Chapter 6

Maintaining Animal Diversity Offsite

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Chapter 6

Maintaining Animal Diversity Offsite

HIGHLIGHTS

- Offsite maintenance of animal diversity includes selective breeding of wild or domestic species and safeguarding genetic diversity through cryopreservation. For wild animals, the programs reinforce rather than replace efforts to maintain diversity onsite. For domestic animals, programs try to maximize usefulness of the animals while preserving their ability to adapt to changing human needs.
- Cryogenic storage could make a considerable contribution to the maintenance of animal diversity. Properly frozen and maintained, sperm and embryos have an expected shelf-life of hundreds of years. Although initial collection and preservation costs are relatively high, subsequent storage costs and space requirements are low.
- The number of individual animals required to start a captive population or a cryogenic store depends on a host of factors. Retaining 99 percent of a source population's genetic diversity for 1,000 generations could require up to 50,000 animals, far too many to be practical under captive management. At a minimum, however, several hundred individual animals are required for captive breeding programs.
- Breeding programs require the international transfer of animals, which risks spreading pests and diseases. For most wild species, regulatory controls are virtually nonexistent. Stringent controls are in place, however, for importing domestic animals. Advances in diagnostic procedures and germplasm transfer technologies are expected to' facilitate the international movement of animals.
- No organized program exists, either in the United States or Internationally, to sam-pie, evaluate maintain, and use available sources of animal germplasr[n]. Such a program is needed, in addition to programs to understand the reproductive processes of wild animals, to develop local expertise in reproductive biology and quantitative genetics, and to increase the number of captive maintenance and breeding facilities.

OVERVIEW

Objectives Offsite Maintenance Efforts

Offsite maintenance of animal diversity is defined as propagation or preservation of animals outside their natural habitat. The programs involve control by humans of the animals chosen to constitute a population and of the mating choices made within that population. The extent of control can vary considerably, but the decision to remove individual animals from a natural habitat implies a major increase in human involvement in propagation of a population.

Captive maintenance of wild species has become progressively more important as increasing numbers of species are threatened or en-

dangered in their natural habitats. These programs can be considered holding actions designed to reinforce rather than replace wild populations. If a natural population is decimated or lost, captive maintenance programs provide a reservoir of individuals to allow reintroduction.

The genetic diversity of the original population must not be lost or seriously reduced during captive maintenance if animals are expected to be able to readapt to life in the wild. Likewise, genetic changes that may be induced during captive maintenance must be minimized. Reciprocal transfers of individuals between wild and captive populations can help reduce genetic pressures. Such exchanges, however, involve the capture of wild animals, and they risk the accidental death of some of them. Therefore, risks should be evaluated carefully before beginning a program of genetic exchanges between wild and captive populations.

For domestic species, all populations are by definition maintained offsite. Most of these animals have existed in association with humans for centuries, and their current genetic diversity is a reflection of this long interaction. Their genes have been manipulated through generations of selective breeding to meet the diverse needs of humans, and this manipulation has led to a wealth of specialized breeds (boxes 6-A and 6-B). Some wild progenitors of domestic species still differ so much from domestic populations that they exist as a reservoir of genetic diversity, but these natural populations are unlikely to contribute much to current commercial stocks through traditional breeding methods.

The aim of programs to maintain genetic diversity in livestock differs somewhat from that for wild animals. In domestic populations, the challenge is to maximize current utility and preserve sufficient diversity to ensure livestock's continued adaptability to changing—and often unforeseen—human needs. In fact, efforts to raise current rates of food production may constitute the greatest threat to future flexibility by concentrating unduly on short-term production goals with attendant losses in genetic diversity that may be important to future generations.

Box 6-A.—Breeds

In its most restrictive form, the designation "breed" is reserved for subpopulations that have both distinctive morphological characteristics and a pedigree-recording system to document the ancestry of individuals within the breed. In practice, however, a breed is often taken to be any differentiated, identifiable domestic subpopulation.

Breeds that are widespread may often be further subdivided into national or regional populations that retain the major characteristics of the parent breed but have become partially differentiated in response to local conditions and selection pressures. Thus, identifiable regional populations exist for Holstein-Friesian dairy cattle in North America and most of the European countries, for Shorthorn beef cattle in nations previously colonized by the British, and for Merino sheep worldwide. Some degree of interbreeding often occurs among such populations, but in most cases they persist and are referred to as "strains" or "stocks" within the breed.

For more industrialized species such as poultry, a few ancestral breeds such as the Leghorn chicken have been used in concert with crossbreeding and selection to develop a wide array of partially differentiated stocks maintained by commercial breeding firms. These stocks are open to genetic manipulation at the discretion of the firms, but they represent another source of differentiated genetic material.

In this assessment, the term breed will be used to describe any differentiated domestic subpopulation and will include both regional strains of major international breeds and identifiable commercial stocks.

Brooding Programs v. Long-Term Cryogonic Storage

Using captive breeding programs to retain a considerable proportion of the genetic diversity of endangered species or rare breeds for

Box 6-B.—Replacement and Genetic ~ *

Traditionally, breed replacement has proceeded on an evolutionary time scale, with gradual changes inbreed composition and provision for maintenance of a wealth of local populations. Recently, however, the pace of breed replacement has accelerated, with thero f genc d tinational breeding companies in poultry and widespread the use of insemination and intensified sire selection in dairy cattle, opportunities h a disseminatfor of germplasm throughout the world. Also, greater standardization of production, marketing, and recording procedures for poultry, swine, and dairy cattle in industrial countries has increasingly promoted replacement of local breeds.

In domestic species, the greatest threat to genetic diversity involves extensive and sometimes indiscriminate crossing of indigenous stocks in developing countries with breeds from North America and Western Europe (3). This crossing stems from needs to increase world food production and from a belief that this goal is b stoclmet iusing possible genetic merit for individual traits (such as milk or egg production). But breeds developed in temperate-zone industrial countries are often not suited to the more restrictive nutritional, management, and disease conditions of developing countries and may be less efficient than indigenous stocks in using available resources. Only recently has the need for comprehensive evaluatio peof the total of imported breeds begun to be recognized in developing countries. Unfortunately, serious dilution of original breeds may have already occurred. Thus, it is not the process of breed replacement per se that is a problem but the rate of replacement and the danger that useful breeds maybe discarded before they can be fairly evaluated.

Regional strains of established breeds are especially vulnerable to lose through intercrossing with more popular strains. Extensive use of Holstein bulls from North America in European Friesian populations threatens serious dilution of the genetic material of these strains. The percentage of Holstein genes in young Friesian bulls entering European artificial insemination programs in *1982* ranged from 8 percent in Ireland to *91* percent in Switzerland and averaged 54 percent for 10 European countries (4).

Genetic diversity can sometimes be reduced in commercial stocks even if population numbers remain large. These losses can occur when selection is intense and control of breeding stock is concentrated in a few large breeding farms (as in the commercial poultry industries] or when artificial insemination allows extensive use of a few selected sires throughout the population (as in the dairy industry). In both cases, the result is increased genetic uniformity within the stock despite the large numbers. Several studies (3,25) have concluded that important losses may be occurring in commercial poultry breeds. Comparable losses have apparently not yet happened in dairy cattle populations. No imminent losses of genetic diversity within major commercial breeds are foreseen for swine, sheep, goats, or beef cattle. Several populations of chickens are currently being maintained without selection and at sufficient population sizes to substantially retard losses in genetic diversity (42). And some artificial insemination organizations retain semen from bulls that have been removed from service. However, these programs do not represent industry or public policy, and parallel programs do not exist for other domestic species.

The loss of endangered species and rare breeds is of particular concern in light of likely future advances in molecular biology and genetics. The ability to extract desirable genes from different species or less productive_ and insert them into domestic animals could have important implications for designing superior animals for specific environmeral conditions. Unfortunately, knowledge of the genetic material of most wildnand: isrudimentary. For instance, it is unclear if adaptive factors suchtas heate and disease a aist:controlled by many o r a few g - , @ = basis yet exists entoal single-gene cof localtibreeds and endangered species.

a substantial period of time requires relatively large numbers of animals. Under the most favorable assumptions, maintenance of 90 to 95 percent of the genetic diversity within a population for 100 to 200 generations would require a captive population of at least several hundred individuals sampled from throughout the range of the species (15,27).

Until relatively recently, zoos have not been concerned with keeping representative levels of genetic diversity within their exhibition stock. Problems in fertility and juvenile survival that often accompany exhaustion of genetic diversity were simply accommodated by obtaining new specimens from the wild. As this became difficult, and in some cases impossible, zoos began to reevaluate their role. The result has been establishment of programs to maintain pedigree information on zoo animals through the International Species Inventory System (ISIS) and to facilitate transfer of individuals among zoos. These efforts help maintain genetic diversity, but existing zoos can support at most 1,000 kinds of terrestrial vertebrates at a minimum population of 250 (2), whereas an estimated 1,500 to 2,000 kinds will be in danger of extinction by the year 2050 (43). The magnitude of the problem will thus outrun currently available facilities for captive breeding.

