countries, and temperate, wealthier ones. The vastly greater number of species in the tropical nations, the much smaller geographic ranges of tropical species on average (*28*), in addition to the high rates of habitat destruction in most of these countries, means that species in the tropics are particularly vulnerable to habitat loss and fragmentation. Similarly, not all parts of the planet will be equally susceptible to the impacts of acid rain, ozone thinning, or greenhouse warming; for example, the effects of greenhouse warming will be much greater at high than low latitudes, except, perhaps, for marine systems (*29*). Other aspects of biogeography are relevant to geographic heterogeneity in biotic vulnerability; on oceanic islands, for example, introduced predators are typically more damaging than on continents (*16, 25, 30*), and introduced animals (goats, pigs, rats, mongooses, snakes, and predatory snails for instance) and plants may have catastrophic effects (*31*).

Although it is difficult to generalize, one can point to some rough principles about the global vulnerability of terrestrial biodiversity (*32*). Habitat loss, fragmentation, and the direct and indirect effects of exotic species are problems everywhere (Fig. 2A), but overharvesting of economically important species is now of greater concern in poorer countries. Pollution and climate pose major threats in the temperate zone nations (Fig. 2B). As discussed below, north-south differences in socioeconomic variables and biogeography mean that conservation tactics must be tailored to the location.

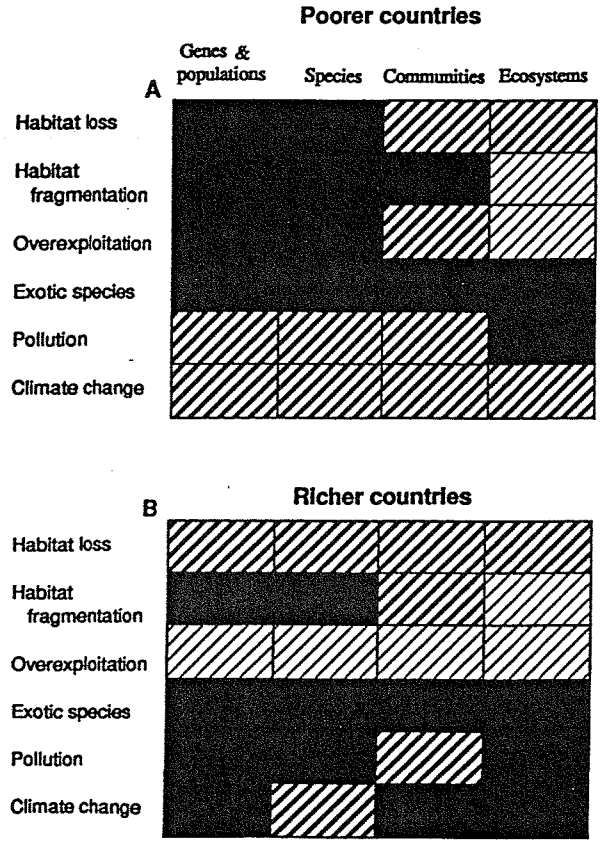


Fig. 2. Relative impacts of factors affecting terrestrial biotic diversity in (**A**) poor and (**B**) rich countries. Shading indicates intensity of impact: solid = highest; thick lines = intermediate; thin lines = lowest. Ecosystems refers to landscape level formations including, for example, mangrove habitats, coral reefs, riverine/riparian systems, forests, and savannas. The distribution of impacts on aquatic and marine systems differs somewhat from those shown here.

The Seven Sources of Biotic Degradation

The six classes of interference may constitute the most obvious proximal causes of biotic attrition, but the more fundamental causes are rooted in the contemporary human condition, especially as they are amplified by the explosive growth in human numbers in the last three centuries (Fig. 1). These more fundamental causes are listed in Table 1. The following brief descriptions of these factors are neither systematic nor exhaustive, but even this superficial treatment demonstrates why simple approaches (such as a network of protected areas alone) will fail.