Recent advances in reproductive biology and cryopreservation may facilitate efforts to preserve genetic diversity. Cryopreservation refers to storage below – 1300 C: water is absent, molecular kinetic energy is low, and diffusion is virtually nil. Thus, storage potential is expected to be extremely long. Storage in liquid nitrogen (— 1960 C) or in the vapor above it (ea. – 1500 C) is a useful technique: Liquid nitrogen is relatively inexpensive, inert, and safer than comparable refrigerants (e.g., liquid hydrogen, liquid oxygen, or freon).

Storage and eventual production of live offspring from frozen semen or embryos have become common for cattle, sheep, goat, buffalo, and horse. The semen of pigs can also be frozen. In 1982, an estimated 10.5 million cattle were produced through artificial insemination with frozen semen. Similarly, bovine embryo transfer has become commercially viable and increasingly involves the use of frozen embryos. Commercial use of frozen semen and embryos is less common in other livestock species, but acceptable results can be achieved. Frozen semen is also regularly used with poultry and with some species of fish (18).

Photo credit. American Breeders Service

This calf, born in 1984, was conceived with semen that had been frozen for 30 years.

Cryopreservation of sperm and embryos of wild species has been much more limited. To date, blackbuck, giant panda, fox, wolf, chimpanzee, and gorilla have been produced from frozen semen (7,9,38); baboon (37) and eland (8), as well as mice, rats, and rabbits, have been produced from frozen embryos. Procedures differ among species, but in theory, semen and embryos from a range of mammalian species can now be successfully frozen.

The contribution of cryopreservation to the maintenance of animal diversity could be tremendous. Properly frozen and maintained, sperm and embryos have an expected shelf-life of hundreds, if not thousands, of years. Although initial collection and preservation costs may be relatively high, subsequent storage costs and space requirements are low, allowing for long-term maintenance of large numbers of individuals and gametes.

These individuals represent a frozen snapshot of the population at the time of collection. If the initial sampling of individuals is done

Photo credit Zoological Society of San Diego

The frozen zoo: Cryogenic storage of cell strains, gametes, and embryos is being undertaken as part of the conservation activities of zoos.

properly, the procedures should allow regeneration of the original population without the genetic changes inherent in the maintenance of captive breeding populations.

The long-term genetic stability of frozen embryos and sperm is a matter of some concern. Freezing and thawing does not appear to increase the mutation rate in these tissues, but long-term exposure to low levels of radiation could be a problem, especially because DNA repair mechanisms would be inoperative at – 1960 C (l). Mouse embryos and semen have been kept *frozen* for at most 10 and 30 years, respectively. However, frozen mouse embryos also have been exposed to augmented levels of radiation equivalent to that experienced in 2,000 years of normal storage without apparent ill effects (16). Normal progeny were produced. Thus, risks of genetic damage from background radiation appear negligible.

Just as captive breeding programs reinforce rather than replace natural populations, cryopreservation efforts reinforce rather than replace captive breeding programs. In wild species, females must still be available to gestate frozen embryos or to provide female gametes in matings involving frozen semen. In domestic species, breeding populations selected for biologically or economically important traits may still be required, but cryogenic storage of individuals from the original population provides a valuable measure of insurance. Periodic

sampling and preservation of gametes or embryos from rare breeds allow a repository of genetic diversity to be maintained.

Two caveats must be kept in mind regarding the role of cryopreservation of gametes and embryos. First, considerable development work is required to extend the techniques to cover the full range of endangered populations. For wild animals, reliable procedures for collecting, freezing, and using semen and embryos have to be developed further and validated for each species or group of species to ensure that sufficient levels of genetic diversity can be regenerated from the frozen store. Preservation technologies for embryos are well developed only in certain domestic mammals. Similar techniques are needed for birds, reptiles, amphibians, fish, and invertebrates.

Second, cryopreservation of gametes and embryos should not bean llth-hour effort to protect seriously endangered species. Restraining wild animals to collect semen or embryos is risky. Some animals die, which entails an unacceptable risk if the species is already rare. Therefore, research and the collection of gametes and embryos from many sources should begin before the populations become endangered.

SAMPLING STRATEGIES

Efficient programs for offsite maintenance of animal genetic diversity require a mechanism for monitoring existing populations—to identify when and if intervention is required—and procedures for sampling threatened populations in a way that ensures desired levels of genetic diversity within the conserved population.

Identification of Candidates for Conservation

Three criteria are generally considered when selecting wild species for captive propagation or preservation (35):

1. Endangerment in the Wild: Information on the status of wild animals is probably best obtained from the Species Conservation Monitoring Unit (SCMU) of the International Union for the Conservation of Nature and Natural Resources at Cambridge University in England. Funding constraints tend to limit the scope and timeliness of SCMU information, however. Local and regional organizations may also provide useful information, but their effectiveness varies widely.
2. Feasibility in captivity: Lack of facilities and expertise may preclude captive breeding or cryogenic storage of some species. The blue whale is an example of a species that cannot be maintained in captivity.
3. Uniqueness: Given limited facilities for captive propagation, programs must try to represent as much available taxonomic diversity as possible. Thus, endangered species that are the only representative of their genus, family, or order would receive high priority.

Subspecies present a special problem. Most wild species have several distinct forms or races, analogous to the breeds found in domestic animals. These subspecies usually cannot all be maintained as discrete breeding populations. Instead, captive propagation programs need to concentrate on one or two representative subspecies or amalgamate several of them into a single interbreeding population. Cryogenic preservation of semen or embryos would facilitate conservation of these identifiable subspecies.

Table 6-1 provides some general guidelines for monitoring and intervention to conserve a natural population. Such an approach has three important advantages:

1, a sample of the source population can be obtained before substantial loss of genetic diversity has occurred;
2, conflict over capture and restraint of rare

Table 6-1 .—General Guidelines for intervention To Conserve Natural Populations

Likelihood of extinction	Number of animals	Action
Possible	fewer than 100,000	At least, serious surveillance of status and trends should be initiated
Probable	fewer than 10,000	Well-managed captive propagation programs should be establ i shed; reproductive technology research should be vigorously conducted; and germinal tissues should be collected for storage while there are an adequate number of animals to use as founders, subjects, and donors
Certain	fewer than 1,000	Off site programs should be intensified while onsite efforts are fortified for a "last stand"; off site programs are imperative
Imminent	fewer than 500	Off site programs become as important as onsite efforts

SOURCE D R Netter and T J Foose, "Concepts and Strategies To Maintain Domestic and Wild Animal Germ Plasm," OTA commissioned paper, 1985

individuals, e.g., the California condor, can be avoided by taking action before extinction is imminent; and

3, if techniques for semen and embryo preservation are not well developed, material can be made available for experimentation,

For the rare breed of a domestic species, identifying candidates for conservation involves assessment of uniqueness, potential economic contribution, and degree of endangerment, Monitoring the status of domestic animal breeds used for food and fiber production is somewhat coordinated by the Food and Agriculture Organisation of the United Nations, under the auspices of the United Nations Environment Programme, Regional efforts are directed by the European Association for Animal Production, the Society for the Advancement of Breeding Researchers in Asia and Oceania, the InterAfrican Bureau for Animal Resources, the International Livestock Centre for Africa, and the Asociacion Latinoamericana de Production Animal (12). Comparable efforts in North America have been less comprehensive and limited to private organizations such as the American Minor Breeds Conservancy.

At least 700 unique strains of cattle, sheep, pigs, and horses have been identified in Europe alone, and 241 of these are considered endangered, under the criteria detailed in table 6-2 (30). public support for maintenance of all these breeds is not feasible, and choices will have to be made. Two considerations have been suggested for choosing among competing domestic breeds (39):

1. the breed exists as a closed population, and a similar population does not exist elsewhere; or
2. the breed exhibits a specific genetic value, such as superiority in some production trait, the existence of a major gene (i.e., a gene with a known effect on some physiological characteristic), or the expression of a unique characteristic of potential importance.

In the selection of threatened breeds, characterization and evaluation are critical first steps (35), Ideally, breeds would be assessed in their native environments and would be evaluated as both pure breeds and as crosses with other indigenous and improved breeds. This evalua-

Table 6-2.—Criteria for Classifying Domestic Breeds as Endangered

Species	Number of active males	Number of active females[a]	
		Stable population	Decreasing population
Cattle	fewer than 20	fewer than 1,000	1,000 to 5,000
Sheep	fewer than 20	fewer than 500	500 to 1,000
Goats	fewer than 20	fewer than 500	500 to 1,000
Pigs	fewer than 20	fewer than 200	200 to 500

[a]The risks associated with a decreasing population were deemed to be greater than those associated with a stable population Therefore, larger numbers were suggested for a decreasing population

SOURCE Adapted from K Maijala, A V Cherekaez, J.M. Devillard, Z. Reklewski, G Rognoni, D.L Simon, and D E. Steane, "Cons servation of Animal Genetic Resources in Europe, Final Report of an E A A P Working Party, " *Livestock Production Science* 11 :3-22, 1984

tion, often lacking for threatened breeds within developing countries, can be extremely important. In the absence of a formal evaluation, bibliographic databases may provide some needed information (12). Following the evaluation, breeds can be put in one of four categories:

1. *Useful under current economic conditions.* Such stocks should be integrated into the production system in a way that uses their genetic material in pure lines, crosses, or selected gene pools. Pure lines should be maintained with selection for net merit in production systems that are characteristic of commercial production within the country of origin or preserved cryogenically if maintenance as a pure line is impossible.
2. *Viable under current economic conditions in relation to other indigenous types, but inferior (in pure lines or in crosses) to improved types; no obvious biological extreme or major gene.* Germplasm preservation in such populations could have two rationales: preservation of frozen semen or embryos to prevent total loss of the germplasm and as insurance during a period of breed replacement with the improved types, or maintenance as pure lines for their cultural-historical value at the option of local governments and producers. A dual philosophy exists here–a unique population should not be discarded until its inferiority is documented, but preservation should not hinder use of improved breeds.
3. *Not competitive under current economic conditions; possesses an extreme phenotype for one or more traits or carries a majorgene.* Such breeds should be conserved cryogenically or as pure lines. Research use should be encouraged, and selection to intensify the extreme phenotype should be considered.
4. *Not competitive with existing adapted types; not a biological extreme; no major genes for production traits. No* particular efforts should be made to conserve such breeds unless they can be documented as unique in their genetic origin. Stocks could move from the second category to this one as more productive breeds prove themselves.