Population growth. The continuous increase in human numbers exacerbates nearly every other environmental problem (*33, 34*). The population reached 1 billion about 1800, and appears to be headed toward 10 billion by 2046 and 12 billion by 2100, according to recent World Bank and United Nations projections. Ecologists argue that such numbers are incompatible with many ecological and evolutionary processes, including the persistence of large predators, the continuation of annual migrations of birds (*35*), speciation in large organisms (*36*), and the protection and maintenance of native biotas in the face of increasing pressure from human beings and

Table 1. Categories of fundamental human factors that contribute to the erosion of biological diversity.

Factor	Example of impact on conservation
Population growth	Population pressures
Poverty	Hunger, deforestation, trade in rare and endangered species, failure of grass roots support
Misperception	Desire for quick results and denial of long-term failures
Anthropocentrism	Lack of support for nonutilitarian causes
Cultural transitions	Unsustainable resource management during colonization and rapid social change
Economics	Failure of planning because of internationalization of markets and erratic pricing of commodities
Policy implementation	Civil disruption, wars, corruption, failure of law enforcement

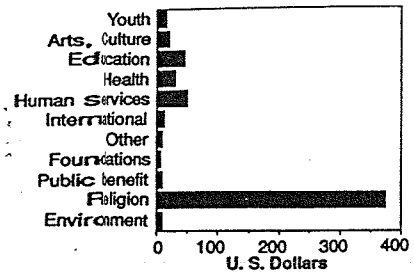


Fig. 3. Average charitable contributions per household in the United States. [Adapted from (*67*)]

introduced species. For nonhuman species, this "demographic winter" will last until human beings decide to reduce their numbers to levels compatible with the restoration of pre-explosion biotic processes (*37*). Human populations are already declining in many industrialized countries.

Poverty. The problem is not merely the shear magnitude of human numbers, however; it is compounded by poverty, the aspirations of people the world over for a better quality of life, and by social and political forces that impede the smooth transition to minimum (let alone "optimal") levels of prosperity, health, and justice (*38*). Disparities in income produce disparities of impacts. The per capita contribution to atmospheric pollution (and, hence, global climate change) is often orders of magnitude higher for citizens of the industrialized countries than for those in poorer nations (*34*), and economic pressures from the former contribute to unsustainable land use practices in the latter. Habitat destruction and extinction, however, will occur most rapidly in the tropics (Fig. 2A), where lack of economic opportunity, demographic momentum, and restrictions on reproductive choice are the engines that power the destruction of life.

It is probable that the price of raising human economic welfare to a standard similar to that in the wealthier countries will be biotic devastation in the tropics on a scale inconsistent with the persistence of wildlands except, perhaps, in remote, nonarable regions (*39*). Ehrlich and Wilson (*40*) point out that the magnitude of human aspirations, including demands on natural resources, if multiplied by the expected increases in human numbers, would require the human co-option of most remaining wildlands for grazing, farming, energy production, mining, transportation, and other uses. Therefore, the loss of most tropical wildlands in the next 50 years or so, an epochal catastrophe for earthly life, appears a virtual inevitability.

Misperception and time scale. Gradual environmental degradation goes almost unnoticed (*41*), whereas governments often overreact to sudden events of lesser overall impact. This short-term mentality is also reflected in current social mores and public policies favoring quick profits and results. The problem is that the benefits of conservation projects can only be measured on a scale of centuries. This difference in time scales between economic development projects and some conservation projects leads to conflicts because the business of conservation is keeping options open, whereas business as usual (economic development) often forecloses them.

Anthropocentrism. Many conservationists argue that current cultural values are antithetical to effective conservation policies, and that a new ethic or a revolutionary change in human consciousness is necessary before significant progress is possible (*42*). There are many calls for less human-centered, more biocentric economic policies. The anthropocentric orientation of most societies (*43*) however, augurs poorly for behavioral revolutions. If charitable donations reflect how Americans rank society's needs, it is evident that humanitarian concerns are dominant; money flows primarily to religious organizations and to medical, cultural, and social welfare causes. Figure 3 shows that only 1.5% of donated monies go to support environmental (nonhuman) groups and causes. This percentage is likely to increase, though, as donors learn about the environmental foundations of physical and social welfare.