Preservation and Collection Considerations

The number of individual animals required to initiate a captive population or a cryogenic store will depend on the nature and extent of the genetic diversity to be maintained, on the population structure in nature, and on the rate at which the captive population reproduces.

The Natural Extent of The Genetic Diversity

Both natural and artificial selection reflect different fitness or reproductive success for individuals carrying different genes and lead to changes in the frequencies of those genes in a population. The diversity of genes in a large, interbreeding population may be quite extensive, with different individuals possessing a somewhat different genetic composition. It is this diversity that enables populations to adapt to environmental changes. Indeed, preserving the evolutionary potential of the species requires the maintenance of these possibly useful genes.

The objective in sampling a source population, then, should be to obtain a group that represents the bulk of its genetic diversity. Fewer animals are required to obtain an adequate initial sample of a population's diversity than are required to ensure continued maintenance of that diversity over time. Thus, 20 to 30 founder animals should provide an adequate sample of the genetic diversity in most interbreeding populations (6,43), but much larger subsequent population sizes are required to prevent erosion of this diversity over time.

In terms of cryopreservation, enough frozen semen to produce 10 live offspring from each of 25 sires, which would require 50 to 100 units of semen per sire, would constitute a good sample of an interbreeding source population (40). The Council for Agricultural Science and Technology recommends production of 40 to 80 offspring from frozen embryos representing 20 or

more unrelated parents (3). Assuming a pregnancy rate of 30 percent and a subsequent survival rate of 80 percent, 167 to 333 frozen embryos would be required for each breed.

For particularly rare breeds, too few individuals may be available to comply with these recommendations. Although a viable population can be established with as few as 4 to 10 animals, such a population may differ considerably in genetic composition from the unendangered source population, and it may have an impaired ability to respond to future changes in environment. Initiation of a captive population with only a few founders could be justified if a reasonable likelihood exists of obtaining additional individuals from the wild at some future point.

Population Structure in Nature

Most domestic and wild populations exist as groups of semi-isolated subpopulations. The extent of this subdivision differs among species and influences the sampling process in developing a captive population. If the population is strongly subdivided, genes present in one subpopulation may be absent in others, and sampling must attempt to include individuals from all major subgroups. According to one calculation, the recommended 20 to 30 founder animals can be decreased by about one-third if the population exists as a small number (2 to 10) of very distinct subpopulations, but it should be increased by about one-third if 50 to 100 distinct subgroups exist (35).

Current assessment of genetic diversity among subpopulations must be based on biochemical, historical, morphological, and ecological criteria. For genes that produce an identifiable protein molecule, genetic differences can be identified by the behavior of the proteins on an electrically charged (electrophoretic) gel. Electrophoretic testing procedures help identify the existence and distribution of various genes in different subpopulations. Rapid advances in molecular biology also hold promise of DNA probes that would directly assess the similarity of DNA molecules among subpopulations. In domestic animals, however, differential selection pressures may result in considerable genetic variation among breeds with similar evolutionary origins.

Roproductive Rate

Reductions in diversity are cumulative over generations in small populations, so the losses associated with a single sampling event are much lower than those that would accumulate over time if the population size remained at the founder number. As soon as a captive breeding population is started, therefore, it should be expanded to a size consistent with continued maintenance of the available genetic diversity. If the reproductive rate is high, maintenance can be achieved rapidly and with only a few founders, If the reproductive rate is low, several intervening generations at limited population size will be required to reach eventual target numbers, and more founders will be needed to assure retention of genetic diversity during this period. Sample sizes for cattle have been suggested to be twice those required for pigs, sheep, and goats, for example (3). One advantage of cryogenic preservation would be that the period of population expansion can be deferred until appropriate facilities and habitat are available,

THE MOVEMENT OF GERMPLASM-DISEASE AND QUARANTINE ISSUES

For many reasons, effective programs for conservation of endangered populations will require extensive international transfer of germplasm. First, facilities, funds, and institutional stability in developing countries maybe insufficient to allow endangered species to be conserved onsite, and animals may have to be transferred to countries better equipped to support captive breeding programs. Second, with wild animals, effective maintenance of genetic

diversity within captive populations will require the international transfer of animals for breeding purposes. And third, the optimum use of domestic animal germplasm for food production depends on the international movement of desirable breeds and strains to countries where they may be useful.

International transport of animal germplasm is accompanied, however, by the risk of introducing and spreading disease agents and vectors, many of which could have an enormous impact on animal productivity. Indeed, transporting animal germplasm without appropriate safeguards could jeopardize the conservation programs for which the germplasm is required. Thus, technologies to facilitate germplasm transfer must also limit the risk of disease transmission.

Many infectious diseases are caused by organisms that do not naturally occur in the United States, and their introduction could have serious effects on U.S. animals. Those causing most concern are foot-and-mouth disease, African swine fever, rinderpest, foreign bluetongue strains, scrapie, fowl plague, velogenic viscerotropic Newcastle disease, and Venezuelan equine encephalomyelitis (20). Current programs to exclude entry of pathogenic organisms vary with the species, disease, and country of origin.

For most wild species (including nonungulate mammals, most birds, reptiles, amphibians, and most fish), regulatory controls to prevent introduction and transfer of hazardous diseases are virtually nonexistent. Except for inspection at the time of entry, movement of such individuals is not restricted. In contrast, entry requirements for domesticated livestock species are quite stringent, especially for those coming from countries that harbor foot-and-mouth disease, rinderpest, scrapie, or velogenic viscerotropic Newcastle disease.

All imported domestic animals are subjected to a variety of diagnostic tests and to varying periods of quarantine in both the country of origin and the United States. Greater control reflects the wide potential dissemination of these animals throughout the livestock industry. Wild ungulates (hoofed mammals) can carry diseases transmissible to livestock and have importation requirements similar to those of domesticated livestock, but also must remain in permanent post-entry quarantine in U.S. Department of Agriculture (USDA)-approved facilities. They can be moved from one USDA-approved zoo to another, however, and their offspring can be transferred to nonregulated facilities.

Current efforts to control introduction of foreign diseases center on combined strategies of blood (serological) testing and quarantine. Some tests are designed to detect antibodies to specific disease organisms and can thereby identify individuals that have been exposed to the disease at some time; other tests maybe used to detect the presence of a specific pathogen. Periods of quarantine support these procedures by allowing an incubation period for animals that may have been infected recently. The tests are conservative, because individuals that have been exposed to a disease but no longer retain the organism still carry antibodies and react positively. However, the procedures also facilitate identification of asymptomatic carriers of the various diseases.

Some serological procedures, such as the complement fixation and the viral neutralization tests, are at times unable to adequately discriminate between pathogenic and nonpathogenie organisms. These limitations have made it very difficult to obtain negative test results for some diseases. Recent advances in diagnostic procedures have yielded tests with much greater accuracy and specificity. Three of the most important are the following:

1. Indirect Immunofluorescence: This procedure can provide very rapid screening of samples for a variety of infectious agents. Although it lacks specificity for some diseases, it greatly facilitates the initial screening process.
2. Enzyme-Linked Immunosorbent Assay (ELISA): The compounds that are produced by a disease organism and that elicit the production of antibodies by the infected individual are called antigens, This test uses carefully selected and purified anti-

gens unique to a given strain of an infectious agent to identify circulating antibodies. It is rapid and can be highly specific. Continued developments in selection and purification of limited amounts of specific antigens using recombinant DNA technology may ultimately make ELISA the preferred serologic testing method for most infectious agents,

3. Complementary DNA Probes: These probes are derived from cloned DNA or RNA of specific infectious agents and can confirm the existence of the infectious agent in tissue samples. The tests would distinguish between animals carrying only antibodies and those that actually carry the infectious agent, The tests would also be of great value in identifying asymptomatic carriers of infectious agents that infect circulating white blood cells without eliciting antibody formation.

In addition to movement of entire animals, increased interest in the international transfer of semen and embryos has produced both opportunities and concerns about disease control. For semen, the risk of disease transmission is usually equated to that associated with the male that produced the semen. When semen is being moved, it undergoes the same tests the donor would undergo if he were being moved. In addition, samples of the semen are usually subjected to various diagnostic tests (44,45),

The risk via either fresh or frozen embryos is less clear, In many cases, infectious agents are attached to the surface of the embryo or found in the associated uterine fluids. Although standard methods of embryo-washing free the embryo of most such organisms (19), it does not remove all of them (e. g., African swine fever) (1 1). Research is thus needed on the feasibility of' purging embryos of undesirable disease agents. Even if the disease organism cannot be disassociated from the embryo, the contaminated germplasm may be rendered noninfectious by highly specific monoclinal antibodies, new antiviral agents, chemical detergents, or immunization of surrogate mothers.

To date, the suitability of embryos for international movement has been equated to the suitability of both parents for such movement, But considerable interest exists in developing procedures that would allow the status of the embryo to be evaluated independently. Such an assessment is likely to become feasible in the future. Indeed, transfer of embryos of wild and domestic animals may ultimately provide the safest means of exchanging germplasm,

Advances in diagnostic procedures and transfer technology should facilitate the international movement of germplasm. For domestic species and wild ungulates, these developments should make foreign breeds more accessible without increasing the risk of introducing disease. Improved serological testing may allow relaxation of the permanent post-entry quarantine now imposed on wild ungulates. For unregulated species, a mechanism for monitoring disease status is needed and should be facilitated by new technologies. These efforts will be particularly important as captive breeding programs enlarge, thereby increasing contact between exotic and indigenous species. Returning individuals from zoos to the wild will also place a premium on ensuring the health status of released individuals.

For improved diagnostic and transfer technologies to be most effective, they must be applied both in the united States and in the countries of origin, Currently, USDA-approved quarantine facilities do not exist in Asia and have only recently been developed in Latin America. To set up such facilities and equip them requires capital inputs—costs that are likely to be borne largely by industrial countries. This approach is reasonable in terms of the ultimate benefits that are expected from global maintenance of animal diversity. The costs of importing animals and semen are currently absorbed by the U.S. importer. Yet this approach ignores societal benefits that accrue from access to foreign domestic animal germplasm and from maintenance of animal diversity as a whole, which argue for a greater U.S. Government role. If widespread maintenance of genetic diversity is the goal, then increased public support for importation, conservation, and use of foreign germplasm is essential.

MAINTENANCE TECHNOLOGIES

Storage Technologies

Cryogenic storage of gametes and embryos introduces a new level of complexity to the procedures already discussed, but it also holds the promise of greatly facilitating conservation of genetic diversity. For both semen and embryos, a critical element for cryopreservation involves development of media to protect cells when they are frozen in liquid nitrogen at – 196° C. Likewise, procedures must be developed to regulate the rate of freezing and thawing of this material in a way that will maintain the integrity of the cells.

Photo credit: Zoological Society of San Diego

In a vial, frozen cells may be stored in suspended animation and later resuscitated. Technologies for storing sperm, ova, and embryos are being developed for domestic and non-domestic species.

The ability to freeze semen successfully resulted from the accidental discovery in 1949 of the cryoprotective action of glycerol. To date, semen has been frozen from at least 200 different species, but little has actually been thawed and tested, Commercial use of artificial insemination with frozen semen is a reality today only for domestic species. Current media for freezing of semen usually include buffering agents, a cryoprotectant such as glycerol, antibiotics, and either egg yolk or milk. Many variations of these media exist, and a somewhat different mix usually must be developed for different species.

The first successful freezing of mammalian embryos with a subsequent live birth was reported in 1972 with mice (50,51). Since then, embryos of 10 mammalian species have been successfully frozen, and the procedure has become routine with the mouse, cow, and rabbit. As with semen, a variety of freezing media and of freezing and thawing procedures are available and are being evaluated. Rapid increases in efficiency have occurred in the bovine embryo transfer industry, and frozen embryos can now be transferred in a manner analogous to artificial insemination. As in the freezing of semen, specific procedures and media appear to be required for each species. Yet the procedure in general rests on a firm mechanistic understanding of the processes responsible for cell injury during freezing, thawing, and dilution. Previous detailed work with mice and primates can act as a model for extension of these techniques to other mammals, Thus, given appropriate research, cryogenic storage of embryos could be developed for a range of species,

Cryopreservation is probably the most promising area of reproduction research today. The potential exists to hold a well-constructed sample of the genetic diversity of a population in suspended animation indefinitely, In practice, frozen semen or embryo storage would probably be used with living populations for a number of reasons: to augment the genetic variation within breeding populations, to allow periodic comparisons between original and current populations, and to validate the viability

Photo credit American Breeders Service

Liquid nitrogen storage vessels (above) contain enough frozen bull semen to inseminate 4.5 million cows. Liquid nitrogen maintains the temperature at –196C C (–320 F).

of the frozen material. Samples from current populations would likewise periodically be added to the frozen store to retain new variants produced by natural selection or mutation. This process would be particularly important in domestic populations, in which selection could make preserved individuals economically obsolete.

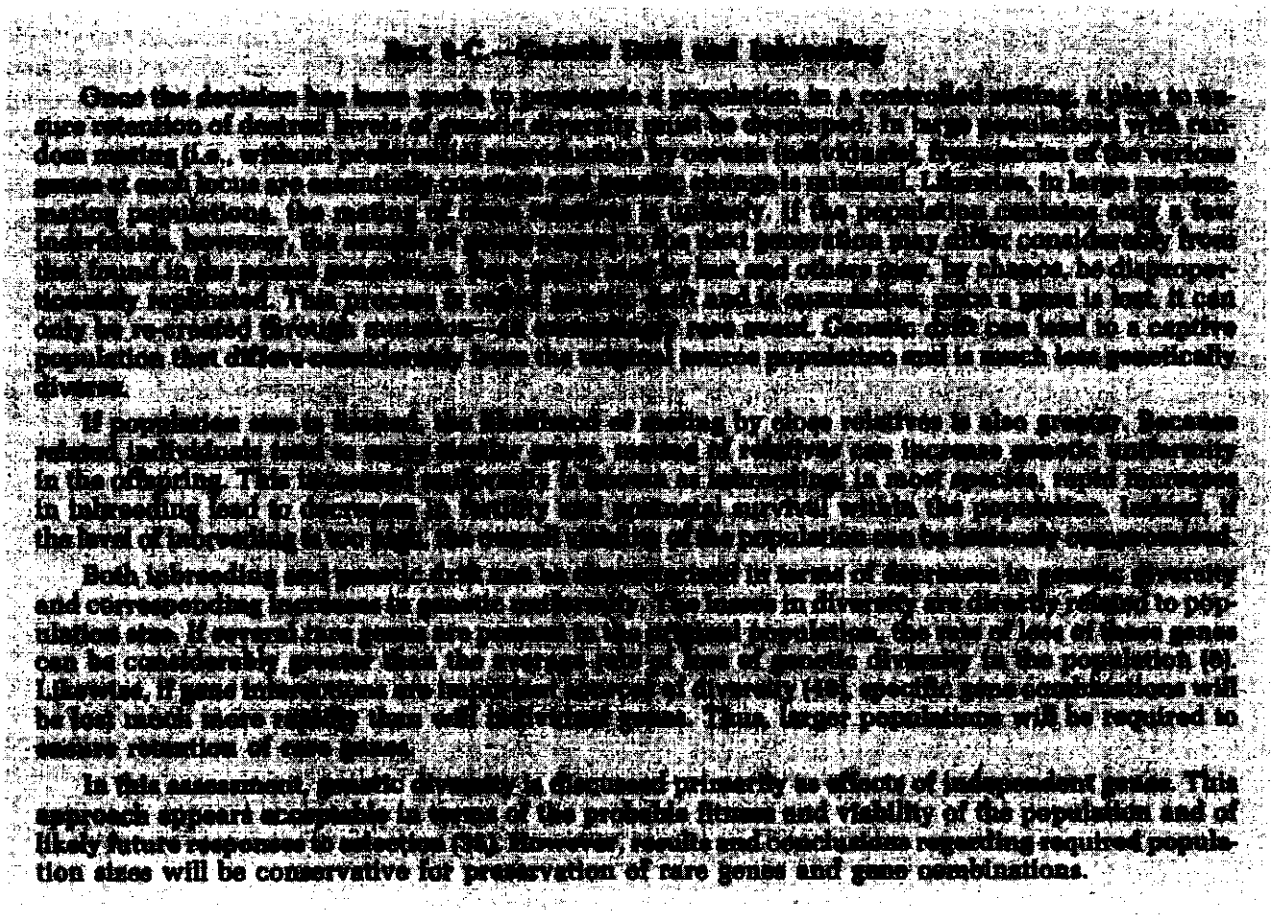
Breeding Technologies

The goals of a propagation program can be defined in terms of how much genetic diversity is to be maintained and for how long. Table 6-3 shows the number of animals required to ensure retention of various proportions of genetic diversity for subsequent generations. Ideally, all of the genetic diversity present in the source population would be maintained indefinitely in the captive population. Table 6-3 suggests that this goal (i. e., retention of 99 percent for 1,000 generations) would require up to 50,000 animals. These numbers are consistent with the guidelines in table 6-1, which suggests natural populations of fewer than 100,000 should be carefully monitored.