Mindful of biases favoring our own species, nearly every book, report, or "strategy" written to promote or guide the conservation of biodiversity presents a list of utilitarian justifications, including the free services and amenities provided by nature (for example, water purification and storage, habitat for fish and livestock, vistas), and the promise of life-extending pharmaceuticals and agro-industrial products that are yet undiscovered in the tissues of organisms (*23*). Unfortunately, the political effectiveness of narrowly utilitarian arguments for large protected areas in the tropics and elsewhere is weak, in part because the promise of long-term economic and health benefits to society as a whole appears abstract to individuals and corporations more concerned with survival and short-term economic gains.

Cultural transitions. The most destructive cultures, environmentally, appear to be those that are colonizing uninhabited territory and those that are in a stage of rapid cultural (often technological) transition (*44*). The cultural groups that appear to be the least destructive to natural systems are those that have been occupying the same place for centuries or more (*45*). Overharvesting of wild animals, of aquatic and marine organisms, and of forests, is predictable, therefore, when human groups (i) have little or no experience in their current geographic setting or (ii) are undergoing integration into the world economy. Wealthy, well-educated, industrialized cultures may have the potential for minimizing environmental damage, but show little promise of this at present. Because most of the world's people are not only poor, but in a transitional phase between traditional agrarian self-sufficiency and a modern, high-input agricultural or industrial-urban society, relatively little value is placed on the protection of nature, and even where nature is highly valued, such valuation is often left out of economic calculus.

Economics. Environmental destruction and the erosion of biological diversity in the tropics and elsewhere is exacerbated by systems of commerce that create demands from the industrialized north for products, the production of which causes massive habitat destruction (*46*). The "cool chain" industry, for example, produces fresh produce such as fruit, vegetables, cut flowers, and mariculture produce (such as, shrimp) in the poorer countries and ships them in refrigerated carriers to the richer countries (*47*). This new industry contributes to the destruction of many habitats including lowland forests, mangrove, estuarine, and reef habitats. Better known are the coffee, sugar cane, banana, cacao, forest products, and cattle industries that account for the loss of a large proportion of tropical forests in developing countries (*23, 48*). In addition, a major contributor to forest and woodland destruction is the cutting of trees for the production of fuel wood and charcoal for domestic cooking and heating uses. Before the international price-fixing agreements among petroleum producers, most people in developing countries could afford to cook with kerosene. Now they must rely on wood, charcoal, and dung, contributing to the deterioration of forests and soils (*49*).

Notwithstanding the grave moral, social, and geopolitical implications of current economic disparities, the redress of such imbalances is unlikely to occur in time to save most seminatural biological systems from massive attrition. Few would question the goals of economic and social justice or their fashionable surrogate, sustainable development, but the premise that a new economic order would, alone, solve the biodiversity crisis (*50*) is suspect. The North American, let alone the Costa Rican experience (*4*), suggests that social justice and other progressive changes cannot protect biological diversity in the face of rapidly changing economic conditions including the internationalization of markets, increasing human numbers, the loss of cultural and ecological traditions, not to mention ethnic and religious conflicts. Even wealthy countries such as the United States and Canada justify the removal of the last

remnants of ancient forests on the grounds of economic necessity; attempts to save that remaining 15% of original forests in the Pacific Northwest have yet to prove successful (*18*). In addition, corruption and bureaucratic inefficiency appear to be virtually indelible.

Policy implementation. There are many reasons for the inability of modern states to enforce laws and implement conservation policies, especially policies that require short-term sacrifices for the sake of long-term benefits. For example, the setting aside and long-term protection of land from the national estate is improbable in societies with many poor or landless people, powerful oligarchies, or corruptible judges and bureaucrats.

In countries where adequate resources are lacking for the protection and management of protected areas, even relatively secure reserves are subject to the removal of trees and to the poaching of game. Most poor nations simply lack the resources to preserve biotic diversity in situ (*51*). Such attrition is frequent during "normal" times (*52*), but during periods of social unrest, the loss of biodiversity can be catastrophic (*53*).