Table 6-3.—Captive Animals Required for a Fixed Proportion of Genetic Diversity Over a Number of Generations

Percentage of aenetic diversitv ~aintained ‘	Number of generations			
	50	100	200	1,000
50	36	72	145	722
75	87	174	348	1,738
90	238	475	949	4,746
95	488	975	1,950	9,748
99	2,488	4,975	9,950	49,750

SOURCE Adapted from D.R Netter and T.J Foose, “Concepts and Strategies To Maintain Domestic and Wild Animal Germ Plasm, ” OTA commissioned paper, 1985



Genetic drift accumulates generation by generation, not year by year, and animal species differ considerably in this regard (box 6-C). For example, 200 years covers perhaps 100 generations of chickens but only 7 to 8 generations of elephants. Yet in most cases, breeding populations should not experience inbreeding rates in excess of 1 percent per generation; to meet this constraint, at least 50 breeding individuals per generation are required.

Manipulation of the breeding structure of a population can have a significant impact on its genetic characteristics. For example, an appropriate level of subdivision of a population can retard the overall rate of genetic loss in the population as a whole: Subdivision increases the rate of loss within each subgroup, but the specific genes that are lost through random drift vary among subpopulations. And the number of genes that can be maintained within the subdivided population will exceed the number that could be maintained in a random-mating population of comparable size. In practice, the minimum size of the subpopulation will depend on the tolerance of the species to the inbreeding rates. Still, some degree of subdivision should be practiced, and the movement of individuals among subpopulations should be fewer than one per generation unless effects of inbreeding become evident. Subdivision also protects the population against disease outbreaks or other disasters that might annihilate any one subpopulation.

The loss of genes from a captive population can also be retarded by controlling mating. In contrast to selection, which presupposes a

differential contribution of different individuals to the next generation, programs that attempt to equalize the contribution of each individual can greatly lower initial rates of genetic loss (21). Likewise, if pedigrees of available individuals are known, matings can be planned in an attempt to equalize the contribution of different lineages. This approach has been used to stabilize founder contributions in a captive population of Speke's gazelle (46). Thus, efforts to record and publish pedigrees of individuals in endangered species (such as the records of ISIS) assume great importance.

For domesticated animals, several population structures and mating systems can be used in conservation programs that are generally not appropriate for wild animals. In many domestic species, the large number of existing breeds (30) precludes conservation of all endangered breeds as pure breeds. One possibility in such cases is to preserve a single breed representative of a group of similar breeds. A better strategy, however, may be to amalgamate into a gene pool individuals from related breeds or from several breeds that excel in a certain characteristic.

Gene-pool populations are designed to conserve genes rather than individual breeds. Thus, several breeds noted for a certain characteristic such as heat tolerance or proliferation might be interbred to provide a single large reservoir of genes for this trait. Although the identity of individual breeds is lost, many genes present in the breeds are retained. Selection to intensify the trait maybe appropriate, depending on the potential or current economic importance of the population. Maintenance of a single interbreeding gene pool is less desirable than of a subdivided population for long-term gene conservation. For domestic species, however, the larger population sizes that are possible in a single gene-pool population are expected to facilitate selection for economically important characters within the population. Simmental cattle representing at least five regional or national strains from Europe were imported into North America in the 1970's, and the current American Simmental population represents a gene pool constituted from these breeds. A gene-pool population of pigs was developed in the early 1970s in Nebraska and used in efforts to increase ovulation rate (53).

A program to not only maintain but also generate genetic diversity in domestic breeds has been suggested (26). In this effort, populations would be selected to generate extreme levels of performance in specific traits. These populations could serve as reservoirs of genetic variation and their characteristics would be well known.

Efficient maintenance of captive populations requires a thorough understanding of the reproductive processes of the species. Optimal use of breeding stock is often facilitated by an ability to manipulate and control these processes. In domestic animals, control of the estrous cycle and ovulation through administration of exogenous hormones has become commonplace, greatly assisting programs of controlled mating, artificial insemination, and embryo transfer. In wild species, however, knowledge of basic reproduction remains limited. Efforts to expand knowledge in this area are largely funded by the private sector and are insufficient.

Infertility is a major problem in many species of zoo animals. It reduces the effective breeding size of captive populations and exacerbates genetic losses. Infertility can often be traced to environmental factors such as light, temperature, nutrition, disease, or social influences. Such problems would be more easily overcome if more were known about basic reproductive processes in wild animals.

General principles underlying control of reproduction are relatively uniform across species, yet the particular hormone levels observed and the release patterns of these hormones are species-specific. A practical, reasonably simple, relatively inexpensive kit for monitoring urine hormone levels has recently been developed (29). This test helps confirm ovulation, predict optimal times for insemination, diagnose reproductive dysfunction, and detect pregnancy. Because the test is based on urine sam-

Photo credit: Zoological Society of San Diego

New technologies in reproductive physiology offer possibilities of producing large numbers of offspring from many vertebrate species. Above, an osmotic pump filled with gonadotropin-releasing hormone is prepared for insertion under the skin of a female iguana. The iguana subsequently entered estrus and ovulated.

pies, it also avoids problems restraining and anesthetizing rare animals.

Although reproductive problems in wild animals can often be solved through management changes, hormone therapy can also be used for infertility arising from age or unknown environmental factors. In particular, gonadotropin-releasing hormone has been used to initiate and maintain estrous cycles and ovulation in monkeys, sheep, and cattle. It is administered through a small osmotic pump implanted beneath the skin and appears to have facilitated the birth of two cubs to a previously subfertile cheetah (28). Similarly, a human fertility drug, clomid, is being considered to support ovulation in female gorillas (9).

Growing pressure for the international movement of animal germplasm will also place an increasing premium on knowledge of reproductive biology. In terms of animal safety, convenience, and disease control, movement of semen and embryos (either fresh or frozen) would be preferable to the movement of animals. Although the techniques to allow collection, preservation, transport, and use of these tissues are relatively well developed in domestic animals, comparable methods do not exist for wild species.

Artificial insemination (A. I.) is the introduction of semen into the female reproductive tract by artificial means. It requires technologies to allow collection of semen from the male, storage of semen until it can be used, identification of females in the proper stage of the estrous cycle, and deposition of semen at the appropriate location in the female reproductive tract. Collection of semen from wild species is usually accomplished by electroejaculation, which involves stimulation of ejaculation by application of a mild, pulsating electrical current through a lubricated rectal probe. The process requires restraint and anesthesia of the male, and semen obtained with this procedure is often less fertile than that obtained in a natural ejaculate. Use of A.I. likewise requires the ability to assess the reproductive status of the female quite accurately, and insemination procedures must be developed that are consistent with the biochemical and physical characteristics of the female reproductive tract.

Although artificial insemination has been attempted in many species of wild animals, it has only been successful in a Iimited number and, in most cases, with one animal in most species. A.I. with frozen semen has been successful with even fewer wild species (see table 6-4) such as the wolf, gorilla, chimpanzee, and giant panda.

Effective use of embryo transfer requires even greater control of an animal's reproductive processes (box 6-D and figure 6-l). Fertilized ova and early embryos are recovered from the

Box 6-D.-Embryo Transfer

Embryo transfer is a well-established practice in the beef and dairy cattle industries. More than 200,000 transfers are performed annually throughout the world, mainly in the United States and Canada. Although the technique was first used with beef cattle, half the transfers are now in dairy cattle. The objective is to increase the number of offspring of cows with valuable genetic traits, such as rapid rates of growth and high levels of milk production. Using this procedure, one valuable cow can produce on average 12 offspring a year.

The procedure involves inducing superovdation in a donor cow using gonadotrophin hormones, so that she will produce six to eight eggs rather than one. The cow is artificially inseminated with semen from a valuable, high-performance bull, and the embryos are collected by nonsurgically flushing the uterus after 6 to 8 days. Embryos that appear viable and healthy by microscopic examination are transferred to recipient cows that are also at the sixth to eighth day of their estrous cycle. Normally one embryo is transferred to each recipient.

Several new technologies hold promise of making the process more efficient and increasing its usefulness to animal agriculture. Among these is the ability to freeze bovine embryos. This procedure is currently used by most embryo transfer companies, and 25 percent of the transfers in the United States are with frozen embryos. Survival of the embryos is not perfect, however: Transfer of unfrozen embryos average a 60-percent pregnancy rate, while frozen and thawed embryos can be expected to yield pregnancy rates of 40 to 50 percent.

Another interesting development in this industry involves cloning bovine embryos. Once developed, this technique would allow the multiplication of large numbers of calves from one valuable embryo. The cloned embryos could be frozen while other embryos from some clonal lines are tested to determine if the line is of high value; valuable ones could be replicated using the frozen clones, providing a powerful tool for livestock improvement. Several research stations are also experimenting with inserting genes for specific productivity traits, such as growth, into embryos before transfer. The application of these new biotechnologies is expected to expand the size and usefulness of the cattle embryo transfer industry.

Although embryo transfer could also be a useful tool in swine production, much of the technology and the industry are not yet well developed. In swine, embryos must be collected and transferred surgically. And the embryos do not survive freezing with present techniques. This procedure therefore has received little use in the swine industry. In addition, the cost of surgically recovering embryos is likely to preclude wide-scale use of this technology in the near future.