Many conservation and development projects are destined to fail in a statistical sense, given their unstable social or political contexts. Wars and the breakdown of civil administration can undermine decades of successful policy implementation. In Africa, recent wars in Ethiopia, Sudan, Liberia, Libya, Morocco, Somalia, South Africa, Zimbabwe, Uganda, Chad, Angola, Mozambique, Rwanda, Burundi, and other countries have led to the partial or complete collapse of nature reserves, the destruction of habitat, and the local extinction of endangered species (*53*). The frequency of events such as wars should be built into the planning processes of responsible agencies and organizations. This is not to say that we should abandon reserves in regions where civil chaos is frequent. Rather, expectations and policies must be tuned to appropriate distributional parameters—for example, to the mean and variance of persistence times of protected areas in similar situations and to the kinds of damage that protected areas are likely to suffer, including the killing of most large animals. The lower the mean and the higher the variance, the greater the emphasis there must be on redundancy, on alternative approaches, and on backup, ex situ projects. It would be prudent, in other words, to think of nature reserves as ephemeral islands, and to plan accordingly.

The human condition is dynamic and unpredictable and will remain so for at least a century, if for no other reasons than the momentum of the population explosion and the unsatisfactory economic and social status for billions of people during the 21st century. The "biotic condition," therefore, will also be tenuous during this interval. Fortunately, conservationists have an increasing number of tools with which to deal with the crisis.

Tactics and Conflicts

The eight paths to biotic survival. What tools are available to protect living nature from humanity? Table 2 presents a brief survey of eight conservation tactics or systems (*5*). The tactics are defined roughly in order of least to most artificial or intrusive.

1) *In situ* refers to those conservation systems based on bounded wild areas with relatively little human disturbance; it includes most protected areas, from wilderness parks to the core areas of biosphere reserves (*54*). Persistence may depend to some extent on the economic benefits, as generated, for example, by tourism, but protected areas tend to degrade, even in the best of circumstances, and few, if any are large enough to maintain viable populations of large predators and omnivores without ex situ supplementation (*16, 19, 26, 55*).

2) *Inter situ* refers to conservation systems or activities in regions where native species still persist, but which are outside the boundaries of established protected areas. Most of the lands belonging to this category are nonarable; typically, they are relatively infertile, cold, steep, rocky, or arid. In the United States, most such regions are administered by the Bureau of Land Management and the U.S. Forest Service.

3) *Extractive reserves* permit certain kinds of resource harvesting on a (theoretically) sustainable basis. Examples include rubber tapping, the collection of edible fruits and nuts, thatch grasses, and, perhaps, even limited logging and hunting. Sustainability of such practices, however, depends on a low population density, a stable economy, and careful management (*56*). In practice there may be little difference between extractive reserves and inter situ projects, except that the latter are more circumscribed.

4) *Ecological restoration projects* refers to intensive management activities intended to increase species richness or productivity in degraded habitats. Among the necessary conditions for such activities are political and institutional stability.

5) *Zooparks* refers to facilities in secure locations where a mix of local and exogenous species can be maintained under seminatural conditions—in other words, sanctuaries for sensitive species of diverse provenance (*57*). The assumptions underlying the establishment of such reserves are that protected areas, in many places, are not viable for social

Table 2. The relative potential significance of eight different conservation systems for the protection and maintenance of natural biological diversity. The "0" indicates little or no role; "X, XX, and XXX" indicate low, moderate, and high significance, respectively. The order of the systems does not imply a ranking of value.

Targets of conservation	Conservation system							
	In situ	Inter situ	Extractive reserves	Restoration projects	Zooparks	Agroecosystems & agroforestry	Living ex situ	Suspended ex situ
Entire systems (ecosystems)								
Processes or functions	XXX	XX	XX	XX	XX	X	0	0
Biosocial (traditional human uses)	X	XX	XXX	XX	X	X	0	0
Biogeographic assemblages	XXX	XX	XX	X	XX	0	0	0
Indigenous and endemic species	XXX	XX	XX	X	XX	X	XX	X
Local populations of species	XXX	XX	XX	X	XX	X	X	X
Genetic variation within species								
Wild relatives of domesticates	XXX	XX	XX	X	X	X	XXX	XX
Traditional domesticated varieties	X	X	X	0	X	XX	X	XXX
Noneconomic genetic variation	XXX	XX	XX	X	X	0	X	X
Ownership	Public & private	Private & public	Public & private	Private & public	Private	Private	Private & public	Private & public

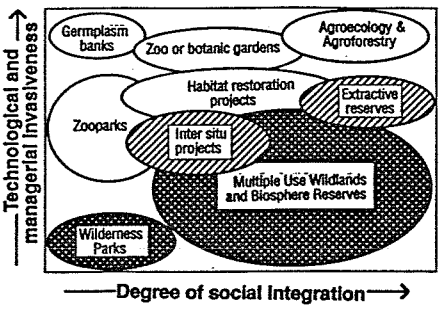


Fig. 4. Descriptive distribution of conservation tactics according to the degree of social integration at the local level, and the degree of technological input or management intensity. Shading indicates relative degree of human interference with natural processes; darker shades indicate less interference. The positions shown for each tactic are meant to suggest the center of the probable zone of action for the tactic. The term "Biosphere Reserves" refers to multiple use, production-oriented projects, with a relatively sacrosanct core protected area.

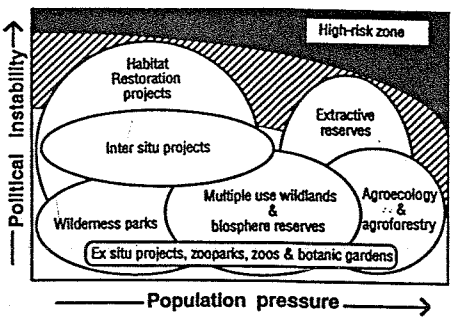


Fig. 5. Prescriptive distribution of conservation tactics based on the probability of increasing population pressure and the likelihood of political instability or violent conflicts. Backup, ex situ facilities are placed in relatively secure, politically stable locations.

or political reasons and the inevitability of highly recombined biotic communities in the future given current rates of species introductions (*58*). This category differs from in situ reserves because of the conscious introducions of target species.

6) *Agroecosystems and agroforestry projects* are highly managed, production-oriented systems with a wide range of dependence on artificial chemical and energy inputs (*59*). The number of native species that can survive in such systems is highly variable, depending mostly on the proximity of garden, farm, and plantation to wildlands, the use of artificial chemical inputs, and the tolerance of farmers to wildlife (*60*).

In addition to zooparks, there are two kinds of ex situ tactics or backup systems (*14*). These are essential where particular reserves are likely to fail or lose significant numbers of their species.

7) *Living ex situ* programs refers to botanical gardens, zoos, aquaria, and similar institutions that maintain and propagate living organisms for noncommercial (education, research, conservation) purposes in a highly controlled, usually urban, context.

8) *Suspended ex situ* programs are completely artificial; living material is metabolically slowed or arrested. Among these projects are germplasm storage facilities such as seed banks, tissue culture collections, and cryopreserved collections of gametes, zygotes, and embryos.

As shown in Table 2, this typology of tactics manifests a current trend—the privatization of conservation. For many reasons, nonprofit groups and individuals increasingly are complementing if not supplanting government agencies in protecting biodiversity. Private zoos, botanical gardens, and others are taking responsibility for the captive propagation of endangered species. Responsibility for the restoration of degraded forest, pastures, and farmlands on both public and private lands is being assumed by private groups. Organizations like The Nature Conservancy and Conservation International are acquiring new sites for protected areas (*61*), though governments are usually the ultimate owners.

Social Context and the Debate over Tactics

Current discussions have tended to oversimplify the diversity in conservation approaches by exaggerating the differences between the so-called species approaches and ecosystem approaches. The former emphasizes the protection, both in situ and ex situ, of endangered, often charismatic vertebrates, whereas the objective of the latter is to set aside and manage natural areas based on systems of landscape classification that will capture as much species and ecological diversity as possible (*62*). Critics of species-level approaches have emphasized the shortcomings of the Endangered Species Act and point out that most of the federal dollars are directed at a few birds and mammals (*62*). Some of these critics argue that success in captive breeding and cryopreservation will lead to complacency about the need for more and better protected areas. Supporters of endangered species might counter that if it were not for the charismatic species, the public appeal of conservation would be much less, that endangered species justify many of the larger protected areas in the United States and elsewhere, and that endangered species legislation is providing the economic leverage to bring developers and government agencies into negotiations about the preservation of large areas of habitat for general biodiversity conservation in the United States (*63*).