Based on the use of embryo transfer in cattle, research on the applicability of this technology for wild species was begun in **1981.** Although the nonsurgical collection techniques are similar, working with exotic species entails several unique problems, such as, the need to administer drugs by dart or pole syringe and the need for anesthesia to perform even the simplest procedures. The ultimate goal was to develop methods for using a common wild species (e.g., the eland antelope) as a surrogate mother for a less common species (e.g., the bongo **antelope).**

In **1983,** an eland calf was born to a surrogate eland mother, becoming the first non-domestic issue of a nonsurgical embryo transfer. A transfer involving a frozen embryo was accomplished soon thereafter. These successes were followed by attempts at interspecies transfer (i.e., a donor and surrogate of different species). Initial efforts for an eland-to-cow transfer were unsuccessful. The eland, however, proved to be a suitable surrogate mother for an embryo collected from a bongo. This first documented nonsurgical embryo transfer between two different species of wild animals indicates that embryos can be gestated by surrogates of differmt species, offering hope for the future of endangered wildlife (figure 6-1).

SOURCE: **Adapted from materials provided by Dr. Neal First, University of Wisconsin and Dr. Betsy Dresser, Cincinnati Wildlife Research Federation.**

F gu e 6 T anscon nen a Embryo T ans e

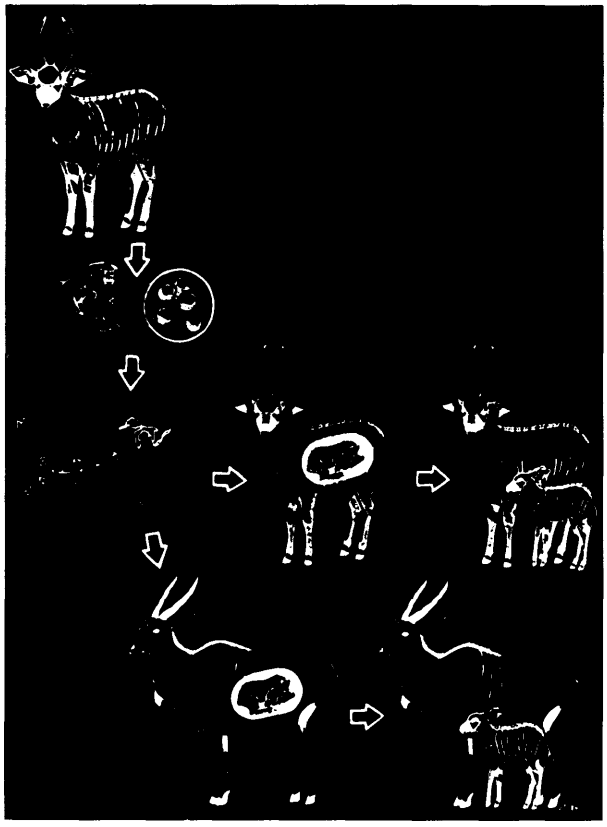


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Photo credit: Zoological Society of San Diego

Collection of sperm samples for artificial insemination and cryogenic storage from non-domestic species is part of many off site conservation programs. Above, an African antelope, the scimitar-horned oryx (Oryx gazella *dairnrnah)* is tranquilized and undergoing semen collection by electroejaculation.

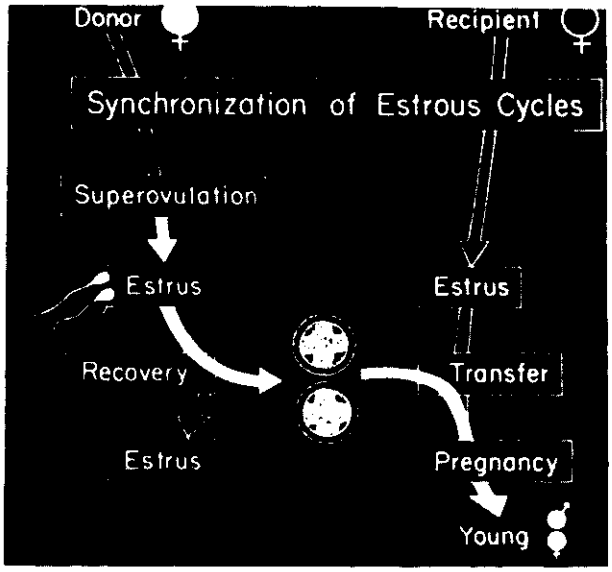
Table 6-4.-Successful Artificial Insemination in Non-domestic Mammals

Guanaco	Gorilla
Llama	Ferret
Black buck	Fox
Bighorn sheep	wolf
Brown brocket deer	Persian leopard
Reindeer	Puma
Red deer	Macaca monkey
Speke's gazelle	Papio baboon
Giant panda	Squirrel monkey
Chimpanzee	

SOURCE: B.L. Dresser, Cincinnati Wildllfe Research Federation, personal communications, Septemtw 19S6.

reproductive tract of a donor female (the genetic mother) and transferred into the tract of a recipient female (the foster mother), in whom the embryos develop into full-term individuals. Successful embryo transfer requires synchronization of the estrous cycles of donor and recipient animals (figure 6-2). In domestic animals this synchrony is usually achieved through exogenous hormone treatment. Donors are induced to produce an excess of eggs (superovulated) by injection of fertility hormones. Superovulation has been fairly successful with hoofed mammals, although the results vary considerably. Optimal drugs and dosages have yet

Figure 6-2.— Embryo Transfer Flowchart



Necessary steps in preparing donor and recipient animals for embryo collection and transfer.

SOURCE: Betsy Dresser, Director of Research, Cincinnati Wildlife Research Federation, 1986

to be identified in most other species. Donors are mated naturally or by artificial insemination, and fertilized eggs are collected from the female tract (surgically and nonsurgically) and transferred (surgically or nonsurgically) to the recipient female.

Development of embryo transfer techniques is important to maintenance of genetic diversity within captive populations, given the considerations of transfer and disease control previously discussed. In addition, surrogate mothers confer passive immunity to offspring developed from transferred embryos. Thus, animals moved into new environments or reintroduced to the wild may benefit from being carried by mothers acclimated to the new environment,

Several more-advanced techniques, studied primarily in domestic animals, hold considerable potential for all species:

- Embryo Culture: This technique involves maintenance of fertilized eggs outside the body during the early stages of embryonic development. The appropriate culture media for development differ among species, but reliable techniques to culture embryos for up to 24 hours exist for cattle, rabbits, mice, sheep, and humans. Successful embryo culture is usually prerequisite to more sophisticated in *vitro* embryo manipulation.
- Embryo Storage: This technology involves holding embryos in arrested development for up to several days, Again, specific storage media must be developed for each species. Embryo storage procedures can greatly facilitate transfer of embryos over long distances and *in vitro* embryo manipulation.
- In ***Vitro*** Egg Maturation: This technique involves the culturing of immature eggs to maturity. Coupled with *in vitro* fertilization, this technique could dramatically increase the number of offspring that a given female might produce. The reproductive lifetime of the female is also lengthened because ova suitable for culturing can be obtained prior to sexual maturity as well as after a female is no longer able to conceive naturally.
- In ***Vitro*** Fertilization: In a few species, it is possible to remove unfertilized ova from a female, mix them with semen *in vitro,* and produce fertilized ova that will develop normally when transferred back into a female, In cases of unexpected death of genetically valuable animals, ova can even be collected from ovaries shortly after death.
- Embryo Splitting: A single embryo can, under the proper conditions, be split into two or four, and each part can subsequently develop into a live offspring, Although the offspring are genetically identical, this process allows a much larger number of offspring to be produced from each embryo collection,
- Interspecific Embryo Transfer: This involves transfer of embryos between related species. Thus, embryos of a rare species could be carried to term by a female of a more common species. This technology has enjoyed some success, but much more research is needed. To date, successful in-

Box 6-E.—Captive Breeding and Przewalski's Horse

Offsite conservation through captive breeding has prevented the extinction of such animal species as the European bison, Peré David's deer, Arabian oryx, and the wild species of horse most closely related to all domestic horse breeds. This latter species, known as Przewalski's horse, has never been domesticated and is known historically to have come from Mongolia and China. Although it is not certain whether it is the direct ancestor of the oldest breeds of domestic horse, this species differs from all domestic horses in possessing a different number of chromosomes, as well as having other genetic variations. First brought to the attention of European scientists in the late 1800s by the Polish explorer N.M. Przewalski, who explored and mapped Mongolia for the Russian czar, the species has a bulky head, erect mane, lack of forelock, and buckskin-dun coloration; it sheds its coat in a manner different from domestic breeds.

This species, *Equus przewalskii*, was already in decline at the time Colonel Przewalski obtained a killed specimen and forwarded the skeleton and skin to the Imperial Zoological Museum at St. Petersburg. The horses depicted on the famous cave paintings at Lascaux in southern France have the same morphological features as Przewalski's horse.

The publication in 1881 of the description of a new species of horse attracted considerable attention from zoologists and horse breeders. By 1902, 52 animals had been captured and were eventually distributed in the Ukraine, Europe, and North America. In Mongolia, young foals were captured shortly after they were born, because attempts to capture adult or even juvenile horses were fruitless. The captured foals were nursed by domestic mares bred to produce their own foals coincident to the capture of the young Przewalski's horses. The Przewalski's horse is thought now to be extinct in its natural habitat. None have been seen since 1967, in spite of annual search efforts by the Joint Soviet-Mongolia Biological Expedition. More than 550 now live in some 70 zoological institutions on all continents except Africa and South America. An international studbook contains the pedigrees of all animals and traces back to 13 individual animals.