Such adversarial discussions, however, often ignore social context. As shown in Fig. 4, conservation tactics can be ranked according to the degree each is integrated into the local human community and the degree that each is dependent on artificial (technological) means and invasive management practices. Implicit is idea that different tactics require different degrees of social and technical sophistication.

A more prescriptive classification is shown in Fig. 5. It distributes the tactics in a plane of human population pressure and political stability. It is based on the untested assertion that the persistence of conservation projects, particularly protected areas, is related to the frequency and degree of political unrest and the rate of population growth. The combination of the two figures suggests that the choice of tactics should be influenced by the probable impact of demographic, economic, and social conditions as discussed above. For example, ex situ tactics are prescribed where political instability is frequent and where population pressure is building.

Much of the debate in the United States over approach and tactics stems from uncertainty and bias about landscape and geography, the importance of socioeconomic conditions and the stability of political structures, confidence in new legislative and legal remedies, and the identity of target organisms. For example, conservationists with experience in the species-rich tropics—where infrastructure is fragile at best, episodes of social chaos inevitable, human populations are doubling every few decades, laws are ignored, and hunting of rare animals and deforestation are a way of subsisting—should support a pluralistic approach that includes ex situ backup for protected areas. On the other hand, those with experience in wealthy, stable, temperate zone regions—where most species have wide geographic ranges and where there exists extensive areas of low productivity, government-owned lands—are more likely to promote systems of protected areas linked by corridors in multiple use zones that can be managed for conservation and sustainable forms of exploitation (*64*). They will also have more faith in legislative remedies and law enforcement. Figure 5 illustrates this tactical pluralism.

Conclusions

Today, the conservation of biodiversity is virtually equivalent to the ex situ protection of wildlands. In the future, however, such reserves will come to be seen actuarially, their life times dependent

on many biogeographic, social, and political factors. Unless a much denser and more secure network of protected areas is established soon, the importance of less appealing alternatives will be greater than conservationists would wish.

This awareness has led some observers to call for a greater emphasis on adjunctive approaches, including inter situ projects—the management of wildlife in nonarable lands outside of traditional reserves (*65*). Though appropriate in certain places, these lands are not immune to overexploitation, desertification, and to other forms of abuse, as the recent history of Tibet, the Sahel of Africa, and the American Southwest have shown. The inter situ tactic is an important backup, however, especially in socially and demographically stable nations and regions. The point is that every tactic has its limitations; sole reliance, for instance, on ecological restoration or on cryopreservation technologies would be premature, if not immoral, because these technologies could protect only a tiny fraction of species diversity for the foreseeable future, especially in tropical seas and forests.

Progress in conservation is hampered by the lack of a clearly articulated public policy on biodiversity. The United States and many other countries lack a coherent conservation strategy. In part, this may stem from confusion about tactics, as discussed above. The United States should join the nations that have developed a national conservation or biodiversity strategy. There is also a need for new institutions such as a National Institutes of the Environment (similar to the National Institutes of Health) to provide intellectual leadership and sustainable funding for planning and research in biodiversity. In addition, a high level review of federal agencies is necessary so that either the authority for the protection of biodiversity is vested in a new agency with clear directives, or the organic acts (if any) of the agencies should be restructured, making conservation a prime directive of the U.S. Forest Service, the Bureau of Land Management, and the National Wildlife Reserve System.

Everywhere, nature reserves must be defended and bolstered by social experimentation in "sustainability." But there is too much at risk to gamble on any one social ideology, theory, or approach. All human institutions are transient expedients, and the conservation systems that are fashionable today will certainly undergo many changes in the next century. Opportunism and tolerance must be the watchwords of the science, the politics, and the art of nature protection (*66*). The issue, therefore, is not the "failure" of conservation; it is whether it can stay the course. During the construction of cathedrals in the Middle Ages, planners and artisans were not dismayed that "success" might require centuries. Like those workers, conservation scientists and practitioners must accommodate their objectives to the social complexity and temporal scale of their enterprise (*67*).

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54. The Biosphere Reserve concept of Unesco's Man and the Biosphere Program is, in part, an attempt to integrate economic development and conservation by sharing the management and benefits of protected areas with local peoples. It attempts to avoid the extremes of banishing people to save nature and banishing nature for the sake of people. It has been difficult to apply in practice [see D. Hales, in (*4*), pp. 139–144]; M. Batisse, *Nat. Resour.* **22**, 1 (1986); S. R. Kellert, *Environ. Conserv.* **13**, 101 (1986).
55. U. S. Seal, in *Endangered Birds: Management Techniques for Preservation of Threatened Species*, S. A. Temple, Ed. (Univ. of Wisconsin Press, Madison, WI, 1978), pp. 303–314; W. D. Newmark, *Nature* **325**, 430 (1987); P. F. Brussard, *Ecol. Appl.* **1**, 6 (1991); R. L. Peters and J. D. Darling, *BioScience* **35**, 707 (1985).
56. L. Silberling, *BioScience* **41**, 284 (1991); J. R. Browder, *ibid.* **40**, 626 (1991); J. R. Browder, *ibid.* **41**, 286 (1991).
57. An example is North American ranches harboring African ungulates. Entrepreneurs might consider the purchase of strategically located islands and other real estate where secure facilities could be located. See also M. E. Soulé, in *Conservation of Threatened Natural Habitats*, A. V. Hall, Ed. (South African National Science Programmes Report 92, CSIR Foundation for Research Development. P.O. Box 395, Pretoria, South Africa), pp. 46–65; M. E. Soulé, in (*4*), pp. 297–303.
58. M. E. Soulé, *Conserv. Biol.* **4**, 233 (1990).
59. M. A. Altieri and L. C. Merrick, in (*22*), pp. 361–369.
60. Although the vast majority of native species currently are unable to survive in intensively managed agricultural zones, especially in the tropics, sound agroecological practices create a healthy environment and contribute to self-sufficiency and the maintenance of crop genetic resources, especially if practiced in an economically and political stable environment. In addition, they may effectively reduce wood-collecting, hunting, and other pressures on nearby wildlands [see M. A. Altieri and D. K. Letourneau, *Crop Protect.* **1**, 405 (1982); S. R. Gliessman, E. R. Garcia, A. M. Amador, *Agro-Ecosystems* **7**, 173 (1982)].
61. Among the most recent and impressive acquisitions by The Nature Conservancy is the purchase in 1990 of the 130,000-ha Gray Ranch in southwestern New Mexico, which includes an entire mountain range (Las Animas).
62. J. M. Scott, B. Csuti, K. Smith, J. E. Estes, S. Caicco, in (*1*), pp. 282–297. See also Part IV of (*1*).
63. See L. A. Greenwalt, in (*1*), pp. 31–36; M. J. Bean, in *ibid.*, pp. 37–42; D. D. Murphy, in *ibid.*, pp. 181–198.
64. R. Reed, *Nat. Areas J.* **7**, 2 (1987); L. D. Harris and J. Eisenberg, in (*4*), pp. 166–181.
65. D. Western, in (*4*), pp. 158–165.
66. Others have called for greater respect for pluralism [D. Western, in (*4*), pp. vi–xv; R. F. Noss, in (*1*), pp. 227–246].
67. *Giving and Volunteering in the United States: Summary of Findings* (Independent Sector, 1828 L Street, NW, Washington, DC, 1988).
68. The manuscript was much improved thanks to the comments of D. B. Botkin, P. R. Ehrlich, D. Goodman, W. P. Gregg, Jr., R. E. Grumbine, D. F. Hales, J. A. McNeely, P. Romans, J. M. Scott, D. Wilcove, and G. Zegers.