Przewalski% homes can interbreed withthdr domestic relative, andtlm hybrids producad by such cross8s were tiegedy incxwpmtad into the R%uwbE 'axar's oavdry ~d.

The disappearance d!theqeoka ww pm#M&ly duatu hunting and, mom important, to restricted access to limited watm suppliti tlmt wam nwncqmlizd by nonmds tifheir MM& ofdonmstic animals. The homes' habitat remab, and plans exist to retire th~ spedaa to ite fornwr range in **Mongolia.** This restoration effort invoivas tha cuop@rative activities of zmbgical parka in the United States, the Soviet Union, the United l&tgdo~ **and West and East Germany. Animaia will be provided by zoological parks ftam their expanding populations.**

The offsite comervatkm ofspeciw and preservation Ofgenstic diveisitythattkwe efforts reprment may play an increasing rob in strategies to pravent ektinatimk. The interaction throtqjh movement of individuals from offdte to onske ciwwrvation fhcilitks mkmea tlm chance of extinction and simultaneously provides access to mm $pecias for wh.wationai and research purposes.

SOURCE: Dr. Oliver Ryder, Research Department, San Diego Zoo.

terspecific embryo transfers have occurred from mouflon (wild sheep) to domestic sheep, gaur to cattle, bongo to eland, zebra to horse, and Przewalski's horse to pony (see box 6-E) (9).

DEVELOPMENT AND UTILIZATION TECHNOLOGIES

The objectives of developing and using genetic diversity differ between wild and almestic animals. For domestic animals, the potential contribution of rare breeds to food and fiber production on an international scale is oof paramount importance. In this context, the most pertinent technologies are those that facilitate the international movement and evalu-

ation of these breeds. Thus, the previously discussed technologies of disease control, artificial insemination, embryo transfer, and cryopreservation of embryos and gametes are extremely important. In particular, aggressive application of state-of-the-art technologies for the control of disease transmission would greatly facilitate use of foreign germplasm.

Equally important, however, is the fact that no organized program exists, either in the United States or elsewhere, to sample, evaluate, preserve, and use available sources of germplasm (3). Current research organizations do not have the resources to evaluate the many unique breeds that exist worldwide. Evaluations of animal germplasm could, however, focus on the present and foreseeable U.S. and world animal production and marketing environments and on the breeds that seem to have the greatest potential for improving animal food and fiber production systems (3).

For wild species, programs of development and utilization are much less clear, The rationale for preservation of such species largely reflects the need to maintain the Earth's ecological structure and, to many individuals, utilization of wild species is inconsistent with this goal. Yet products and processes observed in wild species have been and will continue to be of value to society. Armadillos, for example, provide a unique model of human leprosy. As the understanding of molecular genetics and cellular biology expands, the unique physiological and metabolic processes found in many wild animals are likely to have progressively more important research and development applications.

The domestication of wild animals is an emotional issue. It implies imposition of human control of the mating and husbandry of a previously wild species. To many people, this step is also inconsistent with the preservation of ecological diversity. However, the potential gains from developing adapted populations of previously wild animals to produce food and fiber in harsh or severely restricted environments may be too great to ignore. Thus, populations of red deer in Europe and New Zealand are rapidly becoming domesticated (10), and different species of deer are being crossed to improve production characteristics (32), Eland and oryx in Africa (47), capybara in South America (17), and crocodiles and butterflies in Papua New Guinea (33,34) are also being harvested in semicontrolled programs that may entail domestication of segments of these populations. In such a situation, domestication should not be avoided. Instead, great care must be taken to ensure that protected, viable wild populations are also maintained free of contamination from domesticated subpopulations. Such an approach, though difficult, is necessary to meet the joint goals of food production and maintenance of genetic diversity.

NEEDS AND OPPORTUNITIES

Needs and opportunities for maintaining animal diversity offsite involve both application of available technologies and development of new technologies, Needs differ considerably between wild and domestic animals, and these two groups will be considered separately. For wild animals, many of the needs involve adaptation of techniques that are currently available for domestic animals, In some cases, these adaptations are straightforward. In others, considerable basic research will be required. In almestic animals, efforts to assess and evaluate global genetic resources and facilitate their movement will probably assist in maintaining diversity. Mechanisms to monitor genetic diversity in domestic populations are also badly needed,

Wild Animals

Expertise in Relevant Areas

Maintenance of captive breeding populations oof wild animals requires that breeding programs be based on principles of quantitative genetic management to avoid losses in genetic

diversity. Likewise, a knowledge of the reproductive biology of the species is required to ensure efficient propagation of the animals in captivity. The need for expertise in these areas has increased dramatically as offsite programs have become more common and more complex. Efficient use of animals for genetic purposes requires extensive movement of germplasm among institutions. These efforts are likely to increasingly rely on transfer of semen or embryos, especially at the international level, placing a premium on scientific expertise.

To date, development of expertise in the application of reproductive biology and quantitative genetics management has largely occurred through the initiatives of individual students within traditional reproductive physiology or quantitative genetics programs. In reproductive physiology, programs are usually directed primarily toward domestic animals; efforts to obtain skills applicable to wild species maybe met at best with tolerance or at worst with active discouragement. Still, substantial interest in the reproductive biology of wild animals has been noted, and students of this field are increasingly tolerated. In quantitative genetics, training programs tend to emphasize either the theoretical aspects of quantitative genetics in natural populations or the applied aspects of breeding domestic animals. More opportunities to tailor courses to study of wild populations exist in this area than in reproductive physiology, however.

Fellowships and traineeships in areas that support maintenance of wild animal genetic diversity could be provided on a competitive basis to students in reproductive biology, cryobiology, population genetics, and animal behavior for studies applicable to the genetic and reproductive management of captive populations of wild animals. The program could be administered by the National Science Foundation (NSF). Emphasis would be placed on applying knowledge and theory to managed populations. One advantage of such a program would be sensitization of faculty members to the needs and opportunities in this area.

A grants program to allow selected educational institutions to expand their expertise in supporting maintenance of genetic diversity could be initiated. Grants could be awarded on a competitive basis and could support extension of applied programs to captive wild species. Such a program would be relatively expensive, however, and would tend to concentrate expertise instead of encouraging broad access to needed training.

Facilities for Offsite Maintenance

In recent years, zoo administrators and others have become aware of the need for well-planned breeding programs to ensure maintenance of genetic diversity within captive populations. Substantial theoretical work has gone into developing plans for existing or likely future facilities. The results suggest that today's facilities will not be sufficient to maintain desired levels of diversity. However, zoo personnel appear to have developed mechanisms to make choices (albeit not unanimous choices) among competing possibilities. Still, without additional facilities, losses of diversity appear likely.

Development of captive maintenance and breeding facilities could benefit from additional funding. Such a program would enhance capabilities to preserve biological diversity offsite. Modest levels of funding could have a considerable impact, although substantial funds would be required to address the total problem. Funds could be channeled through the National Zoo in Washington, DC, or through competitive grants to nonprofit zoological parks, Emphasis could be given to species that have limited captive facilities.

Reproductive Biology and Cryopreservation

The reproductive processes of most wild animals are not sufficiently understood to allow optimum rates of reproduction under captive management. This lack of information becomes especially acute in light of increasing interest in artificial insemination and embryo transfer because these technologies require much greater control of the reproductive process. Although the critical elements that control reproduction and cryopreservation in wild species are anal-

ogous to those in domestic animals, important differences exist. Thus, extending available knowledge about domestic animals to wild animals will require accumulation of information unique to each species or group of species. Optimum use of available individuals in programs of captive breeding or cryopreservation will depend on collecting this unique information.

Progress is being made in understanding the reproductive processes of wild animals, but not as quickly as it is needed or as it could be used. Without additional research, many available captive animals will continue to experience suboptimal fertility, and fewer total individuals of all species will be maintained at acceptable population sizes in available facilities. Semen from a number of wild species has already been frozen and exhibits near-normal motility and morphology when thawed, but its ability to result in conception is largely untested. Likewise, successful use of frozen embryos has occurred in only a few species.

A program of competitive grants to support research on the reproductive biology and cryopreservation of wild animals could be initiated. This program could be administered through NSF and would channel funds to both basic studies on the reproductive biology and cryobiology of wild animals and to applied studies of control of reproduction, artificial insemination and embryo transfer. preference could be given to existing programs that emphasize the integration of programs for wild and domestic animals.

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 onsite as well as extramural research with cooperating institutions, The centers could likewise serve as repositories for frozen gametes and embryos from endangered populations as techniques are perfected.

Basic Research in Population Biology and Genetics

Much of the basic theory of population genetics was derived in the first half of the 20th century and was adapted to applications in domestic animal breeding in the 1940s and 1950s. Current interest in developing breeding programs to maintain representative levels of genetic diversity within populations of minimum size has introduced several new program-design questions. These questions relate to such things as the amount and nature of genetic diversity that can be lost without compromising the long-term evolutionary potential of the species, the importance to evolutionary potential of rare genes (which are easily lost by genetic drift), the long-term importance of mutation to maintenance of diversity (22), and the importance of genetic diversity (both among and within species) to maintenance of the integrity of entire ecosystems. In many cases, these questions deal with validation of long-term quantitative genetics theory; answering them will require imaginative syntheses of the disciplines of genetics and ecology.

Some of the needed research is currently being done or has been planned. Without direction, however, it will occur in a piecemeal way, with no assurance that issues of the highest priority will be addressed. A program of competitive grants to support development, extension, and validation of quantitative genetic theory related to questions of maintaining biological diversity could be developed. This program could be administered through NSF and would require less funding (because of fewer equipment needs) than programs in reproductive biology or cryopreservation. Such a program could provide a focus for needed efforts in this area and a mechanism for screening competing proposals to identify those that address areas of highest priority.

Domestic Animals

Objective Assessment of Global Genetic Resources

The potential contributions of indigenous stocks of animal agriculture both in their coun-

try of origin and internationally needs to be assessed. Experience with the prolific Finnish Landrace and Booroola Merino sheep (31,36) and with Sahiwal cattle (24,48) has shown that local, specialized stocks can often have wide utility outside their country of origin. Likewise, comprehensive performance evaluations of crosses of indigenous and imported breeds suggest that local animals may make important contributions to final performance of the crossbreed (5). The use in West Africa of native cattle resistant to trypanosomiasis (23) is an important example. To assess the contributions of such breeds, objective information must be available to potential users. In many cases, some details exist but they are fragmented and difficult to locate and gain access to. In other cases, only anecdotal information is available.

Considerable international awareness of the need for such assessments exists. Efforts to at least list and broadly categorize breed resources have been initiated in Europe (30), Latin America (13), and Eastern Asia and Oceania (41), These efforts have been coordinated by the Food and Agriculture Organization (FAO) of the United Nations (14). Efforts in most of the developing countries have, however, been hampered by insufficient funding to develop electronic databases and library reference facilities. On balance, efforts to date deserve credit and have achieved some successes but are still insufficient.

No comparable assessment of breed resources has been undertaken for North America yet, so commissioning one would indicate support for efforts elsewhere and represent a minimal contribution by North American countries to a global accounting. The assessment could be coordinated by the National Academy of Sciences (NAS) or the U.S. Department of Agriculture (USDA) with technical support from relevant professional societies (American Society of Animal Science, American Dairy Science Association, Poultry Science Association, and Canadian Society of Animal Science) and private agencies (e.g., American Minor Breeds Conservancy). A recently initiated NAS project on global genetic resources could address domestic animal genetic resources and develop options for improving the present efforts.

Limited additional financial and technical support for development of databases and library reference facilities in existing foreign centers could be provided (14). In many cases, funds for microcomputers, software, and reference materials could provide a major improvement in the capabilities of existing institutions at limited cost. Necessary funds and consulting personnel could be channeled through USDA, FAO, or the U.S. Agency for International Development (AID).

Another approach could be the development of an international center for animal genetic resources that would be charged with maintenance of a comprehensive base of information on domestic animal germplasm resources. The center could maintain and update files on the status, trends, and characteristics of domestic breeds worldwide and provide information to potential users of this germplasm. Charges would be made to clients requesting information, but to function properly, considerable public subsidization would probably be required. The center could be a branch of USDA or a part of the National Agricultural Library. This plan has the potential disadvantage of moving responsibility for maintenance of the necessary databases out of national and regional institutions, or at least deemphasizing the roles of such institutions. Such an approach would tend to reduce the emphasis on breed evaluation and preservation at the grassroots level in the countries of origin.

Major new funding to support breed evaluation and characterization efforts could be provided, Even though considerable information already exists on many foreign breeds, the material is often fragmented and limited to only descriptive characteristics. The initiation and support of several major projects to objectively evaluate and compare indigenous breeds to potential imported breeds for the full array of productive traits in the country or region of origin would be a tremendous asset in terms of knowledge of global genetic resources. Funding could be channeled through USDA or AID.

Such a project would require major new funding, incIuding support for development of necessary foreign facilities.

Facilitation of International Movement of Germplasm

Effective use of global germplasm requires that mechanisms exist to facilitate the movement of such resources, This is especially important for specialized breeds in developing countries, such as prolific Chinese pigs (52), which may have utility in crossbreeding programs in industrial countries. The international movement of germplasm is often difficult because of different countries' health-related import-export requirements. This area involves both technologies for actual movement of germplasm (embryo transfer, semen and embryo collection, etc.) and technologies for prevention of disease transmission.

The United States currently maintains facilities for quarantine and disease-testing at Plum Island, NY, and Flemming Key, FL, These stations provide U.S. breeders access to foreign breeds. The approach taken has usually been to provide use of these facilities to importers in the private sector and to require that the cost of importation be borne completely by the importer, Importation of some breeds of sheep and swine has been supported by public (USDA) funds, but these cases are the exceptions. Assessing private sector importers for importation costs does allow the expense to be borne by those likely to receive economic benefit from the sale of imported animals, but it ignores the public benefits likely to accrue from access to foreign germplasm. When the decision to import a breed lies solely within the private sector, preference will be given to more traditional breeds judged to have the most speculative potential while unique breeds of undocumented value will usually be ignored,

USDA and the Animal and Plant Health Inspection Service (APHIS) could be directed to pursue an aggressive program of screening, importation, and evaluation of promising foreign breeds. Such a program would involve both a redirection of existing funds and appropriation of modest new funds. Such a program would recognize the existence of promising foreign breeds and likewise acknowledge that the procurement of these breeds is a matter of public interest. A considerable improvement in U.S. access to foreign germplasm could be accomplished through such a program with existing technology.

New funding for research and development on the diagnosis and neutralization of foreign diseases could be provided to APHIS and other research laboratories through a system of competitive grants. This new funding could be accompanied by a mandate to aggressively pursue importation of promising foreign germplasm into the United States. Objectives of the program would be, first, to validate and apply recently developed technologies for disease diagnosis (ELISA, DNA probes, etc.) and, second, to improve on and extend these technologies. Such a program should be able to accelerate access to foreign germplasm.

The training of foreign professionals in areas that support germplasm transfer could be supported. These areas would include veterinary pathology, reproductive biology, with emphasis on techniques for gamete and embryo collection and transfer, and cryobiology. In many cases, germplasm transfer is limited by insufficient expertise and facilities in the country of origin. An expanded training program for foreign students and professionals would increase the chances that the needed expertise existed onsite. Considerable opportunities for foreign professionals to receive this kind of training already exist, however. A major problem is that students receive sophisticated training in highly technical areas but have insufficient facilities and equipment to put their training to use when they return home.

The development and improvement of foreign centers for transfer of germplasm could be supported. This improvement would require new funding to allow development of centers in major geographical areas of the world. These centers could serve as focuses for a fui[range of considerations relating to maintenance of biological diversity. In particular, equipment

and expertise for collection, preparation for shipment, and preservation of gametes and embryos could be concentrated in such institutions. Facilities for quarantine and diagnostic testing using advanced technologies would greatly facilitate germplasm transfer. To be effective, these centers would have to be well funded and equipped on a continuing basis. Ideally, they would address a range of biological diversity issues, for wild as well as domestic animals, including maintenance of information centers and repositories for cryopreservation of frozen semen and embryos of rare native breeds.

Losses Of Genetic Diversity Among and Within Broods

Indiscriminate crossbreeding of so-called improved breeds from industrial nations coupled with increasing intensification within the poultry, swine, and dairy industries have resulted in reductions in global breed diversity and may lead to substantial losses of rare breeds. Within some of the major commercial breeds of livestock, losses in genetic diversity may also be occurring because of narrow selection goals and intensified use of individual sires and their sons through artificial insemination.

A National Board for Domestic Animal Resources could be established, composed of representatives from USDA, universities, private foundations, and industry. The board could provide a mechanism to coordinate animal germplasm conservation activities. The program could be established through a directive to a lead agency such as USDA and would not require additional legislation. Such aboard would identify potential sources of foreign germplasm for import and monitor the status of genetic diversity within commercial breeds. It could also monitor the status of rare breeds within the United States and make recommendations for their preservation and use. The board could act as a liaison with institutions in other countries and show a U.S. commitment to maintenance of domestic animal biological diversity. It would be primarily advisory in nature but should possess some funding to implement its recommendations to function effectively.

An International Board on Domestic Animal Resources could also be established. This board could provide international coordination of programs, set standards and coordinate the exchange and storage of germplasm, and provide funds to support activities in developing countries, probably at the regional level. Some efforts have already been made in this direction, and the United States could support and expand these efforts.

A program to identify, conserve, and use endangered breeds of potential value worldwide could be developed. It could identify rare breeds of potential value worldwide, with subsequent negotiation of procedures to protect and maintain the genetic integrity of these populations within the country of origin. If maintenance of such populations within the country of origin could not be assured, the United States could support collection and cryogenic storage of gametes and embryos. Semen of all mammalian livestock can be successfully frozen, as can embryos of all mammalian livestock species except the pig. Such storage could be located in this country to ensure maximum safet y of the preserved material, and it would include material that could not be imported as live animals under current animal health regulations. Efforts such as these would require close cooperation with the countries of origin of the various breeds to avoid the perception of exploitation of foreign resources for the sole benefit of the United States.

A program like this could also monitor the status of genetic diversity within commercial populations in the United States. This monitoring would involve interacting with industry to ensure maintenance of genetically diverse poultry control strains, retaining semen from a wide sample of dairy bulls as a reservoir of genetic diversity, and monitoring the status of other species.

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